

Synthesis and Fluorescence Properties of 3,6-Diaminocarbazole-Modified PyrrolidinyI Peptide Nucleic Acid

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Supporting Information

Synthesis of 3,6-dinitrocarbazole (2)

3,6-Dinitrocarbazole was prepared according to Jinzong et al. with slight modification.¹ To a stirring solution of carbazole (2.00 g, 11.96 mmol) in 1,2-dichloroethane (20 mL) at 0 °C, 90% HNO₃ (20 mL) was added dropwise into the solution over 1 h. After completion of the addition, the mixture was warmed to 45 °C for 4 h with vigorous stirring to homogeneous. After the reaction was completed as monitored by TLC, the reaction was cooled down to room temperature followed by addition of water (100 mL). The precipitate was filtered off and washed with water. The crude product was purified by dissolving in a solution of KOH (10 g) in ethanol (125 mL) and water (125 mL). The red solution was filtered and acidified with concentrated HCl. The precipitated product was filtered, washed with water, and dried under vacuum to obtain a yellow solid (2.33 g, 76% yield). ¹H NMR (400 MHz, DMSO-d₆): δ 7.77 (d, *J* = 9.0 Hz, 2H), 8.39 (dd, *J* = 9.0, 2.3 Hz, 2H), 9.50 (d, *J* = 2.3 Hz, 2H), 12.69 (s, 1H).

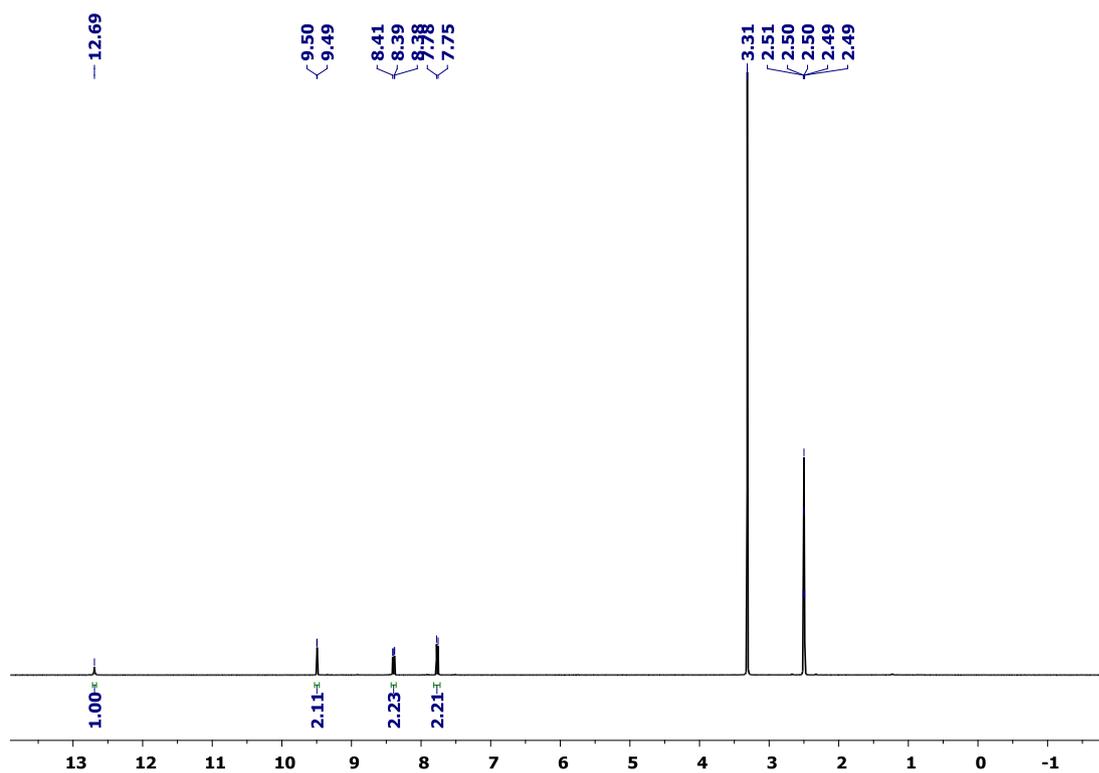


Figure S1. ^1H NMR spectrum (DMSO- d_6 , 400 MHz) of 3,6-diaminocarbazole.

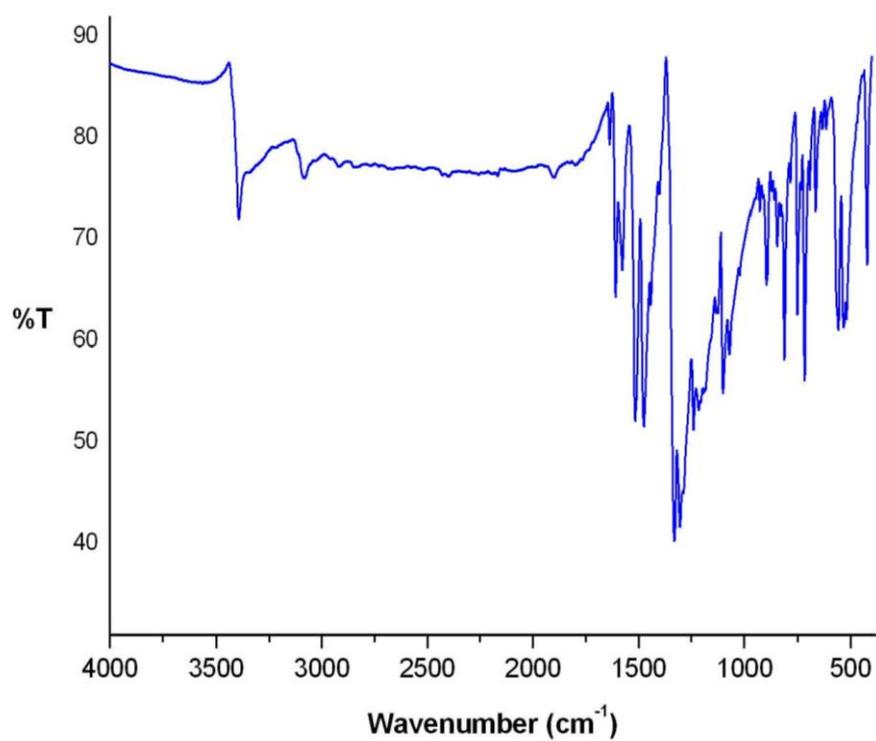


Figure S2. FT-IR spectrum of 3,6-diaminocarbazole.

Synthesis of 3,6-diaminocarbazole (3)

3,6-Diaminocarbazole was prepared according to Maity et al. with slight modification.² To a stirred solution of 3,6-dinitrocarbazole (1.00 g, 3.88 mmol) and anhydrous SnCl₂ (7.35 g, 38.8 mmol) in acetic acid (6 mL) and hydrochloric acid (32 mL) under N₂ atmosphere. The mixture was heated at reflux until the reaction was completed. The reaction mixture was cool down to room temperature, filtered the precipitate and re-dissolved with water. After that, 20% NaOH was added and filtered the occurred precipitate. The precipitated solid was collected by filtration, washed with water and dried under vacuum to obtain the product as a gray solid (0.70 g, 91% yield). ¹H NMR (400 MHz, DMSO-d₆): δ (ppm) 4.45 (s, 4H), 6.64 (dd, *J* = 8.4, 2.0 Hz, 2H), 7.04 (d, *J* = 8.4 Hz, 4H), 10.09 (s, 1H). ¹³C NMR (100 MHz, DMSO-d₆): δ (ppm): 140.2, 133.7, 128.1, 122.9, 114.8, 110.9, 103.8

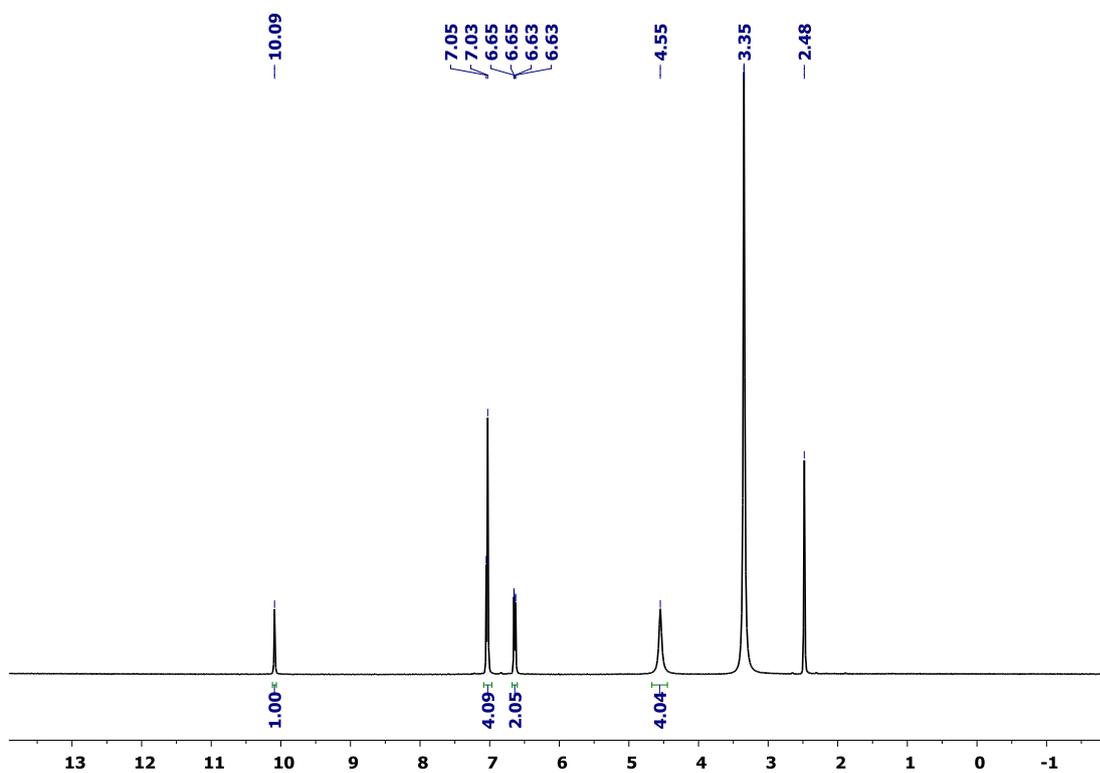


Figure S3. ¹H NMR spectrum (DMSO-*d*₆, 400 MHz) of 3,6-diaminocarbazole.

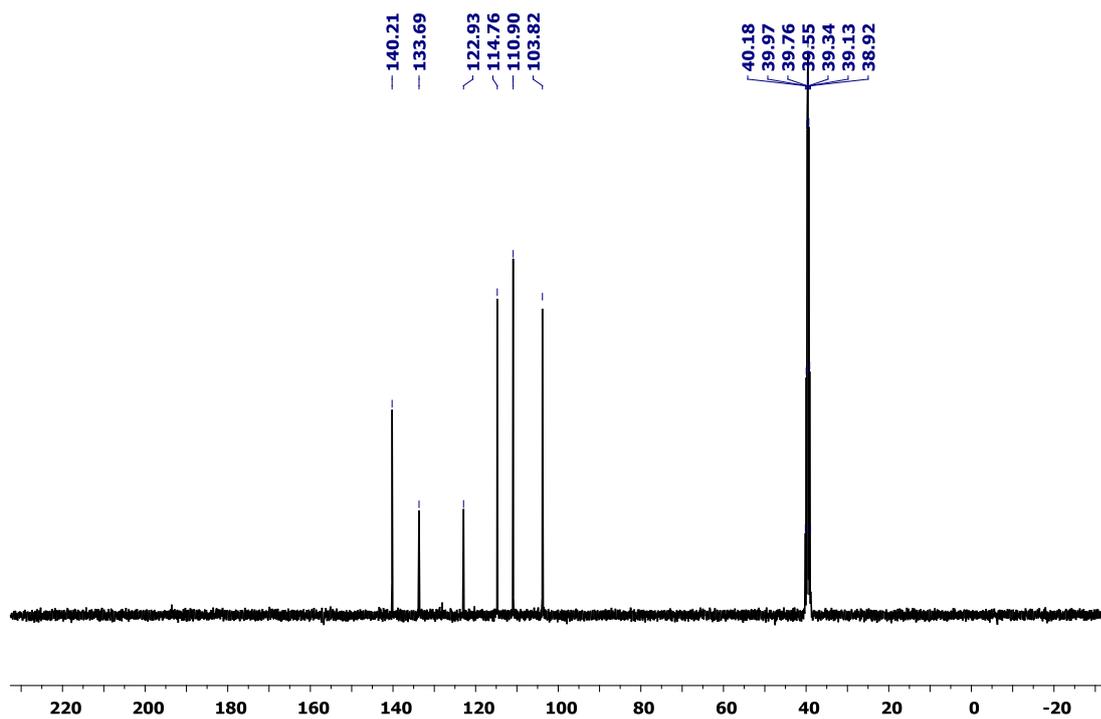


Figure S4. ^{13}C NMR spectrum (DMSO- d_6 , 400 MHz) of 3,6-diaminocarbazole.

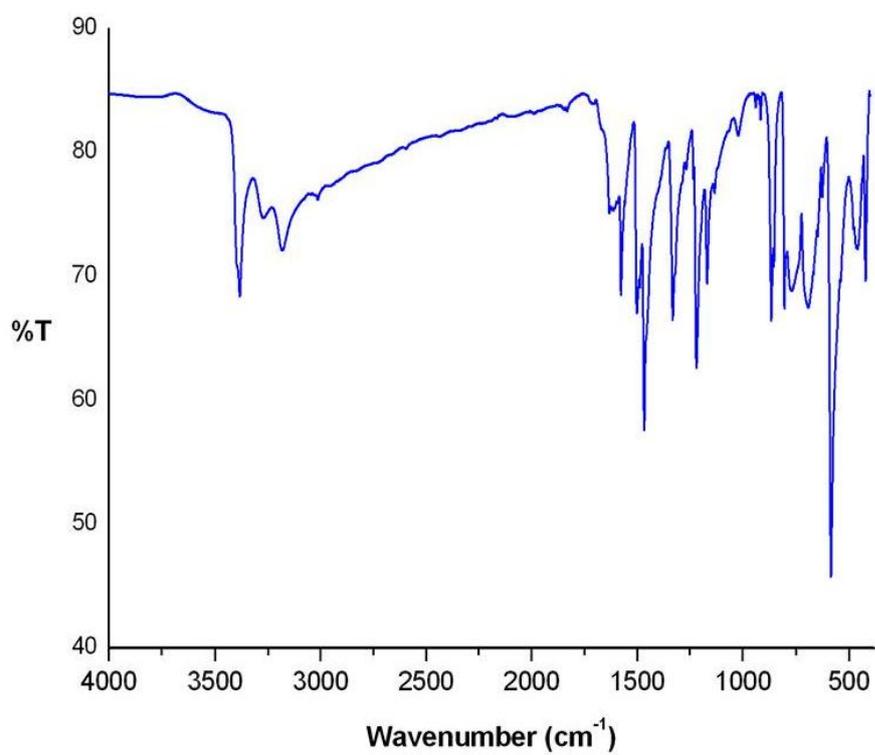


Figure S5. FT-IR spectrum of 3,6-diaminocarbazole

Synthesis of 3,6-Bis(*tert*-butoxycarbonylamino)carbazole (**4**)

To a solution of 3,6-diaminocarbazole (**3**) (0.50 g, 2.53 mmol) and triethylamine (1.06 mL, 7.59 mmol) in methanol (10 mL) was slowly added Boc₂O (1.10 g, 5.06 mmol). The reaction mixture was stirred at room temperature for 24 hours. The solvent was removed by rotary evaporation. Water was added to the residue and the precipitate was collected by filtration, washed with water and dried under vacuum to obtain the crude product as a grey solid which was used for the next step without further purification (1.00 g, 99% yield). ¹H NMR (400 MHz, DMSO-d₆): δ 1.49 (s, 18H), 7.31 (q, *J* = 8.8 Hz, 4H), 8.13 (s, 2H), 9.22 (s, 2H), 10.88 (s, 1H). ¹³C NMR (100 MHz, DMSO-d₆): δ (ppm): 153.2, 136.3, 131.0, 122.2, 118.2, 110.8, 109.4, 78.5, 28.3

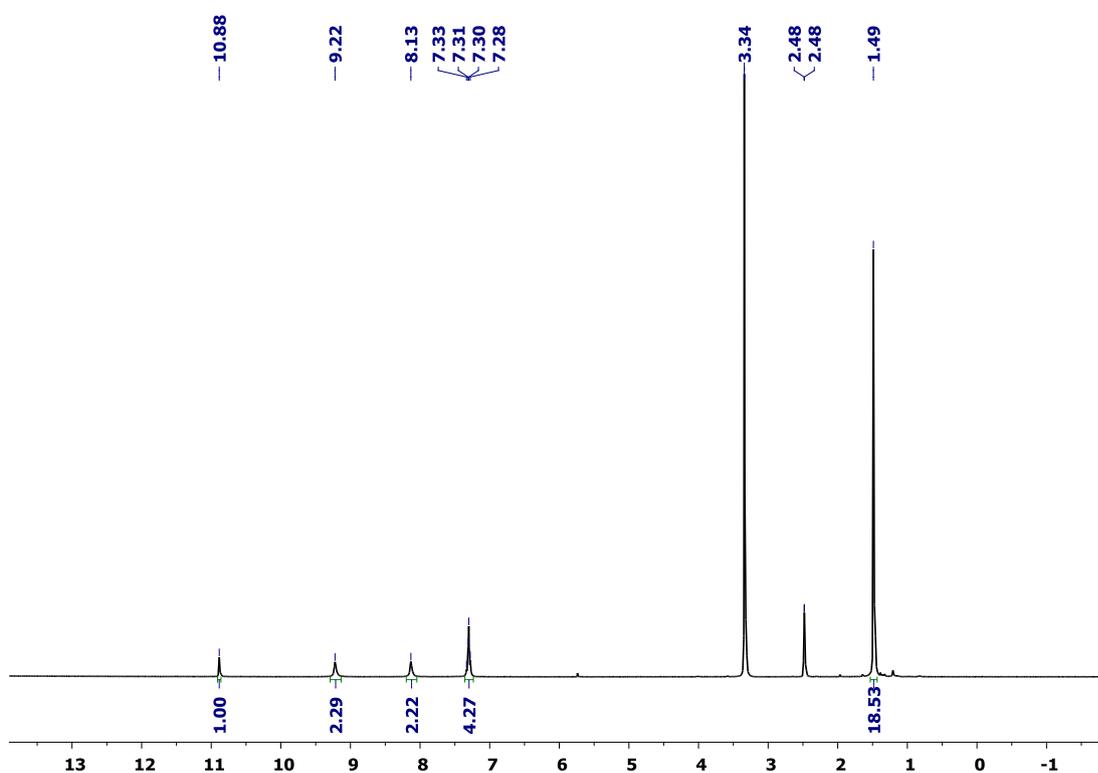


Figure S6. ¹H NMR spectrum (DMSO-*d*₆, 400 MHz) of *N*-Boc 3,6-diaminocarbazole.

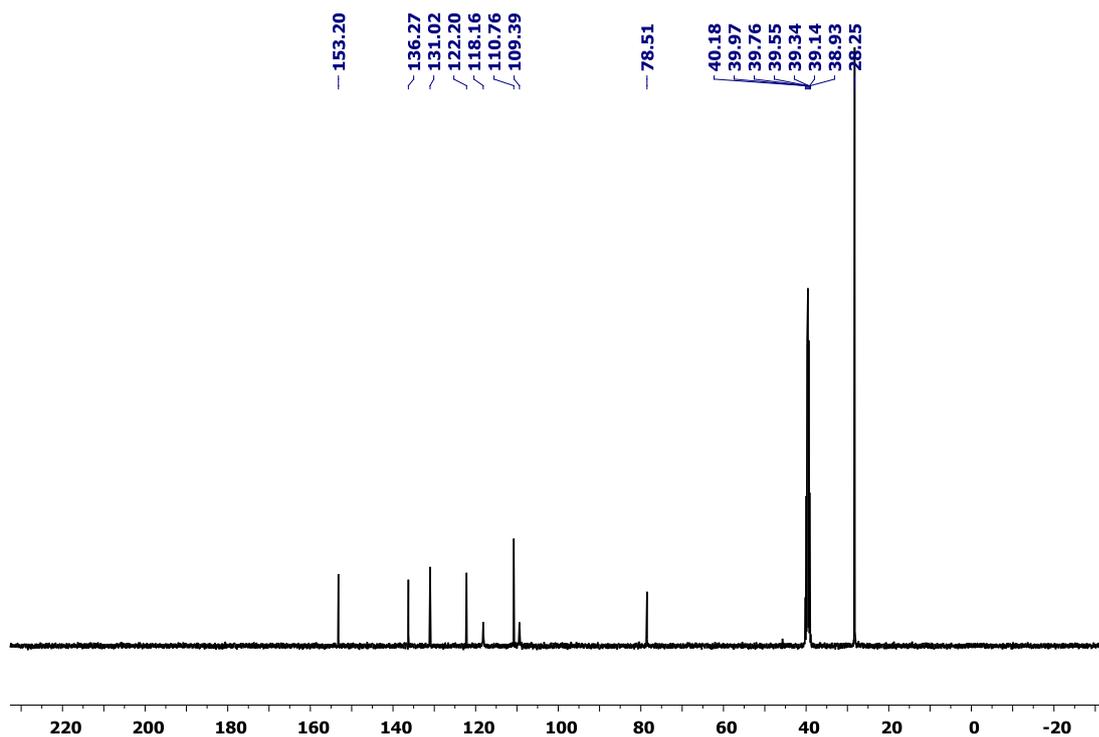


Figure S7. ^{13}C NMR spectrum (DMSO- d_6 , 400 MHz) of *N,N*-Boc 3,6-diaminocarbazole.

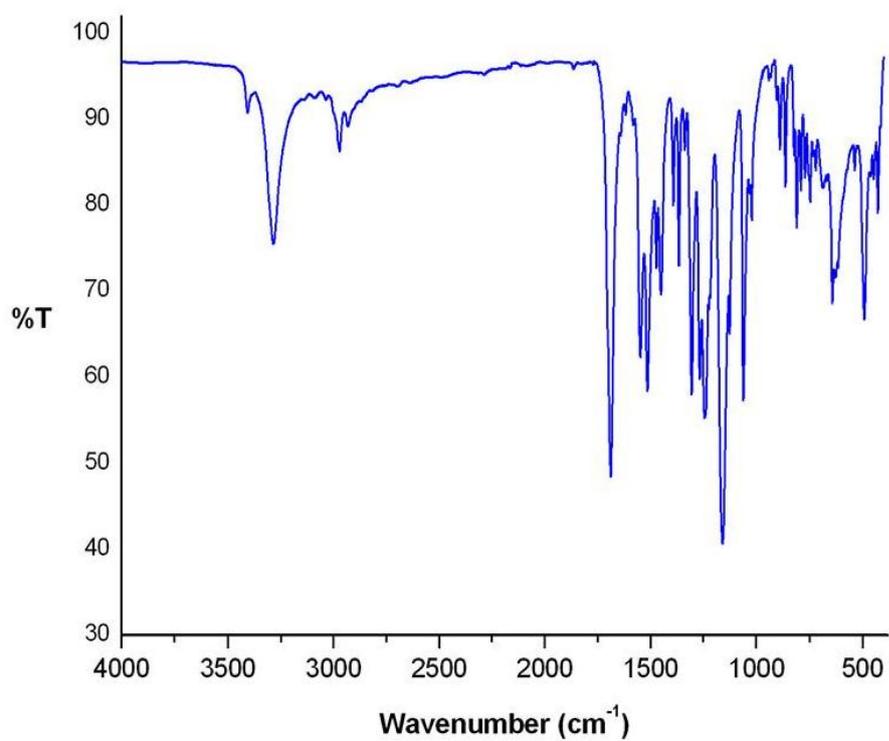


Figure S8. FT-IR spectrum of *N,N*-Boc 3,6-diaminocarbazole.

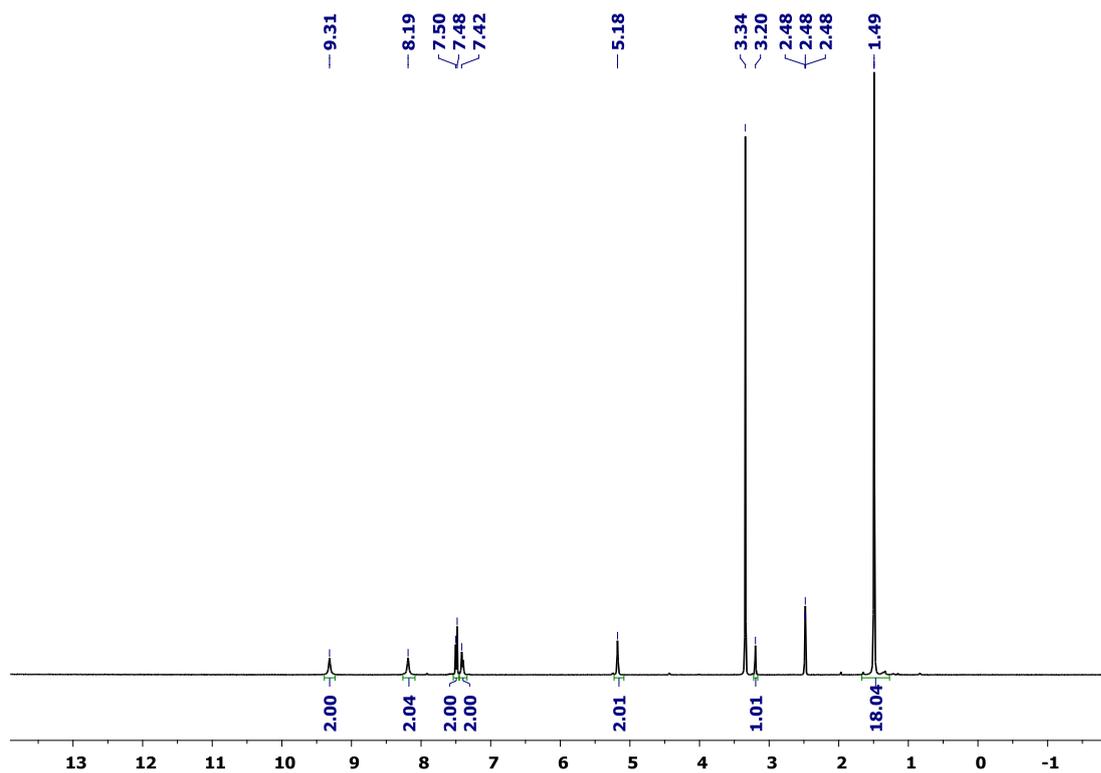


Figure S9. ^1H NMR spectrum (DMSO- d_6 , 400 MHz) of *N*-Propargyl *N,N*-Boc 3,6-diaminocarbazole.

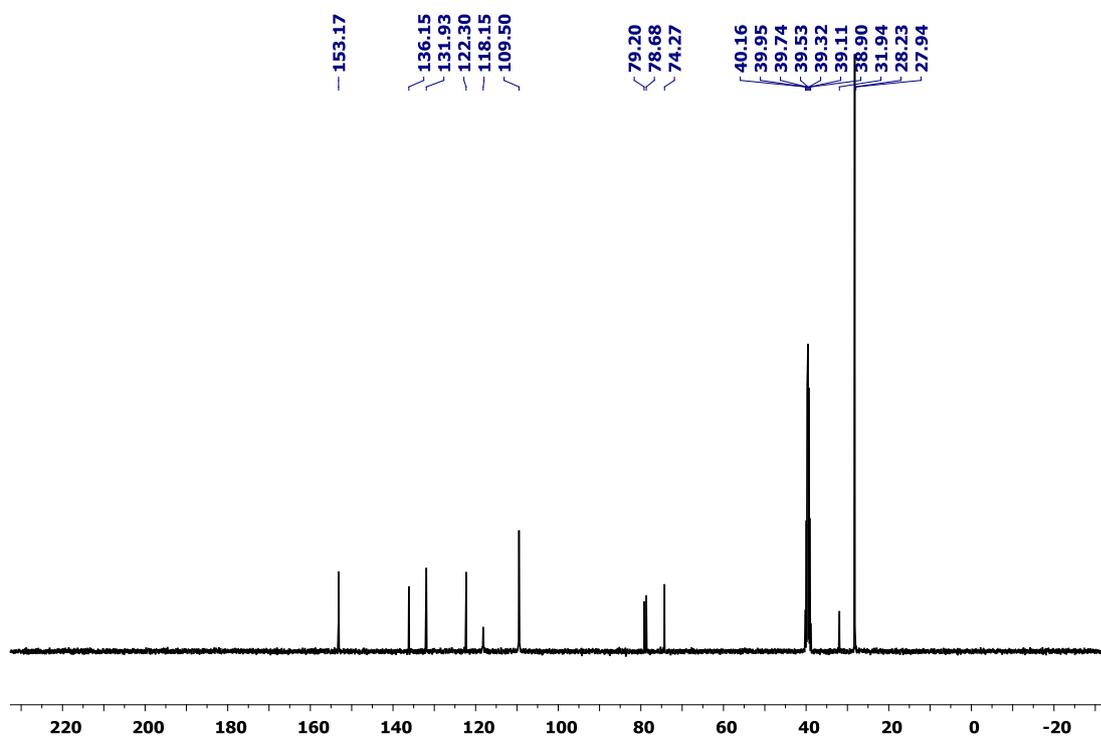


Figure S10. ^{13}C NMR spectrum (DMSO- d_6 , 400 MHz) of *N*-Propargyl *N,N*-Boc 3,6-diaminocarbazole.

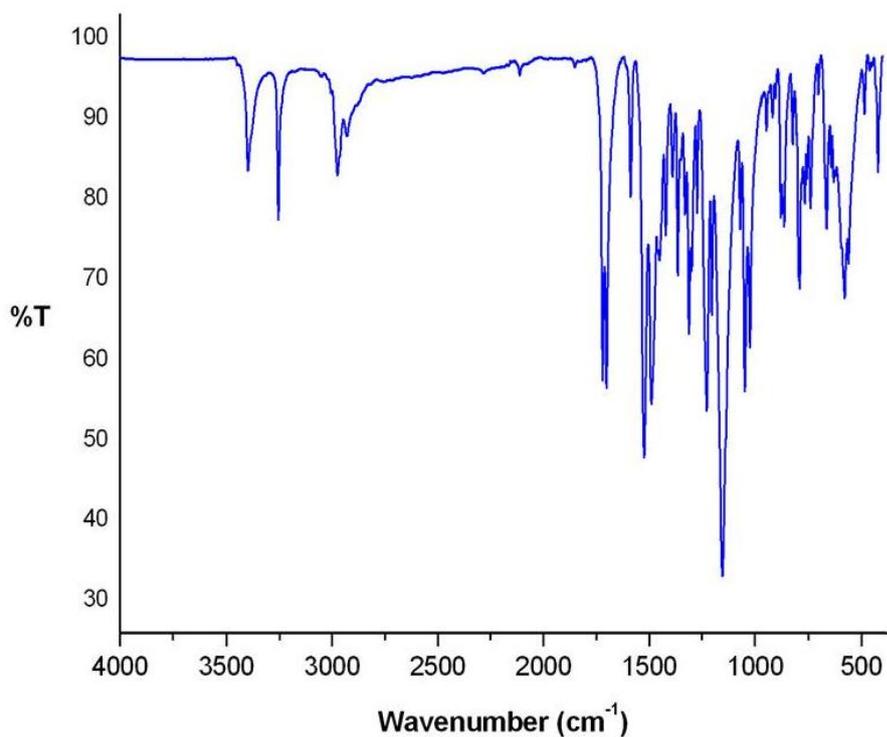


Figure S11. FT-IR spectrum of *N*-Propargyl *N,N*-Boc 3,6-diaminocarbazole.

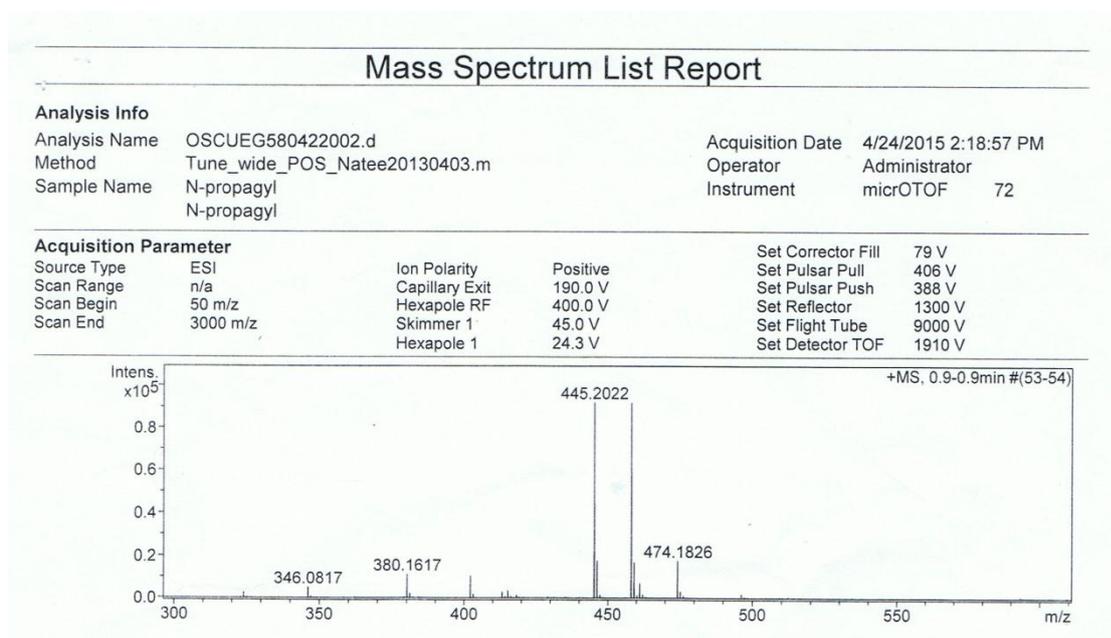


Figure S12. HRMS of *N*-propargyl *N,N*-Boc 3,6-diaminocarbazole.

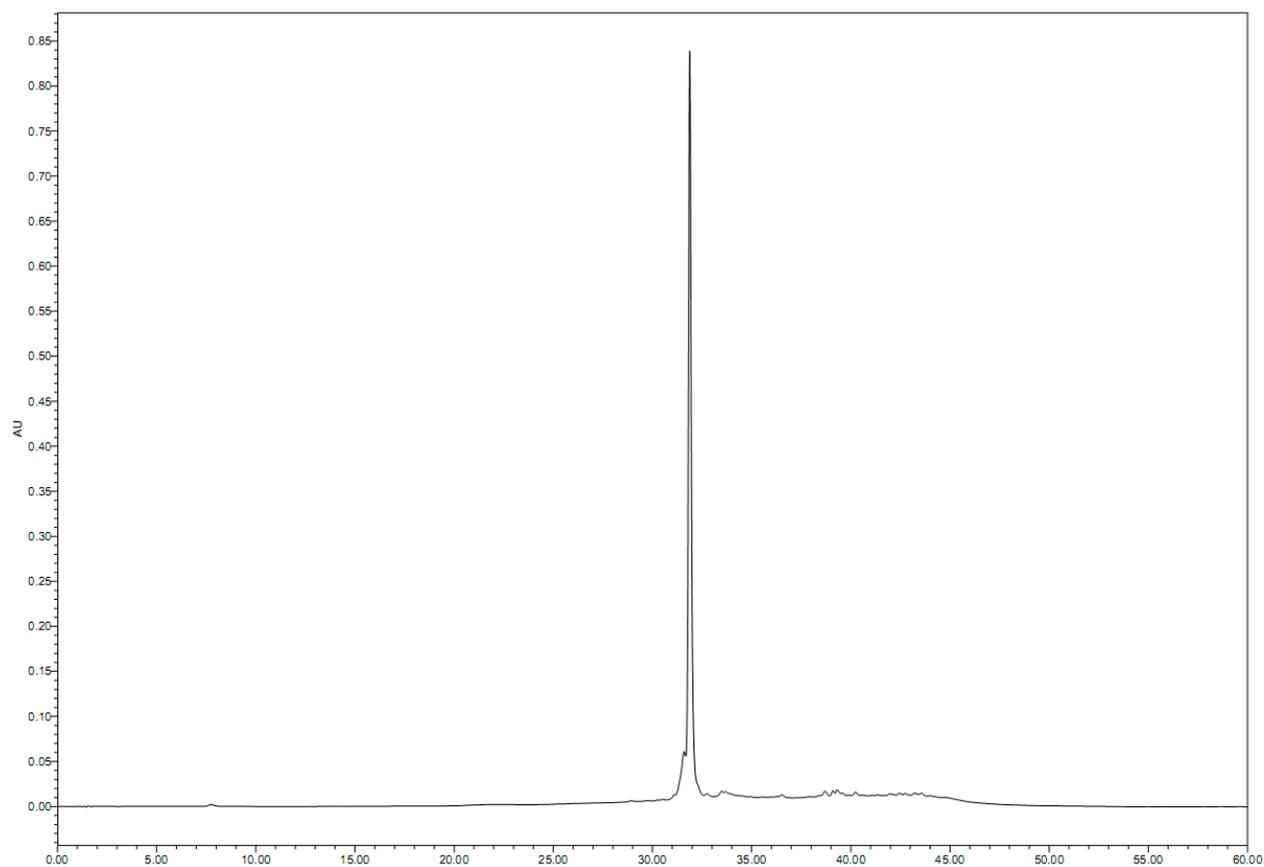


Figure S13. HPLC chromatogram of $T9^{DAC}$ ($t_R = 32.0$ min)

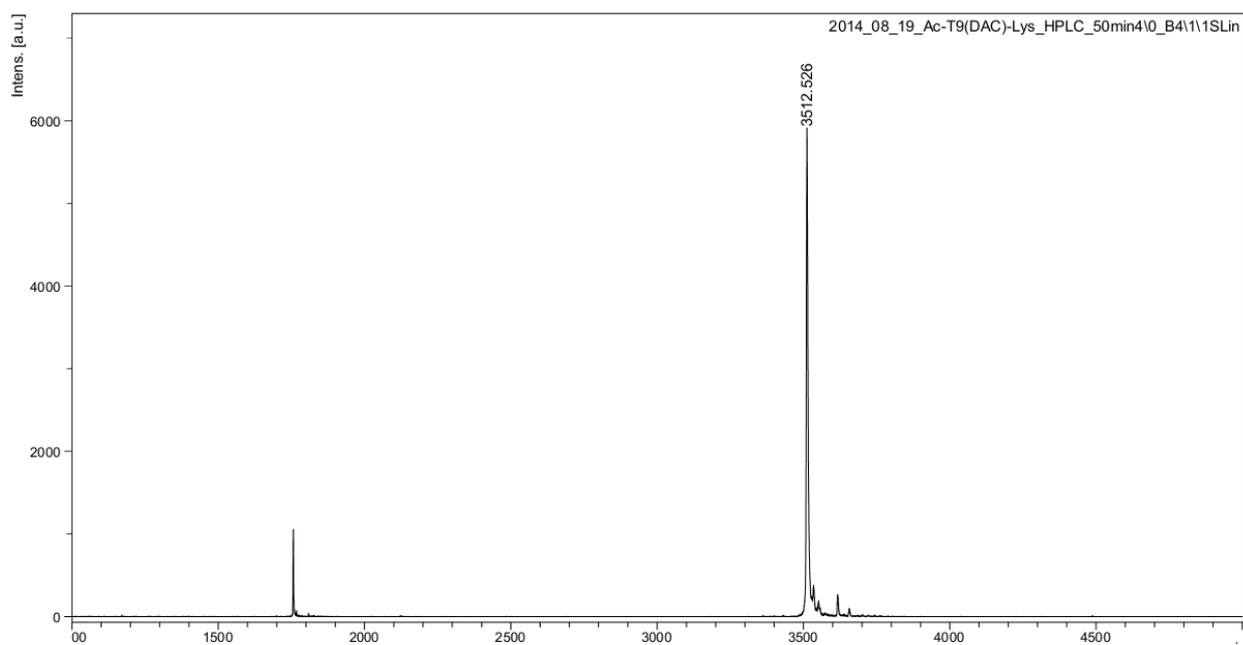


Figure S14. MALDI-TOF mass spectrum of $T9^{DAC}$ (calcd. $m/z = 3513.6$).

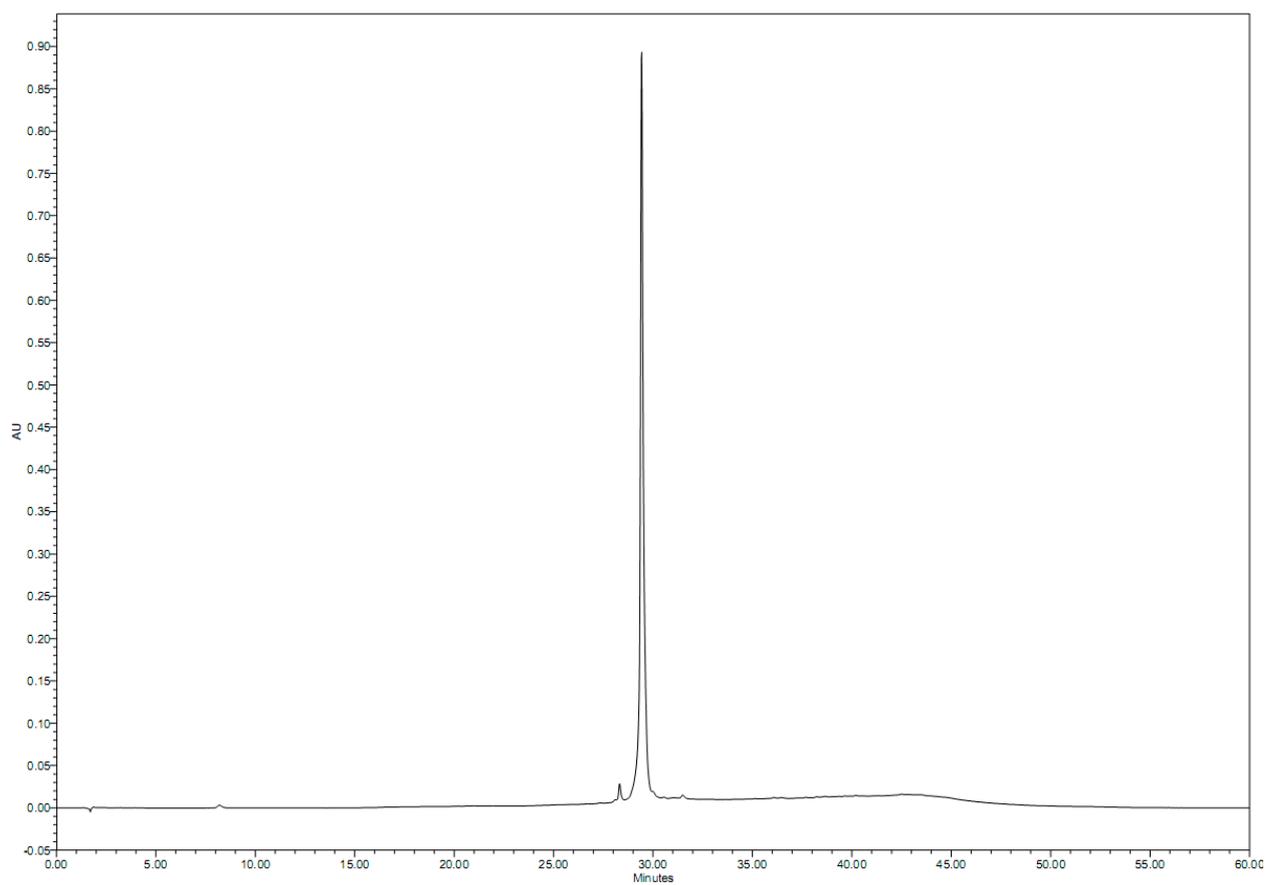


Figure S15. HPLC chromatogram of **M10AT^{DAC}** ($t_R = 29.5$ min)

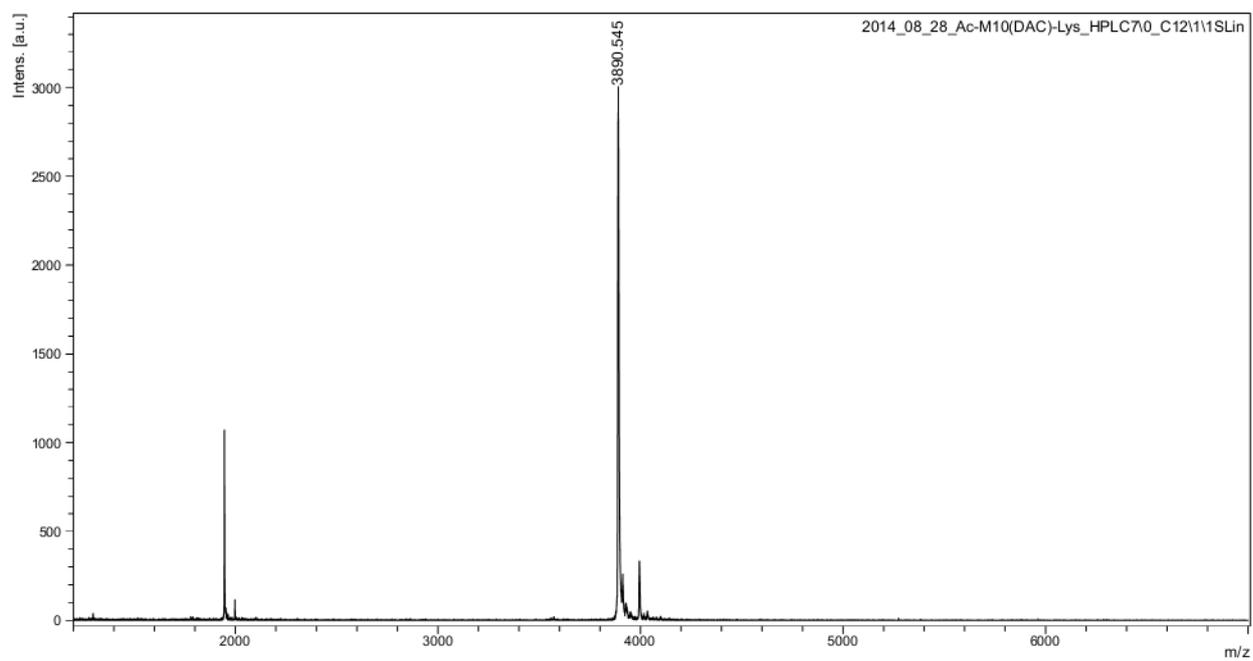


Figure S16. MALDI-TOF mass spectrum of **M10AT^{DAC}** (calcd. $m/z = 3892.8$).

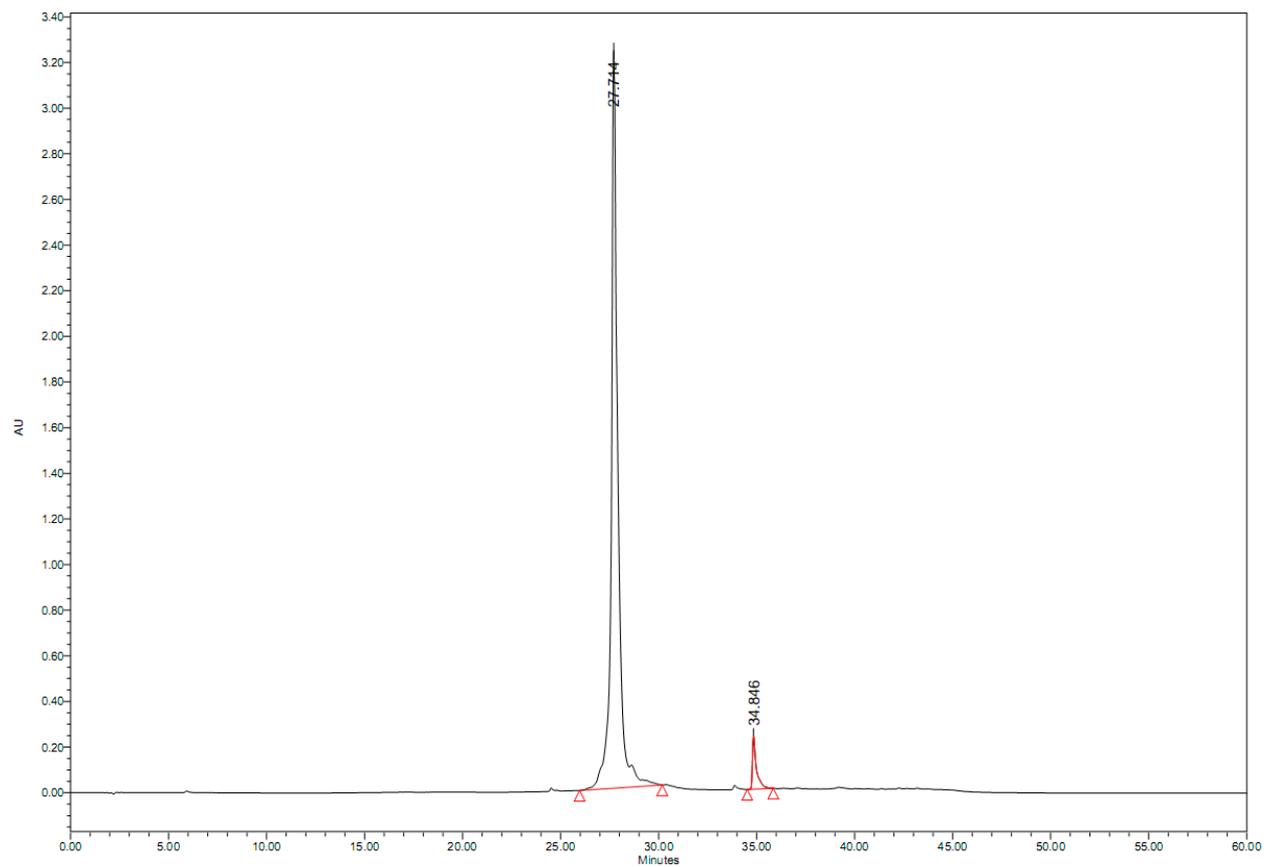


Figure S17. HPLC chromatogram of **M10CG^{DAC}** ($t_R = 27.7$ min)

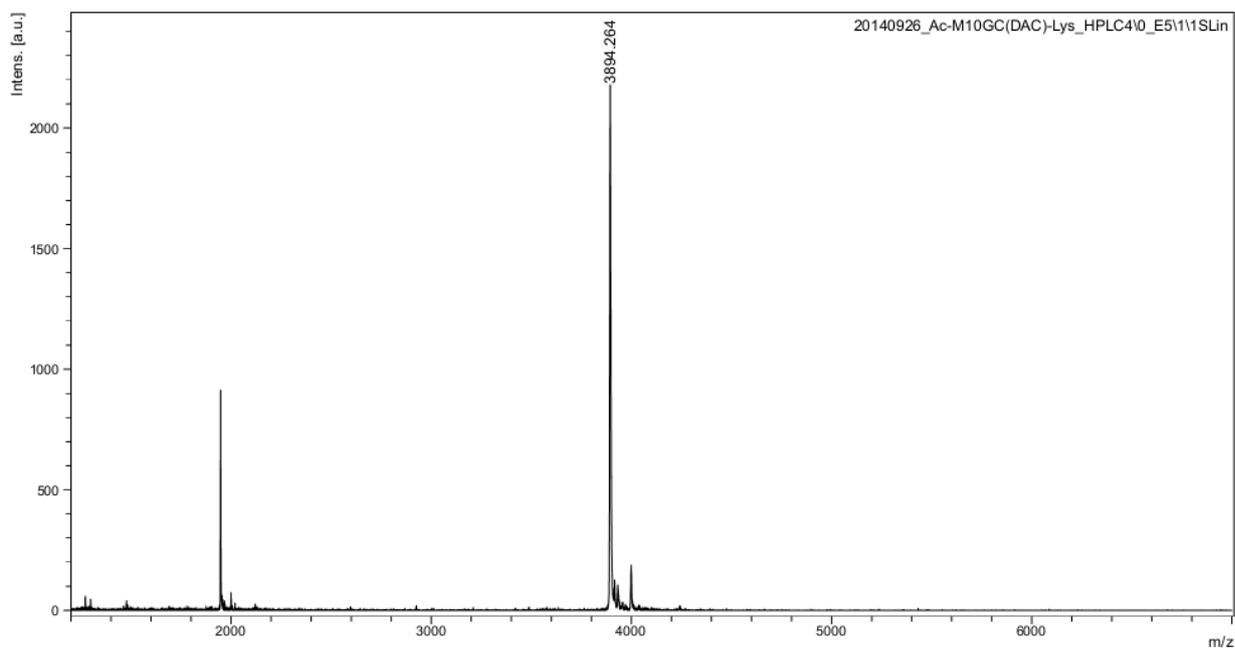


Figure S18. MALDI-TOF mass spectrum of **M10CG^{DAC}** (calcd. $m/z = 3893.8$).

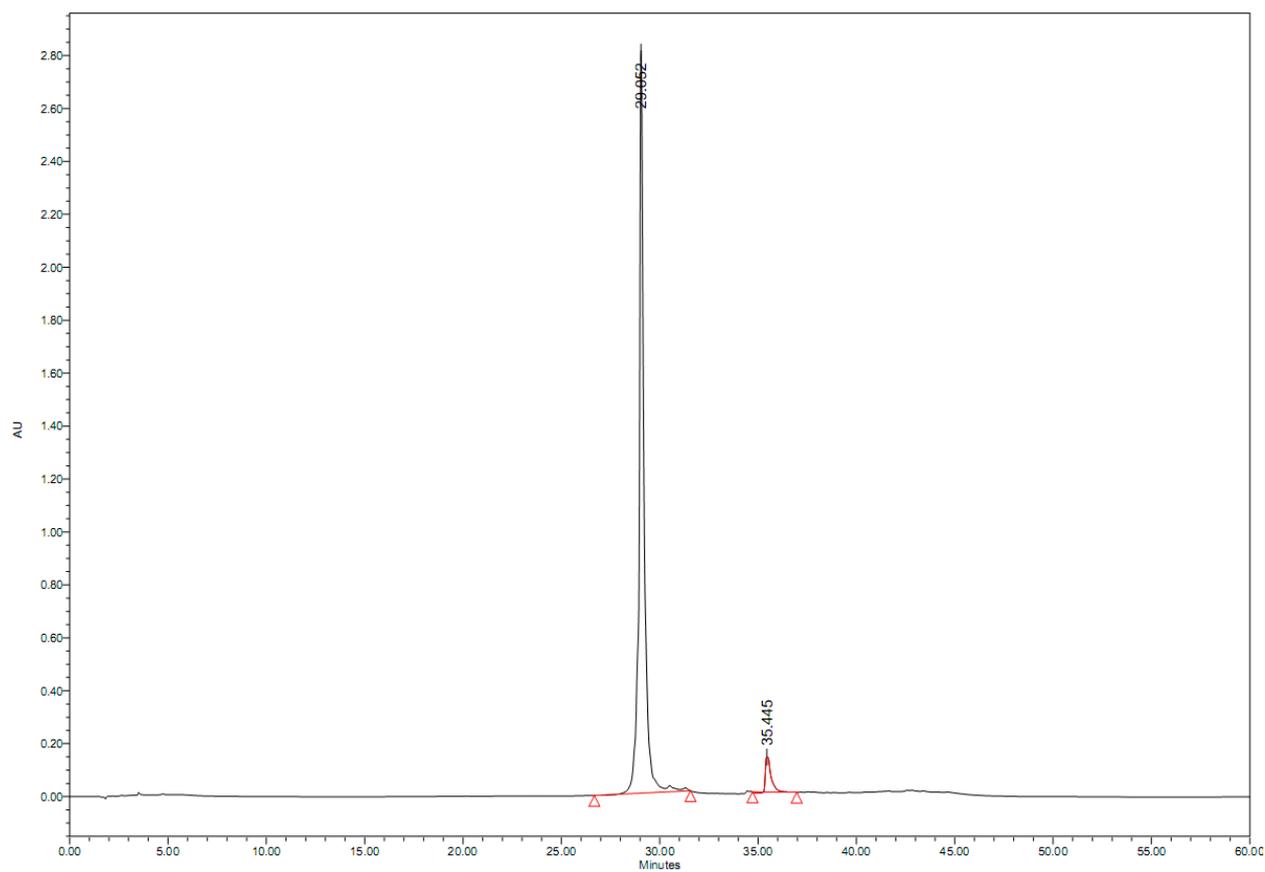


Figure S19. HPLC chromatogram of **DAC-M10** ($t_R = 29.0$ min)

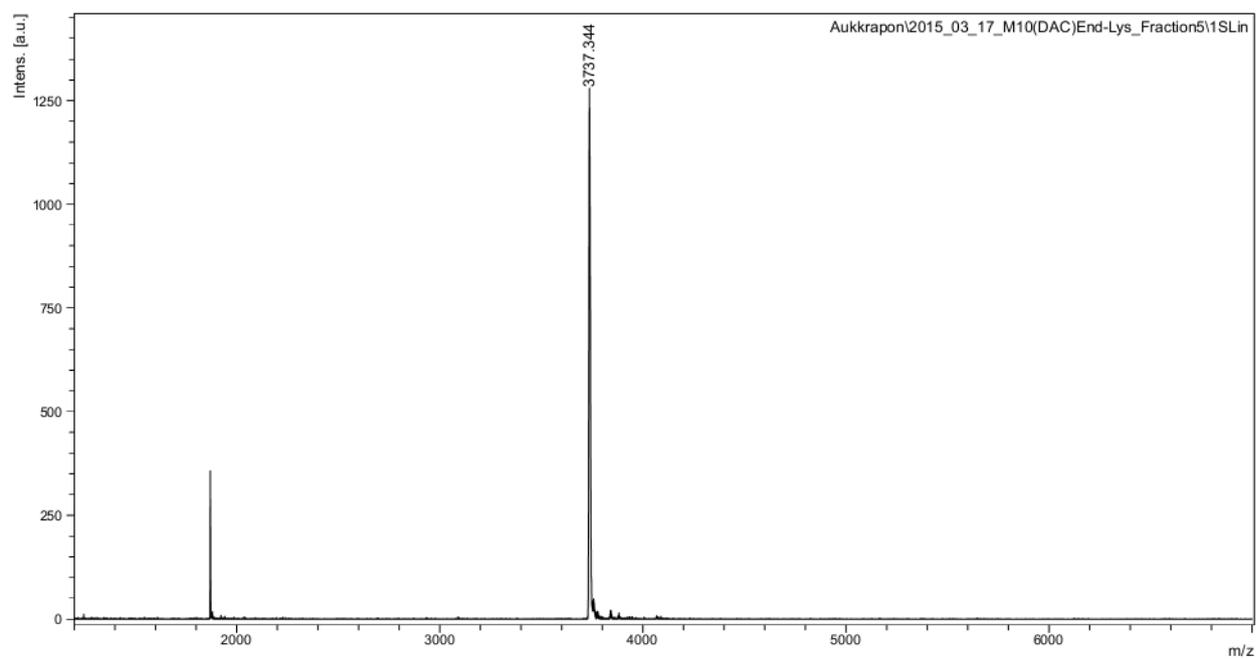
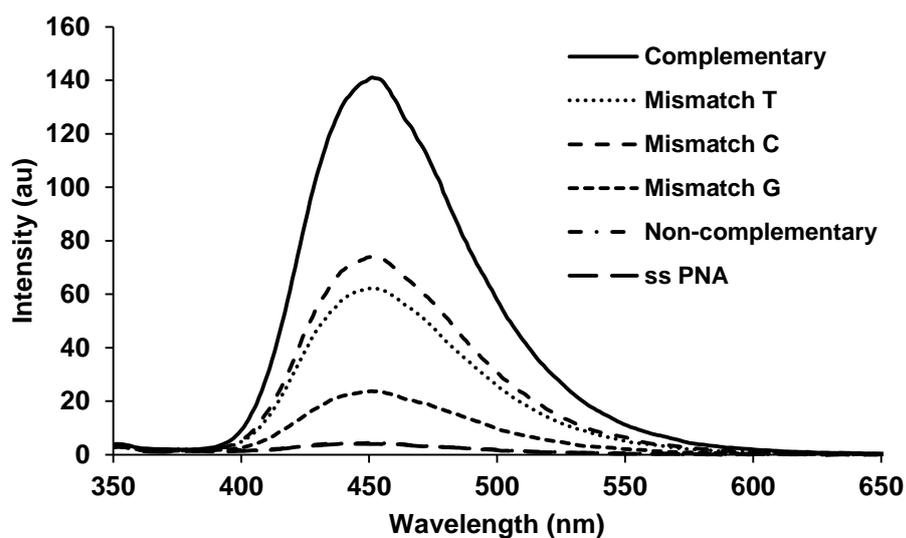
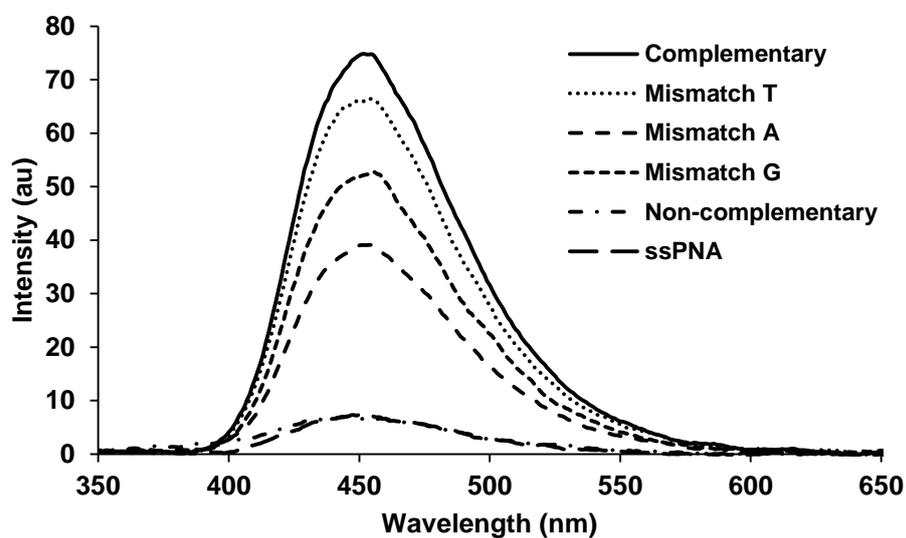


Figure S20. MALDI-TOF mass spectrum of **DAC-M10** (calcd. $m/z = 3738.8$).



(a)



(b)

Figure S21. Fluorescence spectra of DAC-labeled acpPNA with complementary, single mismatch, non-complementary and single strand form of (a) $T9^{DAC}$ (b) $M10GC^{DAC}$. Conditions: 10 mM sodium phosphate buffer, pH 7.0 at 25°C, [PNA] = 1.0 μ M and [DNA] = 1.2 μ M, excitation wavelength = 315 nm. For DNA sequences, see Table 1.

Fluorescence experiments of 3,6-diaminocarbazole with single stranded and double stranded DNA

The fluorescence behavior of free 3,6-diaminocarbazole with single stranded DNA was examined by addition of a concentrated stock solution (125 μM) of an arbitrary DNA sequence (5'-CCA GGG CAT GGT AGA TCA CTG TAC GCC GCG-3') to a solution of 1.0 μM 3,6-diaminocarbazole in 10 mM sodium phosphate buffer, pH 7.0 at 25°C, keeping the volume change minimum. The fluorescence spectra were recorded on a CARY Eclipse Fluorescence spectrophotometer (Varian/Agilent Technologies) at the excitation wavelength of 315 nm. Both excitation and emission slits were set to 5 nm and PMT voltage set to medium.

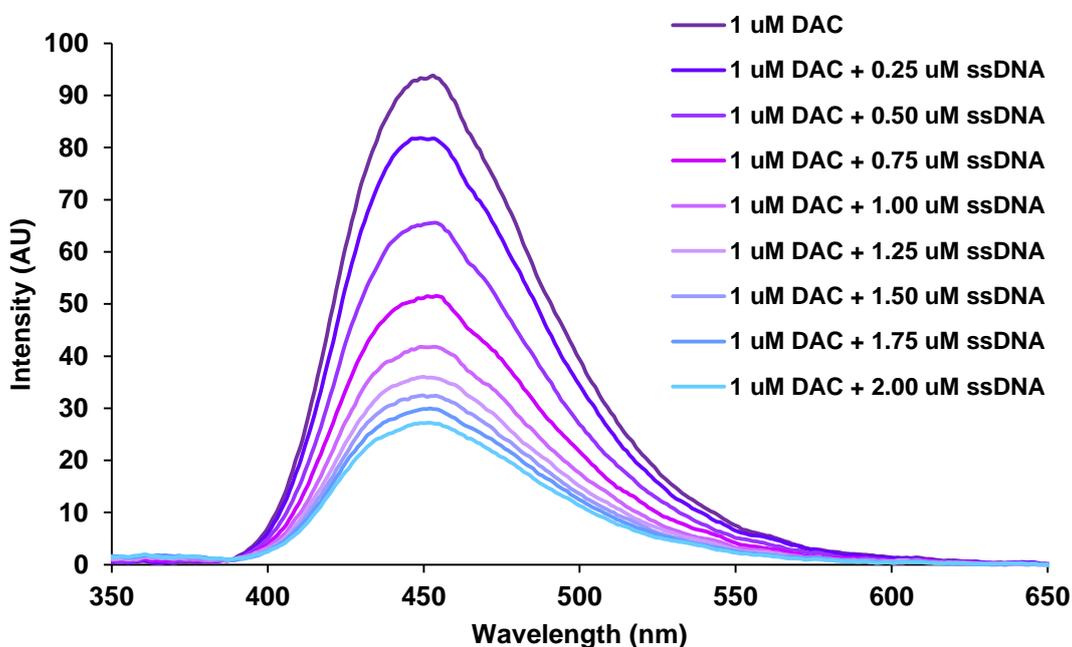


Figure S22. Fluorescence spectra of 3,6-diaminocarbazole (DAC) titrated with single stranded DNA at concentration 0–2 μM in 10 mM phosphate-buffered pH 7.0, $[\text{DAC}] = 1.0 \mu\text{M}$, $\lambda_{\text{ex}} 315 \text{ nm}$.

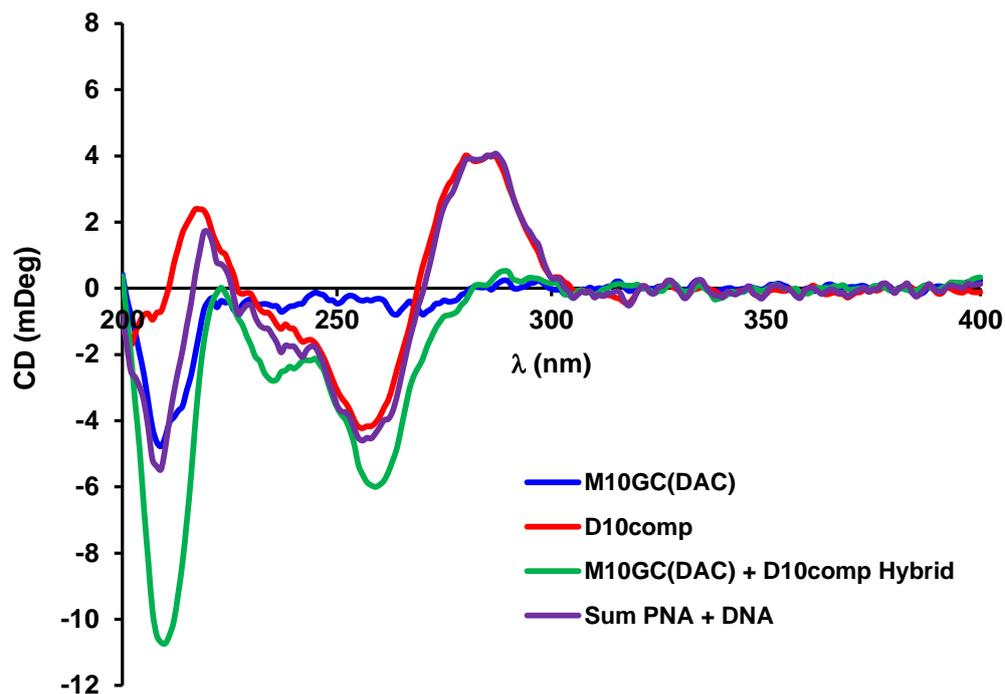


Figure S23. CD spectra of M10GC^{DAC} in the absence and presence of complementary DNA (dAGTGCGCTAC); conditions: 10 mM sodium phosphate buffer, pH 7.0 at 25°C, [PNA] = 2.5 μM and [DNA] = 3.0 μM.

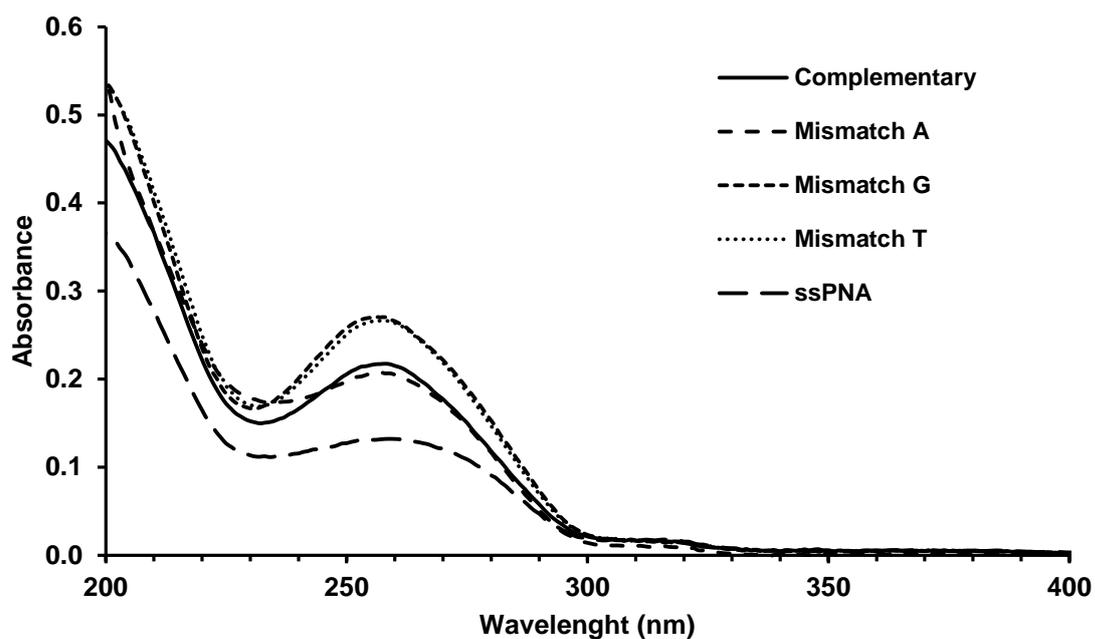


Figure S24. UV spectra of M10GC^{DAC} in the absence and presence of complementary and single mismatched DNA; conditions: 10 mM sodium phosphate buffer, pH 7.0 at 25°C, [PNA] = 1.0 μ M and [DNA] = 1.2 μ M. Complementary DNA = dAGTGCGCTAC, Mismatch A DNA = dAGTGATCTAC, Mismatch G DNA = dAGTGGTCTAC and Mismatch T DNA = dAGTGTTCTAC.

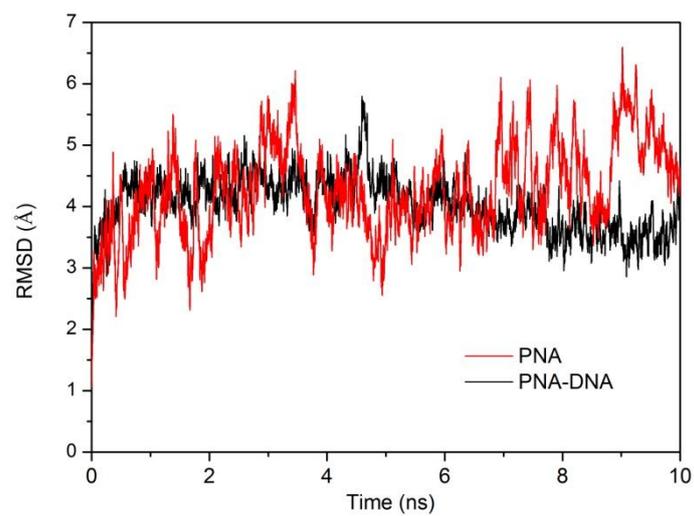


Figure S25. RMSD profiles along 10 ns MD simulations of PNA single strand (red) and PNA·DNA duplex (black).

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