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Supporting Information

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DNA-Catalyzed Reductive Amination**

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Procedures for selection and capture steps during in vitro selection

In the key selection step during each round of selection, the PCR-amplified deoxyribozyme pool (estimated 10 pmol) and 50 pmol of helper oligonucleotide (complementary to the 3′-binding arm of the deoxyribozyme as shown in Fig. 2b) were annealed in 14 µL volume of 5 mM HEPES, pH 7.5, 15 mM NaCl, and 0.1 mM EDTA by heating at 95 °C for 1 min and cooling on ice for 5 min. The sample was brought to final volume of 20 µL containing 50 mM HEPES, pH 7.5, 150 mM NaCl, 2 mM KCl, 40 mM $MgCl_2$, 20 mM $MnCl_2$, and 1 mM CYA tripeptide (added from 50 mM stock solution in DMF) and incubated at 37 °C for 14 h, followed by ethanol precipitation. For the subsequent capture step, 200 pmol of capture oligonucleotide (prepared as described in the Experimental Section) was added, and the sample was annealed in 10 µL volume of 5 mM HEPES, pH 7.5, 15 mM NaCl, and 0.1 mM EDTA by heating at 95 °C for 1 min and cooling on ice for 5 min. The sample was then brought to final volume of 20 µL containing 100 mM NaOAc, pH 5.2, 50 mM $NiCl_2$, and 10 mM $NaCNBH_3$, incubated at 37 °C for 14 h, and separated by 8% PAGE.

Sequences of new deoxyribozymes that catalyze reductive amination

	1–10	11–20	21–30	31–40
8QA124	AAGGGGCAGT	GCTAAACGTG	GACAGGAATC	ATAAGTGTCA
8QA102	......T...	....	A.G.T.GT.T	GGGTAATCTC
8QA103	..T..CAGTG	AGA..CGTAT	.GTCAC.TGT	..GT.GTCAC
8QA107	......T...	..C.......	..A.CCTG.G	GAT.AG...C
8QA111	GG...C.GCG	AT....T..T	.T..T.C.G.	TCTCTGAGGC
8QA115	.G...CAGTG	CTA..CGTGT	ACAGACT.AA	..GGTCTGTC
8QA122	........CG	TGCT..ACGT	.GA....C.T	CGGTTCTC.C
8QA104	.G...TAGTG	C.A..CGTG.	CCTG.TTTGA	TG.TTC.G.C
8QA108	......T...	..C......A	CGA...CT..	..T.CC.A.C
8QA109	...A....CG	TGCT..ACGT	.GA....C.T	CGGTTCTC..

Figure S1. Sequences of new deoxyribozymes that catalyze reductive amination. In addition to the seven deoxyribozymes studied in detail in this report (the first seven entries, including the key 8QA124 deoxyribozyme), several additional deoxyribozyme sequences were identified but not studied in more detail here (the last three entries). The originally random (N_{40}) catalytic region is shown. In each deoxyribozyme, to the 5′-side of the catalytic region is 5′-CCGTCGCCATCTCTTC-3′, and to the 3′-side of the catalytic region is 5′-ATAGTGAGTCGTATTA-3′.

Catalytic activities of the seven new deoxyribozymes

The catalytic activity of the 8QA124 deoxyribozyme is shown in Fig. 2a and is reproduced for comparison in Fig. S2. The activities of the other six studied deoxyribozymes are shown in Fig. S2. Control experiments were also performed. When a noncatalytic template strand that has 40 random nucleotides (N_{40}) was used, or when the the template strand was omitted altogether (untemplated), no product was observed (<0.2% yield in 22 h). When a template strand was included that replaced N_{40} with zero, one, or two unpaired T nucleotides (T_0, T_1, or T_2 template), the yield in 22 h was only 0.6%, 0.4%, or 0.2%, respectively. The T_0 data are plotted as "T_0 control" in Fig. S2. Because 8QA124 has $k_{obs} = 0.37$ h^{-1} and 0.6% in 22 h for the T_0 template is equivalent to 2.7×10^{-4} h^{-1}, a conservative lower limit on the 8QA124 rate enhancement is 1400.

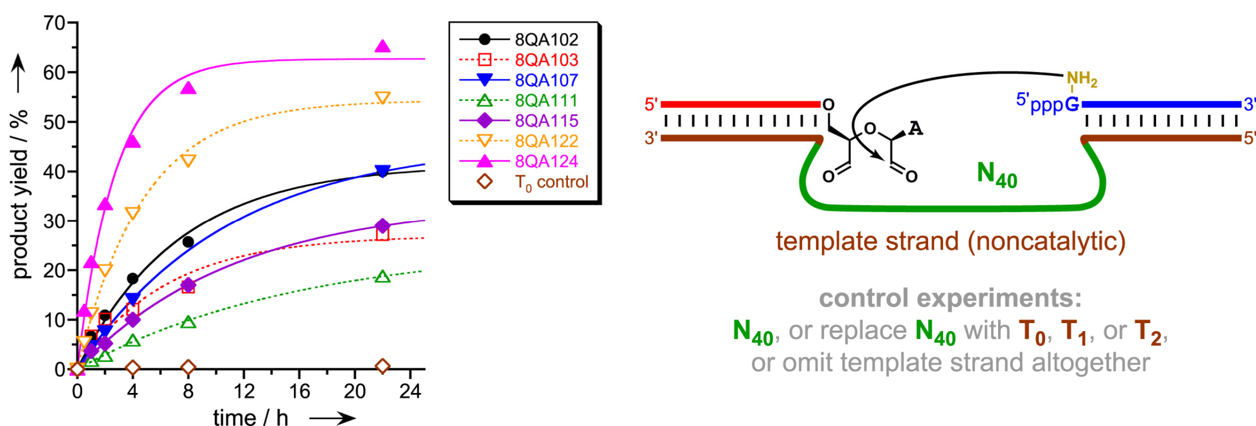

Figure S2. Catalytic activities of the seven new deoxyribozymes studied in this report. The assay procedure is described in the Experimental Section. Incubation conditions: 100 mM NaOAc, pH 5.2, 50 mM $NiCl_2$, and 10 mM $NaCNBH_3$ at 37 °C. See Fig. 2a for the corresponding gel image for 8QA124. k_{obs} values (h^{-1}): 8QA124 0.37; 8QA102 0.14; 8QA103 0.16; 8QA107 0.092; 8QA111 0.062; 8QA115 0.088; 8QA122 0.21. See text for description of the control experiments and resulting data.

Dependence of the catalytic activities on the RNA 5′-terminus

Each of the seven new deoxyribozymes was assayed with RNA substrates that have either 5′-triphosphate, 5′-monophosphate, or 5′-hydroxyl terminus. The results of these assays (Fig. S3) showed that all seven deoxyribozymes function well with 5′-monophosphorylated RNA. In contrast, reactivity varied widely involving the 5′-hydroxyl RNA substrate. 8QA111 has relatively robust activity with 5′-hydroxyl RNA; three of the deoxyribozymes (8QA124, 8QA103, and 8Q115) have low but detectable activity with 5′-hydroxyl RNA; and the remaining three deoxyribozymes (8QA102, 8QA107, and 8QA122) have no detectable activity with 5′-hydroxyl RNA.

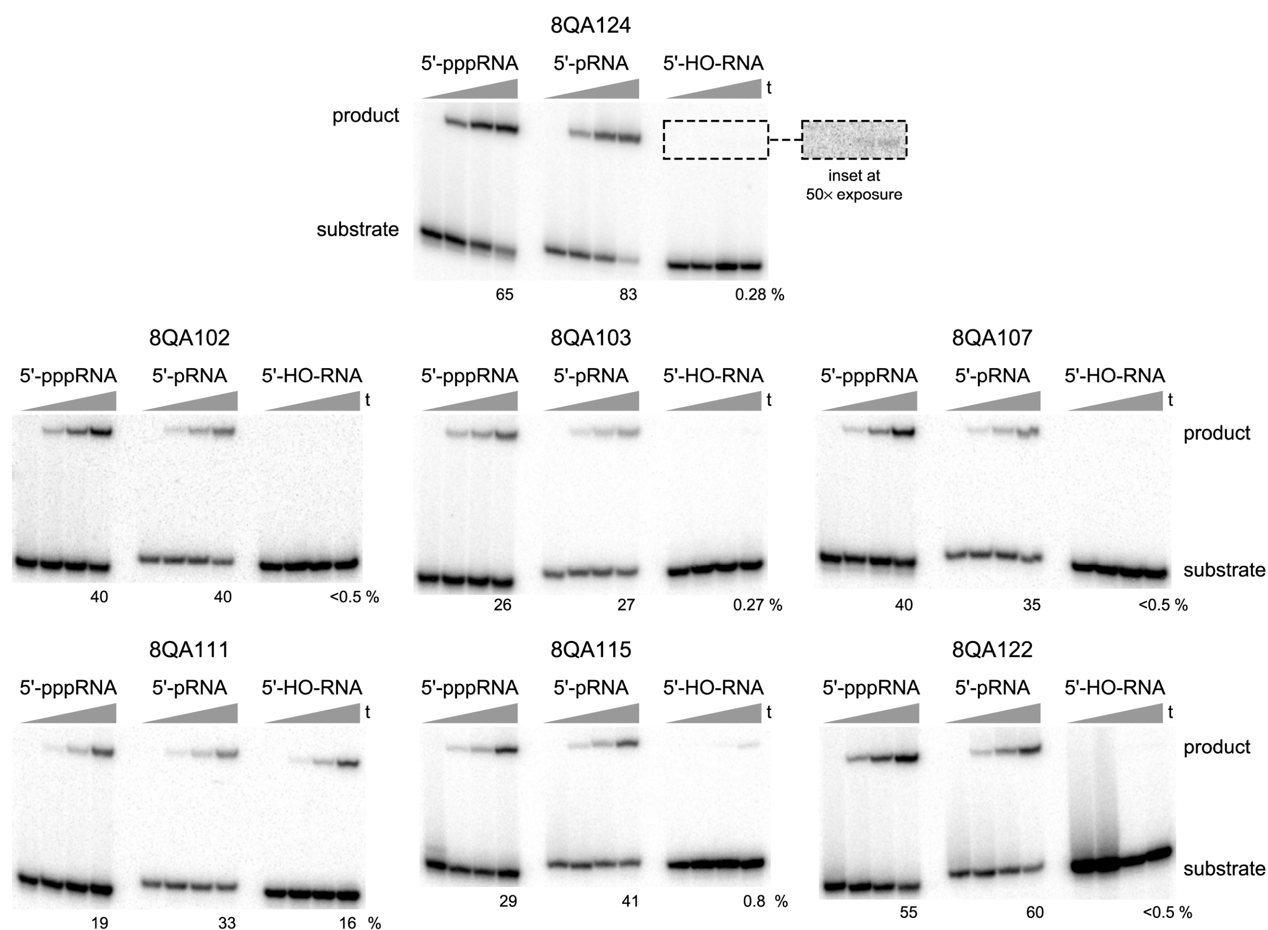

Figure S3. Dependence of catalytic activity on the RNA 5′-terminus for each of the new deoxyribozymes. Incubation conditions: 100 mM NaOAc, pH 5.2, 50 mM $NiCl_2$, and 10 mM $NaCNBH_3$ at 37 °C. t = 30 s, 1 h, 4 h, and 22 h. Yields at the final time point are shown beneath each corresponding lane.

Dependence of the catalytic activities on $NaIO_4$, Ni^{2+}, and $NaCNBH_3$

Each of the seven new deoxyribozymes was shown to require $NaIO_4$ oxidation during preparation of the capture oligonucleotide (Fig. S4). In addition, each of the deoxyribozymes was shown to require both Ni^{2+} as well as $NaCNBH_3$ during the reductive amination step.

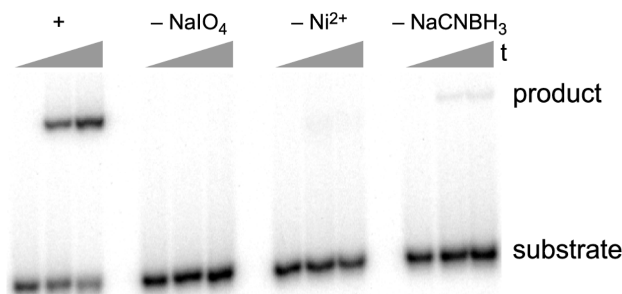

Figure S4. Dependence of the catalytic activities on $NaIO_4$, Ni^{2+}, and $NaCNBH_3$, illustrated with data for 8QA124. Data for each of the other six studied deoxyribozymes was similar (not shown). Incubation conditions: 100 mM NaOAc, pH 5.2, 50 mM $NiCl_2$, and 10 mM $NaCNBH_3$ at 37 °C (omitting Ni^{2+} or $NaCNBH_3$ as indicated). The "– $NaIO_4$ experiment" was performed by omitting $NaIO_4$ during preparation of the capture oligonucleotide (see Experimental Section). t = 30 s, 4 h, and 22 h. The yield for the "– $NaCNBH_3$" experiment at the 22 h time point was 1.3%.

Mass spectrometry analyses of the reaction products ± RNase T1 digestion

Each deoxyribozyme product (as prepared with 5′-monophosphorylated RNA substrate) was analyzed by MALDI-TOF mass spectrometry, both before and after RNase T1 digestion as described in the Experimental Section. Tabulation of the mass spectrometry data is in Table S1.

deoxyribozyme	RNase T1	mass calcd.	mass found	error, % (found – calcd.)
8QA124	– T1	11795.5	11795.9	+0.003
	+ T1 [a]	6878.5	6878.3	–0.003
	+ T1 [b]	6896.5	6896.6	+0.001
8QA102	– T1	11795.5	11793.5	–0.02
	+ T1 [b]	6896.5	6894.5	–0.03
8QA103	– T1	11795.5	11794.6	–0.008
	+ T1 [b]	6896.5	6896.4	–0.001
8QA107	– T1	11795.5	11793.7	–0.02
	+ T1 [b]	6896.5	6894.4	–0.03
8QA111	– T1	11795.5	11793.2	–0.02
	+ T1 [b]	6896.5	6895.3	–0.02
8QA115	– T1	11795.5	11793.6	–0.02
	+ T1 [b]	6896.5	6892.0	–0.07
8QA122	– T1	11795.5	11794.4	–0.009
	+ T1 [b]	6896.5	6895.8	–0.01

Table S1. MALDI mass spectrometry analyses of the reaction products before and after RNase T1 digestion. RNase T1 initially creates a 2′,3′-cyclic phosphate terminus that can be hydrolyzed to 3′-monophosphate, the ratio of these two products depends on the incubation details (T. Uchida & F. Egami, in *The Enzymes* (3rd ed.), ed. P. D. Boyer, Vol. 4, **1971** (New York: Academic Press), pp. 205-250; D. Herschlag et al., *Biochemistry* **1991**, *30*, 4844-4854; F. Kirpekar et al., *RNA* **2000**, *6*, 296-306). Both products were typically observed in our assays. In the table, data is shown for both products (for 8QA124) and for the 3′-monophosphate, which was the more abundant of the two products (for the other deoxyribozymes).

[a] 2′,3′-cyclic phosphate product.

[b] 3′-monophosphate product.

Dependence of activities on presence of the N^2-amino group at the RNA 5′-nucleotide

Each of the seven new deoxyribozymes was assayed with an RNA substrate that has 2′-deoxyinosine as its 5′-terminal nucleotide. In each case, this replacement of the guanine nucleobase with hypoxanthine (which lacks the N^2-amino group of guanine) completely suppressed the catalytic activity (Fig. S5), as expected for DNA-catalyzed reactivity of the guanosine N^2-amine functional group. These experiments were performed using deoxyinosine rather than the ribonucleotide inosine because of the simpler synthetic accessibility of the deoxynucleotide. By itself, the 2′-deoxy modification at the 5′-terminal guanosine nucleotide decreased the catalytic activity to some degree for several of the other deoxyribozymes, although 8QA124 was essentially unaffected. Nevertheless, sufficient activity was retained in all cases to allow a meaningful comparison between 5′-terminal 2′-deoxyinosine and 5′-terminal 2′-deoxyguanosine.

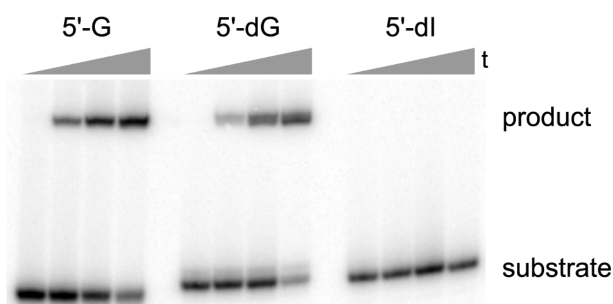

Figure S5. Dependence of catalytic activities on presence of the N^2-amino group at the RNA 5′-nucleotide, illustrated with data for 8QA124. No detectable reactivity (<0.5%) of the RNA substrate that has 2′-deoxyinosine as its 5′-terminal nucleotide was also observed for each of the other six studied deoxyribozymes (data not shown). The RNA substrate was 5′-monophosphorylated for these assays. Incubation conditions: 100 mM NaOAc, pH 5.2, 50 mM $NiCl_2$, and 10 mM $NaCNBH_3$ at 37 °C. t = 30 s, 1 h, 4 h, and 22 h.

MALDI MS/MS data for the 8QA124 reductive amination product

The data in Fig. 3a show the results of MALDI mass spectrometry for the 8QA124 reductive amination product both before and after digestion with RNase T1 to remove the majority of the RNA substrate strand from the product. An MS/MS experiment was also performed, using the 8QA124 product after both DNase digestion (to remove most of the capture oligonucleotide to the 5′-side of the 3′-terminal rA) and RNase T1 digestion. With this digestion product, MS/MS was performed on the pentamer 5′-pATrA–GGp-3′, where the new reductive amination linkage is between the rA and the G (Fig. S6). The results fully support the assigned reductive amination product structure. In particular, we observed a diagnostic peak attributed to deglycosylation of the RNA G1 nucleoside, i.e., cleavage between the G1 ribose and G1 nucleobase. Observation of this peak compels the conclusion that the G1 nucleobase is attached to the 3′-rA of the capture oligonucleotide.

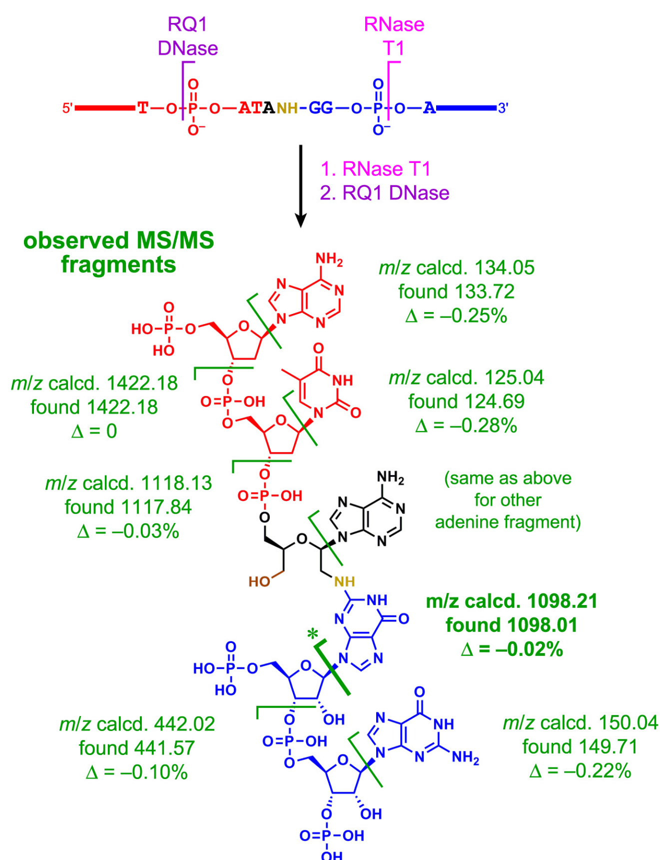

Figure S6. MS/MS data for the 8QA124 reductive amination product fully supports the assigned reductive amination product structure. See text for details. Procedure: A 900 pmol portion of the 8QA124 reductive amination product was treated with 10 units of RQ1 RNase-free DNase (Promega) in 40 mM Tris, pH 8.0, 10 mM $MgSO_4$, and 1 mM $CaCl_2$ in 100 μL total volume at 37 °C for 14 h. The sample was extracted with phenol/chloroform, precipitated with ethanol, and separated by 20% PAGE. Three predominant bands were observed; the middle of these three was excised from the gel, extracted with TEN, and precipitated with ethanol. This DNase-digested product was treated with 20 units of RNase T1 (Ambion) in 50 mM Tris, pH 7.5, and 2 mM EDTA in 40 μL total volume at 37 °C for 14 h. The sample was concentrated to 10 μL by SpeedVac, desalted by C_{18} ZipTip, and analyzed by MALDI mass spectrometry, revealing its identity as the pentamer 5′-pATrA–GGp-3′, where the new reductive amination linkage is between the rA and the G (calcd. 1735.2, found 1733.6, Δ = –0.09%). MS/MS analysis was performed using an UltrafleXtreme TOF/TOF mass spectrometer (Bruker).

Mass spectrometry to validate 3′-aldehyde formation by oxidation of 3′-rA with $NaIO_4$

$NaIO_4$ oxidation of 3′-ribonucleotide-terminated oligonucleotides is extremely well-precedented (e.g., ref. 25). Nevertheless, to provide direct evidence of this oxidation in the present situation, the model hexamer 5′-ACTATrA-3′ (corresponding to the 3′-terminal six nucleotides of the full-length 20 nt 3′-rA oligonucleotide) was analyzed by MALDI mass spectrometry both before and after $NaIO_4$ treatment. The observed mass spectrometry data was fully in accord with periodate oxidation to form a 3′-dialdehyde (Fig. S7a). The oxidized 3'-dialdehyde oligonucleotide was observed partially in the hydrated form, as expected for aldehydes.

The full-length 20 nt 3′-rA oligonucleotide was also assayed by mass spectrometry after $NaIO_4$ oxidation and ethanol precipitation. Only the hydrated form of the 3′-dialdehyde was observed (calcd. 6140.1, found 6138.6, Δ = –0.02%); a similar result was observed after periodate oxidation without ethanol precipitation (calcd. 6140.1, found 6140.0, Δ = –0.002%). Observation of the hydrated form compels the conclusion that oxidation occurred.

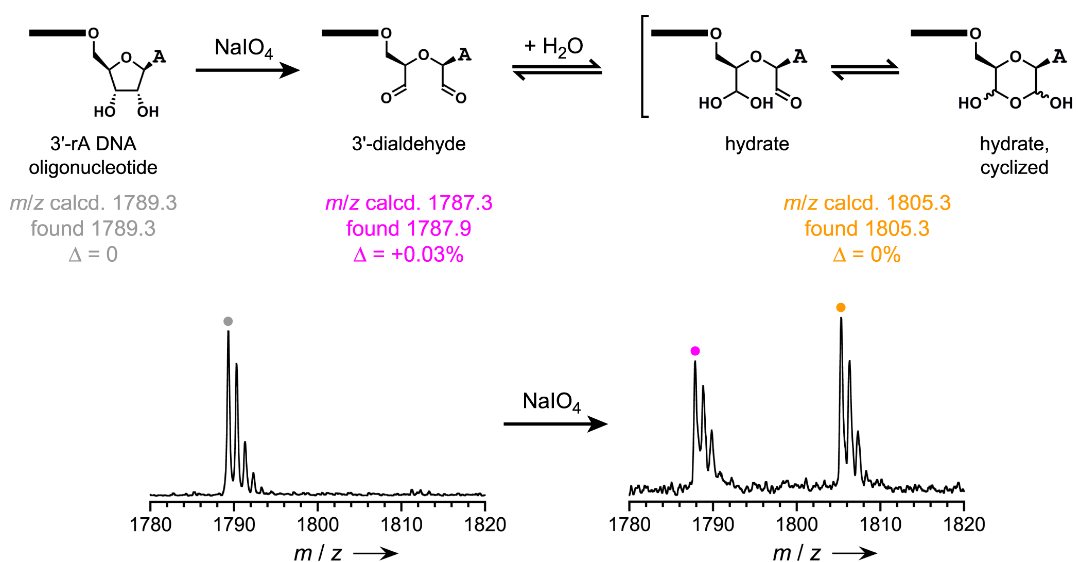

Figure S7. Periodate oxidation of hexamer 3′-rA oligonucleotide assayed by MALDI mass spectrometry. Observed is a mixture of 3′-dialdehyde and its hydrated form, presumably cyclized to the bis-hemiacetal as illustrated. The ratio of 3′-dialdehyde to hydrated form depended on the details of sample preparation (data not shown). Procedure: a 1 nmol portion of the 3′-rA oligonucleotide was oxidized in 100 µL containing 100 mM HEPES, pH 7.5, and 10 mM $NaIO_4$ at 25 °C for 1 h. The sample was desalted by passage through a Sephadex G-10 spin column, concentrated to 10 µL volume by SpeedVac, desalted by C_{18} ZipTip. and assayed by MALDI mass spectrometry.

Mass spectrometry to validate $NaCNBH_3$ reduction of oligonucleotide-aldehydes

As depicted in Fig. 2b and Fig. 3a, the second aldehyde group of the reductive amination product (as derived from the capture oligonucleotide) appears to be reduced to an alcohol under the incubation conditions. Aldehyde reduction by $NaCNBH_3$ under acidic conditions is well-precedented (see main text). Here we experimentally validated this $NaCNBH_3$ reactivity specifically of oligonucleotide-aldehydes.

Because the capture oligonucleotide (20 nt, 3′-dialdehyde after $NaIO_4$ oxidation) is too large for reliable mass spectrometry analysis of its $NaCNBH_3$ reduction reaction, we used the hexamer analog 5′-ACTAT-rA-3′ for mass spectrometry assays. The hexamer was oxidized with $NaIO_4$ (see Fig. S7 on previous page) and then incubated under the reaction conditions that included $NaCNBH_3$ (100 mM NaOAc, pH 5.2, 50 mM $NiCl_2$, and 10 mM $NaCNBH_3$ at 37 °C) for 4 or 15 h. Direct MALDI mass spectrometry analysis clearly demonstrated reduction of one aldehyde group of the oxidized hexamer (Fig. S8). The second aldehyde is not reduced under these conditions, likely due to hemiacetal formation as illustrated.

As shown in Fig. 2b, the complex of RNA substrate, deoxyribozyme, and 3′-dialdehyde capture oligonucleotide is pre-formed, and then the reductive amination process is initiated by addition of Ni^{2+} and $NaCNBH_3$. At that point, the capture oligonucleotide can commence uncatalyzed reduction, but the DNA-catalyzed reaction with the G1 N^2-amine can also occur. The data, especially the time course of uncatalyzed aldehyde reduction as shown in Fig. S8 in comparison with the DNA-catalyzed time courses shown in Fig. 2a and Fig. S2, indicate that the DNA-catalyzed reductive amination reaction is competitive with uncatalyzed aldehyde reduction.

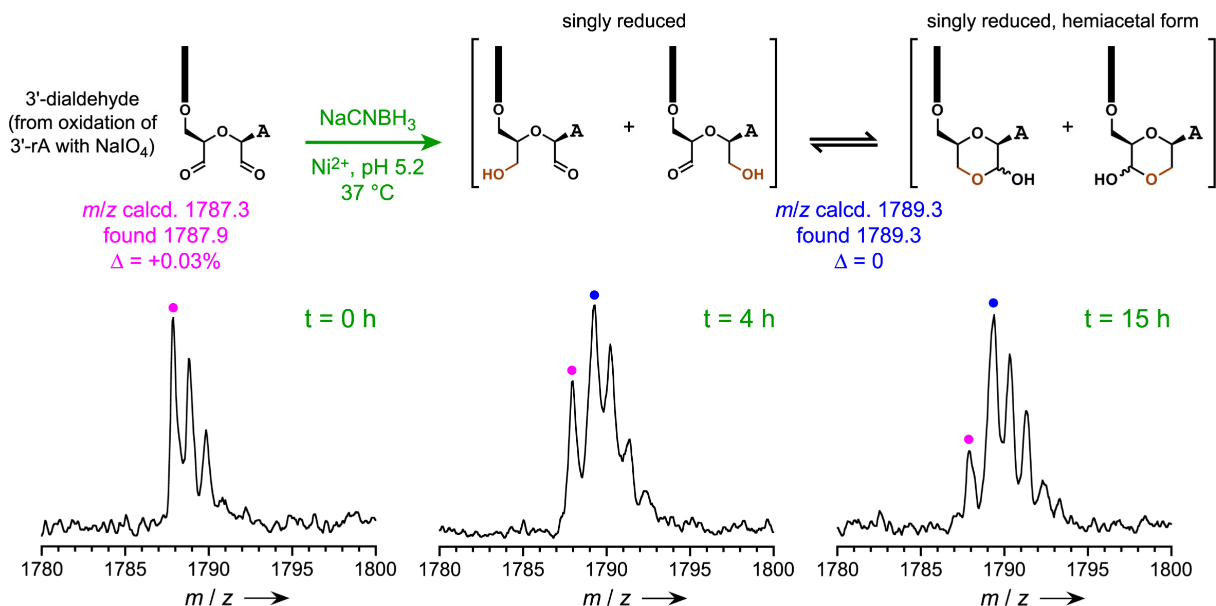

Figure S8. Direct observation by MALDI mass spectrometry of $NaCNBH_3$ aldehyde reduction using an oxidized 3′-rA hexamer oligonucleotide. The expected isotope distribution pattern of each compound (principally N, N+1, and N+2) partially obscures the 2 Da increase in mass upon reduction, but the shift is nevertheless unambiguous. Procedure: Each of two 1 nmol portions of the 3′-rA oligonucleotide was oxidized in 100 µL containing 100 mM HEPES, pH 7.5, and 10 mM $NaIO_4$ at 25 °C for 1 h. Each sample was desalted by passage through a Sephadex G-10 spin column and concentrated to 50 µL volume by SpeedVac. Each sample was then incubated in 100 µL total volume containing 100 mM NaOAc, pH 5.2, 50 mM $NiCl_2$, and 10 mM $NaCNBH_3$ at 37 °C. At the appropriate time, each sample was passed through a Sephadex G-10 spin column, concentrated to 10 µL, desalted by C_{18} ZipTip, and assayed by MALDI mass spectrometry.

Replacement of $NaCNBH_3$ with dimethylamine-borane or 2-picoline-borane

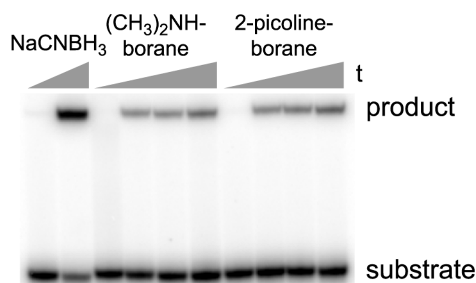

Figure S9. $NaCNBH_3$ can be replaced by dimethylamine-borane (ref. 29a) or 2-picoline-borane (ref. 29b) with substantial retention of catalytic activity. Incubation was performed in 100 mM NaOAc, pH 5.2, 50 mM $NiCl_2$ and 10 mM reducing agent at 37 °C. For stock solutions, dimethylamine-borane was dissolved in water at 200 mM, and 2-picoline-borane was dissolved in 1:1 water:DMF at 200 mM. t = 30 s and 22 h for $NaCNBH_3$; t = 30 s, 4, 8, and 22 h for dimethylamine-borane and 2-picoline-borane. The illustrated data are for 8QA124; similar data was observed for each of the other six studied deoxyribozymes (data not shown). In the illustrated experiment, the yields at the 22 h timepoint for $NaCNBH_3$, dimethylamine-borane, and 2-picoline-borane were 63, 18, and 22%. The key point is not comparison of the absolute yields but simply that borane reagents other than $NaCNBH_3$ still allow a significant reductive amination yield at all. In a control experiment, the 8QA124 yield with $NaCNBH_3$ was comparable when 2.5% DMF was included (data not shown); this was a control experiment for the 2-picoline-borane reaction, which included 2.5% DMF in the final sample to maintain solubility.

Replacement of Ni^{2+} with Mg^{2+}, Ca^{2+}, or Mn^{2+}

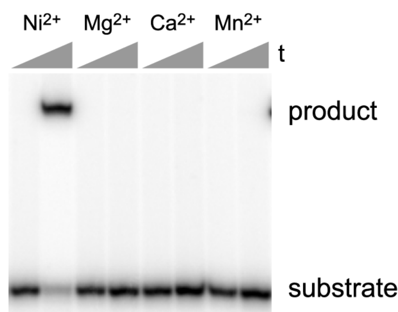

Figure S10. Ni^{2+} cannot be replaced by Mg^{2+}, Ca^{2+}, or Mn^{2+} for any of the deoxyribozymes. Incubation was performed in 100 mM NaOAc, pH 5.2, and 10 mM $NaCNBH_3$ at 37 °C with either 50 mM $NiCl_2$, 40 mM $MgCl_2$, 40 mM $CaCl_2$, or 20 mM $MnCl_2$. t = 30 s and 22 h. The illustrated data are for 8QA124; similar data was observed for each of the other six studied deoxyribozymes (data not shown).