Fluorescent nucleoside analogue displays enhanced emission upon pairing with guanine

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Supporting Information:

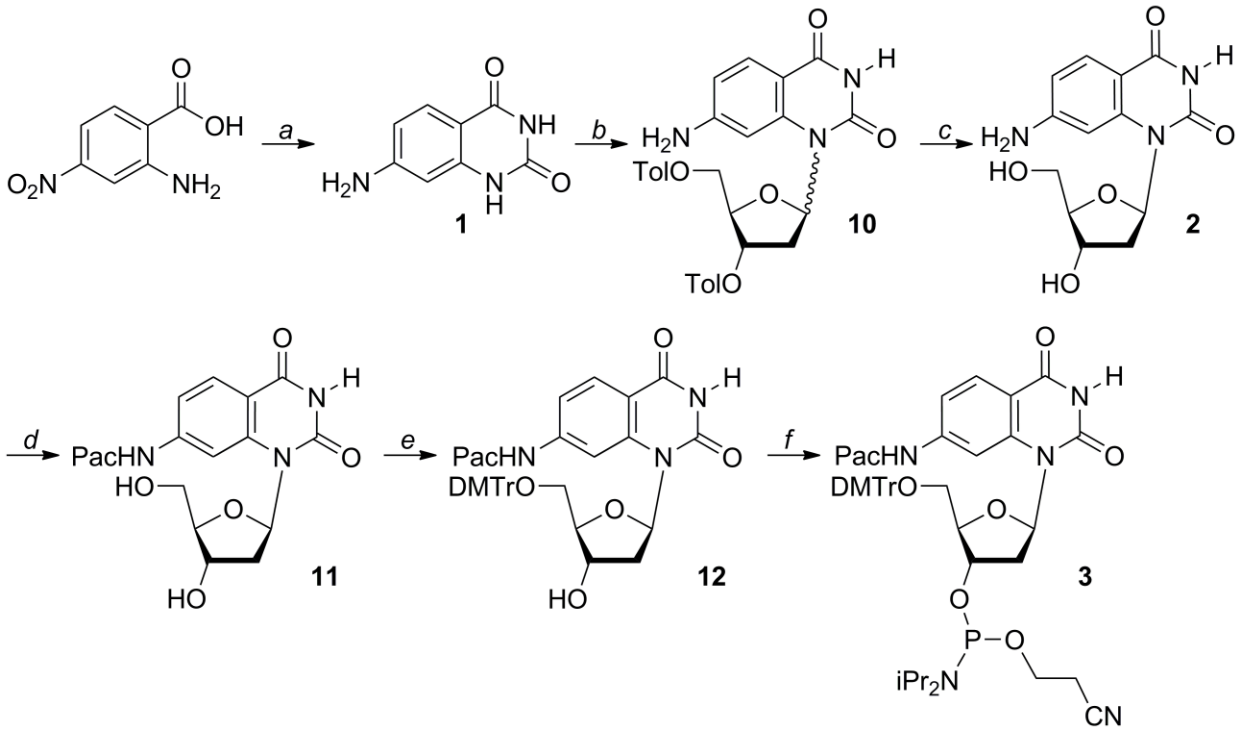
S.1 – Synthetic Procedures and Experimental Data

General Procedures

NMR spectra were recorded on a Varian Mercury 400 MHz spectrometer. Mass spectra were recorded at the UCSD Chemistry and Biochemistry Mass Spectrometry Facility, utilizing either a LCQDECA (Finnigan) ESI with a quadrapole ion trap or a MAT900XL (ThermoFinnigan) FAB double focusing mass spectrometer. UV-Vis spectra were recorded on either a Hewlett Packard 8452A or 8453 Diode Array Spectrometer.

Unless otherwise specified, materials obtained from commercial suppliers were used without further purification. 2-D-3,5-di-*O*-p-toluoyl-α-L-erythro-pentofuranosyl chloride was purchased from Berry & Associates, Inc. Anhydrous pyridine, dichloroethane and acetonitrile were obtained from Fluka. Anhydrous *N,N*-diisopropylethylamine and triethylamine were obtained from Acros. The unmodified oligonucleotides were purchased from Integrated DNA Technologies. Standard phosphoramidites and solutions necessary for solid phase RNA synthesis were purchased from Glen Research. Oligonucleotides were purified by gel electrophoresis and desalted on a Sep-Pak (Waters Corporation). Chemicals for preparing buffer solutions were purchased from Fisher Biotech (enzyme grade). Autoclaved water was used in all fluorescence titrations. NMR solvents were purchased from Cambridge Isotope Laboratories (Andover, MA).

Synthetic Schemes



Scheme S1: Synthesis of **3**. (a) (i) Urea; (ii) $SnCl_2$, conc. HCl, 40%. (b) $(NH_4)_2SO_4$, *N,O*-bis(trimethylsilyl)acetamide, $CF_3SO_3Si(CH_3)_3$, 2-D-3,5-di-*O*-p-toluoyl-α-L-erythro-petofuranosyl chloride, CH_3CN, 80%. (c) conc. NH_4OH, 50%. (d) $(CH_3)_3SiCl$, phenoxyacetic anhydride, H_2O, conc. NH_4OH, pyridine, 75%. (e) DMTrCl, Et_3N, pyridine, 85%. (f) *i*Pr_2NEt, (*i*Pr_2N)P(Cl)O-CH_2CH_2CN, $ClCH_2CH_2Cl$, 65%.

7-Aminoquinazoline-2,4(1*H*,3*H*)-dione (1). Urea (10.0 g, 166.5 mmol) was mixed with 2-amino-4-nitrobenzoic acid (10.0 g, 54.9 mmol). The powder was heated to 200 °C for 4 h. The reaction was cooled to room temperature. Using a coarse frit, the solid was washed with water (250 mL), 5% sodium bicarbonate (200 mL), 10% sodium carbonate (400 mL), dilute sulfuric acid (250 mL), and boiling methanol (300 mL). The solid was stirred in boiling 50% acetic acid (800 mL) for 1 h. The remaining solid was filtered off and the solution was brought back to boiling. The solution was allowed to cool to room temperature and crystals were collected by filtration. The crystals were redissolved in boiling 50% acetic acid. Activated carbon was added to the solution and boiled for 10 min. The solution was filtered hot and allowed to crystallize. The clear yellow crystals, 7-nitroquinazoline-2,4(1*H*,3*H*)-dione, were used without further purification. A suspension of tin chloride (8.20 g, 43.2 mmol) in concentrated HCl (100 mL) was brought to boiling. 7-Nitroquinazoline-2,4(1*H*,3*H*)-dione (2.20 g, 10.6 mmol) was added, and the suspension was refluxed for 5 h. The boiling suspension was filtered with a coarse frit, and the solid was washed with water (500 mL). Product: white solid (3.9 g, 22 mmol, 40 % yield over two steps). ^{1}HNMR (400 MHz, DMSO-d_6): δ 10.88 (s, NH, 1H), 10.82 (s, NH, 1H), 7.62 (d, J = 8.4 Hz, 1H), 6.52 (d, J = 8.4 Hz, 1H), 6.39 (s, 1H); ^{13}CNMR (100 MHz, DMSO-d_6): δ 166.04, 152.84, 151.46, 143.30, 129.12, 111.93, 105.25, 98.90; ESI-MS calculated for $C_8H_7N_3O_3$ $[M+H]^+$ 178.06, found 178.15; m.p. > 300 ºC.

7-Aminoquinazoline-2,4(1*H*,3*H*)-dione deoxyribonucleoside (2). To a suspension of **1** (1.50 g, 8.47 mmol) and ammonium sulfate (0.56 g, 4.2 mmol) in anhydrous acetonitrile (100 mL), *N,O*-bis(trimethylsilyl)acetamide (10.5 mL, 42.3 mmol) was added dropwise under argon. The reaction was stirred at 25 °C for 30 min. 2-D-3,5-di-*O*-p-toluoyl-α-L-erythro-petofuranosyl chloride (3.29 g, 8.46 mmol) was added to the reaction over ice and under argon. TMSOTf (0.77 mL, 4.2 mmol) was dissolved in 1mL of anhydrous acetonitrile and added dropwise over ice. The reaction temperature was raised to room temperature and stirred for 4 h. The reaction was concentrated to an oil, and diluted with dichloromethane (100 mL). The solution was washed with saturated sodium bicarbonate and brine. The organic layer was dried over sodium sulfate. The solvent was removed under reduced pressure, and the crude product was isolated by flash chromatography (0.4-0.8% methanol in dichloromethane). Dioxane (30 mL) was added to **10** (2.00 g, 3.78 mmol) in a pressure tube. Ammonium hydroxide (30%, 70 mL) was added to the tube. The reaction was stirred at 80 °C for 24 h. The solvent was removed under reduced pressure, and the product was isolated by flash chromatography (10% methanol in ethyl acetate). Product: white solid (0.554 g, 1.89 mmol, 40 % over two steps). ^{1}HNMR (400 MHz, DMSO-d_6): δ 11.03 (s, NH, 1H), 7.62 (d, J = 8.4 Hz, 1H), 6.66 (m, 1H), 6.48 (t, J = 7.6 Hz, 1H), 6.42 (d, J_1 = 6.8 Hz, J_2 = 2.0 Hz, 1H), 6.12 (s, NH_2, 2H), 5.21 (d, J = 5.2 Hz, 1H), 4.83 (t, J = 5.2 Hz, 1H), 4.31 (m, 1H), 3.64 (m, 3H), 2.67 (m, 1H), 1.87 (m, 1H); ^{13}CNMR (100 MHz, DMSO-d_6): δ 161.80, 155.23, 151.16, 142.15, 129.82, 110.62, 105.16, 99.03, 87.14, 83.94, 70.70, 62.33, 36.59; ESI-MS calculated for $C_{13}H_{15}N_3O_5$ $[M+H]^+$ 294.11 and $[M+Na]^+$ 316.09, found 293.82 and 316.00, respectively.

7-phenoxyacetylaminoquinazoline-2,4(1*H*,3*H*)-dione deoxyribonucleoside (11). To a suspension of **2** (0.50 g, 1.7 mmol) in anhydrous pyridine (16 mL), chloro trimethylsilane (0.52 mL, 4.1 mmol) was added dropwise under argon. The reaction was stirred at 25 °C for 1 h.

Phenoxyacetic anhydride (0.63 g, 2.2 mmol) was added under argon. The reaction was stirred at 25 °C for 18 h. The reaction was cooled on ice, and cold water (10 mL) was added. The reaction was stirred at 0 °C for 15 min. Cold aqueous ammonium hydroxide (10 mL) was added, and the reaction was stirred at 0 °C for 15 min. The solvent was removed under reduced pressure, and the product was isolated by flash chromatography (2-5% methanol in dichloromethane). Product: white solid (0.547 g, 1.28 mmol, 75 %). ^{1}HNMR (400 MHz, DMSO-d_6): δ 11.51 (s, 1H), 10.39 (s, 1H), 8.104 (s, 1H), 7.94 (d, J = 8.4 Hz, 1H), 7.40 (d, J = 8.8 Hz, 1H), 7.31 (t, J = 7.2 Hz, 2H), 6.99 (m, 3H), 6.59 (t, J = 8.0 Hz, 1H), 5.24 (d, J = 5.2 Hz, 1H), 4.80 (m, 1H), 4.74 (s, 2H), 4.38 (m, 1H), 3.65 (m, 3H), 2.72 (m, 1H), 1.90 (m, 1H); ^{13}CNMR (100 MHz, DMSO-d_6): δ 167.21, 161.68, 158.97, 150.68, 143.34, 136.57, 128.00, 123.25, 113.83, 113.46, 107.52, 87.01, 84.98, 84.55, 72.72, 67.96, 65.02, 61.14, 37.22; ESI-MS calculated for $C_{21}H_{21}N_3O_7$ $[M+K]^+$ 466.10, found 466.04.

5′-Dimethoxytrityl-7*N*-phenoxyacetylaminoquinazoline-2,4(1*H*,3*H*)-dione deoxyribonucleoside (12). Anhydrous pyridine (7.5 mL), anhydrous triethylamine (237 μL, 1.7 mmol) and 4,4′-dimethoxytrityl chloride (0.58 g, 1.7 mmol) were added to **11** (0.50 g, 1.2 mmol) over argon. The reaction was stirred at room temperature for 16 hours and quenched with methanol (1.0 mL). The solvent was removed under reduced pressure, and the product was isolated by flash chromatography (1% triethylamine, 5% methanol, 94 % dichloromethane). Product: off-white solid (0.73 g, 0.96 mmol, 85 % yield). ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.54 (s, 1H), 8.33 (s, 1H), 8.13 (d, J = 8.4 Hz, 1H), 7.42 (d, J = 8.4 Hz, 1H), 7.35−7.30 (m, 12H), 7.19−7.05 (m, 6H), 6.95 (d, J = 7.6 Hz, 1H), 6.84 (t, J = 8.0 Hz, 1H), 6.71 (dd, J_1 = 8.8 Hz, J_2 = 3.2 Hz, 2H), 4.74 (m, 1H), 4.41 (d, J = 14.8 Hz, 1H), 4.24 (d, J = 15.2 Hz, 1H), 4.11 (dd, J_1 = 7.2 Hz, J_2 = 6.8 Hz, 1H), 4.02 (m, 1H), 3.69 (s, 6H), 3.49 (q, J = 7.2 Hz, 1H), 3.42 (dd, J_1 = 5.6 Hz, J_2 = 4.4 Hz, 1H), 3.03 (d, J = 7.2 Hz, 1H); ^{13}CNMR (100 MHz, $CDCl_3$): δ 166.76, 161.29, 158.59, 158.56, 156.98, 150.34, 145.09, 142.80, 141.00, 136.26, 136.23, 130.42, 130.40, 129.82, 128.49, 127.93, 126.91, 122.84, 115.09, 115.05, 113.24, 112.94, 106.99, 86.50, 84.43, 84.00, 72.20, 67.45, 64.52, 60.64, 55.38, 55.36, 53.08, 46.03, 36.64, 29.92; ESI-MS calculated for $C_{42}H_{39}N_3O_9$ $[M+Na]^+$ 752.26, found 752.23.

3′-2-Cyanoethyldiisopropylphosphoramidite-7*N*-phenoxyacetylaminoquinazoline-2,4(1*H*, 3*H*)-dione deoxyribonucleoside (3). Anhydrous dichloroethane (2.0 mL) and *N,N*-diisopropylethylamine (0.28 mL, 1.6 mmol) were added to **12** (0.10 g, 0.14 mmol). The reaction was cooled on ice, and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (38 μL, 0.17 mmol) was added. The reaction was stirred at room temperature for 18 h. The solvent was removed under reduced pressure, and the product was isolated by flash chromatography (1% triethylamine, 15–40% ethyl acetate in hexanes). Product: white foam (0.083 g, 0.088 mmol, 65 % yield). ^{1}HNMR (300 MHz, $CDCl_3$): δ 9.02 (b, 1H), 8.39 (s, 1H), 8.29 (d, J = 11.2 Hz, 1H), 8.19 (d, J = 8.4 Hz, 1H), 7.43−7.31 (m, 12H), 7.20−7.08 (m, 6H), 6.94 (d, J = 7.2 Hz, 1H), 6.89 (d, J = 7.2 Hz, 1H), 6.71 (m, 2H), 4.67 (m, 1H), 4.34 (m, 1H), 4.17 (m, 1H), 3.79 (m, 1H), 3.67 (s, 6H), 3.54 (m, 2H), 3.57 (m, 1H), 2.97 (m, 2H), 2.58 (t, J = 6.0 Hz, 2H), 2.38 (m, 1H), 1.16−1.00 (m, 12H); ^{31}PNMR (162 MHz, $CDCl_3$): δ 150.13, 149.91; ESI-MS calculated for $C_{51}H_{56}N_5O_{10}P$ $[M+Na]^+$ 952.37 and $[M+K]^+$ 968.34, found 952.27 and 968.22, respectively.

S.2 – Crystal Structure of 2

A colorless needle 0.15 × 0.02 × 0.02 mm in size was mounted on a Cryoloop with Paratone oil. Data were collected in a nitrogen gas stream at 100(2) K using phi and omega scans. Crystal-to-detector distance was 60 mm and exposure time was 10 seconds per frame using a scan width of 0.5°. Data collection was 98.1% complete to 67.00° in θ. A total of 6001 reflections were collected covering the indices, –7<=*h*<=8, –9<=*k*<=12, –22<=*l*<=21. 2269 reflections were found to be symmetry independent, with a R_{int} of 0.0245. Indexing and unit cell refinement indicated a primitive, orthorhombic lattice. The space group was found to be P2(1)2(1)2(1). The data were integrated using the Bruker SMART software program and scaled using the SADABS software program. Solution by direct methods (SIR-2004) produced a complete heavy-atom phasing model consistent with the proposed structure. All non-hydrogen atoms were refined anisotropically by full-matrix least-squares (SHELXL-97). All hydrogen atoms were placed using a riding model. Their positions were constrained relative to their parent atom using the appropriate HFIX command in SHELXL-97. The absolute stereochemistry was unambiguously determined to be *R*, *S*, and *R* at C9, C11, and C12 respectively.

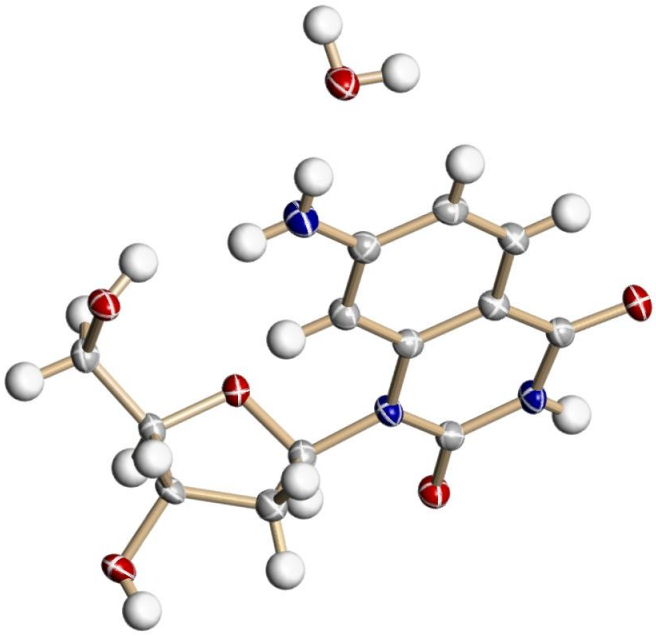


Figure S1 Crystal structure image of **2**.

Table S1. Crystal data and structure refinement for **2**.

X-ray ID	tor41	
Sample/notebook ID	ADDTop	
Empirical formula	C13 H17 N3 O6	
Formula weight	311.30	
Temperature	100(2) K	
Wavelength	1.54178 Å	
Crystal system	Orthorhombic	
Space group	P2(1)2(1)2(1)	
Unit cell dimensions	a = 6.837(2) Å	α= 90°.
	b = 10.470(5) Å	β= 90°.
	c = 18.506(6) Å	γ = 90°.
Volume	1324.7(9) $Å^3$	
Z	4	
Density (calculated)	1.561 Mg/m^3	
Absorption coefficient	1.064 mm^{-1}	
F(000)	656	
Crystal size	0.15 x 0.02 x 0.02 mm^3	
Crystal color/habit	colorless needle	
Theta range for data collection	4.78 to 68.50°.	
Index ranges	–7<=h<=8, –9<=k<=12, –22<=l<=21	
Reflections collected	6001	
Independent reflections	2269 [R(int) = 0.0245]	
Completeness to theta = 67.00°	98.1 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9790 and 0.8567	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2269 / 0 / 208	
Goodness-of-fit on F^2	1.059	
Final R indices [I>2sigma(I)]	R1 = 0.0358, wR2 = 0.0882	
R indices (all data)	R1 = 0.0409, wR2 = 0.0910	
Absolute structure parameter	-0.2(2)	
Extinction coefficient	0.0033(5)	
Largest diff. peak and hole	0.256 and -0.204 $e.Å^{-3}$	

S.3 – Oligonucleotide Synthesis and Purification

The unmodified oligonucleotide was purchased from Thermo Scientific. The modified oligonucleotide was synthesized on a Biosearch Cyclone Plus DNA synthesizer using a 1 μmole scale 500 Å CPG column. Phosphoramidite **3** was site specifically incorporated into the oligonucleotide by trityl-off synthesis of the base oligonucleotide, followed by manual coupling of phosphoramidite **3**. Typically, the modified phosphoramidite was dissolved in 100 μL of anhydrous acetonitrile to give a final concentration of 0.1M. The phosphoramidite solution was pushed into the CPG column via syringe and then 200 μL of 0.45M 1*H*-tetrazole was pushed into the other end of the column via syringe. Coupling reactions, performed twice, were allowed to proceed for 5 minutes (99% coupling efficiency) and were subsequently followed by standard oxidation and capping steps. The rest of the oligonucleotide was synthesized via the standard trityl-off procedure. Upon completion of the oligonucleotide synthesis, the CPG column was treated with 3 mL of 30% aqueous ammonium hydroxide for 2 h at room temperature, mixing via syringe every 1 h. The resulting solution was removed and the CPG column was treated with 1 mL of 30% aqueous ammonium hydroxide at room temperature for 15 min, mixing via syringe every 5 min. The resulting aqueous ammonium hydroxide solutions were consolidated and incubated at 55 ºC for 96 h. The aqueous ammonium hydroxide solution was freeze-dried. The DNA was purified by 20% polyacrylamide gel electrophoresis. The oligonucleotide was visualized by UV shadowing; bands were excised from the gel and extracted with 0.5M sodium acetate buffer (pH 7.0) overnight. The resulting solution was filtered (Bio Rad poly-prep chromatography column) and desalted using a Sep-Pak cartridge (Waters Corporation, MA). The following 260 nm extinction coefficients were used to determine the concentration of oligonucleotides: dG = 11,700, dC = 7,300, dA = 15,400, dU = 10,100, and **2** = 6,000.

S.4 –MALDI-TOF MS of Oligonucleotide

The MW of the modified oligonucleotide was determined via MALDI-TOF MS. 1 μL of a ~200 μM stock solution of the synthesized DNA was combined with 1 μL of 100 mM ammonium citrate buffer (PE Biosystems), 1 μL of a 75 μM DNA standard (5′-GCTGAATACATAAGACG-3′) and 4 μL of saturated 3-hydroxypicolinic acid. The samples were desalted with an ionexchange resin (PE Biosystems) and spotted onto a gold-coated plate where they were air dried. The resulting spectra were calibrated relative to the +1 and +2 ions of the internal DNA standard (m/z: 5225.79 and 2613.40). MALDI-TOF MS calc. for the +1 ion: 4081.65; found 4079.84.

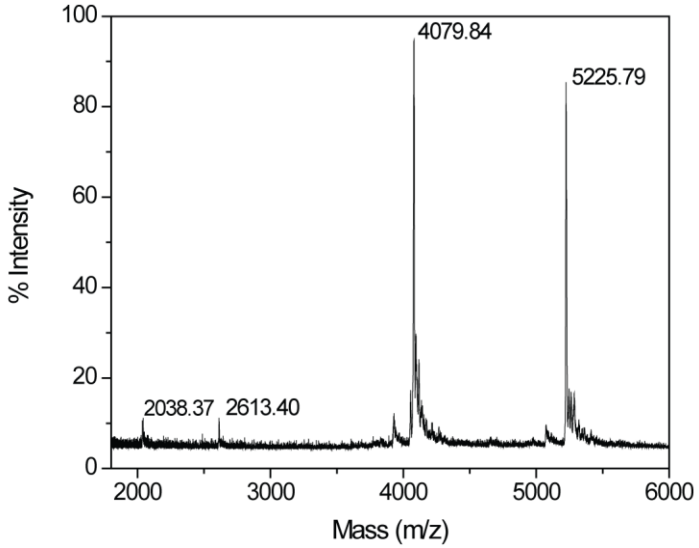


Figure S2: MALDI-TOF MS spectrum of the modified DNA calibrated relative to the +1 and +2 ions of an internal 17-mer DNA standard (m/z: 5225.79 and 2613.40). Calculated mass = 4081.65; observed mass = 4079.84.

S.5 – Photophysical Studies of Oligonucleotides

Steady state fluorescence experiments were carried out at 21°C in a 150 µL quartz fluorescence cell with a path length of 1.0 cm (Hellma GmbH & Co KG, Müllheim, Germany) on a Jobin Yvon Horiba FluoroMax-3 luminescence spectrometer with excitation and emission slit-widths of 8 nm. DNA samples were hybridized by heating to 90 ºC for 5 min and subsequently allowed to cool to room temperature over 2 h prior to measurements. DNA samples were measured at 5×10^{-6} M concentration in 20 mM sodium phosphate buffer (pH 7.0, 500 mM NaCl).

S.6 – Thermal Denaturation Measurements

All hybridizations and UV melting experiments were carried out at 5×10^{-6} M concentration in 20 mM sodium phosphate buffer (pH 7.0, 500 mM NaCl), using a Beckman-Coulter DU® 640 spectrometer with a high performance temperature controller and micro auto six holder. Samples were heated to 90 ºC for 5 min, cooled to room temperature over 2 h, and placed on ice for 30 min prior to measurements. Samples were placed in a stoppered 1.0-cm path length cell (Beckman-Coulter) and a background spectrum (buffer) was subtracted from each sample. Denaturation runs were performed between 25 and 90 ºC at a scan rate of 1.0 ºC min^{-1} with optical monitoring every ºC at 260 nm. Beckman-Coulter software (provided with T_m Analysis Accessory for DU® Series 600 Spectrometers) determined the melting temperatures utilizing the first derivative from the melting profile.

4 5'-GCG ATG **2** GTA GCG-3'
5 3'-CGC TAC **G** CAT CGC-5'
6 3'-CGC TAC **T** CAT CGC-5'
7 3'-CGC TAC **A** CAT CGC-5'
8 3'-CGC TAC **C** CAT CGC-5'
9 5'-GCG ATG **A** GTA GCG-3'

Figure S3: Oligonucleotides used in thermal denaturation experiments.

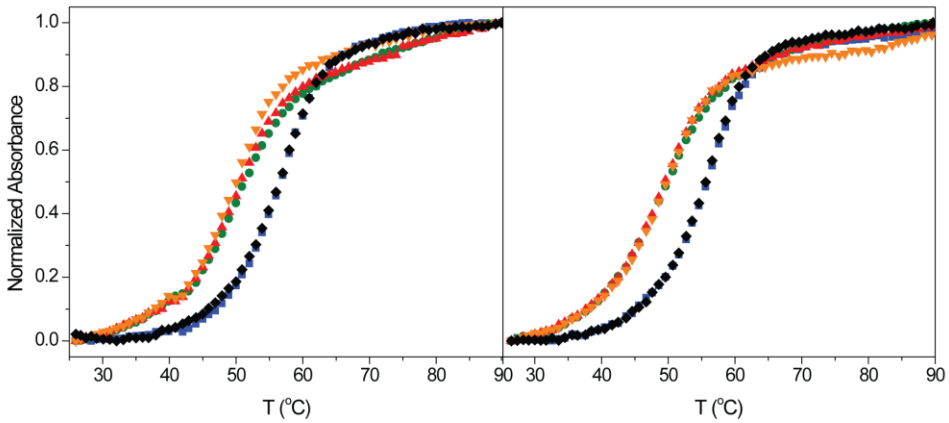


Figure S4: (A) Thermal denaturation curves of **4 • 5** (green), **4 • 6** (orange), **4 •7** (blue), **4 • 8** (red), and **6 • 9** (black). (B) Reverse thermal denaturation curves of **4 • 5** (green), **4 • 6** (orange), **4 •7** (blue), **4 • 8** (red), and **6 • 9** (black).