# Electronic Supplementary Information

## Site-selective depurination by a periodate-dependent deoxyribozyme

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### Details of Experimental Procedures

Materials

DNA oligonucleotides were prepared at IDT (Coralville, IA). All DNA oligonucleotides were purified by denaturing PAGE with running buffer 1× TBE [89 mM each Tris and boric acid and 2 mM EDTA, pH 8.3] as described previously.[1] UDP-GlcNAc was from Sigma, $NaIO_4$ and $NaCNBH_3$ were from Aldrich, and $NiCl_2 \cdot 6H_2O$ was from Fluka.

DNA oligonucleotides

pool complement: CCATCAGGATCAGCT-$N_{70}$-GAAGAGTTCGCCG**X**A (**X** = spacer 18)
primer 1: AACAACAACAAC**X**CCATCAGGATCAGCT
primer 2: GGAGCGGGAAGAGACGGCGAACTCTT
primer 2 (rG16): GGAGCGGGAAGAGAC(rG)G
primer 2 (rG2,rG16): G(rG)AGCGGGAAGAGAC(rG)G
3′-$NH_2$-DNAs: AACAACAACAACATAATACGACTCACTATA**Y** (30-mer), ATACGACTCACTATA**Y** (15-mer) (**Y** = 3′-amino modifier C6)
splint: CCGTCTCTTCCCGCTCCTATAGTGAGTCGTATTA (complementary nucleotides in splint and 3′-$NH_2$-DNAs are underlined; remaining nucleotides in splint are complementary to 5′-terminus of deoxyribozyme)
primer 1 for cloning: TAATTAATTAATTACCCATCAGGATCAGCT
primer 2 for cloning: CGACGGCGAACTCTTC

Preparation of $N_{70}$-DNA pool by primer extension

The starting pool was generated by primer extension with Taq polymerase using the pool complement as template. To ensure that template and primer extension product have different length and can be separated by PAGE, a hexaethylene glycol spacer (spacer 18) was incorporated near the 3′-end of the template. This spacer prevents template extension by Taq polymerase.

In a typical primer extension experiment, 1.4 nmol of primer 2 was incubated with 400 pmol of template (pool complement), 0.4 mM each dNTP, 1× Taq polymerase reaction buffer (New England Biolabs; 20 mM Tris-HCl, pH 8.8, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, 0.1% Triton X-100) in a final volume of 400 µL. Using a GeneAmp PCR System 2400 (Applied Biosystems) instrument, 30 cycles of extension reactions were performed, using the following temperature program: 95 °C, 5 min → [95 °C, 30 sec → 50 °C, 30 sec → 72 °C, 30 sec]$_{30}$ → 72 °C, 5 min → 4 °C, ∞. The DNA products were precipitated by addition of 300 mM NaCl and 3 volumes of ethanol, dissolved in 30 µL of $H_2O$, combined with 30 µL of 80% stop solution [80% formamide, 1x TBE, 50 mM EDTA, 0.025% bromophenol blue, 0.025% xylene cyanol], heated to 95°C for 3 min, placed on ice for 5 min, and separated on an 8% polyacrylamide gel, 7 M urea, 1× TBE, at 30 W for 100 min. The DNA pool was extracted from the gel, precipitated and quantified by UV absorbance ($A_{260}$). Typically, 600 pmol of primer extension product was isolated.

In vitro selection procedure

We previously described the generic in vitro selection strategy that is based on separation of active deoxyribozymes by PAGE.[2] In the key selection step of the selection here designated FN, the deoxyribozyme pool was incubated with 2 mM UDP-GlcNAc in 60 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS), pH 7.0, 150 mM NaCl, 50 mM KCl, 10 mM $MgCl_2$, 10 mM $CaCl_2$, 500 μM $ZnCl_2$, 50 μM $CuCl_2$, and 50 μM $CoCl_2$ at 37 °C for 3 h. The PCR amplification step of the selection procedure and cloning of the deoxyribozymes after round 10 were performed as previously described.[2]

In more detail for the key selection step, the DNA pool was first heated to 95 °C for 3 min in 5 mM MOPS at pH 7.0 and then placed on ice for 5 min. UDP-GlcNAc was added from a 40 mM stock solution, MOPS from a 500 mM stock solution, and NaCl and KCl from a mixed 10× stock solution containing 1.5 M NaCl and 0.5 M KCl. The sample was placed at 50 °C for 5 min and transferred to 37 °C. The metals $Mg^{2+}$, $Ca^{2+}$, $Cu^{2+}$, and $Co^{2+}$ were added from a 10× stock solution containing 100 mM $MgCl_2$, 100 mM $CaCl_2$, 0.5 mM $CuCl_2$, and 0.5 mM $CoCl_2$. The metal $Zn^{2+}$ was added from a 20× stock solution containing 10 mM $ZnCl_2$ in 20 mM $HNO_3$ and 200 mM MOPS at pH 7.0 (this stock solution was freshly prepared from 100 mM $ZnCl_2$ in 200 mM $HNO_3$). The reaction volume was 20 μL (50 μL and 10 μM DNA in the first selection round). The sample was incubated at 37 °C for 3 h (15 h in the first round). Then, EDTA and NaCl were added to final concentrations of ca. 25 mM and 300 mM, respectively, in a final volume of 150 μL (stock solutions were 0.5 M EDTA, pH 8.0, and 3 M NaCl). The DNA was precipitated by addition of 3 volumes (450 μL) of ethanol.

The DNA sample was dissolved in $H_2O$ and treated with 40 mM $NaIO_4$ in 50 mM NaOAc at pH 5.2 at 37 °C for 2 h (reaction volume 20 μL; 40 μL in the first round). Then, NaCl was added to a final concentration of ca. 300 mM. The volume was adjusted to 100 μL by addition of $H_2O$ and the DNA was precipitated with 9 volumes of ethanol. (Usually 3 volumes of ethanol are used for DNA precipitation, but this was found to be insufficient for precipitation after $NaIO_4$ oxidation.)

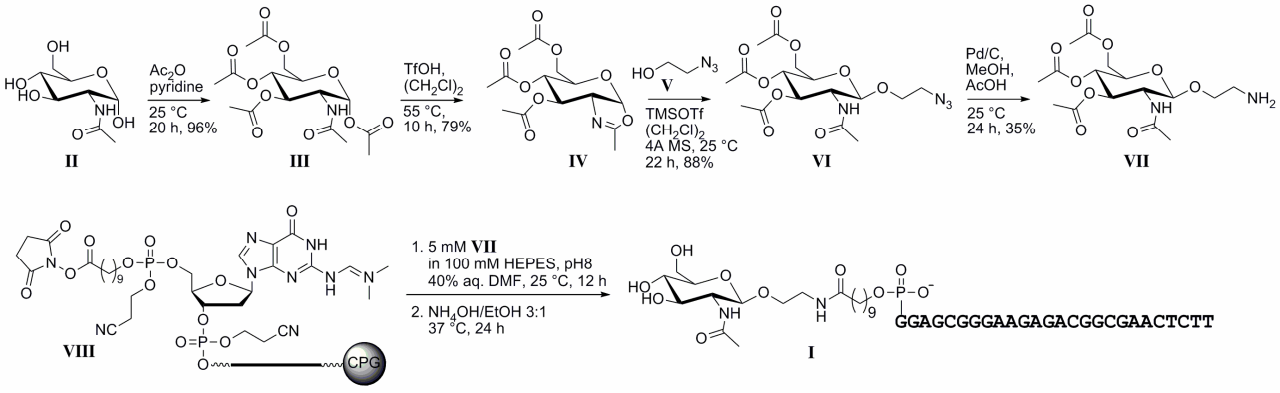
For the reductive amination reaction, the sample was dissolved in $H_2O$. Then, 20 μM 30-mer 3′-$NH_2$-DNA and 15 μM DNA splint (34 nt) were added, and the DNA oligonucleotides were annealed in 100 mM NaOAc, pH 5.2, by heating at 95 °C for 1 min and placing on ice for 3 min. Then, 25 mM $NiCl_2$ and 10 mM $NaCNBH_3$ were added from 4× and 10× stock solutions, respectively.[3] The final reaction volume was 20 μL (60 μL in the first round). The reaction mixture was incubated at 37 °C for 12–14 h. Then, 20 μL of 80% formamide stop solution was added. The sample was heated to 95 °C for 3 min, placed on ice for 5 min, and separated on an 8% denaturing polyacrylamide gel, 7 M urea, 1× TBE, at 30 W for 100 min.

Synthesis of positive control substrate for reductive amination

*N*-Acetylglucosamine (GlcNAc) was covalently attached to the 5′-phosphate of DNA via an amide-containing 13-atom tether (compound **I** in Supplementary Scheme 1). Starting from 2-acetamido-2-deoxy-α-D-glucopyranose (i.e, GlcNAc, **II**), the 2-aminoethyl glycoside **VII** was synthesized in the following four-step procedure. Acetylation of **II** was performed with acetic anhydride in pyridine to give the penta-acetylated carbohydrate **III**, which was transformed into 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-d]-2-oxazoline (**IV**) by treatment with trifluoromethanesulfonic acid in 1,2-dichloro-ethane in 79% yield.[4] Compound **IV** was treated with 2-azidoethanol **V** (which was prepared from 2-chloroethanol and $NaN_3$ as described[5]) in the presence of trimethylsilyl trifluoromethanesulfonate and 4 Å molecular sieves to form the tetraacetylated β-(2-azidoethyl) glycoside **VI** (88% yield), in analogy to a procedure previously described for glycosylation of an oxazoline derivative with an azidoalcohol.[6] Treatment of **VI** with hydrogen over Pd/C in methanol-acetic acid produced the β-(2-aminoethyl)-GlcNAc derivative **VII** in 35% yield after purification by chromatography on silica gel in the presence of triethylamine.

The tetraacetylated, tethered GlcNAc derivative **VII** was used directly for on-bead conjugation to the carboxyl terminus of a 5′-modified DNA that was activated as an *N*-hydroxysuccinimide ester (**VIII**) .The 5′-modification was introduced by solid-phase synthesis using the commercially available 5′-carboxy-modifier C10 phosphoramidite (Glen Research). The conjugation reaction was performed with 5 mM **VII,** which was added from a 50 mM stock solution in dichloromethane to a suspension of the CPG solid-support carrying the modified DNA in 100 mM HEPES (pH 8.0) in 40% aqueous DMF. The reaction mixture was incubated at 25 °C for 12 h. The beads were washed with 50% aqueous DMF and $H_2O$. The GlcNAc-modified DNA was cleaved from the solid-support with concomitant deprotection of the phosphate and nucleobase protecting groups by treatment with $NH_4OH$/EtOH 3:1 at 37 °C for 24 h. The solution was separated from the CPG and evaporated to dryness. The crude product was dissolved in $H_2O$ and desalted on a Sephadex G25 column. Oligonucleotide-containing fractions (as analyzed by UV absorbance, $A_{260}$) were combined, evaporated, and purified on a 20% denaturing polyacrylamide gel, 7 M urea, 1× TBE, at 30 W for 130 min.

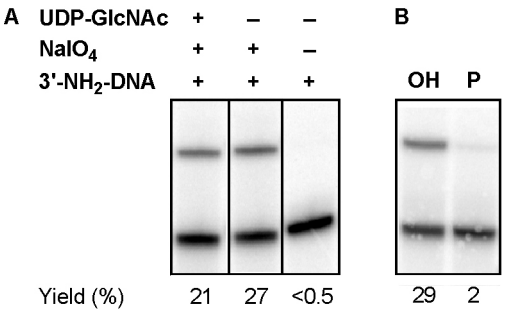
The GlcNAc-conjugated 27-mer DNA **I** (sequence corresponding to primer 2) was characterized by MALDI-MS (*m*/*z* calcd. 8607, found 8615 ± 9) and was used in a primer extension reaction as described above, using the pool complement as template. The 112-mer GlcNAc-DNA was taken through each round of the selection procedure (using **I** as a PCR primer in place of primer 2) to provide a positive control for the reductive amination step (26–44% yield).




**Supplementary Scheme 1**. Synthesis of the GlcNAc-conjugated DNA primer (**I**) for positive control experiments.

Investigating the activity of the uncloned 8FN deoxyribozyme pool

The uncloned DNA pool after 8 rounds of selection was used to investigate the UDP-GlcNAc and $NaIO_4$ requirements for the reductive amination product to be formed (Supplementary Figure 1A). Inclusion of UDP-GlcNAc was not required to observe formation of the reductive amination product, indicating that GlcNAc was not attached to the deoxyribozyme during the selection step. However, $NaIO_4$ incubation was required, indicating that an amine-reactive functional group was formed on the DNA itself. In an additional experiment, the 5′-phosphorylated uncloned 8FN DNA pool was used for the tandem $NaIO_4$ treatment and reductive amination procedure (without UDP-GlcNAc incubation; Supplementary Figure 1B). The presence of the 5′-phosphate greatly reduced the reductive amination yield, suggesting that the DNA-catalyzed reaction occurs near the 5′-terminus of the DNA.




**Supplementary Figure 1**. Investigation of uncloned 8FN deoxyribozyme pool activity. (A) The UDP-GlcNAc incubation, $NaIO_4$ treatment, and reductive amination procedures were performed with the DNA pool after round 8. Three parallel experiments were performed. Lane 1: all three procedural steps were performed. Lane 2: UDP-GlcNAc incubation was omitted. Lane 3: Both UDP-GlNAc incubation and $NaIO_4$ treatment were omitted. (B) 5′-Phosphorylation of the DNA pool after round 8 led to greatly reduced reductive amination yield after $NaIO_4$ treatment (no UDP-GlcNAc incubation was performed for either sample).

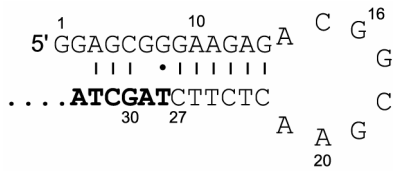
Sequence and secondary structure of the 10FN10 deoxyribozyme

The sequence of the 10FN10 deoxyribozyme is as follows:

5′-*GGAAGAGATGGCGACGGCGAACTCTT*CTAGCTATCCGGTACTAAGGGGCCTGACTCATTAGACCCACACACGGTA-CCCTTTGACTACCTTCGTCCCG*AGCTGATCCTGATGG*-3′

(The nucleotides in italics represent the original primer binding sites present during selection. Primer binding sites for preparation of 2′-ribo-modified 10FN10 variants are underlined.)

The 5′-terminal nucleotides of 10FN10 are expected to form a 6 base-pair stem-loop structure (non-bold nucleotides in Supplementary Figure 2). Secondary structure prediction by mfold suggests that additional base pairs can be formed with nucleotides from the initially random $N_{70}$ region (bold).

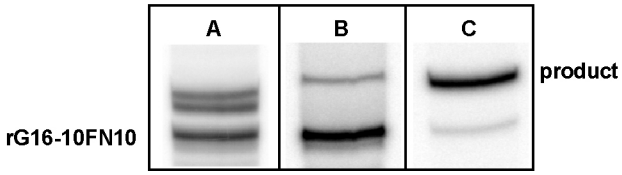



**Supplementary Figure 2.** Secondary structure of the 5′-terminal stem-loop region of the 10FN10 deoxyribozyme. The first 27 nucleotides (non-bold) form the constant primer-binding site. The bold nucleotides are derived from the initially random $N_{70}$ region.

Preparation of the 10FN10 deoxyribozyme and its 2′-ribo-modified variants

The 10FN10 deoxyribozyme and its 2′-ribo-modified variants were prepared by PCR amplification of the 10FN10 region within the plasmid that was isolated by the FastPlasmid® Mini procedure (Eppendorf) from an *E. coli* culture of the 10FN10 clone.

In a typical PCR procedure, 12.5 pmol of primer 1 and 50 pmol of primer 2 (or a 2′-ribo-modified variant) were used to amplify the template (usually 1 µL of a 1:10 dilution of the plasmid miniprep sample or 1 µL of a 10-cycle PCR product) in a 50 µL reaction, using 0.2 mM each dNTP supplemented with α-$^{32}$P-dCTP. Taq polymerase was used in 1× Taq polymerase buffer, and 28 cycles of amplification were performed, using the following temperature program: 95 °C, 5 min → [95 °C, 30 sec → 50 °C, 30 sec → 72 °C, 30 sec]$_{28}$ → 72 °C, 5 min → 4 °C, ∞. The sample was extracted with 50 µL phenol-chloroform-isoamyl alcohol 25:24:1 and mixed with 40 µL 80% stop solution, heated to 95 °C for 3 min, placed on ice for 5 min, and separated on an 8% polyacrylamide gel, 7 M urea, 1× TBE, at 30 W for 100 min. A typical image for preparation of rG16-10FN10 is shown in Supplementary Figure 3A. The lowest migrating band corresponds to rG16-10FN10. The middle band is the tailed complement (originating from primer 1). The upper band is a mixture of both products (not fully denatured) containing mostly the 10FN10 variant, as confirmed by isolation of all three bands and re-analysis on a separate gel.




**Supplementary Figure 3.** (A) 28-cycle PCR product of rG16-10FN10. (B) Reductive amination reaction of $NaIO_4$-treated rG16-10FN10 (yield 11%). (C) 5′-$^{32}$P-labeling of the isolated reductive amination product from (B).

$NaIO_4$ treatment and reductive amination of 10FN10 deoxyribozymes in the absence of UDP-GlcNAc

The internally $^{32}$P-labeled (from α-32P-dCTP during PCR) 10FN10 deoxyribozyme in 50 mM NaOAc, pH 5.2, was treated with 40 mM $NaIO_4$ at 37 °C for 2 h. The DNA was typically 0.5–2.0 µM in a final volume of 10 or 20 µL. The sample was diluted to 50 or 100 µL and NaCl added to a final concentration of 300 mM. The DNA was precipitated by the addition of 9 volumes of ethanol.

The $NaIO_4$-treated DNA was dissolved in $H_2O$, mixed with 3′-$NH_2$-DNA and splint in 100 mM NaOAc, pH 5.2, to give a final concentration of 20 µM 3′-$NH_2$-DNA and 15 µM splint. The sample was heated to 95 °C for 1 min and cooled on ice for 3 min. Then, $NiCl_2$ and $NaCNBH_3$ were added to final concentrations of 25 mM and 10 mM, respectively, in a final volume of 15 or 20 µL. The reaction mixture was incubated at 37 °C for 12–14 h then mixed with 20 µL of 80% stop solution, heated to 95 °C for 3 min, cooled on ice for 5 min, and separated on an 8% denaturing polyacrylamide gel. A typical image for purification of the reductive amination product of rG16-10FN10 with the 15-mer 3′-$NH_2$-DNA is shown in Supplementary Figure 3B.

5′-$^{32}$P-Labeling and alkaline hydrolysis of reductive amination products

The purified reductive amination products were $^{32}$P-labeled at the 5′-end by phosphorylation with T4 polynucleotide kinase (PNK) using γ-$^{32}$P-ATP. The 5′-$^{32}$P-labeled products were purified by PAGE. A typical gel image for $^{32}$P-labeling of the rG16-10FN10 reductive amination product is shown in Supplementary Figure 3C.

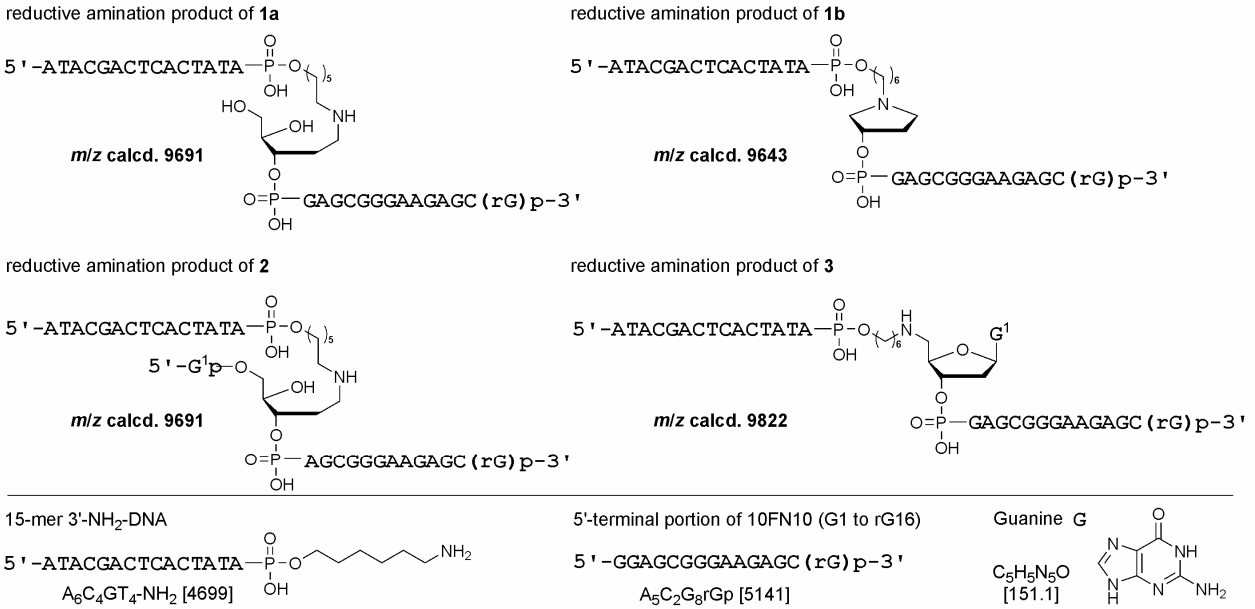
Aliquots of the labeled products were incubated in 100 mM NaOH at 95 °C for 5 min, then mixed with an equal amount of 80% stop solution and analyzed on a 20% polyacrylamide gel, 7 M urea, 1× TBE, at 30 W for 115 min. The gel was dried and exposed to a PhosphorImager Screen. The image was analyzed by ImageQuant software (Molecular Dynamics).

Isolation of reductive amination product and analysis by MALDI-MS

For isolation of the reductive amination product and analysis by MALDI-MS, 560 pmol of rG16-10FN10 was prepared from 2.4 mL of PCR reaction mixture (24 × 100 µL aliquots) using 2.4 nmol of primer 2 (rG16) and 800 pmol of primer 1. 28 cycles of PCR amplification were performed as described above. The product was precipitated by addition of NaCl to 300 mM and 3 volumes of ethanol then purified by PAGE. Oxidation of 500 pmol rG16-10FN10 was performed in 400 µL of 40 mM $NaIO_4$ in 50 mM NaOAc, pH 5.2, at 37 °C for 2 h. The DNA was precipitated by addition of 150 µL 3 M NaCl, 950 µL $H_2O$ and 12 mL ethanol.

The $NaIO_4$-treated sample was dissolved in 81 µL of $H_2O$ and mixed with 20 µL of 1 M NaOAc, pH 5.2. Then, 4 nmol of 15-mer 3′-$NH_2$-DNA and 3 nmol of DNA splint were added. The sample was heated to 95 °C for 1 min and cooled on ice for 2 min. Then, 50 µL of 100 mM $NiCl_2$ and 20 µL of 100 mM $NaCNBH_3$ were added to give a final volume of 200 µL. The reaction mixture was incubated at 37 °C for 12.5 h and precipitated by addition of 20 µL 3 M NaCl and 600 µL of ethanol. The pellet was dissolved in 30 µL $H_2O$, mixed with 30 µl 80% stop solution, heated to 95 °C for 3 min, cooled on ice for 5 min, and separated on an 8% polyacrylamide gel, 7M urea, 1× TBE, at 30 W for 100 min. The reductive amination product was isolated and quantitated by UV absorbance ($A_{260}$). The yield was 30 pmol of reductive amination product (6%), along with 250 pmol (50%) of a mixture of unreacted starting material and depurinated (but not reductively aminated) product.

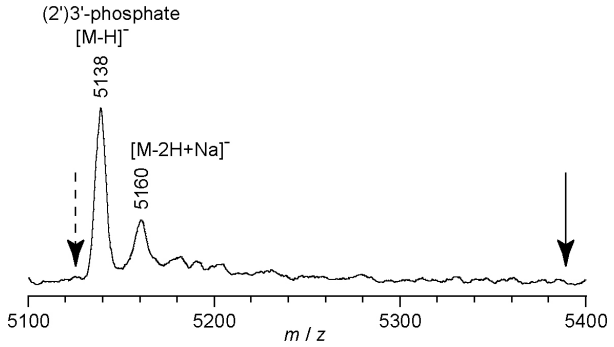
The reductive amination product was dissolved in 4.5 µl $H_2O$ and mixed with 0.5 µL of 100 mM NaOH to give a final NaOH concentration of 10 mM. The sample was heated to 95°C for 45 min and cooled on ice. Sample preparation for MALDI-MS analysis involved desalting via zip-tip and deposition with 3-hydroxypicolinic acid as matrix. MALDI-MS analysis was performed at the UIUC Mass Spectrometry Laboratory using a Voyager-DE STR mass spectrometer (Applied Biosystems) in negative ion mode. Based on the results in the next section, tthe expected *m*/*z* values stated in the manuscript were calculated for cleavage products containing a (2′)3′-phosphate (Supplementary Figure 4).




**Supplementary Figure 4**. Possible reductive amination products **1**–**3** and expected *m*/*z* values for the depurinated and/or oxidized rG16-10FN10 deoxyribozyme after cleavage at rG16. The structures of **1**–**3** are shown in Scheme 1. The products of **1a** and **2** are constitutional isomers and therefore have the same expected *m*/*z* value of 9691; this value was calculated as [(3′-$NH_2$-DNA – H) + (G1 to rG16) – (**G** – H) + 2H] = 4698 + 5141 – 150 + 2 = 9691. The expected *m*/*z* value of 9643 for the reductive amination product of **1b** was calculated as [(3′-$NH_2$-DNA – 2H) + (G1 to rG16) – (**G** – H) – ($CH_3O_2$)] = 4697 + 5141 – 150 – 47 = 9643. The expected *m*/*z* value for the reductive amination product of **3** was calculated as [(3′-$NH_2$-DNA) + (G1 to rG16) – $H_2O$] = 4699 + 5141 – 18 = 9822.

Optimization of alkaline hydrolysis conditions

The alkaline hydrolysis conditions for MALDI-MS analysis were optimized using a short 2′-ribonucleotide-containing oligonucleotide, primer 2 (rG16). Hydrolysis was complete under the conditions described above of 10 mM NaOH, 95 °C, 45 min. The observed *m*/*z* corresponds to the 2′- or 3′-phosphorylated product (no cyclic phosphate detected; *m*/*z* calcd. 5123 for cyclic phosphate, 5141 for open phosphate; found 5138 ± 5); Supplementary Figure 5). Under milder conditions (variation of NaOH concentration and reaction time at 95 °C), cleavage was incomplete and a mixture of cyclic phosphate and open phosphate was observed (data not shown). The optimized conditions were used for hydrolysis of the reductive amination product of rG16-10FN10.




**Supplementary Figure 5**. MALDI-MS of hydrolyzed primer 2 (rG16). The arrows mark the expected peak locations for cyclic phosphate (dashed arrow, *m*/*z* 5123) and unreacted starting material (solid arrow, *m*/*z* 5391). Only the open 2′(3′)-phosphate and not the closed 2′,3′-cyclic phosphate was observed after hydrolysis under the optimized conditions of 10 mM NaOH at 95 °C for 45 min.

Additional experiments for amine-reactive functional group and reductive amination reaction

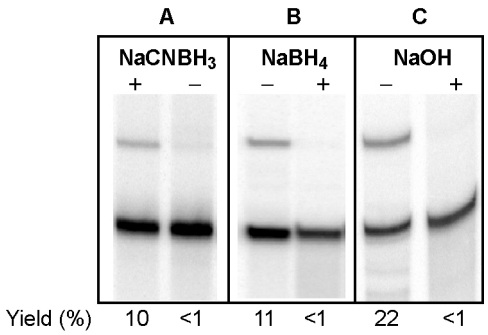
Upon $NaIO_4$ treatment of 10FN10, formation of an amine-reactive functional group that undergoes a reductive amination reaction with the 3′-$NH_2$ oligonucleotide is supported by the following three experiments (Supplementary Figure 6):

**A: $NaCNBH_3$ requirement.** The tandem $NaIO_4$ treatment and reductive amination procedure was performed with 10FN10 with or without inclusion of $NaCNBH_3$ during the latter step. The reactions were performed as described above, replacing 10 mM $NaCNBH_3$ by $H_2O$ in the "–" reaction. Product formation was only observed in the presence of $NaCNBH_3$. This is consistent with formation of an unstable imine intermediate that must be reduced to the amine to survive gel electrophoresis.

**B: $NaBH_4$ reduction.** The internally $^{32}$P-labeled 10FN10 deoxyribozyme was treated with $NaIO_4$ as described above. After precipitation, the product was dissolved in $H_2O$ and 50 mM sodium phosphate, pH 6.2, and 100 mM $NaBH_4$ were added (from 250 mM and 1 M aqueous stock solutions, respectively). The sample was incubated at 25 °C for 2 h and precipitated by addition of 300 mM NaCl and 3 volumes of ethanol. The subsequent reductive amination reaction was performed as described above. In parallel, the reaction was performed without $NaBH_4$ treatment. The $NaBH_4$ treatment suppressed formation of the reductive amination product. This is consistent with the formation of an aldehyde functional group on the DNA that is susceptible to borohydride reduction. All structures **1**–**3** (Scheme 1 in the manuscript) are compatible with this observation.

**C: β-Elimination under alkaline conditions.** The internally $^{32}$P-labeled 10FN10 deoxyribozyme was treated with $NaIO_4$ as described above. After precipitation, the product was dissolved in $H_2O$ and NaOH was added from a 1 M stock solution to give a final concentration of 90 mM NaOH. The sample was heated to 95 °C for 5 min, and the DNA was precipitated by addition of 300 mM NaCl and 9 volumes of ethanol. The subsequent reductive amination reaction was performed as described above. In parallel, the reaction was performed without NaOH treatment. The heating under alkaline conditions after $NaIO_4$ treatment prevented formation of the reductive amination product. This is consistent with β-elimination of

an aldehyde-containing modified deoxyribose moiety, leading to loss of the amine-reactive functional group. All structures **1**–**3** (Scheme 1 in the manuscript) possess a proton α to a carbonyl group that can be removed under alkaline conditions, leading to β-elimination. We also note that the lack of any nonspecific degradation of the DNA in the + lane of Supplementary Figure 6C means that periodate does not induce nonspecific depurination of the DNA, which would lead to cleavage upon base treatment.




**Supplementary Figure 6**. Additional reductive amination experiments. See text for description.

Additional information for alternatives to $NaIO_4$ treatment

**$KIO_4$**: The internally $^{32}$P-labeled 10FN10 deoxyribozyme was treated with 10 mM $KIO_4$ in 50 mM NaOAc, pH 5.2, at 37 °C for 2 h. Precipitation followed by reductive amination with the 30-mer 3′-$NH_2$ oligonucleotide was performed as described above. $KIO_4$ is less soluble in $H_2O$ than $NaIO_4$ (0.42 g/100 g $H_2O$ versus 14.4 g/100 g $H_2O$). Therefore, a saturated $KIO_4$ solution was prepared at 25°C, which is ca. 20 mM ($K_{SP} = 3.7 \times 10^{-4}$ at 25 °C) and used as a 2× stock solution. The reductive amination yield after $KIO_4$ treatment was 10.5%. For comparison, reductive amination after treatment with 10 mM $NaIO_4$ in a parallel experiment resulted in 8.5% yield.

**$NaClO_4$**: The internally $^{32}$P-labeled 10FN10 deoxyribozyme was treated with 40 mM $NaClO_4$ in 50 mM sodium acetate buffer, pH 5.2, at 37 °C for 2 h. Precipitation followed by reductive amination with the 30-mer 3′-$NH_2$ oligonucleotide was performed as described above. The reductive amination yield was 4.0%. For comparison, reductive amination after treatment with 40 mM $NaIO_4$ in a parallel experiment resulted in 21.3% yield. If **1a** is formed in the presence of $NaClO_4$, further oxidiation **1a**→**1b** is not expected, and therefore the reductive amination reaction of the 3′-$NH_2$ oligonucleotide would likely occur with **1a**. However, due to the small amount of available product this has not been tested experimentally.

**$KMnO_4$**: The internally $^{32}$P-labeled 10FN10 deoxyribozyme was treated with 1 mM $KMnO_4$ in 50 mM sodium phosphate buffer, pH 7.4, at 37 °C for 5 min. Then, 100 mM β-mercaptoethanol was added to destroy excess $KMnO_4$, followed by 300 mM NaCl and precipitation with 3 volumes of ethanol. Reductive amination with the 30-mer 3′-$NH_2$ oligonucleotide was performed as described above. The reaction yield was 1.5% (corrected for background signal within the lane). For comparison, in a parallel experiment the reductive amination yield after $NaIO_4$ treatment was 9.8%. If **1a** is formed in the presence of $KMnO_4$, it seems likely that **1a**→**1b** would subsequently occur (and perhaps overoxidation to the carboxylic acid). However, due to the small amount of available product this has not been tested experimentally.

**$H_2O_2$**: The internally $^{32}$P-labeled 10FN10 deoxyribozyme was treated with 40 mM $H_2O_2$ in 50 mM sodium phosphate buffer, pH 7.0, at 37 °C for 2 min. Precipitation followed by reductive amination with the 30-mer 3′-$NH_2$ oligonucleotide was performed as described above, but no product formation was detected (<0.5%). In related experiments, the 10FN10 deoxyribozyme was incubated with 200 mM and 2 M $H_2O_2$, respectively, which led to considerable nonspecific DNA degradation.

**Ammonium persulfate (APS)**: Incubation of the 10FN10 deoxyribozyme with 12.5 mM APS in 40 mM potassium phosphate buffer, pH 5.5, and irradiation with UV light (254 nm) from a handheld UV lamp at 25 °C for 45 min resulted in considerable nonspecific DNA degradation.

References for Supplementary Information

1. Y. Wang and S. K. Silverman, *Biochemistry*, 2003, **42**, 15252-15263.
2. A. Flynn-Charlebois, Y. Wang, T. K. Prior, I. Rashid, K. A. Hoadley, R. L. Coppins, A. C. Wolf and S. K. Silverman, *J. Am. Chem. Soc.*, 2003, **125**, 2444-2454.
3. C. V. Miduturu and S. K. Silverman, *J. Org. Chem.*, 2006, **71**, 5774-5777.
4. S. Nakabayashi, C. D. Warren and R. W. Jeanloz, *Carbohydr. Res.*, 1986, **150**, C7-10.
5. A. Y. Chernyak, G. V. M. Sharma, L. O. Kononov, P. R. Krishna, A. B. Levinsky, N. K. Kochetkov and A. V. Rama Rao, *Carbohydr. Res.*, 1992, **223**, 303-309.
6. P. C. Rensen, S. H. van Leeuwen, L. A. Sliedregt, T. J. van Berkel and E. A. Biessen, *J. Med. Chem.*, 2004, **47**, 5798-5808.