Supplementary Material

Synthesis and Properties of 4'-ThioDNA: Unexpected RNA-like Behavior of 4'-ThioDNA.

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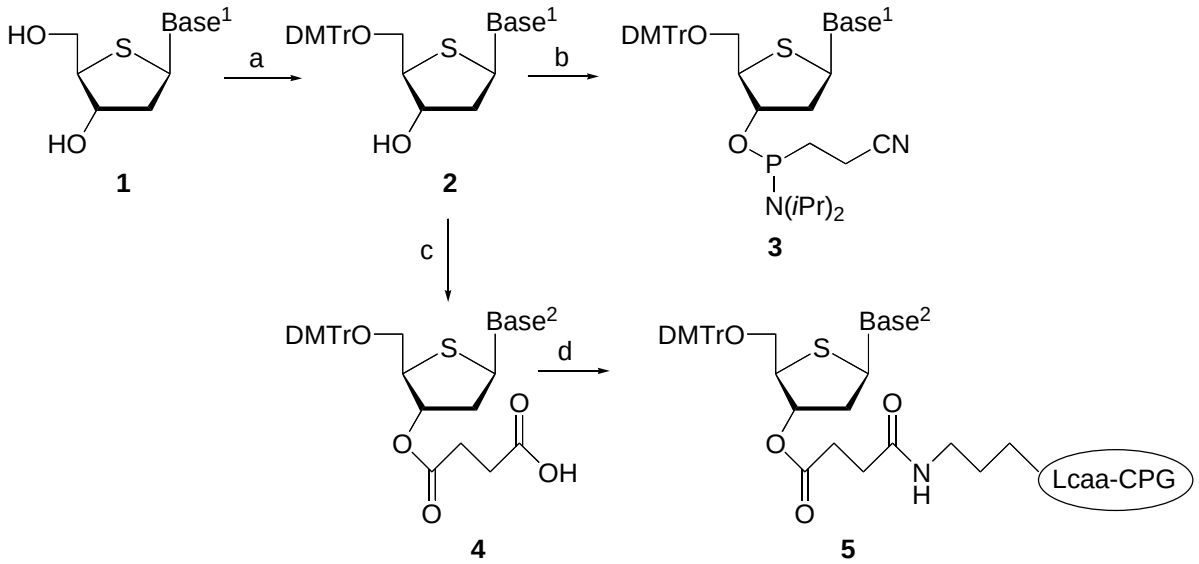
Synthesis of phosphoramidite units and CPG supports for 4'-thioDNA

Scheme-SI. Synthesis of phosphoramidite units and CPG supports for 4'-thioDNA



DMTr = di(4-methoxyphenyl)phenylmethyl

Base[1]

a; Thymin-1-yl
b; N^4-Benzoylcytosin-1-yl
c; N^6-Benzoyladenin-9-yl
d; N^2-(*N*,*N*-Dimethylaminomethylidene)guanin-9-yl

Base[2]

a; Thymin-1-yl
c; N^6-Benzoyladenin-9-yl
d; N^2-(*N*,*N*-Dimethylaminomethylidene)guanin-9-yl

Reagents and conditions: (a) DMTrCl, pyridine; (b) *N*,*N*-diisopropylchlorophosphoramidite, *N*,*N*-diisopropyl ethylamine, CH_2Cl_2, 0 °C; (c) succinic anhydride, triethylamine, 4-dimethylaminopyridine, CH_3CN; (d) 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, Lcaa-CPG, DMF then acetic anhydride, 4-dimethylaminopyridine, pyridine

General Method. Physical data were measured as follows: ^{1}H, ^{13}C NMR and ^{31}P NMR spectra were recorded at 270 or 400 MHz and 100 MHz instruments, respectively, in $CDCl_3$ or DMSO-d_6 as the solvent with tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as s (singlet), d (doublet), t (triplet), m (multiplet), or br (broad). Mass spectra were measured on JEOL JMS-D300 spectrometer. TLC was done on Merck Kieselgel F254 precoated plates. Silica gel used for column chromatography was Merck silica gel 5715.

1-{2-Deoxy-5-*O*-[di(4-methoxyphenyl)phenylmethyl]-4-thio-β-D-ribofuranosyl}thymine (2a).[4] To a solution of **1a** (250 mg, 0.97 mmol) in dry pyridine (14 mL) was added DMTrCl (390 mg, 1.2 mmol), and the mixture was stirred at room temperature for 20 h. The reaction was quenched by addition of MeOH. The reaction mixture was partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$ (three times), 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 $CHCl_3$/MeOH (99:1–49:1), to give **2a** (350 mg, 64% as a yellow form): ^{1}H NMR ($CDCl_3$) δ 8.20 (s, 1H), 7.29-7.44 (m, 10H), 6.85 (d, 4H, J = 8.8 Hz), 6.39 (dd, 1H, J = 6.8, 6.8 Hz), 4.47 (m, 1H), 3.75 (s, 6H), 3.56 (m, 2H), 3.24 (m, 1H), 2.44 (m, 1H), 2.09 (m, 1H), 1.69 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 163.2, 158.6, 150.5, 144.1, 136.1, 135.4, 135.2, 130.0, 128.0, 127.9, 127.1, 113.2, 111.5, 87.1, 76.1, 65.8, 60.7, 60.7, 55.8, 55.3, 42.8, 12.5; FAB-LRMS *m/z* 561 (MH^+); FAB-HMS calcd for $C_{31}H_{33}N_2O_6S$ (MH^+) 561.2059, found 561.2036.

1-{3-*O*-[2-Cyanoethyl(*N*,*N*-diisopropylamino)phosphino]-2-deoxy-5-*O*-[di(4-methoxyphenyl)phenylmetyl]-4-thio-β-D-ribofuranosyl}thymine (3a).[4] To a solution of **2a** (220 mg, 0.39 mmol) in dry CH_2Cl_2 (4.6 mL) were added *N*,*N'*-diisopropylethylamine (0.11 mL, 0.66 mmol), 4-dimethylaminopyridine (5 mg, 0.039 mmol) and 2-cyanoethyl *N*, *N* –diisopropylchloro-phosphoramidite (0.11 mL, 0.50 mmol), and the mixture was stirred at 0 °C for 1 h. After 1 h, 2-cyanoethyl *N*, *N* -diisopropylchlorophosphoramidite (0.048 mL, 0.23 mmol) was added to the mixture and stirred at 0 °C for 2 h. The reaction was quenched by addition of ice. The reaction mixture was partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$ (three times), 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 (2:1–1:1), to give **3a** (200 mg, 67% as a white form): ^{31}P NMR ($CDCl_3$) δ 149.0, 148.8; FAB-LRMS *m/z* 761 (MH^+); FAB-HRMS calcd for $C_{40}H_{50}N_4O_7PS$ (MH^+) 761.3138, found 761.3143.

1-{2-Deoxy-5-*O*-[di(4-methoxyphenyl)phenylmetyl]-3-*O*-succinyl-4-thio-β-D-ribofuranosyl}thymine (4a). To a solution of **2a** (120 mg, 0.21 mmol) in dry acetonitrile (3 mL) were added triethylamine (0.088 mL, 0.63 mmol), 4-dimethylaminopyridine (13 mg, 0.11 mmol) and succinic anhydride (63 mg, 0.63 mmol), and the mixture was stirred at room temperature for 11 h. The reaction was quenched by addition of water. The reaction mixture was partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$ (three times), 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 $CHCl_3$/MeOH (49:1–9:1), to give **4a** (91 mg, 66% as a white form): ^1H NMR ($CDCl_3$) δ 9.48 (s, 1H), 7.24-7.48 (m, 10H), 6.83-6.85 (m, 4H), 6.49 (dd, 1H, *J* = 9.6, 6.2 Hz), 5.51 (s, 1H), 3.79 (s, 6H), 3.64 (m, 1H), 3.50 (dd, 1H, *J* = 5.6, 9.3 Hz), 3.25 (dd, 1H, *J* = 5.8, 9.3 Hz), 2.64-2.71 (m, 4H), 2.52 (dd, 1H, *J* = 6.2, 11.7 Hz), 2.13 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 175.0, 171.2, 163.7, 158.7, 151.1, 144.0, 136.2, 135.2, 135.1, 130.1,128.2, 127.9, 127.1, 113.2, 113.1, 112.2, 87.2, 78.1, 65.3, 61.0, 55.3, 54.0, 40.0, 29.6, 29.1, 12.2; FAB-LRMS *m/z* 661 (MH^+); FAB-HRMS calcd for $C_{35}H_{37}N_2O_9S$ (MH^+) 661.2220, found 661.2217.

1-{2-Deoxy-5-*O*-[di(4-methoxyphenyl)phenylmetyl]-3-*O*-succinyl-4-thio-β-D-ribofuranosyl}thymine unit loaded controlled pore glass support (5a). To a solution of **4a** (91 mg, 0.14 mmol) in DMF (3.5 mL) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (26 mg, 0.14 mmol) and Lcaa-CPG (390 mg, 34 μmol, 89.2 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 4-dimethylaminopyridine and 10% acetic anhydride in pyridine. The resulting solid support was

filtered and washed with MeOH and acetone, and dried under reduced pressure to give **5a**. The loading amount of **5a** was estimated by a DMTr cation assay to be 38.8 μmol/g.

4-*N*-Benzoyl-1-{2-deoxy-5-*O*-[di(4-methoxyphenyl)phenylmethyl]-4-thio-β-D-ribofura nosyl}cytosine (2b).[5] To a solution of **1b** (180 mg, 0.52 mmol) in dry pyridine (7.4 mL) was added DMTrCl (210 mg, 0.62 mmol), and the mixture was stirred at room temperature for 24 h. The reaction was quenched by addition of MeOH. The reaction mixture was partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$ (three times), 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 $CHCl_3$/MeOH (99:1–20:1), to give **2b** (290 mg, 85% as a yellow form): ^{1}H NMR ($CDCl_3$) δ 8.66 (s, 1H), 8.38 (d, 1H, J = 7.5 Hz), 7.88 (m, 2H), 7.26-7.63(m, 14H), 6.88 (m, 4H), 6.35 (dd, 1H, J = 4.3, 7.2 Hz), 4.36 (m, 1H), 3.82 (s, 6H), 3.58-3.52 (m, 2H), 3.41 (m, 1H), 2.68 (br.s, 1H), 2.61 (ddd, 1H, J = 7.2, 14.2, 7.7 Hz), 2.23 (ddd, 1H, J = 4.3, 14.2, 4.5 Hz); ^{13}C NMR ($CDCl_3$) δ 161.8, 158.7, 146.0, 144.1, 135.4, 135.3, 133.1, 130.0, 129.0, 128.1, 128.0, 127.8, 127.5, 127.1, 113.3, 113.1, 87.2, 74.9, 64.4, 62.3, 55.3, 54.9, 43.9; FAB-LRMS *m/z* 650 (MH^+); FAB-HRMS calcd for $C_{37}H_{35}N_3O_6S$ (MH^+) 650.2325, found 650.2333.

4-*N*-Benzoyl-1-{3-*O*-[2-cyanoethyl(*N*,*N*-diisopropylamino)phosphino]-2-deoxy-5-*O*-[d i(4-methoxyphenyl)phenylmetyl]-4-thio-β-D-ribofuranosyl}cytosine (3b).[5] To a solution of **2b** (270 mg, 0.42 mmol) in dry CH_2Cl_2 (5.2 mL) were added *N*,*N'*-diisopropylethylamine (0.15 mL, 0.84 mmol) and 2-cyanoethyl *N*, *N* –diisopropylchlorophosphoramidite (0.14 mL, 0.63 mmol), and the mixture was stirred at 0 °C for 1 h. The reaction was quenched by addition of ice. The reaction mixture was partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$ (three times), 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 (2:1–1:1), to give **3b** (210 mg, 60% as a white form): ^{31}P NMR ($CDCl_3$) δ 149.6, 149.2; FAB-LRMS *m/z* 849 (MH^+); FAB-HRMS calcd for $C_{46}H_{52}N_5O_7PS$ (MH^+) 850.3404, found 850.3397.

6-*N*-Benzoyl-9-{2-deoxy-5-*O*-[di(4-methoxyphenyl)phenylmethyl]-4-thio-β-D-ribofura

nosyl}adenine (2c). To a solution of **1c** (300 mg, 0.79 mmol) in dry pyridine (11 mL) was added DMTrCl (300 mg, 0.87 mmol), and the mixture was stirred at room temperature for 21 h. The reaction was quenched by addition of MeOH. The reaction mixture was partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$ (three times), 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 $CHCl_3$/MeOH (99:1–49:1), to give **2c** (400 mg, 75% as a white form): ^{1}H NMR ($CDCl_3$) δ 8.96 (s, 1H), 8.73 (s, 1H), 8.19 (s, 1H), 8.01 (m, 2H), 7.53 (m, 2H), 7.62 (m, 1H), 7.43 (m, 2H), 7.26-7.34 (m, 7H), 6.85 (m, 4H), 6.29 (dd, 1H, *J* = 5.5, 5.5 Hz), 4.56 (br s, 1H), 3.80 (s, 6H), 3.72 (m, 1H), 3.57-3.65 (m, 2H), 3.46 (m, 1H), 2.58-2.63 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 158.4, 152.2, 151.5, 149.4, 149.3, 144.2, 141.8, 136.0, 135.4, 133.5, 132.6, 129.8, 128.6, 128.0, 127.8, 126.9, 123.7, 123.4, 113.1, 86.8, 75.5, 65.7, 58.7, 55.4, 55.2, 43.2; FAB-LRMS *m/z* 674 (MH^+); FAB-HRMS calcd for $C_{38}H_{35}N_5O_5S$ (MH^+) 674.2438, found 674.2444.

6-*N*-Benzoyl-9-{3-*O*-[2-cyanoethyl(*N*,*N*-diisopropylamino)phosphino]-2-deoxy-5-*O*-[di(4-methoxyphenyl)phenylmetyl]-4-thio-β-D-ribofuranosyl}adenine (3c). To a solution of **2c** (390 mg, 0.58 mmol) in dry CH_2Cl_2 (7.3 mL) were added *N*,*N'*-diisopropylethylamine (0.20 mL, 1.2 mmol) and 2-cyanoethyl *N*, *N* –diisopropylchlorophosphoramidite (0.20 mL, 0.88 mmol), and the mixture was stirred at 0 °C for 50 min. After 50 min, 2-cyanoethyl *N*, *N* -diisopropylchlorophosphoramidite (0.040 mL, 0.23 mmol) was added to the mixture and stirred at 0 °C for 40 min. The reaction was quenched by addition of ice. The reaction mixture was partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$ (three times), 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 (1:1–1:4), to give **3c** (410 mg, 83% as a white form): ^{31}P NMR ($CDCl_3$) δ 149.2; FAB-LRMS *m/z* 874 (MH^+); FAB-HRMS calcd for $C_{47}H_{52}N_7O_6PS$ (MH^+) 874.3516, found 874.3517.

6-*N*-Benzoyl-9-{2-deoxy-5-*O*-[di(4-methoxyphenyl)phenylmetyl]-3-*O*-succinyl-4-thio-β-D-ribofuranosyl}adenine (4c). To a solution of **2c** (135 mg, 0.20 mmol) in dry acetonitrile (2.9

mL) were added triethylamine (0.042 mL, 0.30 mmol), 4-dimethylaminopyridine (12 mg, 0.10 mmol) and succinic anhydride (30 mg, 0.30 mmol), and the mixture was stirred at room temperature for 11 h. The reaction was quenched by addition of water. The reaction mixture was partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$ (three times), 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 $CHCl_3$/MeOH (49:1–24:1), to give **4c** (78 mg, 50% as a white form): ^{1}H NMR ($CDCl_3$) δ 8.72 (s, 1H), 8.25 (s, 1H), 8.04 (d, 2H, *J* = 6.8 Hz), 7.60 (t, 1H, *J* = 9.6 Hz), 7.52 (dd, 2H, *J* = 7.6, 7.6 Hz), 7.45 (d, 2H, *J* = 7.6 Hz), 7.21-7.35 (m, 7H), 6.84 (d, 4H, *J* = 8.8 Hz), 6.42 (dd, 1H, *J* = 6.4, 9.2 Hz), 5.63 (m, 1H), 3.80 (s, 6H), 3.75 (dd, 1H, *J* = 6.0, 8.0 Hz), 3.47 (dd, 1H, *J* = 6.0, 10.0 Hz), 3.40 (dd, 1H, *J* = 8.0, 10.0 Hz), 2.68-2.74 (m, 5H), 2.44 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 171.0, 158.5, 149.9, 144.2, 141.8, 135.5, 135.4, 133.3, 130.0, 128.7, 128.1, 127.9, 127.0, 123.5, 113.3, 86.8, 65.1, 59.3, 55.3, 54.0, 40.8, 29.8, 29.7, 29.3; FAB-LRMS *m/z* 774 (MH^+); FAB-HRMS calcd for $C_{42}H_{40}N_5O_8S$ (MH^+) 774.2598, found 774.2579.

6-*N*-Benzoyl-9-{2-deoxy-5-*O*-[di(4-methoxyphenyl)phenylmetyl]-3-*O*-succinyl-4-thio-β-D-ribofuranosyl}adenine unit loaded controlled pore glass support (5c). To a solution of **4c** (140 mg, 0.17 mmol) in DMF (4.4 mL) was added 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (33 mg, 0.17 mmol) and Lcaa-CPG (490 mg, 44 μmol, 89.2 μmol/g), and the mixture was kept for 40 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 4-dimethylaminopyridine and 10% acetic anhydride in pyridine. The resulting solid support was filtered and washed with MeOH and acetone, and dried under reduced pressure to give **5c**. The loading amount of **5c** was estimated by a DMTr cation assay to be 42.2 μmol/g.

2-*N*-(*N*,*N*-Dimethylaminomethylidene)-9-{2-deoxy-5-*O*-[di(4-methoxyphenyl)phenylmethyl]-4-thio-β-D-ribofuranosyl}guanine (2d). To a solution of **1d** (880 mg, 2.6 mmol) in dry pyridine (37 mL) was added DMTrCl (1.2 g, 3.7 mmol), and the mixture was stirred at room temperature for 18 h. The reaction was quenched by addition of MeOH. The reaction mixture was partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$ (three times), 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 $CHCl_3$/MeOH (99:1–23:1), to give **2d** (1.3 g, 80% as a white form): ^{1}H NMR ($CDCl_3$) δ 8.58 (s, 1H), 8.57 (s, 1H), 7.70 (s, 1H), 7.42 (m, 2H), 7.25-7.34 (m, 13 H), 6.84 (m, 4H), 6.11 (dd, 1H, *J* = 5.8, 6.8 Hz), 4.53 (m, 1H), 3.80 (s, 6H), 3.53-3.58 (m, 2H), 3.55 (m, 1H), 3.18 (s, 3H), 3.09 (s, 3H), 2.72 (m, 1H), 2.52 (ddd, 1H, *J* = 6.8, 13.6, 6.8 Hz), 2.30 (ddd, 1H, *J* = 5.8, 13.6, 4.8 Hz); ^{13}C NMR ($CDCl_3$) δ 158.6, 157.9, 157.5, 156.4, 150.0, 144.2, 136.4, 135.4, 135.3, 129.9, 128.0, 127.1, 120.4, 113.3, 87.0, 76.2, 66.2, 57.1, 55.3, 54.7, 43.7, 41.4, 35.2; FAB-LRMS *m/z* 561 (MH^+); FAB-HRMS calcd for $C_{31}H_{33}N_2O_6S$ (MH^+) 561.2059, found 561.2036.; FAB-LRMS *m/z* 641 (MH^+); FAB-HRMS calcd for $C_{34}H_{37}N_6O_5S$ (MH^+) 641.2546, found 641.2537.

2-*N*-(*N*,*N*-Dimethylaminomethylidene)-9-{3-*O*-[2-cyanoethyl(*N*,*N*-diisopropylamino) phosphino]-2-deoxy-5-*O*-[di(4-methoxyphenyl)phenylmetyl]-4-thio-β-D-ribofuranosyl}guanine (3d). To a solution of **2d** (340 mg, 0.52 mmol) in dry CH_2Cl_2 (6.5 mL) were added *N*,*N'*-diisopropylethylamine (0.18 mL, 1.0 mmol) and 2-cyanoethyl *N*, *N* –diisopropylchlorophosphoramidite (0.18 mL, 0.79 mmol), and the mixture was stirred at 0 °C for 3 h. After 3 h, 2-cyanoethyl *N*, *N* -diisopropylchlorophosphoramidite (0.035 mL, 0.16 mmol) was added to the mixture and stirred at 0 °C for 30 min. The reaction was quenched by addition of ice. The reaction mixture was partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$ (three times), 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 acetone/AcOEt (1:2–1:4), to give **3d** (320 mg, 74% as a white form): ^{31}P NMR ($CDCl_3$) δ 149.2; FAB-LRMS *m/z* 871 (MH^+); FAB-HRMS calcd for $C_{43}H_{53}N_8O_6PS$ (MH^+) 841.3624, found 841.3626.

2-*N*-(*N*,*N*-Dimethylaminomethylidene)-9-{2-deoxy-5-*O*-[di(4-methoxyphenyl)phenyl metyl]-3-*O*-succinyl-4-thio-β-D-ribofuranosyl}guanine (4d). To a solution of **2d** (128 mg, 0.20 mmol) in dry acetonitrile (2.9 mL) were added triethylamine (0.042 mL, 0.30 mmol), 4-dimethylaminopyridine (12 mg, 0.10 mmol) and succinic anhydride (30 mg, 0.30 mmol), and the

mixture was stirred at room temperature for 11 h. The reaction was quenched by addition of water. The reaction mixture was partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$ (three times), 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 $CHCl_3$/MeOH (9:1–4:1), to give **4d** (110 mg, 74% as a yellow form): ^{1}H NMR ($CDCl_3$) δ 8.33 (s, 1H), 8.09 (s, 1H), 7.59 (s, 1H), 7.35 (d, 2H, *J* = 8.0 Hz), 7.11-7.25 (m, 11H), 6.74 (d, 4H, *J* = 8.4 Hz), 6.04 (d, 1H, *J* = 7.6, 7.6 Hz), 5.51 (m, 1H), 3.68 (s, 6H), 3.60 (m, 1H), 3.32 (dd, 1H, *J* = 6.0, 8.4 Hz), 3.17 (dd, 1H, *J* = 7.2, 8.4 Hz), 3.00 (s, 3H), 2.79 (s, 3H), 2.53-2.64 (m, 5H), 2.11 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 176.2, 171.4, 158.6, 158.4, 156.5, 150.8, 144.2, 136.3, 135.4, 135.3, 129.9, 127.9, 127.8, 126.8, 118.9, 113.1, 106.1, 86.6, 65.2, 58.3, 55.2, 53.6, 51.6, 41.5, 40.2, 35.1, 30.1, 29.5; FAB-LRMS *m/z* 741 (MH^+); FAB-HRMS calcd for $C_{38}H_{41}N_6O_8S$ (MH^+) 741.2700, found 741.2726.

2-*N*-(*N*,*N*-Dimethylaminomethylidene)-9-{2-deoxy-5-*O*-[di(4-methoxyphenyl)phenyl metyl]-3-*O*-succinyl-4-thio-β-D-ribofuranosyl}guanine unit loaded controlled pore glass support (5d). To a solution of **4d** (81 mg, 0.11 mmol) in DMF (2.8 mL) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (21 mg, 0.11 mmol) and Lcaa-CPG (310 mg, 28 μmol, 89.2 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 4-dimethylaminopyridine and 10% acetic anhydride in pyridine. The resulting solid support was filtered and washed with MeOH and acetone, and dried under reduced pressure to give **5d**. The loading amount of **5d** was estimated by DMTr cation assay to be 40.7 μmol/g.

Differential scanning calorimetry (DSC) measurements. DSC measurements were performed on a VP-DSC Microcalorimeter (MicroCal, LLC). The solution containing an appropriate oligonucleotide and a complementary sequence (25 μM each) in a buffer of 10 mM sodium cacodylate (pH 7.0) containing 10 mM NaCl was prepared and scanned from 1 to 110 °C at a scan rate 0.5 K/min. The apparent molar heat capacity versus temperature profiles were obtained by

subtracting buffer versus buffer curves from the sample versus buffer curves. The data were normalized with regard to the concentration and sample volume. The excess heat capacity function, ΔC_P, was obtained after baseline subtraction, assuming that the baseline is given by the linear temperature dependence of the native state heat capacity. The process enthalpies, $\Delta H°$, were obtained by integrating the area under the heat capacity versus temperature curves. T_m is the temperature corresponding to the maximum of each DSC peak. The process entropies, $\Delta S°$, were determined by integrating the curve obtained and dividing the heat capacity curve by the absolute temperature, i.e. $\Delta S° = \int(\Delta C_P/T)\Delta T$. The free energies, $\Delta G°$ (37 °C), were determined at T = 310.15K by $\Delta G°$ (37 °C) = $\Delta H° - T\Delta S°$.

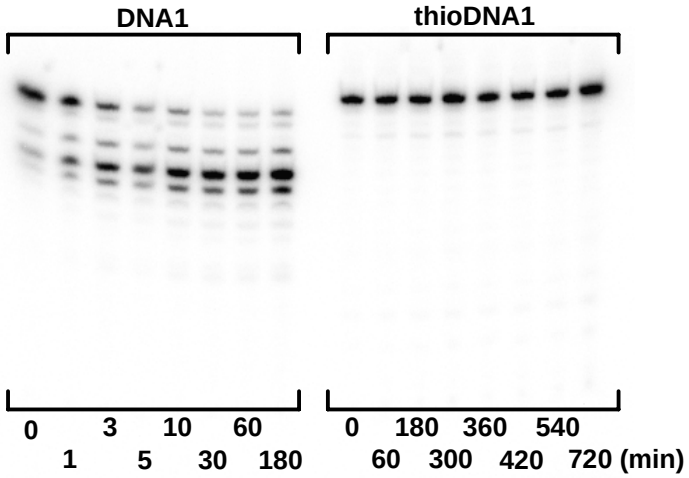
Thermodynamic parameters for duplexes formation determined from DSC measurements[a]

Duplex	$\Delta H°$ (kcal mol^{-1})	$\Delta S°$ (cal mol^{-1} K^{-1})	$\Delta G°$ (37 °C)(kcal mol^{-1})
DNA1:DNA2	–57.1 ± 2.8	–171.3 ± 7.0	–4.95 ± 0.3
thioDNA1:thioDNA2	–108.8 ± 0.6	–338.6 ± 11.1	–5.79 ± 0.7
DNA1:RNA2	–66.2 ± 2.3	–197.6 ± 7.4	–4.90 ± 0.5
thioDNA1:DNA2	–60.2 ± 3.2	–190.7 ± 6.6	–2.58 ± 1.0
thioDNA1:RNA2	–114.9 ± 8.3	–333.0 ± 4.6	–6.44 ± 0.3
DNA4:DNA5	–71.3 ± 1.7	–230.4 ± 1.9	–0.94 ± 1.6
thioDNA4:DNA5	–76.6 ± 0.8	–254.5 ± 3.7	2.31 ± 1.4
thioDNA4:thioDNA5	–58.5 ± 0.2	–201.2 ± 0.2	3.88 ± 0.5

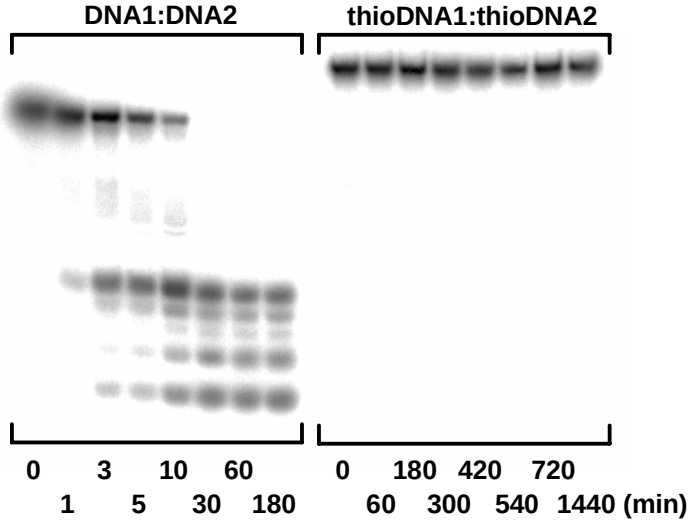
[a]Errors reflect standard deviation from three independent experiments.

Enzymatic stability of single- and double-stranded DNA or 4'-thioDNA

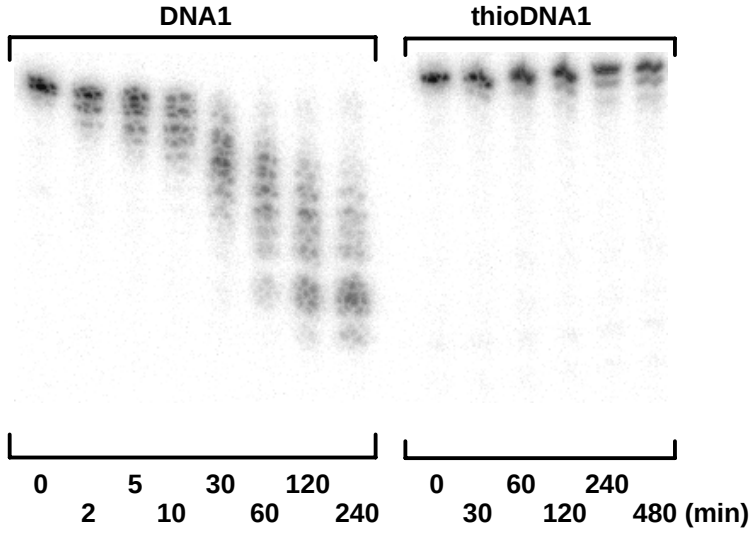
Stability of single-stranded DNA or 4'-thioDNA for DNase I



Stability of double-stranded DNA or 4’-thioDNA for DNase I



Stability of single-stranded DNA or 4’-thioDNA for SVPD



Stability of single-stranded DNA or 4’-thioDNA for 90% human serum