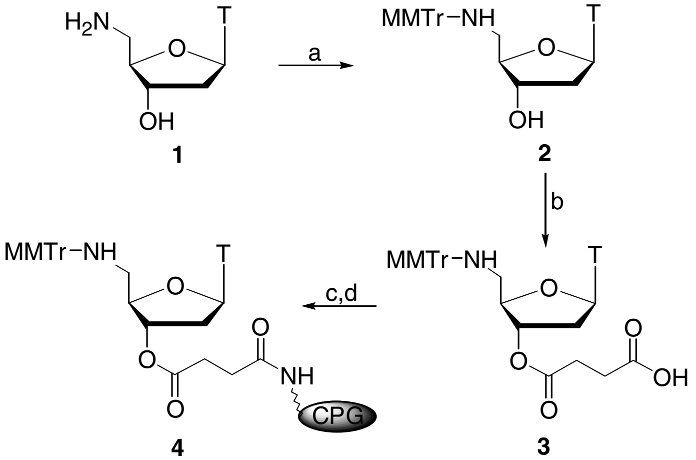
Supporting Material

Solid-Phase Synthesis of Positively Charged Deoxynucleic Guanidine (DNG) Tethering a Hoechst 33258 Analogue: Triplex and Duplex Stabilization by Simultaneous Minor Groove Binding

Putta Mallikarjuna Reddy and Thomas C. Bruice*

Contribution from the Department of Chemistry and Biochemistry
University of California at Santa Barbara, Santa Barbara, CA 9310

Synthetic scheme for loading of 5'- modified monomer on to CPG:



Scheme 1. *Reagents and conditions:* (a) MMTrCl 1.0 equiv., Pyridine, 0 °C – r.t., 6h; (b) Succinic anhydride 0.95 equiv., DMAP, Pyridine, 12h; (c) 4-Nitrophenol, DCC, Pyridine, 1,4-Dioxane; (d) CPG, TEA, DMF, 12h.

5'-Monomethoxytritylamino-2',5'-dideoxythymidine (2). Monomethoxytrityl chloride (4.51 g, 14.6 mmol) in dry pyridine (50 mL) was added drop wise to a solution of **1**[1] (2.35 g, 9.75 mmol) in dry pyridine (100 mL) at room temperature. The reaction mixture was stirred for overnight and solvent was rotoevaporated under reduced pressure. The residue was partitioned between water and DCM, and aqueous layer extracted two more times with DCM. The combined DCM layer was dried and rotoevaporated. The crude product was purified on silica gel column chromatography using 0 - 2% methanol in DCM as eluent, yield 4.5 g (90%). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 1.68 (s, 3H, T-C*H_3*), 2.06 (m, 1H, 2'-H), 2.15 (m, 1H, 2"-H), 2.17 (m, 1H, 5'-H), 2.28 (m, 1H, 5"-H), 2.33 (m, 1H, 3’H), 3.71 (s, 3H, OC*H₃*), 3.81 (m, 1H, 4’-H), 4.19 (m, 1H, 5’-N*H*), 5.24 (d, *J* = 5 Hz, 1H, 3’-OH), 6.14 (t, *J* = 7 Hz, 1H, 1’-H), 6.84 (d, *J* = 9 Hz, 2H, Ar-*H*), 7.17 (m, 2H, Ar-*H*), 7.28 (m, 6H, Ar-*H* & T-C*H*), 7.41 (m, 5H, Ar-*H*), 11.32 (s, N*H*); ^{13}C-NMR (DMSO-d_6, 400 MHz) δ 12.83, 55.62, 70.43, 71.80, 84.08, 86.44, 110.25, 113.71, 126.73, 128.39, 128.97, 130.30, 136.67, 138.43, 146.93, 151.09, 158.06, 164.37; MS (ESI/TOF+) m/z 514.60 (M+H), calculated 514.59 for $C_{30}H_{31}N_3O_5$.

5’-Monomethoxytritylamino-2’,5’-dideoxythymidine-3’-*O*-succinate (**3**). To a stirred solution of **2** (2.06 g, 4 mmol) and DMAP (0.24 g, 2 mmol) in dry pyridine (5 mL) was added succinic anhydride (0.38 g, 3.8 mmol) in portions at room temperature. The reaction mixture was stirred for over night and TLC (10:1 DCM/methanol) indicated the completion of the reaction. Pyridine was removed under reduced pressure and dried under high vacuum. Residue was dissolved in DCM (50 mL) and washed with ice-cold 10% citric acid solution followed by water. DCM layer was dried and rotoevaporated to foam. Residue was redissolved in 5 mL of DCM and excess hexane (100 mL) added slowly with stirring. The precipitated pure product was filtered and dried, yield 2.35 g (95%). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 1.66 (s, 3H, T-CH_3), 2.38 – 2.17 (m, 4H) 2.53 (m, 1H, 5’-H), 2.86 (m, 1H), 3.72 (s, 3H, -OCH_3), 3.71 (m, 1H, 3’-H), 3.99 (m, 1H, 4’-H), 5.30 (m, 1H), 6.14 (t, *J* = 8 Hz, 1H, 1’-H), 6.84 (d, *J* = 9 Hz, 2H, Ar-*H*), 7.18 (m, 2H, Ar-*H*), 7.28 (m, 5H, Ar-*H*), 7.41 (m, 5H, Ar-*H*), 7.61 (s, 1H, N*H*), 11.38 (s, N*H*); ^{13}C-NMR (DMSO-d_6, 400 MHz) δ 12.08, 28.66, 28.80, 35.65, 54.96, 67.80, 74.97, 83.16, 83.68, 93.97, 109.80, 113.05, 126.10, 127.73, 128.31, 129.65, 136.23, 137.70, 146.19, 150.46, 157.42, 163.67, 171.84, 173.40; MS (ESI/TOF+) m/z 614.248 (M+H), calculated 614.250 (M+H) for $C_{34}H_{35}N_3O_8$.

*Preparation of monomer **3** loaded CPG (**4**).* To a solution of **3** (520 mg, 0.85 mmol) and 4-nitrophenol (117 mg, 0.84 mmol) in 1,4-dioxane (4mL) was added DCC (515 mg, 2.5 mmol) portion wise in 5 min at room temperature. After stirring for over night, the precipitated solid was filtered off. Mean while 1 g of long chain alkylamine controlled pore glass (LCAA-CPG) was washed and soaked in DMF (5 mL) for 2 h in SPS tube. The above filtrate and triethylamine (1 mL) were added to the CPG beads. A bright yellow color, due to the release of 4-nitrophenol, was immediately observed. After agitating for 24h at room temperature, the solution was filtered off and beads were washed with DMF and repeated loading one more time to increase the loading percentage. The beads were washed with cupious amounts of DMF, methanol and dichloromethane.

Reference

1. Horwitz, J. P.; Tomson, A. J.; Urbanski. J. A. Chua, J. *J. Org. Chem.* **1962**, *27*, 3045.

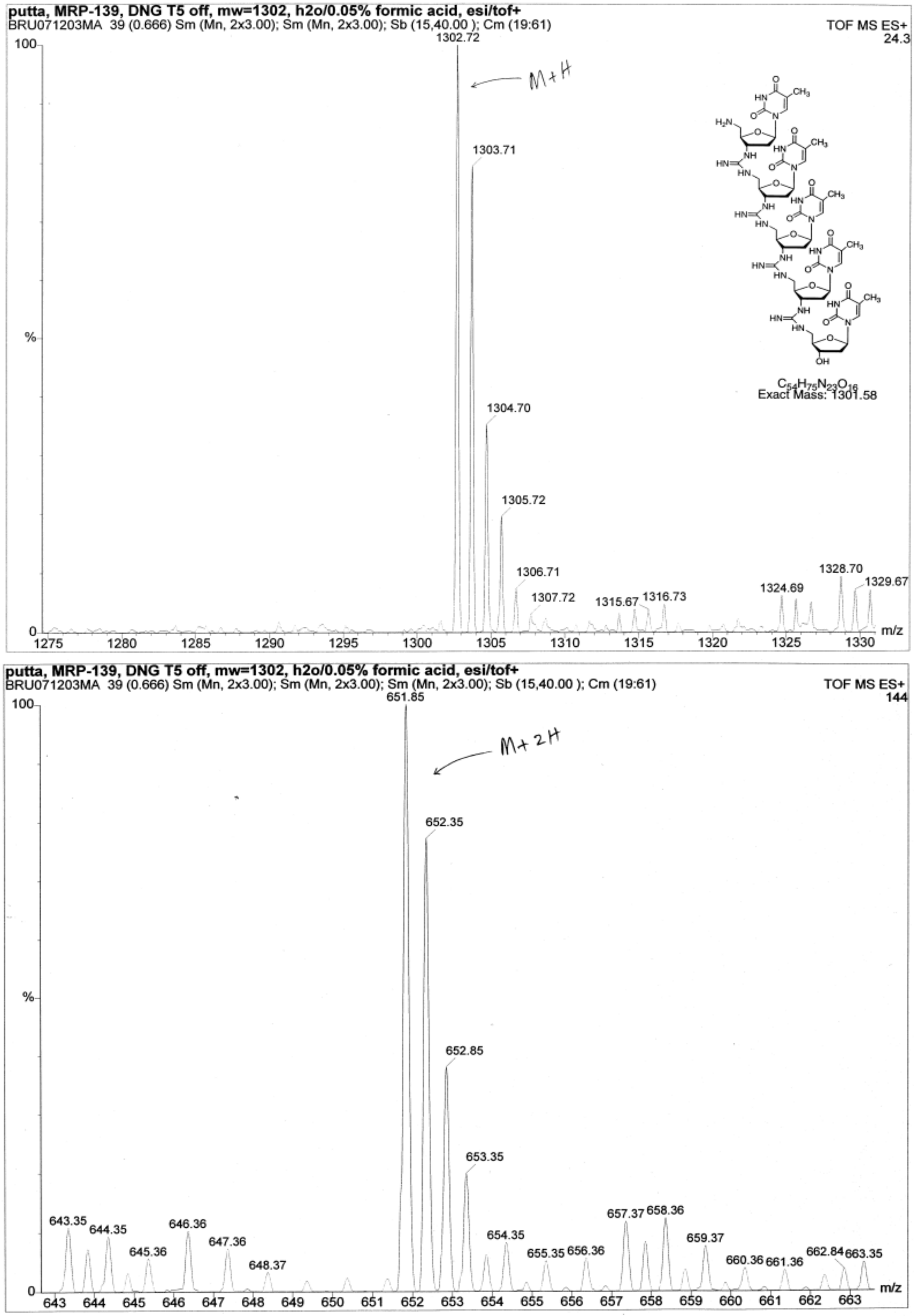


Figure S1: ESI mass spectrum of DNG (**1**); M+H (top) and M+2H (bottom).

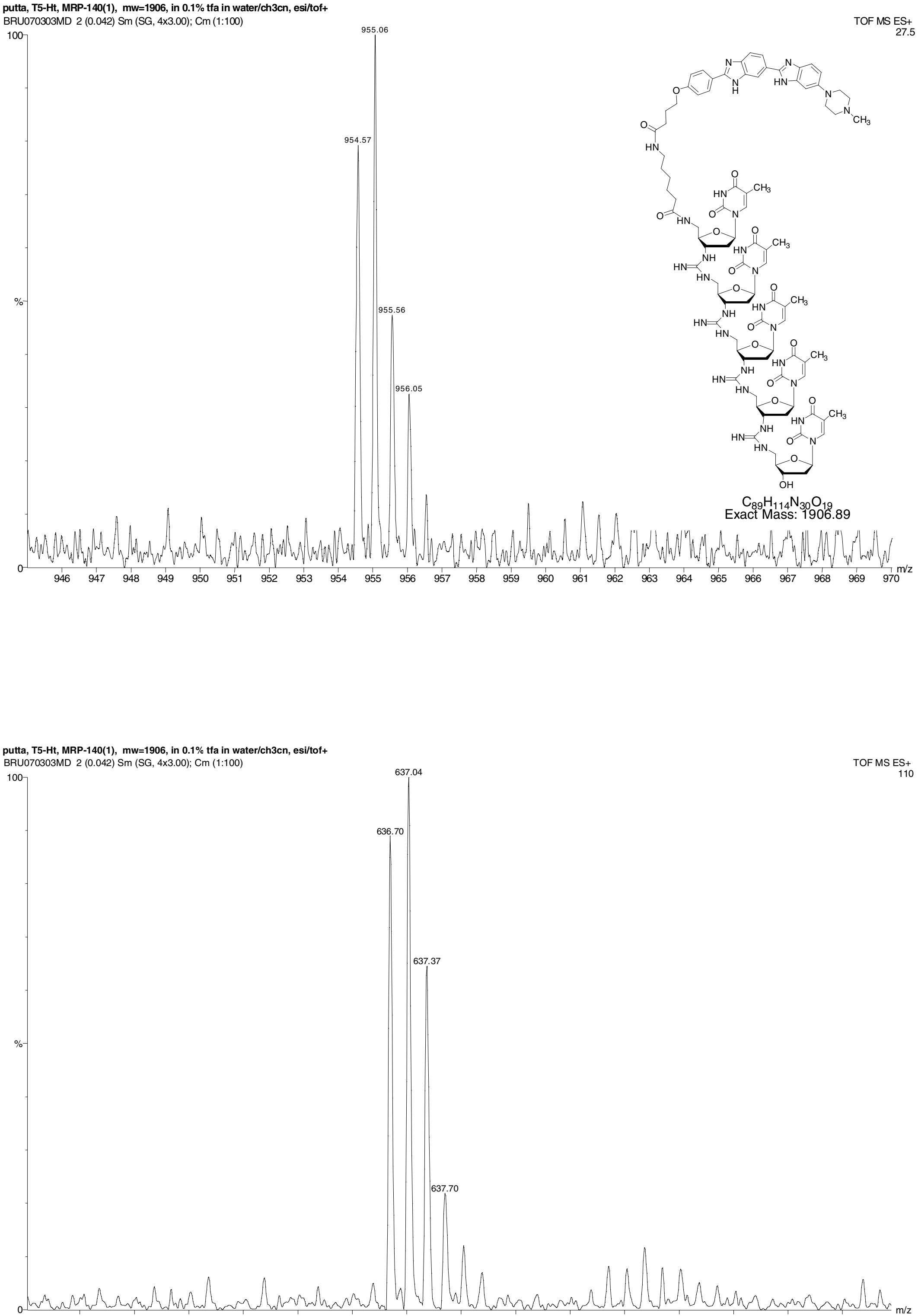


Figure S2: ESI mass spectrum of DNG–Hoechst conjugate (**2**). M+2H (top) and M+3H (bottom).

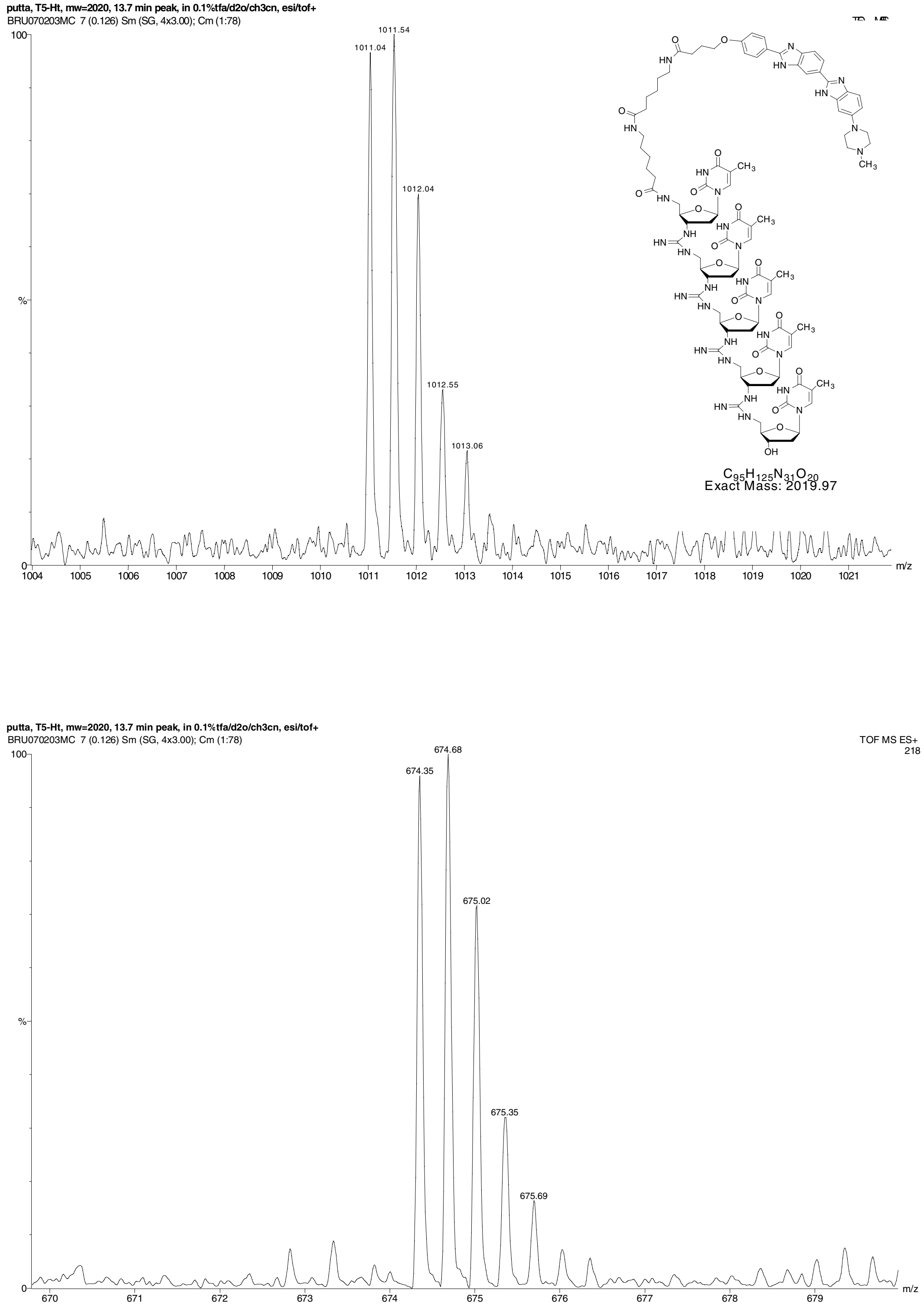


Figure S3: ESI mass spectrum of DNG–Hoechst conjugate (**3**). M+2H (top) and M+3H (bottom).

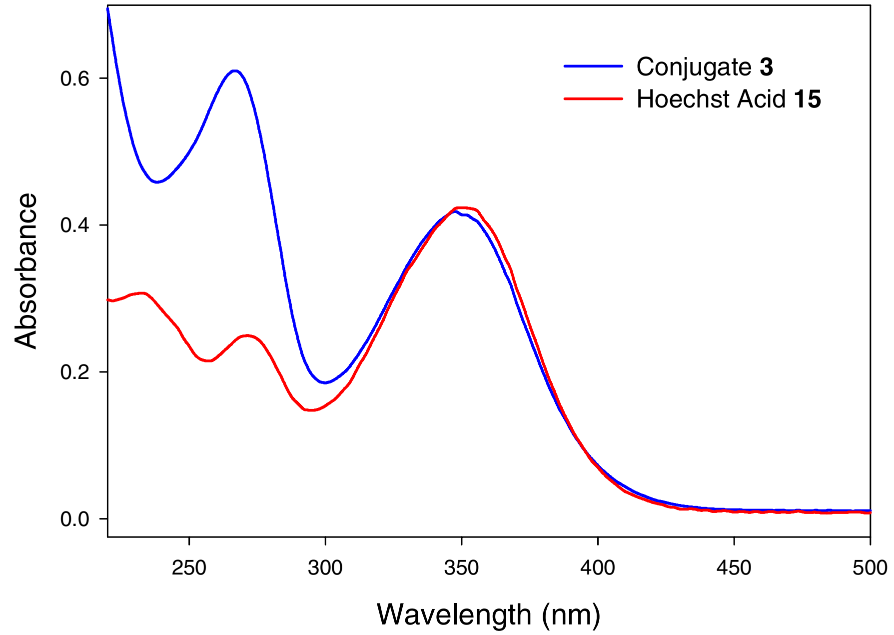


Figure S4. UV spectra of Hoechst 33258 acid (**15**) and DNG–Hoechst conjugate (**3**).

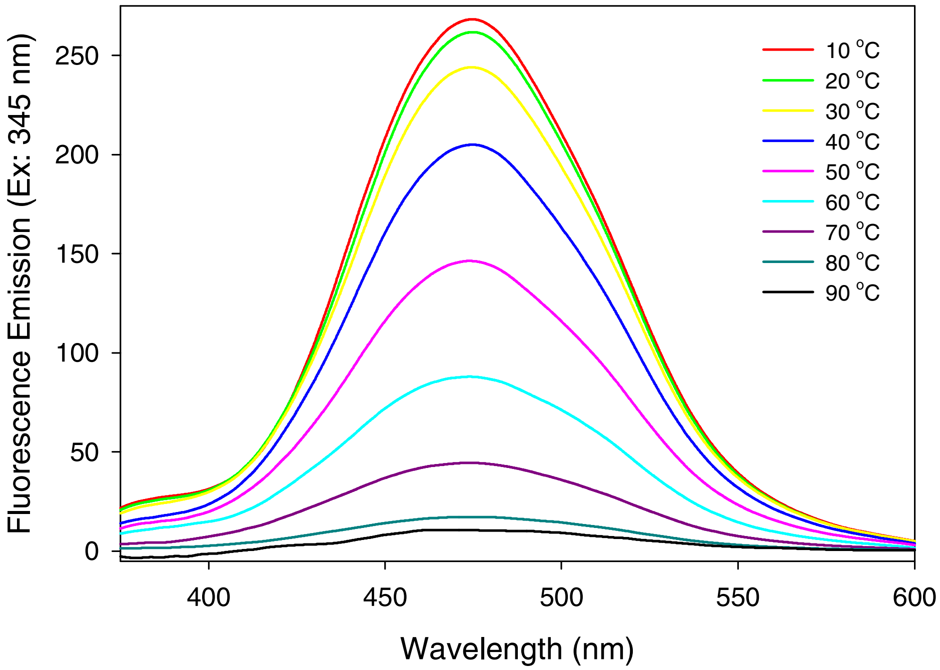


Figure S5. Temperature vs fluorescence emission change of 560 nM DNA▪DNG-H duplex formed by conjugate **3** and 30-mer ssDNA, 5’-CGCCGCGCGCGCCAAAAA CCCGGCGCGCGC-3’.