Reversible Photocontrol of Deoxyribozyme-Catalyzed RNA Cleavage under Multiple-Turnover Conditions


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Synthesis of phosphoramidites

Dichloromethane (DCM) was dried under reflux over calcium hydride, distilled and stored over activated 3Å molecular sieves under argon. DCM was passed through activated basic Al₂O₃ immediately prior to use and NMR’s obtained within 1 hour (¹³C, ³¹P) or 12 hours (¹H) of the CDCl₃ solution being prepared. All other reagents were purchased from commercial suppliers and used without further purification. Silica gel (particle size 30–60 µm) for flash column chromatography was obtained from BDH and dried at 300°C immediately prior to use.

UV-Visible data were obtained with a VARIAN CARY 100 Spectrometer. Nuclear Magnetic Resonance (NMR) spectra were run on Bruker AC-250 and AMX-400 spectrometers.

**o-Phenylazobenzoic acid-N-hydroxysuccinimidy l ester.**

To a stirred suspension of o-phenylazobenzoic acid (452 mg, 2.0 mmol) and N-hydroxysuccinimide (255 mg, 2.2 mmol) in anhydrous DCM (10 ml) N,N'-dicyclohexylcarbodiimide (454 mg, 2.2 mmol) was added at room temperature under argon. After 45 min, precipitated N,N'-dicyclohexylurea was removed by filtration and solvent removed in vacuo. The residual brown solid was purified by silica gel column chromatography eluting with anhydrous DCM to yield the product as a moisture-sensitive brown-orange solid (565 mg, 87%).

**5'-O-(4,4'-dimethoxytrityl)-2'-(N-(o-phenylazo)benzamido)-2'-deoxyuridine.**

To a flask containing 5'-O-(4,4'-dimethoxytrityl)-2'-amino-2'-deoxyuridine (250 mg, 0.46 mmol) and N,N-diisopropylethylamine (DIPEA; 80 µl, 0.47 mmol) added. The reaction was stirred under ambient conditions for a further 6 hours, a further aliquot of DIPEA (100 µl, 0.59 mmol) was added and the reaction stored at 3°C overnight. Excess activated ester was quenched by addition of n-butylamine (190 µl, 1.92 mmol) and stirred with room temperature for 1 hour. The reaction mixture was diluted with ethyl acetate (250 ml) and washed successively with aqueous saturated NaHCO₃ (2 x 150 ml) and saturated NaCl (2 x 150 ml). The organics were dried over anhydrous sodium sulfate, filtered and solvent removed in vacuo. The resultant red gum was purified by silica gel column chromatography, eluting with a gradient of 0–2% (v/v) methanol in DCM containing 1% (v/v) triethylamine to give the title compound as an orange solid (250 mg, 72%).

**NMR data**

1H NMR (300 MHz, CDCl₃, 20°C, TMS) δ = 3.42 (2H, s, 2x CH₂), 7.50 – 7.60 (4H, m, H₃, H₄, H₅, PhN; H₄ ArCO), 7.74 (2H, m, H₂, H₆ PhN), 8.06 (3H, m, H₃, H₅, H₆ ArCO); ¹³C (76 MHz, CDCl₃, 20°C, TMS) δ = 26.2, 119.0, 124.2, 124.40, 129.6, 130.5, 131.3, 132.3, 134.3, 152.7, 152.9, 153.0, 169.4; ES+ MS m/z = 324 (M+H), 346 (M+Na).

**Spectral data**

UV-Visible data were obtained with a VARIAN CARY 100 Spectrometer. Nuclear Magnetic Resonance (NMR) spectra were run on Bruker AC-250 and AMX-400 spectrometers.

**ACKNOWLEDGEMENTS**

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**Reversible photocontrol of deoxyribozyme-catalyzed RNA cleavage under multiple turnover conditions**

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**Synthesis of phosphoramidites**

Dichloromethane (DCM) was dried under reflux over calcium hydride, distilled and stored over activated 3Å molecular sieves under argon. Triethylamine and n-butylamine were dried under reflux over calcium hydride and distilled immediately prior to use; N,N-diisopropylethylamine (DPEA) was similarly purified but stored over activated 3Å molecular sieves for up to 6 weeks. DME was obtained as anhydrous solvent over molecular sieves from Fluka. Dry MeOH was obtained by distillation from magnesium turnings and stored over activated 3Å molecular sieves. Tetrahydrofuran (THF) was distilled from sodium/benzophenone, stored over activated 3Å molecular sieves and used within 7 days. Acid-free ethyl acetate was prepared by prewashing with saturated Na₂CO₃, CDCl₃ was passed through activated basic Al₂O₃ immediately prior to use and NMR’s obtained within 1 hour (¹³C, ³¹P) or 12 hours (¹H) of the CDCl₃ solution being prepared. All other reagents were purchased from commercial suppliers and used without further purification. Silica gel (particle size 30–60 µm) for flash column chromatography was obtained from BDH and dried at 300°C immediately prior to use.

UV-Visible data were obtained with a VARIAN CARY 100 Spectrometer. Nuclear Magnetic Resonance (NMR) spectra were run on Bruker AC-250 and AMX-400 spectrometers.

**o-Phenylazobenzoic acid-N-hydroxysuccinimidy l ester.**

To a stirred suspension of o-phenylazobenzoic acid (452 mg, 2.0 mmol) and N-hydroxysuccinimide (255 mg, 2.2 mmol) in anhydrous DCM (10 ml) N,N'-dicyclohexylcarbodiimide (454 mg, 2.2 mmol) was added at room temperature under argon. After 45 min, precipitated N,N'-dicyclohexylurea was removed by filtration and solvent removed in vacuo. The residual brown solid was purified by silica gel column chromatography eluting with anhydrous DCM to yield the product as a moisture-sensitive brown-orange solid (565 mg, 87%).

**5'-O-(4,4'-dimethoxytrityl)-2'-(N-(o-phenylazo)benzamido)-2'-deoxyuridine.**

To a stirred suspension of o-phenylazobenzoic acid-N-hydroxysuccinimidy l ester (260 mg, 0.80 mmol) in anhydrous DMF (5 ml) at room temperature was added n-butylamine (10 µl, 0.10 mmol) and the reaction mixture maintained under these conditions for a further 10 min. This solution was transferred under argon to a flask containing 5'-O-(4,4'-dimethoxytrityl)-2'-amino-2'-deoxyuridine (250 mg, 0.46 mmol) and N,N-diisopropylethylamine (DPEA; 80 µl, 0.47 mmol) added. The reaction was stirred under ambient conditions for a further 6 hours, a further aliquot of DPEA (100 µl, 0.59 mmol) was added and the reaction stored at 3°C overnight. Excess activated ester was quenched by addition of n-butylamine (190 µl, 1.92 mmol) and stirred with room temperature for 1 hour. The reaction mixture was diluted with ethyl acetate (250 ml) and washed successively with aqueous saturated NaHCO₃ (2 x 150 ml) and saturated NaCl (2 x 150 ml). The organics were dried over anhydrous sodium sulfate, filtered and solvent removed in vacuo. The resultant red gum was purified by silica gel column chromatography, eluting with a gradient of 0–2% (v/v) methanol in DCM containing 1% (v/v) triethylamine to give the title compound as an orange solid (250 mg, 72%).

**1H NMR data**

1H NMR (300 MHz, CDCl₃, 20°C, TMS) δ = 3.42 (2H, s, 2x CH₂), 7.50 – 7.60 (4H, m, H₃, H₄, H₅, PhN; H₄ ArCO), 7.74 (2H, m, H₂, H₆ PhN), 8.06 (3H, m, H₃, H₅, H₆ ArCO); ¹³C (76 MHz, CDCl₃, 20°C, TMS) δ = 26.2, 119.0, 124.2, 124.40, 129.6, 130.5, 131.3, 132.3, 134.3, 152.7, 152.9, 153.0, 169.4; ES+ MS m/z = 324 (M+H), 346 (M+Na).
5′-O-(4,4′-dimethoxytrityl)-2′-O-(N-(o-phenylbenzamido)-2′-deoxyuridine-3′-O-(2-cyanoethyl-N,N-diisopropyl-aminooxy)-phosphoramidite.

To a stirred solution of 5′-O-(4,4′-dimethoxytrityl)-2′-O-(N-(o-phenylbenzamido)-2′-deoxyuridine (180 mg, 0.24 mmol) and DIPEA (90 µl, 0.98 mmol) in anhydrous DCM (4 ml) and THF (1 ml) at room temperature was added 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite (86 µl, 0.53 mmol) dropwise over 3 min. The reaction was maintained under these conditions for a further 30 min and then quenched following addition of anhydrous methanol (60 µl, 1.48 mmol) and DIPEA (60 µl, 0.65 mmol) and stirring for a further 10 min. The reaction mixture was diluted with acid-free ethyl acetate (50 ml) and washed successively with aqueous saturated NaHCO₃ (2 x 50 ml) and saturated NaCl (1 x 50 ml). The organics were dried over anhydrous sodium sulfate, filtered and solvent removed in vacuo. The resultant solid was purified by silica gel column chromatography, eluting with 2% (v/v) solutions of triethylamine in 3:1 DCM:40-60 petroleum ether; pure DCM and 3:1 DCM: ethyl acetate. The title compound was isolated as a brown foam (194 mg, 0.20 mmol, 85 %).

The resultant red gum was purified by silica gel column chromatography, eluting with a gradient of 0 - 2% (v/v) methanol in DCM containing 1% (v/v) triethylamine to give the title compound as an orange solid (250 mg, 72 %). ³¹H NMR (300 MHz, CDCl₃, 20°C, TMS) δ = 3.40 - 1.90 (m, 2x(C₆H₅), 1.93 and 2.17 (2H, 2xm, CH₂CN), 2.31 – 3.59 (9H, 2xCH₂O), 4.25 and 4.37 (1H, 2xm, HOCH₂O), 4.53 and 4.73 (1H, 2xd, 5.0, 11.4, 5.3, 10.6, 6.25), 5.23 and 5.32 (1H, 2xm, H₂), 5.43 (1H, d, J = 8.2 Hz, H₅), 6.34 (1H, d, J = 8.2 Hz, H₁′), 6.88 (4H, d, J = 8.9 Hz, DMTrH α- to OMe), 7.20 – 7.33 (¶7H, m, DMTr-H), 7.97 (2H, m, PhH α to N₃), 8.24 (2H, dd, J = 1.5, 6.0 Hz, ArH α to p to C(O), N₃), 8.69 (1H, s, ArH α to C(O), N₃); ³¹C NMR (76 Mz, CDCl₃, 20°C, TMS) δ = 26.1, 123.5, 123.6, 125.3, 130.0, 130.2, 130.4, 132.6, 133.0, 152.7, 153.0, 161.4, 169.6; MS (ES+) m/z = 324 (M+H), 346 (M+Na)

m-Phenylbenzeneoic acid-N-hydroxysuccinimidyld ester.

To a stirred suspension of m-phenylbenzeneoic acid (452 mg, 2.0 mmol) and N-hydroxysuccinimide (255 mg, 2.2 mmol) in anhydrous DCM (10 ml) N,N′-dicyclohexykarbodiimide (454 mg, 2.2 mmol) was added at room temperature under argon. After 45 min, the precipitated urea was removed by filtration and the solvent removed in vacuo. The residual orange solid was purified by silica gel column chromatography eluting with anhydrous DCM to yield the pure product as a moisture-sensitive orange solid (612 mg, 95%). ³¹H NMR (300 MHz, CDCl₃, 20°C, TMS) δ = 2.95 (4H, s, 2x CH₂), 7.53 (3H, m, PhH), 7.67 (1H, t, J = 8 Hz, ArH m to N₃), 7.97 (2H, m, PhH α to N₃), 8.24 (2H, dd, J = 1.5, 6.0 Hz, ArH α to p to C(O), N₃), 8.69 (1H, s, ArH α to C(O), N₃); ³¹P NMR (121 MHz, CDCl₃, 20°C, 85% H₃PO₄) δ = 149.6, 147.9

5′-O-(4,4′-dimethoxytrityl)-2′-O-(N-(m-phenylbenzamido)-2′-deoxyuridine.

To a stirred solution of m-phenylbenzeneoic acid-N-hydroxysuccinimidyld ester (260 mg, 0.80 mmol) in anhydrous DMF (5 ml) at room temperature was added n-butylamine (10 µl, 0.10 mmol) and the reaction mixture maintained under these conditions for a further 10 min. The reaction mixture was transferred under argon to a flask containing 5′-O-(4,4′-dimethoxytrityl)-2′-amino-2′-deoxyuridine (250 mg, 0.46 mmol) and N,N-diisopropylethylamine (DIPEA: 80 µl, 0.47 mmol) added. The reaction was stirred under ambient conditions for a further 6 hours, a further aliquot of DIPEA (100 µl, 0.59 mmol) was added and the reaction stored at 3°C overnight. Excess activated ester was quenched by addition of n-butylamine (190 µl, 1.92 mmol) and stirring at room temperature for 1 hour. The reaction mixture was diluted with ethyl acetate (250 ml) and washed successively with aqueous saturated NaHCO₃(2 x 150 ml) and saturated NaCl(2 x 150 ml). The organics were dried over anhydrous sodium sulfate, filtered and solvent removed in vacuo. The resultant red gum was purified by silica gel column chromatography, eluting with a gradient of 0 - 2% (v/v) methanol in DCM containing 1% (v/v) triethylamine to give the title compound as an orange solid (250 mg, 72 %). ³¹H NMR (300 MHz, CDCl₃, 20°C, TMS) δ = 3.40 - 2.00 (2H, m, H₅, H₅'), 3.77 (6H, s, 2xOCH₃), 4.22 (1H, s, H₄'''), 4.62 (1H, d, J = 4.8 Hz, H₄), 4.89 (1H, m, H₃''), 5.43 (1H, d, J = 8.1 Hz, H₃), 6.28 (1H, d, J = 8.4 Hz, H₁′), 6.84 (4H, d, J = 9.0 Hz, DMTr-H α- to OMe), 7.20 – 7.33 (9H, m, DMTr-H, NHC(O)AB), 7.40 - 7.46 (6H, m, DMTr-H, AB-H), 7.58 (1H, d, J = 7.5 Hz, AB-H), 7.67 (1H, d, J = 8.1 Hz, H₆), 7.80 - 7.94 (4H, m, AB-H), 8.30 (1H, s, AB-H); ³¹C NMR (101 MHz, CDCl₃, 20°C, TMS) δ = 55.7, 58.0, 64.1, 72.2, 86.1, 86.9, 87.6, 103.6, 113.8, 122.3, 123.4, 127.6, 128.5, 128.6, 129.8, 129.9; 130.6; 131.8; 134.7; 135.5; 135.7; 140.5; 144.5; 151.9; 152.8; 152.9; 159.1; 163.5; 167.9; HRMS (ESI) 753.2791 (calc.753.2799 for MH⁺)
**5'-O-(4,4'-dimethoxytrityl)-2'-O-(N-(m-phenylazobenzoamido)-2'-deoxyuridine-3'-O-(2-cyanoethyl-N,N-diisopropylamino)-phosphoramidite.**

To a stirred solution of 5'-O-(4,4'-dimethoxytrityl)-2'-O-(N-(m-phenylazobenzoamido)-2'-deoxyuridine (134 mg, 0.18 mmol) and DIPEA (67 µl, 0.73 mmol) in anhydrous DCM (3 ml) and THF (0.5 ml) at room temperature, was added 2-cyanoethyl-N,N-diisopropylaminohlorophosphoramidite (57 µl, 0.36 mmol) dropwise over 3 min. The reaction was maintained under these conditions for a further 30 min and then quenched following addition of anhydrous methanol (90 µl, 2.22 mmol) and DIPEA (90 µl 0.98 mmol) and stirring for a further 10 min. The reaction mixture was diluted with acidified ethyl acetate (50 ml) and washed successively with aqueous saturated NaHCO₃ (2 x 50 ml), saturated NaCl (1 x 50 ml). The organics were dried over anhydrous sodium sulfate, filtered and solvent removed in vacuo. The resultant solid was purified by silica gel column chromatography, eluting with 2% (v/v) solutions of triethylamine in 3:1 DCM:40-60 petroleum ether; pure DCM and 3:1 DCM : ethyl acetate. The title compound was isolated as an orange foam (160 mg, 0.17 mmol, 93%).

**p-Phenylazobenzoic acid-N-hydroxy succinimidyl ester.**

To a stirred suspension of p-phenylazobenzoic acid (452 mg, 2.0 mmol) and N-hydroxsuccinimide (255 mg, 2.2 mmol) in anhydrous DCM (10 ml) N,N-dicyclohexylcarbodiimide (454 mg, 2.2 mmol) was added at room temperature. After 45 min, the precipitated urea was removed by filtration and the solvent removed in vacuo. The residual orange solid was purified by silica gel column chromatography eluting with anhydrous DCM to yield the pure product as a filtration and the solvent removed under argon. After 45 min, the precipitated urea was removed by filtration and the solvent removed in vacuo. The resultant solid was purified by silica gel column chromatography, eluting with 2% (v/v) solutions of triethylamine in 3:1 DCM:40-60 petroleum ether; pure DCM and 3:1 DCM : ethyl acetate. The title compound was isolated as an orange solid (250 mg, 72%).
To a stirred solution of 5'-O-(4,4'-dimethoxytrityl)-2'-N-(p-phenylazo)benzamido)-2'-deoxyuridine-3'-O-(2-cyanoethyl-N,N-
diisopropylamino)-phosphoramidite,

DIPEA (125 µl, 1.36 mmol) and THF (2 ml) at room temperature was added 2-cyanoethyl-N,N-
diisopropylaminochlorophosphoramidite (106 µl, 0.66 mmol) dropwise over 3 min. The reaction was maintained under these conditions for a further 30 min and then quenched following addition of anhydrous methanol (60 µl, 1.48 mmol) and DIPEA (60 µl 0.65 mmol) and stirring for a further 10 min. The reaction mixture was diluted with acid-free ethyl acetate (250 ml) and washed successively with aqueous saturated NaHCO₃ (2 x 250 ml), saturated NaCl (1 x 150 ml). The organics were dried over anhydrous sodium sulfate, filtered and solvent removed in vacuo. The resultant solid was purified by silica gel column chromatography, eluting with 2% (v/v) solutions of triethylamine in 3:1 DCM: 40-60 petroleum ether; pure DCM and 3:1 DCM: ethyl acetate. The title compound was isolated as an orange foam (308 mg, 0.32 mmol, 97 %).

**Synthesis of oligonucleotides**

Oligosynthesis was carried out on Beckman Oligo 1000M and Expedite 8909 DNA/RNA synthesizers on a 1 µmol scale using standard DNA and RNA phosphoramidites from Link Technologies. Standard nucleobase protecting groups and 2'-O-TBDMS-protection were used. Benzylthiotetrazole (0.2 M) was used as activating agent. Oxidation was performed using 8:1:1 THF:pyridine:H₂O containing either 50 mM or 20 mM I₂ for, respectively, Beckman- or Expedite-run syntheses. Unmodified DNA and RNA were synthesized DMT-on and deprotected and purified according to standard procedures. The coupling time was extended (5-6 min) for 2'-azobenzene-modified amidites. Deprotection of azobenzene-modified oligonucleotides was performed with 1.5 ml of an anhydrous mixture of NH₃/MeOH (dry MeOH saturated with gaseous ammonia at -10°C) overnight at room temperature. The decanted supernatant was evaporated and the crude oligonucleotides purified by RP-HPLC (column: Hichrom KR100-5C18; buffer A: 0.1 M TEAAc, pH 6.5, 5% (v/v) MeCN; buffer B: 0.1 M TEAAc, pH 6.5, 65% (v/v) MeCN) monitoring absorbance in the range 200 – 400 (or 600) nm with a diode array detector. After evaporation of the buffer the oligonucleotides were desalted using standard SepPak protocols (Waters) and concentrations determined using UV absorbance at 260 nm.

**MALDI-TOF analysis of DNA-azobenzene conjugates**

<table>
<thead>
<tr>
<th>DNA oligomer</th>
<th>Mass (calc.)</th>
</tr>
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<tbody>
<tr>
<td>DRo</td>
<td>8438.79 (8440.46)</td>
</tr>
<tr>
<td>DRm</td>
<td>8434.24 (8440.46)</td>
</tr>
<tr>
<td>DRp</td>
<td>8434.17 (8440.46)</td>
</tr>
<tr>
<td>ACC1oGGTA</td>
<td>2620.29 (2617.51)</td>
</tr>
<tr>
<td>ACC1mGGTA</td>
<td>2619.58 (2617.51)</td>
</tr>
<tr>
<td>ACC1pGGTA</td>
<td>2626.46 (2617.51)</td>
</tr>
</tbody>
</table>

**Photoswitching of DNA-azobenzene conjugates**

For the irradiation a Medium Pressure Hg Arc-lamp (100 W, Engelhard Hanovia of Canada Ltd.) was utilized. A band-pass filter was used for irradiation at 366 nm (4.13 W) and a cutoff filter was used for irradiation >400 nm (435 nm, 3.36 W) in combination with a water filter (1 cm) to prevent warming of the samples during prolonged irradiation.

As the high concentrations of deoxyribozyme and labeled substrate used prevented full kinetic analysis, all constructs were assayed at a fixed set of standard conditions (20 µM substrate, 2 µM deoxyribozyme), and from the measured initial rates, apparent first order rate constants of the Z- and E-isomers were determined.
UV analysis of $E \rightarrow Z$ photoswitching following irradiation at 366 nm and 435 nm

**ACC1oGGTA**

**KEY**
- UV spectrum of 8-mer following HPLC purification
- UV spectrum of 8-mer following irradiation at 366 nm for 8 min
- UV spectrum of 8-mer following irradiation at 366 nm for 8 min and incubation at 26°C for 6 h
- UV spectrum of 8-mer following irradiation at 435 nm for 2 min

**ACC1mGGTA**

**KEY**
- UV spectrum of 8-mer following HPLC purification
- UV spectrum of 8-mer following irradiation at 366 nm for 8 min
- UV spectrum of 8-mer following irradiation at 366 nm for 8 min and incubation at 26°C for 6 h
- UV spectrum of 8-mer following irradiation at 435 nm for 2 min

**ACC1pGGTA**

**KEY**
- UV spectrum of 8-mer following HPLC purification
- UV spectrum of 8-mer following irradiation at 366 nm for 5 min
- UV spectrum of 8-mer following irradiation at 366 nm for a further 3 min
HPLC analysis of $E \rightarrow Z$ photoswitching efficiency following irradiation at 366 nm for 8 min

Conditions for DR$_m$ and DR$_p$: Hichrom KR100-5C18 with UV detection eluting with buffer A: 0.1 M TEAAc, pH 6.5, solvent C: MeCN, monitored at 260 nm; gradient indicated by purple line.

Conditions for ACC$_{10}$GGTA: Hichrom KR100-5C18 eluting with buffer A: 0.1 M TEAAc, pH 6.5, buffer B: 0.1 M TEAAc, pH 6.5, 65% (v/v) MeCN, monitored at 260 nm; gradient indicated by turquoise line.

**DR$_m$**

**KEY**

14.69 min Z-DR$_m$ (75%)
15.36 min E-DR$_m$ (25%)

**DR$_p$**

**KEY**

14.64 min Z-DR$_p$ (61%)
15.60 min E-DR$_p$ (39%)

**ACC$_{10}$GGTA**

**KEY**

15.44 min Z-ACC$_{10}$GGTA (86%)
19.87 min E-ACC$_{10}$GGTA (14%)
Activity assays for 10-23 deoxyribozymes in the PAGE format

For the measurements of RNA-cleavage activities under multiple turnover conditions the deoxyribozyme construct (final concentration 2 µM or 1µM) was added to a buffered solution containing 50 mM Tris, pH 7.5, 40 mM NaCl and 25 mM MgCl$_2$ (all final concentrations). Preirradiation of these mixtures was performed at 366 nm for 10 min and at 435 nm for 2 min in clear eppendorf tubes. The reaction was initiated by addition of the deoxyribozyme solutions to the substrate RNA (20 µM), yielding a total volume of 50 or 100 µl. Incubation was performed in the absence of light at 26°C, except for irrDRp, which was incubated under continuous irradiation at 366 nm (temperature under these conditions was determined to be 26°C). After appropriate time intervals aliquots (10 µl) were withdrawn, mixed with a stop solution (6 M urea, 50 mM EDTA) and frozen at -20°C. The samples were loaded on a 15% (w/v) (3% cross-linked) denaturing polyacrylamide sequencing gel and run at 900 V for 2 hr. The gel was then removed from the plates and transferred onto a gel documentation system (Versadoc, Biorad) and analyzed. Quantitation of the bands was performed with the accompanying software (QuantityOne) such that the intensity of the product bands was set in relation to the sum of the intensities of the product and substrate bands which resulted in relative cleavage values.

Activity assays for 10-23 deoxyribozymes using RP-HPLC analysis

For the measurements of RNA-cleavage activities under multiple turnover conditions the deoxyribozyme construct (final concentration 2 µM) was added to a buffered solution containing 50 mM Tris, pH 7.5, 40 mM NaCl and 25 mM MgCl$_2$ (all final concentrations). Preirradiation of these mixtures was performed at 366 nm for 10 min and at 435 nm for 2 min in clear eppendorf tubes. The reaction was initiated by addition of the deoxyribozyme solutions to the substrate RNA (20 µM), yielding a total volume of 50 µl. Incubation was performed in the absence of light at 26°C, except for irrDRp, which was incubated under continuous irradiation at 366 nm (temperature under these conditions was determined to be 26°C). After 1.5 hr an aliquot (45 µl) was removed and quenched with 10 µl 250 mM EDTA. For the injection 40 µl 0.1 M TEAAc were added and the samples analyzed on RP-HPLC (column: Hichrom KR100-5C18, buffer A: 0.1 M TEAAc, pH 6.5; buffer B: 0.1 M TEAAc, pH 6.5, 65% (v/v) MeCN; UV detection at 260 nm; gradient indicated by turquoise line). Quantitation was performed by determining peak areas for the two product peaks and set in relation to the sum of the peak areas of the product and substrate peaks which resulted in relative cleavage values.
HPLC activity assays

Cleavage of unlabeled RNA substrate after 90 min by $d\alpha$DRs and irrDRs monitored by HPLC.