Supplementary Material (ESI) for Chemical Communications

SUPPORTING INFORMATION

Identification of Efficient and Sequence Specific Bimolecular Artificial Ribonucleases by a Combinatorial Approach

*Marcin Kalek, Peter Benediktson, Birte Vester, Jesper Wengel**

Table of contents for Supporting Information

S2. Synthesis of monomers (Scheme S1).

S7. Synthesis and purification of oligonucleotides.

S8. MALDI-MS of synthesized oligonucleotides (Table S1).

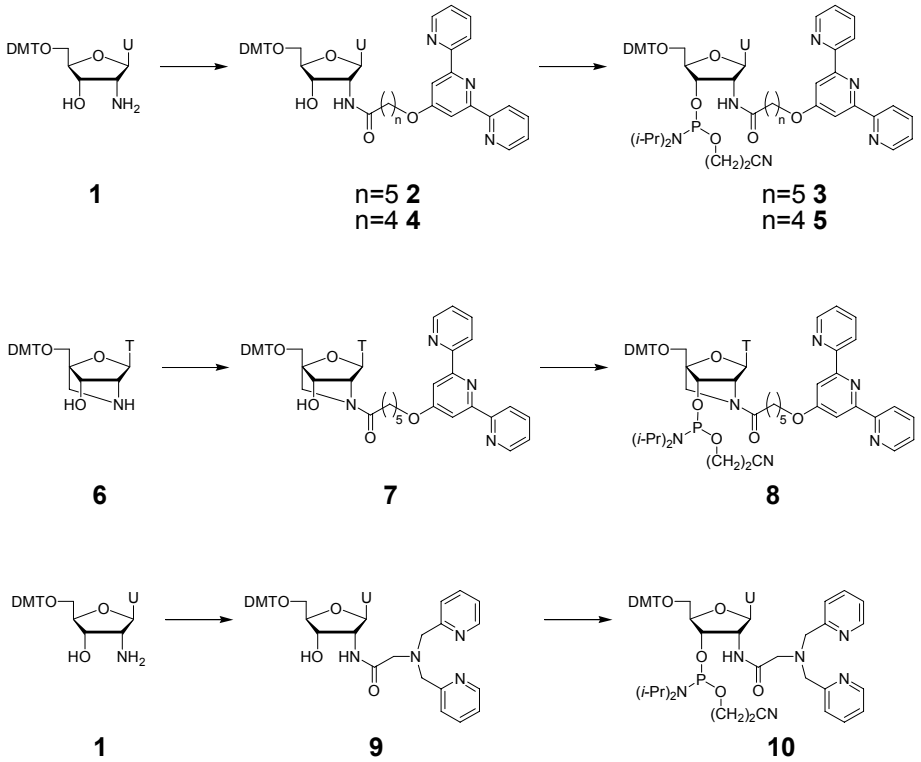
S9. Experimental details of the RNA cleavage reactions.

S10. RNA cleavage by oligonucleotides DNA1-**N** and DNA2-**N** (Figure S1).

S10. Thermal denaturation studies (Table S2).

S11. References.

Synthesis of monomers



Scheme S1. Synthesis of phosphoramidites for incorporation of monomers **X**, **Y**, **Z** and **P**.

General

All reagents and solvents were of analytical grade and obtained from commercial suppliers and used without further purification except for CH_2Cl_2, which was distilled prior to use. Anhydrous CH_2Cl_2 and anhydrous toluene were dried through storage over activated 4Å molecular sieves. Reactions were monitored by thin layer chromatography (TLC) using silica gel (Merck, Silica gel 60) or neutral aluminum oxide (Merck, Aluminum oxide 60) coated plates with indicator. Column chromatography was performed on silica gel (Merck, Silica gel 60, 0.063-0.200 mm) or neutral aluminum oxide (Merck, Aluminum oxide 90 active neutral, 0.063-0.200 mm). After chromatography fractions containing product were pooled, evaporated to dryness and dried under vacuum for 12 hours. NMR spectra were recorded on a Varian Gemini 2000

300MHz instrument. Chemical shifts are reported in ppm relative to TMS (^{1}H; internal), solvents peaks (^{13}C) and H_3PO_4 (^{31}P; external). Assignments of the NMR signals are based on 2D correlation experiments. Signals in the NMR spectra originating from terpyridine, *N*,*N*-bis(2-pyridylmethyl)glycyl and dimetoxytrityl moieties are indicated with Ter, Pyr and DMT subscripts, respectively. High resolution mass spectra (HRMS) were recorded in positive mode on an IonSpec Fourier Transform MALDI mass spectrometer. Starting compounds **1**,[1] **6**,[2] 5-(2,2':6',2''-terpyridin-4'-yloxy)pentanoic acid,[3] 6-(2,2':6',2''-terpyridin-4'-yloxy)hexanoic acid[3] and *N*,*N*-bis(2-pyridylmethyl)glycine[4] were prepared as described in the literature.

2'-Amino-2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-2'-*N*-(6-(2,2':6',2"-terpyridin-4'-yloxy)hexanoyl)uridine (2). A mixture of 6-(2,2':6',2''-terpyridin-4'-yloxy)hexanoic acid[3] (165 mg, 0.45 mmol) and nucleoside **1**[1] (370 mg, 0.68 mmol) was co-evaporated with anhydrous toluene (2 × 5 mL) and dried *in vacuo*. *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (108 mg, 0.56 mmol) and anhydrous CH_2Cl_2 (10 mL) were added, and the mixture was stirred for 24 hours at rt under an atmosphere of nitrogen. The reaction mixture was diluted with CH_2Cl_2 (15 mL) and washed with brine (2 × 10 mL). The aqueous phases were combined and extracted with $CHCl_3$ (3 × 10 mL). The combined organic phase was dried over Na_2SO_4, filtered and evaporated to dryness. The resulting residue was purified by column chromatography (neutral Al_2O_3, 0-10% MeOH in CH_2Cl_2, v/v) to give amide derivative **2** (289 mg, 72%) as a white solid material. TLC: R_f 0.8 (neutral Al_2O_3, 10% MeOH in CH_2Cl_2, v/v). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.53 (2H, d, H3$_{Ter}$, H3"$_{Ter}$, $J_{3\text{-}4} = J_{3"\text{-}4"}$ = 7.8 Hz), 8.51 (2H, dd, H6$_{Ter}$, H6"$_{Ter}$, $J_{6\text{-}5} = J_{6"\text{-}5"}$ = 4.7 Hz, $J_{6\text{-}4} = J_{6"\text{-}4"}$ = 1.8 Hz), 7.87 (2H, s, H3'$_{Ter}$, H5'$_{Ter}$), 7.76 (2H, td, H4$_{Ter}$, H4"$_{Ter}$, $J_{4\text{-}3} = J_{4"\text{-}3"} = J_{4\text{-}5} = J_{4"\text{-}5"}$ = 7.8 Hz, $J_{4\text{-}6} = J_{4"\text{-}6"}$ = 1.8 Hz), 7.56 (1H, d, H6, $J_{6\text{-}5}$ = 8.2 Hz), 7.32-6.70 (16H, m, DMT, H5$_{Ter}$, H5"$_{Ter}$, N*H*-2'), 6.07 (1H, d, H1', $J_{1'\text{-}2'}$ = 8.3 Hz), 5.41 (1H, d, H5, $J_{5\text{-}6}$ = 8.2 Hz), 4.61 (1H, app. q, H2', $J_{2'\text{-}1'} = J_{2'\text{-}3'} = J_{2'\text{-NH}}$ = 8.3 Hz), 4.35 (1H, d, H3', $J_{3'\text{-}2'}$ = 8.3 Hz), 4.20-4.10 (3H, m, H4', Hε), 3.67 (6H, s, 2×OC*H*$_3$), 3.30-3.24 (2H, m, H5'a, H5'b), 2.19 (2H, t, Hα, $J_{\alpha\text{-}\beta}$ = 6.5 Hz), 1.77 (2H, quintet, Hδ, $J_{\delta\text{-}\gamma} = J_{\delta\text{-}\varepsilon}$ = 6.2 Hz), 1.67 (2H, quintet, Hβ, $J_{\beta\text{-}\gamma} = J_{\beta\text{-}\alpha}$ = 6.5 Hz), 1.45 (2H, quintet, Hγ, $J_{\gamma\text{-}\beta} \sim J_{\gamma\text{-}\delta}$ = 6.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.80 (*C*O(2')), 167.13 (C4'$_{Ter}$), 163.13 (C4), 158.57 (C4$_{DMT}$, C4'$_{DMT}$), 156.66 (C2'$_{Ter}$, C6'$_{Ter}$), 155.96 (C1$_{Ter}$,

C1"$_{Ter}$), 151.13 (C2), 148.62 (C6$_{Ter}$, C6"$_{Ter}$), 144.10 (C1"$_{DMT}$), 140.11 (C6), 137.11 (C4$_{Ter}$, C4"$_{Ter}$), 135.26 and 135.11 (C1$_{DMT}$, C1'$_{DMT}$), 130.00 (C2$_{DMT}$, C2'$_{DMT}$, C6$_{DMT}$, C6'$_{DMT}$), 128.06 and 127.96 (C2"$_{DMT}$, C6"$_{DMT}$ and C3"$_{DMT}$, C5"$_{DMT}$), 127.00 (C4"$_{DMT}$), 123.96 (C5$_{Ter}$, C5"$_{Ter}$), 121.71 (C3$_{Ter}$, C3"$_{Ter}$), 113.23 (C3$_{DMT}$, C3'$_{DMT}$, C5$_{DMT}$, C5'$_{DMT}$), 107.47 (C3'$_{Ter}$, C5'$_{Ter}$), 102.90 (C5), 86.97 (Ar$_3$*C*O$_{DMT}$), 86.68 (C1'), 85.82 (C4'), 71.22 (C3'), 68.49 (Cε), 63.67 (C5'), 55.45 (C2'), 55.20 and 55.16 (2×O*C*H$_3$), 36.05 (Cα), 27.92 (Cδ), 25.93 (Cγ), 25.10 (Cβ). HRMS: *m/z* 913.3547 ([M+Na]$^+$, $C_{51}H_{50}N_6O_9Na^+$ calcd. 913.3532).

2'-Amino-3'-*O*-(2-cyanoethoxy(diisopropylamino)phosphino)-2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-2'-*N*-(6-(2,2':6',2"-terpyridin-4'-yloxy)hexanoyl)uridine (3). Amide derivative **2** (225 mg, 0.25 mmol) was co-evaporated with anhydrous toluene (2 × 5 mL) and dried *in vacuo*. Anhydrous CH_2Cl_2 (5 mL) and diisopropylethylamine (437 μL, 323 mg, 2.50 mmol) were added. The resulting solution was stirred at rt under an atmosphere of nitrogen, and 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (85 μL, 90 mg, 0.38 mmol) was added dropwise. After 1 hour, the reaction mixture was diluted with CH_2Cl_2 (10 mL), washed with sat. aq. $NaHCO_3$ (2 × 5 mL), with brine (5 mL) and dried by filtration through a layer of Na_2SO_4. After evaporation to dryness, the residue was purified by column chromatography (neutral Al_2O_3, 0-3% MeOH in CH_2Cl_2, v/v containing 2 % pyridine) to give phosphoramidite **3** (261 mg, 95%) as a white solid material. TLC: R_f 0.7 (neutral Al_2O_3, 5% MeOH in CH_2Cl_2). ^{31}P NMR (121.5 MHz, $CDCl_3$): δ 151.25 and 150.00 HRMS: *m/z* 1113.4556 ([M+Na]$^+$, $C_{60}H_{67}N_8O_{10}PNa^+$ calcd. 1113.4610).

2'-Amino-2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-2'-*N*-(5-(2,2':6',2"-terpyridin-4'-yloxy)pentanoyl)uridine (4). Amide derivative **4** (245 mg, 49%) was obtained as a white solid material starting form nucleoside **1**[1] (486 mg, 0.86 mmol), 5-(2,2':6',2''-terpyridin-4'-yloxy)pentanoic acid[3] (200 mg, 0.57 mmol) and *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (137 mg, 0.71 mmol), following the same procedure as described for the synthesis of amide **2**. TLC: R_f 0.71 (neutral Al_2O_3, 10% MeOH in CH_2Cl_2, v/v). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.57 (2H, d, H3$_{Ter}$, H3"$_{Ter}$, $J_{3\text{-}4} = J_{3"\text{-}4"} = 7.7$ Hz), 8.56 (2H, m, H6$_{Ter}$, H6"$_{Ter}$), 7.90 (2H, s, H3'$_{Ter}$, H5'$_{Ter}$), 7.83 (2H, td, H4$_{Ter}$, H4"$_{Ter}$, $J_{4\text{-}3} = J_{4"\text{-}3"} = J_{4\text{-}5} = J_{4"\text{-}5"} = 7.7$ Hz, $J_{4\text{-}6} = J_{4"\text{-}6"} = 1.5$ Hz), 7.56 (1H, d, H6, $J_{6\text{-}5} = 8.3$ Hz),

7.36-6.73 (16H, m, DMT, $H5_{Ter}$, $H5''_{Ter}$, N*H*-2'), 6.07 (1H, d, H1', $J_{1'-2'}$ = 8.1 Hz), 5.49 (1H, d, H5, J_{5-6} = 8.3 Hz), 4.55 (1H, app. q, H2', $J_{2'-1'} = J_{2'-3'} = J_{2'-NH}$ = 8.1 Hz), 4.40 (1H, dd, H3', $J_{3'-2'}$ = 8.1 Hz, $J_{3'-4'}$ = 1.8 Hz), 4.30-4.16 (3H, m, H4', Hδ), 3.75 (6H, s, 2×OCH_3), 3.37-3.30 (2H, m, H5', H5''), 2.39 (2H, t, Hα, $J_{\alpha-\beta}$ = 6.2 Hz), 2.08-1.72 (4H, m, Hβ, Hγ). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.41 (*C*O(2')), 166.86 ($C4'_{Ter}$), 162.99 (C4), 158.54 ($C4_{DMT}$, $C4'_{DMT}$), 156.69 ($C2'_{Ter}$, $C6'_{Ter}$), 155.93 ($C2_{Ter}$, $C2''_{Ter}$), 150.92 (C2), 148.62 ($C6_{Ter}$, $C6''_{Ter}$), 144.17 ($C1''_{DMT}$), 140.11 (C6), 137.20 ($C4_{Ter}$, $C4''_{Ter}$), 135.36 and 135.21 ($C1_{DMT}$, $C1'_{DMT}$), 129.99 ($C2_{DMT}$, $C2'_{DMT}$, $C6_{DMT}$, $C6'_{DMT}$), 128.06 and 127.92 ($C2''_{DMT}$, $C6''_{DMT}$ and $C3''_{DMT}$, $C5''_{DMT}$), 126.99 ($C4''_{DMT}$), 124.03 ($C5_{Ter}$, $C5''_{Ter}$), 121.77 ($C3_{Ter}$, $C3''_{Ter}$), 113.20 ($C3_{DMT}$, $C3'_{DMT}$, $C5_{DMT}$, $C5'_{DMT}$), 107.50 ($C3'_{Ter}$, $C5'_{Ter}$), 102.83 (C5), 87.21 (C1'), 86.81 (Ar_3CO_{DMT}), 85.69 (C4'), 70.40 (C3'), 68.40 (Cδ), 63.63 (C5'), 55.45 (C2'), 55.17 (2×O*C*H_3), 34.94 (Cα), 27.49 (Cγ), 22.40 (Cβ). HRMS: *m/z* 899.3398 ($[M+Na]^+$, $C_{51}H_{50}N_6O_9Na^+$ calcd. 899.3375).

2'-Amino-3'-*O*-(2-cyanoethoxy(diisopropylamino)phosphino)-2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-2'-*N*-(5-(2,2':6',2''-Terpyridin-4'-yloxy)pentanoyl)uridine (5). Phosphoramidite **5** (206 mg, 61%) was obtained as a white solid starting material from amide derivative **4** (195 mg, 0.22 mmol) and 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (98 μL, 104 mg, 0.44 mmol) following the same procedure as described for **3**. TLC: R_f 0.63 (neutral Al_2O_3, 5% MeOH in CH_2Cl_2, v/v). ^{31}P NMR (121.5 MHz, $CDCl_3$): δ 149.17 and 148.17. HRMS: *m/z* 1099.4415 ($[M+Na]^+$, $C_{62}H_{69}N_8O_{10}PNa^+$ calcd. 1099.4454).

2'-Amino-5'-*O*-(4,4'-dimethoxytrityl)-2'-*N*,4'-methylene-2'-*N*-(6-(2,2':6',2''-terpyridin-4'-yloxy)hexanoyl)thymidine (7). Amide derivative **7** (231 mg, 72%) was obtained as a white solid material starting form nucleoside **6**[2] (200 mg, 0.35 mmol), 6-(2,2':6',2''-terpyridin-4'-yloxy)hexanoic acid[3] (191 mg, 0.53 mmol) and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (88 mg, 0.46 mmol), following the same procedure as described for the synthesis of amide **2**. TLC: R_f 0.67 (neutral Al_2O_3, 10% MeOH in CH_2Cl_2, v/v). ^{1}H NMR (300 MHz, *d*$_6$-DMSO, major rotamer): δ 8.59-8.54 (4H, m, $H3_{Ter}$, $H3''_{Ter}$, $H6_{Ter}$, $H6''_{Ter}$), 7.92 (2H, s, $H3'_{Ter}$, $H5'_{Ter}$), 7.83 (2H, td, $H4_{Ter}$, $H4''_{Ter}$, $J_{4-3} = J_{4''-3''} = J_{4-5} = J_{4''-5''}$ = 7.5 Hz, $J_{4,4''-6,6''}$ = 1.7 Hz), 7.56 (1H, s, H6), 7.40-6.77 (16H, m, DMT, $H5_{Ter}$, $H5''_{Ter}$, N*H*-2'), 6.29 (1H, b, 3'O*H*), 5.43

(1H, s, H1'), 4.47 (1H, m, H2'), 4.32-4.20 (3H, m, H3', Hε), 3.78 and 3.77 (6H, s, 2×OCH_3), 3.62 (1H, d, H5'a, $J_{5'a\text{-}5'b}$ = 10.9 Hz), 3.55 (1H, d, H5"a, $J_{5"a\text{-}5"b}$ = 11.1 Hz), 3.45 (1H, d, H5'b, $J_{5'b\text{-}5'a}$ = 10.9 Hz), 3.43 (1H, d, H5"b, $J_{5"b\text{-}5"a}$ = 11.1 Hz), 2.55 (2H, t, Hα, $J_{\alpha\text{-}\beta}$ = 7.5 Hz), 2.00-1.85 (4H, m, Hβ, Hδ), 1.65 (2H, quintet, Hγ, $J_{\gamma\text{-}\beta} = J_{\gamma\text{-}\delta}$ = 6.8 Hz), 1.38 (3H, s, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.08 (*C*O(2')), 167.12 (C4'$_{Ter}$), 163.53 (C4), 158.68 (C4$_{DMT}$, C4'$_{DMT}$), 156.68 (C2'$_{Ter}$, C6'$_{Ter}$), 155.94 (C2$_{Ter}$, C2"$_{Ter}$), 150.00 (C2), 148.86 (C6$_{Ter}$, C6"$_{Ter}$), 144.25 (C1"$_{DMT}$), 137.21 (C4$_{Ter}$, C4"$_{Ter}$), 135.25 and 135.24 (C1$_{DMT}$, C1'$_{DMT}$), 134.23 (C6) 130.05 and 130.0 (C2$_{DMT}$, C6$_{DMT}$, and C2'$_{DMT}$, C6'$_{DMT}$), 128.06 and 127.99 (C2"$_{DMT}$, C6"$_{DMT}$ and C3"$_{DMT}$, C5"$_{DMT}$), 127.11 (C4"$_{DMT}$), 123.98 (C5$_{Ter}$, C5"$_{Ter}$), 121.77 (C3$_{Ter}$, C3"$_{Ter}$), 113.28 (C3$_{DMT}$, C3'$_{DMT}$, C5$_{DMT}$, C5'$_{DMT}$), 110.24 (C5), 107.72 (C3'$_{Ter}$, C5'$_{Ter}$), 87.93 (Ar$_3$*C*O$_{DMT}$), 87.41 (C1'), 86.66 (C4'), 69.39 (C3'), 68.62 (Cε), 63.89 (C2'), 59.08 (C5'), 55.23 (2×O*C*H$_3$), 51.21 (C5"), 34.72 (Cα), 27.63 (Cδ), 26.35 (Cγ), 24.35 (Cβ), 12.35 (*C*H$_3$). HRMS: *m/z* 939.3657 ([M+Na]$^+$, $C_{53}H_{52}N_6O_9Na^+$ calcd. 939.3688).

2'-Amino-3'-*O*-(2-cyanoethoxy(diisopropylamino)phosphino)-5'-*O*-(4,4'-dimethoxytrityl)-2'-*N*,4'-methylene-2'-*N*-(6-(2,2':6',2''-terpyridin-4'-yloxy)hexanoyl)thymidine (8). Phosphoramidite **8** (156 mg, 61%) was obtained as a white solid starting material from amide derivative **7** (213 mg, 0.23 mmol) and 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (77 μL, 81 mg, 0.34 mmol) following the same procedure as described for **3**. TLC: R_f 0.56 and 0.81 (neutral Al_2O_3, 5% MeOH in CH_2Cl_2, v/v). ^{31}P NMR (121.5 MHz, $CDCl_3$): δ 149.46 and 147.71. HRMS: *m/z* 1139.4823 ([M+Na]$^+$, $C_{62}H_{69}N_8O_{10}PNa^+$ calcd. 1139.4767).

2'-Amino-2'-*N*-[*N*,*N*-bis(2-pyridylmethyl)glycyl]-2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)uridine (9). Amide derivative **9** (900 mg, 76%) was obtained as a white solid material starting form nucleoside **1**[1] (819 mg, 1.50 mmol), *N*,*N*-bis(2-pyridylmethyl)glycine[4] (579 mg, 2.25 mmol) and *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (361 mg, 1.88 mmol), following the same procedure as described for the synthesis of amide **2**, except that purification was performed by silica gel column chromatography (0-6% MeOH in CH_2Cl_2). TLC: R_f 0.59 (silica, 15% MeOH in CH_2Cl_2, v/v). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.82 (1H, d, N*H*-2', $J_{NH\text{-}2'\text{-}2'}$ = 10.0 Hz), 8.55 (2H, dd, H6$_{Pyr}$, H6'$_{Pyr}$, $J_{6\text{-}5} = J_{6'\text{-}5'}$ = 4.8 Hz, $J_{6\text{-}4} = J_{6'\text{-}4'}$ = 1.1 Hz), 7.79 (1H, d, H6,

$J_{6\text{-}5}$ = 8.2 Hz), 7.62 (2H, t, $H4_{Pyr}$, $H4'_{Pyr}$, $J_{4\text{-}3} = J_{4\text{-}5} = J_{4'\text{-}3'} = J_{4'\text{-}5'}$ = 7.6 Hz), 7.43 (2H, d, $H3_{Pyr}$, $H3'_{Pyr}$, $J_{3\text{-}4} = J_{3'\text{-}4'}$ = 7.6 Hz), 7.34-8.85 (15H, m, DMT, $H5_{Pyr}$, $H5'_{Pyr}$), 6.30 (1H, d, H1', $J_{1'\text{-}2'}$ = 8.7 Hz), 5.37 (1H, d, H5, $J_{5\text{-}6}$ = 8.2 Hz), 5.00 (1H, m, H2'), 4.44 (1H, d, H3', $J_{3'\text{-}2'}$ = 4.7 Hz), 4.32 (1H, s, H4'), 3.85 (2H, d, 2×NC$H_a$$H_b$Ar, $J_{a\text{-}b}$ = 13.5 Hz), 3.78-3.69 (6H, m, 2×OCH_3, 2×NC$H_a$$H_b$Ar), 3.46 (2H, s, H5'), 3.40 (1H, d, C$_\alpha$$H_a$$H_b$, $J_{a\text{-}b}$ = 16.4 Hz), 3.20 (1H, d, C$_\alpha$$H_a$$H_b$, $J_{b\text{-}a}$ = 16.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.39 (*C*O(2')), 163.14 (C4), 158.62 ($C4_{DMT}$, $C4'_{DMT}$), 157.02 ($C2_{Pyr}$, $C2'_{Pyr}$), 150.87 (C2), 149.42 ($C6_{Pyr}$, $C6'_{Pyr}$), 144.11 ($C1''_{DMT}$), 140.32 (C6), 137.15 ($C4_{Pyr}$, $C4'_{Pyr}$), 135.29 and 135.08 ($C1_{DMT}$, $C1'_{DMT}$), 130.13 ($C2_{DMT}$, $C2'_{DMT}$, $C6_{DMT}$, $C6'_{DMT}$), 128.18 and 127.98 ($C2''_{DMT}$, $C6''_{DMT}$ and $C3''_{DMT}$, $C5''_{DMT}$), 127.05 ($C4''_{DMT}$), 124.00 ($C5_{Pyr}$, $C5'_{Pyr}$), 122.79 ($C3_{Pyr}$, $C3'_{Pyr}$), 113.25 ($C3_{DMT}$, $C3'_{DMT}$, $C5_{DMT}$, $C5'_{DMT}$), 102.67 (C5), 87.12 (C1'), 85.45 (Ar_3CO_{DMT}), 84.84 (C4'), 72.51 (C3'), 63.99 (C5'), 59.74 and 58.41 (2×N*C*H_2Ar), 55.45 (C2'), 55.18 (2×O*C*H_3), 46.07 (Cα).

2'Amino-2'-*N*-(*N*,*N*-bis(2-pyridylmethyl)glycyl)-3'-*O*-(2-cyanoethoxy(diisopropylamino)phosphino)-2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)uridine (10). Phosphoramidite **10** (858 mg, 81%) was obtained as a white solid starting material from amide derivative **9** (851 mg, 1.08 mmol) and 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (306 μL, 324 mg, 1.37 mmol) following the same procedure as described for **3** except that purification was performed by silica gel column chromatography (0-5% MeOH in CH_2Cl_2, containing 2% pyridine). TLC: R_f 0.62 (silica gel, 15% MeOH in CH_2Cl_2, v/v). ^{31}P NMR (121.5 MHz, $CDCl_3$): δ 150.7 and 151.3.

Synthesis and purification of oligonucleotides

Synthesis of oligonucleotides was performed in 0.2 μmol scale using an automated DNA synthesizer (PerSpective Biosystems Expedite 8909). Oligonucleotides bearing a modified monomer at the 3'-end were synthesized using universal support (Glen Research, Universal Support II). Standard cycle procedures were applied for unmodified phosphoramidites using 0.25 M solution of 4,5-dicyanoimidazole as activator. In case of phosphoramidite **8** (for incorporation of monomer **Z**) and LNA phosphoramidites (monomers $\mathbf{A^L}$ and $\mathbf{T^L}$) both coupling time and oxidation time were extended to 30 min

and 1 min, respectively. To accomplish incorporation of monomers **X** and **Y** (phosphoramidites **3** and **5**, respectively), a double coupling procedure with extended coupling time (2 × 30 min) was performed. Incorporation of monomer **P** (phosphoramidite **10**) required the use of 0.5 M pyridinium hydrochloride solution in acetonitrile as activator, together with double coupling procedure and extended coupling time (2 × 30 min). Stepwise coupling yields, as determined by a spectrophotometric DMT^+ assay, were >99% for standard and LNA phosphoramidites, >98% for phosphoramidite **8**, >85% for phosphoramidites **3** and **5**, and >40% for phosphoramidite **10**.

Removal of the nucleobase protecting groups and cleavage from solid support was effected using standard conditions (32% aqueous ammonia for 12 h at 55 °C). When universal support was used the cleavage was performed by 12 h treatment with saturated methanolic ammonia solution at rt, followed by addition of 32% aqueous ammonia and heating for 12 h at 55 °C.

Purification of all oligonucleotides was performed by DMT-ON RP-HPLC using a Waters Prep LC 4000 HPLC machine equipped with an Xterra MS C18-column (10 μm, 300 mm × 7.8 mm). The following eluent system was used: A-buffer: 95% 0.1 M $Et_3NH{\cdot}HCO_3$, 5% CH_3CN; B-buffer: 25% 0.1 M $Et_3NH{\cdot}HCO_3$, 75% CH_3CN. Gradient: 0-5 min isocratic hold of 100% A-buffer, followed by a linear gradient to 55% B-buffer over 75 min at a flow rate of 1.0 mL/min. Fractions containing pure oligonucleotides were collected and evaporated on a speed-vac, followed by detritylation (80% aq. AcOH, 20 min), precipitation (abs. EtOH, −18 °C, 12 h) and washing with abs. EtOH.

Unmodified RNA was obtained from a commercial supplier.

MALDI-MS of synthesized oligonucleotides

The composition of oligonucleotides was verified by MALDI-MS analysis (Table S1) whereas the purity (>80%) was verified by ion-exchange HPLC using a LaChrom L-7000 system (VWR International) equipped with a Gen-Pak Fax column (100 mm × 4.6 mm). The following eluent system was used: A-buffer: 25 mM Tris-Cl, 1 mM EDTA, pH 8.0; B-buffer: 1 M NaCl. Gradient: 0-5 min isocratic hold of 95% A-buffer, followed by a linear gradient to 70% B-buffer over 41 min at a flow rate of 0.75 mL/min.

Table S1. MALDI-MS of synthesized ONs.

Code	Sequence	Found *m/z*	Calc. *m/z*
DNA1-**X**	5'-d(CA$^{\mathbf{L}}$C CA$^{\mathbf{L}}$A C**X**)	2742	2740
DNA1-**Y**	5'-d(CA$^{\mathbf{L}}$C CA$^{\mathbf{L}}$A C**Y**)	2726	2726
DNA1-**Z**	5'-d(CA$^{\mathbf{L}}$C CA$^{\mathbf{L}}$A C**Z**)	2766	2767
DNA1-**P**	5'-d(CA$^{\mathbf{L}}$C CA$^{\mathbf{L}}$A C**P**)	2635	2635
DNA2-**X**	5'-d(**X**CT T$^{\mathbf{L}}$CC A$^{\mathbf{L}}$CA)	3036	3035
DNA2-**Y**	5'-d(**Y**CT T$^{\mathbf{L}}$CC A$^{\mathbf{L}}$CA)	3022	3023
DNA2-**Z**	5'-d(**Z**CT T$^{\mathbf{L}}$CC A$^{\mathbf{L}}$CA)	3062	3062
DNA2-**P**	5'-d(**P**CT T$^{\mathbf{L}}$CC A$^{\mathbf{L}}$CA)	2931	2930

Experimental details of the RNA cleavage reactions

5'-End-labeling of the RNA substrate. The RNA substrate (1 pmol), [γ-^{32}P] ATP (1 μCi) and T4 polynucleotide kinase were incubated for 60 min at 37 ºC in a buffer containing 70 mM Tris HCl pH 7.6, 10 mM $MgCl_2$ and 5 mM DTT. After denaturating the enzyme by heating to 80 ºC for 15 min., the labeled RNA was mixed with non-labeled RNA (499 pmol) and the volume was adjusted with water to 100 μL (obtained stock solution with final RNA concentration of 5 μM was used in the cleavage experiments).[5]

RNA cleavage. All RNA cleavage reactions were performed at 37ºC in 100 mM NaCl, 20 mM Tris HCl pH 7.4, with an RNA concentration of 1 μM. The reactions were carried out with concentrations of oligonucleotide cleaving agents and Cu^{2+} (added as $CuCl_2$ solution) as stated in the communication with total reaction volumes of 5-15 μL. Prior to starting the reaction by addition of the Cu^{2+} solution, denaturation was performed by heating to 80 ºC for 2 min. Aliquots (1 μL) were taken at specific times and the cleavage reactions were stopped by addition of EDTA and formamide followed by heating to 80 ºC for 2 min. Samples were analyzed on 20% urea polyacrylamide gel.[5]

RNA cleavage by oligonucleotides DNA1-N and DNA2-N

The ability of oligonucleotides DNA1-**N** and DNA2-**N** to individually cleave the RNA target was studied. As seen from the results depicted in Figure S1, this resulted in much lower cleavage efficiencies relative to combined used of DNA1-**N** and DNA2-**N**, providing an evidence for RNA cleavage cooperativity for the bimolecular artificial ribonuclease system.

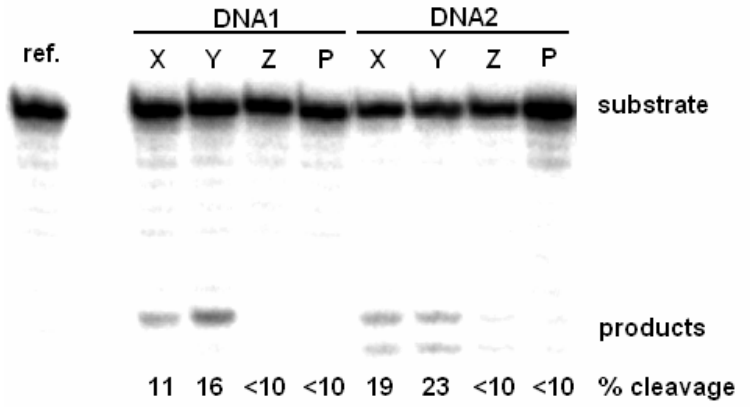


Figure S1. Denaturating gel electrophoresis of cleavage reactions catalyzed by either DNA1-**N** or DNA2-**N** (16 h; $c_{\text{DNAx-N}}$ = 4 μM; $c_{\text{Cu2+}}$ = 8 μM; c_{RNA} = 1 μM; 37ºC).

Thermal denaturation studies

Concentrations of oligonucleotides were calculated using the following extinction coefficients (OD_{260}/μmol): G, 10.5; A, 13.9; T/U, 7.9; C, 6.6; terpyridine, 8.0;[6] *N*,*N*-bis(2-pyridylmethyl), 6.0 (2×OD_{260}/μmol for pyridine[7]). Oligonucleotides (1.0 μM of each strand) and Cu^{2+} (added as $CuCl_2$ solution) were thoroughly mixed in T_m-buffer (100 mM NaCl, pH 7.0 adjusted with 10 mM NaH_2PO_4/5 mM Na_2HPO_4) and denaturation was performed by heating at 80 for 2 min which was followed by cooling to the starting temperature of the experiment. Quartz optical cells with a path-length of 1.0 cm were used. Thermal denaturation temperatures (T_m values/°C) were measured on a Perkin Elmer Lambda 35 UV/VIS spectrometer equipped with a PTP-6 Peltier temperature programmer and determined as the maximum of the first derivative of the thermal denaturation curve (A_{260} vs. temperature). A temperature ramp of 1.0 °C/min was used in

all experiments. Reported thermal denaturation temperatures are an average of two measurements within ±1.0 °C.

Table S2. Thermal denaturation temperatures (T_m values (ºC)) of duplexes formed between DNA1-**Y**, DNA2-**Y** and complementary RNA at different concentration of Cu^{2+}.

		Cu^{2+} per duplex	
Code	Sequence	1 eq.	5 eq.
DNA1-**Y**	5'-d(C**A**$^{\mathbf{L}}$C C**A**$^{\mathbf{L}}$A C**Y**)	52.5	nd[a]
RNA	3'-r(GUG GUU GAA GAA GGU GU)		
DNA2-**Y**	5'-d(**Y** CT**T**$^{\mathbf{L}}$ CC**A**$^{\mathbf{L}}$ CA)	53.5	nd[a]
RNA	3'-r(GUG GUU GAA GAA GGU GU)		
DNA1-**Y**	5'-d(C**A**$^{\mathbf{L}}$C C**A**$^{\mathbf{L}}$A C**Y**)		
DNA2-**Y**	5'-d(**Y** CT**T**$^{\mathbf{L}}$ CC**A**$^{\mathbf{L}}$ CA)	53.0[b]	54.0[b]
RNA	3'-r(GUG GUU GAA GAA GGU GU)		

[a] not determined; [b] single transition observed

References

1. McGee, D. P. C.; Vaughn-Settle, A.; Vargeese, C.; Zhai, Y. *J. Org. Chem.*, 1996, **61**, 781-785.
2. Singh, S. K.; Kumar, R.; Wengel J. *J. Org. Chem.*, 1998, **63**, 10035-10039.
3. Anders, P. R.; Lunkwitz, R.; Pabst, G. R.; Bohn, K.; Wouters, D.; Schmatloch, S.; Schubert, U. S. *Eur. J. Org. Chem.*, 2003, 3769-3776.
4. Cox, D. D.; Benkovic, S. J.; Bloom, L. M.; Bradley, F. C.; Nelson, M. J.; Que Jr., L.; Wallick, D. E. *J. Am. Chem. Soc.*, 1998, **110**, 2026-2032.
5. Sambrook, J.; Fritsch, E. F.; Maniatis, T. "Molecular cloning: A laboratory manual." 1989, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
6. Martin, R. B.; Lissfelt, J. A. *J. Am. Chem. Soc.* 1956, **78**, 938-940.
7. "Handbook of Chemistry and Physics", Weast, R. C., Ed., 1974-1975, 55th ed. CRC Press, Cleveland, Ohio.