Fluorescent 1,10-phenanthroline-containing oligonucleotides distinguish between perfect and mismatched base pairing

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Supporting Information:

Supplementary Information

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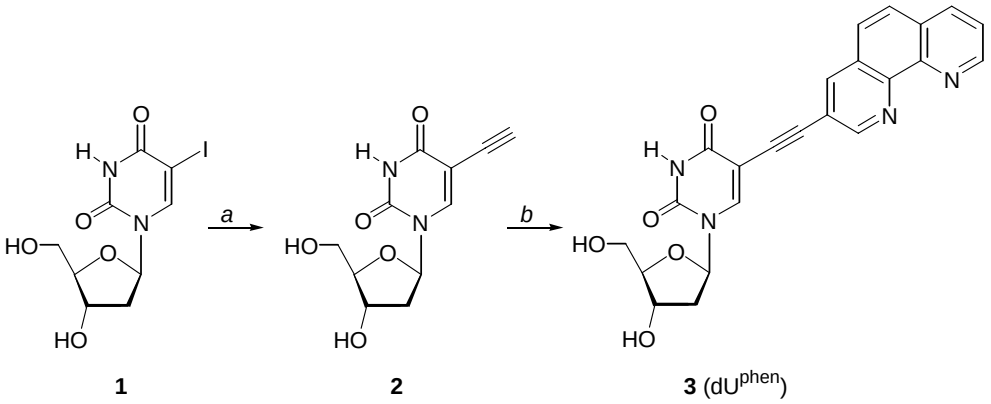
General Procedures. NMR spectra were recorded on a Varian Mercury 300 MHz or Varian Mercury 400 MHz spectrometer. Mass spectra were measured at the UCSD Mass Spectrometry Facility, utilizing an ESI with a quadrapole ion trap: manufactured by ThermoFinnigan Corp. High Resolution mass spectra were measured at the Scripps Research Institute. UV-Vis spectra were recorded on a Hewlett Packard 8452 A Diode Array Spectrophotometer. Unless otherwise specified, materials were obtained from commercial suppliers and used without further purification. Anhydrous DMF was obtained from Fluka. Anhydrous acetonitrile and triethylamine were each freshly distilled from calcium hydride. All oxygen and water sensitive reactions were carried out under argon. Unless otherwise stated, all reactions were carried out at room temperature.

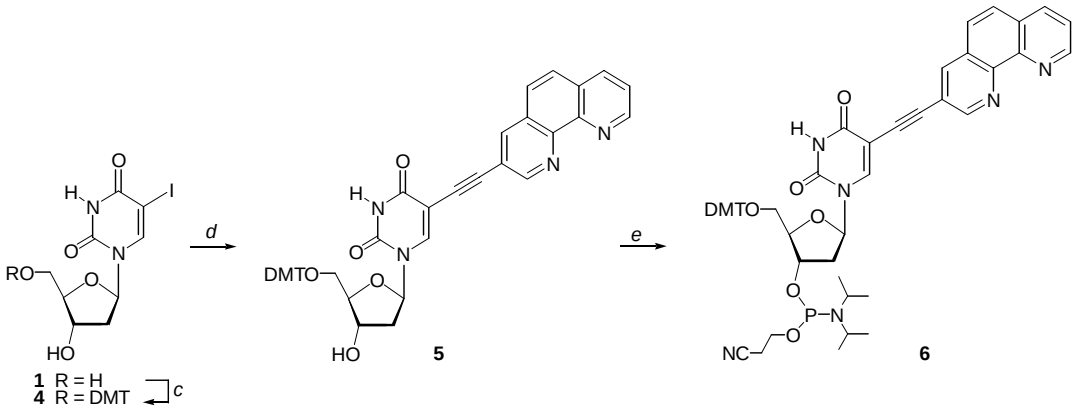
Scheme S1. Synthesis of 5-[(1,10-phenanthrolin-3-yl)ethynyl]-2'-deoxyuridine **3.**



[a] Reagents and conditions: (a) (i) $(CF_3CO)_2O$; (ii) Me_3Si-C≡CH, $(Ph_3P)_4Pd$, CuI, DMF, Et_3N, 95–98%; (iii) K_2CO_3, MeOH, 75–92%; (b) 3-bromo-1,10-phenanthroline, $(dppf)PdCl_2 \cdot CH_2Cl_2$, CuI, DMF, Et_3N, 72–84%.

5-[(1,10-phenanthrolin-3-yl)ethynyl)]-2'-deoxyuridine 3. 3-bromo-1,10-phenanthroline (120 mg, 0.37 mmol), $(dppf)PdCl_2$ (28 mg, 0.034 mmol), and CuI (1.5 mg, 0.017 mmol), were dissolved in a mixture of DMF/Et_3N (4:1, 1.25 mL). Separately, 5-ethynyl-2'-deoxyuridine (0.16 mg, 0.45 mmol) was dissolved in a mixture of DMF/Et_3N (4:1, 1.25 mL) and was cannulated into the reaction mixture. The solution was sonicated for 18 h, then concentrated. The crude mixture was purified by flash chromatography over 50 mL silica gel (2-25% CH_3OH/2% Et_3N/CH_2Cl_2). Following the concentration of product containing fractions, the pale yellow solid was washed with H_2O (3 mL) and acetonitrile (3 mL). The solvents were removed to yield 192 mg of a pale yellow powder (51% yield). IR (KBr) 2216 cm^{-1} (-C≡C-) ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.79 (s, 1H, N-3), 7.79-9.11 (m, 7H, phen), 6.15 (dd, J_1= 3.0, J_2= 0.6 Hz, 1H, H-1'), 5.29 (d, J=2 Hz, 1H, OH-3'), 5.24 (s, 1H, OH-5'), 4.28 (m, 1H, H-3'), 3.83 (m, 1H, H-4'), 3.65 (m, 1H, H-5'), 2.19 (m, 1H, H-2'); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 161.53, 151.09, 150.52, 149.57, 145.30, 144.97, 144.28, 138.11, 136.46, 129.06, 127.86, 127.80, 126.35, 123.77, 118.69, 97.67, 89.11, 87.71, 86.92, 85.07, 69.88, 60.80, 40.27; UV(H_2O) λ_{max} nm (ε × 10^{-4}) 248 (1.9), 260 (1.8), 278 (2.0), 296 (1.8), 330 (2.0), 345 (1.8); MALDI-FTMS calcd for $C_{23}H_{18}N_4O_5$ $[M+H]^+$ 431.1350, found 431.1344.

Scheme S2. Synthesis of 3-dimethoxytrityl-5-[(1,10-phenanthrolin-3-yl)ethynyl]-2'-deoxyuridine phosphoramidite **6**.

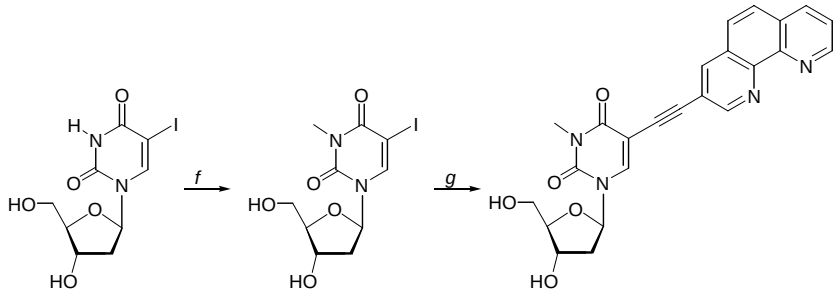


Reagents and conditions: c) 4,4'-dimethoxytrityl chloride (DMT-Cl), DMAP, pyridine, Et_3N, 92%; (d) 3-ethynyl-1,10-phenanthroline, (dppf)$PdCl_2$•CH_2Cl_2, CuI, DMF, Et_3N, 73–76%; (e) (iPr$_2$N)$_2$POCH_2CH_2CN, 1*H*-tetrazole, CH_3CN 81%.

DMT-dU^{phen} 5. The DMT-protected nucleoside **4** (1.014 g, 1.54 mmol), (dppf)$PdCl_2$ (102 mg, 0.077 mmol), and CuI (8.6 mg, 0.042 mmol) were dissolved in a degassed solution of DMF/Et_3N (4:1, 22 mL). In a separate flask, 3-ethynyl-1,10-phenanthroline (346 mg, 1.69 mmol) was dissolved in a degassed solution of DMF/Et_3N (4:1, 22 mL) and cannulated into the above mixture. The reaction was sonicated for 18 h, then concentrated. Following removal of the solvents in vacuo, the crude solid was purified by flash chromatography over 250 mL silica gel (0-8% CH_3OH/CH_2Cl_2). The product-containing fractions were concentrated, brought up in 300 mL CH_2Cl_2, then washed with a saturated solution of sodium bicarbonate (1 × 25 mL) and water (2 × 25 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated to yield 967 mg of a pale yellow powder (86% yield). IR (KBr) 2216 cm^{-1} (-C≡C-); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.11 (d, J=3.0 Hz, 1H, 9-phen), 8.9 (s, 1H, 2-phen), 8.45 (s, 1H, H-6), 8.16 (m, 2H, 4-phen), 8.15 (d, J=1.0 Hz, 1H, 7-phen), 7.75 (dd, J_1= 12.0, J_2= 2.0 Hz, 2H, 5,6-phen), 7.59-7.61 (m, 1H, 8-phen), 6.45 (dd, J_1= 3.0, J_2= 0.5 Hz, 1H, H-1'), 4.65 (m, 1H, H-3'), 4.17 (m, 1H, H-4'), 3.55 (s, 3H, -OCH_3), 3.53 (s, 3H, -OCH_3), 3.46 (m, 1H, H-5'), 2.64 (m, 1H, H-2'); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 162.24, 158.60, 151.72, 150.46, 149.91, 145.79, 144.64, 144.37, 143.39, 138.51, 136.08, 135.65, 129.97, 129.93, 128.88, 128.10, 127.91, 127.42, 127.04, 126.97, 126.17, 123.19, 119.00, 113.38, 99.83, 90.55, 87.06, 86.82, 86.09, 84.72, 72.07, 63.48, 55.04, 41.83; ESI-MS calcd for $C_{23}H_{18}N_4O_6$ $[M+H]^+$ 733, found 733.

DMT-dU^{phen}-amidite 6. Protected nucleoside **5** (100 mg, 0.137 mmol) was dissolved in anhydrous CH_2Cl_2 (2 mL) and treated with 1*H*-tetrazole (9 mg, 0.130 mmol) in anhydrous CH_3CN (0.5 mL). 2-cyanoethyl-*N*,*N*,*N*',*N*'-tetraisopropyl-diphosphoramidite (54 mg, 0.18 mmol) was added to the solution, which was stirred for 1 h. The solution was diluted in cold CH_2Cl_2/0.25% Et_3N (300 mL) and washed with a saturated solution of sodium bicarbonate (2 × 25 mL) and brine (1 × 25 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography over a silica gel (40 mL). The column was eluted first with 0.5% MeOH/0.25% Et_3N/CH_2Cl_2 to remove impurities. The product was then cleanly eluted with 3% CH_3OH/CH_2Cl_2 to yield 57 mg of a pale yellow foam (45% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) 9.14 (d, *J*=4.0 Hz, 1H, 9-phen), 8.85 (s, 1H, 2-phen), 8.52 (s, 1H, H-6), 8.19 (m, 2H, 4-phen), 7.72 (d, *J*=1.0 Hz, 1H, 7-phen), 7.59 (dd, J_1= 12.0, J_2= 2.0 Hz, 2H, 5,6-phen), 7.45-7.48 (m, 1H, 8-phen), 6.36 (dd, J_1= 3.0, J_2= 0.5 Hz, 1H, H-1'), 4.67 (m, 1H, H-3'), 4.20 (m, 1H, H-4'), 3.51-3.59 (m, 10H, 6H from, -OCH_3, 1H from H-5', 2H from -cyanoethyl, 1H from isopropyl), 3.39 (m, 1H, H-5'), 2.61 (t, *J*= 9.0 Hz, 2H, O-CH_2-CH_2CN), 2.51 (m, 1H, H-2'), 1.15 (s, 9H, N-CH(CH_3)$_2$). 1.05 (s, 3H, N-CH(CH_3)$_2$); ^{31}P NMR ($CDCl_3$, referenced to an internal 80% H_3PO_4 capillary, 269 MHz) δ 148.9, 148.5; ESI-MS calcd for $C_{23}H_{18}N_4O_6$ $[M+H]^+$ 933, found 933, [M+Na] calcd 955, found 955.

Scheme S3. Synthesis of 3-methyl-5-[(1,10-phenanthrolin-3-yl)ethynyl]-2'-deoxyuridine **16**.



1 **17** **15**

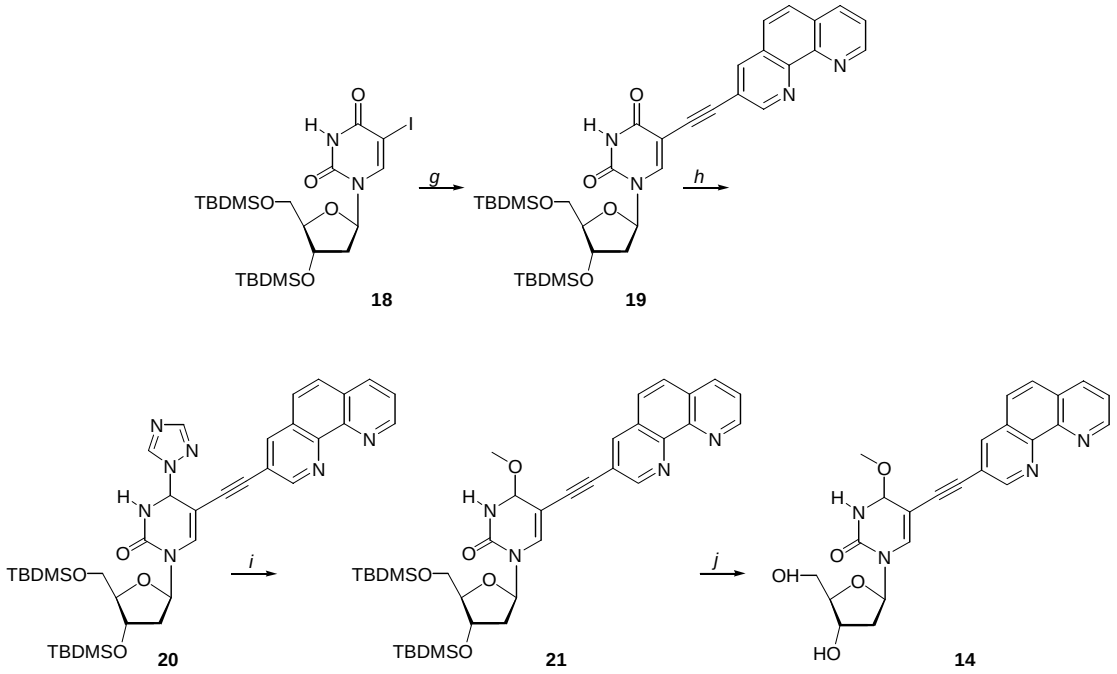
Reagents and conditions: (f) trimethylphosphate, H_2O 72%; (g) 3-ethynyl-1,10-phenanthroline, (dppf)$PdCl_2$•CH_2Cl_2, CuI, DMF, Et_3N, 73–76%.

3-methyl-5-iodo-uridine 17. 5-Iodo-2'-deoxyuridine **1** (350 mg, 1.0 mmol) and trimethylphosphate (5 mL, 30 mmol) were stirred in water (15 mL, pH 10, NaOH) at 37 ºC for 48 h. After the solvent was remove in vacuo, the crude material was purified over 75 mL silica gel (15% CH_3OH/CH_2Cl_2) to give 270 mg of a white solid (73% yield). ^{1}H-NMR (300 MHz, D_2O) δ 8.21 (s, 1H, H-6), 6.09 (dd, J_1= 2.7, J_2= 0.6 Hz, 1H, H-1'), 4.28 (m, 1H, H-3'), 3.88 (m, 1H, H-4'), 3.66 (m, 1H, H-5'), 3.18 (s, 3H, H-N3), 2.23 (m, 1H, H-2'); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 161.06, 151.16, 144.31, 88.04, 86.92, 70.59, 68.33, 61.38, 41.03, 29.98; ESI-MS calcd for $C_{10}H_{13}IN_2O_5$ $[M+H]^+$ 368.9, found 368.7.

3-methyl-5-[(1,10-phenanthrolin-3-yl)ethynyl)]-2'-deoxyuridine 15. The *N*-methylated nucleoside **17** (140 mg, 0.37 mmol), (dppf)$PdCl_2$ (16 mg, 0.020 mmol), and CuI (4.0 mg, 0.060 mmol) were dissolved in a degassed solution of DMF/Et_3N (4:1, 1.25 mL). Separately, 3-ethynyl-1,10-phenanthroline was dissolved in a degassed mixture of DMF/Et_3N (4:1, 1.25 mL), and was cannulated into the reaction mixture. The reaction mixture was heated to 80 ºC and stirred for 3 days, then concentrated. The crude mixture was purified by flash chromatography over 75 mL silica gel (2-20% CH_3OH/CH_2Cl_2, yielding 72 mg of a pale yellow powder (49% yield). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.77-9.11 (m, 7H, phen), 6.17 (dd, J_1= 6.0, J_2= 2.7 Hz, 1H, H-1'), 5.31 (br, 2H, OH-3', 5'), 4.27 (m, 1H, H-3'), 3.84 (m, 1H, H-4'), 3.66 (m, 1H, H-5'), 3.21 (s, 3H, CH_3N), 2.02 (m, 1H, H-2'); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 160.95, 151.32, 151.18, 149.94, 145.44, 144.42, 144.28, 138.14, 136.46, 129.26, 128.01, 127.35, 126.67, 123.11, 118.93, 97.04,

89.16, 87.51, 86.63, 82.53, 70.01, 60.80, 42.76, 40.27; MALDI-FTMS calcd for $C_{24}H_{20}N_4O_5$ $[M+H]^+$ 445.1506, found 445.1520.

Scheme S4. Synthesis of 4-methoxy-5-[(1,10-phenanthrolin-3-yl)ethynyl]-2'-deoxyuridine **14**



Reagents and conditions: (g) 3-ethynyl-1,10-phenanthroline, (dppf)$PdCl_2$•CH_2Cl_2, CuI, DMF, Et_3N, 73–76%; (h) triazole, Et_3N, $POCl_3$, CH_3CN, 89%; (i) DBU, CH_3OH, 19%; (j) TBAF, THF 88%.

3', 5'-O-TBDMS-5-iodouridine 18. A solution of 5-iodo-2′-deoxyuridine **1** (1.0 g, 4.1 mmol) in DMF (12 mL) was treated with imidazole (1.2 g, 9.1 mmol) and the suspension was stirred until solvation was complete. *Tert*-butyldimethylsilyl chloride (1.4 g, 9.08 mmol) was then added and the solution was stirred for 14 h. The reaction mixture was concentrated to 6 mL, then diluted with water (6 mL). The product was extracted into ether (10 mL) and the organic layer was washed with 1M $NaHCO_3$ (3 mL) followed by 1M NH_4Cl (3 mL). The ether was dried over Na_2SO_4 and evaporated to yield 1.46 g of a white solid. This compound was used without further purification (89% crude yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.04 (s, 1H, H-6), 6.25 (dd, J_1= 6.0, J_2= 3.1 Hz, 1H, H-1'), 4.37-4.38 (m, 1H, H-3'), 3.97 (m, 1H, H-4'), 3.73-3.89 (m, 1H, H-5'), 1.97-2.19 (m, 1H, H-2'), 0.87 (m, 18H, *t*-Bu), 0.06-0.14 (m, 12H, $SiCH_3$); ^{13}C-NMR (100 MHz,

DMSO-d_6) δ 160.11, 149.74, 143.78, 86.95, 84.48, 71.85, 69.91, 62.53, 39.93, 25.99, 25.67, 18.15, 17.73, -4.72, -4.87, -5.20, -5.22; ESI-MS calcd for $C_{21}H_{39}IN_2O_5Si_2$ $[M+H]^+$ 583, found 583, $[MNa^+]$ calcd 605, found 605, $[M-H]^-$ 581, calcd found 581, $[M+Cl]^-$ calcd 617, found 617.

5-[(1,10-phenanthrolin-3-yl)ethynyl)]- 3', 5'-O-TBDMS -2'-deoxyuridine 19.

The TBDMS-protected nucleoside **18** (0.32 g, 0.54 mmol), (dppf)$PdCl_2$ (23 mg, 0.030 mmol), and CuI (3.1 mg, 0.060 mmol) were dissolved in a degassed solution of DMF/Et_3N (4:1, 1.25 mL). Separately, 3-ethynyl-1,10-phenanthroline was dissolved in a degassed solution of DMF/Et_3N (4:1, 1.25 mL), and cannulated into the above mixture. After stirring for 16 h, the reaction mixture was concentrated. The residue was taken up in CH_2Cl_2 (25 mL), washed with water (10 mL), 1M EDTA (10 mL, pH=8), and again with water. The crude solid was purified by flash chromatography over 60 mL silica gel (0-4% CH_3OH/CH_2Cl_2) yielding 100 mg of a pale yellow powder (28% yield). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.19 (s, 1H, H-6) 7.65-9.18 (m, 7H, phen), 6.33 (dd, J_1= 6.3, J_2= 3.1 Hz, 1H, H-1'), 4.41 (m, 1H, H-3'), 4.00 (m, 1H, H-4'), 3.75-3.96 (m, 1H, H-5'), 2.07-2.31 (m, 1H, H-2'). ^{13}C-NMR (100 MHz, DMSO-d_6) δ 160.97, 150.44, 150.09, 149.02, 144.84, 143.62, 143.37, 137.83, 136.07, 128.73, 127.58, 127.40, 125.95, 123.48, 118.23, 96.98, 87.04, 86.94, 86.43, 84.89, 71.45, 62.30, 40.30, 25.85, 25.67, 18.07, 17.73, -4.67, -4.85, -5.33, -5.38.; ESI-MS calcd for $C_{35}H_{46}N_4O_5Si_2$ $[M+H]^+$ 659, found 659, $[MNa^+]$ calcd 681, found 681, $[M-H]^-$ 657, calcd found 657, $[M+Cl]^-$ calcd 693, found 693.

4-(1,2,4-triazol-1-yl)-5-[(1,10-phenanthrolin-3-yl)ethynyl)]-3', 5'-O-TBDMS -2'-deoxyuridine 20. Triazole (0.52 g, 9.1 mmol) was dissolved in dry acetonitrile (10 mL) at 0 ºC. $POCl_3$ (0.455 mmol) was added dropwise at 0 ºC, followed by a dropwise addition of Et_3N (0.73 mL, 9.9 mmol) and the mixture was stirred at 0 ºC for 45 min. A solution of the nucleoside **19** (100 mg, 0.15 mmol) in dry acetonitrile (10 mL) was cannulated into the reaction mixture. The reaction mixture was stirred for 3 h, then diluted with 1M $NaHCO_3$ (10 mL). The product was then extracted into dichloromethane (25 mL). The organic layer was washed with 1M $NaHCO_3$ (7 mL), and water (7 mL), then dried over Na_2SO_4, and evaporated to yield 114 mg of a yellow solid (89% yield). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.29 (s, 1H, H-5'), 8.80 (s, 1H, H-3'), 7.67-9.22 (m, 7H,

phen), 6.29 (dd, J_1= 6.2, J_2= 3.2 Hz, 1H, H-1'), 4.41 (m, 1H, H-3'), 4.13 (m, 1H, H-4'), 3.78-4.05 (m, 1H, H-5'), 2.09-2.74 (m, 1H, H-2'), 0.87-0.77 (m, 18H, t-Bu), 0.06-0.14 (m, 12H, SiMe); ESI-MS calcd for $C_{37}H_{47}N_7O_4Si_2$ $[M+H]^+$ 710, found 710, $[M+Cl]^-$ calcd 744, found 744.

4-methoxy-5-[(1,10-phenanthrolin-3-yl)ethynyl)]-3', 5'-O-TBDMS -2'-deoxyuridine 21. A solution of the 4-triazole nucleoside **20** (0.15 g, 0.34 mmol) in dry acetonitrile (3 mL) was treated with DBU (56.1 mg, 0.37 mmol) and methanol (17 mL, 6.7 mmol). The mixture was stirred for 5 h, and then was neutralized by adding glacial acetic acid. The solution was concentrated and the residue was purified by flash chromatography over 75 mL silica gel (50% hexanes/ethyl acetate), yielding 30 mg of a white solid (19% yield). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.65-8.43 (m, 7H, phen), 7.71 (s, 1H, H-6), 6.31 (dd, J_1= 6.2, J_2= 3.2 Hz, 1H, H-1'), 4.32-4.35 (m, 1H, H-3'), 4.09 (s, 3H, O-Me), 3.71-3.94 (m, 2H, H-4', H-5'), 2.40-2.45 (m, 1H, H-2'), 0.85-1.87 (m, 18H, t-Bu), 0.02-0.07 (m, 12H, SiMe); ESI-MS calcd for $C_{21}H_{39}IN_2O_5Si$ $[M+H]^+$ 673, found 673, $[MNa^+]$ calcd 695, found 695, $[M-H]^-$ 671, calcd found 671, $[M+Cl]^-$ calcd 707, found 707.

4-methoxy-5-[(1,10-phenanthrolin-3-yl)ethynyl)]-2'-deoxyuridine 14. To a solution of the *O*-Me-nucleoside **21** (14 mg, 0.021 mmol) in THF (1.0 mL), TBAF (1M in THF, 0.05 mL) was added dropwise, and the solution was stirred for 16 h. The reaction solution was then diluted with ethyl acetate (2 mL) and washed with water (1 mL). The organic phase was dried over Na_2SO_4 and concentrated to give 14 mg pale of a yellow solid (88% yield). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.55-9.00 (m, 7H, phen), 6.07 (dd, J_1= 3.0, J_2= 2.8 Hz, 1H, H-1'), 4.27-4.29 (m, 1H, H-3'), 4.01 (s, 3H, -OCH_3), 3.96 (m, 1H, H-4'), 3.74-3.96 (m, 1H, H-5'), 2.16-2.49 (m, 1H, H-2'); ^{13}C (101 MHz, DMSO-d_6) δ 161.07, 151.42, 150.66, 149.57, 145.30, 144.97, 144.28, 138.11, 136.05, 130.37, 127.52, 127.10, 125.09, 124.69, 118.13, 97.67, 89.11, 87.71, 86.92, 85.65, 69.44, 65.71, 61.59, 40.63; MALDI-FTMS calcd for $C_{24}H_{20}N_4O_5$ $[M+H]^+$ 445.1506, found 445.1497.

Oligonucleotide Synthesis and Characterization

Oligonucleotides were synthesized using standard solid-phase phosphoramidite chemistry as previously described (Hurley & Tor, *J. Am. Chem. Soc.*, **1998**, *120*, 2194; Hurley & Tor, *J. Am. Chem. Soc*., **2002**, *124*, 3749). The coupling efficiency of the encorporation of the dU^{phen}-containing nucleoside into the oligonucleotide was 45%. Purification was accomplished by preparative polyacrylamide gel electrophoresis and reversed phase HPLC. To verify the integrity and composition of the dU^{phen}-containing strands **7** and **9**, the oligonucleotides were enzymatically digested as previously described, and HPLC analysis of the resulting nucleoside mixture was employed to verify the presence of the intact dU^{phen}-containing nucleoside in the modified oligonucleotides (Table S1).

Table S1. The Enzymatic Digestion of dU^{phen}-containing Oligonucleotides **7** and **9**.

Sequence	dC	DG	dT	dA	dU^{phen}
9	4.0 (4.0)	4.0 (4.5)	5.0 (5.6)	5.0 (5.0)	1.0 (0.5)
7	4.0 (4.0)	4.0 (4.5)	4.0 (4.5)	6.0 (6.2)	1.0 (0.3)

As the dU^{phen} nucleoside was exposed to multiple cycles of DNA synthesis, confirmation that no degradation of the nucleosides had occurred was essential. In the enzymatic digestions of **7** and **9**, the dU^{phen}-containing nucleoside elutes cleanly as a single peak, with the exact same retention time and UV-Vis absorption spectra as the nucleoside standard **3**. The lack of dU^{phen}-containing nucleoside degradation products in the digestion of **9** confirms that the modified nucleoside is stable toward multiple cycles of DNA synthesis. The lower-than-expected dU^{phen} content determined for each strand may be due to the hydrophobicity of the nucleoside, resulting in binding of the free nucleoside to the digestion filter prior to HPLC injection.

Thermal Denaturation Studies

All UV melting experiments were carried out in 100 mM NaCl, 10 mM sodium phosphate buffer at pH 7.0 using a Varian Cary 1E Spectrophotometer with a temperature-controlled

cell compartment. Samples (double stranded concentration: 1-2 μM) were heated to 90 ºC for 5 min and cooled to room temperature for 2-3 h before measurements. Samples were placed in a stoppered 1.0-cm path length cell. Heating runs were performed between 25 and 90 ºC at a scan rate of 0.5 ºC min^{-1} with the optimal monitoring at 260 nm. T_m values were determined by a computer fit of the melting data, followed by calculation of the first derivative of the resulting melting curve. The melting temperature (T_m) values derived from the UV melting profiles are listed in Table S2. Uncertainty in T_m values is estimated to be ±0.5 ºC. (This value reflects the instrumentation error and not other potential experimental errors that are difficult to estimate. *Note, however, that the measurements under identical conditions have been performed to draw qualitative and comparative information between the oligonucleotides*.)

Table S2. Spectroscopically Measured T_m Values (260 nm) for Duplexes of Unmodified and Phenanthroline Containing Oligonucleotides[a]

DNA duplex	T_m (ºC)
7•8	60
9•10	57
9•11	57
9•12	54
9•13	56
10•16[b]	62

[a] Conditions: 1-2 μM duplex in 10 mM sodium phosphate pH 7.0, 100 mM NaCl.

[b] **16** 3'-A GCT AGT CAT ACT GAT CGT-5' Control strand without dU^{phen}

Steady-State Fluorescence Experiments

Steady-state fluorescence experiments were determined with excitation at 333 nm using a Perkin Elmer LS 50B luminescence spectrophotometer, with slit widths of 5 nm and a scan rate of 400 nm/min. $\lambda_{ex,\,max}$ for the phenanthroline containing nucleotides, degassed and at a concentration of 2.6 μM, were obtained by scanning the emission spectra in the region of 350-600 nm. Each of the samples were prepared by dissolving a known amount of the nucleoside in DMSO. 10μL of the DMSO solution was then diluted into 990 μL of the appropriate solvent. The emission maxima of the nucleotides are listed below in Table S3. The oligonucleotides were degassed and measured at a duplex concentration of 1.3 μM in 100 mM NaCl, 10 mM sodium phosphate buffer at pH 7.0. To study the

temperature dependence on emission, data was collected over 5 ºC increments, starting at 35 ºC. Samples were allowed to equilibrate at each temperature for 10 min prior to collection of emission spectra. The temperature dependence of the emission maxima for the oligonucleotides is listed in Table S3/ Figure 3.

Solvent Polarity Measurements

The dependence of emission energies on solvent polarity was determined in five different solvents: diethyl ether, dichloromethane, methanol, acetonitrile, and 100 mM NaCl, 10 mM sodium phosphate buffer at pH 7.0. (note: each sample contained 1% DMSO to increase the solubility of the free nucleosides) Emission spectra were converted from wavelength (nm) to frequency (cm^{-1}) units. $E_T(30)$ values of each of the three free nucleosides were experimentally determined for each solvent mixture using Reichardt's salt (C. Reichardt, *Chem. Rev.* **1994**, 94, 2319-2358).

Table S3. Spectroscopically Measured Emission Maxima (cm^{-1}) for Phenanthroline Containing Nucleotides in Various solvent Conditions.[a]

Solvent	$E_T(30)$	**3** (dU^{phen})	**14** ($^{OMe}dU^{phen}$)	**15** ($^{NMe}dU^{phen}$)
15%CH_3CN/H_2O	60.4	24225	24158	24295
CH_3OH	55.0	24002	24190	24086
CH_3CN	48.7	24681	24767	24591
CH_2Cl_2	41.2	25913	26048	25896
Diethyl ether	9.1	25761	25375	25981

[a] All solvent mixtures for $E_T(30)$ measurements included 1% DMSO.

List of Abbreviations

DBU	1,8-diazobicyclo[5.4.0]undec-7-ene
DMF	dimethylformamide
$(dppf)PdCl_2$	[1,1'-bis(diphenylphosphino)ferrocene]palladium(II)chloride
THF	tetrahydrofuran
TBAF	tetrabutylammonium fluoride

Figure S1. Normalized Emission Spectra of **3**, **14**, and **15**.

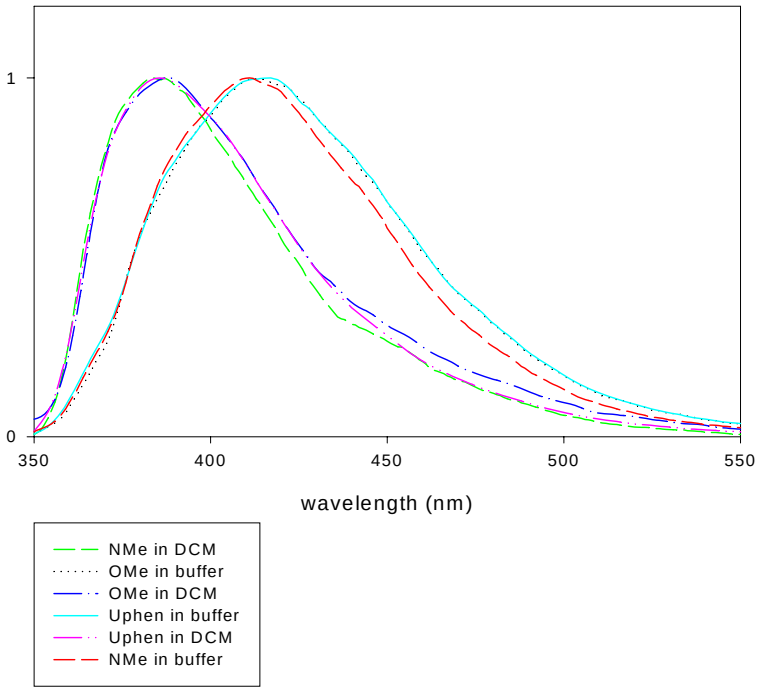


Figure S2. Normalized Absorption Spectra of **3**, **14**, and **15**.

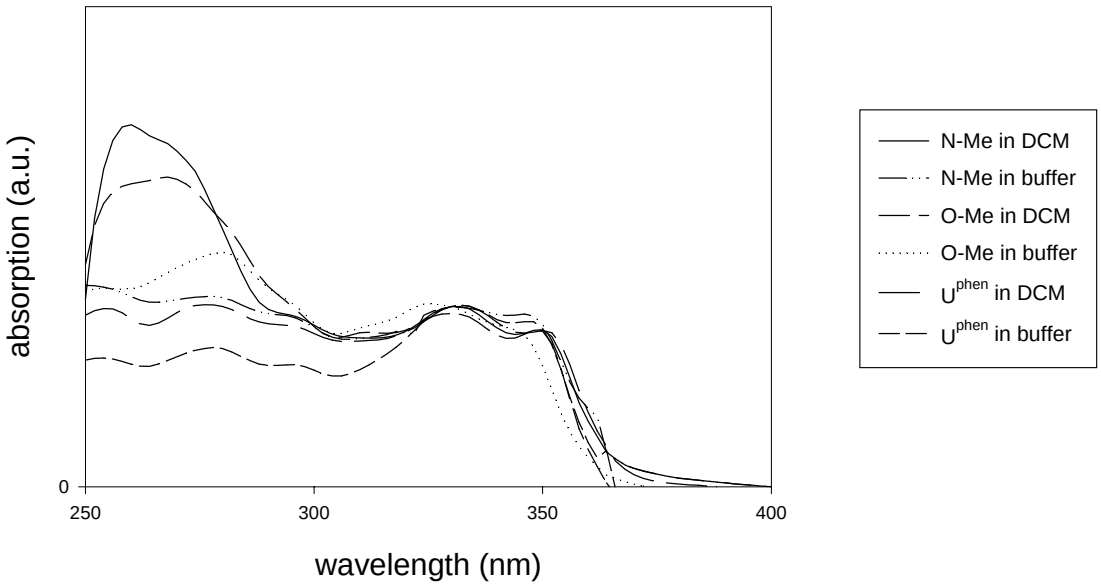


Figure S3. Emission Spectra of Duplex **7·8** as a Function of Temperature from 35 ºC (blue) to 75 ºC (olive).

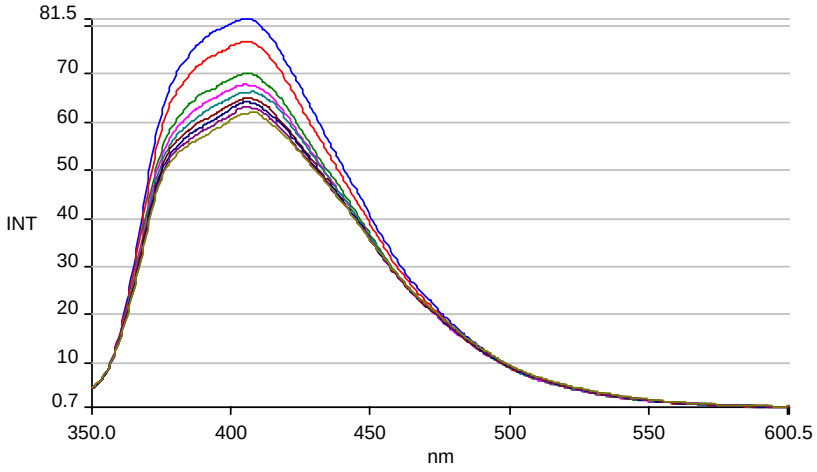


Figure S4. Emission-monitored thermal denaturation curves of **9·10** and **9·13** with excess **13**.

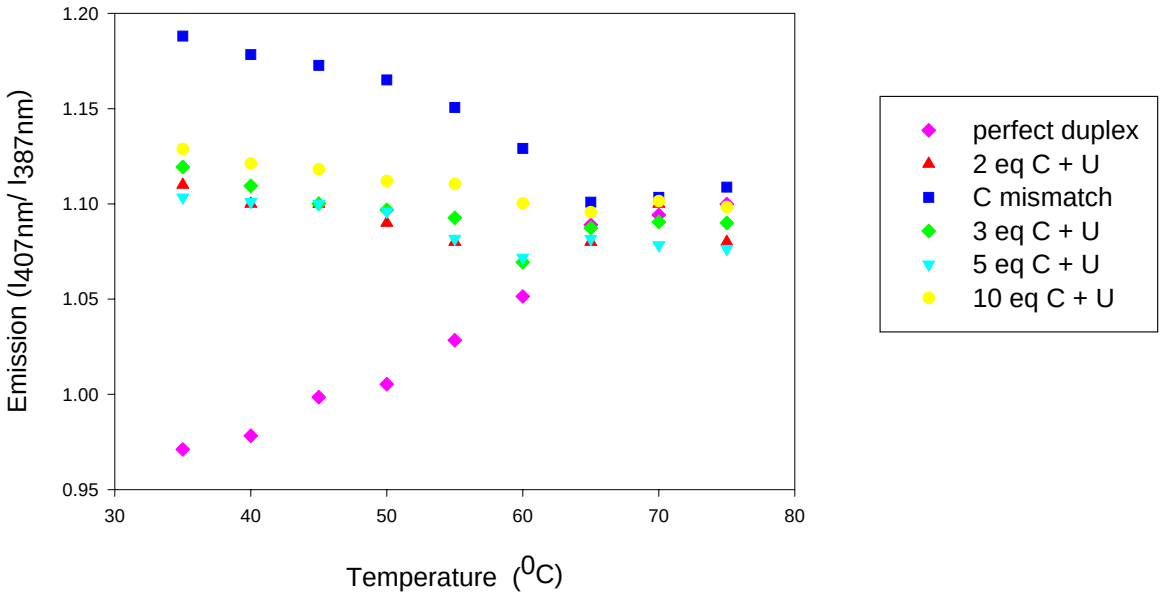
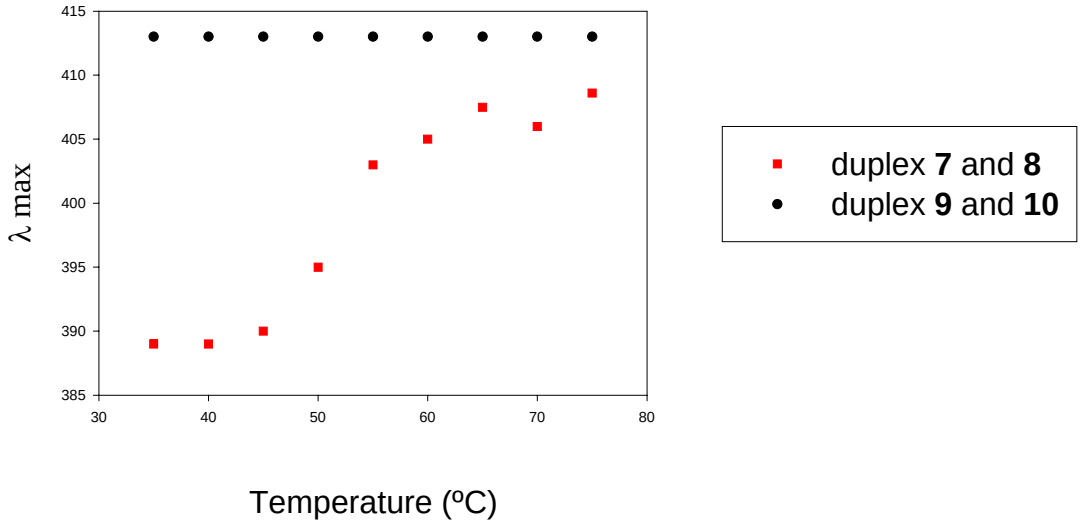


Figure S5. Temperature vs. λ_{max} for Duplexes **7·8** and **9·10**.



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