CHEMBIOCHEM

Supporting Information

DOI: 10.1002/cbic.200900232

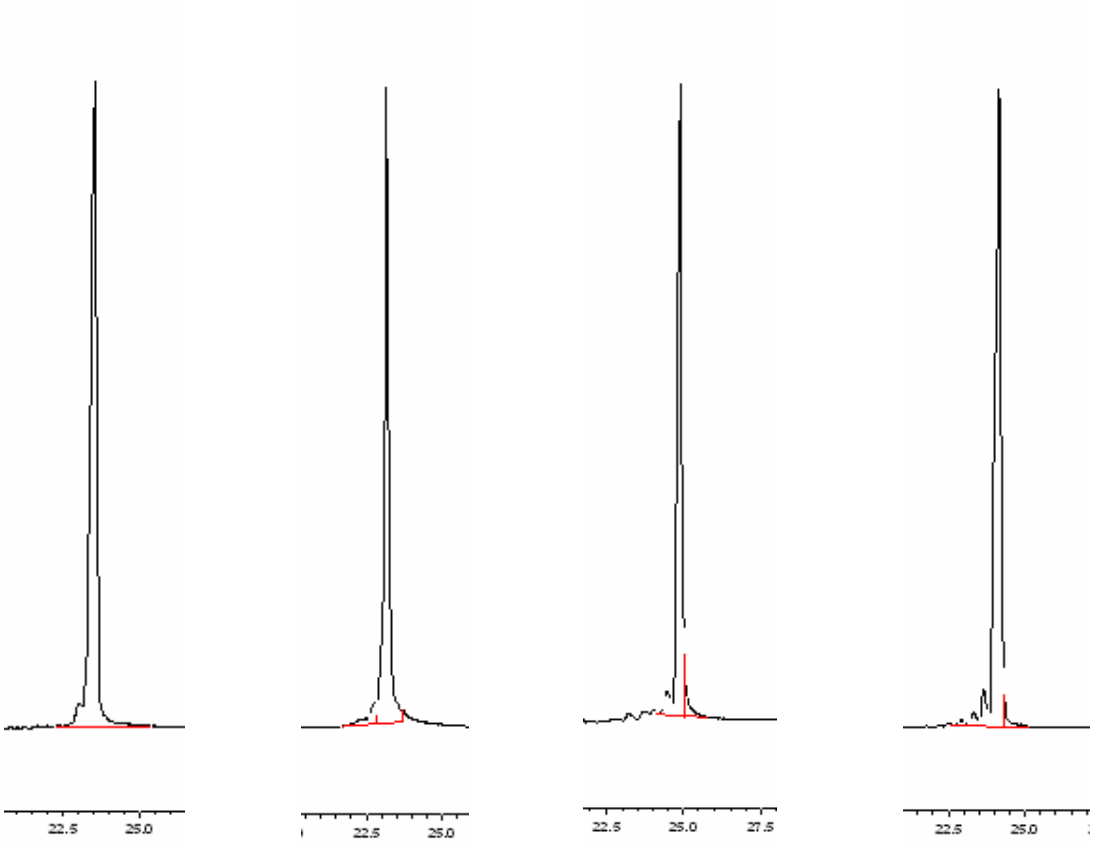
Supporting Information

for

Potent Triple Helix Stabilization by 5',3'-Modified Triplex Forming Oligonucleotides

Nouha Ben Gaied, Zhengyun Zhao, Simon R. Gerrard,
Keith R. Fox, and Tom Brown*

TFO-11	TFO-14	TFO-15	TFO-16
S-AnthThr	**S-MeAcPyr**	**S-DAEdR**	**S-Pam**



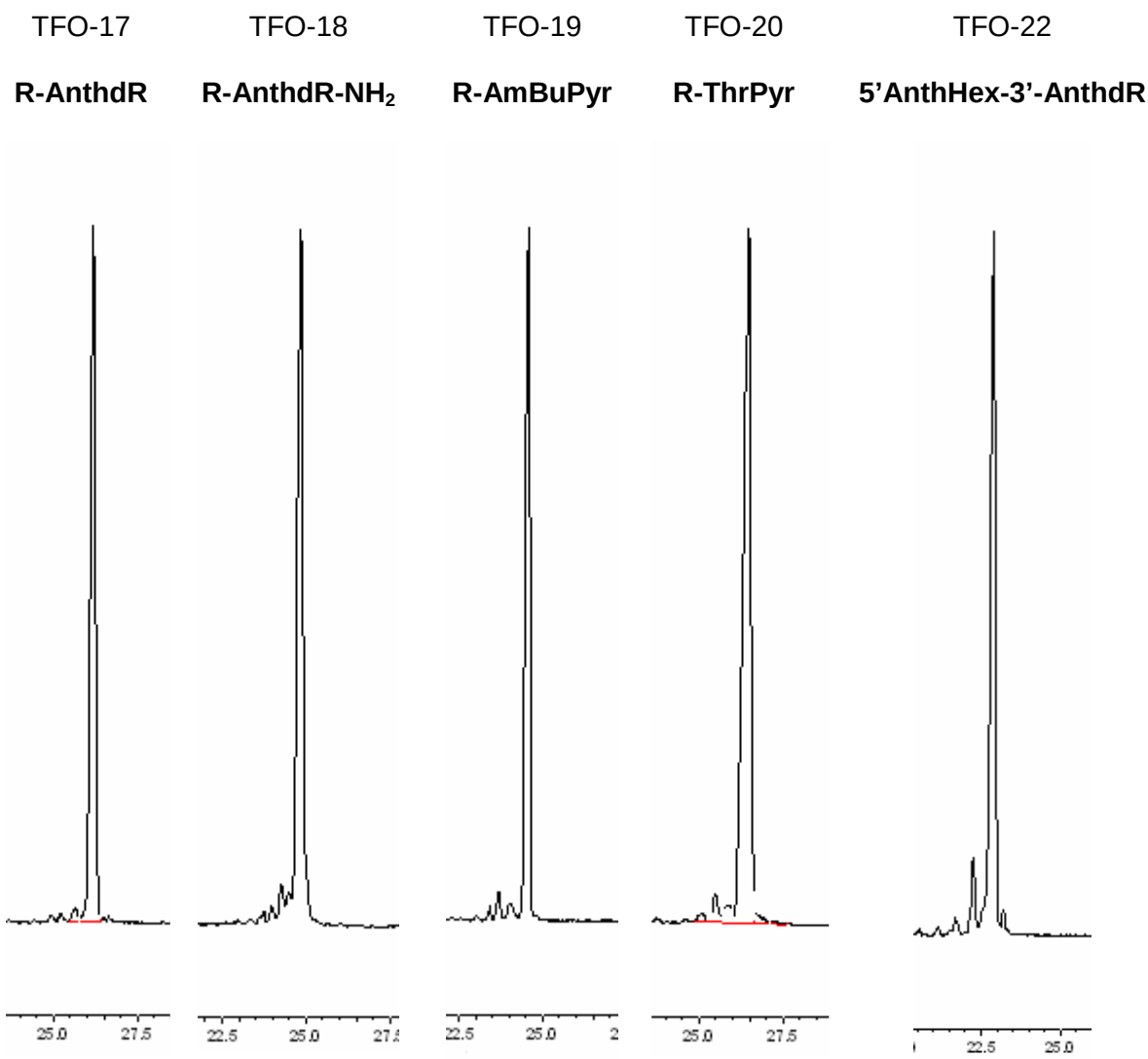
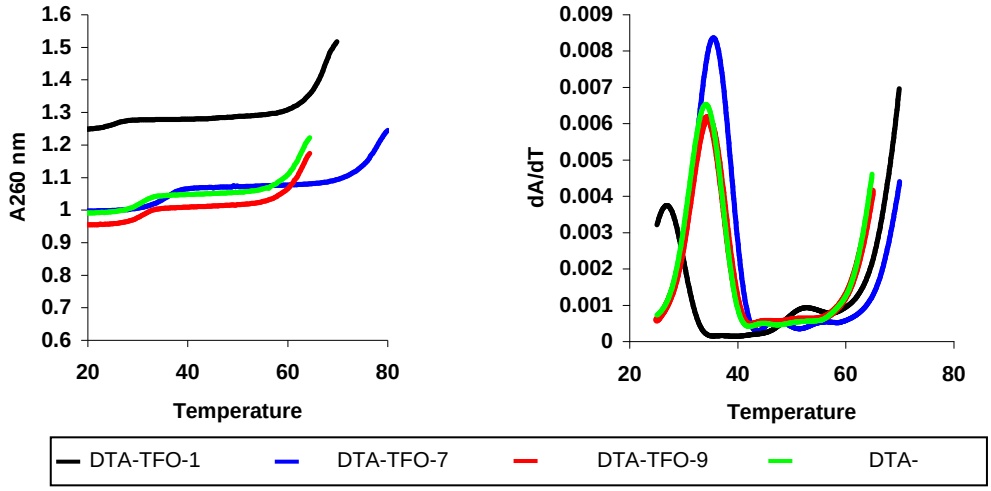
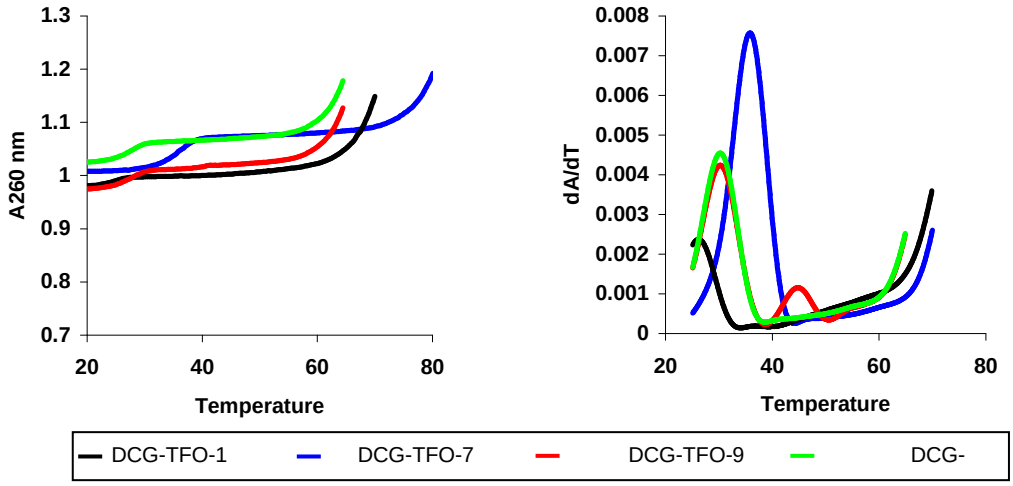


Figure S1. Representative Capillary electrophoresis (CE) analysis of modified oligonucleotides (TFO-11, TFO14-20 and TFO-22) performed after HPLC purification. The purity of oligonucleotides was confirmed by injection (0.4 OD/100 µL) of each sample individually on ssDNA 100-R Gel. Tris-Borate 7M Urea were used (kit N°477480) on a Beckman coulter P/ACE™ MDQ Capillary Electrophoresis system using 32 Karat software. UV-254, injection voltage 10.0kV and separation voltage 9.0 kV (45.0 min duration). The x-axis shows time in min and the y-axis is UV absorbance at 254 nm.

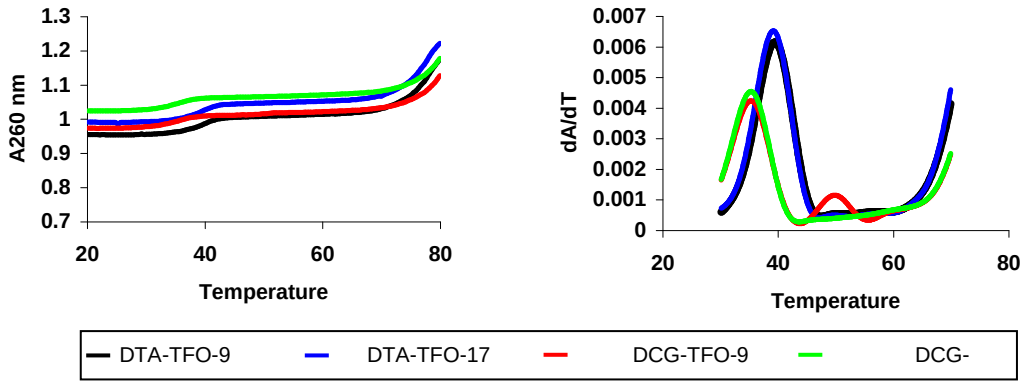
A)


A260 nm
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
20
40
60
80
Temperature
dA/dT
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
20
40
60
80
Temperature
DTA-TFO-1
DTA-TFO-7
DTA-TFO-9
DTA-

B)


A260 nm
1.3
1.2
1.1
1
0.9
0.8
0.7
20
40
60
80
Temperature
dA/dT
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
20
40
60
80
Temperature
DCG-TFO-1
DCG-TFO-7
DCG-TFO-9
DCG-

C)


A260 nm
1.3
1.2
1.1
1
0.9
0.8
0.7
20
40
60
80
Temperature
dA/dT
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
20
40
60
80
Temperature
DTA-TFO-9
DTA-TFO-17
DCG-TFO-9
DCG-

D)

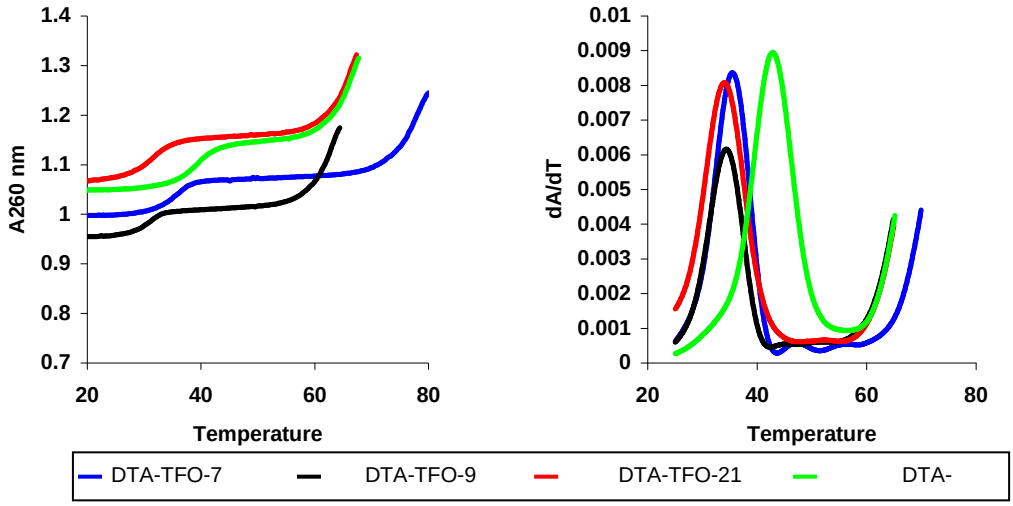


Figure S2. Representative UV melting curves and derivatives. 3'-Anthraquinone TFOs with TA and CG base pair duplexes. Spectra were recorded on a Cary 400 Scan UV-visible spectrophotometer (Varian) at a ratio of 3/1 (TFO/Duplex) in 10 mM phosphate buffer, 200 mM NaCl at pH 6.2, measured at 260 nm. T_m values were calculated using Cary Win UV thermal application software. A) Effect of 3'-modification on TA recognition. UV melting study performed between DTA and TFO-1 (T-P), TFO-7 (T-AnthdR), TFO-9 (S-AnthdR), TFO-17 (R^{An}-AnthdR) at pH 6.2. B) Effect of 3'-modification on CG recognition. UV melting study performed between DCG and TFO-1 (T-P), TFO-7 (T-AnthdR), TFO-9 (S-AnthdR), TFO-17 (R^{An}-AnthdR) at pH 6.2. C) Direct comparison of 3'-anthraquinone TFOs on TA or CG base pairs. UV melting study was performed between DTA and DCG and TFOs 9 (S-AnthdR) & 17 (R^{An}-AnthdR) at pH 6.2. D) Effect of 5' and 5'-3'-anthraquinone on TA recognition. UV melting study performed between DTA and TFO-7 (T-AnthdR), TFO-9 (S-AnthdR), TFO-21 (5'-AnthHex) and TFO-22 (5'-AnthHex-3'-AnthdR) at pH 6.2.

Table S1. Mass spectra of triplex forming oligonucleotides (TFOs) were recorded by negative mode electrospray on a Fisons VG platform spectrometer in water with tri-isopropylamine (2 µL of 1% solution in MeOH). Duplexes were analysed by MALDI-TOF using a ThermoBioAnalysis Dynamo MALDI-TOF spectrometer in positive ion with oligo-dT as reference.

Code number	Sequence	MW required	MW found
DTA	5′-GTGTTAGGAAGAGAAAAAAGAAtAGGT H 3′-CACAATCCTTCTCTTTTTTCTTaTCCA	16961	16960
DAT1	5′-GTGTTAGGAAGAGAAAAAAGAAaAGGT H 3′-CACAATCCTTCTCTTTTTTCTTtTCCA	16961	16958
DAT2	5′-GTGTTAGGAAGAGAAAAAAGAAaTGGT H 3′-CACAATCCTTCTCTTTTTTCTTtACCA	16961	16959
DCG	5′-GTGTTAGGAAGAGAAAAAAGAAcAGGT H 3′-CACAATCCTTCTCTTTTTTCTTgTCCA	16962	16948
DGC1	5′-GTGTTAGGAAGAGAAAAAAGAAgAGGT H 3′-CACAATCCTTCTCTTTTTTCTTcTCCA	16962	16959
DGC2	5′-GTGTTAGGAAGAGAAAAAAGAAgTGGT H 3′-CACAATCCTTCTCTTTTTTCTTcACCA	16962	16963
TFO-1	TCCTTCTCTTTTTTCTTT-P	5475	5482
TFO-2	TCCTTCTCTTTTTTCTTC-P	5460	5461
TFO-3	TCCTTCTCTTTTTTCTTS-P	5625	5630
TFO-4	TCCTTCTCTTTTTTCTTR^{An}-P	5632	5633
TFO-5	TCCTTCTCTTTTTTCTTC-AnthdR	5864	5866
TFO-6	TCCTTCTCTTTTTTCTTC-ThrPyr	5761	5764
TFO-7	TCCTTCTCTTTTTTCTTT-AnthdR	5881	5885
TFO-8	TCCTTCTCTTTTTTCTTT-ThrPyr	5776	5783
TFO-9	TCCTTCTCTTTTTTCTTS-AnthdR	6028	6035
TFO-10	TCCTTCTCTTTTTTCTTS-AnthdR-NH_2	6143	6147
TFO-11	TCCTTCTCTTTTTTCTTS-AnthThr	5886	5896
TFO-12	TCCTTCTCTTTTTTCTTS-AmBuPyr	6050	6054
TFO-13	TCCTTCTCTTTTTTCTTS-ThrPyr	5922	5926
TFO-14	TCCTTCTCTTTTTTCTTS-MeAcPyr	5936	5945
TFO-15	TCCTTCTCTTTTTTCTTS-DAEdR	5793	5802
TFO-16	TCCTTCTCTTTTTTCTTS-Pam	5940	5948
TFO-17	TCCTTCTCTTTTTTCTTR^{An}-AnthdR	6036	6033
TFO-18	TCCTTCTCTTTTTTCTTR^{An}-AnthdR-NH_2	6150	6151
TFO-19	TCCTTCTCTTTTTTCTTR^{An}-AmBuPyr	6059	6063
TFO-20	TCCTTCTCTTTTTTCTTR^{An}-ThrPyr	5931	5935
TFO-21	AnthHex-TCCTTCTCTTTTTTCTTT-P	5976	5979
TFO-22	AnthHex -TCCTTCTCTTTTTTCTTT-AnthdR	6377	6379
TFO-23	AnthHex -TCCTTCTCTTTTTTCTTT-ThrPyr	6272	6276