Electronic Supplementary Information for

A firmly hybridizable, DNA-like architecture with DAD/ADA- and ADD/DAA-type nonnatural base pairs as an extracellular genetic candidate

Wataru Shirato, Junya Chiba* and Masahiko Inouye*

Graduate School of Pharmaceutical Sciences, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan

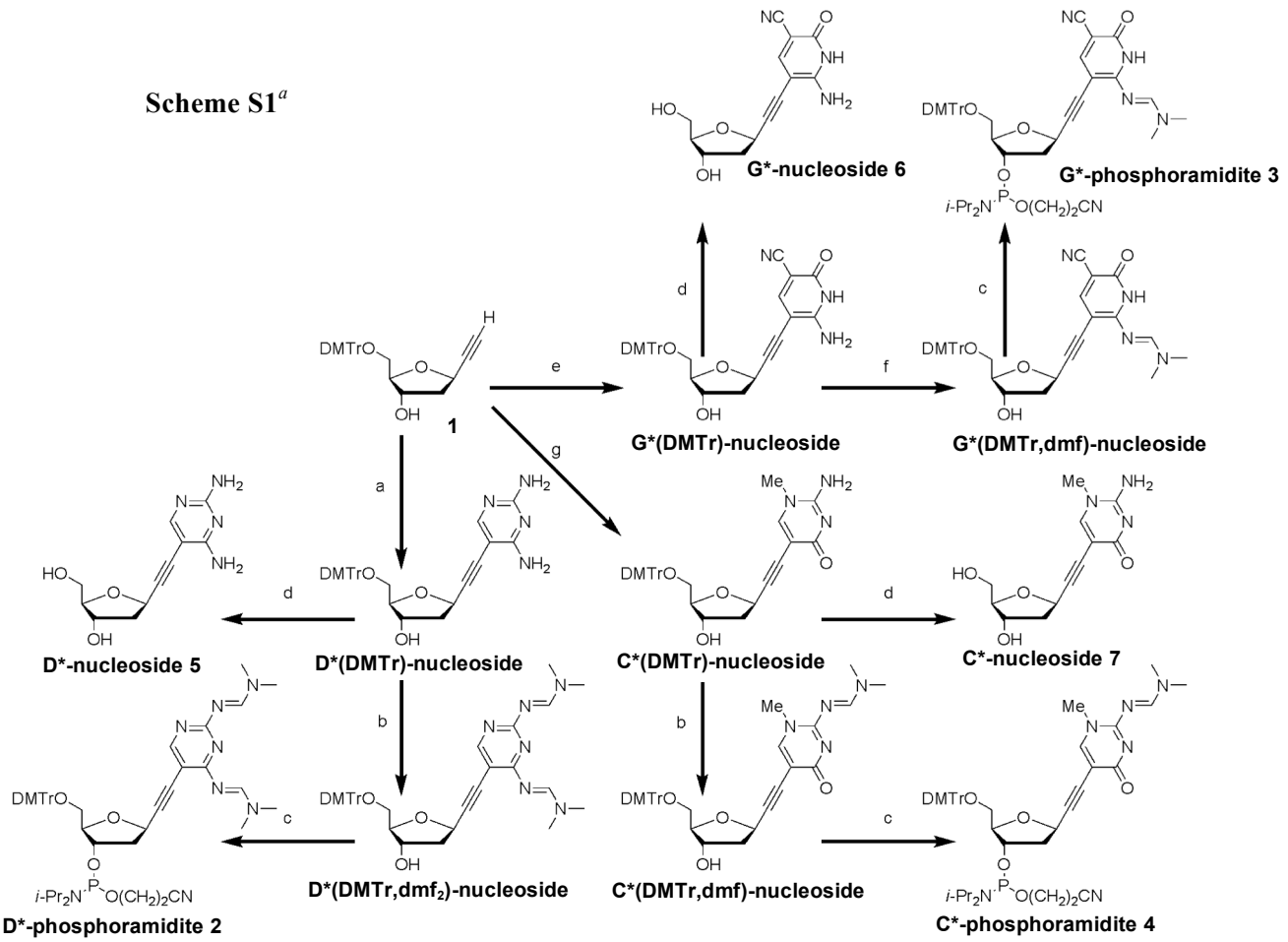
Contents

1. Synthetic Procedures.

General. ^{1}H and ^{13}C NMR spectra were obtained at 300 and 75 MHz, respectively, on a Varian Gemini 300 spectrometer. IR spectra were measured on a JASCO-FT/IR-460 plus spectrometer. UV-vis spectra were obtained on a JASCO V-560 UV/VIS spectrophotometer. ESI-HRMS analyses were carried out on a JEOL JMS-T100LC mass spectrometer. Melting points were determined with Yanako MP-500D and not corrected.

Materials. Halogenated nonnatural bases, 2,6-diamino-5-iodopyrimidine (D*-I)[s1] was known but synthesized by newly developed procedures. The following compounds, (5-iodo-1-methylisocytosine) (C*-I),[s2] (2*R*,3*S*,5*R*)-2-(4,4'-dimethoxytrityloxymethyl)-5-ethynyl-3-hydroxytetrahydrofuran (**1**)[s3] and T* phosphoramidite[s4] were prepared according to literature procedures. Other materials were all commercially available. The corresponding phosphoramidites (**2**, **3** and **4**) and the nucleosides (**5**, **6** and **7**) of the nonnatural bases were prepared according to the following Scheme S1.

Scheme S1[a]



[a](a) 1) D*-I, $PdCl_2(PPh_3)_2$, CuI, DMF, $(Me_3Si)_2NH$, 2) NH_3aq, MeOH; (b) $Me_2NCH(OMe)_2$, MeOH; (c) *i*-$Pr_2NP(Cl)O(CH_2)_2CN$, *i*-Pr_2NEt, CH_2Cl_2; (d) CCl_3COOH, CH_2Cl_2; (e) 1) G*-I, $PdCl_2(PPh_3)_2$, CuI, DMF, $(Me_3Si)_2NH$, 2) pyridine, H_2O, NH_3aq; (f) $Me_2NCH(OMe)_2$, DMF; (g) C*-I, $PdCl_2(PPh_3)_2$, CuI, DMF, $(Me_3Si)_2NH$, 2) pyridine, H_2O, NH_3aq;.

D*-I: 2, 6-Diamino-5-iodopyrimidine. This compound was synthesized by a procedure previously reported[s1] with several modifications as below. 2,6-Diaminopyrimidine (25 g, 0.227 mol) was suspended into $AcOH/H_2O/H_2SO_4$ (300 + 75 + 10 mL). To the reaction mixture were added I_2 (23 g, 0.18 mol) in ethanol (100 mL) and $NaIO_4$ (9.71g, 0.045 mmol) in H_2O (50 mL). The mixture was stirred at 60˚C for 1 h and then poured into H_2O (500 mL). To the aqueous solution was added $Na_2S_2O_3$, and the solution was adjusted to pH 8.0 with NaOH. The resulting precipitate was filtered and washed with H_2O. Recrystallization from (DMF/ether) gave pure **D*-I** (39.1 g, 73%) as a white powder. This product was identical to the title compound previously reported.[s1]

D*(DMTr)-nucleoside:

(2*R*,3*S*,5*R*)-5-(2,4-Diaminopyrimidin-5-ylethynyl)-2-(4,4'-dimethoxytrityloxymethyl)-3-hydroxytetrahydrofuran. A mixture of **D*-I** (435 mg, 1.84 mmol), **1**[s3] (680 mg, 1.53 mmol), $PdCl_2(PPh_3)_2$ (32.1 mg, 0.046 mmol), and CuI (5.9 mg, 0.031 mmol) in $(Me_3Si)_2NH$/DMF (6.1 + 6.1 mL) was stirred under an argon atmosphere at 75 ˚C for 2 h. The reaction mixture was diluted with EtOAc/THF (1 : 1) and washed with ice-cold 10% NaCl, ice-cold 1% citric acid, 5% Na_2CO_3, and saturated NaCl aqueous solutions subsequently. The organic phase was dried over Na_2SO_4 and evaporated. The residue was diluted with MeOH (40 mL) and conc. NH_4OH (100 mL). After stirring at room temperature for overnight, the diluted mixture was additionally stirred at 50˚C for 3 h. After removal of the MeOH, the residue was diluted with EtOAc and wash with saturated NaCl aqueous solution three times. The organic phase was dried over Na_2SO_4 and evaporated. The residue was chromatographed (SiO_2; eluent, $CHCl_3$/MeOH = from 50 : 1 to 50 : 2) to give **D*(DMTr)-nucleoside** (780 mg, 92%) as a colorless foam. Mp 101–103 ˚C; IR (KBr) 3468, 3328, 3197, 2931, 2220, 1608, 1541, 1175, 1033, 828 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.90 (s, 1 H), 7.45-7.43 (m, 2 H), 7.35-7.16 (m, 7 H), 6.82 (d, *J* = 9.0 Hz, 4 H), 5.21 (s, 2 H), 5.10 (s, 2 H), 5.07-5.02 (m, 1 H), 4.43-4.41 (m, 1 H), 4.00-3.98 (m, 1 H), 3.74 (s, 6 H), 3.23-3.20 (m, 2 H), 2.35-2.24 ppm (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 163.4, 161.3, 159.4, 158.2, 144.5, 135.7, 135.6, 129.8, 127.9, 127.6, 126.6, 112.9, 94.6, 91.6, 86.2, 85.9, 78.3, 74.1, 68.2, 64.4, 55.1, 42.4 ppm; HRMS calcd for MH^+, $C_{32}H_{33}N_4O_5$: 553.2451; found 553.2459.

D*(DMTr,dmf$_2$)-nucleoside:

(2*R*,3*S*,5*R*)-2-(4,4'-Dimethoxytrityloxymethyl)-5-[2,4-bis(dimethylamidino)pyrimidine-5-ylethynyl]-3-hydroxytetrahydrofuran. A mixture of **D*(DMTr)-nucleoside** (193 mg, 0.35 mmol) and $Me_2NCH(OMe)_2$ (0.46 mL, 3.50 mmol) in MeOH (5 mL) was stirred at room temperature for 16 h and concentrated. The residue was dried under reduced pressure and used in the next step without further purification.

D*-phosphoramidite 2. To a dry CH_2Cl_2 (2.3 mL) solution of **D*(DMTr,dmf$_2$)-nucleoside** (230 mg, 0.35 mmol) were added *i*-$Pr_2NP(Cl)O(CH_2)_2CN$ (0.18 mL, 0.7 mmol) and *i*-Pr_2NEt (0.25 mL, 1.22 mmol) at room temperature under argon atmosphere. The reaction mixture was stirred for 90 min at the same temperature, and to the reaction mixture was added MeOH. After removal of the solvent, the residue was diluted with EtOAc. The organic solution was washed with 10% Na_2CO_3 and brine subsequently, dried over $MgSO_4$, and concentrated. The residue was chromatographed (SiO_2; eluent EtOAc/MeOH = from 1 : 0 ~ 50 : 1) to give a diastereomer mixture of **2** (130 mg, 43% over two steps) as a colorless foam. Further purificiation was perfomed by reverse phase HPLC (eluent, MeOH). Mp 79–84 ˚C; IR (KBr) 2965, 2930, 2871, 2837, 2251, 2224, 1626, 1558, 1509, 2378, 1250, 1177, 1105, 977, 754 cm^{-1}; ^1H

NMR ($CDCl_3$) δ 8.77 (s, 1 H), 8.64 (s, 1 H), 8.27 (s, 1 H), 7.49-7.45 (m, 2 H), 7.38-7.16 (m, 7 H), 6.80-6.77 (m, 4 H), 5.09-5.04 (m, 1 H), 4.58-4.43 (m, 1 H), 4.18-4.13 (m, 1 H), 3.79-3.52 (m, 4 H), 3.75 (s, 6 H), 3.21-3.13 (m, 2 H), 3.15 (s, 3 H), 3.12 (s, 3 H), 3.05 (s, 3 H), 2.97 (s, 3 H), 2.59 (t, J = 6.6 Hz, 1 H), 2.45 (t, J =6.6 Hz, 1 H), 2.41-2.31 (m, 2 H), 1.19-1.08 ppm (m, 12 H); ^{13}C NMR ($CDCl_3$) δ 168.4, 164.8, 161.0, 158.1, 156.7, 144.7, 135.9, 135.8, 130.0, 128.1, 127.6, 126.5, 117.3, 112.9, 104.2, 92.9, 86.0, 85.5, 85.3, 81.0, 68.7, 64.2, 58.4, 58.3, 58.2, 58.1, 55.1, 53.4, 43.2, 43.1, 41.2, 41.1, 40.8, 35.1, 34.7, 24.7, 24.6, 24.5, 24.4, 20.3, 20.2 ppm; HRMS calcd for MH^+, $C_{47}H_{60}N_8O_6P$: 863.4373; found 863.4378.

D*-nucleoside 5. A CH_2Cl_2 (15 mL) solution of **D*(DMTr)-nucleoside** (200 mg, 0.32 mmol) and trichloroacetic acid (294 mg, 1.8 mmol) was stirred for 1 h at room temperature. The reaction mixture was quenched by the addition of Et_3N (1 mL) and then evaporated. The residue was purified by reverse-phase HPLC on a Chemcobond 5-ODS-H column (10×150 mm) with an eluent of 5 mM ammonium formate and a linear gradient of acetonitrile (0-40 min, 0-30% CH_3CN) at a flow rate of 3.0 mL/min to give **5** (50 mg, 55%) as an yellow solid. Mp 80 ˚C; IR (KBr) 3340, 3205, 2942, 2361, 2340, 2224, 1660, 1630, 1596, 1543, 1466, 1350, 1350, 1271, 1086, 1040 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.81 (s, 1 H), 6.44 (br, 2 H), 6.31 (s, 2 H), 5.10-4.97 (m, 1 H), 4.87-4.70 (m, 2 H), 4.15-4.10 (m, 1 H), 3.64-3.60 (m, 1 H), 3.41-3.38 (m, 2 H), 2.14-2.00 ppm (m, 2 H); ^{13}C NMR (DMSO-d_6) δ 163.5, 162.0, 159.1, 94.2, 89.2, 87.3, 79.0, 71.7, 67.3, 62.0, 41.9 ppm; HRMS calcd for MH^+, $C_{11}H_{15}N_4O_3$: 251.1144; found 251.1135; UV (H_2O, 25 ˚C) ε_{260} = 12460 $Lmol^{-1}cm^{-1}$.

G*-I: 6-(Acethylamino)-3-cyano-5-iodo-2-pyridone. A suspension of 6-(acethylamino)-3-cyano-2-pyridone[s5] (2.1g, 11.85 mmol) in *i*PrOH (50 mL) were added NIS (3.2g, 14.22 mmol) at room temperature. The reaction mixture was stirred for 5 h at the same temperature and concentrated. To the mixture was added water, and the resulting precipitate was filtered and washed with water to give **G*-I** (1.6 g, 44%) as a yellow powder. Mp >218 ˚C (decompose); IR (KBr) 3229, 3055, 2228, 1647, 1589, 1550, 1200 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 11.16 (s, 1 H), 8.47 (s, 1 H), 6.75 (s, 2 H), 2.11 ppm (s, 3 H); ^{13}C NMR (DMSO-d_6) δ 171.4, 167.2, 161.2, 150.8, 150.3, 116.2, 99.2, 24.7 ppm; HRMS calcd for MNa^+, $C_8H_6N_3O_2INa$: 325.9402; found 325.9395.

G*(DMTr)-nucleoside:
(2*R*,3*S*,5*R*)-5-(6-Amino-3-cyano-2-pyridone-5-ylethynyl)-2-(4,4'-dimethoxytrityloxymethyl)-3-hydroxytetrahydrofuran. A mixture of **G*-I** (736 mg, 2.43 mmol), **1**[s3] (1.3 g, 2.92 mmol), $PdCl_2(PPh_3)_2$ (51 mg, 0.07 mmol), and CuI (9.2 mg, 0.048 mmol) in $(Me_3Si)_2NH$ /DMF (9.7 + 9.7 mL) was stirred under an argon atmosphere at 80 ˚C for 4 h. The reaction mixture was diluted with EtOAc and washed with saturated NaCl, 1% citric acid, saturated $NaHCO_3$, and saturated NaCl aqueous solutions subsequently. The organic phase was dried over Na_2SO_4 and evaporated. The residue was diluted with Pyridine (24 mL), H_2O (9.7 mL) and conc. NH_4OH (24 mL). The diluted mixture was stirred at room remperature overnight and concentrated under reduced pressure. The residue was chromatographed (SiO_2; eluent, $CHCl_3$/MeOH = from 50 : 1 to 50 : 3) to give **G*(DMTr)-nucleoside** (1.0 g, 71%) as a yellow foam. Mp 128–132 ˚C; IR (KBr) 3337, 3197, 2931, 2215, 1647, 1508, 1249, 1176, 1033, 828, 755 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 11.52 (br, 1 H), 7.60 (s, 1H), 7.43-7.36 (m, 2 H), 7.31-7.15 (m, 7 H), 6.89-6.81 (m, 4 H), 5.13 (s, 2 H), 4.93 (t, J = 7.2 Hz, 1 H),

4.20-4.09 (m, 1 H), 3.93-3.82 (m, 1 H), 3.73 (s, 6 H), 3.05-2.96 (m, 2 H), 2.24-2.05 ppm (m, 2 H); ^{13}C NMR (DMSO-d_6) δ 171.2, 162.6, 159.6, 157.8, 156.4, 149.4, 144.7, 135.5, 135.4, 129.6, 129.5, 127.6, 126.4, 117.3, 113.0, 93.0, 85.4, 85.3, 79.1, 72.1, 67.6, 64.5, 54.9, 41.8, 22.5 ppm; HRMS calcd for MNa^+, $C_{34}H_{31}N_3O_6Na$: 600.2111; found 600.2112.

G*(DMTr,dmf)-nucleoside:
(2*R*,3*S*,5*R*)-2-(4,4'-Dimethoxytrityloxymethyl)-5-[6-(dimethylamidino)-3-cyano-2-pyridone-5-ylethynyl]-3-hydroxy tetrahydrofuran. A mixture of **G*(DMTr)-nucleoside** (312 mg, 0.54 mmol) and $Me_2NCH(OMe)_2$ (0.72 mL, 5.4 mmol) in DMF (9 mL) was stirred at room temperature for 8 h and concentrated. The residue was dried under reduced pressure and used in the next step without further purification.

G*-phosphoramidite 3. To a dry CH_2Cl_2 (4 mL) solution of **G*(DMTr,dmf)-nucleoside** (340 mg, 0.54 mmol) were added *i*-$Pr_2NP(Cl)O(CH_2)_2CN$ (0.24 mL, 1.08 mmol) and *i*-Pr_2NEt (0.47 mL, 2.70 mmol) at room temperature under argon atmosphere. The reaction mixture was stirred for 90 min at the same temperature, concentrated, and diluted with EtOAc. The organic solution was washed with saturated $NaHCO_3$ twice and saturated NaCl aqueous solutions subsequently. The organic phase was dried over Mg_2SO_4, evaporated, and chromatographed (SiO_2; eluent EtOAc/Hexane = from 1 : 1 to 1 : 0) to give a diastereomer mixture of **3** (280 mg, 59% over two steps) as a yellow foam. Further purificiation was perfomed by reverse phase HPLC (eluent, CH_3CN). Mp 54–59 ˚C; IR (KBr) 3453, 2967, 2932, 2838, 2251, 2217, 1645, 1559, 1508, 1388, 1250, 1178, 1032, 977, 829 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.49 (s, 1 H), 7.67 (s, 1 H), 7.47-7.44 (m, 2 H), 7.36-7.18 (m, 7 H), 6.82-6.78 (m, 4 H), 5.00-4.95 (m, 1 H), 4.54-4.50 (m, 1 H), 4.13-4.09 (m, 1 H), 3.77 (s, 6 H), 3.74-3.44 (m, 4 H), 3.28-3.15 (m, 2 H), 3.18 (s, 3 H), 3.14 (s, 3 H), 2.60 (t, J = 6.3 Hz, 1 H), 2.45 (t, J = 6.3 Hz, 1 H), 2.34-2.24 (m, 2 H), 1.19-1.03 ppm (m, 12 H); ^{13}C NMR ($CDCl_3$) δ 163.1, 158.3, 158.2, 156.8, 150.9, 144.6, 135.8, 135.7, 130.0, 128.2, 128.1, 127.6, 126.6, 117.3, 116.8, 116.5, 113.0, 95.4, 91.5, 90.5, 86.0, 85.4, 80.2, 68.5, 64,3, 58.4, 58.3, 58.2, 58.1, 58.0, 55.2, 45.4, 45.3, 43.3, 43.1, 41.7, 41.5, 35.0, 24.7, 24.7, 24.6, 24.5, 23.1, 23.0, 22.9, 20.5, 20.3, 20.2, 20.1 ppm; HRMS calcd for MH^+, $C_{46}H_{54}N_6O_7P$: 833.3792; found 833.3793.

G*-nucleoside 6. A CH_2Cl_2 (10 mL) solution of **G*(DMTr)-nucleoside** (108 mg, 0.19 mmol) and 3% trichloroacetic acid in CH_2Cl_2 (4 mL) was stirred for 30 min at room temperature. To the reaction mixture was added THF, and the resulting precipitate was filtered and washed with CH_2Cl_2 to give **6** (16 mg, 31%) as a yellow powder. Mp >177 ˚C (decompose); IR (KBr) 3333, 3202, 2933, 2219, 1773, 1647, 1591, 1278, 1087, 1043, 673 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 11.5 (bs, 1 H), 7.76 (s, 1 H), 7.11 (br, 2 H), 5.08-5.02 (m, 1 H), 4.88-4.74 (m, 2 H), 4.18-4.10 (m, 1 H), 3.66-3.61 (m, 1 H), 3.40-3,33 (m, 2 H), 2.10-1.98 ppm (m, 2 H); ^{13}C NMR (DMSO-d_6) δ 159.5, 156.4, 149.5, 117.4, 93.0, 87.3, 79.1, 77.5, 72.0, 71.6, 67.2, 62.0, 41.7 ppm; HRMS calcd for MNa^+, $C_{13}H_{13}N_3O_4Na$: 298.0804; found 298.0804; UV (H_2O, 25 ˚C) ε_{260} = 5470 Lmol^{-1}cm^{-1}.

C*(DMTr)-nucleoside:
(2*R*,3*S*,5*R*)-5-(2-Amino-1-methypyrimidin-4-on-5-ylethynyl)-2-(4,4'-dimethoxytrityloxymethyl)-3-hydroxytetrah ydrofuran. A mixture of **C*-I**^{s2} (610 mg, 2.43 mmol), **1**^{s3} (915 mg, 2.06 mmol), $PdCl_2(PPh_3)_2$ (77 mg, 0.11 mmol),

and CuI (9.15 mg, 0.048 mmol) in $(Me_3Si)_2NH$ /DMF (12 + 24 mL) was stirred under an argon atmosphere at 70 ˚C for 6 h. The reaction mixture was diluted with AcOEt and washed with saturated NaCl, 1% citric acid, saturated $NaHCO_3$, and saturated NaCl aqueous solutions subsequently. The organic phase was dried over Na_2SO_4 and evaporated. The residue was diluted with pyridine (30 mL), H_2O (12 mL), and conc. NH_4OH (30 mL). The diluted mixture was stirred at room temperature for 12 h. After removal of the solvent, the residue was chromatographed (SiO_2; eluent, $CHCl_3$/MeOH = from 50 : 1 to 50 : 2) to give **C*(DMTr)-nucleoside** (1.0 g, 86%) as a colorless foam. Mp 107–110 ˚C; IR (KBr) 3335, 3195, 2932, 2835, 2229, 1693, 1508, 1249, 1175, 1032, 829, 754 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.16 (d, J = 13.8 Hz, 1 H), 7.44-7.40 (m, 2 H), 7.31-7.07 (m, 7 H), 6.75 (d, J = 8.4 Hz, 4 H), 6.01 (s, 2 H), 5.08-4.98 (m, 1 H), 4.38-4.31 (m, 1 H), 3.99-3.93 (m, 1 H), 3.67 (s, 6 H), 3.22-3.09 (m, 2 H), 2.30-2.19 ppm (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 162.8, 158.1, 155.0, 144.6, 135.8, 135.6, 129.9, 128.0 127.6, 126.5, 112.9, 91.9, 86.0, 78.5, 73.7, 68.2, 64.6, 55.1, 42.2, 38.9 ppm; HRMS calcd for MH^+, $C_{33}H_{34}N_3O_6$: 590.2267; found 590.2284.

C*(DMTr,dmf)-nucleoside:
(2*R*,3*S*,5*R*)-2-(4,4'-Dimethoxytrityloxymethyl)-5-[2-(dimethylamidino)-1-methylpyrimidin-4-on-5-ylethynyl]-3-hydroxytetrahydrofuran. A mixture of **C*(DMTr)-nucleoside** (1.0 g, 1.77 mmol) and $Me_2NCH(OMe)_2$ (0.71 mL, 5.31 mmol) in MeOH (5 mL) was stirred at room temperature for 8 h and concentrated. The residue was dried under reduced pressure and used in the next step without further purification.

C*-phosphoramidite 4. To a dry CH_2Cl_2 (30 mL) solution of **C*(DMTr,dmf)-nucleoside** (1.1 g, 1.77 mmol) were added *i*-$Pr_2NP(Cl)O(CH_2)_2CN$ (0.8 mL, 3.5 mmol) and *i*-Pr_2NEt (1.54 mL, 8.85 mmol) at room temperature under argon atmosphere. The reaction mixture was stirred for 90 min at the same temperature, concentrated, and chromatographed (SiO_2; eluent EtOAc/Acetone = 4 :1) to give a diastereomer mixture of **4** (630 mg, 41% over two steps) as a colorless foam. Further purificiation was perfomed by reverse phase HPLC (eluent, CH_3CN). Mp 97–102 ˚C; IR (KBr) 3429, 2966, 2931, 2837, 2251, 2228, 1643, 1626, 1490, 1415, 1346, 1250, 1178, 1032, 829, 791, 753, 585 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.80 (s, 1 H), 7.53-7.49 (m, 2 H), 7.40-7.18 (m, 7 H), 7.04 (d, J = 7.5 Hz, 2 H), 6.84-6.79 (m, 4 H), 5.07 (t, J = 7.6, 1 H), 4.59-4.50 (m, 1 H), 4.13-4.08 (m, 1 H), 3.77 (s, 6 H), 3.72-3.52 (m, 4 H), 3.31-3.23 (m, 1 H), 3.27 (d, J = 7.5 Hz, 3 H), 3.22-3.09 (m, 1 H), 3.21 (s, 3 H), 3.09 (s, 3 H), 2.60 (t, J = 6.4 Hz, 1 H), 2.43 (t, J = 6.4 Hz, 1 H), 2.44-2.34 (m, 2 H), 1.17-1.06 ppm (m, 12 H) ; ^{13}C NMR ($CDCl_3$) δ 169.6, 159.1, 158.2, 157.7, 146.5, 144.9, 136.1, 136.0, 130.1, 128.3, 128.2, 127.7, 126.5, 113.0, 100.6, 92.4, 88.0, 85.2, 68.9, 64.2, 58.4, 58.2, 55.2, 43.4, 43.2, 41.4, 38.6, 35.3, 24.6, 24.5, 20.5 ppm; HRMS calcd for MH^+, $C_{45}H_{55}N_6O_7P$: 823.3948; found 823.3946.

C*-nucleoside 7. A CH_2Cl_2 (1 mL) solution of **C*(DMTr)-nucleoside** (67 mg, 0.12 mmol) and 3% trichloroacetic acid in CH_2Cl_2 (2 mL) was stirred for 30 min at room temperature. To the reaction mixture was added THF, and the resulting precipitate was filtered and washed with CH_2Cl_2 to give **7** (12 mg, 38%) as a white powder. Mp 144–147 ˚C; IR (KBr) 3369, 3177, 2933, 2233, 1707, 1333, 1082, 1042, 831 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.74 (s, 1 H), 7.06 (br, 2 H), 5.07-5.05 (m, 1 H), 4.78-4.73 (m, 2 H), 4.16-4.08 (m, 1 H), 3.65-3.61 (m, 1 H), 3.40-3,34 (m, 2 H), 2.02-1.94 ppm (m, 2H); ^{13}C NMR (DMSO-d_6) δ 167.5, 154.6, 147.1, 101.4, 90.5, 87.4, 79.5, 71.6, 67.0, 62.1, 42.1, 38.2 ppm; HRMS calcd for MH^+, $C_{12}H_{16}N_3O_4$: 266.1141; found 266.1142; UV (H_2O, 25 ˚C) ε_{260} = 5090 $Lmol^{-1}cm^{-1}$.

Synthesis of artificial DNA olgomers. The artificial DNA oligomers were synthesized by use of phosphoramidites **2**, **3**, **4**, and the previously reported T*-phosphoroamidite[s4] on an Applied Biosystems 392 synthesizer using standard β-cyanoethylphosphoramidite chemistry with the coupling reaction time of 15 min. The solid support (Universal Support II® or III®), which allows for 3' placement of nonnatural nucleosides, was purchased from Glen Research. After automated synthesis, the oligomers were removed from the solid support with 2 M ammonia methanol solution at 30 ºC for 30 min and deprotected with concentrated NH_4OH at 40 ºC for 8 h. The oligomers were then purified by reverse-phase HPLC using a $5C_{18}$-AR-II column (4.6 x 150 mm) with an eluent of 5 mM ammonium formate and the following CH_3CN percentages of linear gradient (0–60 min, 3–18%) at a flow rate of 1.0 mL/min.

2. Measurements.

MALDI-TOF mass measurements. MALDI-TOF mass spectra were recorded on a Bruker-Daltonics-Autoflex mass spectrometer operating in the negative ion mode with 3-hydroxypicolinic acid as a matrix (see Figure S2). 5'-d(T*D*D*T*D*T*D*T*T*T*D*): calcd for $[M-H]^-$, $C_{115}H_{130}N_{30}O_{58}P_9$: 3138.58; found 3138.20, $d(D^*)_{16}$: calcd for $[M-H]^-$, $C_{176}H_{208}N_{64}O_{78}P_{15}$: 4932.04; found 4930.07, 5'-d(T*D*D*T*G*C*D*T*T*T*D*): calcd for $[M-H]^-$, $C_{117}H_{130}N_{30}O_{58}P_9$: 3162.58; found 3162.88, 5'-d(T*G*D*T*G*C*D*T*C*D*): calcd for $[M-H]^-$, $C_{119}H_{130}N_{30}O_{58}P_9$: 3186.58; found 3185.74, 5'-d(T*D*T*T*G*C*T*D*T*T*): calcd for $[M-H]^-$, $C_{119}H_{130}N_{26}O_{62}P_9$: 3194.55; found 3194.02, 3'-d(D*T*D*D*C*G*D*T*D*D*): calcd for $[M-H]^-$, $C_{115}H_{130}N_{34}O_{54}P_9$: 3130.61; found 3129.37, 5'-d(D*T*D*D*C*G*D*T*D*D*): calcd for $[M-H]^-$, $C_{115}H_{130}N_{34}O_{54}P_9$: 3130.61; found 3129.60, 5'-d(T*D*T*T*C*C*T*D*T*T*): calcd for $[M-H]^-$, $C_{118}H_{132}N_{26}O_{62}P_9$: 3184.56; found 3183.31.

UV and T_m Measurements. UV-vis spectra and T_m melting curves (1.0 ºC/1.0 min) were obtained by JASCO V-560 UV/vis spectrophotometer with a peltier and a temperature controller in a temperature range from 10 to 70 °C. The T_m values were determined from the maxima of the first derivatives of the melting curves measured in a buffer solution: 10 mM Hepes (pH 7.0), 10 mM $MgCl_2$, 100 mM NaCl. Errors were estimated at ± 1.0 ºC. Concentrations of the solutions containing artificial DNAs were determined based on the molar extinction coefficients at 260 nm (e_{260}) of the artificial nucleoside monomers **5**, **6**, **7**, and the previously reported T*-nucleoside[s4] (see SI Text).

CD Measurements. CD spectra were recorded using a JASCO-J-720WI spectropolarimeter with a temperature controller at 10, 20, 30, 40, 50, 60, and 70 °C in a buffer solution: 10 mM Hepes (pH 7.0), 10 mM $MgCl_2$, 100 mM NaCl.

Titration Experiments. Titration curves for artificial DNAs were obtained by monitoring a specified wavelength of CD. In entry 5, for example, 3.0 mL of a $d(D^*)_{16}$ solution (4.0 μM with 10 mM Hepes (pH 7.0), 10 mM $MgCl_2$, and 100 mM NaCl) was prepared, and CD measurement of the solution was carried out at 5 ºC using a quartz cell of 1 cm pathlength. Separately, 200 μL of a $d(T^*)_{16}$ solution (200 μM in the same buffer) was then prepared, and 12.0 μL of the $d(T^*)_{16}$ solution (0.2 equivalent against the $d(D^*)_{16}$) was added to the $d(D^*)_{16}$ solution in the quartz cell. The mixed solution was heated to 70 ºC, annealed to 5 ºC for 1 h, and then CD measurement was performed at 5 ºC. A series of the operations were repeated for all the ratios of $[d(T^*)_{16}]/[d(D^*)_{16}]$ = 0, 0.2, 0.4, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2,

1.3, 1.4, 1.6, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, and 2.4. The normalized CD changes at 308 nm were plotted against $[d(T^*)_{16}]/[d(D^*)_{16}]$ (Figure 2C).

Job's Plots. Job's plots for artificial DNAs were obtained by monitoring a specified wavelength of CD or UV. In entry 11, for example, 10 mL of a 5'-d(T*D*T*T*G*C*T*D*T*T*) solution (1.0 μM with 10 mM Hepes (pH 7.0), 10 mM $MgCl_2$, and 100 mM NaCl) and 10 mL of a 3'-d(D*T*D*D*C*G*D*T*D*D*) solution (1.0 μM in the same buffer) were prepared. The 5'-d(T*D*T*T*G*C*T*D*T*T*) solutions of 0, 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 μL were mixed in micro test tubes with 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100, and 0 μL of the 3'-d(D*T*D*D*C*G*D*T*D*D*) solutions, respectively. All the mixed solutions were heated to 70 ºC, followed by slow cooling to 25 ºC over 30 min. UV-vis measurements of all of the solutions were carried out at 25 ºC using a quartz cell of 1 cm pathlength. Against [5'-d(T*D*T*T*G*C*T*D*T*T*)]/ ([5'-d(T*D*T*T*G*C*T*D*T*T*)] + [3'-d(D*T*D*D*C*G*D*T*D*D*)]) were plotted the normalized UV changes for the mixtures of a 5'-d(T*D*T*T*G*C*T*D*T*T*) and 3'-d(D*T*D*D*C*G*D*T*D*D*) at 305 nm. The changes were corrected for by subtracting sum of the intensities for each strand at the same concentrations (Figure 3A).

References for ESI

S1 P. M. Pelphrey, V. M. Popov, T. M. Joska, J. M. Beierlein, E. S. D. Bolstad, Y. A. Fillingham, D. L. Wright and A. C. Anderson, *J. Med. Chem.*, 2007, **50**, 940.

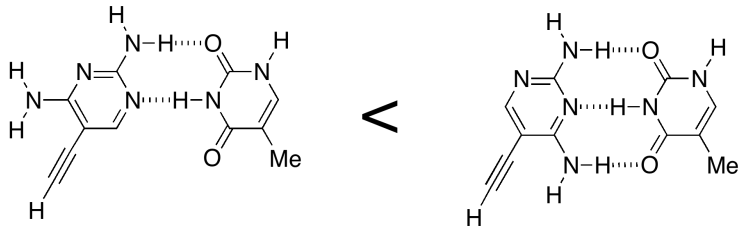
S2 K. W. Wellington, H. C. Ooi and S. A. Benner, *Nucleosides, Nucleotides Nucleic Acids*, 2009, **28**, 275.

S3 J. Chiba, S. Takeshima, K. Mishima, H. Maeda, Y. Nanai, K. Mizuno and M. Inouye, *Chem. Eur. J.*, 2007, **13**, 8124; M. Takase, T. Morikawa, H. Abe and M. Inouye, *Org. Lett.*, 2003, **5**, 625.

S4 Y. Doi, J. Chiba, T. Morikawa and M. Inouye, *J. Am. Chem. Soc.*, 2008, **130**, 8762.

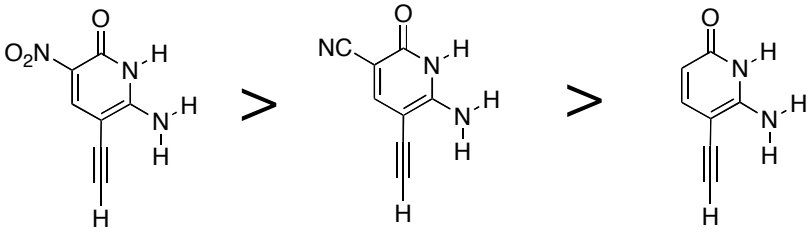
S5 M. Inouye, K. Kim and T. Kitao, *J. Am. Chem. Soc.*, 1992, **114**, 778.

A



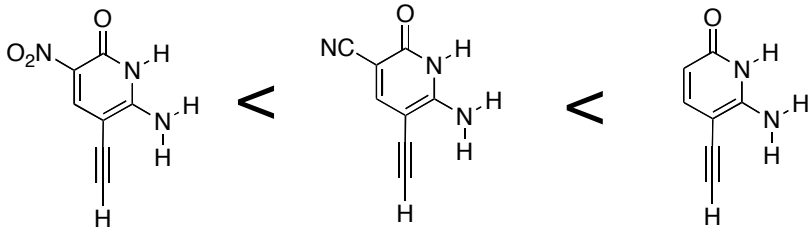
More stable by 9.2 kJ/mol

B



LUMO: Susceptibility for nucleophilic attack

C



HOMO: Susceptibility for oxidation

Chart S1. (A) Comparison of "sum of electronic and zero-point energies" in DFT calculations (RB3LYP/6-311+G) for the D*/T* base pair with a DA/AD-type double hydrogen-bonding (left: –905.121265 Hartree) and a DAD/ADA-type triple hydrogen-bonding (right: –905.124776 Hartree). (B) Comparison of LUMO energies in DFT calculations (RB3LYP/6-311G+(d, 2p)) for 3-nitro-substituted (left: –0.09641 Hartree), 3-cyano-substituted (center: –0.07793 Hartree), and 5-ethynyl-6-amino-2-pyrimidone (right: –0.04540 Hartree). (C) Comparison of HOMO energies in DFT calculations (RB3LYP/6-311G+(d, 2p)) for 5-ethynyl-6-amino-2-pyrimidone (right: –0.21084 Hartree), 3-cyano-substituted (center: –0.23186 Hartree), and 3-nitro-substituted (left: –0.24036 Hartree).

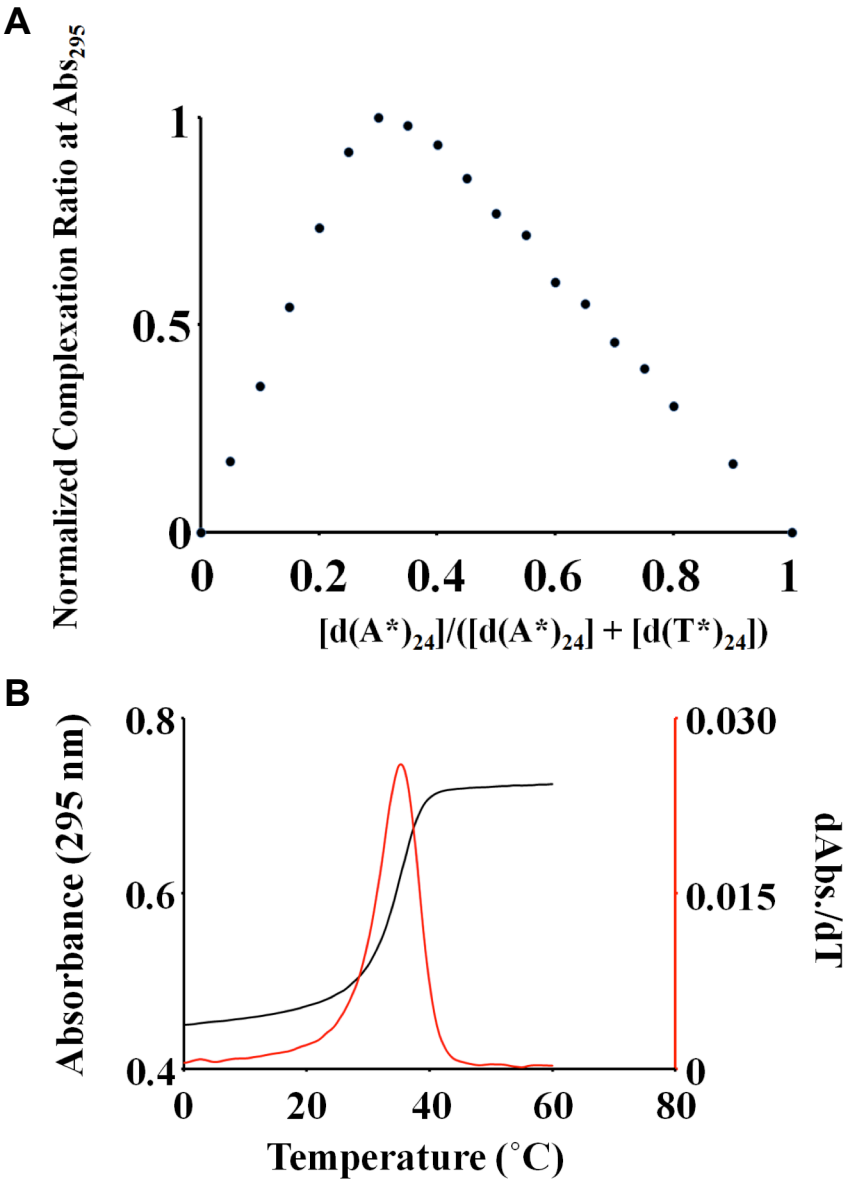


Figure S1. (A) Job's plot ($[d(A^*)_{24}] + [d(T^*)_{24}]$ = 2.0 μM at 0 ºC) and (B) the thermal denaturation study ($[d(A^*)_{24}]$ = 1.0 μM and $[d(T^*)_{24}]$ = 2.0 μM) monitored by UV (295 nm) for the triplex in 10 mM Hepes (pH 7.0), 10 mM $MgCl_2$, and 100 mM NaCl.

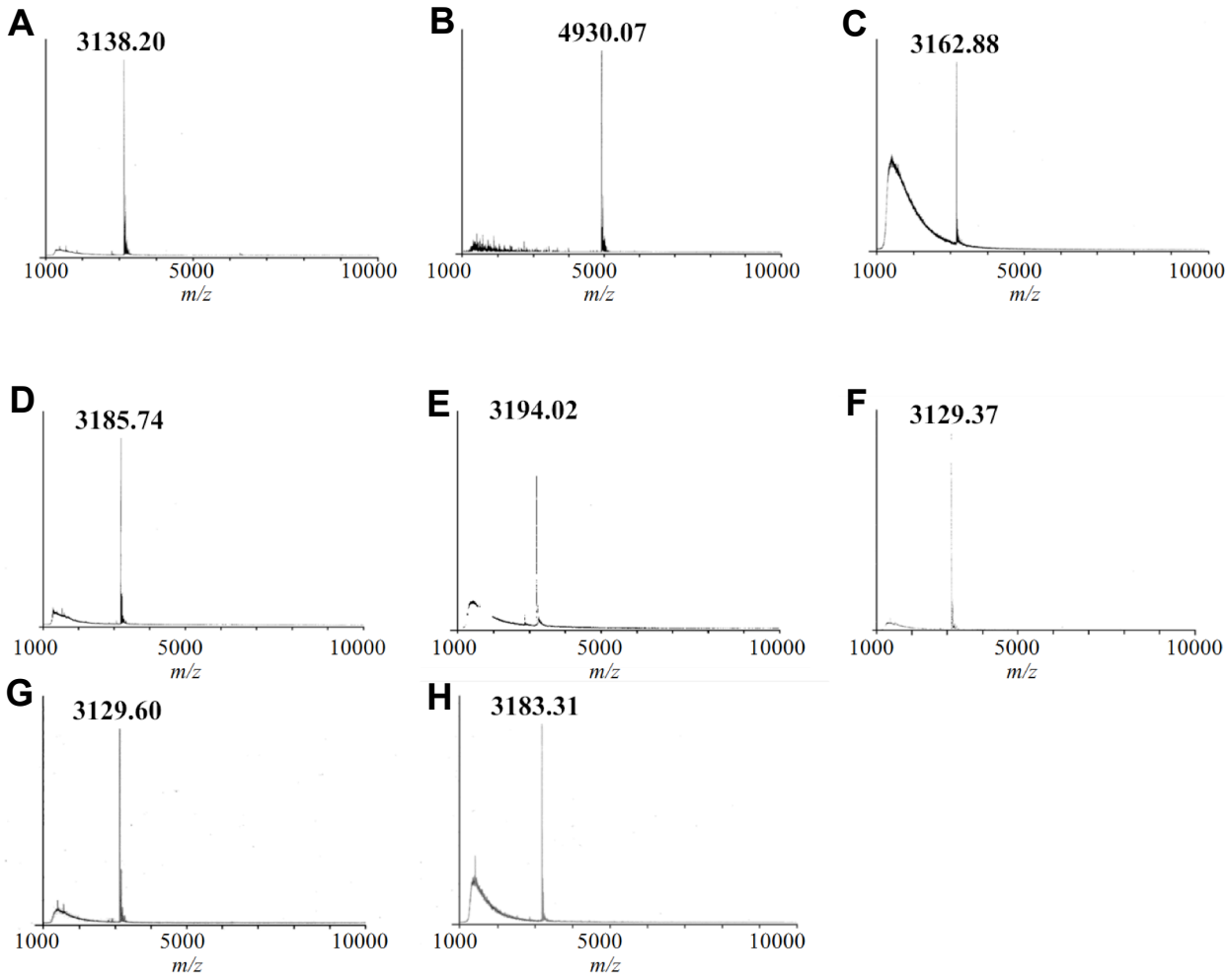


Figure S2. MALDI-TOF mass spectra (negative ion mode) for (A) 5'-d(T*D*D*T*D*T*D*T*T*T*D*), (B) $d(D^*)_{16}$, (C) 5'-d(T*D*D*T*G*C*D*T*T*T*D*), (D) 5'-d(T*G*D*T*G*C*D*T*C*D*), (E) 5'-d(T*D*T*T*G*C*T*D*T*T*), (F) 3'-d(D*T*D*D*C*G*D*T*D*D*), (G) 5'-d(D*T*D*D*C*G*D*T*D*D*), and (H) 5'-d(T*D*T*T*C*C*T*D*T*T*). See also **MALDI-TOF mass measurements** (page S7).

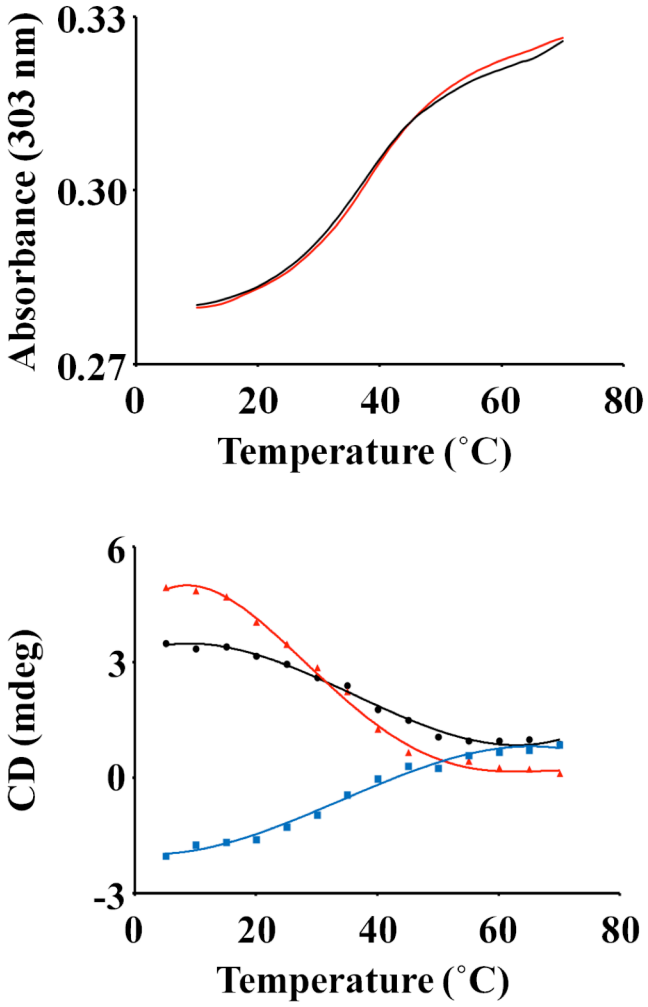


Figure S3. Absorbance and CD data for 5'-d(T*D*D*T*D*T*D*T*T*D*) (2.0 μM) in 10 mM Hepes (pH 7.0), 10 mM $MgCl_2$, 100 mM NaCl. (A) UV melting (red) and annealing (black) curves monitored at 303 nm. Melting temperatures were obtained at 39 ± 1.0 ºC from the curves. (B) CD melting curves monitored at 258 (red), 286 (blue), and 313 nm (black) in Figure 1B. Melting temperatures were obtained at 38.5 ± 1.0 ºC from the curves.

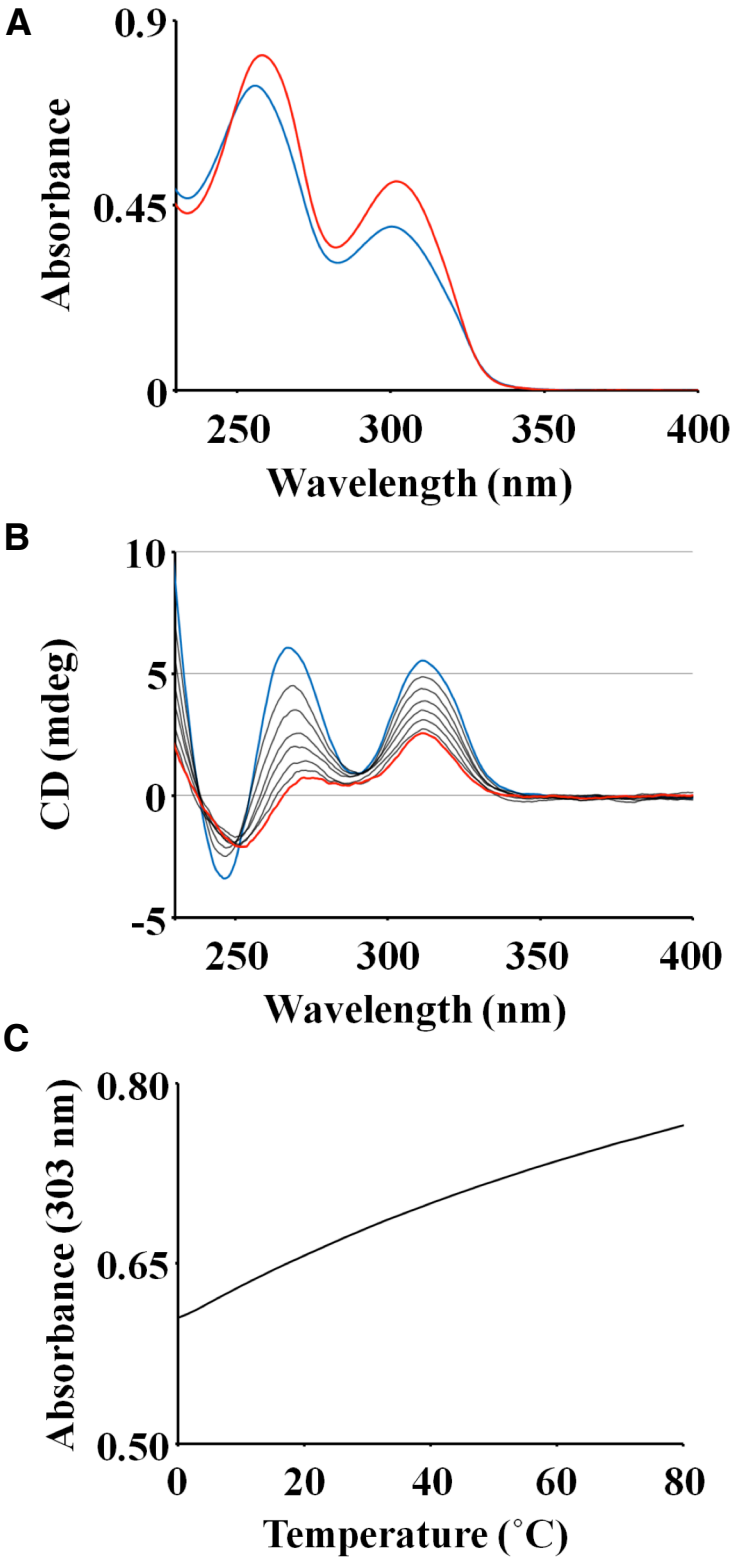


Figure S4. UV-vis and CD data for homooligomer $d(D^*)_{16}$ (2.0 μM) in 10 mM Hepes (pH 7.0), 10 mM $MgCl_2$, 100 mM NaCl. (A) UV spectra at 0 (blue) and 70 ºC (red). (B) CD spectra at 0 (blue), 10, 20, 30, 40, 50, 60, and 70 ºC (red). (C) Absorbance monitored at 303 nm.

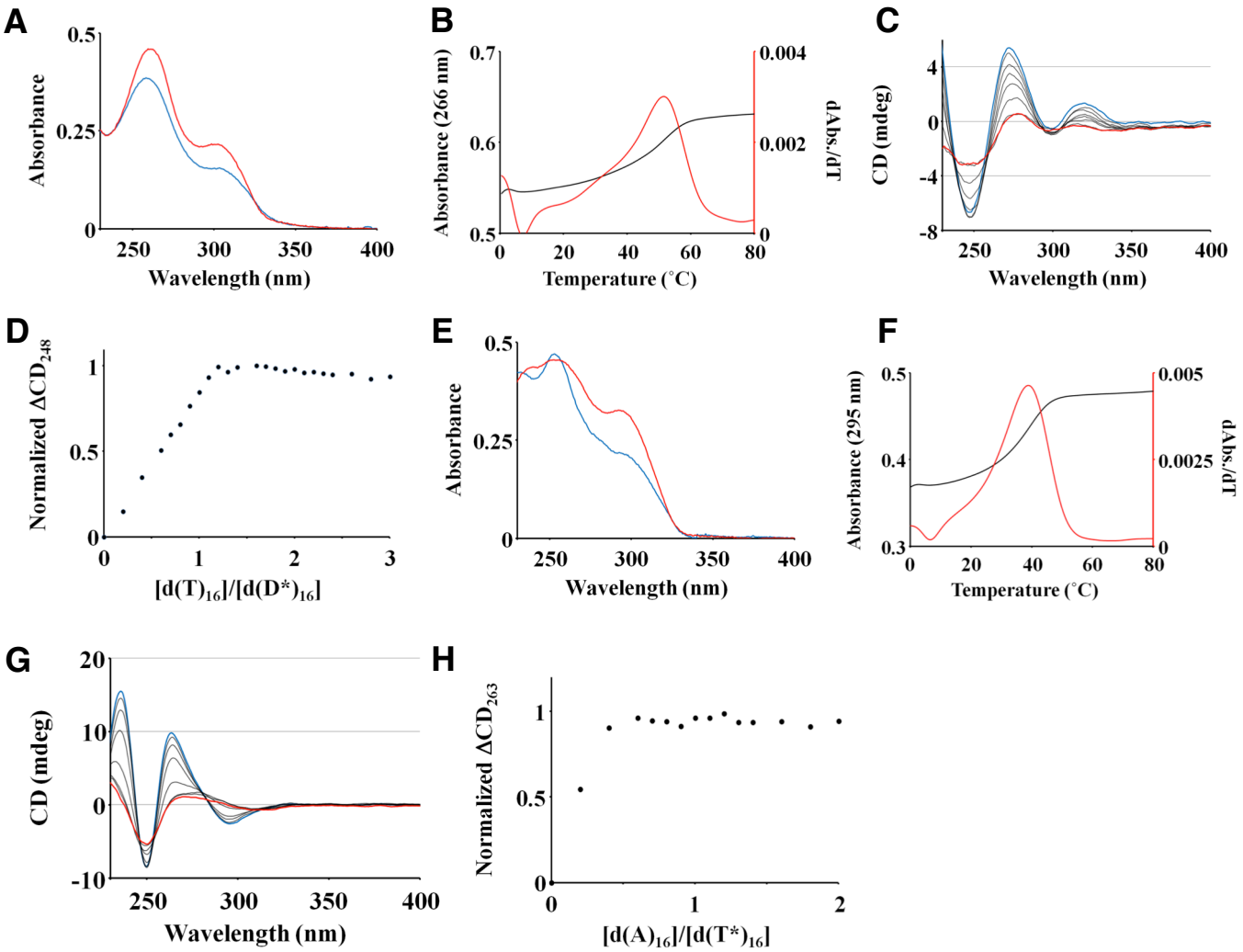


Figure S5. UV-vis and CD data of hetero-duplex ($d(D^*)_{16}/dT_{16}$, [duplex] = 1.0 μM) and hetero-triplex ($d(T^*)_{16}/dA_{16}$/ $d(T^*)_{16}$, [triplex] = 1.0 μM) in 10 mM Hepes (pH 7.0), 10 mM $MgCl_2$, 100 mM NaCl. (A) UV spectra at 0 (blue) and 70 ºC (red) for $d(D^*)_{16}/dT_{16}$. (B) UV melting curve monitored at 266 nm for $d(D^*)_{16}/dT_{16}$. (C) CD spectra at 0 (blue), 10, 20, 30, 40, 50, 60, and 70 ºC (red) for $d(D^*)_{16}/dT_{16}$. (D) The titration plot of $d(D^*)_{16}$ and dT_{16} monitored by CD at 248 nm. $[d(D^*)_{16}]$ = 1.0 μM, $[dT_{16}]/[d(D^*)_{16}]$ = 0, 0.2, 0.4, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.6, 2.8 and 3.0. (E) UV spectra at 0 (blue) and 70 ºC (red) for $d(T^*)_{16}/dA_{16}$/ $d(T^*)_{16}$. (F) UV melting curve monitored at 295 nm for $d(T^*)_{16}/dA_{16}$/ $d(T^*)_{16}$. (G) CD spectra at 0 (blue), 10, 20, 30, 40, 50, 60, and 70 ºC (red) for $d(T^*)_{16}/dA_{16}$/ $d(T^*)_{16}$. (H) The titration plot of $d(T^*)_{16}$ and dA_{16} monitored by CD at 263 nm. $[d(T^*)_{16}]$ = 1.0 μM, $[dA_{16}]/[d(T^*)_{16}]$ = 0, 0.2, 0.4, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.6, 1.8, 1.9, and 2.0.

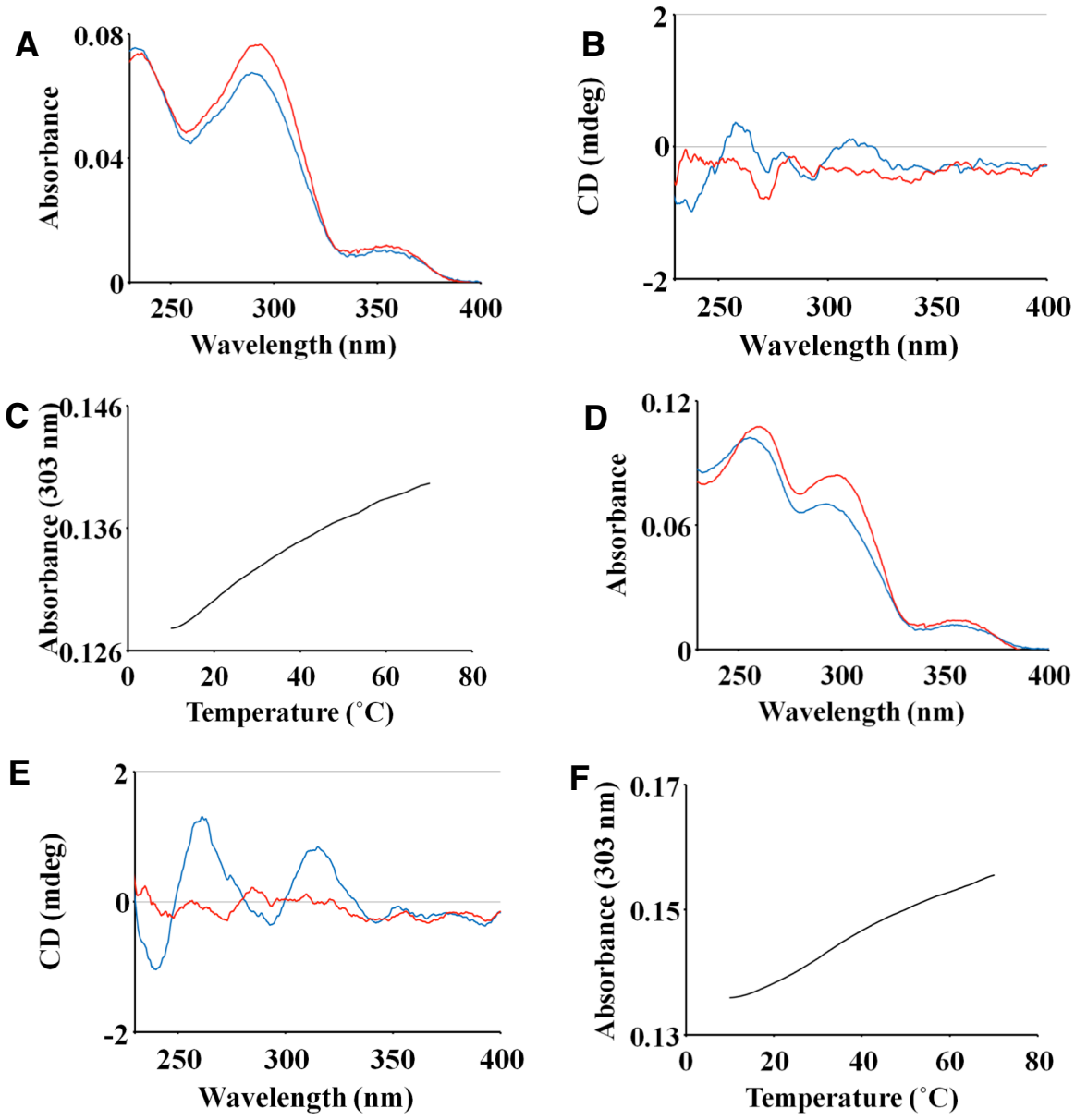


Figure S6. UV-vis and CD data of single-stranded 5'-d(T*D*T*T*G*C*T*D*T*T*) (A–C, 1.0 μM) and 3'-d(D*T*D*D*C*G*D*T*D*D*) (D–F, 1.0 μM) in 10 mM Hepes (pH 7.0), 10 mM $MgCl_2$, 100 mM NaCl. (A) UV-vis spectra at 10 (blue) and 70 ºC (red). (B) CD spectra at 10 (blue) and 70 ºC (red). (C) Absorbance monitored at 303 nm. (D) UV-vis spectra at 10 (blue) and 70 ºC (red). (E) CD spectra at 10 (blue) and 70 ºC (red). (F) Absorbance monitored at 303 nm.

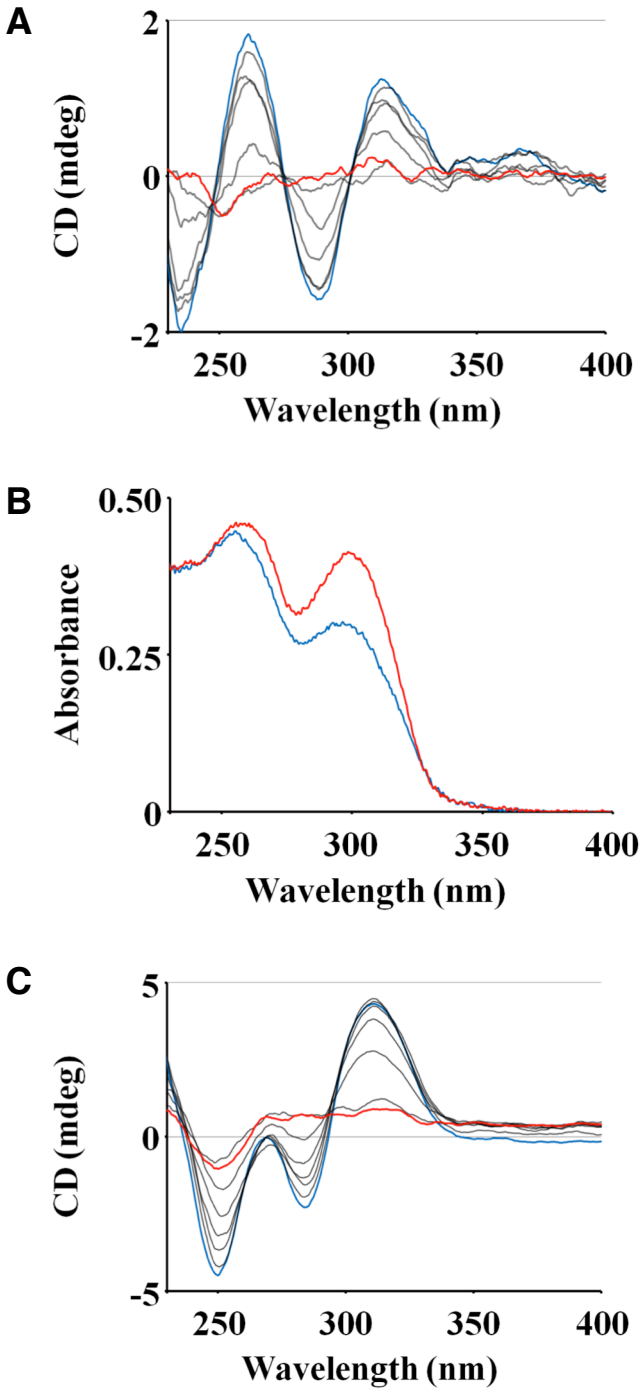


Figure S7. UV-vis and CD data in 10 mM Hepes (pH 7.0), 10 mM $MgCl_2$, 100 mM NaCl. (A) CD spectra of 5'-d(T*D*T*T*G*C*T*D*T*T*) and 3'-d(D*T*D*D*C*G*D*T*D*D*) at 10 (blue), 20, 30, 40, 50, 60, and 70 ºC (red), [duplex] = 1.0 μM. (B) UV-vis spectra of $d(D^*)_{16}$ and $d(T^*)_{16}$ at 10 (blue) and 70 ºC (red), $[d(D^*)_{16}] = [d(T^*)_{16}]$ = 1.0 μM. (C) CD spectra of $d(D^*)_{16}$ and $d(T^*)_{16}$ at 10 (blue), 20, 30, 40, 50, 60, and 70 ºC (red), $[d(D^*)_{16}] = [d(T^*)_{16}]$ = 1.0 μM.

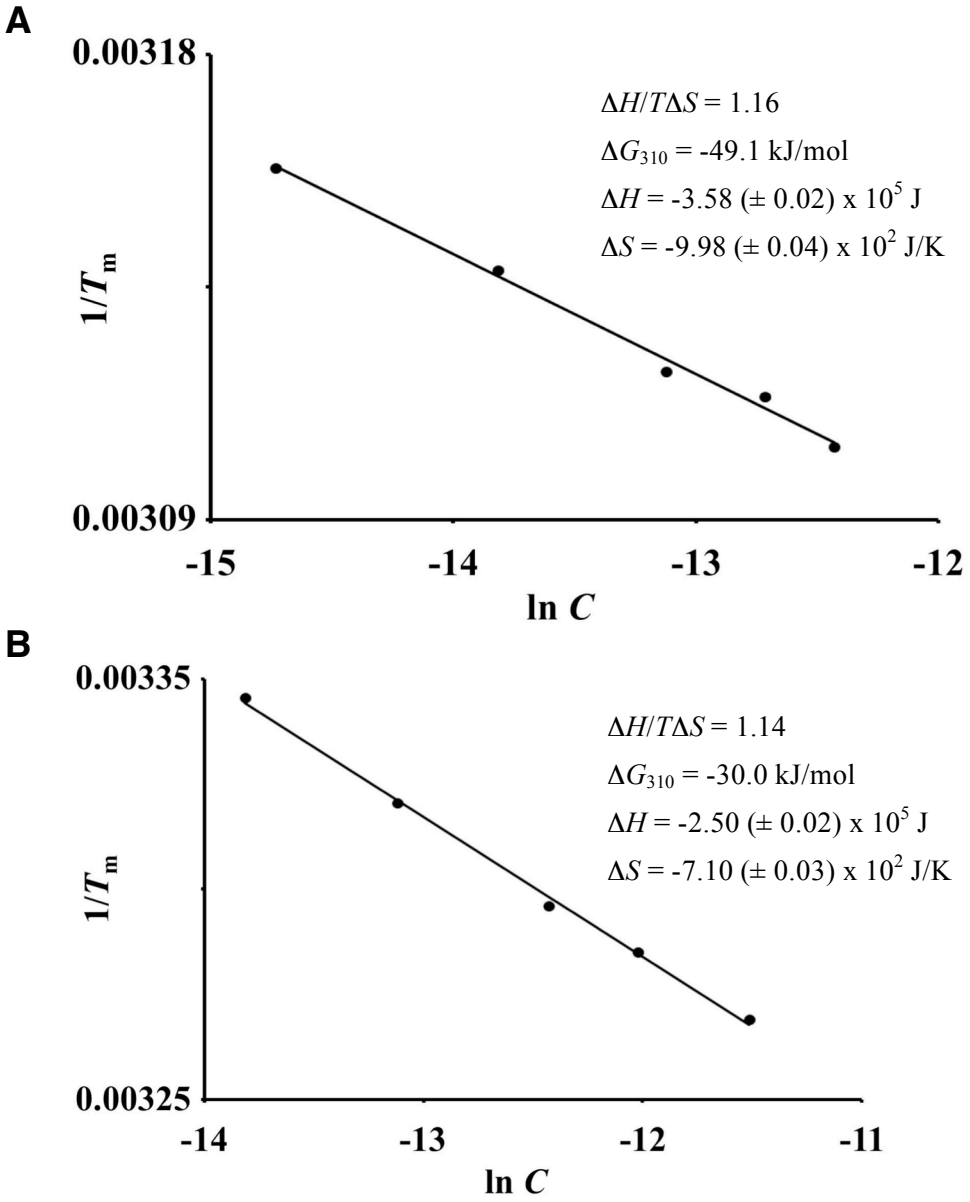


Figure S8. Plot of $1/T_m$ versus ln C (C is the duplex concentration) for (A) 5'-d(T*D*T*T*G*C*T*D*T*T*) and 3'-d(D*T*D*D*C*G*D*T*D*D*) and (B) 5'-d(TATTGCTATT) and 3'-d(ATAACGATAA) in 10 mM Hepes (pH 7.0), 10 mM $MgCl_2$, 100 mM NaCl. From the slope of the line and the y-axis intercept (ln C = 0), ΔH and ΔS were determined from the equation $1/T_m = R/\Delta H$ ln C + (ΔS – R ln 4)/ ΔH and ΔG_{310} is calculated from the equation $\Delta G = \Delta H - T\Delta S$ at 37 ºC.