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Expanding the Catalytic Repertoire of DNAzymes by Modified Nucleosides

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Abstract: Modified nucleoside triphosphates (dNTPs) are a versatile platform for the generation of high-density functionalized nucleic acids. The enzymatic polymerization of dNTPs allows the introduction of sensible functionalities that might not be compatible with the standard automated synthesis of oligonucleotides. Their application to *in vitro* selections, an elegant chemical approach to Darwinian evolution, delivers modified aptamers and catalytic nucleic acids with potentially enhanced properties. This review article highlights some recent synthetic examples of dNTPs bearing functionalities that are either found in the active site of protein enzymes or have been employed in organocatalysis and further underscores their usefulness in the development of some modified catalytic nucleic acids with special emphasis on M^{2+}-independent RNA-cleaving DNAzymes.

Keywords: Base-modified DNA · DNAzymes · Enzyme-mimetic · Nucleoside triphosphates · SELEX

1. Introduction

DNA is often thought to be a functionally devoid entity merely acting as the repository of genetic information. However, this view is certainly simplistic and deceiving as underscored by the staggering increase of nucleic acid-based applications developed over the last decades. Moreover, thinking of nucleic acids (and DNA specifically) as catalytic entities might cause some bafflement, especially when considering that in biology the predominant catalysts are proteins. Notwithstanding the confusion this idea might infuse, SELEX[1] and related combinatorial methods of *in vitro* selections have allowed for the discovery of myriad unnatural catalytic RNAs (ribozymes)[2] and DNAs (DNAzymes or Dz).[3–7] Indeed, catalytic nucleic acids have been crafted to catalyze a surprising variety of reactions including DNA hydrolysis,[8,9] porphyrin metallation,[10] thymine dimer photoreversion,[11,12] carbon-carbon bond formation (Diels-Alder[13–15] and aldol condensation[16]) and RNA-cleavage.[17–20] However, despite this respectable catalytic repertoire, RNA-cleaving DNAzymes are still the best described and most fully characterized catalytic DNA molecules, mainly because of the ease of selection[17,20] and their potential applications as biosensors[21–24] and therapeutic gene silencing agents through sequence-specific mRNA degradation.[7,25] Thus, natural[26] and especially artificial Darwinian evolution processes[27] have led to the identification of numerous catalytic nucleic acids that might be pivotal for many applications. Unfortunately, nucleic acid based catalysts generally, and DNAzymes specifically, still compare unfavorably to protein enzymes in terms of catalytic efficiency and variety of reactions catalyzed. Moreover, catalytic nucleic acids have a limited array of functional groups, especially when compared to the impressive arsenal used by protein enzymes. This dearth of functionality is often blamed for their rather limited catalytic efficiency along with a limited number of reaction classes catalyzed.

The paucity of functional groups is often mitigated by the use of divalent metal cations (M^{2+}) as cofactors. Indeed, most of the selected DNAzymes require M^{2+} cations for optimal activity and, at higher concentrations (*e.g.* 10–25 mM Mg^{2+}) can display remarkable diffusion-controlled catalytic efficiencies (k_{cat}/K_m ~10^9 $M^{-1}min^{-1}$) that rival protein enzymes.[18,28] However, while high M^{2+} concentrations ensure optimal catalytic activity of many deoxyribozymes, this strong necessity concurrently undermines their efficiency for potential *in vivo* applications.

A way to circumvent these drawbacks is by grafting additional functional groups on the nucleobases of known DNAzymes in order to compensate for lower M^{2+} concentrations and/or the lack of reactive functionalities that could support efficient catalysis. In this context, the appendage of amino acid-like moieties[29,30] on nucleotides of the catalytic core or substrate binding regions of known DNAzymes (Dz10-23 and 8-17) or the incorporation of intercalators covalently attached to the phosphate backbone[31] resulted in modified DNAzymes with improved kinetic properties. Whilst this strategy might lead to an enhancement of the catalytic efficiency of DNAzymes, promote catalysis in M^{2+}-independent media, and increase the resistance towards nucleases, inherent knowledge of the role of each nucleotide involved in the catalytic core would be considered important to guaranteeing success in this approach. Indeed, many DNAzymes are sensitive to even minute modifications and alterations made to their catalytic core often lead to a total ablation of catalytic activity.[32,33] Consequently, the effect of modified nucleotides on the cleavage efficiency has to be studied systematically and combinatorially, rather than by rationally designing better catalysts through the appendage of chemical functionalities.

The enzymatic polymerization of modified nucleoside 5'-triphosphates (dNTPs) *in lieu* of their natural counterparts represents a very alluring and versatile way to generate modified nucleic acids.[34,35] This methodology has been successfully applied to the elaboration of highly functionalized nucleic acids,[36–40] aptamers,[41–46] and ribozymes.[47–50] Examples of modified DNAzymes are also known. Indeed, trading the natural dTTP for a C5-imidazole-functionalized nucleoside triphosphate in the *in vitro* selection process, led to the identification of a Zn^{2+}-dependent

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DNAzyme that could sequence-specifically cleave RNA substrates under multiple turnover conditions.[51] More recently, the synergy of two dNTPs embellished with imidazole and primary cationic amine side chains was exploited in the selection of an RNA-cleaving DNAzyme that operates in M^{2+}-independent media.[52] The success of *in vitro* selection experiments with modified dNTPs heavily relies on their acceptance by template-dependent DNA polymerases for the synthesis of the functionalized dsDNA under investigation, which in turn must be a competent template in a PCR in order to reinstate an unmodified DNA population that will be engaged in subsequent rounds of selection.[53]

The purpose of this article is to describe the aim of my current and future work in this emerging subject. To do so, the progress made in the *in vitro* selection of modified DNAzymes and the concomitant (yet) unsolved challenges will be described. More precisely, the synthesis of modified dNTPs adorned with amino acid-like side chains mimicking the residues found at the active sites of numerous protein enzymes will be portrayed. Moreover, the derivatization of dNTPs with functionalities of crucial importance in the field of organocatalysis will also be mentioned. Finally, the steps ultimately leading to the disclosure of DNAzymes will be highlighted and the properties of some recently selected modified deoxyribozymes outlined.

2. Synthesis of Modified Nucleoside Triphosphates

A wealth of examples of modified dNTPs has inundated the recent literature[34] and most of the modifications are usually appended at the C(5) position of the pyrimidine ring or anchored at the N(7) of a 7-deazapurine nucleotide.[54,55] These sites are particularly advantageous since the side chains will protrude into the major groove of dsDNA and will not disrupt Watson-Crick base-pairing. In addition, dNTPs modified at these locations have proven to be good substrates for DNA polymerases both in primer-extension reactions and PCR.[56] However, alterations are not restricted to these particular sites and successful enzymatic polymerizations of 8-substituted purine nucleotides[39,55,57] and base-modified dNTPs[58,59] have also been reported.

Even though diverse methods exist for the synthesis of nucleoside triphosphates,[60] most rely on multistep 'one-pot' processes that involve the trapping of an activated monophosphate derivative by the pyrophosphate ion. Indeed, in the Yoshikawa procedure,[61,62] the unprotected nucleoside is treated directly with phosphorous oxychloride ($POCl_3$), which yields a highly reactive phosphorodichloridate intermediate that can be reacted *in situ* with the tributylammonium salt of pyrophosphate to afford the corresponding triphosphate. The procedure developed by Ludwig and Eckstein is another elegant method that often leads to high yields and few side products.[63] Briefly, the 3'-O-protected nucleosides are reacted with salicyl phosphorochlorite, which is active enough to specifically phosphorylate the hydroxyl groups. Addition of pyrophosphate then displaces the salicylate and subsequent oxidation with I_2 of the intermediate phosphite yields the corresponding triphosphate. Finally, modified dNTPs are often obtained by derivatization of commercially available or readily synthesized precursors. In this context, appendage of the modifications can be achieved by Sonogashira or Suzuki coupling reactions with the corresponding iodo- and bromo-functionalized dNTPs.[40,64] Moreover, functional side chains can be introduced by amide bond formation by reacting the corresponding activated esters with a primary amine supported by the linker arm of a dNTP.[65,66]

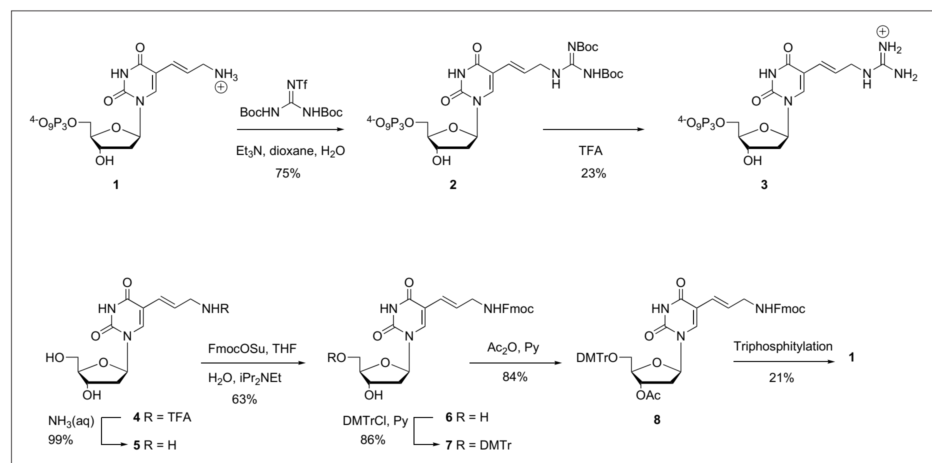
The synthesis of the guanidinylated triphosphate dU^{ga}TP **3** (Scheme 1), which was engaged in an *in vitro* selection using *three* modified dNTPs, is a good illustration of the strategy involving the derivatization of a precursor dNTP.[67] Indeed, dNTP **3** was obtained by guanidinylation of the commercially available dU^{aa}TP **1**, followed by deblocking of the Boc-protecting groups by TFA. Recently, an optimized synthetic route for dU^{aa}TP **1** was elaborated (Scheme 1), which included a protection group swap from TFA to a more suitable and labile Fmoc.[68] The triphosphitylation was then performed by application of the Ludwig and Eckstein protocol.

Furthermore, dU^{aa}TP **1** proves to be a versatile stepping-stone for the generation of modified triphosphates since it reacts smoothly with a variety of *N*-hydroxysuccinimide esters (Scheme 2).[65] By application of this methodology, a *cis*-aminoproline residue could be installed on dNTP **9**, which can be used in selections for DNAzymes that could catalyze the aldol, Mannich, and/or Michael reactions.[69] Similarly, a dipeptide residue could be deposited on the linker arm of dNTP **10**.[68] Considering that prolinamides and proline-based dipeptides, especially when in synergy with the amino acid phenylalanine, are revealed to be potent organocatalysts for a broad range of reactions, the use of dNTP **10** could lead to DNAzymes that act as organocatalysts. Finally, dNTP **11**, obtained by application of the same synthetic route, bears a guanidinocarbonyl pyrrole (Gcp) moiety, which was conceived as an improved motif for the binding of oxyanions[70,71] and was soon revealed to be an effulgent synthon. Indeed, by weaving an extended hydrogen bond network, Gcp-units introduced in a synthetic polypeptide showed transition-state stabilization and acid-base catalysis.[72] Thus, dNTP **11** is an alluring candidate for the development of peptide-cleaving DNAzymes, especially when used in conjunction with dNTPs bearing carboxylic acid residues and/or with M^{2+}.[73]

The compatibility of the modified dNTPs **9–11** with *in vitro* selection methods is currently being evaluated.

3. *In vitro* Selection of Modified DNAzymes

The identification of modified DNAzymes occurs by application of an *in vitro* selection protocol that is highly similar to the one used for unmodified nucleic acids and is highlighted in Scheme 3 for an RNA-cleaving DNAzyme.[7,18,74] Briefly, the initial oligonucleotide population consists of a template containing a randomized region (*e.g.* N_{20} or N_{40}) flanked by fixed



Scheme 1. Synthesis of modified nucleoside triphosphates **1** (dU^{aa}TP) and **3** (dU^{ga}TP).

Scheme 2. Derivatization of dU^{aa}TP **1** with NHS-esters.

Scheme 3. Schematic illustration of a typical *in vitro* selection using modified dNTPs to generate RNA-cleaving DNAzymes.

regions that are necessary for the docking of the PCR primers. After annealing of the 5'-biotinylated primer comprising the scissile ribonucleoside substrate onto the template strand, the modified dNTP along with the three natural triphosphates are then enzymatically polymerized on the primer-template duplex. After immobilization of the modified dsDNA on streptavidin magnetic particles, the unmodified template strand is removed. The resulting modified ssDNA is then subjected to the suitable reaction conditions and only the catalytically active sequences will elute from the solid support. The catalytically active single stranded modified DNAs are then amplified by application of a double nested PCR amplification method and used as templates for subsequent rounds of selection.[18,74] Higher catalytic efficiencies of the resulting DNAzymes can be enticed by increasing the stringency of the selection through variation of one or more parameters (*e.g.* incubation time, buffer composition, temperature, *etc.*). The efficiency can be gauged by monitoring the cleavage activity of the populations of the generations throughout the selection process. Individual DNA sequences are cloned and subsequently sequenced when increases in the stringency are of no avail and the activity stalls and reaches a plateau. The most active clone is then pinpointed by an initial kinetic survey of the resulting individual sequences and used for further characterization or converted to a *trans*-acting (*i.e.* intermolecularly) species.

3.1 *Dz10-13: A Highly Selective DNAzyme Sensor for Hg^{2+}*

Application of the aforementioned selection protocol with dA^{im}TP **13** (Scheme 4) and dU^{aa}TP **1** *in lieu* of their natural counterparts, led to the discovery of Dz9$_{25}$-11c.[74] This DNAzyme, which could be converted to a *trans*-cleaving species Dz9$_{25}$-11t,[75] catalyzes the M^{2+}-independent cleavage of a single embedded ribophosphodiester linkage and acts as a good mimic of RNase A through the combined use of the cationic amine and imidazole residues of the side chains which bestow acid-base catalysis and electrostatic stabilization of the negative charge build-up respectively (*vide infra*).[76,77] Moreover, the catalytic activity of Dz9$_{25}$-11c (Fig. 1A) was found to be heavily impaired by the presence of Hg^{2+} due to the binding of the metal cation to the soft imidazole ligands of the side chains, thus opening up the possibility of using Dz9$_{25}$-11c as a mercuric sensor.[78] However, for a Hg^{2+}-sensor to be effective, the following criteria must be fulfilled: high sensitivity in order to detect even trace amounts of mercury (picomolar to low nanomolar concentrations), water solubility, impeccable selectivity with no interference from other cations such as Cu^{2+} and functioning in the 'turn-on' mode (*i.e.* increase of the signal in the presence of the analyte).[21,79] Since the catalytic activity of Dz9$_{25}$-11c is vitiated by the presence of Cu^{2+} and only operates in a 'turn-off' mode, an *in vitro* selection for a more effective DNAzyme sensor for Hg^{2+} was carried out. The selection protocol was similar to the one used for Dz9$_{25}$-11, albeit with a longer randomized region (N$_{40}$ vs. N$_{20}$) to allow for the incorporation of a sufficient number of modified nucleotides such that both binding to the Hg^{2+} and catalysis would be ensured.[80] In addition, a strong negative selection was included to avoid the leakage of sequences that could self-cleave in the presence of Mg^{2+}, Cu^{2+} and Zn^{2+} or in the absence of M^{2+} in the population of later generations. Cloning of the population of the 10th generation and an initial kinetic assessment of the resulting individual molecules led to the disclosing of Dz10-13 (Fig. 1B), which self-cleaves at a rate of k_{obs} ~0.04 min^{-1} in the presence of 5 μM Hg^{2+} (*i.e.* the concentration used in the selection buffer). In addition, Dz10-13 supported cleavage over a broad range of Hg^{2+} concentrations and a K_d of ~1 μM was estimated. The mercuric cations adopt a dichotomic role in that they are necessary for activity but simultaneously inhibit catalysis at higher Hg^{2+} concentrations. Moreover, when the Hg^{2+} cations were swapped for one of 16 plausible competing metal cations, no catalytic activity could be observed, demonstrating the high selectivity of Dz10-13 for mercuric cations. Not surprisingly, when the modified dNTPs were replaced by their natural counterparts, no catalytic activity could be discerned, hence showing that the modified nucleosides are pivotal and vital for catalysis. In addition, when dA^{imp}TP **14** (Scheme 4), which has an additional methylene in the linker arm connecting the imidazole moiety to the nucleobase, was polymerized on the template instead of dA^{im}TP **13**, the catalytic activity was completely abolished as well, revealing that even minute alterations to the chemical environment of Dz10-13 has fatal consequences for catalysis.

3.2 *RNA-cleaving DNAzymes in the Absence of M^{2+}*

Artificial ribonucleases have received considerable attention due to their high potential for numerous biotechnological applications including their use as catalytic antisense oligonucleotides. In this context DNAzymes have been upheaved as prime candidates. The most prominent and proficient DNAzymes, Dz10-23 and Dz8-17, cleave RNA with a remarkable efficiency and selectivity.[18] However, the range of M^{2+}-concentrations required for the optimal activity of these DNAzymes exceeds by far that found in cells and thus impedes their efficacy for the selective catalytic cleavage of intracellular mRNA. $Dz9_{25}$-11 was selected to circumvent this constraining requirement for high M^{2+}-concentrations and indeed, displays remarkable kinetic properties for M^{2+}-independent RNA-cleavage: k_{obs} ~0.3 min^{-1} for self-cleavage at 13 °C and k_{cat} ~0.03 min^{-1} under multiple turnover conditions at 25 °C.[74–76] However, despite these encouraging assets, the low temperature optima and the insufficient catalytic rates at 37 °C were still cause for concern. Moreover, since reselecting using templates of various lengths failed to provide an improved catalytic motif, it was deemed that increasing the chemical diversity rather than the sequence space might be beneficial in the *in vitro* selection process. Accordingly, the simultaneous enzymatic polymerization of *three* modified dNTPs bearing an imidazole ($dA^{im}TP$ **13**, Scheme 4), a cationic guanidine ($dU^{ga}TP$ **3**, Scheme 1) and a cationic amine ($dC^{aa}TP$ **15**, Scheme 4) generated an initial population that was used in an *in vitro* selection that culminated with the discovery of Dz9-86 (Fig. 1C).[81] This highly functionalized DNAzyme self-cleaves an embedded ribocytosine with a rate constant k_{obs} = 0.13 min^{-1} which compares to that observed for $Dz9_{25}$-11c at 13 °C (k_{obs} ~0.3 min^{-1}). Even though Dz9-86 was selected at room temperature, the rate constant increased steadily with the temperature before reaching a maximum at ~37 °C. This behavior deviates markedly from the temperature dependence of $Dz9_{25}$-11 (both the *cis* and *trans*-cleaving species) where the rate constant significantly diminished with increasing temperature. The pH-rate profile of Dz9-86 adopted a typical bell-shape that is consistent with a two-step protonation-deprotonation mechanism, most likely involving two imidazole residues engaged in general acid and general base catalysis. Moreover, Dz9-86 showed a high tolerance to physiologically relevant metal ions since for instance Mg^{2+} and Ca^{2+} had little effect on the rate of self-cleavage. On the other hand, whereas a certain number of transition metals completely abolished the catalytic activity of Dz9-86, they did so at a physiologically irrelevant concentration (0.5 mM). Unsurprisingly, Hg^{2+} tightly bound the soft imidazole ligands (K_d^{APP} ~80 nM) and killed the catalytic activity. Ultimately, Dz9-86 was completely indifferent to the nature and the concentration of the monovalent salt employed, an effect possibly emerging from the stabilizing effect of the cationic guanidinium residues. While the overall properties of Dz9-86 represent a major improvement compared to those of $Dz9_{25}$-11, especially in terms of cleavage under physiologically relevant conditions (*i.e.* low salt concentrations, pH 7.4, 37 °C and presence of Mg^{2+} and Ca^{2+}), the rate constants for self-cleavage still should be improved and Dz9-86 needs to be engineered to a *trans*-cleaving species capable of multiple turnover.[81] Consequently, an *in vitro* selection was initiated utilizing greater sequence space (N_{40} vs. N_{20}) in order to favor potentially more active sequences with longer catalytic motifs, while maintaining the same chemical diversity *via* the combined use of the three modified dNTPs **3**, **13** and **15**.[67] A strong catalytic activity was observed at an early stage of the selection (3% cleavage after 60 min in round 2 already), which steadily increased throughout the generations. After cloning and sequencing of the 10th generation, Dz10-66 arose as the most potent of all the individual clones analyzed (Fig. 1D). The 44 nucleotide-long DNAzyme Dz10-66c presented the highest M^{2+}-independent rate for self-cleavage of a single ribophosphodiester linkage (k_{obs} >0.5 min^{-1}) reported for any type of nucleic acid catalyst. As observed in the case of Dz9-86, the rate constant further increased with temperature to reach a staggering 0.6 min^{-1} at 37 °C. Moreover, analysis of the bell-shaped pH-rate profile yielded pK_a values of 7.5 and 6.6, which are consistent with a two-step protonation-deprotonation mechanism. Although these values are lower than those found for Dz9-86, they still hint at the possible intervention of imidazole residues in

Scheme 4. Chemical structures of **12** ($dA^{imm}TP$), **13** ($dA^{im}TP$), **14** ($dA^{imp}TP$), **15** ($dC^{aa}TP$) and **16**.

Fig. 1. Sequence and hypothetical 2D structures of DNAzymes: A) $Dz9_{25}$-11t; B) Dz10-13; C) Dz9-86; D) Dz10-66t. Bold-face letters represent modified nucleosides and only the sequence of the catalytic core is explicitly indicated. X = C during the selection (cognate substrate) and X = G for multiple turnover experiments (near-cognate substrate).

general acid and general base catalysis. The beneficial stabilization of the cationic guanidinium residues was observed when Dz10-66c was assayed in a minimal buffer with no added salt (*i.e.* 1 mM EDTA, 5 mM phosphate, pH 7.4), conditions under which only protein enzymes such as RNase A are able to demonstrate any form of catalytic activity, since Dz10-66c maintained a baffling high rate constant of k_{obs} = 0.1 min^{-1}. Dz10-66c was then enzymatically converted to a *trans*-cleaving species Dz10-66t (Fig. 1D) by means of a primer extension reaction in which a biotinylated template and a truncated primer were used. Surprisingly, Dz10-66t was unable to cleave its cognate substrate (X = C in Fig. 1D) and strong catalytic activity was only detected when the dC·dC^{aa} mismatch was replaced by the corresponding Watson-Crick dG·dC^{aa} base pair (X = G, Fig. 1D) and the length of the substrate was slightly increased.[67] A catalytic efficiency (k_{cat}/K_m) of 6×10^5 $M^{-1}min^{-1}$ was observed under multiple turnover conditions at room temperature and in the absence of M^{2+}, an efficiency that compared with that of some Mg^{2+}-dependent DNAzymes. Moreover, addition of 0.2 mM Mg^{2+} had little effect on the second-order rate constant, but was a necessary additive for catalysis at 37 °C.

The choice of the nature of the linker arm is of crucial importance since it can affect both the catalytic efficiency of a modified DNAzyme and the substrate properties of the modified dNTPs for polymerases.[82] Indeed, while longer linker arms might ensure a good uptake by polymerases in a PCR and a primer-template extension reaction, shorter linkers could lessen the entropic penalty occasioned by the forced positioning of the modification and hence lead to catalytically more active nucleic acids. Furthermore, the stereochemistry and the chemical nature of the linker arm (*e.g.* alkane vs. alkynes or alkenes) might have a dramatic repercussion on the polymerase uptake of certain modified dUTPs,[82] but at the same time modified dATPs are indifferent to this issue.[83] The question of the effect of the length of the linker arm on the efficiency of a catalytic nucleic acid was tackled with the selection of Dz20-49.[84] Indeed, while dA^{imm}TP **12** (which has one methylene unit less in the linker arm than **13**) was used instead of dA^{im}TP **13**, all aspects and conditions that had been used in the selection of Dz10-66c were maintained, thus hoping to generate a good mimic of Dz10-66c that would reveal the effect of the length of the linker arm on catalysis. The build-up of activity was rather sluggish and after 20 rounds of selection, cloning and sequencing allowed for the identification of DNAzyme Dz20-49, which self-cleaves a single ribophosphodiester linkage with a rate constant k_{obs} ~$3\cdot10^{-3}$ min^{-1} in the absence of M^{2+}; a ~200 fold decrease in activity as compared to Dz10-66c.[84] Despite using a modified dNTP with a shortened linker arm that could adequately guide and conformationally restrict the catalytically active imidazole residues into a favorable position, a depletion *rather* than an enhancement of the catalytic activity was observed for Dz20-49.

Finally, a recent effort was devoted to the use of the phenol-modified dNTP **16** in an *in vitro* selection of an RNA-cleaving DNAzyme.[66] The resulting DNAzyme, Dz11-17PheO, self-cleaves a single embedded ribocytosine residue with a rate constant of k_{obs} = 0.2 min^{-1} in the presence of 1 mM Zn^{2+} and 10 mM Mg^{2+} at 24 °C. The appreciable catalytic activity of Dz11-17PheO strongly depends on the presence of the tyrosine-like residues of the modified nucleosides and was inhibited by the presence of Hg^{2+} and Eu^{3+} probably through the formation of T-Hg^{2+}-T base pairs and phenoxide-Eu^{3+} interactions respectively.[66]

4. Conclusions and Future Direction

The heart of my present work resides in the synthesis of modified nucleoside triphosphates for their further use in selections to generate DNAzymes with enhanced catalytic properties and a broader palette of reactions. The examples of *in vitro* selected modified DNAzymes are scarce so far and their catalytic activity is sometimes comparable but often only marginally superior to that of unmodified nucleic acid catalysts. However, in light of the results obtained for M^{2+}-independent RNA-cleaving DNAzymes briefly described in this review, the use of modified nucleoside triphosphates in the context of *in vitro* selections will hopefully help to fulfill one of the 'Holy Grails' of chemical biology, namely the extension of the catalytic repertoire of (nucleic acid) catalysts and increase their rate enhancements to a comparable level to that of protein enzymes.[85,86]

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[1] G. F. Joyce, *Angew. Chem. Int. Ed.* **2007**, *46*, 6420.
[2] G. F. Joyce, *Annu. Rev. Biochem.* **2004**, *73*, 791.
[3] S. K. Silverman, *Nucleic Acids Res.* **2005**, *33*, 6151.
[4] S. K. Silverman, *Chem. Commun.* **2008**, *30*, 3467.
[5] D. A. Baum, S. K. Silverman, *Cell. Mol. Life Sci.* **2008**, *65*, 2156.
[6] K. Schlosser, Y. Li, *Chem. Biol.* **2009**, *16*, 311.
[7] S. K. Silverman, *Angew. Chem. Int. Ed.* **2010**, *49*, 2.
[8] Y. Xiao, M. Chandra, S. K. Silverman, *Biochemistry* **2010**, *49*, 9630.
[9] M. Chandra, A. Sachdeva, S. K. Silverman, *Nat. Chem. Biol.* **2009**, *5*, 718.
[10] Y. Li, D. Sen, *Nat. Struct. Biol.* **1996**, *3*, 743.
[11] D. J. Chinnapen, D. Sen, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 65.
[12] R. E. Thorne, D. J. Chinnapen, G. S. Sekhorn, D. Sen, *J. Mol. Biol.* **2009**, *388*, 21.
[13] B. Seelig, A. Jäschke, *Chem. Biol.* **1999**, *6*, 167.
[14] B. Seelig, S. Keiper, F. Stuhlmann, A. Jäschke, *Angew. Chem. Int. Ed.* **2000**, *39*, 4576.
[15] M. Chandra, S. K. Silverman, *J. Am. Chem. Soc.* **2008**, *130*, 2936.
[16] S. Fusz, A. Eisenfuhr, S. G. Srivatsan, A. Heckel, M. Famulok, *Chem. Biol.* **2005**, *12*, 941.
[17] R. R. Breaker, G. F. Joyce, *Chem. Biol.* **1994**, *1*, 223.
[18] S. W. Santoro, G. F. Joyce, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 4262.
[19] J. Li, W. Zheng, A. H. Kwon, Y. Lu, *Nucleic Acids Res.* **2000**, *28*, 481.
[20] K. Schlosser, Y. Li, *ChemBioChem* **2010**, *11*, 866.
[21] J. Liu, Y. Lu, *Angew. Chem. Int. Ed.* **2007**, *46*, 7587.
[22] J. Liu, A. K. Brown, X. Meng, D. M. Cropek, J. D. Istok, D. B. Watson, Y. Lu, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 2056.
[23] M. M. Ali, S. D. Aguirre, H. Lazim, Y. Li, *Angew. Chem. Int. Ed.* **2011**, *50*, 3751.
[24] X.-B. Zhang, R.-M. Kong, Y. Lu, *Annu. Rev. Anal. Chem.* **2011**, *4*, 105.
[25] C. R. Dass, *Trends Pharmacol. Sci.* **2004**, *25*, 395.
[26] J. A. Doudna, T. R. Cech, *Nature* **2002**, *418*, 222.
[27] R. R. Breaker, *Nature* **2004**, *432*, 838.
[28] M. Bonaccio, A. Credali, A. Peracchi, *Nucleic Acids Res.* **2004**, *32*, 916.
[29] D. Smuga, K. Majchrzak, E. Sochacka, B. Nawrot, *New J. Chem.* **2010**, *34*, 934.
[30] C. H. Lam, D. M. Perrin, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5119.
[31] H. Asanuma, H. Hayashi, J. Zhao, X. Liang, A. Yamazawa, T. Kuramochi, D. Matsunaga, Y. Aiba, H. Kashida, M. Komiyama, *Chem. Commun.* **2006**, 5062.
[32] Z. Zaborowska, J. P. Fürste, V. A. Erdmann, J. Kurreck, *J. Biol. Chem.* **2002**, *43*, 40617.
[33] S. Schubert, D. C. Gül, H.-P. Grunert, H. Zeichhardt, V. A. Erdmann, J. Kurreck, *Nucleic Acids Res.* **2003**, *31*, 5982.
[34] M. Hocek, M. Fojta, *Org. Biomol. Chem.* **2008**, *6*, 2233.
[35] S. Franzen, *Curr. Opin. Mol. Ther.* **2010**, *12*, 223.
[36] O. Thum, S. Jäger, M. Famulok, *Angew. Chem. Int. Ed.* **2001**, *40*, 3990.
[37] S. Jäger, G. Rasched, H. Kornreich-Leshem, M. Engeser, O. Thum, M. Famulok, *J. Am. Chem.*

Soc. **2005**, *127*, 15071.
[38] S. Jäger, M. Famulok, *Angew. Chem. Int. Ed.* **2004**, *43*, 3337.
[39] P. Capek, H. Cahová, R. Pohl, M. Hocek, C. Gloeckner, A. Marx, *Chem. Eur. J*. **2007**, *13*, 6196.
[40] A. Macícková-Cahová, R. Pohl, P. Horáková, L. Havran, J. Capek, M. Fojta, M. Hocek, *Chem. Eur. J*. **2011**, *17*, 5833.
[41] J. A. Latham, R. Johnson, J. J. Toole, *Nucleic Acids Res.* **1994**, *22*, 2817.
[42] T. R. Battersby, D. N. Ang, P. Burgstaller, S. C. Jurczyk, M. T. Bowser, D. D. Buchanan, R. T. Kennedy, S. A. Benner, *J. Am. Chem. Soc*. **1999**, *121*, 9781.
[43] M. M. Masud, M. Kuwahara, H. Ozaki, H. Sawai, *Bioorg. Med. Chem*. **2004**, *12*, 1111.
[44] A. Shoji, M. Kuwahara, H. Ozaki, H. Sawai, *J. Am. Chem. Soc*. **2007**, *129*, 1456.
[45] K. Ohsawa, T. Kasamatsu, J.-I. Nagashima, K. Hanawa, M. Kuwahara, H. Ozaki, H. Sawai, *Anal. Sci.* **2008**, *24*, 167.
[46] J. D. Vaught, C. Bock, J. Carter, T. Fitzwater, M. Otis, D. Schneider, J. Rolando, S. Waugh, S. K. Wilcox, B. E. Eaton, *J. Am. Chem. Soc.* **2010**, *132*, 4141.
[47] T. M. Tarasow, S. L. Tarasow, B. E. Eaton, *Nature* **1997**, *389*, 54.
[48] T. W. Wiegand, R. C. Janssen, B. E. Eaton, *Chem. Biol*. **1997**, *4*, 675.
[49] D. Nieuwlandt, M. West, X. Cheng, G. Kirschenheuter, B. E. Eaton, *ChemBioChem* **2003**, *4*, 651.
[50] K. T. Gagnon, S.-Y. Ju, M. B. Goshe, E. S. Maxwell, S. Franzen, *Nucleic Acids Res.* **2009**, *37*, 3074.
[51] S. W. Santoro, G. F. Joyce, K. Sakthivel, S. Gramatikova, C. F. Barbas, *J. Am. Chem. Soc.* **2000**, *122*, 2433.
[52] A. V. Sidorov, J. A. Grasby, D. M. Williams, *Nucleic Acids Res*. **2004**, *32*, 1591.
[53] R. Kranaster, A. Marx, *ChemBioChem* **2010**, *11*, 2077.
[54] A. Baccaro, A. Marx, *Chem. Eur. J.* **2010**, *16*, 218.
[55] C. Lam, C. Hipolito, D. M. Perrin, *Eur. J. Org. Chem*. **2008**, 4915.
[56] D. Loakes, P. Holliger, *Chem. Commun*. **2009**, 4619.
[57] D. M. Perrin, T. Garestier, C. Hélène, *Nucleosides Nucleotides* **1999**, *18*, 377.
[58] S. G. Srivatsan, Y. Tor, *Chem Asian J*. **2009**, *4*, 419.
[59] M. Kimoto, T. Mitsui, S. Yokoyama, I. Hirao, *J. Am. Chem. Soc.* **2010**, *132*, 4988.
[60] K. Burgess, D. Cook, *Chem. Rev.* **2000**, *100*, 2047.
[61] M. Yoshikawa, T. Kato, T. Takenishi, *Tetrahedron Lett.* **1967**, 5065.
[62] V. Borsenberger, M. Kukwikila, S. Howorka, *Org. Biomol. Chem*. **2009**, *7*, 3826.
[63] J. Ludwig, F. Eckstein, *J. Org. Chem*. **1989**, *54*, 631.
[64] P. Kielkowski, R. Pohl, M. Hocek, *J. Org. Chem*. **2011**, *76*, 3457.
[65] K. Sakthivel, C. F. Barbas, *Angew. Chem. Int. Ed.* **1998**, *37*, 2872.
[66] C. H. Lam, C. J. Hipolito, M. Hollenstein, D. M. Perrin, *Org. Biomol. Chem*. **2011**, DOI: 10.1039/c1030xx00000x.
[67] M. Hollenstein, C. J. Hipolito, C. H. Lam, D. M. Perrin, *ChemBioChem* **2009**, *10*, 1988.
[68] M. Hollenstein, **2011**, manuscript in preparation.
[69] B. List, *Tetrahedron* **2002**, *58*, 5573.
[70] C. Schmuck, T. Rehm, L. Geiger, M. Schäfer, *J. Org. Chem.* **2007**, *72*, 6162.
[71] T. Rehm, C. Schmuck, *Chem. Commun*. **2008**, 801.
[72] N. J. V. Lindgren, L. Geiger, J. Razkin, C. Schmuck, *Angew. Chem. Int. Ed.* **2009**, *48*, 6722.
[73] J. Suh, *Acc. Chem. Res.* **2003**, *36*, 562.
[74] D. M. Perrin, T. Garestier, C. Hélène, *J. Am. Chem. Soc.* **2001**, *123*, 1556.
[75] L. Lermer, Y. Roupioz, R. Ting, D. M. Perrin, *J. Am. Chem. Soc*. **2002**, *124*, 9960.
[76] R. Ting, J. M. Thomas, L. Lermer, D. M. Perrin, *Nucleic Acids Res.* **2004**, *32*, 6660.
[77] R. Ting, J. M. Thomas, D. M. Perrin, *Can. J. Chem*. **2007**, *85*, 313.
[78] J. M. Thomas, R. Ting, D. M. Perrin, *Org. Biomol. Chem.* **2004**, *2,* 307.
[79] J. Liu, Z. Cao, Y. Lu, *Chem. Rev.* **2009**, *109*, 1948.
[80] M. Hollenstein, C. Hipolito, C. Lam, D. Dietrich, D. M. Perrin, *Angew. Chem. Int. Ed*. **2008**, *47*, 4346.
[81] M. Hollenstein, C. J. Hipolito, C. H. Lam, D. M. Perrin, *Nucleic Acids Res*. **2009**, *37*, 1638.
[82] S. E. Lee, A. V. Sidorov, T. Gourlain, N. Mignet, S. J. Thorpe, J. A. Brazier, M. J. Dickman, D. P. Hornby, J. A. Grasby, D. M. Williams, *Nucleic Acids Res*. **2001**, *29*, 1565.
[83] T. Gourlain, A. V. Sidorov, N. Mignet, S. J. Thorpe, S. E. Lee, J. A. Grasby, D. M. Williams, *Nucleic Acids Res.* **2001**, *29*, 1898.
[84] C. J. Hipolito, M. Hollenstein, C. H. Lam, D. M. Perrin, *Org. Biomol. Chem*. **2011**, *9*, 2266.
[85] T. M. Penning, J. M. Jez, *Chem. Rev*. **2001**, *101*, 3027.
[86] Y. Gao, Q. Wang, Y. Zhang, B. Xu, *Chem. Soc. Rev*. **2010**, *39*, 3425.