Supplementary Material

Synthesis and Characterization of 2'-Modified-4'-ThioRNA: A Comprehensive Comparison of Nuclease Stability.

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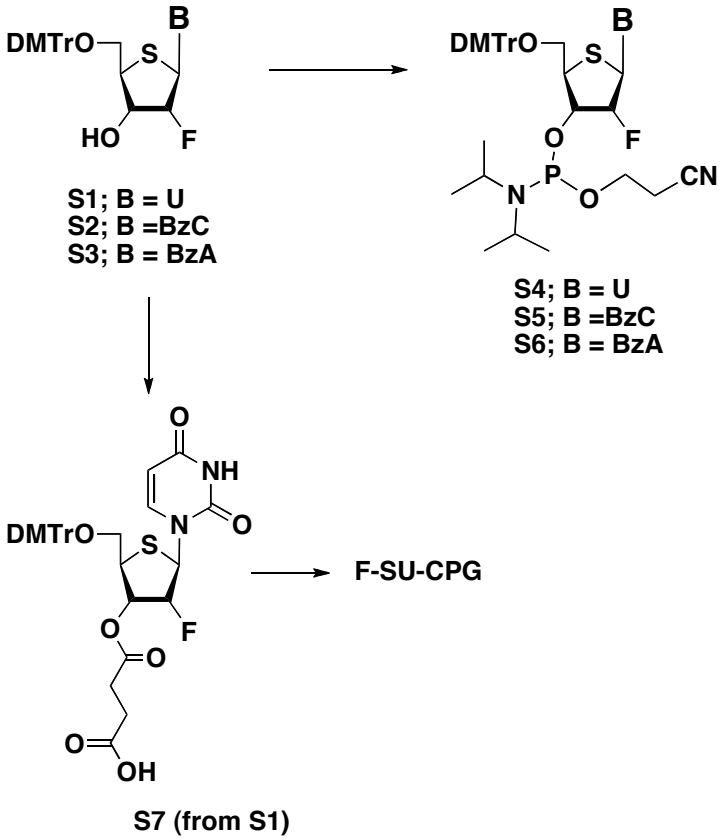
Synthesis of phosphoroamidite units and CPG supports



Scheme S1; Synthesis of phosphoramidite units and CPG for F-SRNA

*1-[3-O-{2-Cyanoethoxy(diisopropylamino)phosphino}-5-O-(4,4'-dimethoxytrityl)-2-deoxy-2-fluoro -4-thio-β-D-ribofuranosyl]uracil (**S4**)*. To a solution of **S1** (508 mg, 0.90 mmol) in dry CH_2Cl_2 (9 mL) were added *N*,*N*-diisopropylethylamine (314 μL, 1.5 mmol), DMAP (11 mg, 0.090 mmol) and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (300 μL, 1.3 mmol), and the reaction mixture

was stirred for 3 h at room temperature. The reaction was quenched by addition of ice. The reaction mixture was diluted with AcOEt, which was washed with H_2O (twice) and saturated brine. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by a silica gel column, eluted with hexane/AcOEt (1:1), to give **S4** (510 mg, 74%) as a yellow foam: FAB-LRMS *m/z* 765 (MH^+); FAB-HRMS calcd for $C_{39}H_{47}FN_4O_7PS$ (MH^+) 765.2887, found 775.2900; ^{31}P NMR ($CDCl_3$) d: 151.6 and 151.3.

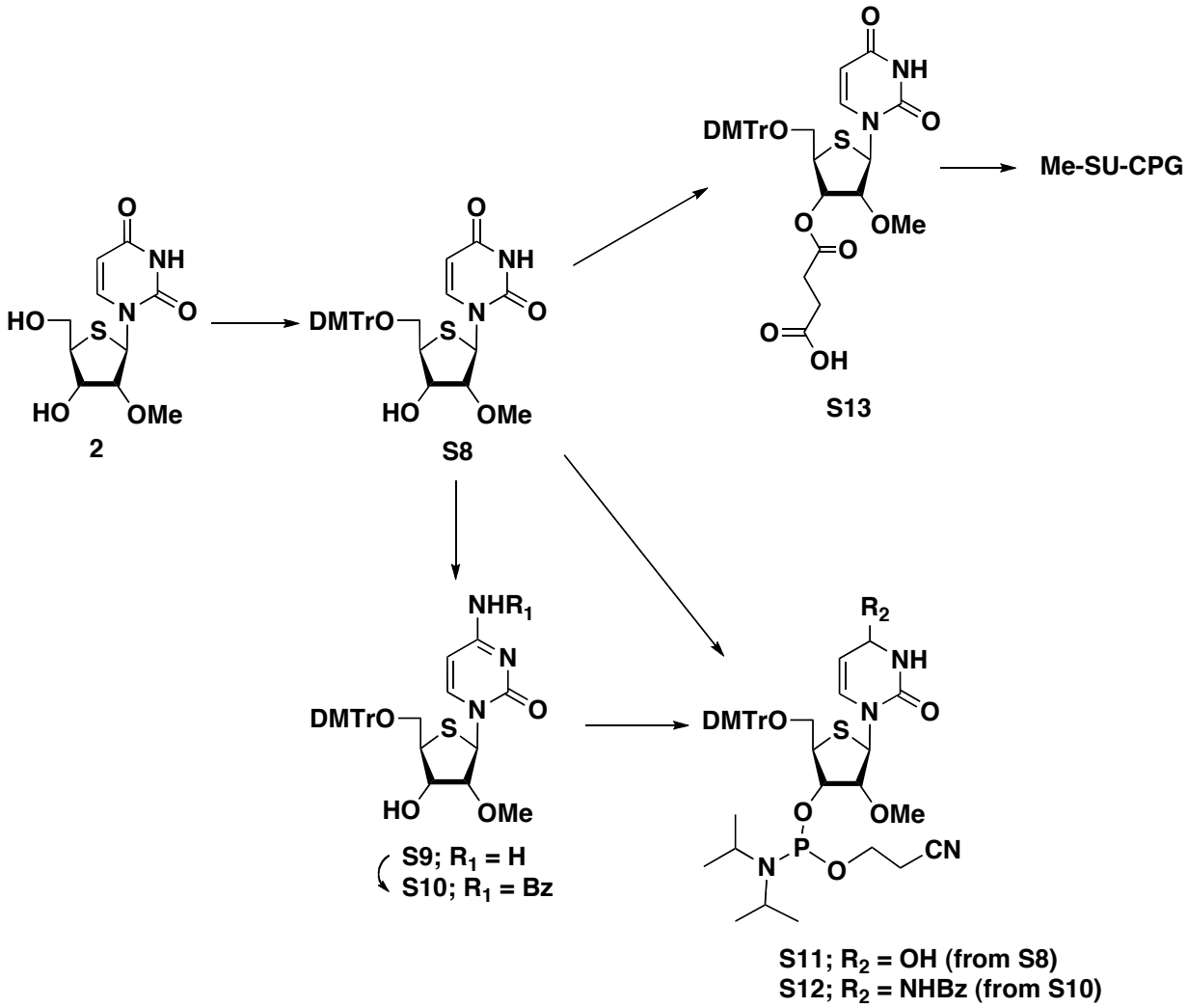
*N^4-Benzoyl-1-[3-O-{2-cyanoethoxy(diisopropylamino)phosphino}-5-O-(4,4'-dimethoxytrityl)-2-deoxy-2-fluoro-4-thio-β-D-ribofuranosyl]cytosine (**S5**)*. In the similar manner as described for **S4**, **S2** (440 mg, 0.66 mmol) in dry CH_2Cl_2 (7 mL) was treated with *N,N*-diisopropylethylamine (200 μL, 1.1 mmol), DMAP (8 mg, 65 μmol) and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (190 μL, 0.85 mmol) to give **S5** (480 mg, 84%) as a white foam: FAB-LRMS *m/z* 868 (MH^+); FAB-HRMS calcd for $C_{46}H_{52}FN_5O_7PS$ (MH^+) 868.3309, found 868.3321; ^{31}P NMR ($CDCl_3$) δ: 151.9 and 150.4.

*N^6-Benzoyl-9-[3-O-{2-cyanoethoxy(diiopropylamino)phosphino}-5-O-(4,4'-dimethoxytrityl)-2-O-deoxy-2-fluorol-4-thio-β-D-furanosyl]adenine (**S6**)*. In the similar manner as described for **S4**, **S3** (620 mg, 0.89 mmol) in dry CH_2Cl_2 (9 mL) was treated with *N,N*-diisopropylethylamine (260 μL, 1.5 mmol), DMAP (11 mg, 89 μmol) and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (258 μL, 1.15 mmol) to give **S6** (660 mg, 83%) as a white foam: FAB-LRMS *m/z* 892 (MH^+); FAB-HRMS calcd for $C_{47}H_{52}FN_7O_6PS$ (MH^+) 892.3422, found 992.3429; ^{31}P NMR ($CDCl_3$) d: 151.7 and 151.5.

*1-[5-O-(4,4'-Dimethoxytrityl)-2-deoxy-2-fluoro-3-O-succinyl-4-thio-β-D-ribofuranosyl]uracil (**S7**)*. To a solution of **S1** (120 mg, 0.21 mmol) in dry acetonitrile (3 mL) were added Et_3N (88 μL, 0.63 mmol), succinic anhydride (63 mg, 0.63 mmol) and DMAP (13 mg, 0.11 mmol), and the reaction mixture was stirred for 2 h at room temperature. The reaction mixture was diluted with $CHCl_3$, which was washed with H_2O and saturated aqueous NH_4Cl, followed by brine. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by a silica gel column,

eluted with MeOH in $CHCl_3$ (0–2%), to give **S7** (72 mg, 52%) as a white foam: ^{1}H NMR ($CDCl_3$) δ: 10.5 (br s, 1 H), 8.24 (d, 1H, *J* = 8.0 Hz), 7.46–7.28 (m, 9 H), 6.86–6.83 (m, 4 H), 6.21 (dd, 1 H, *J* = 2.8 and 12.5 Hz), 5.38–5.31 (m, 2 H), 5.17–5.06 (ddd, 1 H, *J* = 2.8 and 49.7 Hz), 3.79 (s, 6 H), 3.77 (m, 1 H), 3.52 (dd, 1H, *J* = 2.8 and 10.8 Hz), 3.45 (dd, 1 H, *J* = 4.0 and 10.8 Hz), 2.79–2.56 (m, 3 H), 2.42 (m, 1 H).

Synthesis of ***F-SU-CPG***. To a solution of **S7** (72 mg, 0.11 mmol) in dry DMF (3 mL) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI•HCl, 21 mg, 0.11 mmol) and LCA-CPG (212 mg, 0.027 mmol, 130 μmol/g), and the mixture was kept for 42 h at room temperature. The solid support was filtered and washed with pyridine. The remaining amino groups were capped by treatment with 0.1 M DMAP and 10% Ac_2O in pyridine. The resulting solid support was filtered and washed with EtOH and acetone, and dried under reduced pressure to give 2'-deoxy-2'-F-4'-thiouridine unit loaded CPG support. The loading amount of **F-SU-CPG** was estimated by DMTr cation assay to be 33.0 μmol/g.



Scheme S2; Synthesis of phosphoramidite units and CPG for Me-SRNA

*1-[5-O-(4,4'-Dimethoxytrityl)-2-O-methyl-4-thio-β-D-ribofuranosyl]uracil (**S8**)*. To a solution of **2** (745 mg, 2.7 mmol) in dry pyridine (20 mL) was added DMTrCl (1.4 g, 4.1 mmol), and the reaction mixture was stirred for 2 h at room temperature. The reaction was quenched by addition of ice. The mixture was concentrated *in vacuo*. The residue was diluted with AcOEt, which was washed with H_2O and saturated aqueous $NaHCO_3$, followed by brine. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by a silica gel column, eluted with hexane/AcOEt (1:1–1:2), to give **S8** (1.53 g, quant.) as a white foam: FAB-LRMS *m/z* 599 (MNa^+); FAB-HRMS calcd for $C_{31}H_{32}N_2NaO_7S$ (MNa^+) 599.1828, found 599.1826; ^1H NMR ($CDCl_3$) δ: 8.21 (br s, 1 H), 8.11 (d, 1 H, *J* = 8.2 Hz), 7.44–7.25 (m, 9 H), 6.89–6.84 (m, 4 H), 6.02 (d, 1 H, *J* = 2.4 Hz), 5.45 (d, 1 H, *J* = 8.2 Hz), 4.14–4.09 (m, 1 H), 3.80 (s, 6 H), 3.76 (m, 1 H),

3.60 (s, 3 H), 3.56–3.51 (m, 3 H); ^{13}C NMR ($CDCl_3$) δ: 158.8, 144.5, 141.3, 135.4, 130.3, 130.3, 128.3, 128.0, 127.2, 113.3, 102.4, 87.9, 87.2, 73.1, 62.3, 62.2, 58.6, 55.3, 51.1.

*1-[3-O-{2-Cyanoethoxy(diisopropylamino)phosphino}-5-O-(4,4'-dimethoxytrityl)-2-O-methyl-4-thio-β-D-ribofuranosyl]uracil (**S11**)*. In the similar manner as described for **S4**, **S8** (500 mg, 0.87 mmol) in dry CH_2Cl_2 (11 mL) was treated with *N,N*-diisopropylethylamine (260 μL, 1.5 mmol), DMAP (10 mg, 0.087 mmol) and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (250 μL, 1.1 mmol) to give **S11** (590 mg, 87%) as a white foam: FAB-LRMS *m/z* 777 (M^+); FAB-HRMS calcd for $C_{40}H_{50}N_4O_8PS$ (MH^+) 777.3087, found 777.3082; ^{31}P NMR ($CDCl_3$) δ: 151.2 and 150.9.

*1-[5-O-(4,4'-Dimethoxytrityl)-2-O-methyl-4-thio-β-D-ribofuranosyl]cytosine (**S9**)*. To a solution of **S8** (1.2 g, 2.1 mmol) in dry acetonitrile (20 mL) were added Et_3N (860 μL, 6.2 mmol), Ac_2O (590 μL, 6.2 mmol) and DMAP (24 mg, 0.21 mmol), and the reaction mixture was stirred for 20 min at room temperature. The reaction was quenched by addition of ice. The solvent was removed *in vacuo*, and the residue was diluted with AcOEt. The organic layer was washed with H_2O and saturated aqueous $NaHCO_3$, followed by brine. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was dissolved in dry acetonitrile (20 mL), and Et_3N (860 μL, 6.2 mmol), 2,4,6-triisopropylbenzenesulfonyl chloride (1.9 g, 6.2 mmol) and DMAP (760 mg, 6.2 mmol) were added to the solution. The mixture was stirred for 1 h at room temperature. After the starting material was consumed, concentrated NH_4OH (28%, 30 mL) was added, and the reaction mixture was kept for 9 h at room temperature. The whole mixture was concentrated *in vacuo*. The residue was diluted with H_2O, and the aqueous layer was extracted with $CHCl_3$ (x 3). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by a silica gel column, eluted with MeOH in $CHCl_3$ (0–5%), to give **S9** (1.1 g, 88% in 2 steps) as a yellow foam: FAB-LRMS *m/z* 576 (MH^+); FAB-HRMS calcd for $C_{31}H_{34}N_3O_6S$ (MH^+) 576.2169, found 576.2144; ^{1}H NMR (DMSO-d_6) δ: 7.83 (d, 1 H, *J* = 7.3 Hz), 7.40–7.23 (m, 11 H), 6.91–6.88 (m, 4 H), 5.96 (d, 1 H, *J* = 4.3 Hz), 5.66 (d, 1 H, *J* = 7.3 Hz), 5.21 (d, 1 H, *J* = 6.2 Hz), 4.04–4.01 (m, 1 H), 3.73 (s, 6 H), 3.59 (t, 1 H, *J* = 4.3 Hz), 3.49–3.45 (m, 1 H), 3.41–3.38 (M, 1 H), 3.34 (s, 3 H),

3.29–3.26 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ: 165.3, 158.1, 155.3, 144.8, 141.7, 135.4, 135.3, 129.8, 127.9, 127.7, 126.7, 113.2, 94.3, 86.3, 85.8, 79.1, 71.1, 64.2, 61.5, 57.5, 55.0, 50.1.

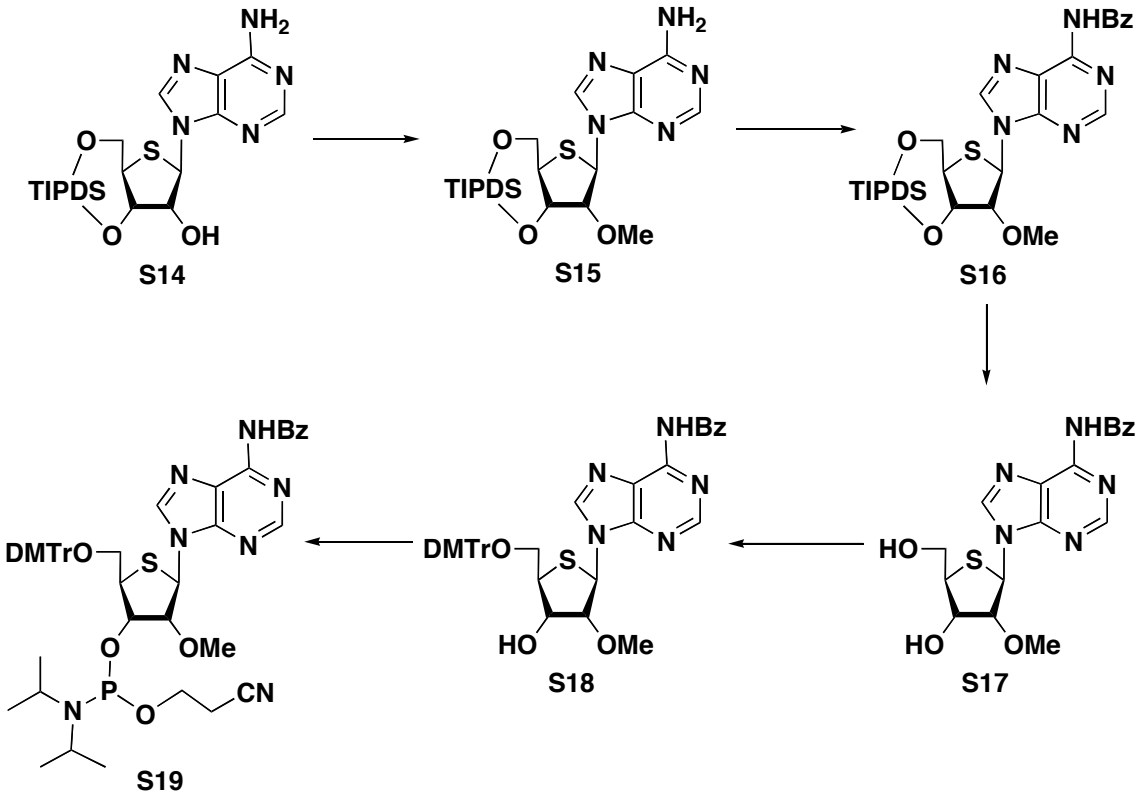
*N^4-Benzoyl-1-[5-O-(4,4'-dimethoxytrityl)-2-O-methyl-4-thio-β-D-ribofuranosyl]cytosine (**S10**)*. To a solution of **S9** (190 mg, 0.34 mmol) in DMF (3.5 mL) was added Bz_2O (115 mg, 0.51 mmol), and the reaction mixture was stirred at 50 ˚C. After 4.5 h, additional Bz_2O (15 mg, 68 μmol) was added to the reaction mixture, and the mixture was stirred for additional 1.5 h at the same temperature. The reaction was quenched by addition of saturated aqueous $NaHCO_3$. The mixture was partitioned between AcOEt and H_2O. The separated organic layer was washed with H_2O and saturated aqueous $NaHCO_3$, followed by brine. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by a silica gel column, eluted with MeOH in $CHCl_3$ (0–1%), to give **S10** (220 mg, 95%) as a yellow foam: FAB-LRMS *m/z* 680 (MH^+); FAB-HRMS calcd for $C_{38}H_{38}N_3O_7S$ (MH^+) 680.2430, found 680.2409; ^{1}H NMR ($CDCl_3$) δ: 8.73–8.27 (m, 2 H), 7.89 (d, 2 H, *J* = 7.5 Hz), 7.64–7.24 (m, 13 H), 6.88 (m, 4 H), 6.14 (s, 1 H), 4.18 (m, 1 H), 3.83 (s, 6 H), 3.82–3.80 (m, 1 H), 3.72 (s, 3 H), 3.60–3.53 (m, 3 H), 2.44 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ: 166.5, 162.4, 158.7, 155.5, 146.8, 144.3, 135.7, 133.2, 130.3, 129.0, 128.4, 128.1, 127.8, 127.2, 113.3, 97.0, 87.6, 87.1, 72.7, 64.0, 62.1, 58.5, 55.3, 51.1

*N^4-Benzoyl-1-[3-O-{2-cyanoethoxy(diisopropylamino)phosphino}-5-O-(4,4'-dimethoxytrityl)-2-O-methyl-4-thio-β-D-ribofuranosyl]cytosine (**S12**)*. In the similar manner as described for **S4**, **S10** (1.5 g, 2.2 mmol) in dry CH_2Cl_2 (22 mL) was treated with *N,N*-diisopropylethylamine (768 μL, 4.4 mmol), DMAP (26 mg, 0.22 mmol) and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (736 μL, 3.3 mmol) to give **S12** (1.2 mg, 64%) as a white foam: FAB-LRMS *m/z* 880 (MH^+); FAB-HRMS calcd for $C_{47}H_{55}N_5O_8PS$ (MH^+) 880.3509, found 880.3524; ^{31}P NMR ($CDCl_3$) δ: 151.3 and 150.9.

*1-[5-O-(4,4'-Dimethoxytrityl)-2-O-methyl-3-O-succinyl-4-thio-β-D-ribofuranosyl]uracil (**S13**)*. In the similar manner as described for **S7**, **S8** (120 mg, 0.21 mmol) in dry acetonitrile (3 mL) was treated with Et_3N (88 μL, 0.63 mmol), succinic anhydride (63 mg, 0.63 mmol) and DMAP (13 mg,

0.11 mmol) to give **S13** (100 mg, 71%) as a white foam: FAB-LRMS *m/z* 699 (MNa^+); FAB-HRMS calcd for $C_{35}H_{36}N_2NaO_{10}S$ (MNa^+) 699.2989, found 699.1989; ^1H NMR ($CDCl_3$) δ: 10.8 (br s, 1 H), 8.35 (d, 1H, *J* = 8.0 Hz), 7.41–7.21 (m, 9 H), 6.85–6.80 (m, 4 H), 5.92 (d, 1 H, *J* = 2.3 Hz), 5.36 (d, 1 H, *J* = 8.0 Hz), 5.09 (dd, 1 H, *J* = 3.4 and 9.1 Hz), 3.89–3.87 (m, 1 H), 3.79 (s, 6 H), 3.78 (m, 1 H), 3.59 (dd, 1 H, *J* = 3.5 and 10.3 Hz), 2.83–2.67 (m, 2 H), 2.52–2.47 (m, 1 H), 2.30–2.25 (m, 1 H); ^{13}C NMR (DMSO-d_6) δ: 173.7, 171.9, 163.2, 158.7, 151.2, 145.1, 141.2, 135.7, 135.6, 130.3, 128.4, 128.2, 127.4, 113.8, 102.9, 86.6, 83.3, 79.7, 72.0, 64.1, 61.7, 58.5, 55.5, 48.6, 29.2.

Synthesis of ***Me-SU-CPG***. To a solution of **S13** (54 mg, 0.080 mmol) in dry DMF (2 mL) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI•HCl, 15 mg, 0.080 mmol) and LCA-CPG (220 mg, 0.020 mmol, 90 μmol/g), and the mixture was kept for 52 h at room temperature. The solid support was filtered and washed with pyridine. The remaining amino groups were capped by treatment with 0.1 M DMAP and 10% Ac_2O in pyridine. The resulting solid support was filtered and washed with EtOH and acetone, and dried under reduced pressure to give **Me-SU-CPG**. The loading amount of 2'-*O*-methyl-4'-thiouridine unit was estimated by DMTr cation assay to be 59.9 μmol/g.



Scheme S3; Synthesis of phosphoramidite units for Me-SRNA

*9-[2-O-Methyl-3,5-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-β-D-ribofuranosyl]adenine (**S15**)*. To a mixture of **S14** (3.0 g, 5.7 mmol) and methyl iodide (1.8 mL, 28 mmol) in dry DMF (57 mL) was added NaH (60% in mineral oil, 180 mg, 17 mmol) at –40 ˚C. After being stirred for 2 h at the same temperature, the reaction was quenched by addition of saturated aqueous NH_4Cl. The solvent was removed *in vacuo*. The residue was diluted with $CHCl_3$, which was washed with H_2O and saturated aqueous NH_4Cl, followed by brine. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by a silica gel column, eluted with MeOH in $CHCl_3$ (0–2%), to give **S15** (2.5 mg, 80%) as a white foam: FAB-LRMS *m/z* 540 (MH^+); FAB-HRMS calcd for $C_{23}H_{42}N_5O_4SSi_2$ (MH^+) 504.2496, found 504.2491; ^{1}H NMR ($CDCl_3$) δ: 8.44 (s, 1 H), 8.37 (s, 1 H), 5.87 (s 1 H), 5.64 (br s, 2 H), 4.45 (dd, 1 H, *J* = 3.4 and 10.2 Hz), 4.16 (dd, 1 H, *J* = 2.8 and 12.6 Hz), 4.06 (d, 1 H, *J* = 12.6 Hz), 3.92 (d, 1 H, *J* = 3.4 Hz), 3.74 (s, 3 H), 3.72 (dd, 1 H, *J* = 2.8 and 10.2 Hz), 1.15–0.18 (m, 28 H); ^{13}C NMR ($CDCl_3$) δ: 155.3, 153.0, 149.8, 139.9, 120.3, 87.1, 72.3, 59.5, 59.4, 57.8, 49.4, 17.4, 17.3, 17.0, 17.0, 16.9, 13.2, 13.1, 13.0, 12.3.

*N^6-Benzoyl-9-[2-O-methyl-3,5-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-thio-β-D-ribofurano syl]adenine (**S16**)*. To a solution of **S15** (320 mg, 0.60 mmol) in dry pyridine (4 mL) was added BzCl (350 μL, 3.0 mmol), and the reaction mixture was stirred for 40 min at room temperature. The reaction was quenched by addition of saturated aqueous $NaHCO_3$. The mixture was partitioned between AcOEt and H_2O. The separated organic layer was washed with H_2O and saturated aqueous $NaHCO_3$, followed by brine. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was dissolved in MeOH (6 mL), and NaOMe (28% in MeOH, 10 μL, 0.052 mmol) was added to the solution. After being stirred for 2 h at 0 ˚C, additional NaOMe (28% in MeOH, 600 μL, 3.1 mmol) was added and the reaction mixture was stirred additional 3 h at the same temperature. The reaction was quenched by addition of pyridine/AcOH (0.4 mL:0.35 mL). The mixture was concentrated *in vacuo*. The residue was diluted with $CHCl_3$, which was washed with H_2O and saturated aqueous $NaHCO_3$, followed by brine. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by a silica gel column, eluted with hexane/AcOEt (2:1), to give **S16** (246 mg, 64%) as a white foam: FAB-LRMS *m/z* 644 (MH^+); FAB-HRMS calcd for $C_{30}H_{46}N_5O_5SSi_2$ (MH^+) 644.2758, found 644.2751; ^{1}H NMR ($CDCl_3$) δ: 9.02 (br s, 1 H), 8.85 (s, 1 H), 8.62 (s, 1 H), 8.04–8.01 (m, 2 H), 7.64–7.52 (m, 3 H), 5.95 (s, 1 H), 4.46 (s, 1 H, *J* = 3.2 and 9.6 Hz), 4.17 (dd, 1 H, *J* = 3.0 and 12.7 Hz), 4.07 (d, 1 H, *J* = 12.7 Hz), 3.76 (s, 3 H), 3.74 (dd, 1 H, *J* = 3.0 and 9.6 Hz), 1.06–0.85 (m, 28 H); ^{13}C NMR ($CDCl_3$) δ: 164.6, 152.7, 151.4, 149.5, 142.2, 133.6, 132.7, 128.8, 127.8, 123.5, 87.0, 72.3, 59.7, 59.5, 57.7, 49.5, 17.4, 17.3, 17.3, 17.0, 17.0, 16.9, 16.9, 13.2, 13.1, 12.9, 12.3.

*N^6-Benzoyl-9-(2-O-methyl-4-thio-β-D-ribofuranosyl)adenine (**S17**)*. To a solution of **S16** (130 mg, 0.20 mmol) in THF (2 mL) containing AcOH (23 μL, 0.40 mmol) was added TBAF (1 M in THF, 0.40 mL, 0.40 mmol) at 0 ˚C. After being stirred for 40 min at the same temperature, the reaction mixture was concentrated *in vacuo*. The residue was purified by a silica gel column, eluted with MeOH in $CHCl_3$ (0–2%), to give **S17** (80 mg, quant.) as a white foam: FAB-LRMS *m/z* 402 (MH^+); FAB-HRMS calcd for $C_{18}H_{20}N_5O_4S$ (MH^+) 402.1245, found 402.1246; ^{1}H NMR (DMSO-d_6) δ: 11.2 (br s, 1 H), 8.83 (s, 1 H), 8.75 (s, 1 H), 8.03 (d, 2 H, *J* = 7.4 Hz), 7.64 (t, 1 H, *J* = 7.4 Hz) 7.54 (t, 2 H, *J* = 7.4 Hz), 6.12 (d, 1 H, *J* = 6.8 Hz), 5.44 (d, 1 H, *J* = 4.5 Hz), 5.26 (t, 1 H,

J = 5.7 Hz), 4.48–4.43 (m, 2 H), 3.85–3.80 (m, 1 H), 3.67–3.62 (m, 1 H), 3.38–3.35 (m, 1 H), 3.33 (s, 3 H); ^{13}C NMR (DMSO-d_6) δ: 165.6, 152.3, 151.7, 150.4, 143.3, 133.3, 132.5, 128.5, 125.7, 85.7, 70.1, 62.9, 59.3, 57.7, 53.8.

N^6*-Benzoyl-9-[-5-O-(4,4'-dimethoxytrityl)-2-O-methyl-4-thio-β-D-furanosyl]adenine (**S18**)*. In the similar manner as described for **S8**, **S17** (240 mg, 0.60 mmol) in dry pyridine (4 mL) was treated with DMTrCl (305 mg, 0.90 mmol) to give **S18** (380 mg, 90%) as a white foam: FAB-LRMS *m/z* 704 (MH^+); FAB-HRMS calcd for $C_{39}H_{38}N_5O_6S$ (MH^+) 704.2543, found 704.2538; ^{1}H NMR ($CDCl_3$) δ: 9.31 (br s, 1 H), 8.73 (s, 1 H), 8.31 (s, 1 H), 8.00 (d, 2 H, *J* = 7.4 Hz), 7.58–7.21 (m, 12 H) 6.85–6.83 (m, 4 H), 6.11 (d, 1 H, *J* = 3.4 Hz), 4.28–4.26 (m, 1 H), 4.15 (t, 1 H, *J* = 3.4 Hz), 3.78 (s, 6 H), 3.73–3.70 (m, 1 H), 3.63–3.60 (m, 1 H), 3.55 (s, 3 H), 3.48 (dd, 1 H, *J* = 6.8 and 9.6 Hz), 3.14 (br d, 1 H, *J* = 7.4 Hz); ^{13}C NMR ($CDCl_3$) δ: 164.6, 158.5, 152.5, 151.6, 144.3, 142.1, 135.4, 133.4, 132.7, 130.0, 129.9, 128.7, 128.1, 128.0, 127.8, 127.8, 126.9, 113.1, 87.4, 86.6, 73.6, 64.5, 59.8, 58.7, 55.1, 50.7.

N^6*-Benzoyl-9-[3-O-{2-cyanoethoxy(diiopropylamino)phosphino}-5-O-(4,4'-dimethoxytrityl)-2-O-methyl-4-thio-β-D-furanosyl]adenine (**S19**)*. In the similar manner as described for **S4**, **S18** (274 mg, 0.39 mmol) in dry CH_2Cl_2 (4 mL) was trated with *N,N*-diisopropylethylamine (115 μL, 0.66 mmol), DMAP (5 mg, 39 μmol) and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (113 μL, 0.50 mmol) to give **S19** (273 mg, 67%) as a white foam: FAB-LRMS *m/z* 904 (MH^+); FAB-HRMS calcd for $C_{48}H_{55}N_7O_7PS$ (MH^+) 904.3621, found 904.3625; ^{31}P NMR ($CDCl_3$) δ: 151.2 and 151.1.

CD spectra of duplexes consist of ON1s:cDNA1 and ON2s:cDNA2

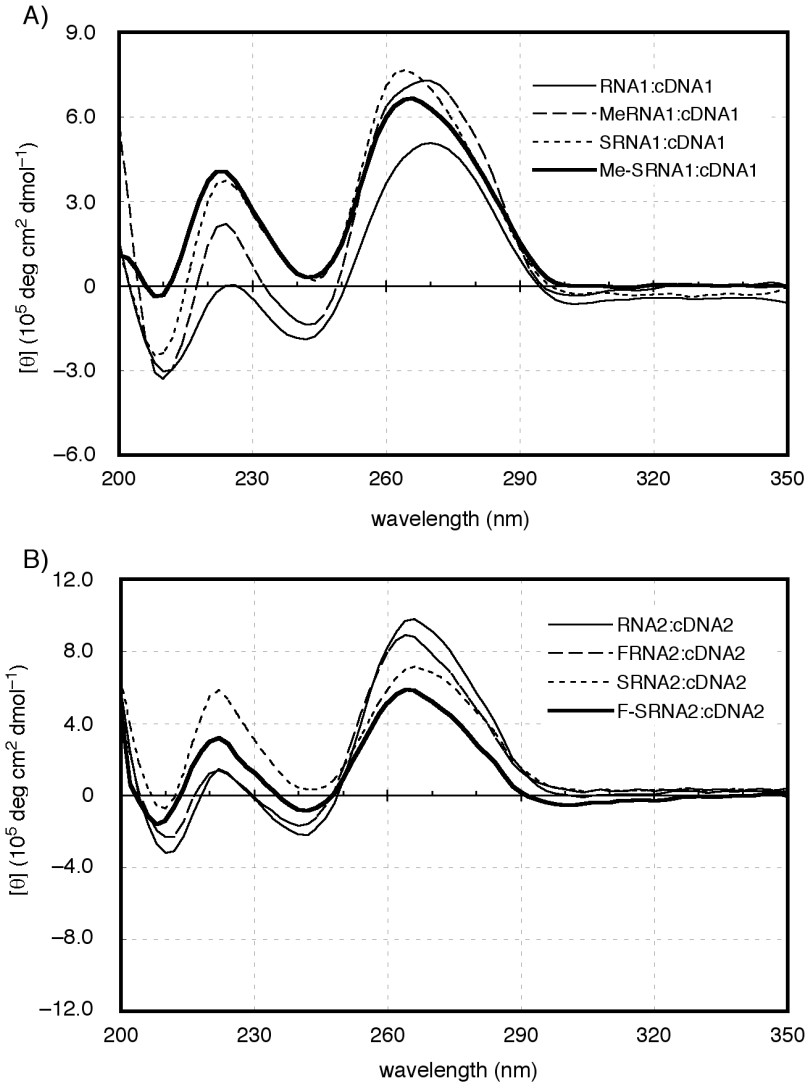


Figure S1; CD spectra of duplexes consist of ON1s:cDNA1 (A) and ON2s:cDNA2 (B).

Nuclease stability. The results of PAGE analyses of ONs in S1 nuclease was shown in Figure S2, and those of ONs in SVPD was shown in Figure S3. Figure S4 showed hydrolysis pattern of SRNA1 and SRNA2 in 50% human plasma. In order to determine the cleavage site, the sequence markers obtained by treatment of each SRNA with 50 mM aq. Na_2CO_3 (pH 9.0) were also electrophoresed.

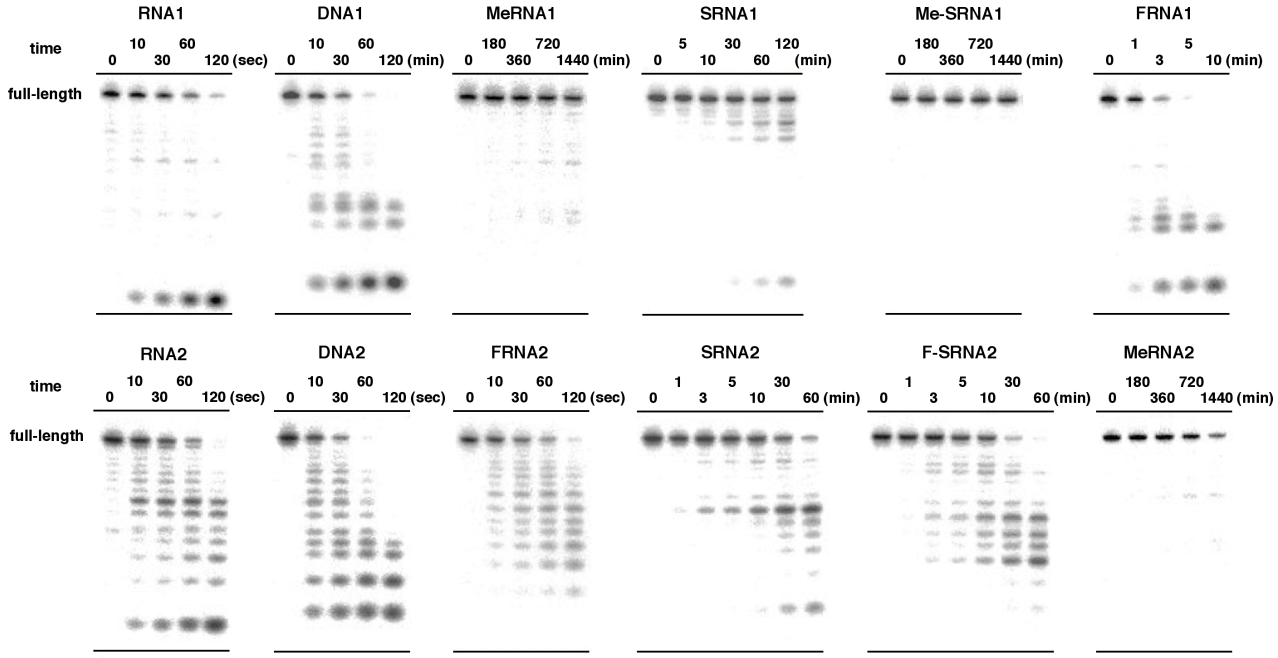


Figure S2; *Hydrolysis pattern of ONs in S1 nuclease. See Materials and Methods for experimental conditions.*

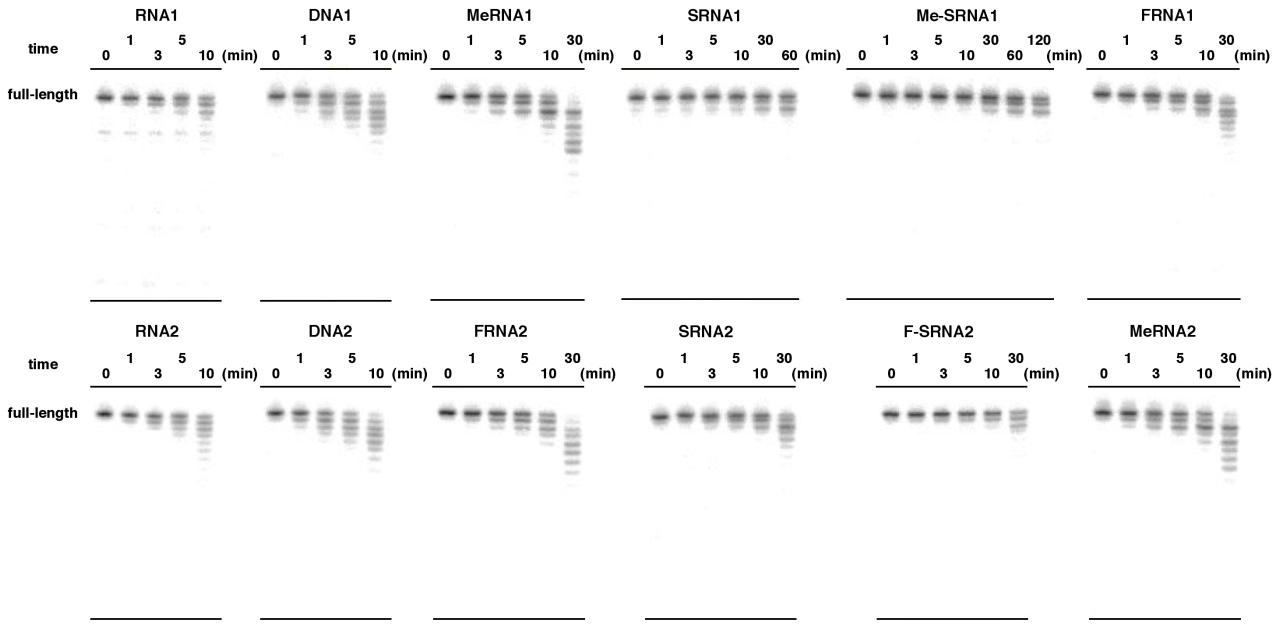


Figure S3; *Hydrolysis pattern of ONs in SVPD. See Materials and Methods for experimental conditions.*

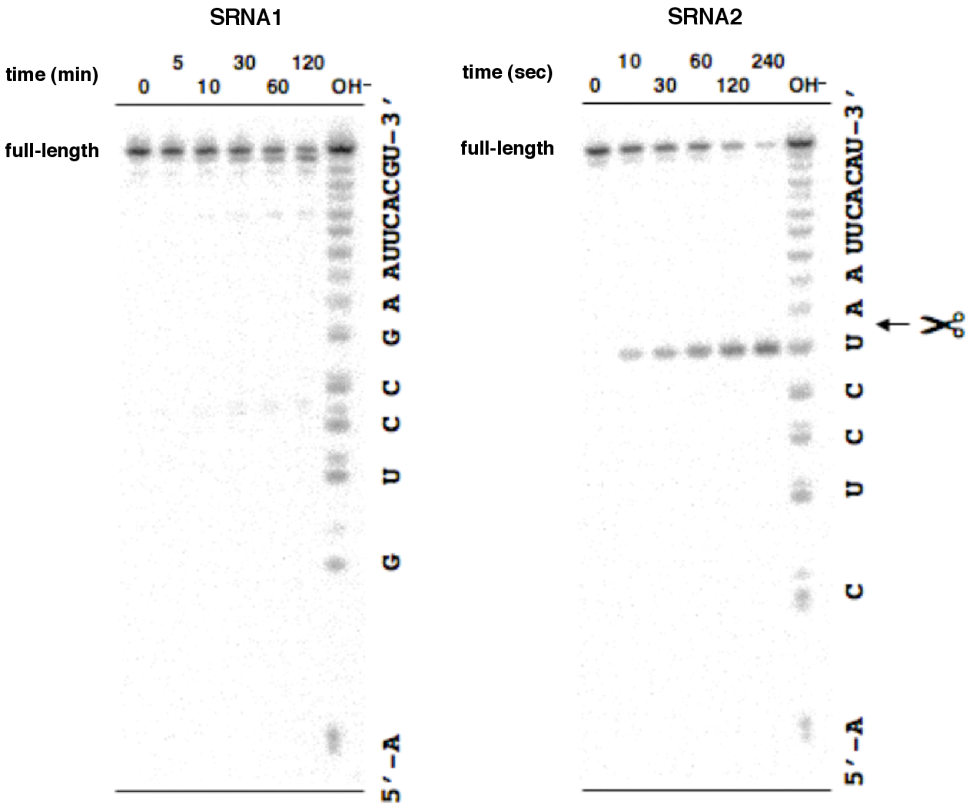


Figure S4; *Hydrolysis pattern of SRNA1 and SRNA2 in 50% human plasma. See Materials and Methods for experimental conditions.*