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ELECTRONIC SUPPLEMENTARY INFORMATION

A novel pyrimidine tetrad contributing to stabilize tetramolecular G-quadruplex structures

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- HYDROGEN BONDING PATTERNS (G-TETRAD AND T-TETRAD)
- CD SPECTRA
- NOESY SPECTRA (**BR** AND **TH**)
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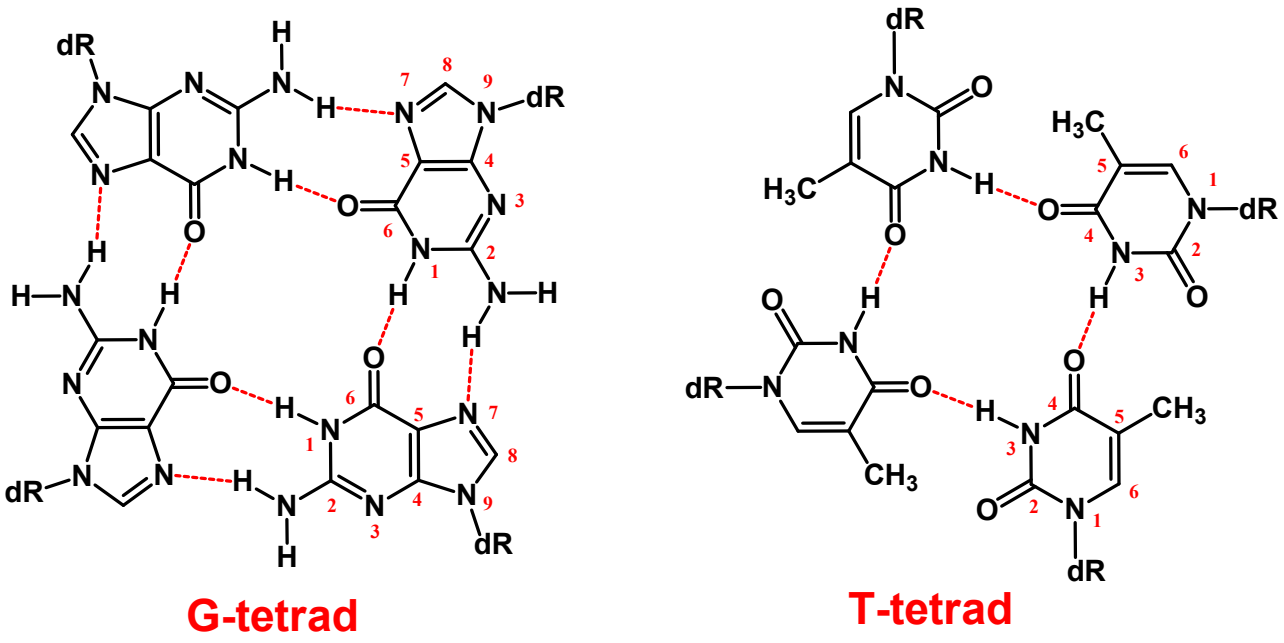

Figure S1: Hydrogen bond patterns of a G-tetrad and a T-tetrad.

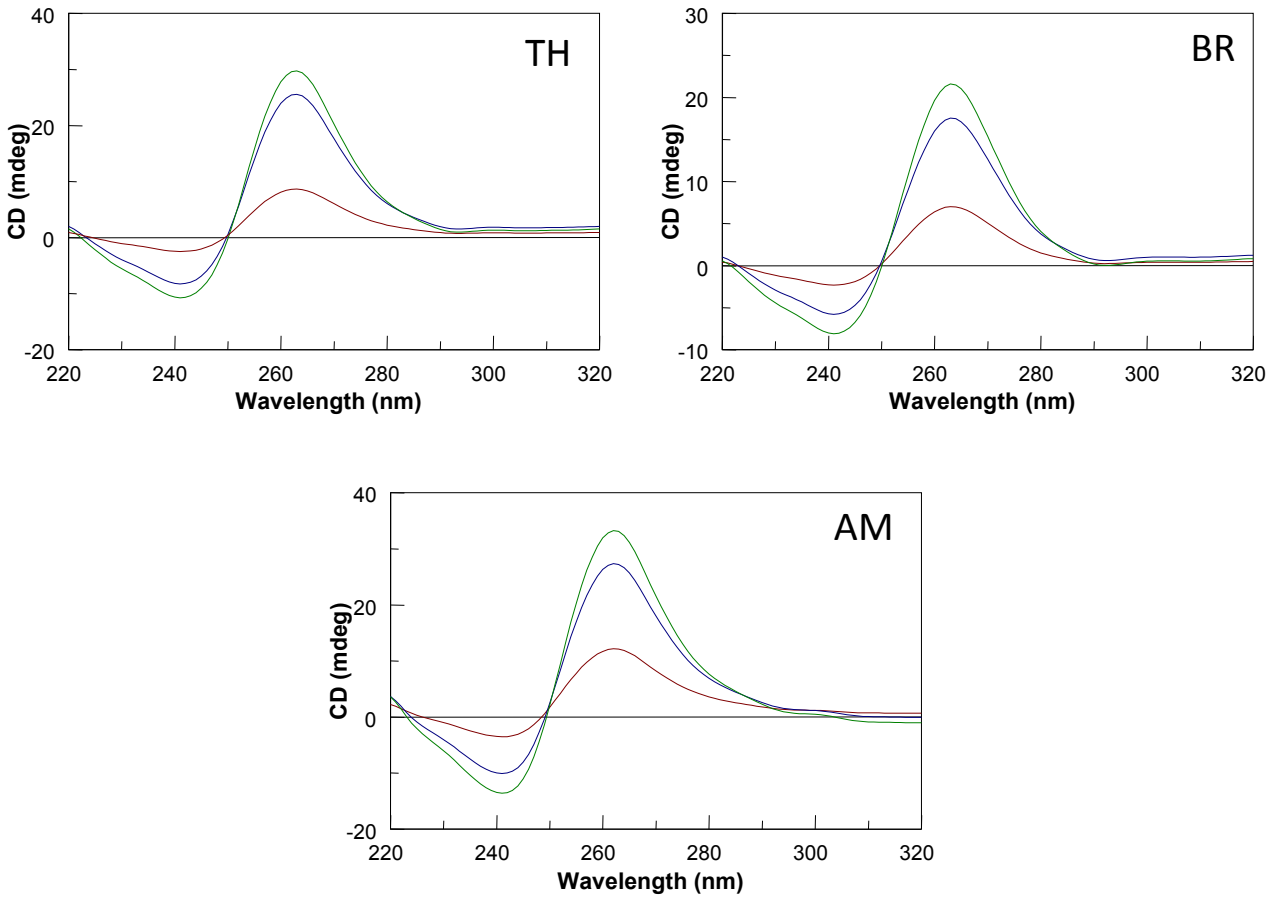

Figure S2. CD spectra at 20°C of investigated G-quadruplexes in potassium buffer solutions (20 mM KH_2PO_4/K_2HPO_4, pH 7.0) at different KCl concentration: 10 mM (red), 100 mM (blue), 300 mM (green). ODN strand concentration: 100 μM.

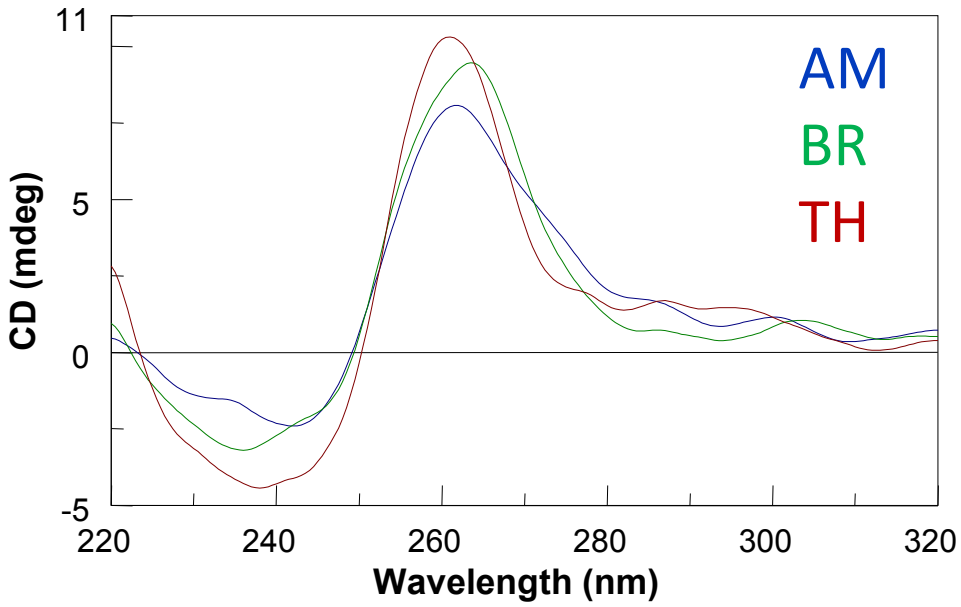

Figure S3. CD spectra at 20°C of investigated G-quadruplexes at 100 μM ODN strand concentration in a buffer solution 20 mM NaH_2PO_4/Na_2HPO_4, 100 mM NaCl (pH 7.0).

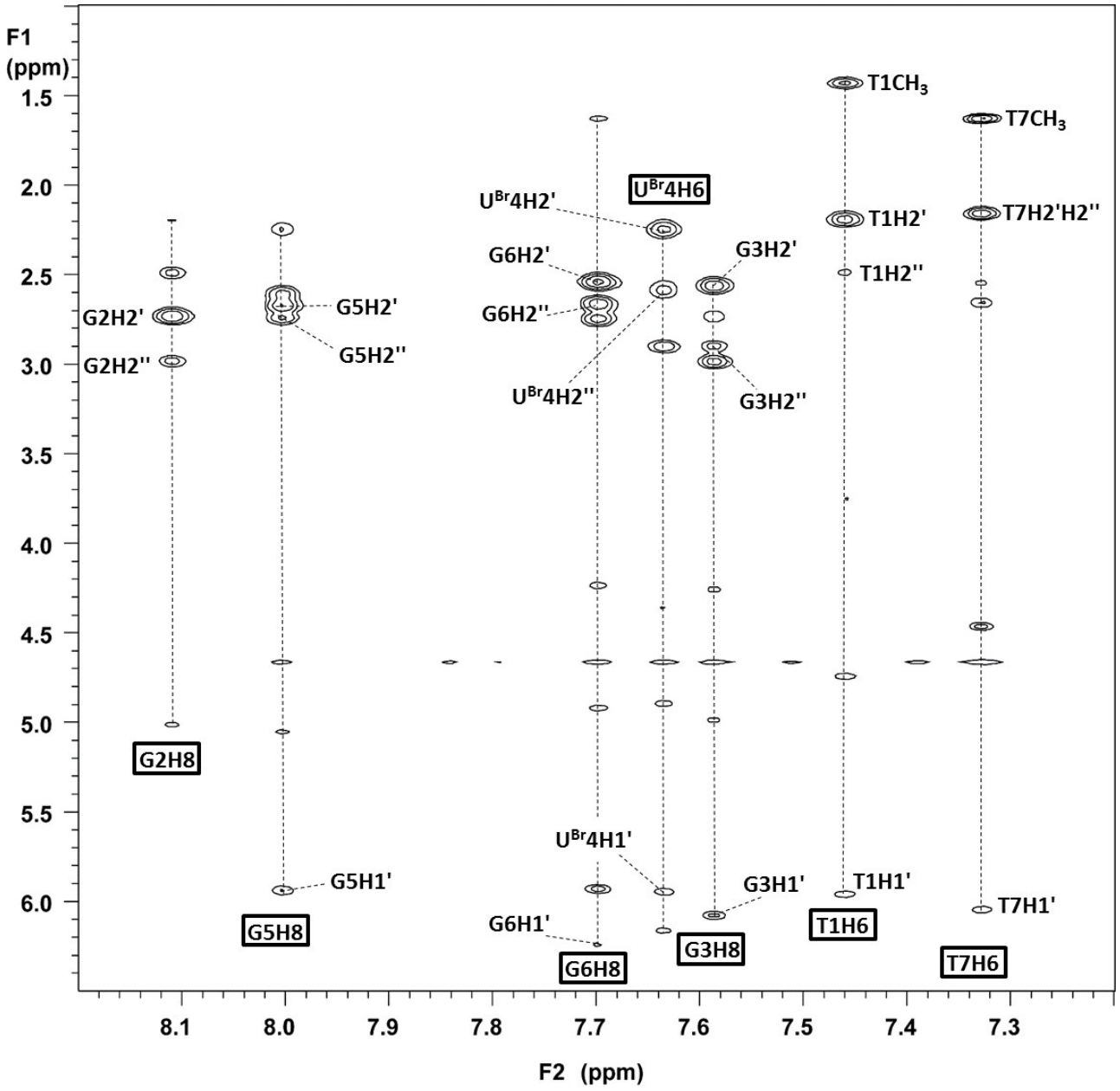

Figure S4: Expanded region of 2D NOESY spectrum of G-quadruplex **BR** (500 MHz; 25°C; strand concentration 1.5 mM; 20 mM KH_2PO_4/K_2HPO_4, 100 mM KCl and 0.2 mM EDTA, pH 7.0 in H_2O/D_2O 9:1; total volume 0.6 ml; mixing time 180 ms) correlating bases H8/H6 and sugar protons.

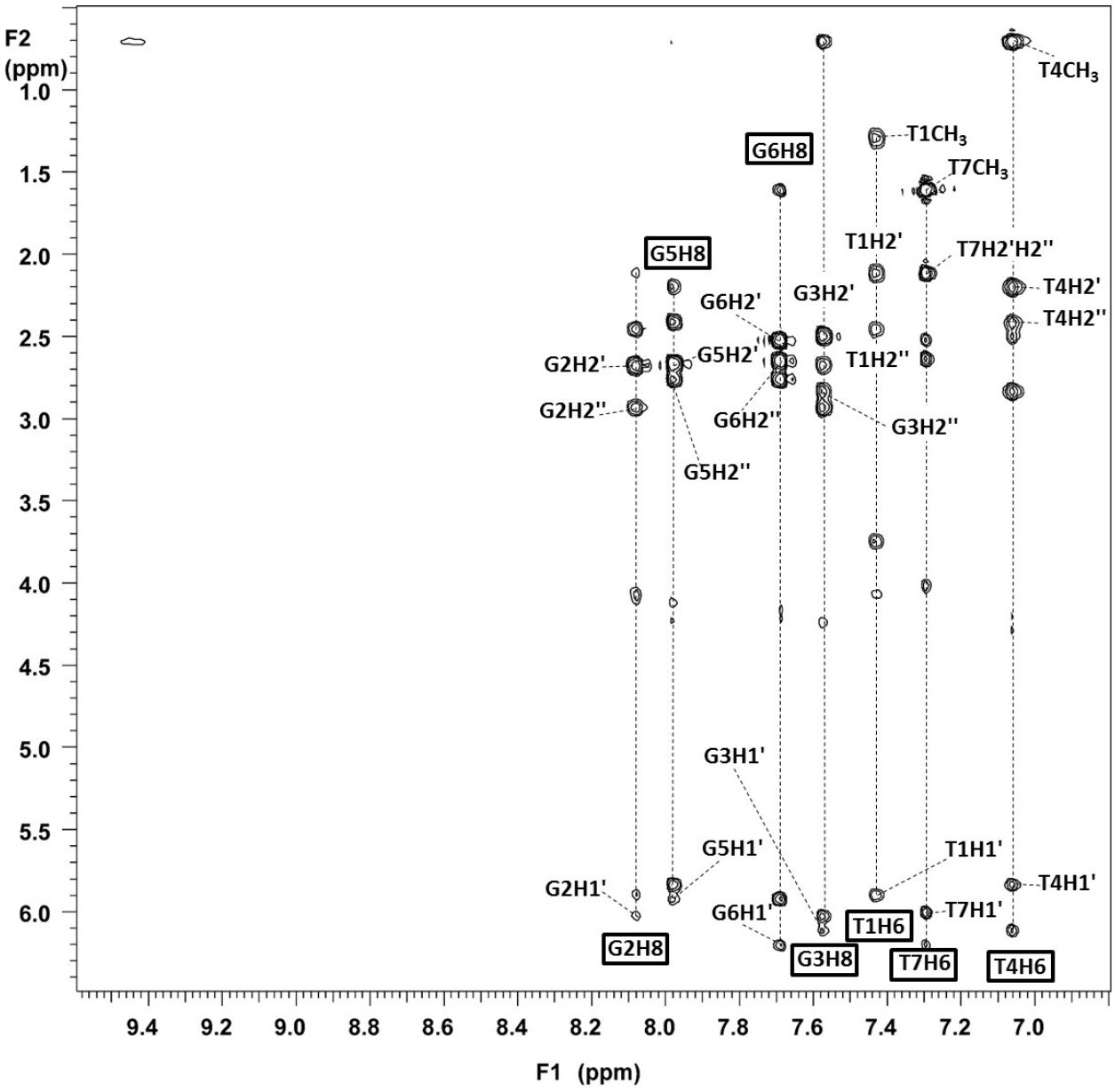

Figure S5: Expanded region of 2D NOESY spectrum of G-quadruplex **TH** (500 MHz; 25°C; strand concentration 1.5 mM; 20 mM KH_2PO_4/K_2HPO_4, 100 mM KCl and 0.2 mM EDTA, pH 7.0 in H_2O/D_2O 9:1; total volume 0.6 ml; mixing time 180 ms) correlating bases H8/H6 and sugar protons.

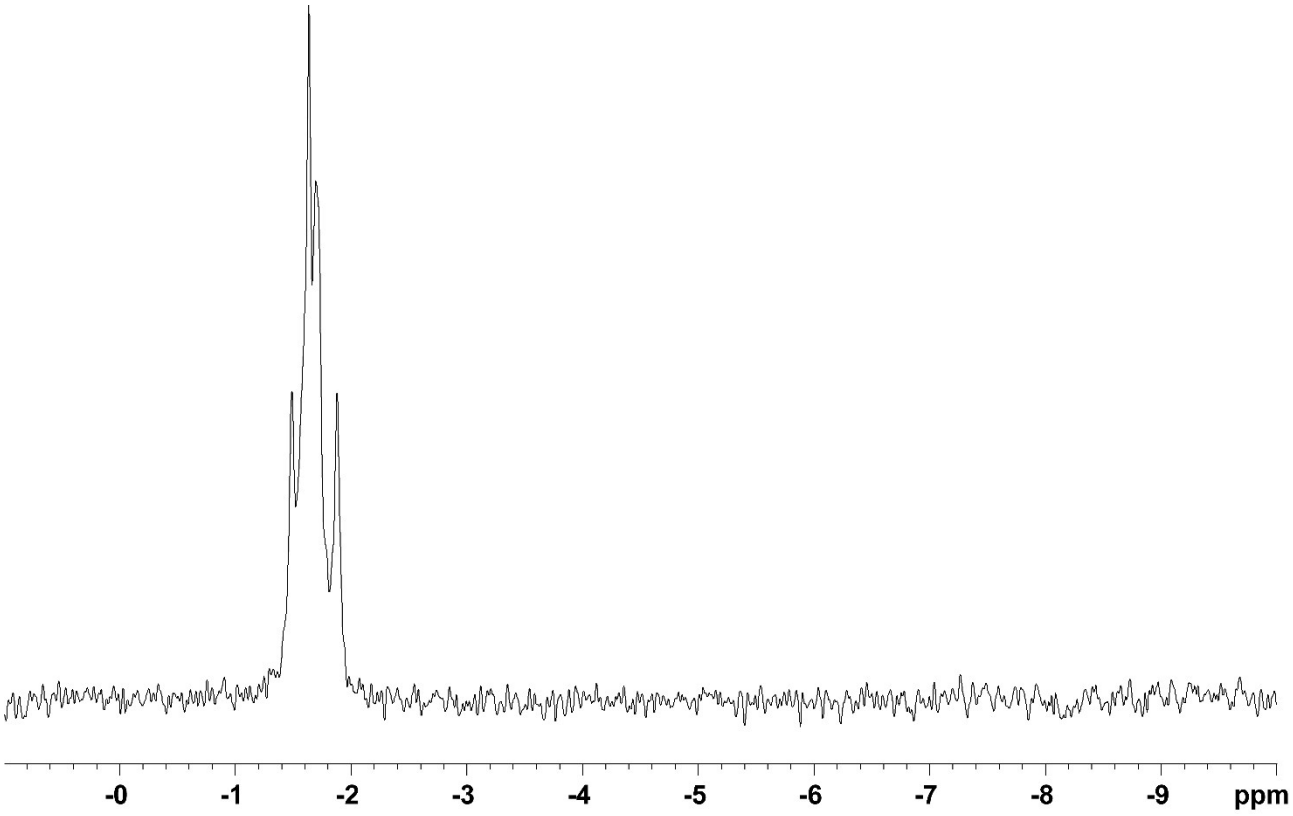

Figure S6: Proton decoupled ^{31}P-NMR spectrum of **AM** (500 MHz, T = 25°C, 20 mM KH_2PO_4, 100 mM KCl, 0.2 mM EDTA, pH=7).

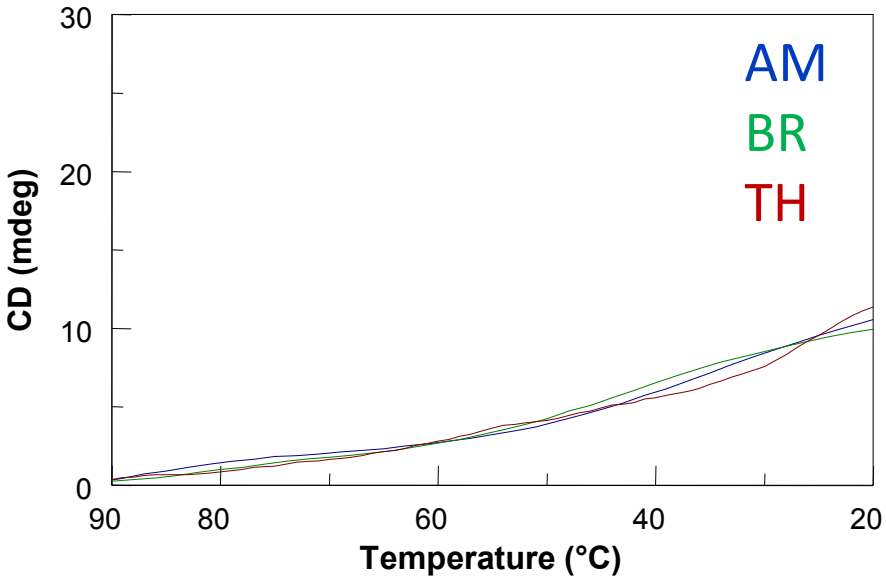

Figure S7. CD annealing profiles registered as a function of temperature from 90°C to 20°C for all investigated G-quadruplexes at their maximum Cotton effect wavelengths. CD data were recorded in a 0.1 cm pathlength cuvette with a scan rate of 10°C/h at 100 μM ODN strand concentration in a buffer solution 20 mM KH_2PO_4/K_2HPO_4, 100 mM KCl (pH 7.0).

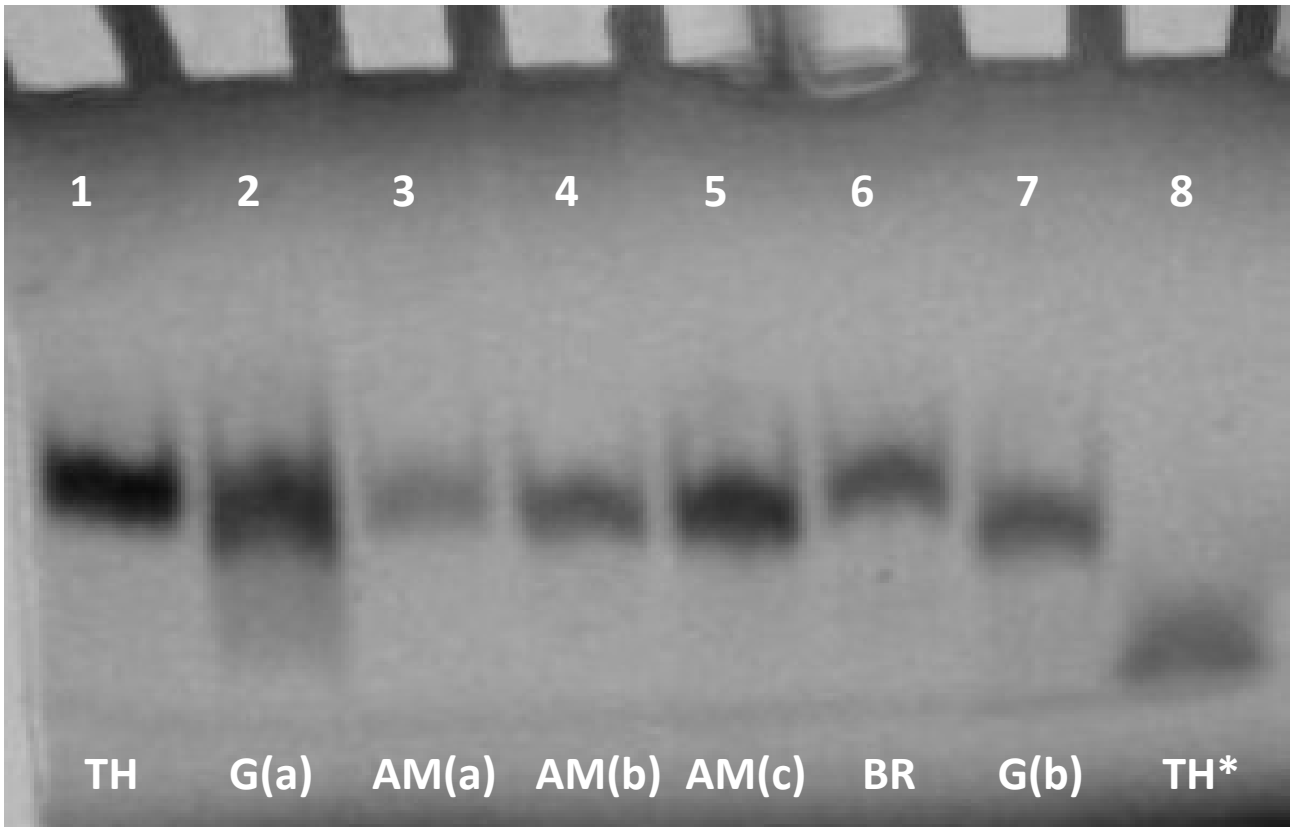

Figure S8: Non-denaturing polyacrylamide gel electrophoresis of the investigated G-quadruplex stuctures and $[d(TG_5T)]_4$ as a reference. Lane 1: **TH**, [ODN] ≈ 1.5 mM; lanes 2 and 7 $[d(TG_5T)]_4$, [ODN] ≈ 1.5 mM G(a) and 1 mM G(b), respectively; lanes 3, 4 and 5: **AM**, [ODN] ≈ 0.5 mM AM(a), 1 mM AM(b) and 1.5 mM AM(c), respectively; lane 6: **BR**, [ODN] ≈ 1 mM; lane 8 (single strand reference): **TH** denatured by pre-treatment with LiOH (40 mM, 5' at 80°C) followed by neutralization with 40 mM HCl and immediate loading on the gel, [ODN] ≈ 1 mM.

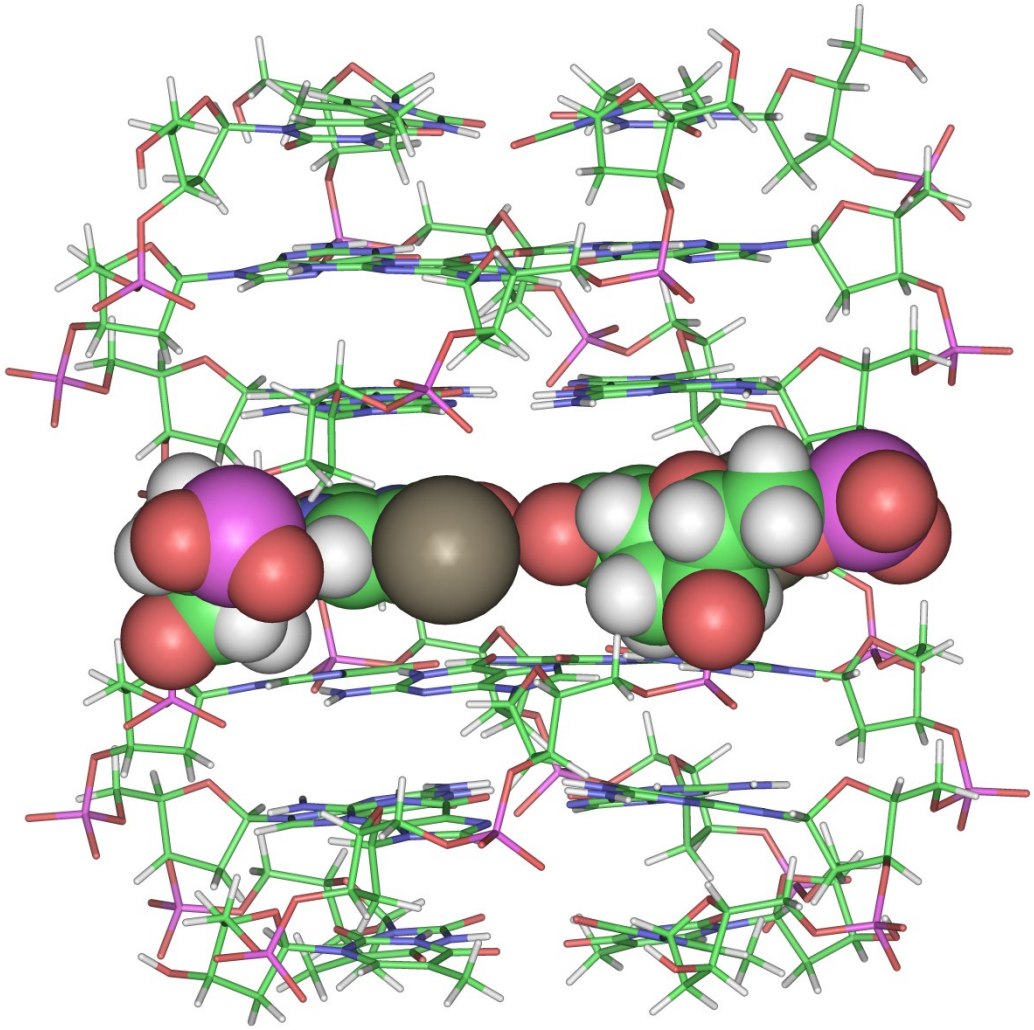

Figure S9: Side view of stick model of G-quadruplex **BR**. The U^{Br}-tetrad is reported in CPK. Heavy atoms are shown with different colors (carbons, green; nitrogens, blue; oxygens, red; hydrogens, white; phosphorus, purple; bromine, dark grey).

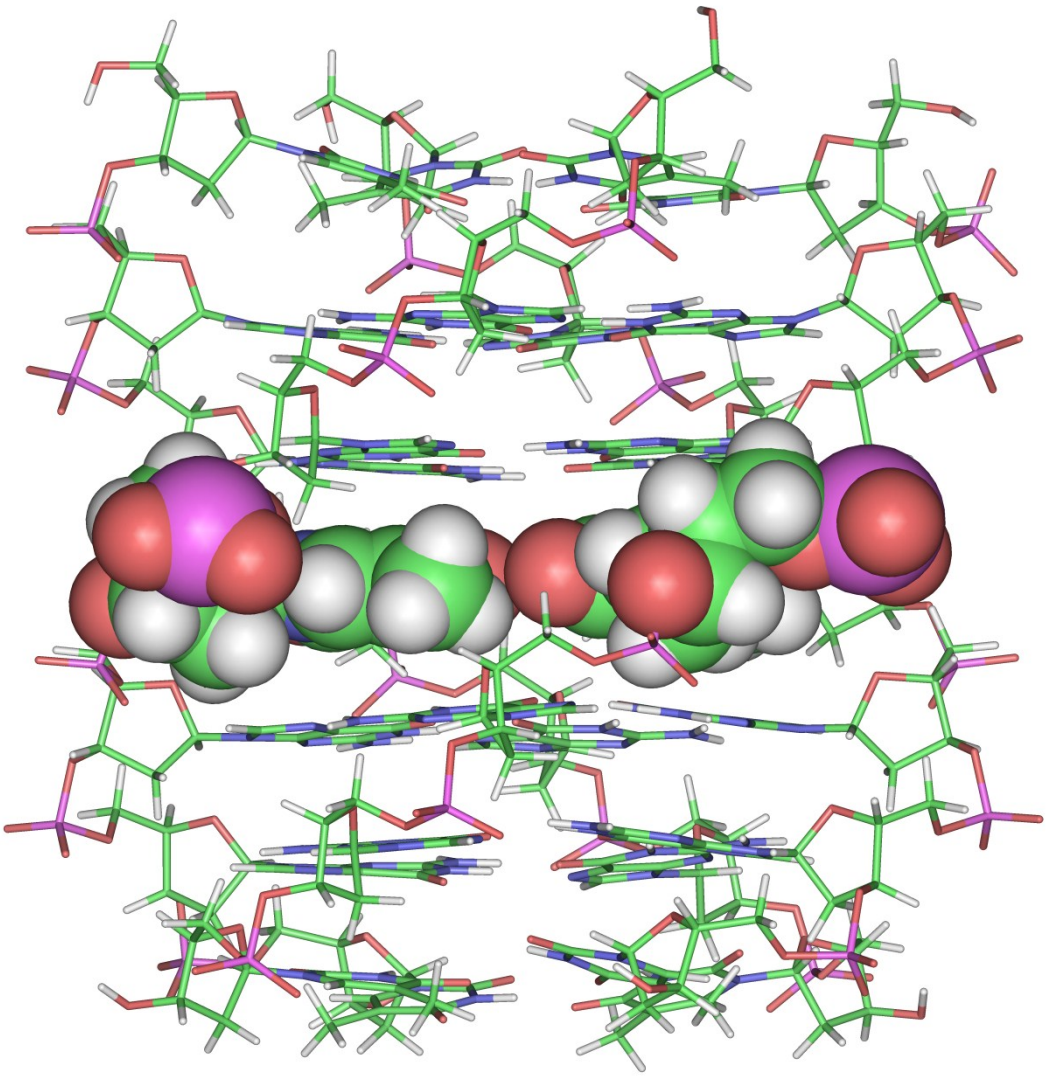

Figure S10: Side view of stick model of G-quadruplex **TH**. The T-tetrad is reported in CPK. Heavy atoms are shown with different colors (carbons, green; nitrogens, blue; oxygens, red; hydrogens, white; phosphorus, purple).

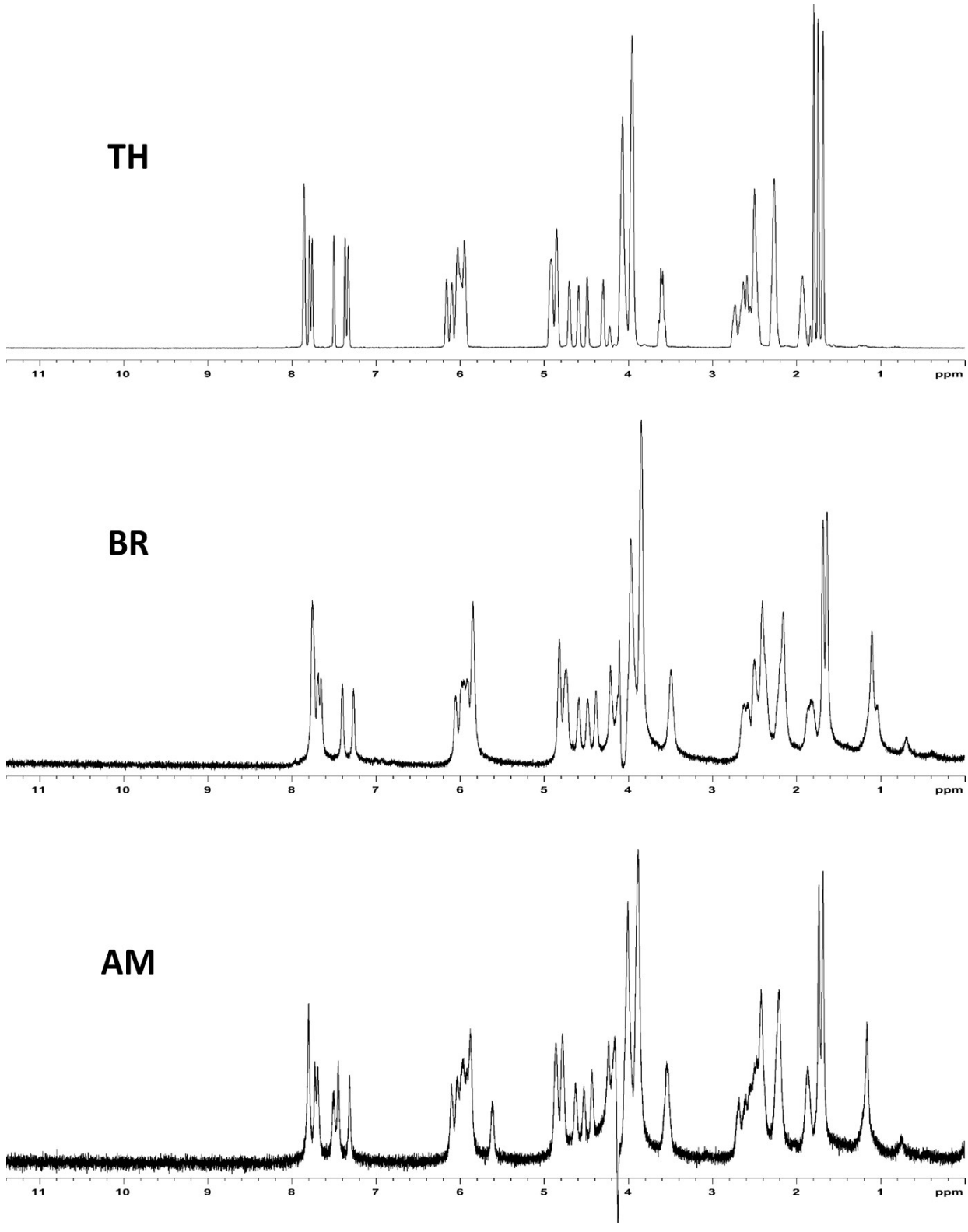

Figure S11: High-resolution ^{1}H-NMR spectra (500 MHz, D_2O, 80°C, no salt) of **TH**, **BR** and **AM**.

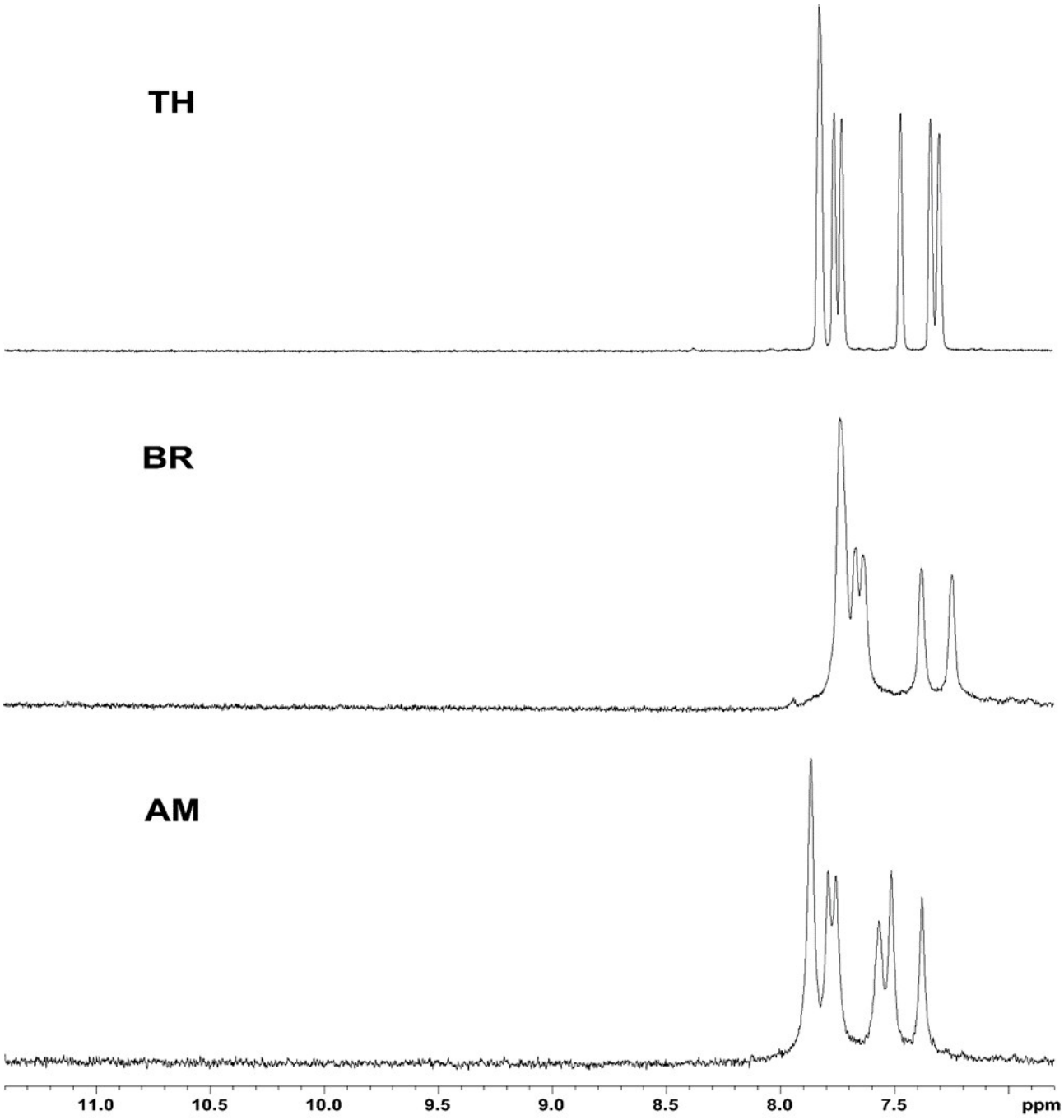

Figure S12: Imino and aromatic proton regions of the high-resolution ^{1}H-NMR spectra (500 MHz, D_2O, 80°C, no salt) of **TH**, **BR** and **AM**.

5'-$T_1G_2G_3U^{NH_2}{}_4G_5G_6T_7$-3' (AM)								
	H8/H6	H1'	H2'/H2''	H3'	H4'	H5'/H5''	CH_3/NH_2	NH
T_1	7.31	6.04	2.18	4.49	4.23	4.04	1.66	-
G_2	7.64	6.03	2.75/3.00	5.01	4.27	N.D.	-	11.53
G_3	7.58	6.18	2.45/2.87	4.98	4.24	N.D.	-	11.28
$U^{NH_2}{}_4$	7.21	5.82	2.13/2.45	4.84	4.38	N.D.	4.48	10.75
G_5	8.13	6.03	2.77/2.89	5.09	4.27	4.13	-	11.22
G_6	7.64	6.20	2.57/2.63	4.11	N.D.	N.D.	-	11.04
T_7	7.31	6.04	2.18	4.23	4.04	4.04	1.66	-

5'-$T_1G_2G_3U^{Br}{}_4G_5G_6T_7$-3' (BR)								
	H8/H6	H1'	H2'/H2''	H3'	H4'	H5'/H5''	CH_3	NH
T_1	7.46	5.96	2.20/2.50	4.75	N.D.	4.10/3.76	1.44	-
G_2	8.11	6.08	2.73/3.00	5.02	4.41	4.12/4.08	-	11.66
G_3	7.59	6.16	2.57/2.90	4.99	4.50	4.27/4.01	-	11.28
$U^{Br}{}_4$	7.64	5.95	2.26/2.60	4.90	4.36	4.25	-	-
G_5	8.00	5.93	2.67/2.75	5.06	4.41	4.25/4.13	-	11.25
G_6	7.70	6.24	2.55/2.66	4.92	4.49	4.24	-	11.00
T_7	7.33	6.04	2.17	4.47	4.22	4.06/3.64	1.64	-

5'-$T_1G_2G_3T_4G_5G_6T_7$-3' (TH)								
	H8/H6	H1'	H2'/H2''	H3'	H4'	H5'/H5''	CH_3	NH
T_1	7.43	5.90	2.13/2.46	4.71	4.08	3.76	1.30	-
G_2	8.08	6.03	2.68/2.93	5.00	4.40	4.12/4.07	-	11.56
G_3	7.57	6.11	2.50/2.86	4.99	4.47	4.24/4.08	-	11.23
T_4	7.06	5.84	2.21/2.43	4.89	4.30	4.20/4.11	0.72	9.44
G_5	7.98	5.94	2.67/2.78	5.04	4.43	4.24/4.13	-	11.23
G_6	7.69	6.21	2.54/2.65	4.93	4.47	4.20/4.03	-	10.99
T_7	7.30	6.01	2.13	4.46	4.20	4.05/4.01	1.61	-

Table S1: Proton chemical shifts assignment for G-quadruplex structures formed by ODNs **AM**, **BR** and **TH** (500 MHz, T = 25°C) in 20 mM KH_2PO_4/K_2HPO_4, 100 mM KCl and 0.2 mM EDTA (pH 7.0).