Phosphoserine Lyase Deoxyribozymes: DNA-Catalyzed Formation of Dehydroalanine Residues in Peptides

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Oligonucleotides and oligonucleotide conjugates

DNA oligonucleotides and oligonucleotide conjugates were obtained from Integrated DNA Technologies (Coralville, IA) or prepared by solid-phase synthesis on an ABI 394 instrument using reagents from Glen Research. All oligonucleotides and conjugates were purified by 7 M urea denaturing PAGE with running buffer 1× TBE (89 mM each Tris and boric acid and 2 mM EDTA, pH 8.3), extracted from the polyacrylamide with TEN buffer (10 mM Tris, pH 8.0, 1 mM EDTA, 300 mM NaCl), and precipitated with ethanol as described previously.[1]

Deoxyribozyme sequences were as follows (76 nt; initially random N_{40} regions underlined; each is 39 nt, presumably due to a fortuitous deletion by Taq polymerase in an unidentified selection round):

DhaDz1 5′-CGAATTAAGACTGAATTCACAGGTGGCGGATGTAGTTTACCCGTTTTCTGTAGAGCCATAAGAAACGAGATATTCC-3′

DhaDz2 5′-CGAATTAAGACTGAATTCACAGGATTCAAAGTGGCTGTCAGAAGTTGGTGAGGGAAGATAAGAAACGAGATATTCC-3′

Synthesis of peptides and DNA-anchored peptides

All amino acid monomers including those for phosphoserine (pSer, S^P), homocysteine (Hcy), and *S*-methylcysteine (Cys^{Me}, C^{Me}) were obtained from Chem-Impex (Wood Dale, IL). Peptides were prepared by solid-phase synthesis using Fmoc Rink amide MBHA resin from Chem-Impex, with *N,N,N′,N′*-tetramethyl-*O*-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate (HATU) as coupling agent.[2]

Synthesis of the peptide $AAAS^PAA$ was performed at 0.2 mmol scale, initiated using 260 mg of Rink amide resin with a loading capacity of 0.77 mmol/g. All steps were monitored by ninhydrin test. For each coupling, 5 equivalents (1.0 mmol) of Fmoc-amino acid, 4.9 equivalents (373 mg, 0.98 mmol) of HATU, and 10 equivalents (350 μL, 2.0 mmol) of *N*,*N*-diisopropylethylamine (DIPEA) were mixed in 5 mL of anhydrous DMF. The coupling reaction was initiated by adding this mixture to the resin and agitating by bubbling with nitrogen for 60 min, followed by washing with DMF (3 × 10 mL). The N-terminus of the newly installed amino acid was deprotected by agitating the resin in 20% piperidine in DMF (5 mL) under nitrogen for 5 min a total of three times, each time washing with DMF (3 × 5 mL). The peptide was cleaved from the solid support by stirring the resin in a separate vial with a solution containing 5 mL of trifluoroacetic acid (TFA), 125 μL of water, and 50 μL of triisopropylsilane for 90 min. The liquid solution was separated from the resin by filtration. This solution was dried on a rotary evaporator, providing a solid. To this material 20 mL of cold diethyl ether was added, and the peptide was obtained as a white solid that was filtered and purified by HPLC.

Synthesis of each of the peptides $AAAC^{Me}AA$ and Ac-IS^PVWQDWGAHR(Hcy)T (compstatin precursor) was performed as described above, with the following procedural modifications. Synthesis was performed at 0.1 mmol scale, initiated using 303 mg of 0.33 mmol/g (Hcy) or 130 mg of 0.77 mmol/g (C^{Me}) Rink amide resin. For each coupling of a standard amino acid, 5 equivalents (0.5 mmol) of Fmoc-amino acid, 4.9 equivalents (187 mg, 0.49 mmol) of HATU, and 10 equivalents (175 μL, 1.0 mmol) of DIPEA were mixed in 3 mL of anhydrous DMF and agitated with the resin for 60 min. For Fmoc-*S*-trityl-L-homocysteine (Hcy) or Fmoc-*S*-methyl-L-cysteine (C^{Me}), 2 equivalents (0.2 mmol) of Fmoc-amino acid, 1.96 equivalents (75 mg, 0.20 mmol) of HATU, and 4 equivalents (70 μL, 0.4 mmol) of DIPEA were mixed in 3 mL of anhydrous DMF and agitated with the resin for 120 min. The compstatin peptide was capped via acetylation of its N-terminus by agitating the resin in 50 equivalents (462 μL, 5 mmol) of acetic anhydride and 25 equivalents of DIPEA (435 μL, 2.5 mmol) in 5 mL of DMF under nitrogen for 30 min. The peptide was cleaved from the solid support by stirring the resin in a separate vial with a solution containing 3 mL of TFA, 100 μL of water, 100 μL of ethanedithiol (for compstatin), and 40 μL of triisopropylsilane for 90 min.

The $AAAS^PAA$ peptide was coupled to the DNA anchor oligonucleotide via the N-terminal α-amino group with a linkage created by reductive amination (Figure S1A). The DNA anchor oligonucleotide was 5′-GGAATATCTCGTTTCTTAT-HEG-rA-3′. This sequence includes a hexa(ethylene glycol) [HEG] spacer, which provides structural flexibility and may induce the emergent deoxyribozymes to function well with free (unanchored) peptide substrates, and a 3′-terminal ribonucleotide, which enables the reductive amination process. Coupling of peptide to DNA anchor was performed using the previously described procedure.[2]

To prepare the DNA-anchored AAA(Dha)AA peptide, a precursor peptide incorporating *S*-methylcysteine, Cys^{Me} (C^{Me}), was synthesized. The $AAAC^{Me}AA$ peptide was conjugated to the 3′-terminal ribonucleotide of the DNA anchor oligonucleotide as described above. Then, a 50 µL sample containing 500 pmol of DNA-anchored $AAAC^{Me}AA$, 100 mM HEPES, pH 7.5, and 10 mM $NaIO_4$ was incubated at room temperature for 1 h to oxidize the thioether to the sulfone. The sulfone product was precipitated by addition of 220 µL of water, 30 µL of 3 M NaCl, and 900 µL of ethanol. The sulfone product was dissolved in 20 µL of 10 mM NaOH, pH 12.0, and incubated at 37 °C for 4 h to form the final elimination product, DNA-anchored AAA(Dha)AA. This final product was precipitated by addition of 250 µL of water, 30 µL of 3 M NaCl, and 900 µL of ethanol. MALDI mass spectrometry for DNA-anchored $AAAC^{Me}AA$ peptide *m*/*z* calcd. 6913.9, found 6915.5, Δ = +0.02%. DNA-anchored AAA(Dha)AA elimination product *m*/*z* calcd. 6865.8, found 6864.8, Δ = –0.01%.

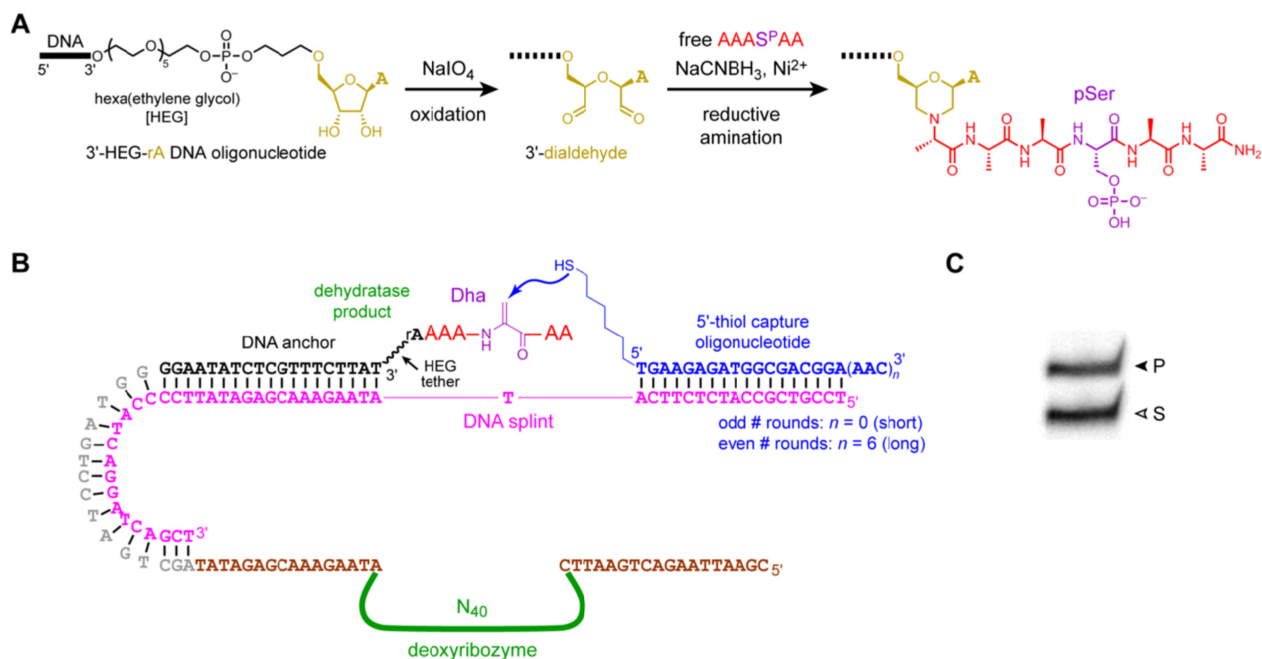

Figure S1. (A) Synthesis of DNA-anchored $AAAS^{P}AA$ peptide by attachment of the DNA anchor to the N-terminal α-amino group, with the linkage created by reductive amination. (B) Capture step during in vitro selection. (C) Validation of the capture reaction using the short 5′-thiol capture oligonucleotide (S = substrate, P = product).

Procedure for validation of Dha capture reaction during in vitro selection process. The 5′-terminus of the DNA-anchored $AAAC^{Me}AA$ precursor peptide was ligated to the 3′-terminus of the random deoxyribozyme pool using a DNA splint and T4 DNA ligase (see *Procedure for ligation step*, below). The ligated product was oxidized and eliminated as described above, forming DNA-anchored AAA(Dha)AA linked to the deoxyribozyme pool. A 10 µL sample containing 0.1 pmol of DNA-anchored AAA(Dha)AA linked to the pool, 20 pmol of capture splint, and 100 pmol of the short 5′-thiol capture oligonucleotide (*n* = 0 in Figure S1B) in 100 mM Na_2CO_3, pH 9.2, was incubated at 37 °C for 12 h, with result shown in Figure S1C.

<u>In vitro selection procedure</u>

The selection procedure, cloning, and initial screening of individual clones were performed essentially as described previously.[2] An overview of the key selection and capture steps of each round is shown in Figure 2. A depiction of the capture step with nucleotide details and validation data is shown in Figure S1. The random deoxyribozyme pool was 5′-CGAATTAAGACTGAATTC-N_{40}-ATAAGAAACGAGATATAGCTGATCC-TGATGG-3′. PCR primers were 5′-CGAATTAAGACTGAATTC-3′ (forward primer) and 5′-(AAC)$_4$XCCATCAG-ATCAGCT-3′, where X is the HEG spacer to stop Taq polymerase (reverse primer). In each round, the

ligation step to attach the deoxyribozyme pool at its 3′-end with the 5′-end of the DNA-anchored hexapeptide substrate was performed using a DNA splint and T4 DNA ligase. The ligation splint sequence was 5′-ATAAGAAACGAGATATTCCTCCATCAGGATCAGCTATATCTCGTTTCTTAT-3′, where the underlined T is included to account for the untemplated A nucleotide that is added at the 3′-end of each PCR product by Taq polymerase. This T nucleotide was omitted from the splint used for ligation of the initially random N_{40} pool, which was prepared by solid-phase synthesis without the untemplated A. The length of the capture oligonucleotide was alternated between shorter (odd-numbered rounds) and longer (even-numbered rounds), to avoid selection of particular DNA sequences that migrate aberrantly at a fixed position on the gel. Nucleotide sequences of the DNA anchor oligonucleotide, the deoxyribozyme binding arms, the splint oligonucleotide, and the two 5′-thiol capture oligonucleotides are shown in Figure S1B.

Procedure for ligation step in round 1. A 34 µL sample containing 1 nmol of DNA pool, 850 pmol of DNA splint, and 750 pmol of 5′-phosphorylated DNA-hexapeptide substrate was annealed in 5 mM Tris, pH 7.5, 15 mM NaCl, and 0.1 mM EDTA by heating at 95 °C for 3 min and cooling on ice for 5 min. To this solution was added 4 µL of 10× T4 DNA ligase buffer (Fermentas) and 2 µL of 5 U/µL T4 DNA ligase (Fermentas). The sample was incubated at 37 °C for 12 h and purified by 8% PAGE.

Procedure for ligation step in subsequent rounds. A 16 µL sample containing the PCR-amplified DNA pool (~5–10 pmol), 25 pmol of DNA splint, and 50 pmol of 5′-phosphorylated DNA-hexapeptide substrate was annealed in 5 mM Tris, pH 7.5, 15 mM NaCl, and 0.1 mM EDTA by heating at 95 °C for 3 min and cooling on ice for 5 min. To this solution was added 2 µL of 10× T4 DNA ligase buffer (Fermentas) and 2 µL of 1 U/µL T4 DNA ligase (Fermentas). The sample was incubated at 37 °C for 12 h and purified by 8% PAGE.

Procedure for selection step in round 1. The selection experiment was initiated with 200 pmol of the ligated N_{40} pool. A 20 µL sample containing 200 pmol of ligated N_{40} pool was annealed in 5 mM HEPES, pH 7.5, 15 mM NaCl, and 0.1 mM EDTA by heating at 95 °C for 3 min and cooling on ice for 5 min. The selection reaction was initiated by bringing the sample to 40 µL total volume containing 70 mM HEPES, pH 7.5, 1 mM $ZnCl_2$, 20 mM $MnCl_2$, 40 mM $MgCl_2$, and 150 mM NaCl. The sample was incubated at 37 °C for 14 h.

Procedure for selection step in subsequent rounds. A 10 µL sample containing ligated pool was annealed in 5 mM HEPES, pH 7.5, 15 mM NaCl, and 0.1 mM EDTA by heating at 95 °C for 3 min and cooling on ice for 5 min. The selection reaction was initiated by bringing the sample to 20 µL total volume containing 70 mM HEPES, pH 7.5, 1 mM $ZnCl_2$, 20 mM $MnCl_2$, 40 mM $MgCl_2$, and 150 mM NaCl. The sample was incubated at 37 °C for 14 h.

Procedure for preparation of 5′-thiol capture oligonucleotide. The 5′-thiol capture oligonucleotide (short or long version as appropriate; Figure S1B) was prepared by DTT reduction of the precursor oligonucleotide (prepared using the Glen Research thiol modifier C6 S-S) immediately prior to each use. A 50 µL sample containing 2 to 5 nmol of precursor oligonucleotide in 100 mM HEPES, pH 7.5, and 25 mM DTT was incubated at 37 °C for 2 h. To remove excess DTT, the sample was precipitated by adding 220 µL of water, 30 µL of 3 M NaCl, and 900 µL of ethanol.

Procedure for capture step in round 1. To the 40 µL selection sample was added 6 µL of 0.5 M EDTA, pH 8.0 (3 µmol). The DNA was precipitated by adding 224 µL of water, 30 µL of 3 M NaCl, and 900 µL of ethanol. The sample was then brought to 40 µL total volume containing 300 pmol of capture splint, and 500 pmol of short 5′-thiol capture oligonucleotide in 100 mM Na_2CO_3, pH 9.2 and incubated at 37 °C for 12 h. The sample was loaded directly on 8% PAGE.

Procedure for capture step in subsequent rounds. To the 20 µL selection sample was added 3 µL of 0.5 M EDTA, pH 8.0 (1.5 µmol). The DNA was precipitated by adding 247 µL of water, 30 µL of 3 M NaCl, and 900 µL of ethanol. The sample was then brought to 10 µL total volume containing 20 pmol of capture splint, and 100 pmol of the appropriate 5′-thiol capture oligonucleotide (long for even-numbered rounds, short for odd-numbered rounds) in 100 mM Na_2CO_3, pH 9.2 and incubated at 37 °C for 12–14 h. The sample was loaded directly on 8% PAGE.

Procedure for PCR. In each selection round, two PCR reactions were performed, 10-cycle PCR followed by 30-cycle PCR. First, a 100 µL sample was prepared containing the PAGE-purified selection product, 200 pmol of forward primer, 50 pmol of reverse primer, 20 nmol of each dNTP, and 10 µL of 10× Taq polymerase buffer (1× = 20 mM Tris-HCl, pH 8.8, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, and 0.1% Triton X-100). This sample was cycled 10 times according to the following PCR program: 94 °C for 2 min, 10× (94 °C for 30 s, 47 °C for 30 s, 72 °C for 30 s), 72 °C for 5 min. Taq polymerase was removed by phenol/chloroform extraction. Second, a 50 µL sample was prepared containing 1 µL of the 10-cycle PCR product, 100 pmol of forward primer, 25 pmol of reverse primer, 10 nmol of each dNTP, 20 µCi of α-^{32}P-dCTP (800 Ci/mmol), and 5 µL of 10× Taq polymerase buffer. This sample was cycled 30 times according to the following PCR program: 94 °C for 2 min, 30× (94 °C for 30 s, 47 °C for 30 s, 72 °C for 30 s), 72 °C for 5 min. Samples were separated by 8% PAGE. The forward and reverse single-stranded PCR products were separable because formation of the reverse product was initiated using the reverse primer, which contains a nonamplifiable spacer that stops Taq polymerase.

Procedure for cloning and initial screening. Using 1 µL of a 1:1000 dilution of the 10-cycle PCR product from selection round 9, 25-cycle PCR was performed using the analogous procedure as described above for 30-cycle PCR, omitting α-^{32}P-dCTP and using primers 5′-CGAATTAAGACTGAATTC-3′ (forward primer, 25 pmol) and 5′-TAATTAATTAATTACCCATCAGGATCAGCT-3′ (reverse primer, 25 pmol), where the extensions with TAA stop codons in each frame were included to suppress false negatives in blue-white screening.[3] The PCR product was purified on 2% agarose and cloned using a TOPO TA cloning kit (Invitrogen). Initial screening of individual deoxyribozyme clones was performed using DNA strands prepared by PCR from miniprep DNA derived from individual *E. coli* colonies. The miniprep DNA samples were first assayed by digestion with EcoRI to ascertain the presence of the expected insert. The concentration of each PAGE-purified deoxyribozyme strand was estimated from the UV shadowing intensity relative to suitable standards. Each screening assay used ~0.1 pmol of 5′-^{32}P-radiolabeled DNA-anchored AAAS^{P}AA substrate and at least 20 pmol of deoxyribozyme in the single-turnover assay procedure described in a subsequent section of this document (here using 1.0 mM $ZnCl_2$).

Selection progression

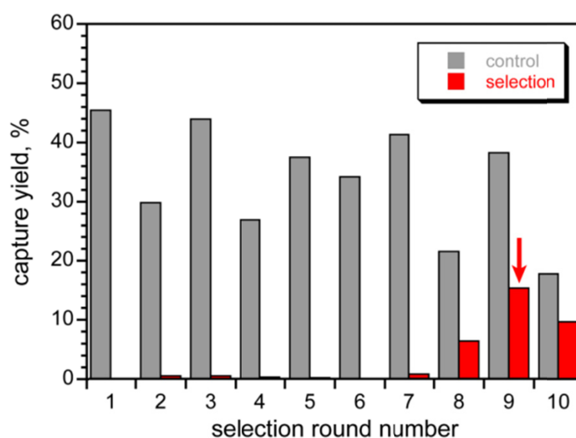

Figure S2. Progression of the in vitro selection experiment. In each round, "control" refers to the yield for the splint capture reaction using the DNA-anchored AAA(Dha)AA substrate, and "selection" refers to the yield for the splint capture reaction using the deoxyribozyme pool for that round. The arrow marks the cloned round. Round 1 pool yield was not measured because the pool was not radiolabeled. Higher capture yield was observed with the short 5′-thiol oligonucleotide ($n = 0$ in Figure S1B; 18-mer) as compared with the long 5′-thiol oligonucleotide ($n = 6$ in Figure S1B; 54-mer). This outcome was attributed to some of the long 5′-thiol oligonucleotide lacking the intended 5′-thiol group, likely due to relatively inefficient coupling of the thiol phosphoramidite (due to partial hydrolysis) along with poor 12% PAGE resolution for such a lengthy oligonucleotide. After this selection experiment was concluded and individual deoxyribozymes were already identified, we prepared a sequence variant of the long 5′-thiol oligonucleotide by splint ligation starting with the 20% PAGE-purified short 5′-thiol oligonucleotide. This variant of the long 5′-thiol oligonucleotide had capture yield equivalent to that of the short 5′-thiol oligonucleotide (ca. 40%).

Single-turnover deoxyribozyme assay procedure (Figure 3 and Figure S3A)

The 5′-end of DNA-anchored AAAS^{P}AA was radiolabeled using γ-^{32}P-ATP and Optikinase (USB), which lacks the 3′-phosphatase activity that we observed also dephosphorylates the pSer side chain. A 10 μL sample containing 0.1 pmol of 5′-^{32}P-radiolabeled DNA-anchored AAAS^{P}AA and 20 pmol of deoxyribozyme was annealed in 5 mM HEPES, pH 7.5, 15 mM NaCl, and 0.1 mM EDTA by heating at 95 °C for 3 min and cooling on ice for 5 min. The DNA-catalyzed reaction was initiated by bringing the sample to 20 μL total volume containing 70 mM HEPES, pH 7.5, 1.5 or 2.0 mM $ZnCl_2$ if included, 20 mM $MnCl_2$ if included, 40 mM $MgCl_2$ if included, and 150 mM NaCl, and the sample was incubated at 37 °C. Note that the Zn^{2+} concentration was carefully optimized for each deoxyribozyme. At appropriate time points, 2 μL aliquots were quenched with 5 μL stop solution (80% formamide, 1× TBE [89 mM each Tris and boric acid and 2 mM EDTA, pH 8.3], 50 mM EDTA, 0.025% bromophenol blue, 0.025% xylene cyanol). Samples were separated by 20% PAGE and quantified with a PhosphorImager. Values of k_{obs} were obtained by fitting the yield versus time data directly to first-order kinetics; i.e., yield = Y•$(1 - e^{-kt})$, where $k = k_{obs}$ and Y is the final yield. Each k_{obs} value is reported with error calculated as the standard deviation from the indicated number of independent determinations.

Multiple-turnover procedure with malachite green dye assay (Figure 4A)

A 20 μL sample containing 2 nmol of deoxyribozyme, 2.5 nmol of free DNA anchor oligonucleotide, and 80 nmol of AAAS^{P}AA hexapeptide in 70 mM HEPES, pH 7.5, 6 or 8 mM $ZnCl_2$ as appropriate, 20 mM $MnCl_2$, 40 mM $MgCl_2$, and 150 mM NaCl was incubated at 37 °C. Note that the Zn^{2+} concentration was carefully optimized for each deoxyribozyme. At each time point (0–96 h), 2 μL of the sample was frozen on dry ice and stored at –80 °C until further processing. To the sample was added 98 μL of water and 20 μL of malachite green assay solution (BioAssay Systems POMG-25H). After incubation at room temperature for 30 min, the sample was diluted with 180 μL of water, and the absorbance at 620 nm was measured (NanoDrop 2000c, Thermo Scientific). Standard curves for inorganic phosphate (P_i) were

obtained using solutions containing known amounts of P_i and all other assay components with 2 nmol of an unrelated, catalytically inactive 74 nt DNA oligonucleotide instead of the deoxyribozyme. Numbers of turnovers were calculated directly from the ratio of mole amounts of released P_i and deoxyribozyme.

Multiple-turnover procedure with LC-MS assay (Figure 4B and Figure S3B)

A 20 µL sample containing 2 nmol of deoxyribozyme, 2.5 nmol of free DNA anchor oligonucleotide, and 80 nmol of peptide in 70 mM HEPES, pH 7.5, 6 or 8 mM $ZnCl_2$ (optimized), 20 mM $MnCl_2$, 40 mM $MgCl_2$, and 150 mM NaCl was incubated at 37 °C for 96 h. To remove the deoxyribozyme before LC-MS analysis, a filtration step was performed. An Amicon Ultra-0.5 mL 3 kDa centrifugal filter (EMD Millipore) was washed with 400 µL of water by centrifugation at 14000 × *g* for 10 min. To the sample was added 400 µL water, and the combined solution was transferred to the filter, which was centrifuged at 14000 × *g* for 20 min. Another 400 µL of water was passed through the filter at 14000 × *g* for 20 min. Finally, 400 µL of 5% aqueous acetonitrile was passed through the filter at 14000 × *g* for 20 min. The combined filtrates were evaporated to dryness, and redissolved in 40 µL of water. The sample was analyzed by LC-MS on an Acquity UPLC instrument with a Phenomenex Jupiter C_{18} column (5 µm, 0.1 × 150 mm) attached to an electrospray ionization mass spectrometer (Q-TOF Synapt-G1 Waters in positive ion scan mode using the manufacturer's suggested parameters) and an isocratic gradient of 1% solvent A (0.1% formic acid in acetonitrile) and 99% solvent B (0.1% formic acid in water) at 0.18 mL/min for 10 min.

Single-turnover procedure with HPLC assay (Figure 5B)

A 20 µL sample containing 2 nmol of deoxyribozyme, 2.5 nmol of free DNA anchor oligonucleotide, and 1 nmol of peptide in 70 mM HEPES, pH 7.5, 4 mM $ZnCl_2$ (optimized), 20 mM $MnCl_2$, 40 mM $MgCl_2$, and 150 mM NaCl was incubated at 37 °C for 96 h. The sample was quenched with 3 µL of 0.5 M EDTA, pH 8.0. To reverse formation of potential peptide disulfide dimers, 4 µL of 1 mM tris(2-carboxyethylphosphine) (TCEP; 4 nmol) was added, and the sample was incubated at 25 °C for 15 min. Because compstatin does not pass well through the Amicon filter, the deoxyribozyme was not removed by filtration. The sample was analyzed by HPLC on a Beckman System Gold instrument with a Phenomenex Kinetex C_{18} column (2.6 µm, 4.6 × 150 mm) and a gradient of 15% solvent A (acetonitrile) and 85% solvent B (0.1% trifluoroacetic acid in water) at 0 min to 30% solvent A and 70% solvent B at 60 min with flow rate of 0.5 mL/min.

The HPLC-purified intermediate was cyclized by base treatment. The intermediate was evaporated to dryness, redissolved in 40 µL of 100 mM Tris, pH 8.0, and incubated at 25 °C for 4 h (shorter times not attempted). The sample was analyzed by HPLC as above.

Mass spectrometry procedures

To prepare the DNA-anchored AAA(Dha)AA using a deoxyribozyme, a 10 µL sample containing 50 pmol of DNA-anchored AAAS^PAA substrate and 100 pmol of DhaDz1 (or DhaDz2) was annealed in 5 mM HEPES, pH 7.5, 15 mM NaCl, and 0.1 mM EDTA by heating at 95 °C for 3 min and cooling on ice for 5 min. The DNA-catalyzed elimination reaction was initiated by bringing the sample to 20 µL total volume containing 70 mM HEPES, pH 7.5, 1.5 mM $ZnCl_2$ (or 2.0 mM $ZnCl_2$), 20 mM $MnCl_2$, 40 mM $MgCl_2$, and 150 mM NaCl. The sample was incubated at 37 °C for 16 h and quenched with 5 µL of 0.5 M EDTA, pH 8.0 (2.5 µmol), desalted by Millipore C_{18} ZipTip, and analyzed by MALDI mass spectrometry. Data were acquired on a Bruker UltrafleXtreme MALDI-TOF mass spectrometer with matrix 3-hydroxypicolinic acid in positive ion mode at the UIUC School of Chemical Sciences Mass Spectrometry Laboratory (instrument purchased with support from NIH S10RR027109A).

For the MALDI mass spectrometry analyses of Figure 5C, the HPLC-purified precursor, intermediate, or product was evaporated to dryness, redissolved in a total volume of 40 µL containing 100 mM Tris, pH 8.0, 25 mM IAA, and 100 µM TCEP, and incubated at 25 °C for 2 h in the dark. The sample was desalted and analyzed by MALDI mass spectrometry as described above, except with 2,5-dihydroxybenzoic acid as matrix.

Single-turnover and multiple-turnover data for the DhaDz2 deoxyribozyme

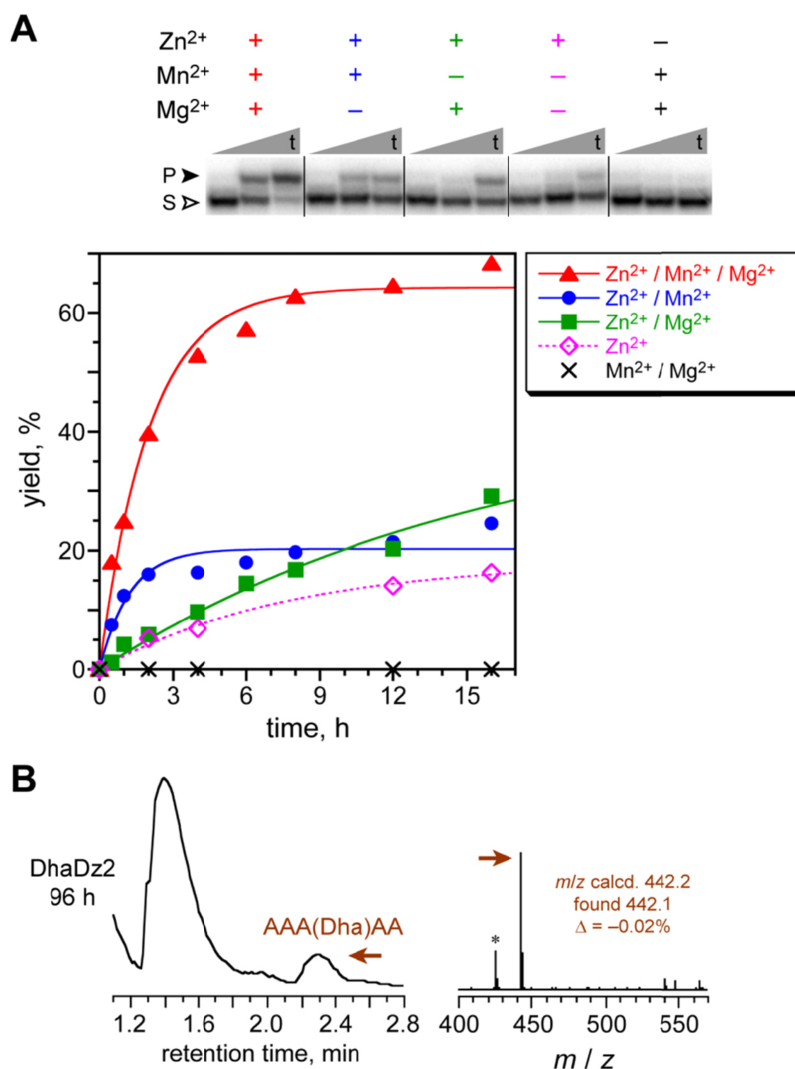

Figure S3. Single-turnover and multiple-turnover data for the DhaDz2 deoxyribozyme. (A) Single-turnover pSer lyase activity of the DhaDz2 deoxyribozyme with DNA-anchored peptide AAASPAA. S = pSer substrate; P = Dha product. Incubation conditions: 70 mM HEPES, pH 7.5, combinations of 2.0 mM $ZnCl_2$, 20 mM $MnCl_2$, and 40 mM $MgCl_2$ as appropriate, and 150 mM NaCl at 37 °C (10 nM 5′-^{32}P-radiolabeled DNA-anchored peptide, 1 µM DhaDz2). The PAGE image shows timepoints at t = 30 s, 4 h, and 16 h. The Zn^{2+} concentration of 2.0 mM was optimal at 20 mM Mn^{2+} and 40 mM Mg^{2+}. (B) Multiple-turnover pSer lyase activity of the DhaDz2 deoxyribozyme with free peptide AAAS^{P}AA. LC-MS experiment as in Figure 4B, but with 8 mM Zn^{2+}. The observed 10% yield is equivalent to 4.0 turnovers.

References for Supporting Information

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