Supplementary Information

In-Stem Thiazole Orange Reveals the Same Triplex Intermediate for pH and Thermal Unfolding of I-Motif

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Materials and Methods

All chemicals were purchased from Aldrich, Alfa Aesar or Merck. Standard reagents and CPG columns (1 μmol) for DNA syntheses were purchased from Glen Research. All reagents and chemicals were used as purchased without further purification. TO phosphoramidite was synthesized in laboratory using previously reported method.[1] TO modified DNA sequences were synthesized by standard solid phase phosphoramidite chemistry on a MerMade-4 DNA synthesizer with slight modification during the TO phosphoramidite coupling.[2] Modified oligonucleotides were purified by reverse phase HPLC (Figure S7) and characterized by ESI-MS (Figure S8). HPLC purified unmodified DNA oligonucleotide **cHT22** was purchased from Sangon (Shanghai, China).

Circular Dichroism (CD) spectra were recorded on a Jasco J-810 CD Spectrophotometer equipped with a temperature controller with the scan speed of 200 nm/min. Each measurement was an automatic average of 3 repeated scans at 25 °C. The UV-thermal denaturation experiments were performed in a Shimadzu UV-2550 UV-vis spectrophotometer by measuring the change of absorbance at 260 nm with increasing temperature from 15 to 80 °C, with a ramp rate of 0.5 °C/min. All fluorescence spectra were recorded on Cary Eclipse fluorescence spectrophotometer equipped with a temperature controller. Fluorescence emission spectra were obtained at 20 °C with excitation wavelength at 490 nm. Fluorescence melting data were obtained by measuring the change in fluorescent emission intensity at 535 nm and 580 nm with increasing temperature from 15 to 80 °C, using ramp rate of 0.5 °C/min. Due to the formation of exciplex, the fluorescence thermal denaturation of 4TO and 16TO was monitored both at 535 nm and 580 nm. DNA samples used for all measurements were 2 μM in 180 mM NaCl, 20 mM sodium cacodylate at various pHs (5.0 to 7.4).

Figure S1. Circular Dichroism Spectra of HT-iM and TO-iM at pH 5.0.

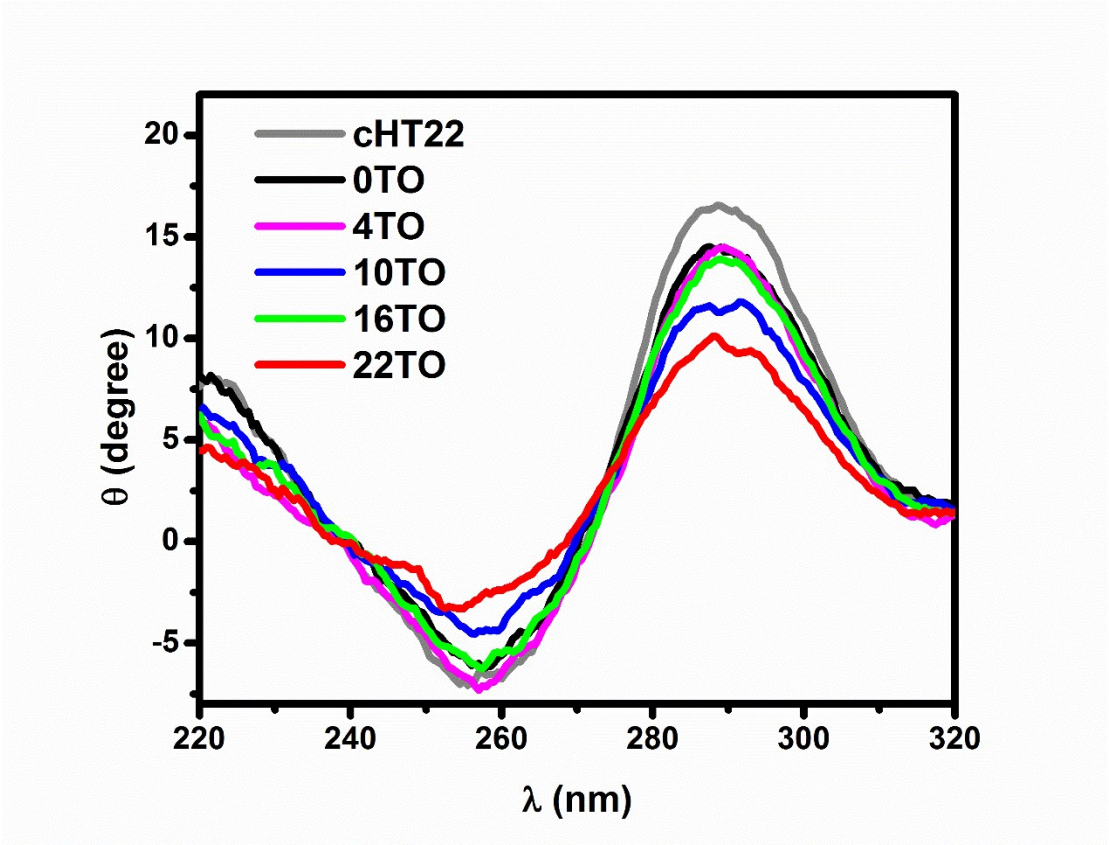


Figure S2. Temperature dependent static fluorescence studies for TO modified DNAs at pH 5.0

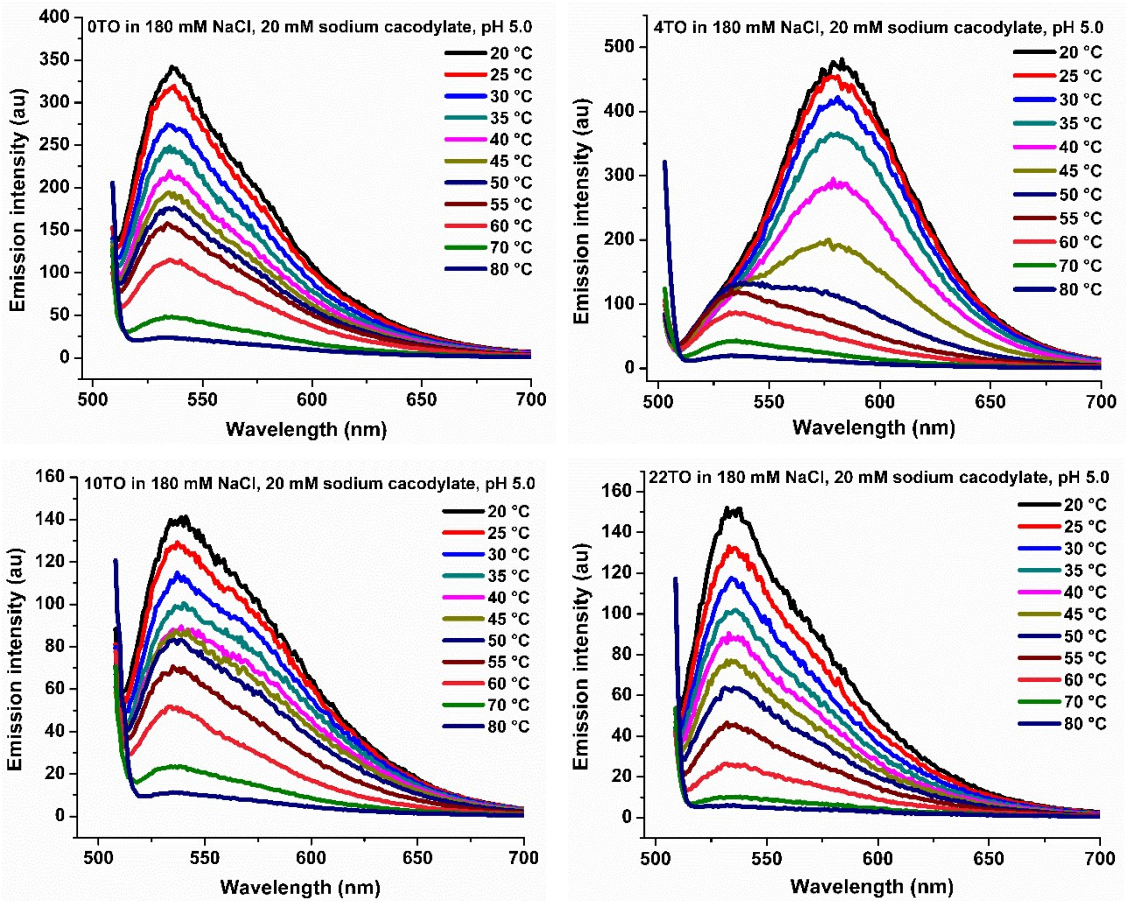


Figure S3. Thermal denaturation (UV-T_m) spectra of for unmodified and all TO modified DNAs at 260 nm at pH 5.0

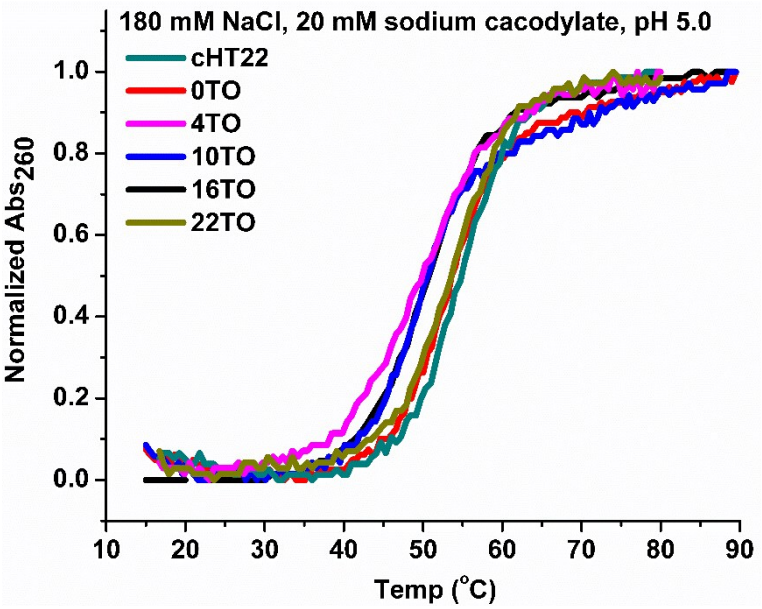


Figure S4. 1st derivatives of fluorescence thermal denaturation (FL-T_m) for all TO modified DNAs at 535 nm at pH 5.0

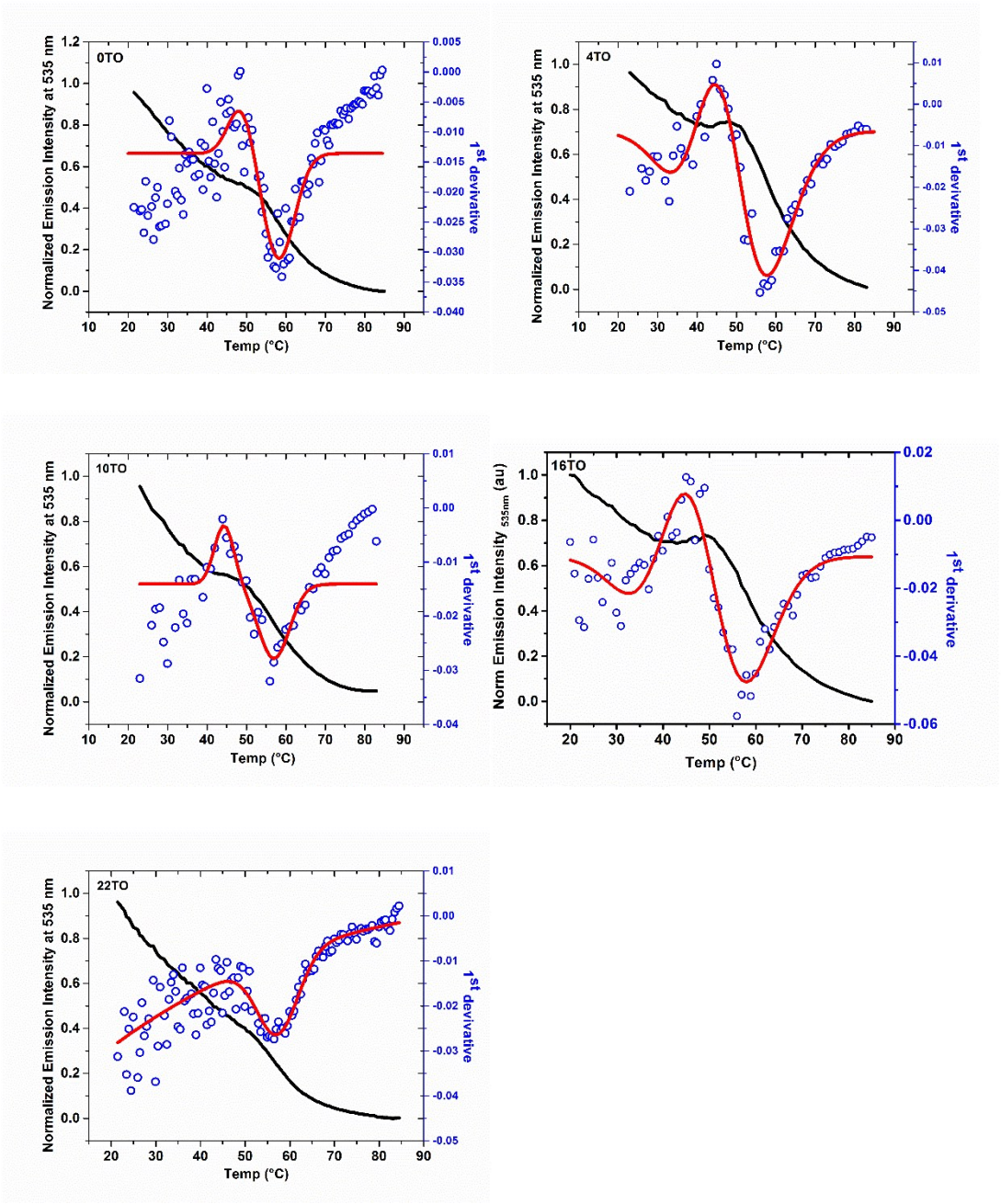
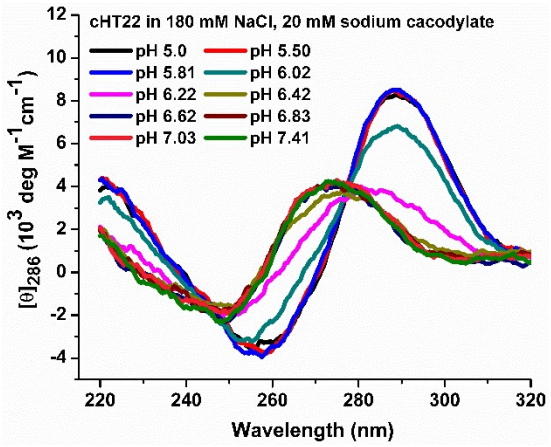
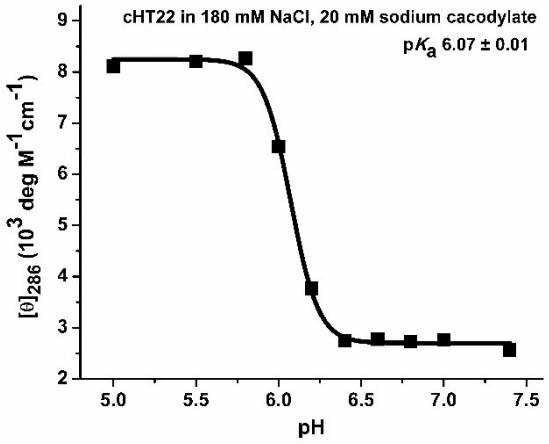
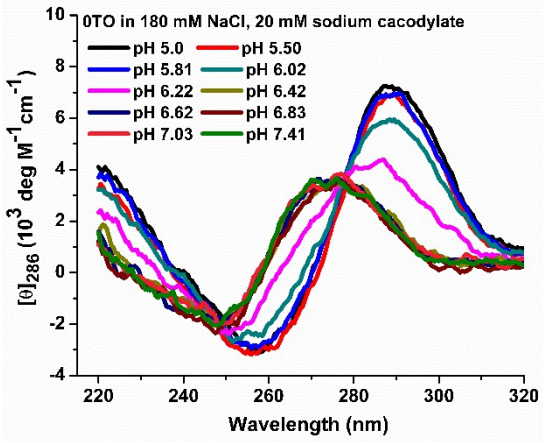
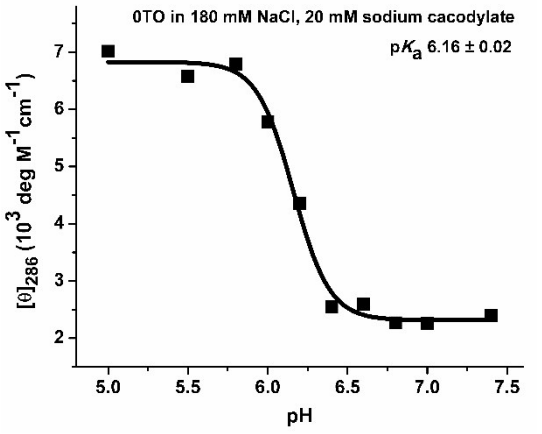


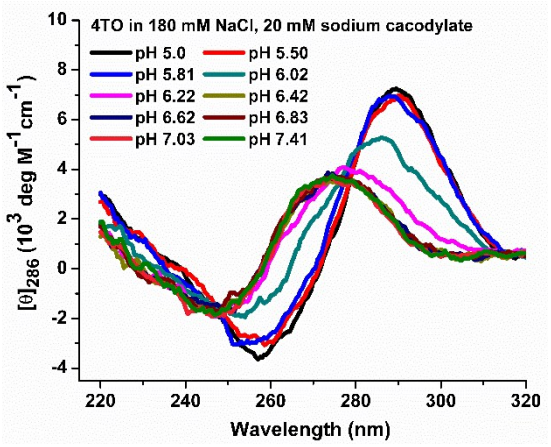
Figure S5: pH dependent CD spectra and p*K*a determination for all TO-DNAs and **cHT22**

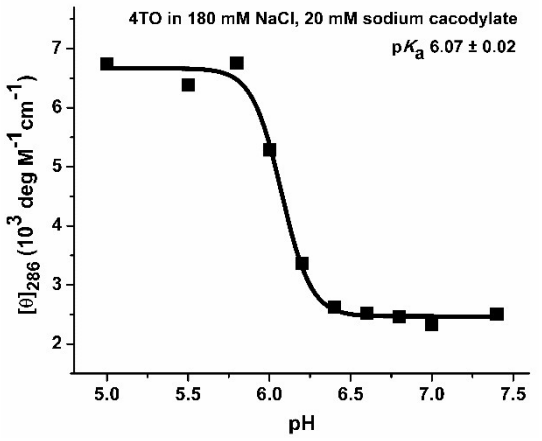


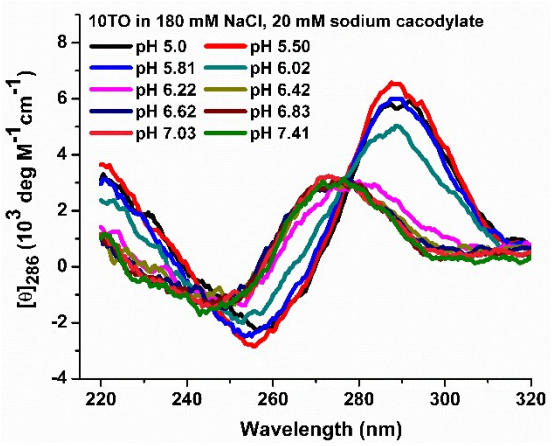


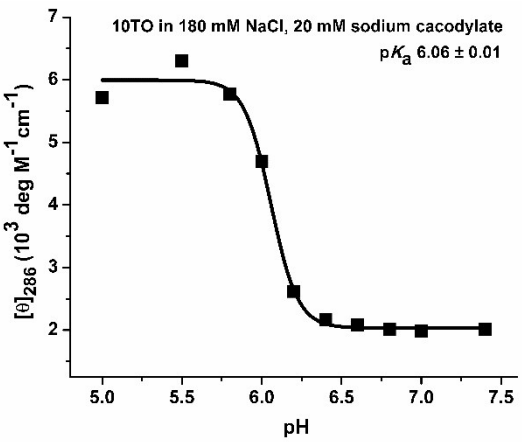


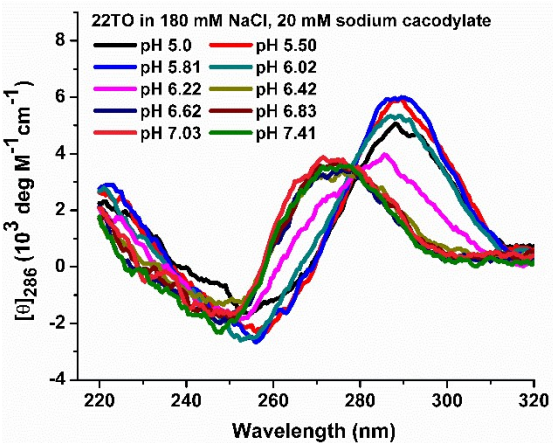







10TO in 180 mM NaCl, 20 mM sodium cacodylate
pH 5.0
pH 5.50
pH 5.81
pH 6.02
pH 6.22
pH 6.42
pH 6.62
pH 6.83
pH 7.03
pH 7.41
[θ]286 (10^3 deg M^-1cm^-1)
Wavelength (nm)


10TO in 180 mM NaCl, 20 mM sodium cacodylate
pKa 6.06 ± 0.01
[θ]286 (10^3 deg M^-1cm^-1)
pH


22TO in 180 mM NaCl, 20 mM sodium cacodylate
pH 5.0
pH 5.50
pH 5.81
pH 6.02
pH 6.22
pH 6.42
pH 6.62
pH 6.83
pH 7.03
pH 7.41
[θ]286 (10^3 deg M^-1cm^-1)
Wavelength (nm)

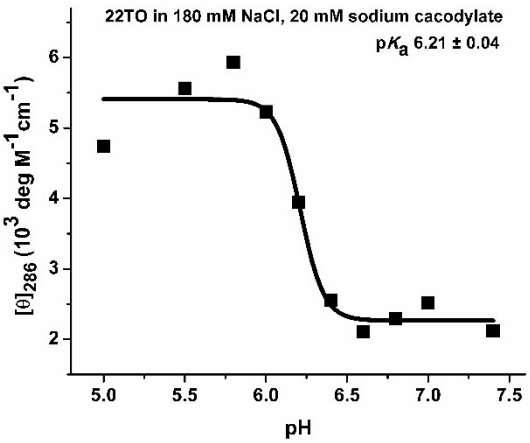

22TO in 180 mM NaCl, 20 mM sodium cacodylate
pKa 6.21 ± 0.04
[θ]286 (10^3 deg M^-1cm^-1)
pH

Figure S6: pH dependent static fluorescence spectra and intermediate pH determination for all **0TO, 4TO and 10TO**.

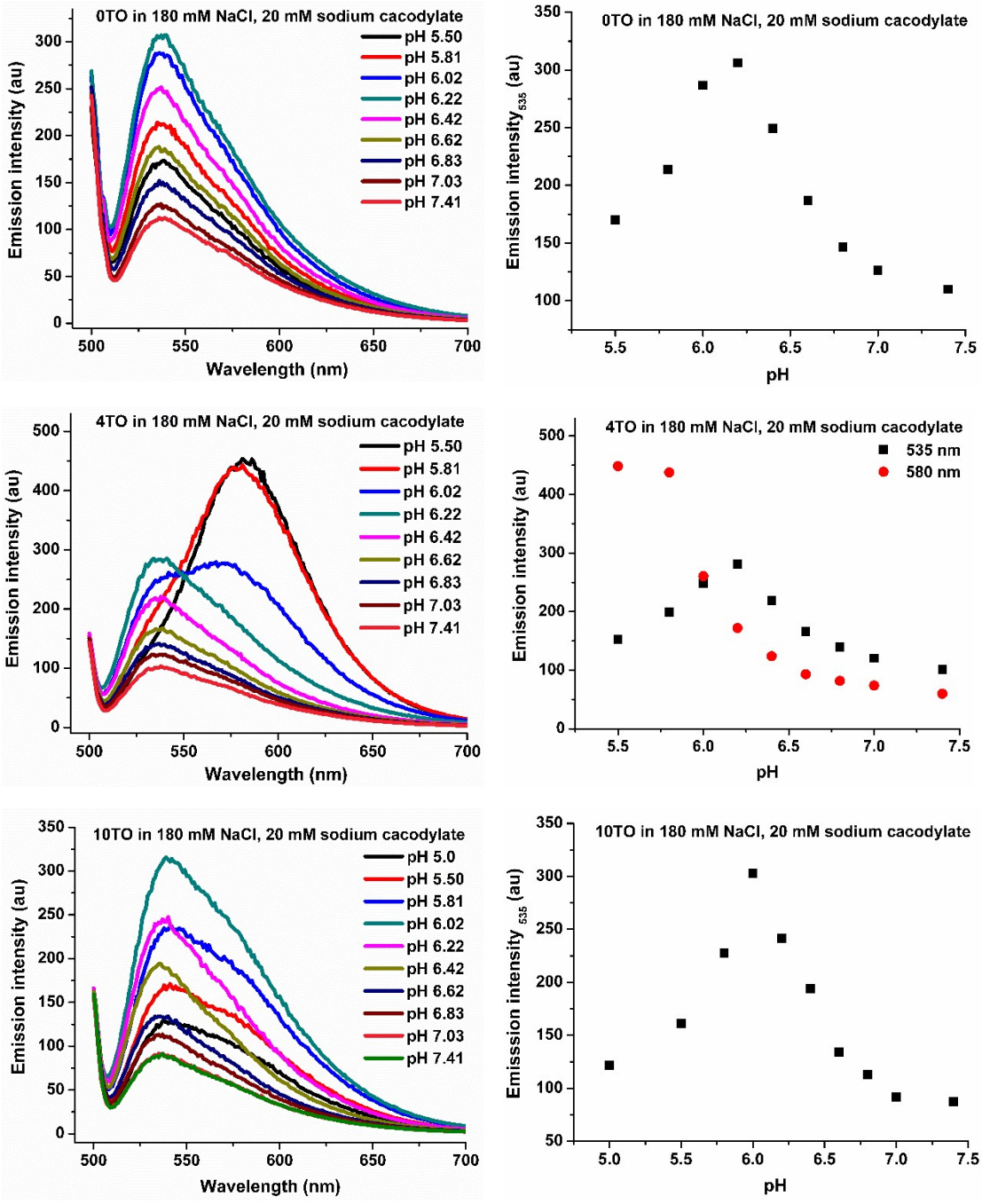


Figure S7: HPLC profiles for TO-DNAs

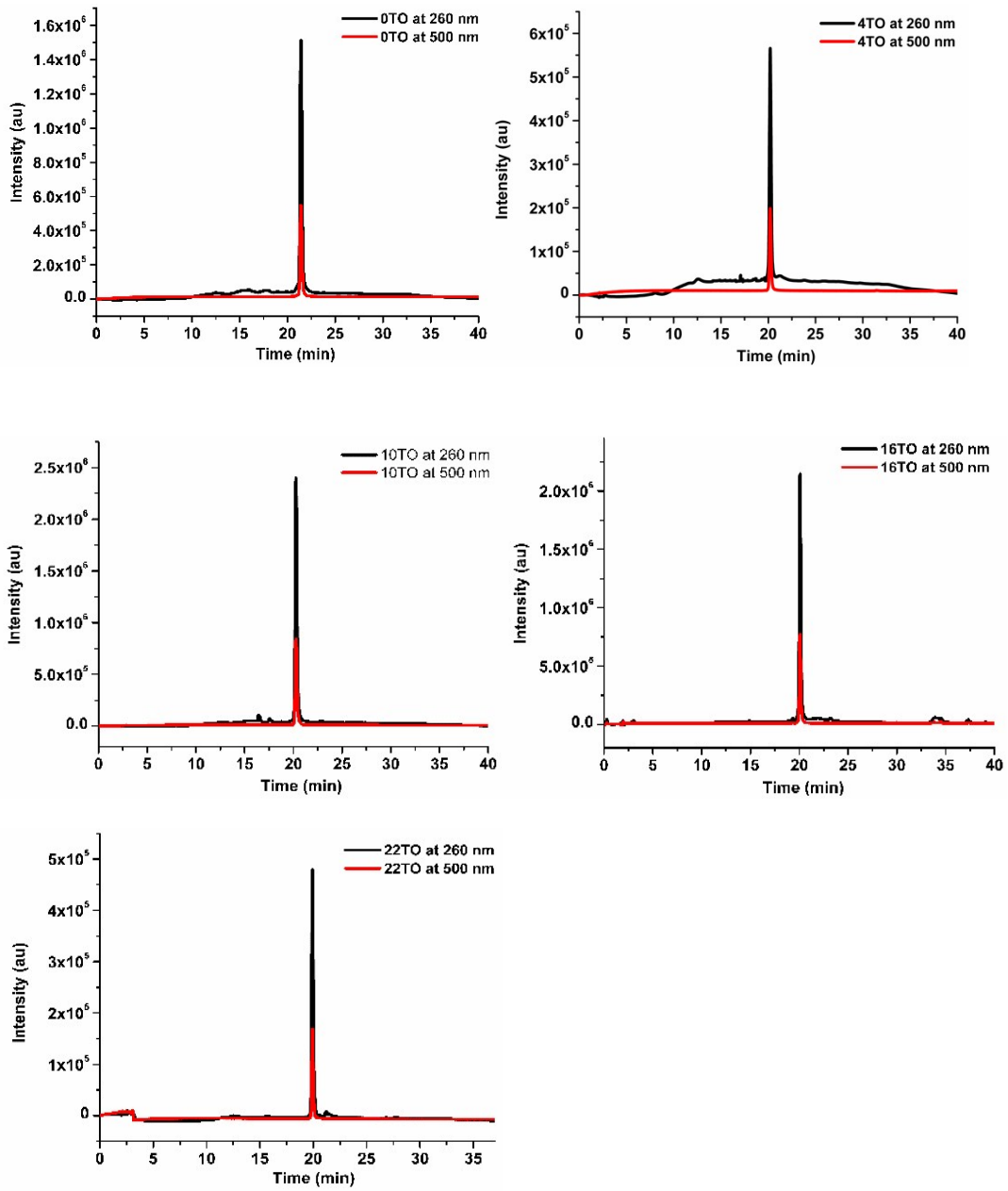
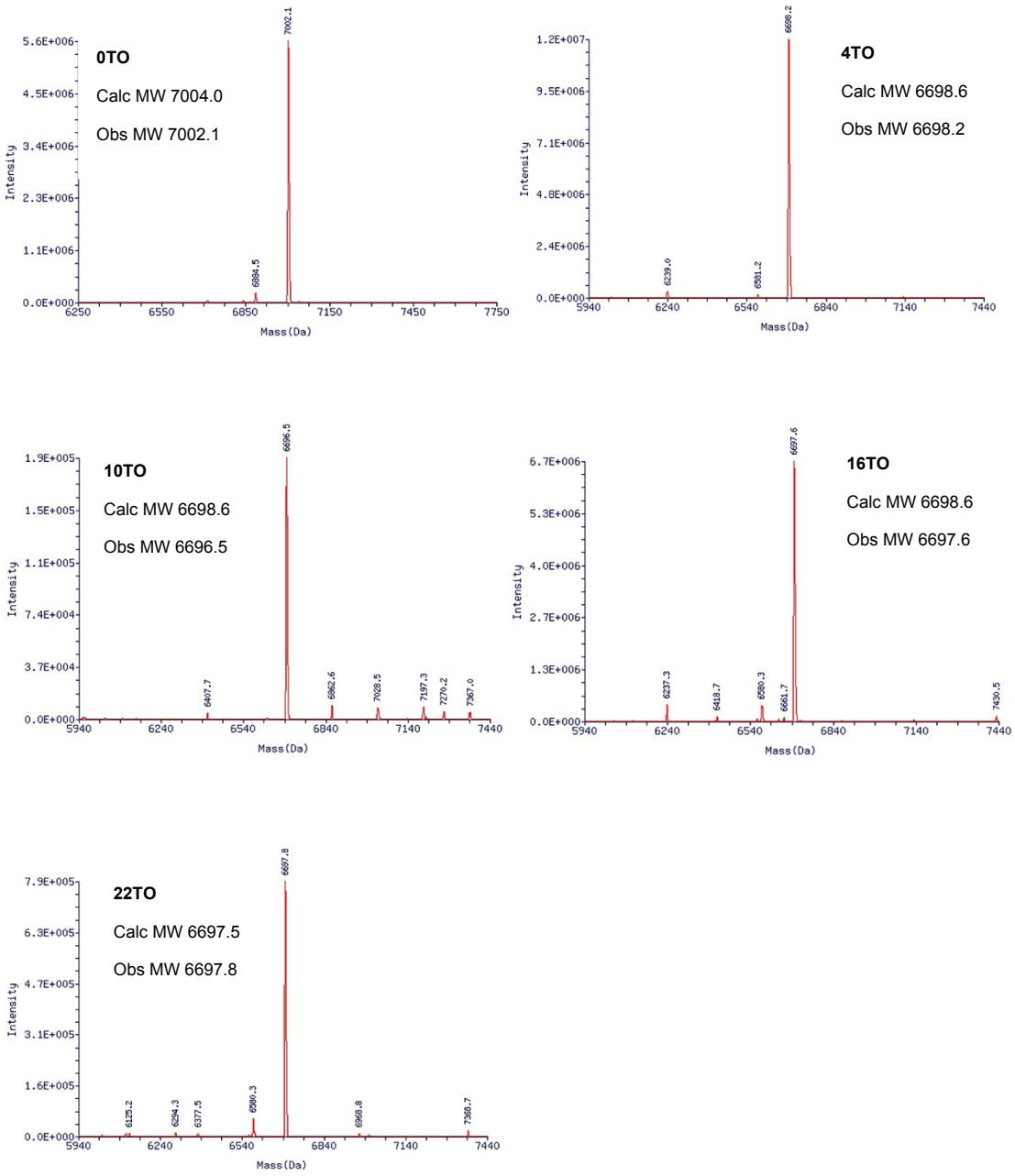


Figure S8: ESI-MS for TO modified DNAs



References

1. Hara, Y.; Fujii, T.; Kashida, H.; Sekiguchi, K.; Liang, X.; Niwa, K.; Takase, T.; Yoshida, Y.; Asanuma, H. *Angew. Chem. Int. Ed. Engl.* **2010**, *49*, 5502-5506.
2. Xu, B.; Wu, X.; Yeow, E. K.; Shao, F. *Chem. Commun.*, **2014**, *50*, 6402-6405.