ELECTRONIC SUPPLEMENTARY INFORMATION

IMPROVED THROMBIN BINDING APTAMER ANALOGUES CONTAINING INVERSION OF POLARITY SITES: STRUCTURAL EFFECTS OF EXTRA-RESIDUES AT THE ENDS

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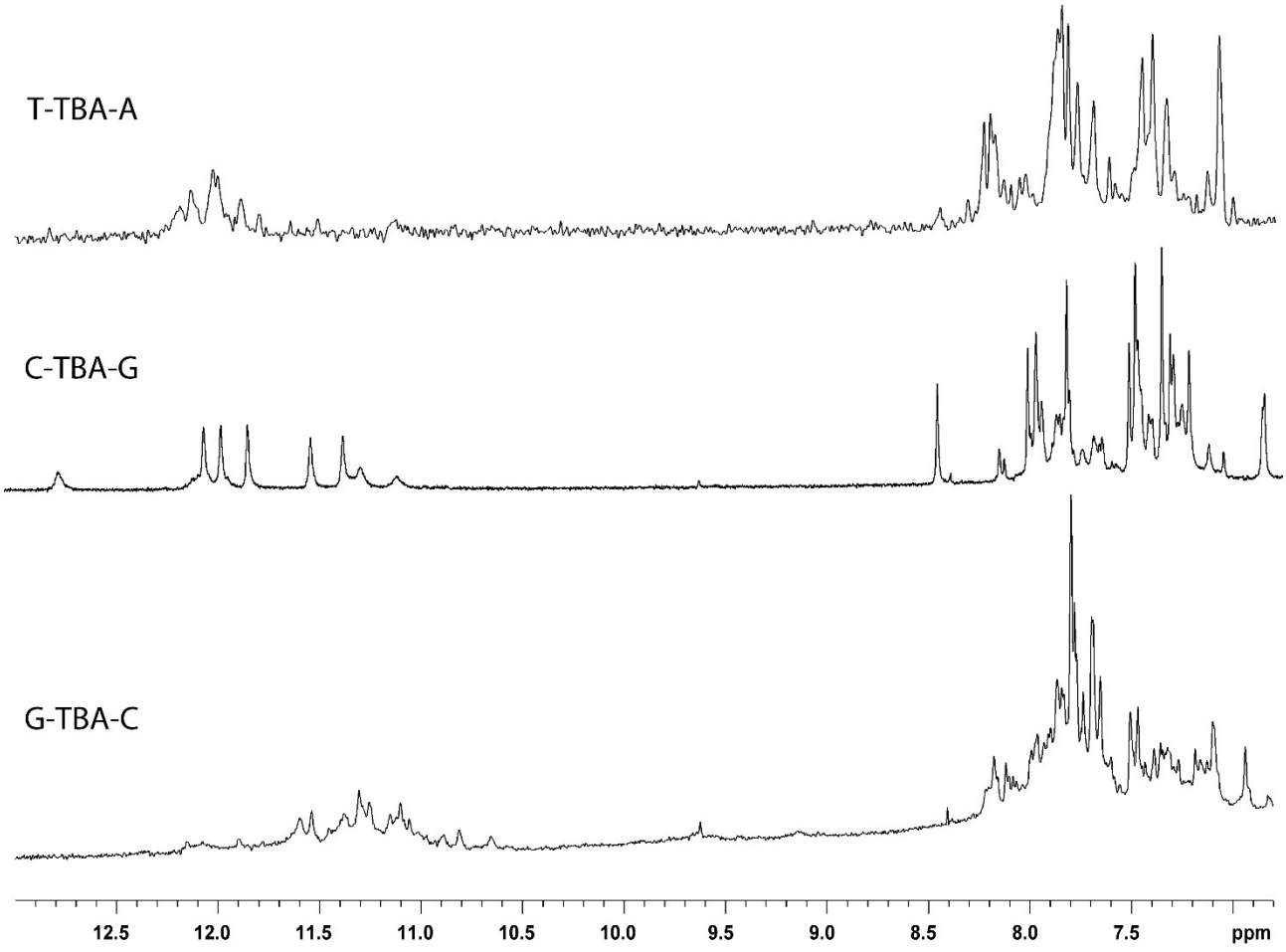
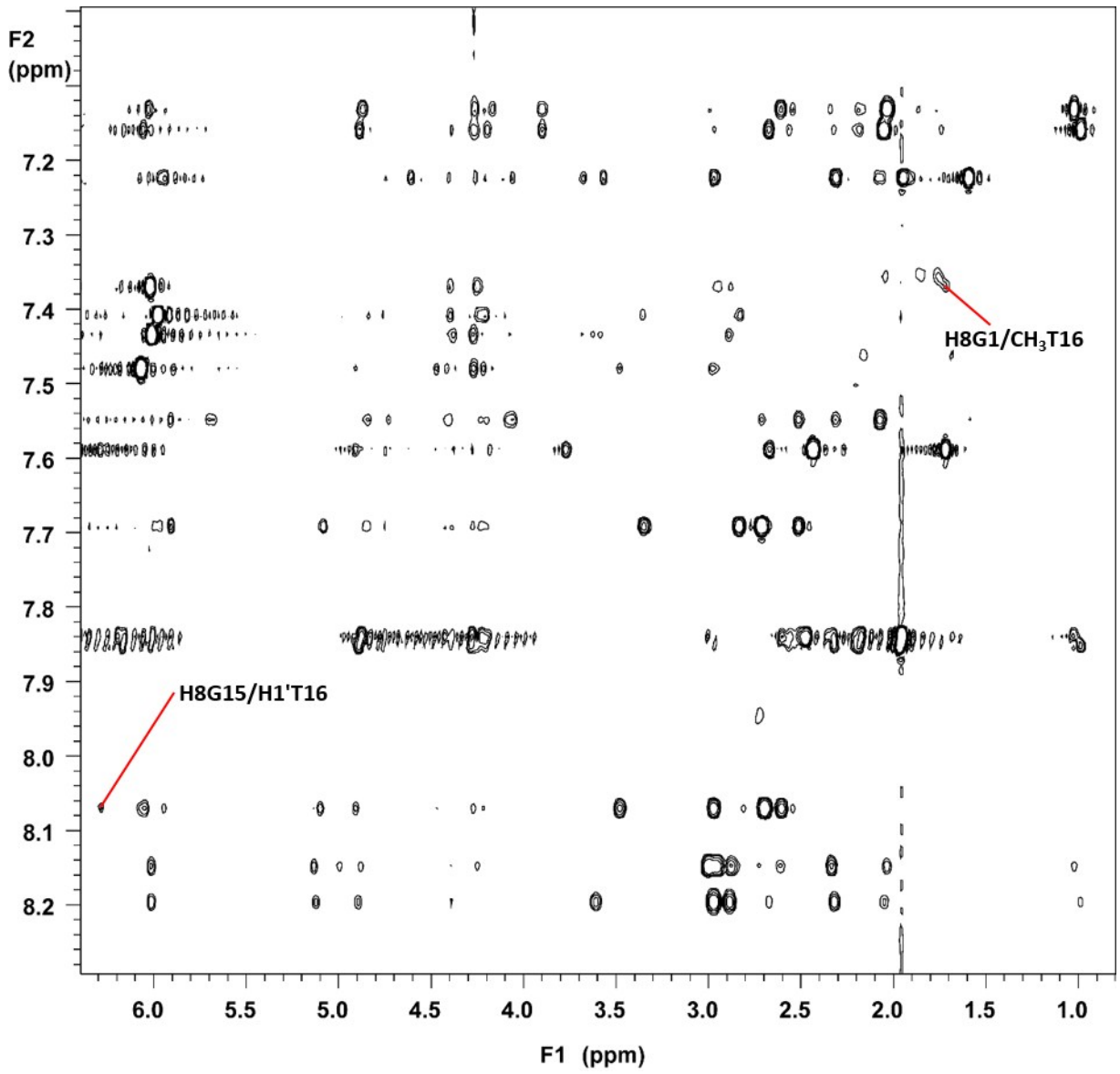


Fig. S1. Aromatic and imino proton regions of the ^{1}H-NMR spectra (700 MHz, T = 25°C) of the TBA analogues T-TBA-A, C-TBA-G and G-TBA-C in PBS buffer solution and 0.2 mM EDTA (pH 7.0).

TBA-T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(ppm)
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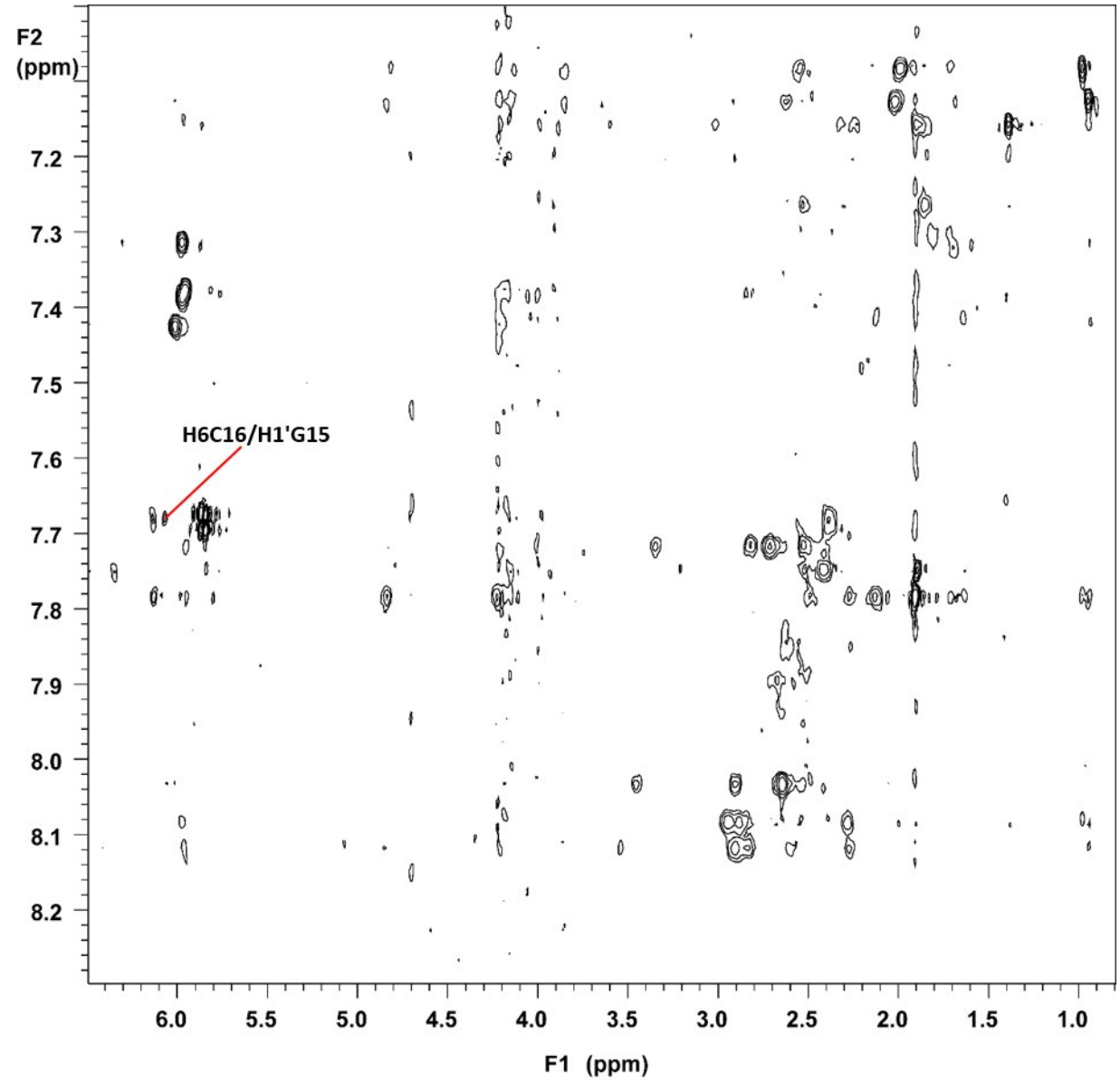
7.3
7.4
7.5
7.6
7.7
7.8
7.9
8.0
8.1
8.2
H8G1/CH3T16
H8G15/H1'T16
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
F1 (ppm)

TBA-C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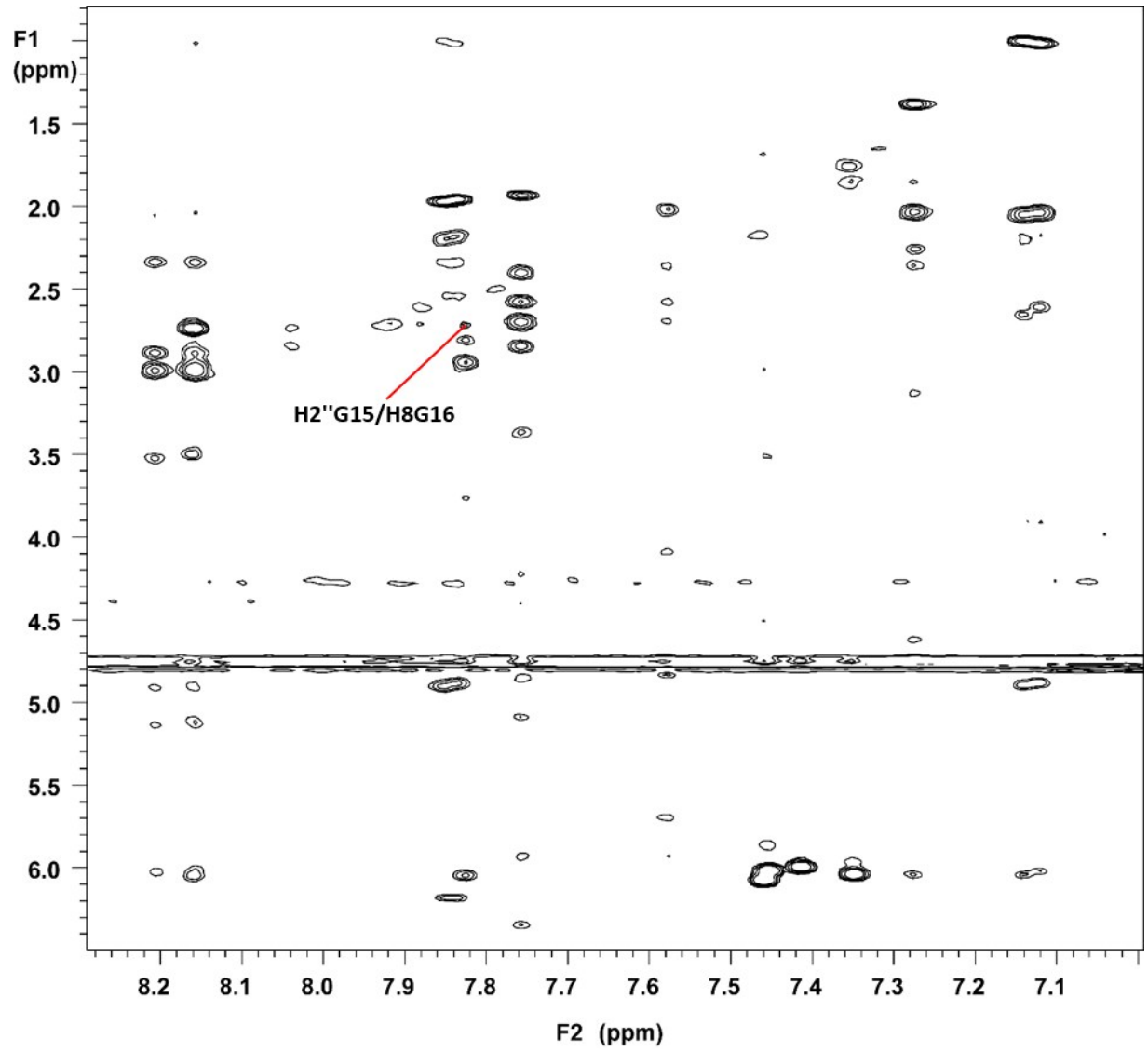
7.5
7.6
7.7
7.8
7.9
8.0
8.1
8.2
H6C16/H1'G15
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
F1 (ppm)

TBA-G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5.5
6.0
H2''G15/H8G16
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
F2 (ppm)

A-TBA-T

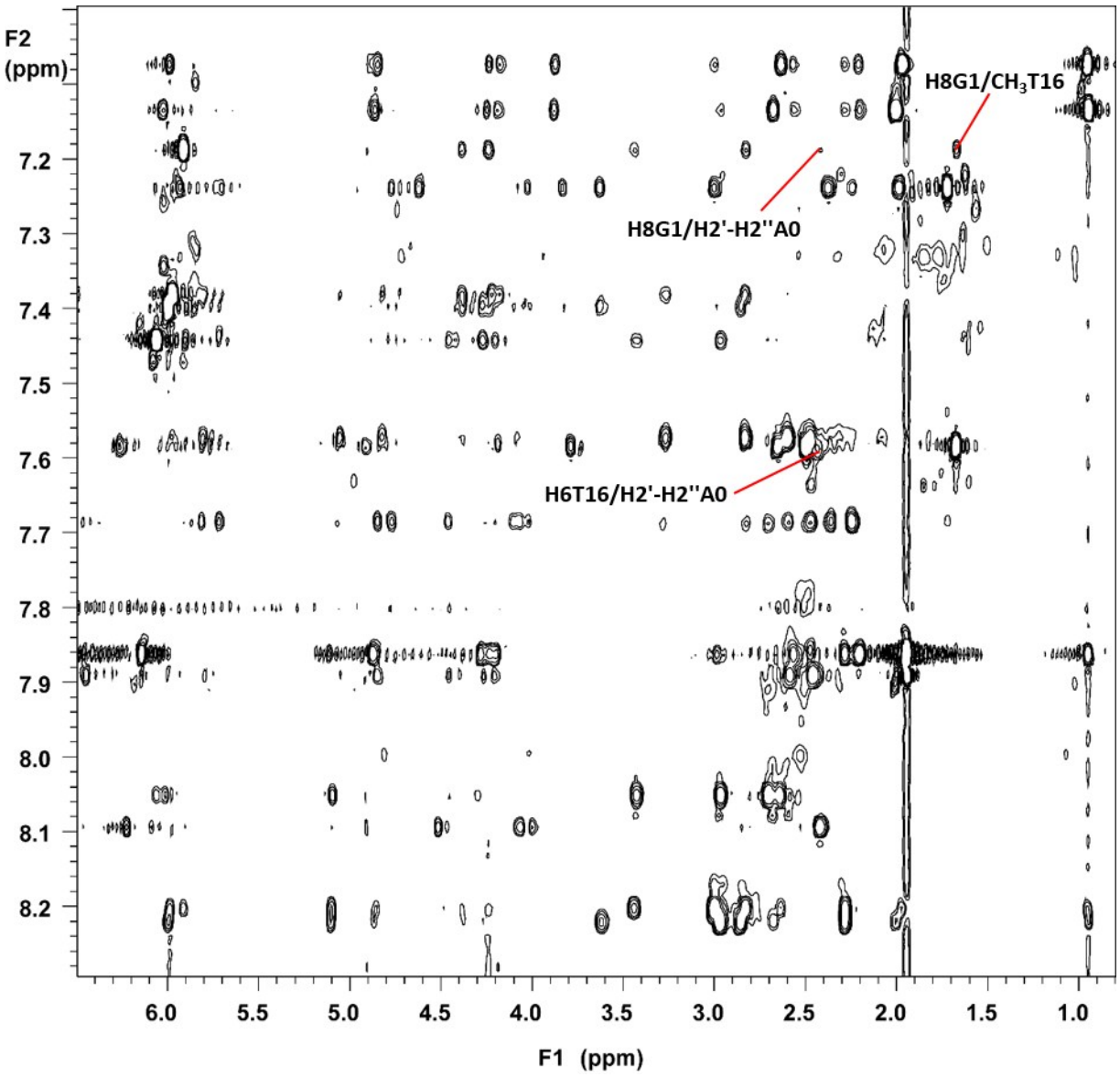

Fig. S2. Expanded region of 2D NOESY spectra of TBA-T, TBA-C, TBA-G and A-TBA-T (700 MHz; 25°C; strand concentration 2 mM in PBS buffer solution **(H_2O/D_2O 9:1)** and 0.2 mM EDTA (pH 7.0)**; total volume 0.6 ml; mixing time 180 ms) correlating bases H8/H6 and anomeric with sugar protons. The NOEs involving the extra-residue are labelled.**

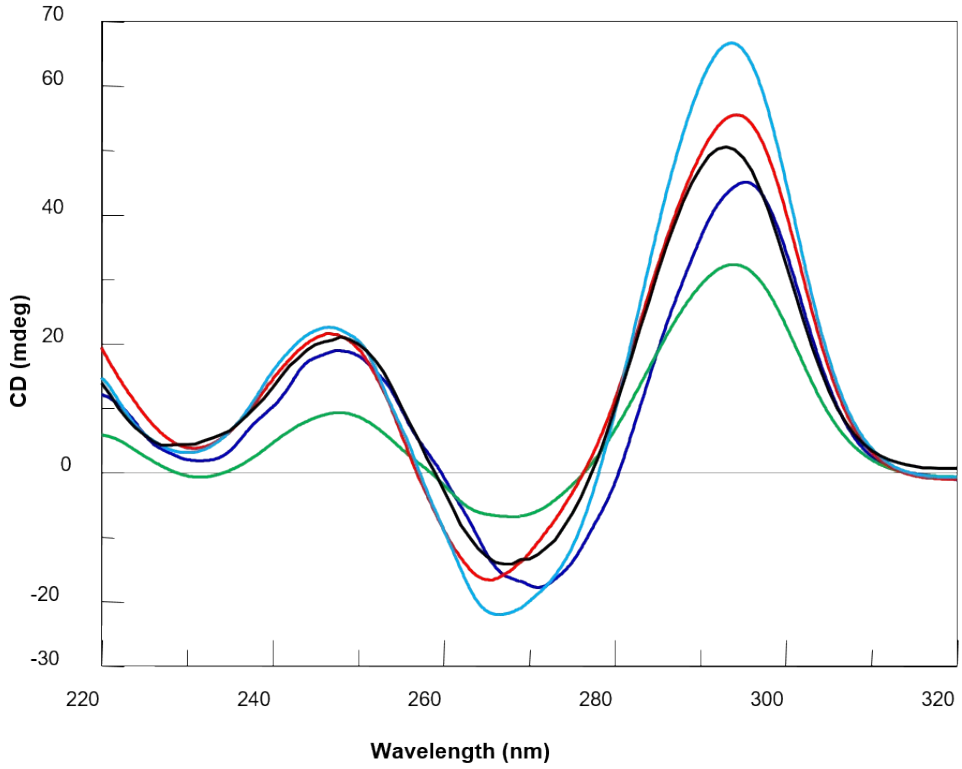

Fig. S3. CD spectra at 20°C (buffer solution 10 mM KH_2PO_4/K_2HPO_4, 70 mM KCl, pH 7.0) of TBA (black), TBA-G (red), TBA-A (dark blue), TBA-C (light blue), TBA-T (green).

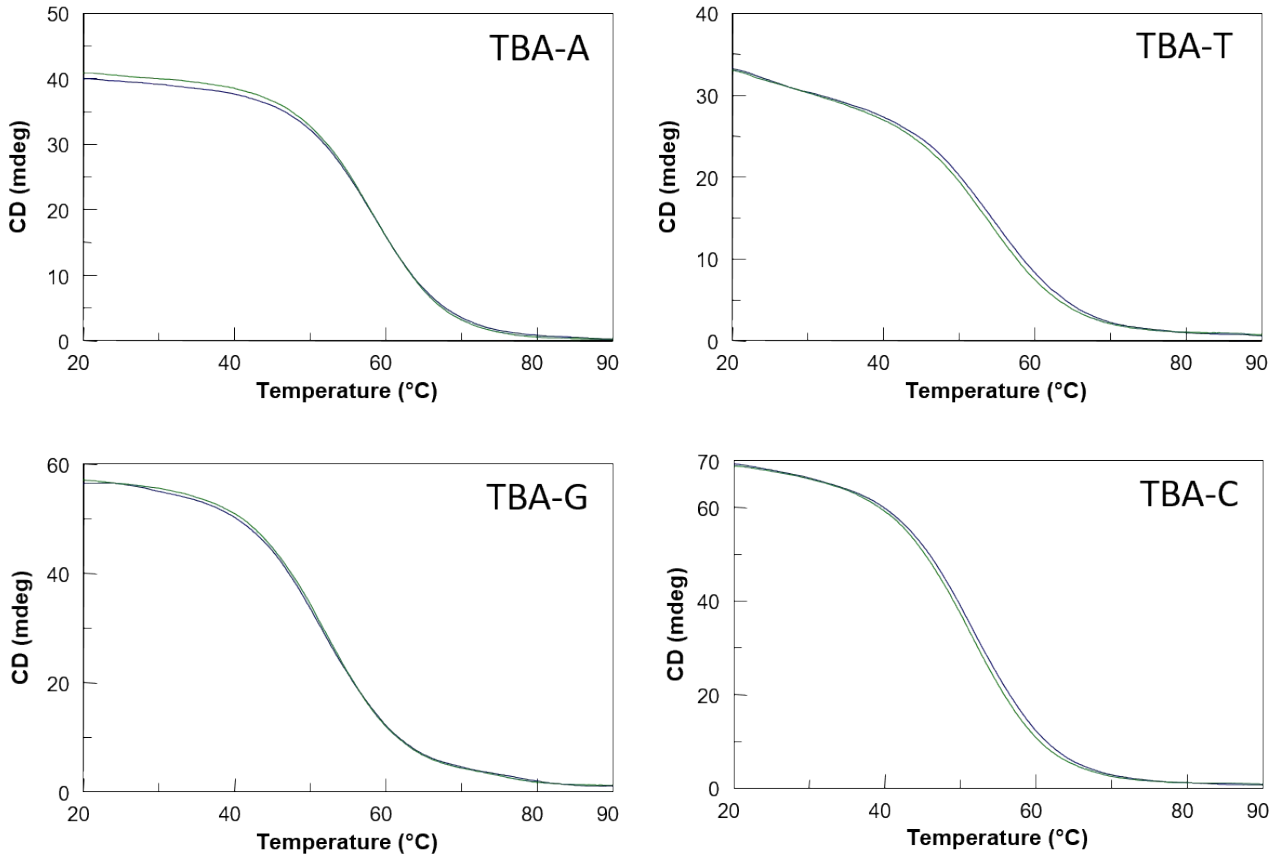

Fig. S4. CD melting (blue) and annealing (green) curves of modified TBAs registered as a function of temperature (range: 20-90°C) for all G-quadruplexes at their maximum Cotton effect wavelengths (294-295 nm). The CD data were recorded in a 0.1 cm pathlength cuvette at 100 µM ODN strand concentration in a buffer solution 10 mM KH_2PO_4/K_2HPO_4, 70 mM KCl (pH 7.0), with a scan rate of 0.5°C/min.

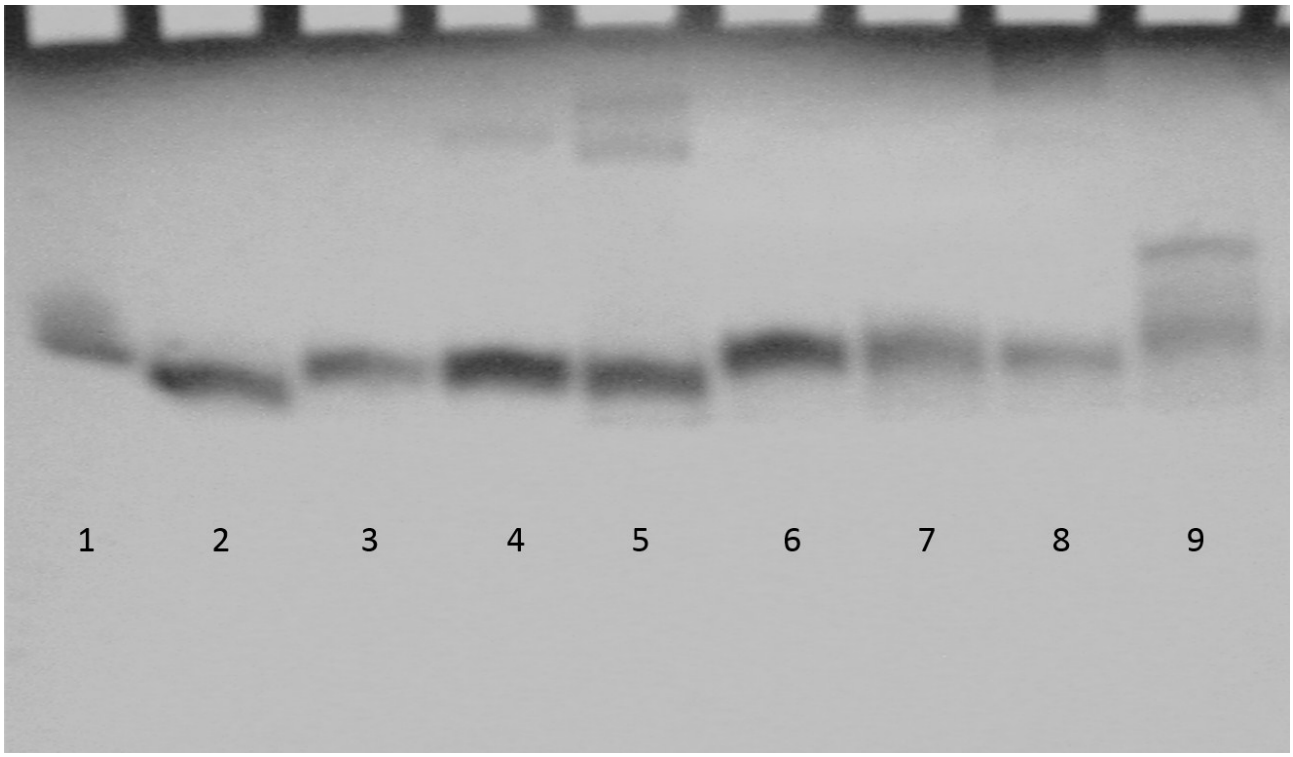

Fig. S5. Polyacrylamide non-denaturing gel electrophoresis of the TBA analogues and their natural counterpart. See Table 1 and the main text for details and experimental procedures. Lane 1: TBA; lane 2: TBA-A; lane 3: TBA-G: lane 4: TBA-T; lane 5: TBA-C; lane 6: A-TBA-T; lane 7: T-TBA-A; lane 8: C-TBA-G; lane 9: G-TBA-C.

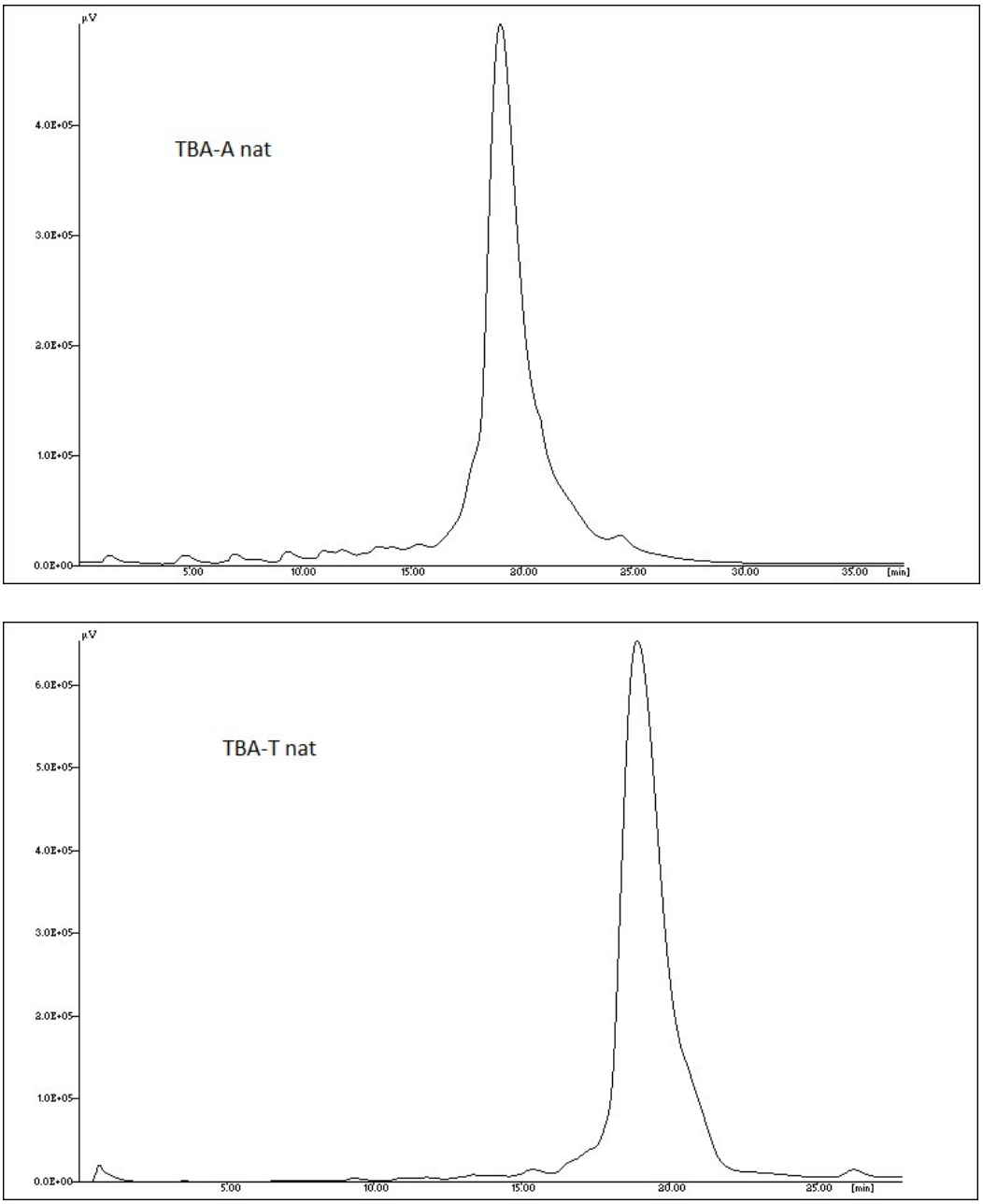

Fig. S6. HPLC chromatograms of purified TBA-A nat and TBA-T nat. See experimental section for details.

A-TBA-T $A_0$5'-5'$G_1G_2T_3T_4G_5G_6T_7G_8T_9G_{10}G_{11}T_{12}T_{13}G_{14}G_{15}$3'-3'$T_{16}$							
	H8/H6	H1'	H2'/H2"	H3'	H4'	H5'/H5"	CH_3/H2
A_0	8.10	6.23	2.43	4.52	4.45	4.07/4.00	7.81
$\underline{G}_1$	7.19	5.91	2.83/3.44	4.90	-	4.39/4.24	
G_2	8.21	5.99	2.28/3.00	5.10	4.86	4.39/4.24	
T_3	7.86	6.14	2.20/2.56	4.87	-	4.28/4.22	1.95
T_4	7.08	5.99	1.97/2.64	4.85	4.24	4.17/3.87	0.95
$\underline{G}_5$	7.38	5.98	2.84/3.27	4.82	4.39	4.22/4.18	
G_6	7.58	5.81	2.47/2.60	5.05	4.38	4.08	
T_7	7.89	6.45	2.46/2.58	4.85	4.45	4.26/4.20	1.95
G_8	7.69	5.71	2.25/2.36	4.77	4.46	4.08/4.02	
T_9	7.24	5.93	1.99/2.38	4.62	4.02	3.83/3.63	1.72
$\underline{G}_{10}$	7.40	6.00	2.86/3.62	-	4.39	4.28/4.24	
G_{11}	8.22	6.00	2.28/2.97	5.10	4.38	-	
T_{12}	7.86	6.14	2.20/2.56	4.87	-	4.28/4.22	1.95
T_{13}	7.14	6.03	2.01/2.68	4.86	4.25	4.19/3.88	0.95
$\underline{G}_{14}$	7.44	6.06	2.97/3.42	-	4.44	4.27/4.20	
G_{15}	8.05	6.01	2.64/2.70	5.09	4.30	-	
T_{16}	7.59	6.27	2.50/2.65	4.92	4.18	3.79	1.67

TBA-G	5'$G_1G_2T_3T_4G_5G_6T_7G_8T_9G_{10}G_{11}T_{12}T_{13}G_{14}G_{15}$3'-3'$G_{16}$						
	H8/H6	H1'	H2'/H2"	H3'	H4'	H5'/H5"	CH_3
$\underline{G}_1$	7.35	6.04	2.90	4.92	4.40	4.25	
G_2	8.16	6.05	2.36	5.15	-	-	
T_3	7.83	6.18	2.19/2.56	4.90	4.29	4.22	1.95
T_4	7.12	6.03	2.06/2.61	4.88	-	4.17/3.91	1.01
$\underline{G}_5$	7.41	6.00	2.85/3.39	4.87	4.27	-	
G_6	7.76	5.93	2.58/2.70	5.08	4.51	4.21	
T_7	7.76	6.34	2.43/2.58	4.84	4.42	4.21	1.94
G_8	7.58	5.70	2.04/2.37	4.73	-	4.10	
T_9	7.27	6.04	2.05/2.26	4.63	3.62	3.52/3.13	1.38
$\underline{G}_{10}$	7.45	6.02	2.91/3.53	4.92	4.40	4.27	
G_{11}	8.21	6.03	2.35/3.01	5.14	4.40	-	
T_{12}	7.85	6.18	2.20/2.56	4.89	4.29	4.22	1.97
T_{13}	7.14	6.05	2.07/2.66	4.89	-	4.20/3.91	1.00
$\underline{G}_{14}$	7.46	6.06	2.99/3.51	4.92	4.27	-	
G_{15}	8.16	6.05	2.63/2.73	4.98	4.36	-	
G_{16}	7.82	6.05	2.82/2.95	5.04	4.29	3.77	

TBA-C	5'$G_1G_2T_3T_4G_5G_6T_7G_8T_9G_{10}G_{11}T_{12}T_{13}G_{14}G_{15}$3'-3'$C_{16}$						
	H8/H6	H1'	H2'/H2"	H3'	H4'	H5'/H5"	CH_3/H5
$\underline{G}_1$	7.31	5.97	2.88	4.94	-	-	
G_2	8.09	5.96	2.29/2.94	5.08	-	-	
T_3	7.79	6.13	2.14/2.49	-	-	-	1.91
T_4	7.09	5.96	1.99/2.55	-	-	-	0.98
$\underline{G}_5$	7.38	5.96	2.83/3.35	4.82	-	-	
G_6	7.72	5.95	2.53/2.72	5.06	-	-	
T_7	7.75	6.35	2.42/2.52	-	-	-	1.90
G_8	7.27	5.59	1.86/2.32	4.67	-	-	
T_9	7.16	5.86	2.25/2.32	-	-	-	1.40
$\underline{G}_{10}$	7.39	5.97	2.84/3.55	4.86	-	-	
G_{11}	8.12	5.96	2.27/2.90	5.08	-	-	
T_{12}	7.79	6.13	2.14/2.49	-	-	-	1.91
T_{13}	7.13	5.96	2.02/2.62	4.84	-	-	0.95
$\underline{G}_{14}$	7.43	6.01	2.91/3.45	4.87	-	-	
G_{15}	8.04	6.06	2.41/2.65	5.01	-	-	
C_{16}	7.68	6.13	2.38/2.56	-	-	-	5.85

TBA-A	5'$G_1G_2T_3T_4G_5G_6T_7G_8T_9G_{10}G_{11}T_{12}T_{13}G_{14}G_{15}$3'-3'$A_{16}$						
	H8/H6	H1'	H2'/H2"	H3'	H4'	H5'/H5"	CH_3/H2
$\underline{G}_1$	7.20	6.05	2.80/2.94	5.01	4.24	4.00	
G_2	8.12	5.96	2.01/2.31	5.12	-	-	
T_3	7.82	6.17	2.17/2.31	4.87	-	4.26/4.21	1.94
T_4	7.09	6.00	2.00/2.57	4.86	4.25	4.16/3.89	0.98
$\underline{G}_5$	7.44	5.98	2.79/3.31	4.84	4.48	4.22	
G_6	7.84	6.10	2.52/2.79	5.12	-	-	
T_7	7.62	6.32	2.52/2.56	4.85	4.26	4.20/4.14	1.92
G_8	7.51	6.11	2.52	5.23	4.44	4.15	
T_9	7.01	6.07	1.87/2.10	5.24	4.47	3.45/3.35	0.83
$\underline{G}_{10}$	7.48	6.05	2.91/3.71	5.11	4.92	4.41	
G_{11}	8.26	6.07	2.35/3.04	5.14	-	-	
T_{12}	7.87	6.18	2.23/2.59	4.90	4.29	4.23	1.97
T_{13}	7.07	6.00	2.02/2.64	4.89	4.27	4.19/3.91	1.00
$\underline{G}_{14}$	7.50	6.13	3.04/3.51	4.91	4.30	-	
G_{15}	8.21	6.16	2.67/2.86	5.05	-	4.30/4.21	
A_{16}	8.33	6.21	2.91/2.97	5.11	-	-	7.78

TBA-T	5'$G_1G_2T_3T_4G_5G_6T_7G_8T_9G_{10}G_{11}T_{12}T_{13}G_{14}G_{15}$3'-3'$T_{16}$						
	H8/H6	H1'	H2'/H2"	H3'	H4'	H5'/H5"	CH_3
$\underline{G}_1$	7.37	6.02	2.88/2.95	5.00	4.40	4.25/4.03	
G_2	8.15	6.02	2.33/2.99	5.13	-	4.25	
T_3	7.84	6.20	2.18/2.55	4.89	-	4.27/4.19	1.95
T_4	7.13	6.03	2.04/2.61	4.87	4.47	4.17/3.90	1.02
$\underline{G}_5$	7.41	5.98	2.83/3.35	4.85	4.40	4.22	
G_6	7.69	5.91	2.52/2.71	5.09	-	4.22	
T_7	7.84	6.43	2.48/2.60	4.86	-	-	1.96
G_8	7.55	5.70	2.07/2.31	4.73	4.41	4.21/4.07	
T_9	7.22	5.96	1.94/2.31	4.61	-	3.68/3.57	1.59
$\underline{G}_{10}$	7.44	6.01	2.88/3.60	4.90	4.39	4.27/4.11	
G_{11}	8.20	6.02	2.32/2.97	5.12	-	4.28	
T_{12}	7.85	6.17	2.19/2.56	4.89	4.47	4.27/4.19	1.96
T_{13}	7.16	6.06	2.05/2.67	4.88	4.47	4.19/3.90	0.98
$\underline{G}_{14}$	7.48	6.07	2.97/3.48	4.91	4.48	4.27/4.21	
G_{15}	8.07	6.06	2.61/2.70	5.10	4.48	4.27/4.22	
T_{16}	7.59	6.28	2.44/2.67	4.90	4.19	3.77	1.72

Table S1. Proton chemical shifts assignment for G-quadruplex structures formed by ODNs A-TBA-T, TBA-G, TBA-C, TBA-A, TBA-T (700 MHz; 25°C) in PBS buffer solution **(H_2O/D_2O 9:1)** and 0.2 mM EDTA (pH 7.0)**. Deoxyguanosines adopting *syn* glicosydic conformation are underlined. The hyphens indicate values not determined.**

Names	T_m (°C) (± 1)	ΔT_m (°C)	$\Delta H_{v.H.}$ (kJ/mol)[a]	$\Delta S_{v.H.}$ (kJ/mol K)[a]
TBA	50	-	154	0.48
TBA-A	59	+9	194	0.58
TBA-T	56	+6	174	0.53
TBA-G	52	+2	175	0.54
TBA-C	53	+3	172	0.53

[a] The errors on $\Delta H_{v.H.}$ and $\Delta S_{v.H.}$ are within the 10 %.

Table S2. Thermodynamic parameters of some modified aptamers and their natural counterpart (TBA) in potassium buffer obtained from the van't Hoff analysis of the CD melting curves (see text for details).