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Keiper, S., & Vyle, J. (2006). Reversible Photocontrol of Deoxyribozyme-Catalyzed RNA Cleavage under Multiple-Turnover Conditions. *Angewandte Chemie International Edition*, 45(20), 3306-3309.  
<https://doi.org/10.1002/anie.200600164>

**Published in:**  
Angewandte Chemie International Edition

**Document Version:**  
Peer reviewed version

**Queen's University Belfast - Research Portal:**  
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Supporting Information

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

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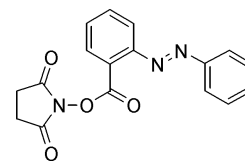
## Synthesis of phosphoramidites

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 (DIPEA) was similarly purified but stored over activated 3Å molecular sieves for upto 6 weeks. DMF 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 Na<sub>2</sub>CO<sub>3</sub>. CDCl<sub>3</sub> was passed through activated basic Al<sub>2</sub>O<sub>3</sub> immediately prior to use and NMR's obtained within 1 hour (<sup>31</sup>P, <sup>13</sup>C) or 12 hours (<sup>1</sup>H) of the CDCl<sub>3</sub> 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.

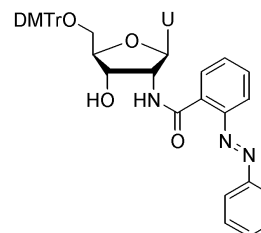
### *o*-Phenylazobenzoic acid-*N*-hydroxysuccinimidyl ester.

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%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 20°C, TMS) δ = 2.92 (4H, s, 2 x CH<sub>2</sub>), 7.50 – 7.60 (4H, m, H<sub>3</sub>, H<sub>4</sub>, H<sub>5</sub>, PhN<sub>2</sub>; H<sub>4</sub> ArCO), 7.74 (2H, m, H<sub>2</sub>, H<sub>6</sub> PhN<sub>2</sub>), 8.06 (3H, m, H<sub>3</sub>, H<sub>5</sub>, H<sub>6</sub> ArCO); <sup>13</sup>C (76 MHz, CDCl<sub>3</sub>, 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)



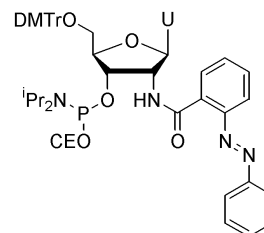
### 5'-*O*-(4,4'-dimethoxytrityl)-2'-(*N*-(*o*-phenylazo)benzamido)-2'-deoxyuridine.

To a stirred solution of *o*-phenylazobenzoic acid-*N*-hydroxysuccinimidyl 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 (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 NaHCO<sub>3</sub> (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 %). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 20°C, TMS) δ = 3.42 (2H, ψd, H<sub>5'</sub>, H<sub>5''</sub>), 3.79 (6H, s, 2x CH<sub>3</sub>O), 4.17 (1H, m, H<sub>4'</sub>), 4.52 (1H, dd, <sup>3</sup>*J*(H,H) = 1.8, 5.7 Hz, H<sub>3'</sub>), 5.03 (1H, m, H<sub>2'</sub>), 5.45 (1H, d <sup>3</sup>*J*(H,H) = 8.1 Hz, H<sub>5</sub>), 6.18 (1H, d <sup>3</sup>*J*(H,H) = 7.8 Hz, H<sub>1'</sub>), 6.85 (4H, d <sup>3</sup>*J*(H,H) = 8.4 Hz, DMTrH *o* to OMe), 7.29 – 7.60 (14H, m, DMTr-H, AB-H), 7.70 (1H, d <sup>3</sup>*J*(H,H) = 8.1 Hz, H<sub>6</sub>), 7.77 (1H, m, AB-H), 7.96 (2H, m, AB-H), 8.31 (1H, m, AB-H), 9.17 (1H, d <sup>3</sup>*J*(H,H) = 7.8 Hz, AB-H); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>, 20°C, TMS) δ = 54.3, 55.6, 62.8, 70.2, 84.5, 86.0, 86.2, 101.7, 112.3, 115.2, 122.1, 122.7, 126.1, 127.0, 127.2, 128.4, 128.4, 129.2, 129.6, 130.3, 130.4, 131.0, 131.2, 134.3, 134.5, 139.5, 143.3, 148.8, 149.8, 151.3, 157.7, 162.2, 165.6; HRMS (ESI) 753.2789 (calc.753.2799 for MH<sup>+</sup>).



5'-O-(4,4'-dimethoxytrityl)-2'-(N-(o-phenylazo)benzamido)-2'-deoxyuridine-3'-O-(2-cyanoethyl-N,N-diisopropyl-amino)-phosphoramidite.

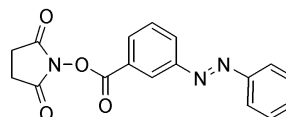
To a stirred solution of 5'-O-(4,4'-dimethoxytrityl)-2'-(N-(o-phenylazo)benzamido)-2'-deoxyuridine (180 mg, 0.24 mmol) and DIPEA (90  $\mu$ l, 0.98 mmol) in anhydrous DCM (4 ml) and THF (1 ml) at room temperature was added 2-cyanoethyl-N,N-diisopropylaminochlorophosphoramidite (86  $\mu$ l, 0.53 mmol) 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  $\mu$ l, 1.48 mmol) and DIPEA (60  $\mu$ l, 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 NaHCO<sub>3</sub> (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 a brown foam (194 mg, 0.20 mmol, 85 %). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 20°C, TMS)  $\delta$  = 0.80 – 1.10 (¶12H, m 2 x (CH<sub>3</sub>)<sub>2</sub>), 1.93 and 2.17 (2H, 2xm, CH<sub>2</sub>CN), 3.21 – 3.59 (¶6H, m, H5', H5'', (-CH<sub>2</sub>)<sub>2</sub>N, CH<sub>2</sub>O), 3.81 (6H, 2xs, 2xCH<sub>3</sub>O), 4.25 and 4.37 (1H, 2xs, H4'), 4.55 and 4.73 (1H, 2xdd, 5.0, 11.4, 5.3, 10.6, H2'), 5.23 and 5.32 (1H, 2xm, H3'), 5.43 (1H, d <sup>3</sup>J (H,H) = 8.2 Hz, H5), 6.34 (1H,  $\psi$ t, <sup>3</sup>J (H,H) = 8.2 Hz, H1'), 6.88 (4H, d <sup>3</sup>J (H,H) = 8.9 Hz, DMTrH *o*- to OMe), 7.30 – 7.70 (¶12H, m, DMTrH, NHC(O)AB AB-H) 7.73 and 7.79 (1H, 2xd <sup>3</sup>J (H,H) = 9.3, 9.3 Hz, H6), 7.87 (1H, m, AB-H) 8.00 (2H, m, AB-H) 8.36 and 8.40 (1H, 2xm, AB-H), 8.85 and 9.06 (1H, 2xd, <sup>3</sup>J (H,H) = 8.2 Hz AB-H); <sup>31</sup>P NMR (121 MHz, CDCl<sub>3</sub>, 20°C, 85% H<sub>3</sub>PO<sub>4</sub>)  $\delta$  = 149.6, 147.9

m-Phenylazobenzoic acid-N-hydroxysuccinimidyl ester.

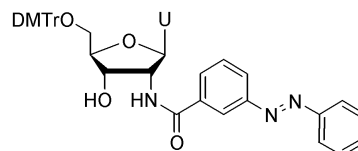
To a stirred suspension of *m*-phenylazobenzoic acid (452 mg, 2.0 mmol) and N-hydroxysuccinimide (255 mg, 2.2 mmol) in anhydrous DCM (10 ml) N,N'-dicyclohexylcarbodiimide (454 mg, 2.2 mmol) was added at room temperature under argon. After 45 min, the precipitated urea was removed by filtration and the 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%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 20°C, TMS)  $\delta$  = 2.95 (4H, s, 2x CH<sub>2</sub>), 7.53 (3H, m, PhH), 7.67 (1H, t <sup>3</sup>J (H,H) = 7.8 Hz, ArH *m* to N<sub>2</sub>), 7.97 (2H, m, PhH *o* to N<sub>2</sub>), 8.24 (2H, dd *J* = 1.5, 6.0 Hz, ArH *o*, *p* to C(O), N<sub>2</sub>), 8.69 (1H, s, ArH *o* to C(O), N<sub>2</sub>); <sup>13</sup>C NMR (76 Mz, CDCl<sub>3</sub>, 20°C, TMS)  $\delta$  = 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)

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

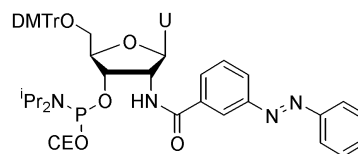
To a stirred solution of *m*-phenylazobenzoic acid-N-hydroxysuccinimidyl ester (260 mg, 0.80 mmol) in anhydrous DMF (5 ml) at room temperature was added *n*-butylamine (10  $\mu$ l, 0.10 mmol) and the reaction mixture maintained under these conditions for a further 10 min. The reaction mixture was transferred 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  $\mu$ l, 0.47 mmol) added. The reaction was stirred under ambient conditions for a further 6 hours, a further aliquot of DIPEA (100  $\mu$ l, 0.59 mmol) was added and the reaction stored at 3°C overnight. Excess activated ester was quenched by addition of *n*-butylamine (190  $\mu$ l, 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 NaHCO<sub>3</sub> (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 %). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 20°C, TMS)  $\delta$  = 3.40 (2H, m, H5', H5''), 3.77 (6H, s, 2xOCH<sub>3</sub>), 4.22 (1H, s, H4'), 4.62 (1H, d <sup>3</sup>J (H,H) = 4.8 Hz, H2'), 4.89 (1H, m, H3'), 5.43 (1H, d <sup>3</sup>J (H,H) = 8.1Hz, H5), 6.28 (1H, d <sup>3</sup>J (H,H) = 8.4 Hz, H1'), 6.84 (4H, d <sup>3</sup>J (H,H) = 9.0 Hz, DMTr-H *o*- to OMe), 7.20 – 7.33 (¶7H, m, DMTr-H, NHC(O)AB), 7.40 – 7.46 (6H, m, DMTr-H, AB-H), 7.58 (1H, d <sup>3</sup>J (H,H) = 7.5 Hz, AB-H), 7.67 (1H, <sup>3</sup>J (H,H) = 8.1 Hz, H6), 7.80 – 7.94 (4H, m, AB-H), 8.30 (1H, s, AB-H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>, 20°C, TMS)  $\delta$  = 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; 129.5; 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<sup>+</sup>)

5'-O-(4,4'-dimethoxytrityl)-2'-(N-(m-phenylazo)benzamido)-2'-deoxyuridine-3'-O-(2-cyanoethyl-N,N'-diisopropylamino)-phosphoramidite.

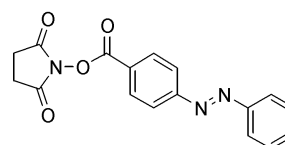
To a stirred solution of 5'-O-(4,4'-dimethoxytrityl)-2'-(N-(m-phenylazo)benzamido)-2'-deoxyuridine (134 mg, 0.18 mmol) and DIPEA (67  $\mu$ l, 0.73 mmol) in anhydrous DCM (3 ml) and THF (0.5 ml) at room temperature was added 2-cyanoethyl-N,N'-diisopropylaminochlorophosphoramidite (57  $\mu$ l, 0.36 mmol) 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  $\mu$ l, 2.22 mmol) and DIPEA (90  $\mu$ l 0.98 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  $\text{NaHCO}_3$  (2 x 50 ml), saturated  $\text{NaCl}$  (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 %).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ , 20°C, TMS)  $\delta$  = 1.00 – 1.35 ( $\text{¶}12\text{H}$ , m 2 x  $(\text{CH}_3)_2$ ), 2.31 and 2.44 (2H, 2xm,  $\text{CH}_2\text{CN}$ ) 3.44 (2H, m,  $\text{H}5'$ ,  $\text{H}5''$ ) 3.45 - 3.80 (4H, m,  $(-\text{CH}_2)_2\text{N}$ ,  $\text{CH}_2\text{O}$ ), 3.80 (6H, s, 2x $\text{CH}_3\text{O}$ ), 4.30 and 4.45 (1H, 2xs,  $\text{H}4'$ ), 4.56 and 4.75 (1H, 2xdd,  $\text{H}2'$ ), 4.97 and 5.13 (1H, 2xm,  $\text{H}3'$ ), 5.50 (1H,  $\psi\text{t}$ ,  $\text{H}5$ ), 6.26 (1H,  $\psi\text{t}$ ,  $\text{H}1'$ ), 6.87 (4H, 2xd  $^3J$  (H,H) = 8.9 Hz,  $\text{DMTrH}$  *o*- to OMe), 6.97 and 7.14 (1H, 2xm,  $\text{NH}(\text{C}(\text{O})\text{AB})$ ), 7.20 – 7.80 ( $\text{¶}14\text{H}$ , m,  $\text{DMTrH}$ , AB-H,  $\text{H}6$ ), 7.90-8.15 (4H, m, AB-H), 8.32 (1H, s, AB-H);  $^{31}\text{P}$  NMR (121 MHz,  $\text{CDCl}_3$ , 20°C, 85%  $\text{H}_3\text{PO}_4$ )  $\delta$  = 149.4, 148.1

p-Phenylazobenzoic acid-N-hydroxysuccinimidyl ester.

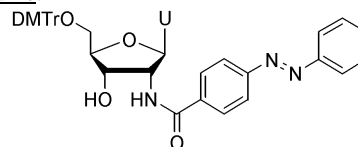
To a stirred suspension of *p*-phenylazobenzoic acid (452 mg, 2.0 mmol) and *N*-hydroxysuccinimide (255 mg, 2.2 mmol) in anhydrous DCM (10 ml) *N,N'*-dicyclohexylcarbodiimide (454 mg, 2.2 mmol) was added at room temperature under argon. After 45 min, the precipitated urea was removed by filtration and 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 (544 mg, 84%).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ , 20°C, TMS)  $\delta$  = 2.94 (4H, s, 2x  $\text{CH}_2$ ), 7.55 (3H, m,  $\text{PhH}$ ), 7.97 (2H, m,  $\text{PhH}$  *o* to  $\text{N}_2$ ), 8.02 (2H, d  $^3J$  (H,H) = 8.4 Hz  $\text{ArH}$  *o* to  $\text{C}(\text{O})$ ), 8.30, (2H, d  $^3J$  (H,H) = 8.4 Hz,  $\text{ArH}$  *o* to  $\text{N}_2$ ),  $^{13}\text{C}$  NMR (101 Mz,  $\text{CDCl}_3$ , 20°C, TMS)  $\delta$  = 25.7, 123.0, 123.3, 126.5, 129.2, 131.7, 132.1, 152.4, 156.1, 161.4, 169.2;  $\text{Cl}^+$  MS  $m/z$  324 (MH $^+$ )

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

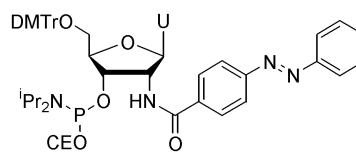
To a stirred solution of *p*-phenylazobenzoic acid-N-hydroxysuccinimidyl ester (260 mg, 0.80 mmol) in anhydrous DMF (5 ml) at room temperature was added *n*-butylamine (10  $\mu$ l, 0.10 mmol) and the reaction mixture maintained under these conditions for a further 10 min. The reaction mixture was transferred 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  $\mu$ l, 0.47 mmol) added. The reaction was stirred under ambient conditions for a further 6 hours, a further aliquot of DIPEA (100  $\mu$ l, 0.59 mmol) was added and the reaction stored at 3°C overnight. Excess activated ester was quenched by addition of *n*-butylamine (190  $\mu$ l, 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  $\text{NaHCO}_3$  (2 x 150 ml) and saturated  $\text{NaCl}$  (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 %).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ , 20°C, TMS)  $\delta$  = 3.42 (2H, m,  $\text{H}5'$ ,  $\text{H}5''$ ), 3.75 (6H, s, 2x $\text{OCH}_3$ ), 4.23 (1H, s,  $\text{H}4'$ ), 4.65 (1H, m,  $\text{H}2'$ ), 4.77 (1H, m,  $\text{H}3'$ ), 5.47 (1H, d  $^3J$  (H,H) = 8.2 Hz,  $\text{H}5$ ), 6.20 (1H, d  $^3J$  (H,H) = 8.1 Hz,  $\text{H}1'$ ), 6.89 (4H, d  $^3J$  (H,H) = 9.0 Hz,  $\text{DMTrH}$  *o*- to OMe), 7.20 – 7.43 ( $\text{¶}10\text{H}$ , m,  $\text{DMTrH}$ ,  $\text{NH}(\text{C}(\text{O})\text{AB})$ ), 7.48 (3H, m, AB-H) 7.67 (1 H, d,  $^3J$  (H,H) = 8.2 Hz,  $\text{H}6$ ), 7.85-7.95 (6H, m, AB-H);  $^{13}\text{C}$  NMR (63 MHz,  $\text{CDCl}_3$ , 20°C, TMS)  $\delta$  = 55.7, 57.9, 64.2, 72.1, 86.3, 87.1, 87.6, 103.6, 113.8, 123.3, 123.6, 127.6, 128.5, 128.6, 128.7, 129.5, 130.6, 132.0, 135.4, 135.6, 135.7, 140.5, 144.5, 152.0, 152.9, 154.8, 159.1, 163.7, 167.8;  $\text{FAB}^+$  MS  $m/z$  754 (MH $^+$ )

5'-O-(4,4'-dimethoxytrityl)-2'-(N-(p-phenylazo)benzamido)-2'-deoxyuridine-3'-O-(2-cyanoethyl-N,N-diisopropylamino)-phosphoramidite.

To a stirred solution of 5'-O-(4,4'-dimethoxytrityl)-2'-(N-(p-phenylazo)benzamido)-2'-deoxyuridine (250 mg, 0.33 mmol) and DIPEA (125  $\mu$ l, 1.36 mmol) in anhydrous DCM (5 ml) and THF (2 ml) at room temperature was added 2-cyanoethyl-N,N-diisopropylaminochlorophosphoramidite (106  $\mu$ l, 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  $\mu$ l, 1.48 mmol) and DIPEA (60  $\mu$ l, 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 NaHCO<sub>3</sub> (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 %). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 20°C, TMS)  $\delta$  = 0.95 – 1.25 ( $\eta$ 12H, m 2 x (CH<sub>3</sub>)<sub>2</sub>), 2.33 and 2.48 (2H, 2xm, CH<sub>2</sub>CN) 3.36 (2H, m, H5', H5'') 3.40 - 3.80 (4H, m, (-CH<sub>2</sub>)<sub>2</sub>N, CH<sub>2</sub>O), 3.80 (6H, s, 2xCH<sub>3</sub>O), 4.30 and 4.46 (1H, 2xs, H4'), 4.53 and 4.69 (1H, 2xdd <sup>3</sup>J (H,H) = 6.8 Hz, H2'), 4.97 and 5.09 (1H, 2xm, H3'), 5.49 (1H,  $\psi$ t, H5), 6.22 and 6.29 (1H, 2xd <sup>3</sup>J (H,H) = 6.8 Hz, H1'), 6.82 and 6.83 (4H, 2xd <sup>3</sup>J (H,H) = 6.8 Hz, DMTrH *o*- to OMe), 6.97 and 7.14 (1H, 2xm, NHC(O)AB), 7.30 – 7.55 ( $\eta$ 12H, m, DMTrH, AB-H), 7.72 and 7.81 (1H, 2xd <sup>3</sup>J (H,H) = 8.2 Hz, H6), 7.85 – 7.90 (6H, m, AB-H); <sup>31</sup>P NMR (101 MHz, CDCl<sub>3</sub>, 85% H<sub>3</sub>PO<sub>4</sub>)  $\delta$  = 152.1, 149.8; FAB+ MS m/z 853 (M-<sup>1</sup>Pr<sub>2</sub>N), 883 (M-OCE), 954 (M+H)

Synthesis of oligonucleotides

Oligosynthesis was carried out on Beckman Oligo 1000M and Expedite 8909 DNA/RNA synthesizers on a 1  $\mu$ mol 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:H<sub>2</sub>O containing either 50 mM or 20 mM I<sub>2</sub> 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 NH<sub>3</sub>/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

DNA oligomer	Mass (calc.)
DRo	8438.79 (8440.46)
DRm	8434.24 (8440.46)
DRp	8434.17 (8440.46)
ACC1oGGTA	2620.29 (2617.51)
ACC1mGGTA	2619.58 (2617.51)
ACC1pGGTA	2626.46 (2617.51)

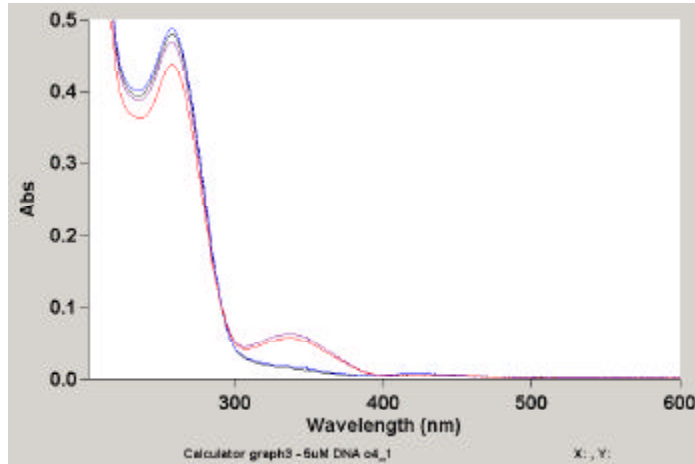
Photoswitching of DNA-azobenzene conjugates

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  $\mu$ M substrate, 2  $\mu$ M 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

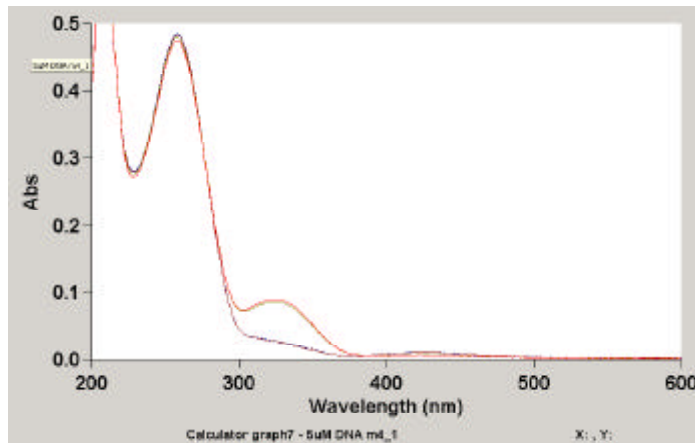
ACC1oGGTA



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

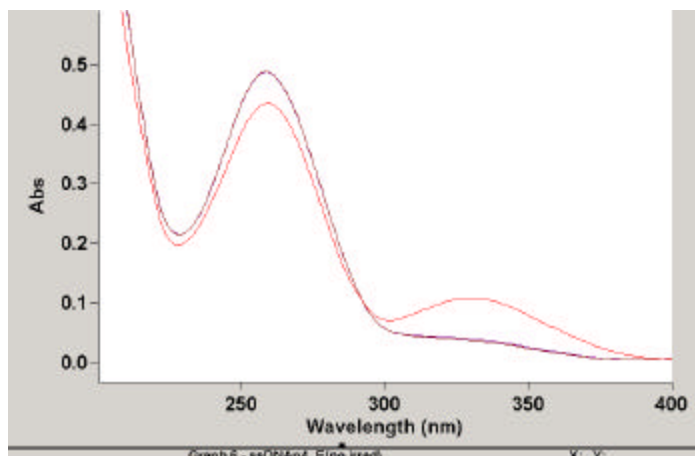
ACC1mGGTA



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

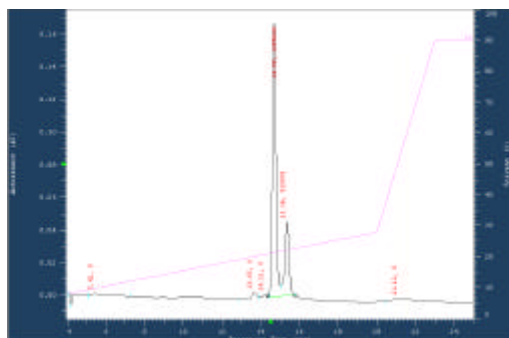
- UV spectrum of 8-mer following HPLC purification
- UV spectrum of 8-mer following irradiation at 366 nm for 5 min
- UV spectrum of 8-mer following irradiation at 366 nm for a further 3 min

HPLC analysis of  $E \rightarrow Z$  photoswitching efficiency following irradiation at 366 nm for 8 min

Conditions for DRm and DRp: 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 ACC1oGGTA: 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.

**DRm**

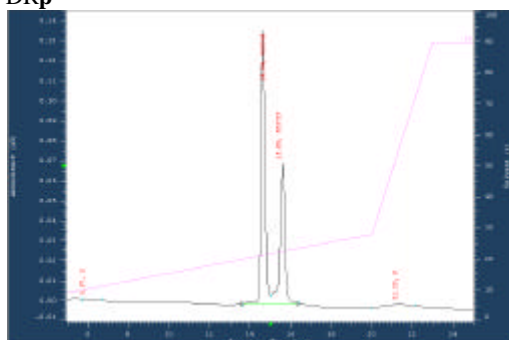


KEY

14.69 min *Z*-DRm (75%)

15.36 min *E*-DRm (25%)

**DRp**

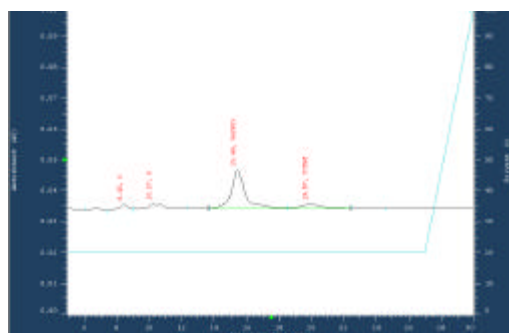


KEY

14.64 min *Z*-DRp (61%)

15.60 min *E*-DRp (39%)

**ACC1oGGTA**



KEY

15.44 min *Z*-ACC1oGGTA (86%)

19.87 min *E*-ACC1oGGTA (14%)



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

### **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  $\mu$ M or 1 $\mu$ M) was added to a buffered solution containing 50 mM Tris, pH 7.5, 40 mM NaCl and 25 mM MgCl<sub>2</sub> (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  $\mu$ M), yielding a total volume of 50 or 100  $\mu$ l. 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  $\mu$ l) 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.

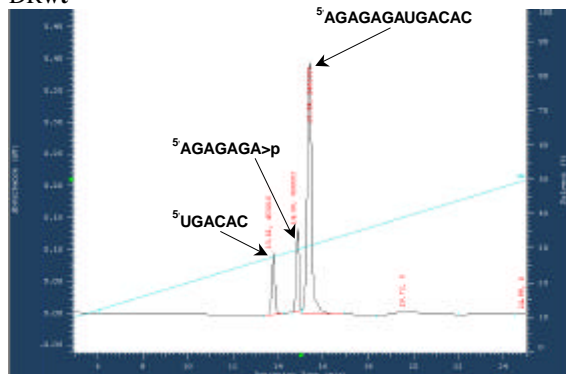
### **Activity assays for 10-23 deoxyribozymes using RP-HPLC analysis**

For the measurements of RNA-cleavage activities under multiple turnover conditions the deoxyribozyme construct (final concentration 2  $\mu$ M) was added to a buffered solution containing 50 mM Tris, pH 7.5, 40 mM NaCl and 25 mM MgCl<sub>2</sub> (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  $\mu$ M), yielding a total volume of 50  $\mu$ l. 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  $\mu$ l) was removed and quenched with 10  $\mu$ l 250 mM EDTA. For the injection 40  $\mu$ l 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.

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

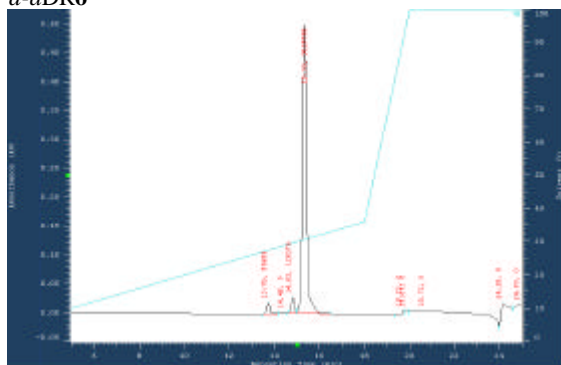
## HPLC activity assays

DRwt

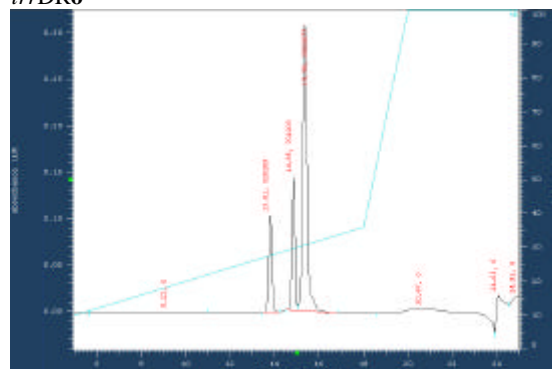


Cleavage of unlabeled RNA substrate after 90 min by *d*-aDRs and *irr*DRs monitored by HPLC.

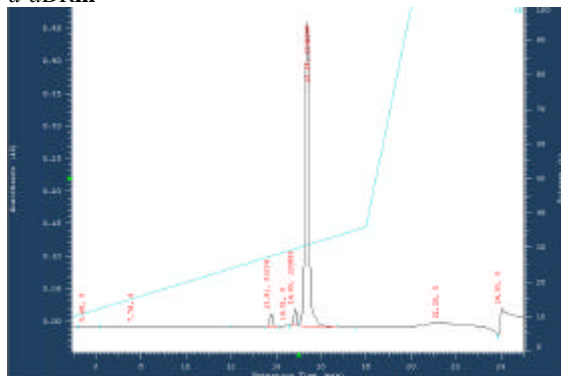
*d*-aDRo



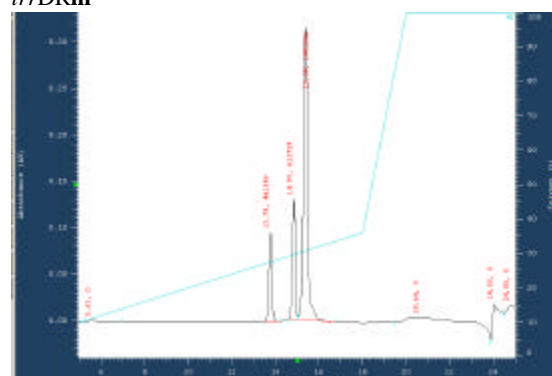
*irr*DRo



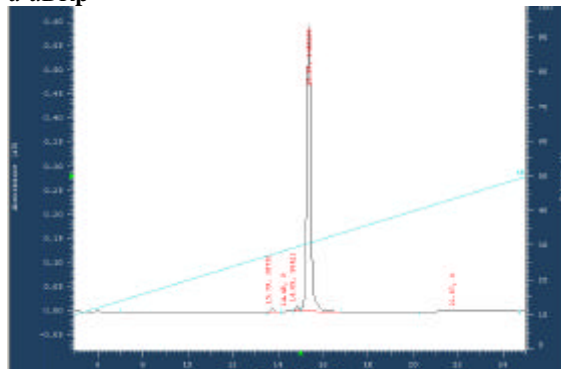
*d*-aDRm



*irr*DRm



*d*-aDRp



*irr*DRp

