Lanthanide cofactors accelerate DNA-catalyzed synthesis of branched RNA

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General Information

Standard 5'-O-DMT-protected 3'-β-cyanoethyl phosphoramidites of \( N^6 \)-benzoyl-2'-deoxyadenosine, \( N^4 \)-acetyl-2'-deoxycytidine, \( N^2 \)-(N,N-dimethylamino)methylene-2'-deoxyguanosine, and thymidine were from SAFC/Sigma-Aldrich. 5'-O-DMT-2'-O-TOM-protected 3'-β-cyanoethyl phosphoramidites of \( N^6 \)-acetyladenosine, \( N^4 \)-acetylcytidine, \( N^2 \)-acetylguanosine, and uridine were from ChemGenes. Benzylthiotetrazole (BTT) was from Carbosynth (UK). Ribonucleotide triphosphates (NTPs) for in vitro transcription reactions were from Jena Bioscience, and \( \alpha ^-32 \)P-dATP (3000 Ci/mmol) and \( \gamma ^-32 \)P-ATP (3000 Ci/mmol) was from Perkin Elmer. T4 RNA ligase, T4 polynucleotide kinase (PNK), and DNase I were from Fermentas, and Klenow fragment was from Jena Bioscience.

Table S1. Oligonucleotides used in this study

a. RNA substrates

1. **Acceptor**  
5' -UUAAAACGACUCAUCUAUA-3'

1i. **Inactive acceptor**  
5' -UUAAUCGACUCACU(dA)UA-3'

2. **Donor**  
5' -pppGGAAGAGAUGGCGACGG-3'

2s. **Short donor**  
5' -pppGGAAGAGAUGG-3'

2i. **Inactive donor**  
5' -pppGGAAGAGAUGGCGACGG-3'

2a. **Alternative donor**  
5' -pppGAAUAACAUCCACACCA-3'

Branch-site nucleotides in 1 are marked in bold letters (A8 for 7S11, U14 for 9F13 and A15 for 9F7).

b. Deoxyribozymes

9F7 for 2  
5' -CAACGTGGCCATCTCAATGAGGTTTGGGCAGGGATTATGATTATATACACTCCCGGATAGTGTATTA-3'

9F7 for 2s  
5' -CAACATCTCAATGAGGTTTGGGCAGGGATTATGATTATATACACTCCCGGATAGTGTATTA-3'

9F7min for 2a  
5' -CAACGTGGCCATCTCAATGAGGTTTGGGCAGGGATTATGATTATATACACTCCCGGATAGTGTATTA-3'

9F7min-1 for 2  
5' -CAACGTGGCCATCTCAATGAGGTTTGGGCAGGGATTATGATTATATACACTCCCGGATAGTGTATTA-3'

9F7min-2 for 2  
5' -CAACGTGGCCATCTCAATGAGGTTTGGGCAGGGATTATGATTATATACACTCCCGGATAGTGTATTA-3'

9F7min-3 for 2  
5' -CAACGTGGCCATCTCAATGAGGTTTGGGCAGGGATTATGATTATATACACTCCCGGATAGTGTATTA-3'

9F13 for 2  
5' -CAACGTGGCCATCTCAATGAGGTTTGGGCAGGGATTATGATTATATACACTCCCGGATAGTGTATTA-3'

9F13 for 2s  
5' -CAACATCTCAATGAGGTTTGGGCAGGGATTATGATTATATACACTCCCGGATAGTGTATTA-3'

9F13min for 2a  
5' -CAACGTGGCCATCTCAATGAGGTTTGGGCAGGGATTATGATTATATACACTCCCGGATAGTGTATTA-3'

9F13min for 2s  
5' -CAACATCTCAATGAGGTTTGGGCAGGGATTATGATTATATACACTCCCGGATAGTGTATTA-3'

7S11 for 2  
5' -CAACGTGGCCATCTCAATGAGGTTTGGGCAGGGATTATGATTATATACACTCCCGGATAGTGTATTA-3'

The bold nucleotides represent the catalytic loop regions. The non-bold nucleotides constitute the binding arm for the RNA substrates. The underlined nucleotides in 7S11 form a paired region with the donor RNA in the 3HJ structure.

c. Templates and promoter strand for in vitro transcription of donor strands

Template for 2  
5' -CGTGGCCATCTCAATGAGGTTTGGGCAGGGATTATGATTACAG-3'

Template for 2a  
5' -CGTGGCCATCTCAATGAGGTTTGGGCAGGGATTATGATTACAG-3'

Template for 2s  
5' -CGTGGCCATCTCAATGAGGTTTGGGCAGGGATTATGATTACAG-3'

T7 promoter  
5' -CTGTAATACGACTATA-3'

d. Template DNA for 3'-\( \gamma ^-32 \)P-labeling of deoxyribozyme libraries

T-9F7  
5' -GTTAATACGACTACCC-3'

T-9F13  
5' -GTTAATACGACTACCC-3'

e. complementary DNA for HPLC analysis of Tb\( ^3 \) effect on triphosphorylated donor

Complement for 2  
5' -CGTGGCCATCTCAATGAGGTTTGGGCAGGGATTATGATTACAG-3'
Unmodified DNA oligonucleotides were purchased from Sigma Aldrich and purified by denaturing PAGE (20% or 15% polyacrylamide). The RNA donor strands were prepared by in vitro transcription using T7 RNA polymerase and synthetic DNA templates. The RNA acceptor substrates and CoMA pools were prepared by solid-phase synthesis, following our previously described procedures.¹

Figure S1. Ligation kinetics of 9F7-catalyzed ligation of acceptor RNA 1 and donor RNA 2 in 50 mM HEPES buffer pH 7.5, 150 mM NaCl, 2 mM KCl, 37°C, and the indicated concentration of Mg²⁺ or Mn²⁺.

Figure S2. a) Ligation kinetics of 9F7-catalyzed ligation with magnesium and europium; compare Figure 1c,d for comparable data with Tb³⁺ and Gd³⁺. b) Ligation kinetics of 9F7-catalyzed ligation with magnesium and lanthanum, cerium and ytterbium. The data for ligation in absence of lanthanides (only 80 mM Mg²⁺) is plotted for comparison.
Figure S3. Dependence of ligation yield on concentration of a) Gd$^{3+}$, b) Eu$^{3+}$, c) La$^{3+}$ and Ce$^{3+}$, in presence of 80 mM Mg$^{2+}$, at pH 7.5, 37°C. Yields are determined by quantification of $^{32}$P-images after gel electrophoresis. Fits to the equation: fraction ligated = \( Y_0 + Y_a \cdot \frac{[\text{Ln}^{3+}]^n}{([\text{Ln}^{3+}])_{1/2,a}^n + [\text{Ln}^{3+}]^n} \) \cdot \{1-\frac{[\text{Ln}^{3+}]^m}{([\text{Ln}^{3+}])_{1/2,b}^m + [\text{Ln}^{3+}]^m}\}. Fit values are summarized in Table S2. Compare Figure 2 (in manuscript) for comparable data with terbium. The fit equation describes a model with independent binding sites for lanthanide ions that activate or inhibit the deoxyribozyme. A similar model with \( n \) activating and \( m \) inhibitory lanthanide ions was used by Silverman and coworkers to describe the lanthanide dependent activity of DNA-catalyzing deoxyribozymes.\(^{[4]}\) Here, the model is expanded to additionally address the “background” activity of 9F7 with divalent metal ions (\( Y_0 \)).

Table S2. Fit values for Figure 2 and S3.

<table>
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<tr>
<th>Titration</th>
<th>reaction time</th>
<th>( Y_0 )</th>
<th>( Y_a )</th>
<th>[Ln$^{3+}$]_{1/2,a}</th>
<th>n</th>
<th>[Ln$^{3+}$]_{1/2,b}</th>
<th>m</th>
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<td>0.02</td>
<td>0.91</td>
<td>71</td>
<td>18</td>
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<tr>
<td></td>
<td>5 h</td>
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<td>162</td>
<td>5</td>
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<tr>
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<tr>
<td></td>
<td>60 min</td>
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<td>0.95</td>
<td>-</td>
<td>-</td>
<td>97</td>
<td>2.2</td>
</tr>
<tr>
<td>Gd$^{3+}$ with 80 mM Mg$^{2+}$</td>
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<td>0.76</td>
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<td>32</td>
<td>164</td>
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<td>Eu$^{3+}$ with 80 mM Mg$^{2+}$</td>
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<td>0.008</td>
<td>0.76</td>
<td>81</td>
<td>7</td>
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<tr>
<td></td>
<td>5 h</td>
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<td>0.33</td>
<td>69</td>
<td>14</td>
<td>192</td>
<td>7</td>
</tr>
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</table>

1h incubation at 37°C in presence of
50mM HEPES pH 7.5, 150mM NaCl, 2mM KCl
1µM complexes

no Mg$^{2+}$
no Tb$^{3+}$
80mM Mg$^{2+}$
no Tb$^{3+}$
100µM Tb$^{3+}$
80mM Mg$^{2+}$
200µM Tb$^{3+}$

Figure S4. HPLC analysis of the fate of triphosphate donor RNA in presence of Tb$^{3+}$. RNA 2 (labeled pppR) was hybridized to its complementary DNA to resemble the deoxyribozyme binding arm. Anion exchange HPLC on Dionex-DNAPac PA200, 80°C. Quantification via area integration of UV$^{260}$ trace, pR is the diphosphorylated analog of RNA 2, and pR is the 5’-monophosphorylated analog of RNA 2. Identification was performed via MS and coinjection with authentic samples.
Figure S5. HPLC analysis of preparative ligation reactions. Conditions: donor (1) : acceptor (2) : deoxyribozyme (E) = 1 : 1 : 1; 80 mM Mg\(^{2+}\), 100 µM Tb\(^{3+}\), 37°C. Concentration of pre-ligation complex and incubation time as indicated for each panel. Anion exchange HPLC on Dionex-DNAPac PA200, 80°C. Quantification via area integration of UV\(_{260}\) trace.

Figure S6. Phosphorothioate effects and metal ion rescue attempts for 9F7-catalyzed ligation of RNAs 1 and 2 with gamma phosphorothioate. See manuscript text for details.
**Figure S7.** Phosphorothioate effects and metal ion rescue attempts for 9F7-catalyzed ligation of RNAs 1 and alternative donor 2a (containing only one single G on 5' end) with alpha and gamma phosphorothioate. Comparison of Cd²⁺ and Tb³⁺ effect on ligation of PO and PS donor substrate in presence of 20 mM Mn²⁺. The alternative substrate 2a is ligated 10-fold more slowly than original substrate 2. 1 mM Cd²⁺ and 10 µM Tb³⁺ have comparable 3-fold effect for acceleration of alpha PS. In contrast, the ligation of PO substrate 2a is not affected by 1 mM Cd²⁺ but 10-fold accelerated by 10 µM Tb³⁺.

**Figure S8** continued on next page
Figure S8. Full hydrolysis gels (a) and interference values (b) for 9F7 CoMA. a) left: The full CoMA hydrolysis gel for separation after ligation with 20 mM Mn$^{2+}$. Middle and right: The hydrolysis pattern of active and unselected libraries for the 5 conditions applied in this study. For each mutant library, the active and inactive portions, as well as the unselected library were subjected to hydrolysis and the products were resolved on denaturing sequencing gel. In all cases, hydrolysis was performed with 10 mM freshly made NaOH by heating at 95°C for 10 min. (A water control was done for each hydrolysis sample and was run aside the sample shown in the left gel, labeled “–”). b) The interference value for each library at indicated conditions was calculated from volume analysis of PhosphorImages. Interference value = (intensity in unseparated library) / (intensity in active fraction).

Figure S9. Kinetic data for shortened variants of 9F7 (based on CoMA results) at 20 mM Mn$^{2+}$, 37°C. $k_{rel}$ was defined with the ratio of $k_{obs}$ for shortened variants over $k_{obs}$ for 9F7 wt.
**Figure S10.** Kinetic data for 9F7wt and 9F7min, including Tb$^{3+}$ acceleration with Mn$^{2+}$ and Mg$^{2+}$ (bottom right is same as in Figure 5; here reproduced for comparison)

**Figure S11.** Rescue of 2'-OH effect by 100 µM Tb$^{3+}$ in presence of 80 mM Mg$^{2+}$. Compare Figure 6 for data with Mn$^{2+}$/Mn$^{2+}$+Tb$^{3+}$. 
Figure S12. Graphical representation of CoMA results for the 9F7 deoxyribozyme, comparing mutation tolerance in absence and presence of Tb$^{3+}$ (left: with 2 mM Mn$^{2+}$, right: with 80 mM Mg$^{2+}$). The color code is explained in the figure. The double line between A18 and C34 indicates the position of dispensable nucleotides that tolerated all mutations under all conditions.

Figure S13. Left: Luminescence spectrum of Tb$^{3+}$ bound to DNA (excitation at 284 nm). Middle & right: Time course of luminescence response upon Tb$^{3+}$ titration in presence of 10 or 100 mM Mg$^{2+}$. These plots show excerpts for the data presented in Figure 7a, highlighting the three different modes of binding kinetics (< 20 µM, 20-50 µM, > 60µM) and demonstrating the importance of minimally 10 min equilibration time before taking the intensity reading. The arrows point at aliquot additions to reach the stated concentration (µM). Data acquisition started 10s after each aliquot addition.

Table S3. Fit values for Figure 7a,b.

<table>
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<tr>
<th>Divalent Metal Ion</th>
<th>$I_{\text{max}}$</th>
<th>$[\text{Tb}^{3+}]_{1/2}$ (µM)</th>
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</tr>
</thead>
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<tr>
<td>Mn$^{2+}$</td>
<td></td>
<td></td>
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<tr>
<td>1 mM</td>
<td>245</td>
<td>69</td>
<td>2.4</td>
</tr>
<tr>
<td>2 mM</td>
<td>167</td>
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</tr>
<tr>
<td>5 mM</td>
<td>145</td>
<td>86</td>
<td>2.8</td>
</tr>
<tr>
<td>10 mM</td>
<td>82</td>
<td>101</td>
<td>2.7</td>
</tr>
<tr>
<td>50 mM</td>
<td>101</td>
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<td>Mg$^{2+}$</td>
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</tr>
<tr>
<td>1 mM</td>
<td>508</td>
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<tr>
<td>10 mM</td>
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<tr>
<td>20 mM</td>
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<tr>
<td>100 mM</td>
<td>373</td>
<td>139</td>
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</table>

Data were fit to the equation: $I = I_{\text{max}} \cdot \frac{[\text{Tb}^{3+}]^n}{([\text{Tb}^{3+}]_{1/2}^n + [\text{Tb}^{3+}]^n)}$. 
**Figure S14.** Tb$^{3+}$ luminescence experiments with two inactive complexes, to check for differences in luminescence response when triphosphate on the donor or 2'-OH at the branch-site is absent. (black: with 1 and 2; blue: with 1i and 2.

**Table S4.** Summary of ligation yields of G4 mutants of 9F7min.

<table>
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<tr>
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<th>nucleotide at position 4</th>
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<td></td>
<td>G</td>
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<tr>
<td>20 mM Mn$^{2+}$</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>0.95</td>
</tr>
<tr>
<td>2 mM Mn$^{2+}$ + 10 µM Tb$^{3+}$</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>0.95</td>
</tr>
<tr>
<td>80 mM Mg$^{2+}$</td>
<td>0.23</td>
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<tr>
<td>80 mM Mg$^{2+}$ + 100 µM Tb$^{3+}$</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
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</tr>
</tbody>
</table>

**Figure S15.** Line graph analysis of DMS probing data (compare Figure 8). In the absence of Mn$^{2+}$ (left) all guanosines are equally accessible to DMS in all three complexes. The reduced intensity of G4-G15 with respect to G16 and G17 in the folded complex c in the presence of Mn$^{2+}$ (right) is clearly visible. In complex b, the protection is still visible but less pronounced. (- and + indicate electrophoresis direction)
Figure S16. Comparison of sequences and branch-site context for 9F13[2] and 7S11[3]. bp = base-paired regions responsible for formation of 3-helix junction structure in 7S11.

Figure S17. Kinetic analysis of 9F13-catalyzed RNA ligation with 20 mM Mn²⁺ and effect of Tb³⁺. Fraction ligated vs. time data were fit to the equation fraction ligated = Y_{max} \cdot [a_1 \cdot (1-e^{-k_1 \cdot t}) + (1-a_1) \cdot (1-e^{-k_2 \cdot t})]. [Table: a_2 = 1-a_1].

Figure S18. CoMA results for 9F13. a) Hydrolysis gel after separation of active and inactive library fraction, ligation with 20 mM Mn²⁺ at 37°C. b) Interference values for all four libraries. The OH effect is plotted separately as the bottom graph (only at T5 and A38 severe inhibition was found in the presence of the 2’-OH tag), all other positions can be analyzed for the mutation effect.
Figure S19. Schematic of 9F13 deoxyribozyme. Nucleotides marked green in the full length wt sequence tolerate all mutations and are dispensable for activity. The minimized version 9F13min retains two green nucleotides between essential sections to maintain proper folding into an active conformation. Kinetic analysis shows 4-fold improved reaction kinetics for 9F13min. Ligation at pH 7.5, 37°C with 20 mM Mn²⁺ (note the slower reaction compared to data shown for 9F13wt in Fig S15 is due to the shorter binding arm of the donor substrate 2s used).

Figure S20. Kinetics of 7S11-catalyzed ligation of RNAs 1 and 2 at pH 7.5, 37°C using Mn²⁺ or Mg²⁺ as cofactor. Shown are gel images and kinetic curves for optimal Tb³⁺ concentrations in comparison to absence of Tb³⁺. The available data in the presence of Tb³⁺ do not allow accurate determination of $k_{obs}$ (estimates given in the Figure). The acceleration effect of Tb³⁺ is therefore ~20-fold with Mg²⁺, and ~50-fold with Mn²⁺.
**Table S5.** Tb\(^{3+}\)-stock solutions used for luminescence titrations. At every titration step, 1\(\mu\)L of the respective stock solution was added (sample volume at start of the experiment 600 \(\mu\)L).

<table>
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<tr>
<th>No</th>
<th>Final Tb(^{3+}) concentration (µM)</th>
<th>Individual stock conc. for each titration step (µM)</th>
<th>Sample volume after each addition (µL)</th>
<th>Dilution factor</th>
<th>Preparation of individual stock conc.</th>
<th>Volume of parent Tb(^{3+}) stock soln. (µL)</th>
<th>H(_2)O (µL)</th>
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**References for supporting information**


