Supplementary Information

Regulation of Telomeric i-motif Stability by 5-methylcytosine and 5-hydroxymethylcytosine Modification

Baochang Xu[‡], Gitali Devi[‡] and Fangwei Shao*

Division of Chemistry and Biological Chemistry, Nanyang Technological University, 21 Nanyang Link, Singapore 637371. Fax: (65)6791-1961; Tel: (65) 6592-2511;
E-mail: fwshao@ntu.edu.sg

Table of Contents

Experimental

Materials and Methods

All the chemicals were purchased from Aldrich, Alfa Aesar or Merck and used as received without further purification. Phosphoramidites of unmodified, and 5mC bases, CPG columns (1 μmol) and standard reagents for DNA synthesis were purchased from Glen Research. 5hmC phosphoramidites were synthesized at lab.[1] All the DNA oligonucleotides listed in Table 1 were synthesized using MerMade-4 DNA synthesizer.[1] These are purified by RP-HPLC in a Shimadzu HPLC instrument equipped with a Varian Dynamax 250×10 mm C18 reverse phase column. Either triethylammonium acetate (TEAA) or ammonium acetate buffer has been used as the elution buffer. The concentrations of oligonucleotides were determined by UV absorbance at 260 nm on a Cary100 UV-Visible Spectrophotometer at room temperature. Samples used for CD and UV measurements were 3 μM of DNA in 50 mM sodium cacodylate buffer with indicated pH, unless otherwise stated. The UV melting curves were obtained by monitoring UV absorbance at 260 nm with continuous elevation of temperature from 20 to 90 ^{o}C with a ramp rate of 0.5 ^{o}C/min in a Beckman Coulter DU 800 UV/Visible Spectrophotometer. Melting temperatures (T_m) of native and epigenetic modified i-motifs were obtained by sigmoidal fitting of UV melting curves. CD spectra were recorded on a Jasco J-810 CD spectrophotometer equipped with a temperature controller with the scan speed of 200 nm/min at 20 ^{o}C. Each measurement was an automatic average of 10 scans. Acid dissociation constants (p*K*a) of native and i-motifs with epigenetic cytosines were calculated by sigmoidal fitting the plots of CD signals at 286 nm against pHs. Standard deviations (SD) of melting temperatures and dissociation constants are calculated over at least three parallel experiment repeats.

Table S1. Properties of substituent at C5 position of pyrimidine ring in cytosine.

Nucleobase	C5 substituent	Electronic substituent Constant[1]	p*Ka*[2]	Hydrophobicity[3]	Size[4] (Å^3)
C	H	0	4.3	0.00	10.5
mC	CH_3	-0.07	4.4	0.56	30.5
hmC	CH_2OH	0	3.5	-1.03	40.8

[1]. C. Hansch, A. Leo, S. H. Unger, K. H. Kim, D. Nikaitan and E. J. Lien, *J. Med. Chem*. 1973, **16**, 1207. [2] R. M. C. Dawson, D. C. Elliott and W. H. Elliott, K. M. Jones, *Data for Biochemical Research*, *3rd ed.* Oxford, Clarendon Press, 1989. [3] The hydrophobic substituent constant is derived from partitioning studies of substituted benzenes between octanol and water, and negative value for hydroxymethyl substituent represents less hydrophobicity. Data from C. Hansch, A. Leo, S. H. Unger, K. H. Kim, D. Nikaitan and E. J. Lien, *J. Med. Chem.* 1973, **16**, 1207. [4] The volume is determined by linking the 5-position substituent to a single hydrogen atom and calculating the total volume. Data from C. S. Nabel, H. J. Jia, Y. Ye, L. Shen, H. L. Goldschmidt, J. T. Stivers, Y. Zhang and R. M. Kohli, *Nat. Chem. Biol*. 2012, **8**, 751.

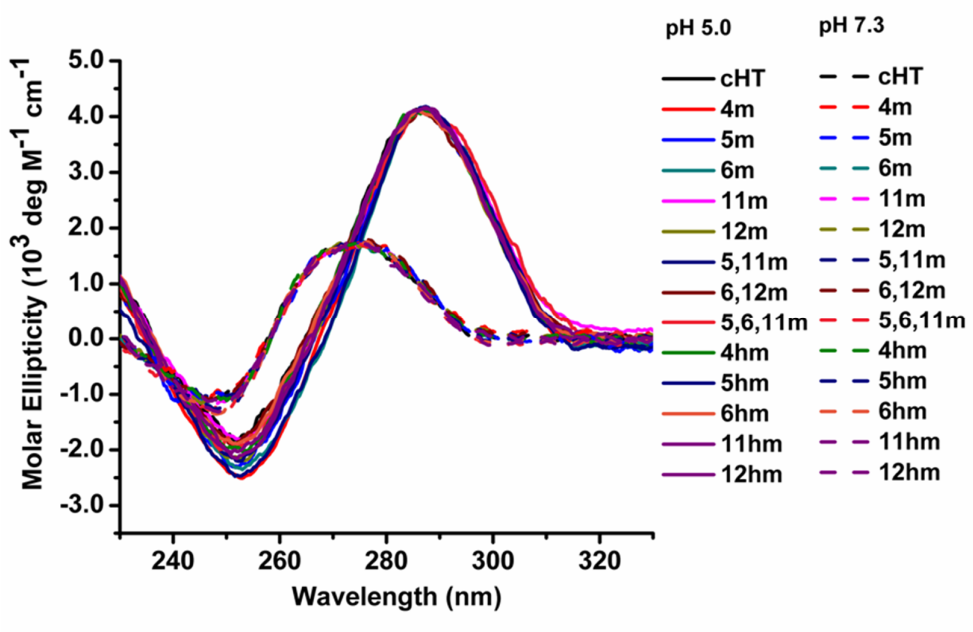


Figure S1. CD spectra of **cHT**, **Nm** and **Nhm** series of DNA at pH 5 (solid lines) and pH 7.3 (dash lines) at room temperature.

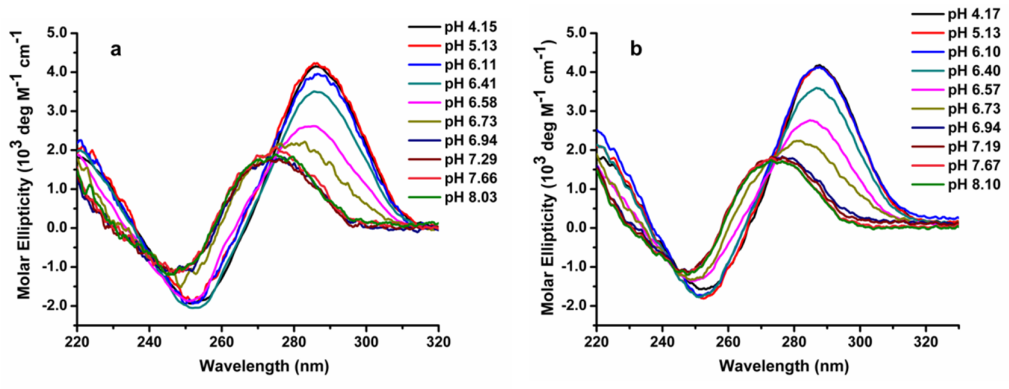


Figure S2. pH dependent CD spectra of (a) **cHT** and (b) **5m** in aqueous buffer.

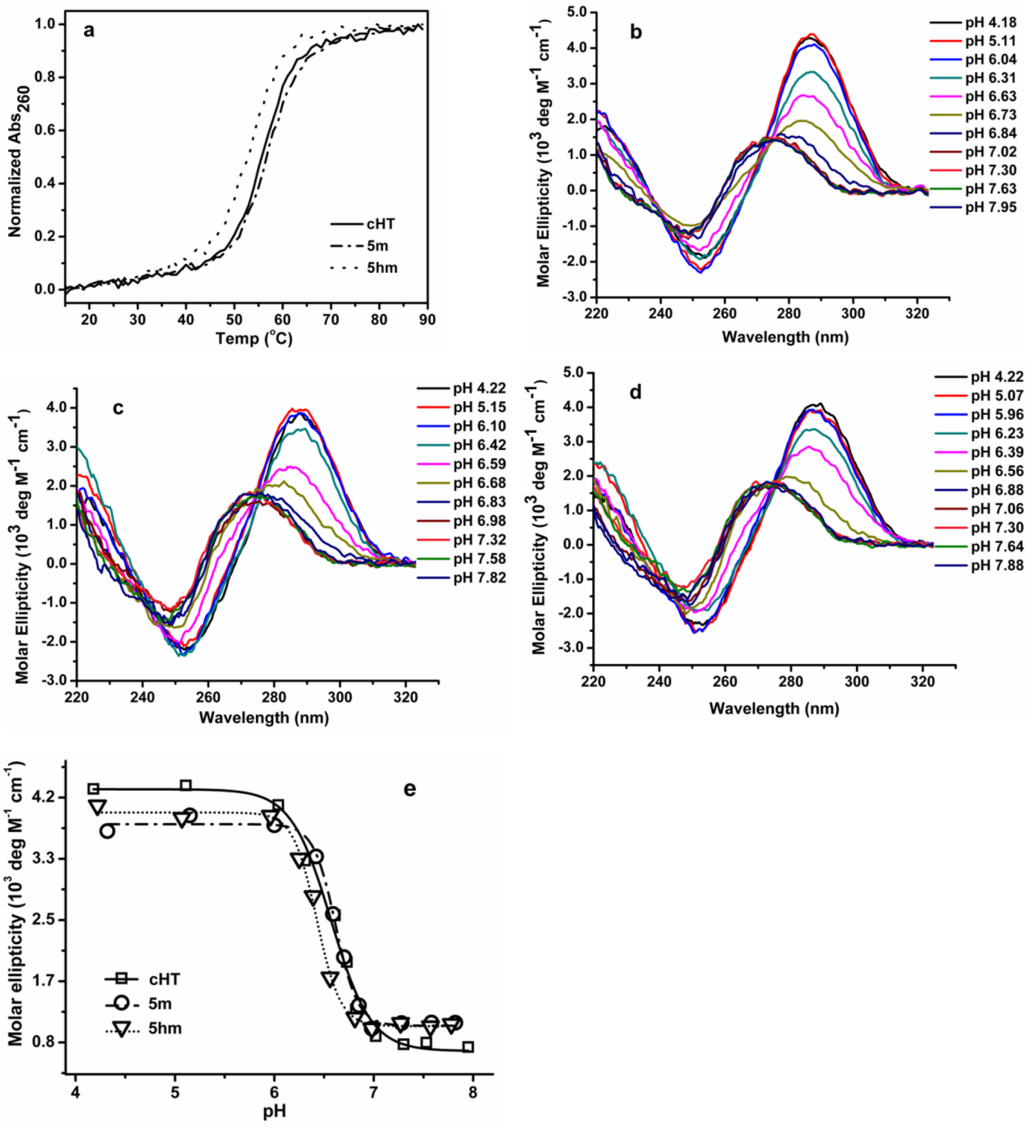
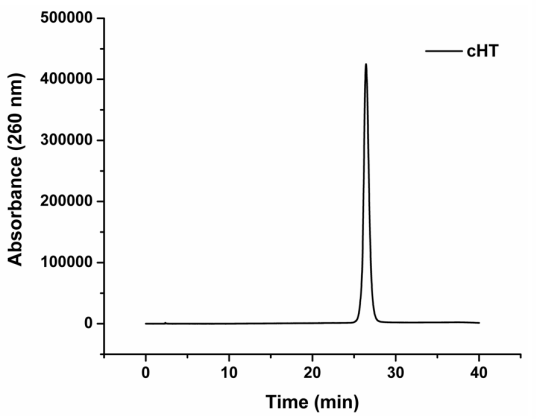
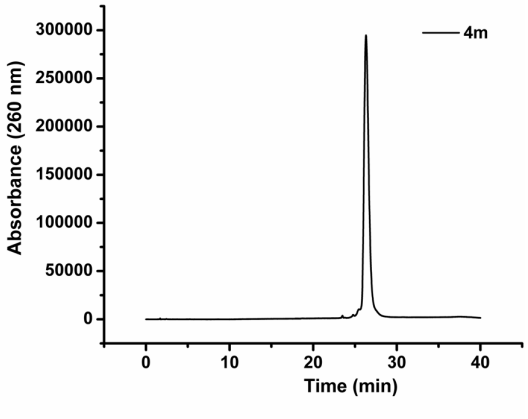
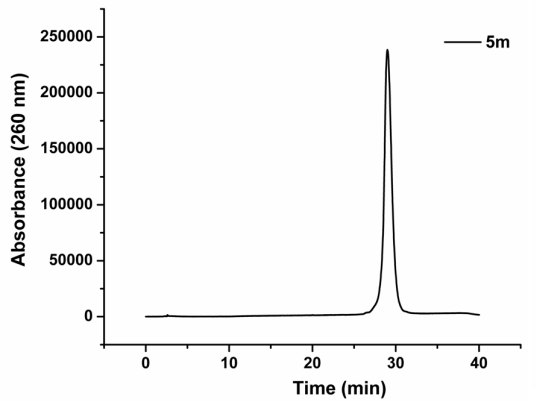
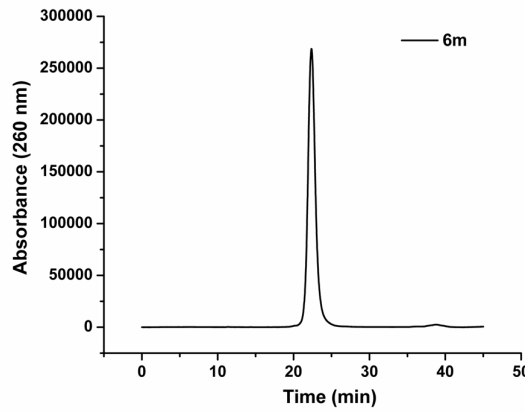


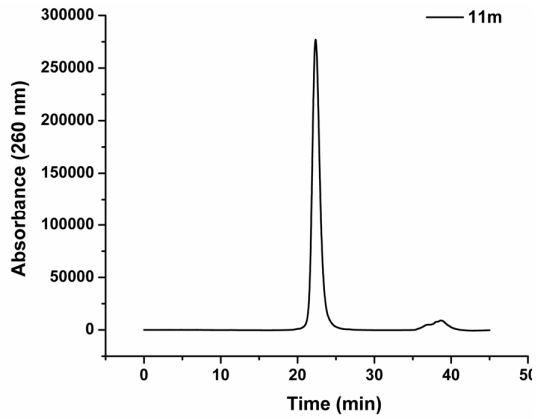
Figure S3. Thermal denaturation of **cHT**, **5m** and **5hm** (a); pH-dependent CD spectra of **cHT** (b), **5m** (c) and **5hm** (d); pH-dependent dissociation of i-motif (e) in presence of 20% (wt%) Ficoll 400, pH 5.0.

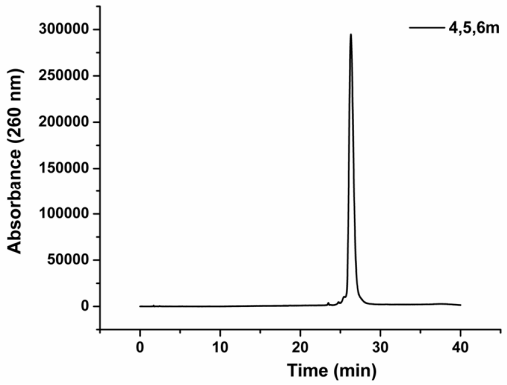

cHT
Absorbance (260 nm)
Time (min)


4m
Absorbance (260 nm)
Time (min)


5m
Absorbance (260 nm)
Time (min)


6m
Absorbance (260 nm)
Time (min)


11m
Absorbance (260 nm)
Time (min)


4,5,6m
Absorbance (260 nm)
Time (min)

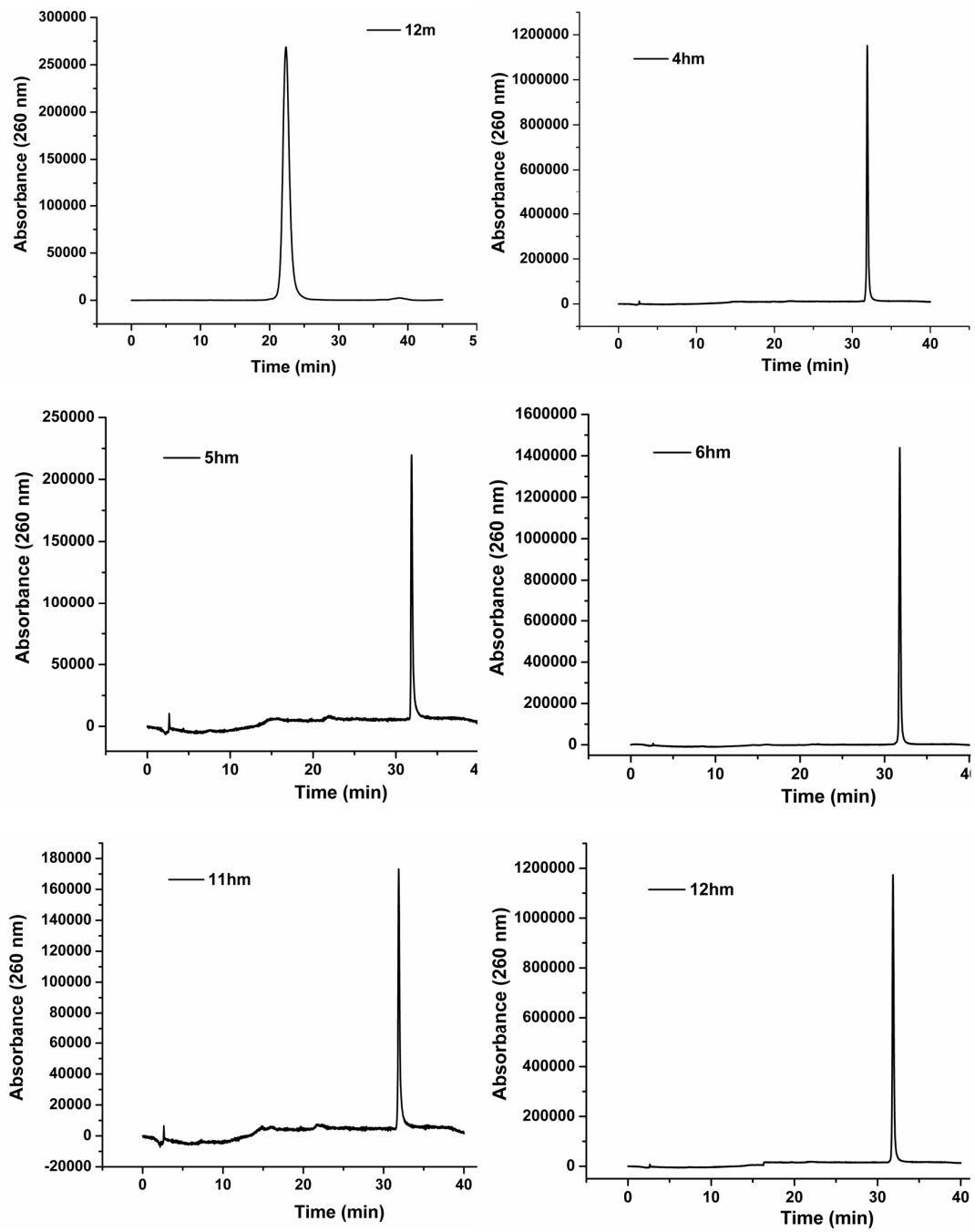
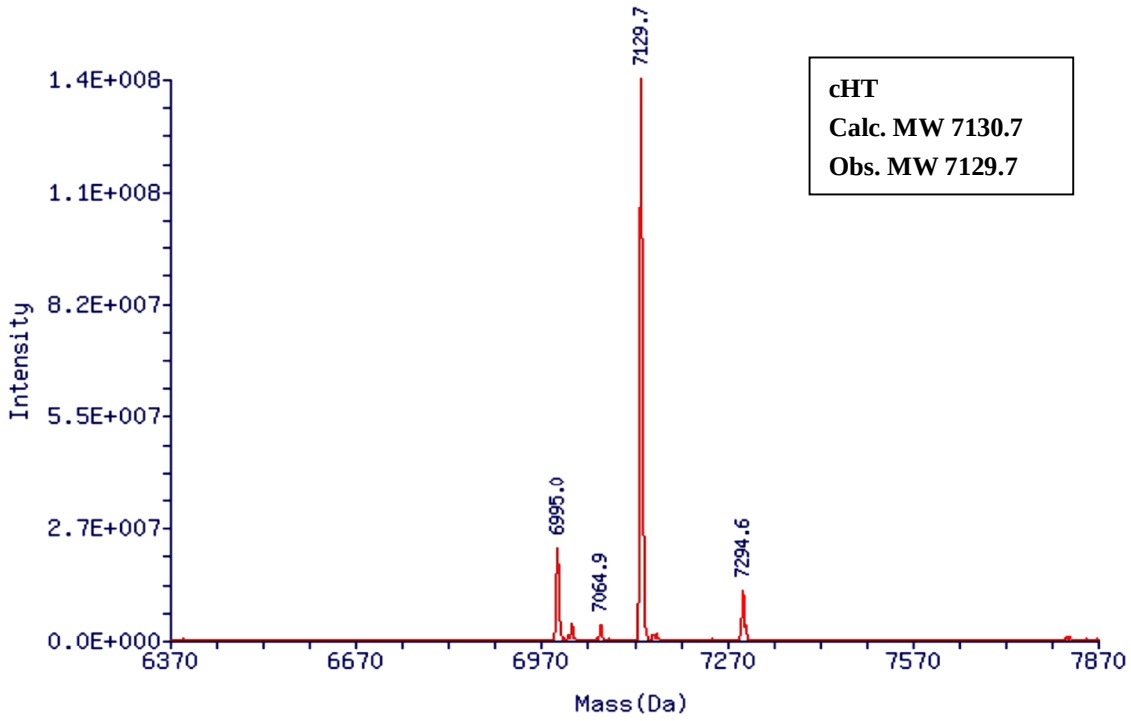
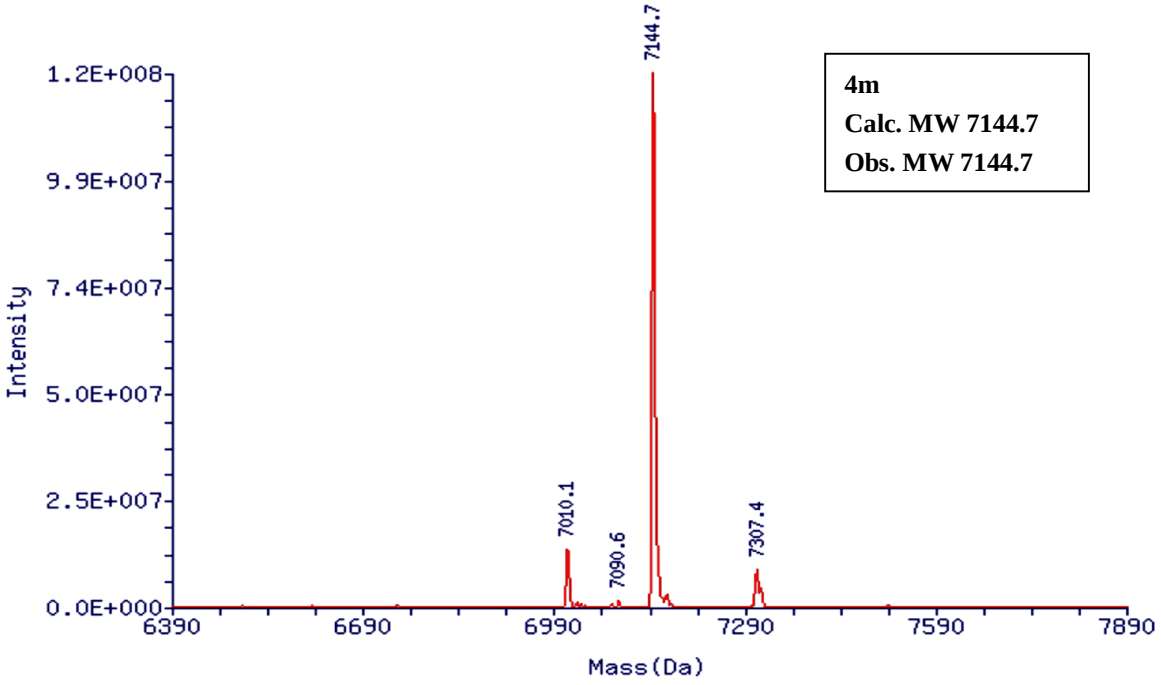
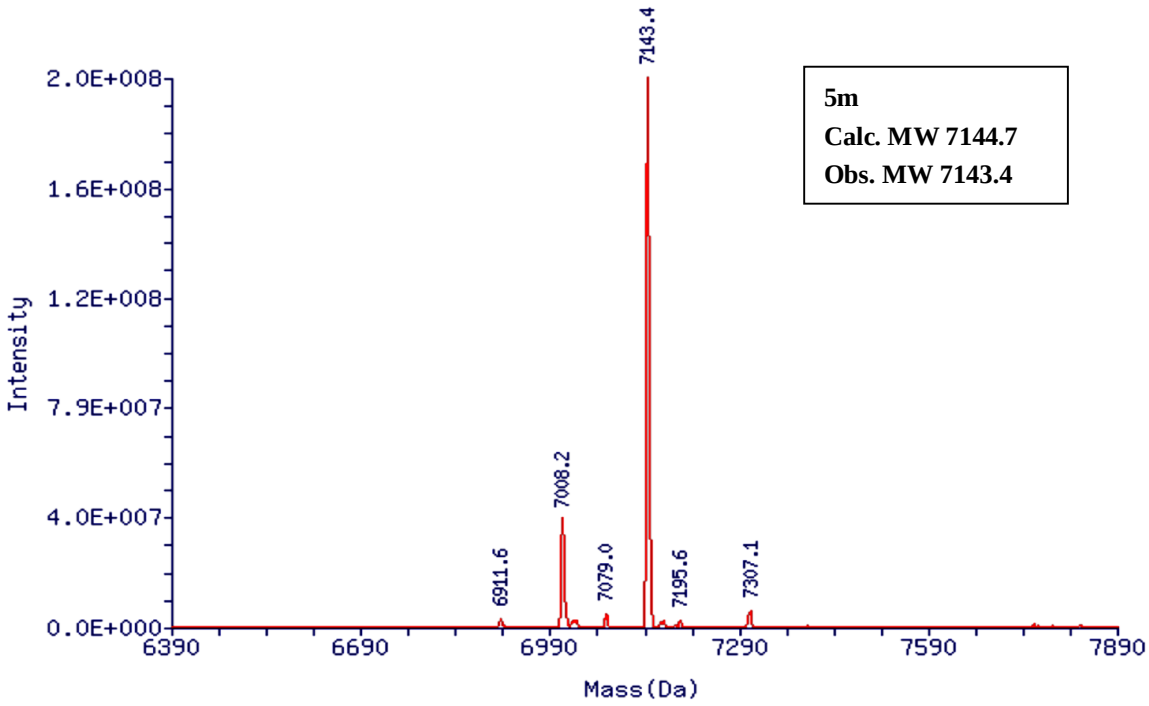
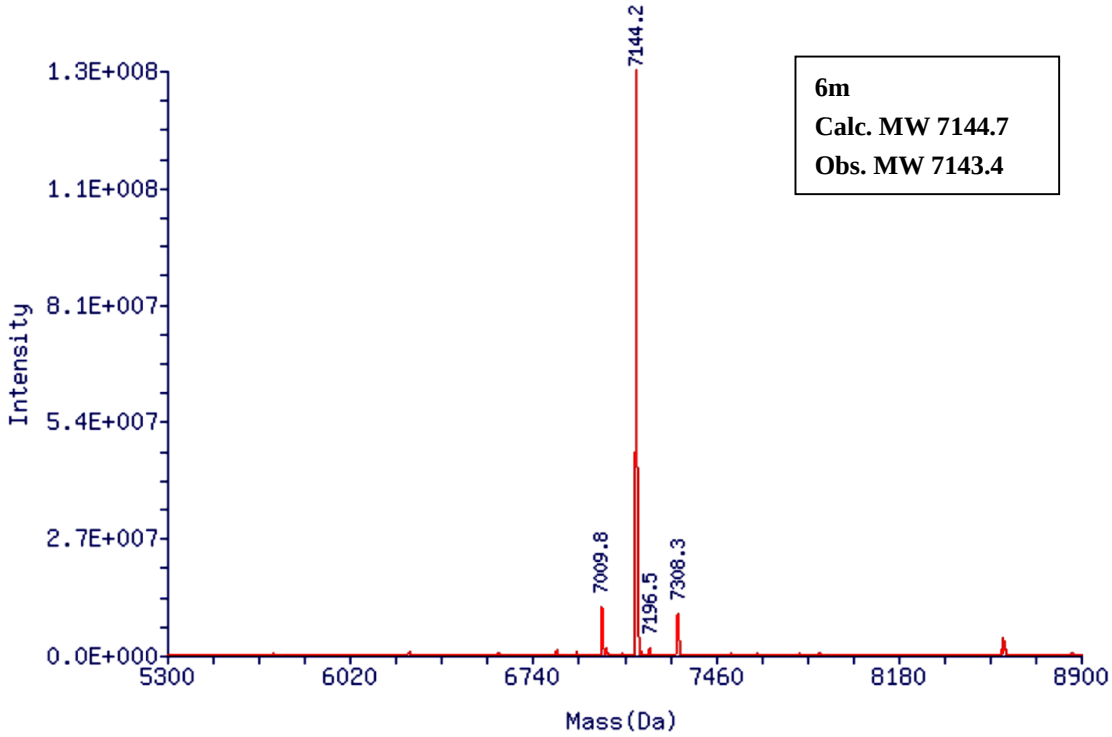


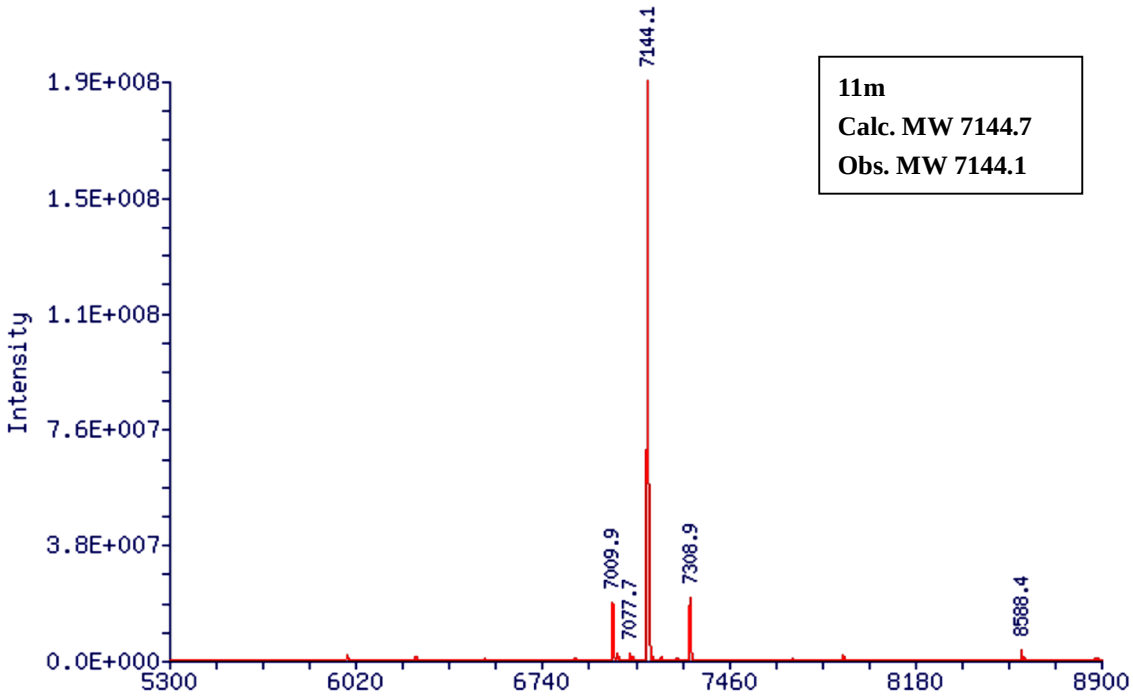
Figure S4: HPLC profiles for **cHT**, **Nm** and **Nhm** DNAs

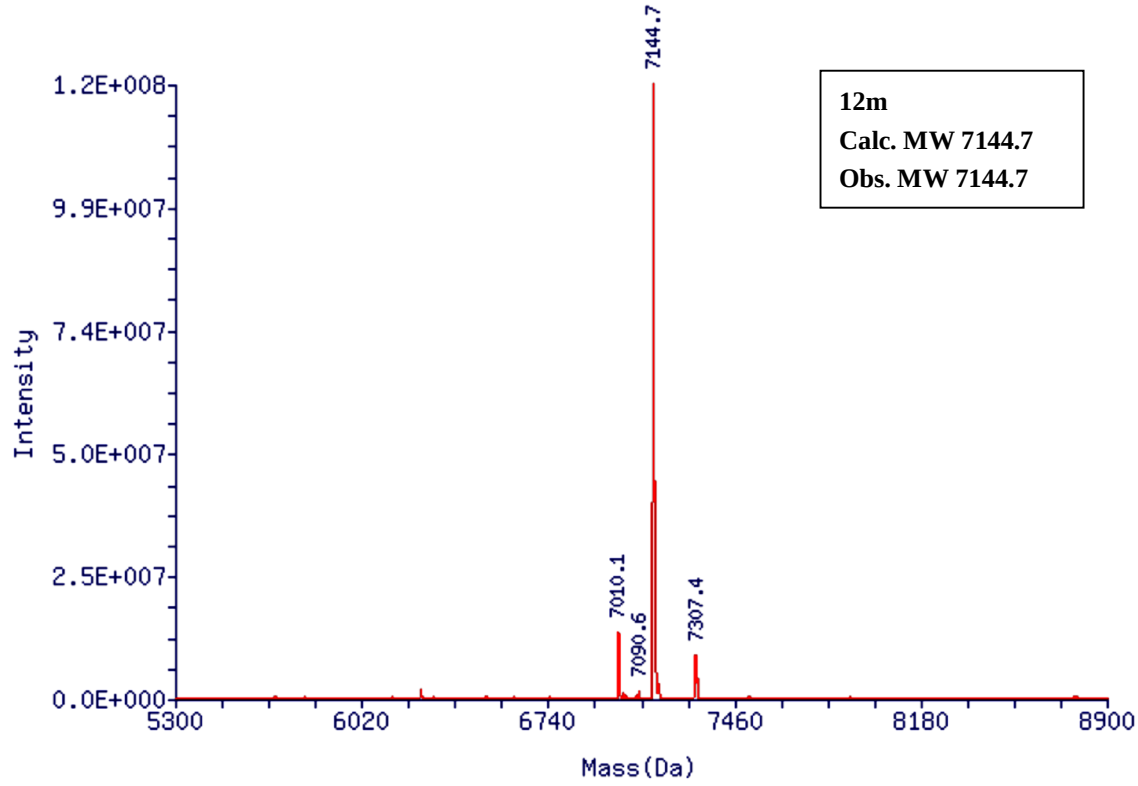

cHT
Calc. MW 7130.7
Obs. MW 7129.7
7129.7
6995.0
7064.9
7294.6
1.4E+008
1.1E+008
8.2E+007
5.5E+007
2.7E+007
0.0E+000
Intensity
6370
6670
6970
7270
7570
7870
Mass(Da)

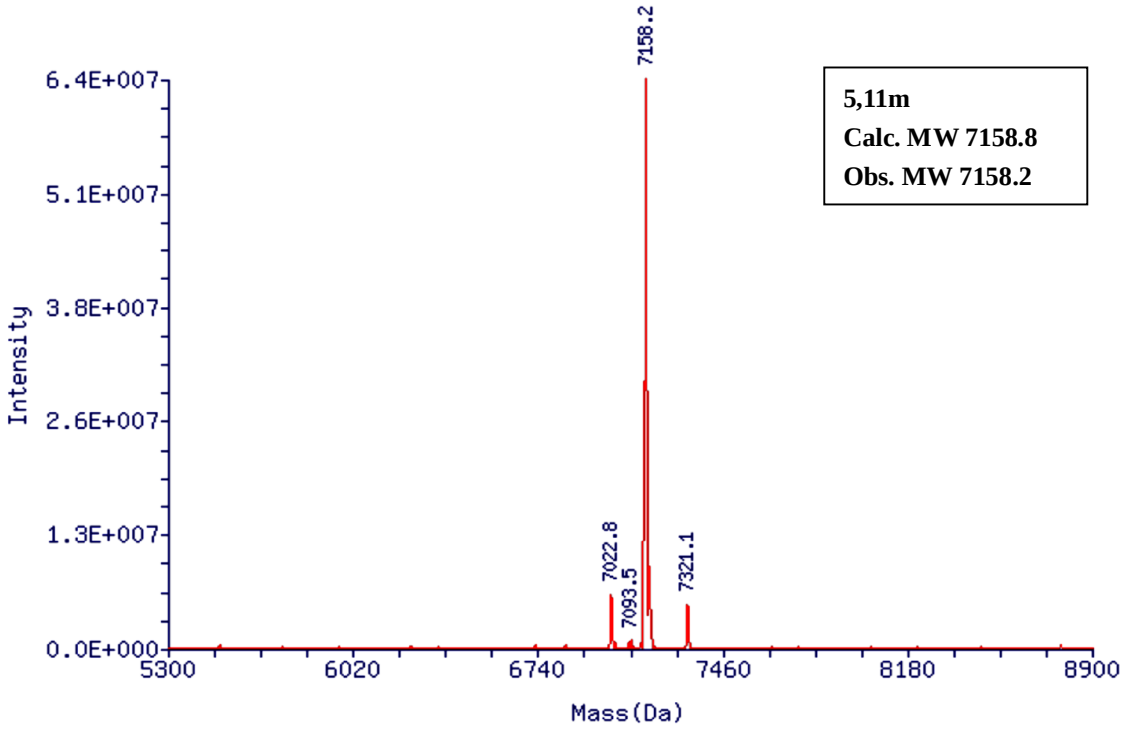

4m
Calc. MW 7144.7
Obs. MW 7144.7
7144.7
7010.1
7090.6
7307.4
1.2E+008
9.9E+007
7.4E+007
5.0E+007
2.5E+007
0.0E+000
Intensity
6390
6690
6990
7290
7590
7890
Mass(Da)

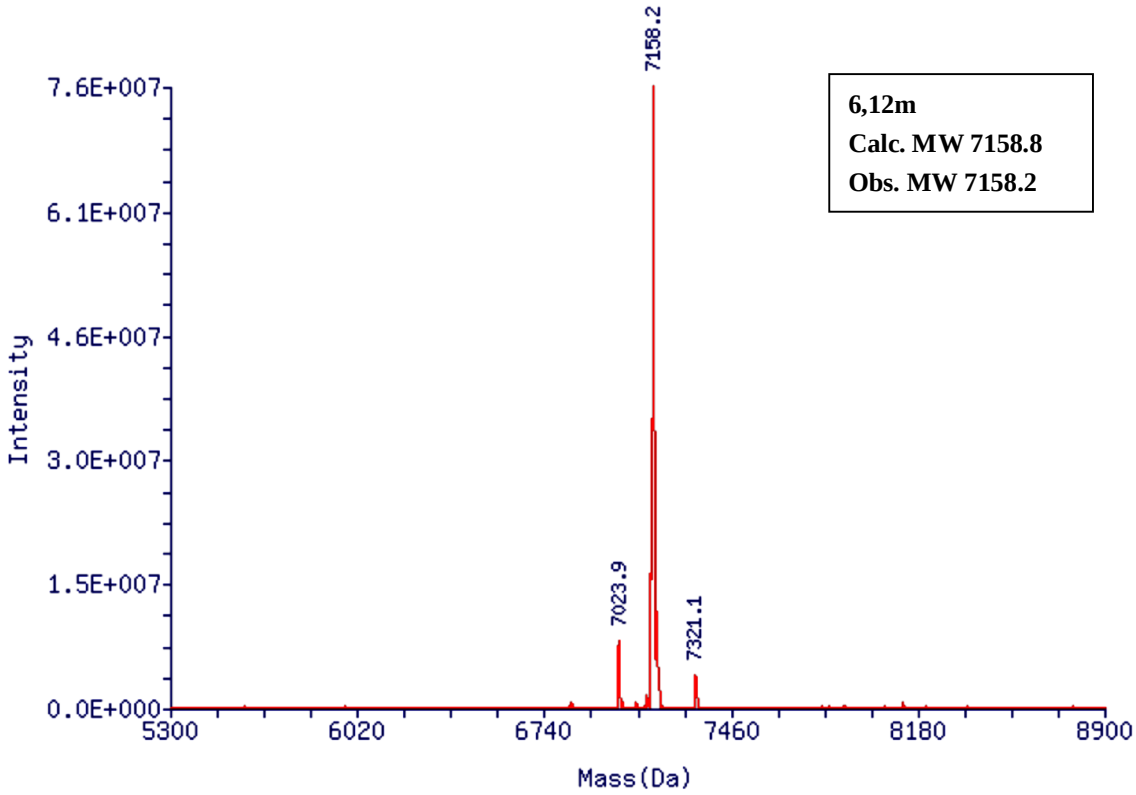

5m
Calc. MW 7144.7
Obs. MW 7143.4
Intensity
2.0E+008
1.6E+008
1.2E+008
7.9E+007
4.0E+007
0.0E+000
6911.6
7008.2
7079.0
7143.4
7195.6
7307.1
6390
6690
6990
7290
7590
7890
Mass(Da)

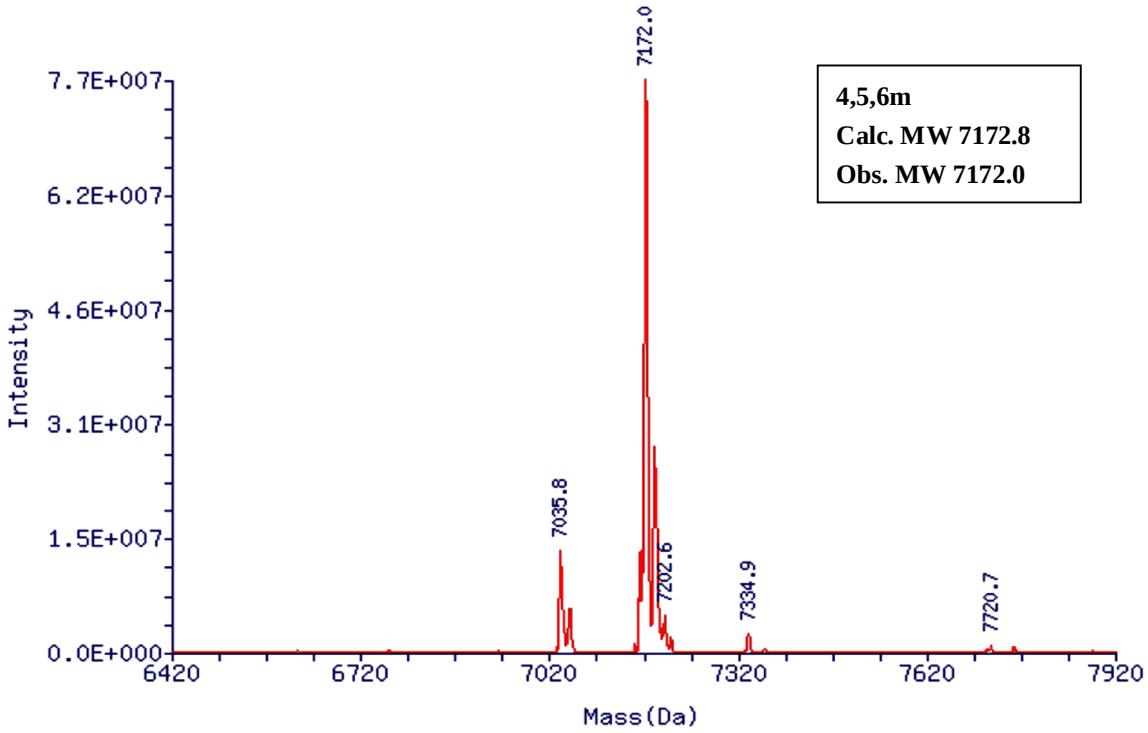

6m
Calc. MW 7144.7
Obs. MW 7143.4
Intensity
1.3E+008
1.1E+008
8.1E+007
5.4E+007
2.7E+007
0.0E+000
7009.8
7144.2
7196.5
7308.3
5300
6020
6740
7460
8180
8900
Mass(Da)

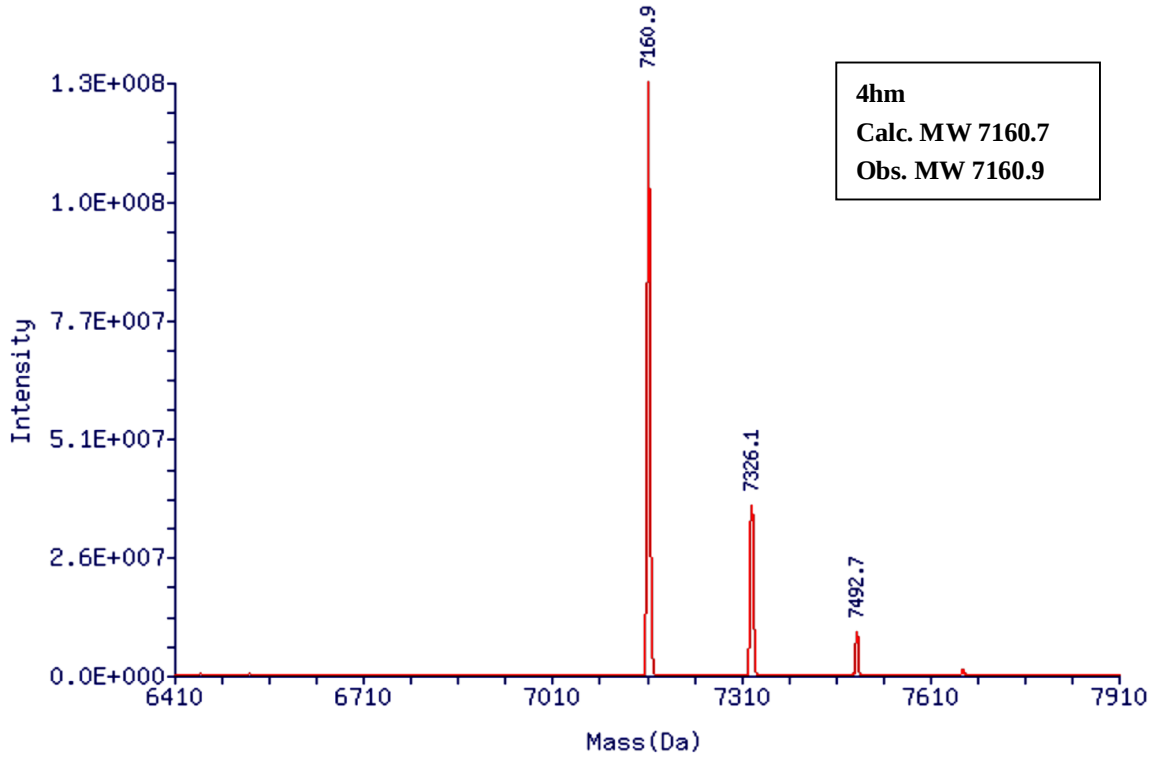

11m
Calc. MW 7144.7
Obs. MW 7144.1
Intensity
1.9E+008
1.5E+008
1.1E+008
7.6E+007
3.8E+007
0.0E+000
5300
6020
6740
7460
8180
8900
7144.1
7009.9
7077.7
7308.9
8588.4

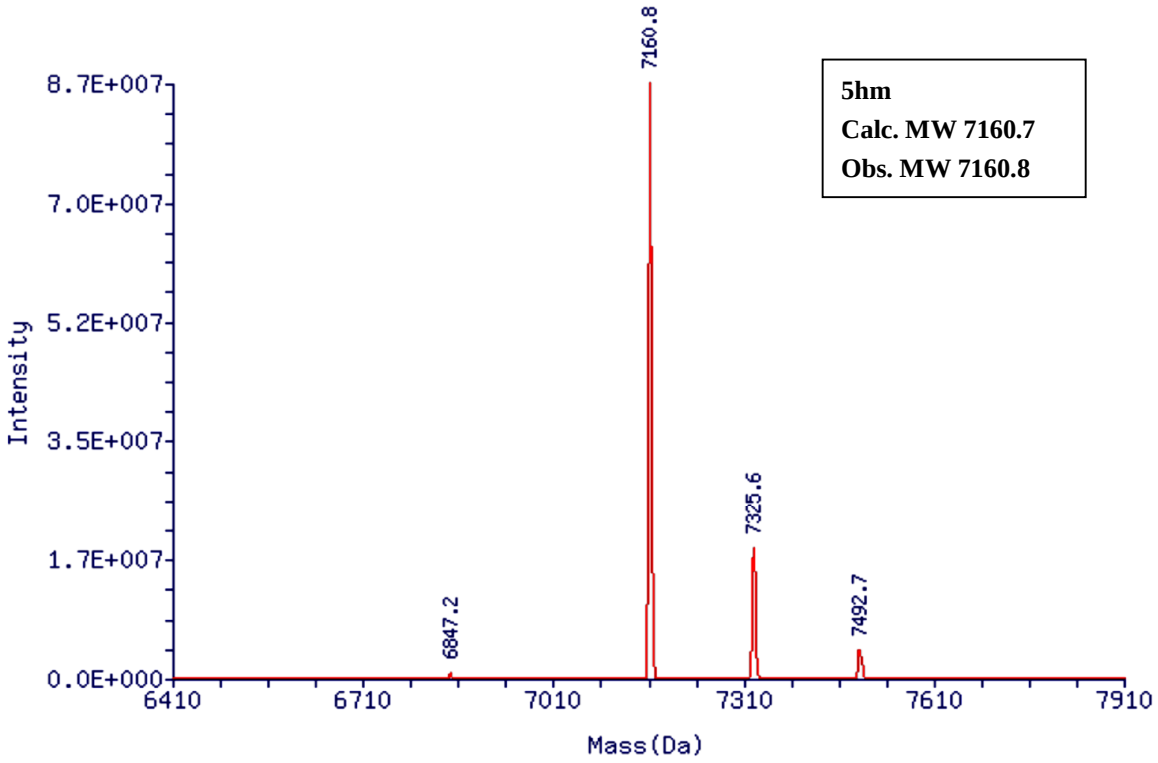

12m
Calc. MW 7144.7
Obs. MW 7144.7
Intensity
1.2E+008
9.9E+007
7.4E+007
5.0E+007
2.5E+007
0.0E+000
5300
6020
6740
7460
8180
8900
Mass(Da)
7144.7
7010.1
7090.6
7307.4

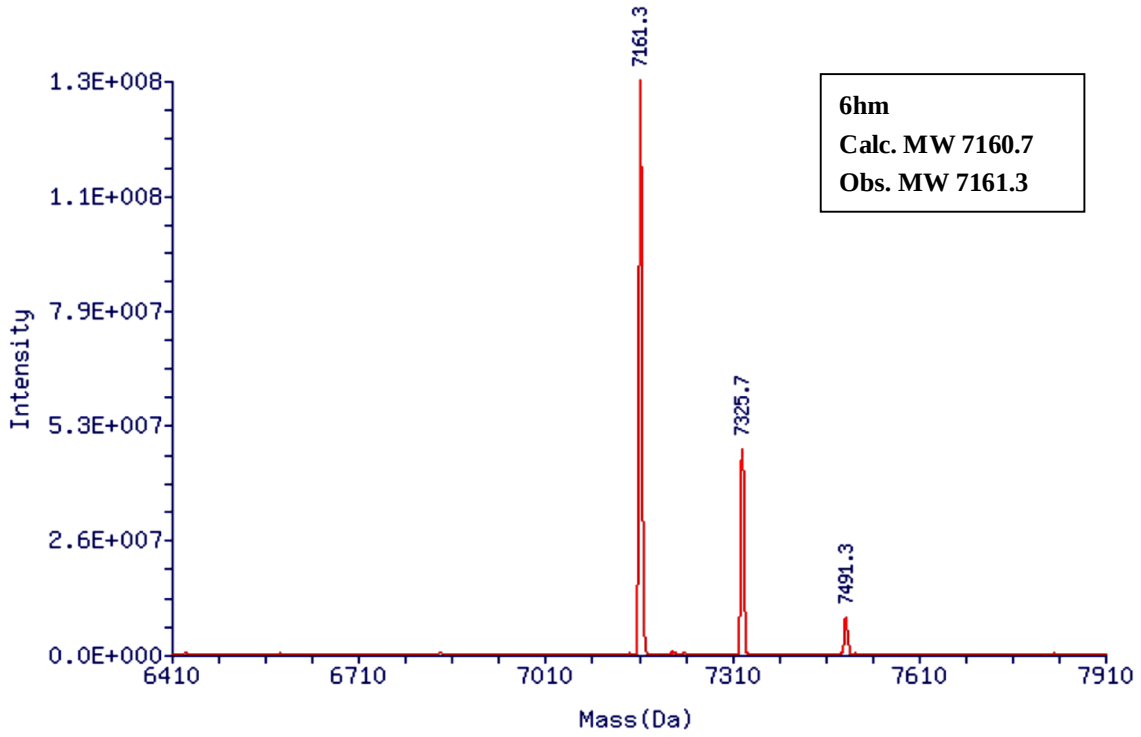

5,11m
Calc. MW 7158.8
Obs. MW 7158.2
7158.2
7022.8
7093.5
7321.1
6.4E+007
5.1E+007
3.8E+007
2.6E+007
1.3E+007
0.0E+000
Intensity
5300
6020
6740
7460
8180
8900
Mass(Da)


6,12m
Calc. MW 7158.8
Obs. MW 7158.2
7158.2
7023.9
7321.1
7.6E+007
6.1E+007
4.6E+007
3.0E+007
1.5E+007
0.0E+000
Intensity
5300
6020
6740
7460
8180
8900
Mass(Da)


7172.0
4,5,6m
Calc. MW 7172.8
Obs. MW 7172.0
7.7E+007
6.2E+007
4.6E+007
3.1E+007
1.5E+007
0.0E+000
Intensity
7035.8
7202.6
7334.9
7720.7
6420
6720
7020
7320
7620
7920
Mass(Da)


7160.9
4hm
Calc. MW 7160.7
Obs. MW 7160.9
1.3E+008
1.0E+008
7.7E+007
5.1E+007
2.6E+007
0.0E+000
Intensity
7326.1
7492.7
6410
6710
7010
7310
7610
7910
Mass(Da)


5hm
Calc. MW 7160.7
Obs. MW 7160.8
7160.8
7325.6
7492.7
6847.2
8.7E+007
7.0E+007
5.2E+007
3.5E+007
1.7E+007
0.0E+000
Intensity
6410
6710
7010
7310
7610
7910
Mass(Da)


6hm
Calc. MW 7160.7
Obs. MW 7161.3
7161.3
7325.7
7491.3
1.3E+008
1.1E+008
7.9E+007
5.3E+007
2.6E+007
0.0E+000
Intensity
6410
6710
7010
7310
7610
7910
Mass(Da)

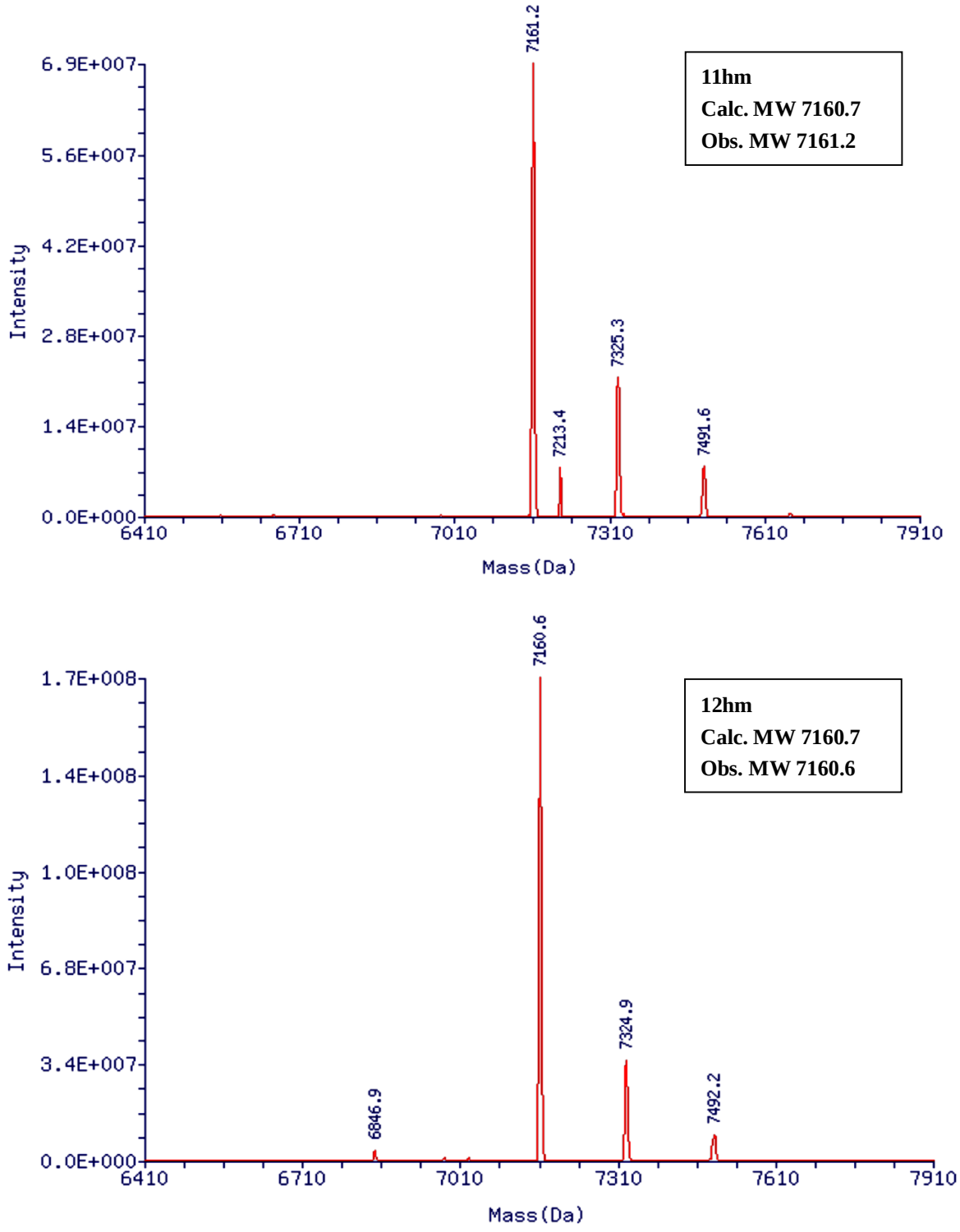


Figure S5: ESI-MS for **cHT**, **Nm** and **Nhm** DNAs

Reference

1. S. Xuan, Q. Wu, L. Cui, D. Zhang and F. Shao, *Bioorganic & medicinal chemistry letters*, 2015, 25, 1186-1191.