Supplemental Figures and Tables

for

The origin of the high stability of 3'-terminal uridine tetrads. The contributions of hydrogen bonding, stacking interactions and steric factors evaluated using modified oligonucleotide analogs.

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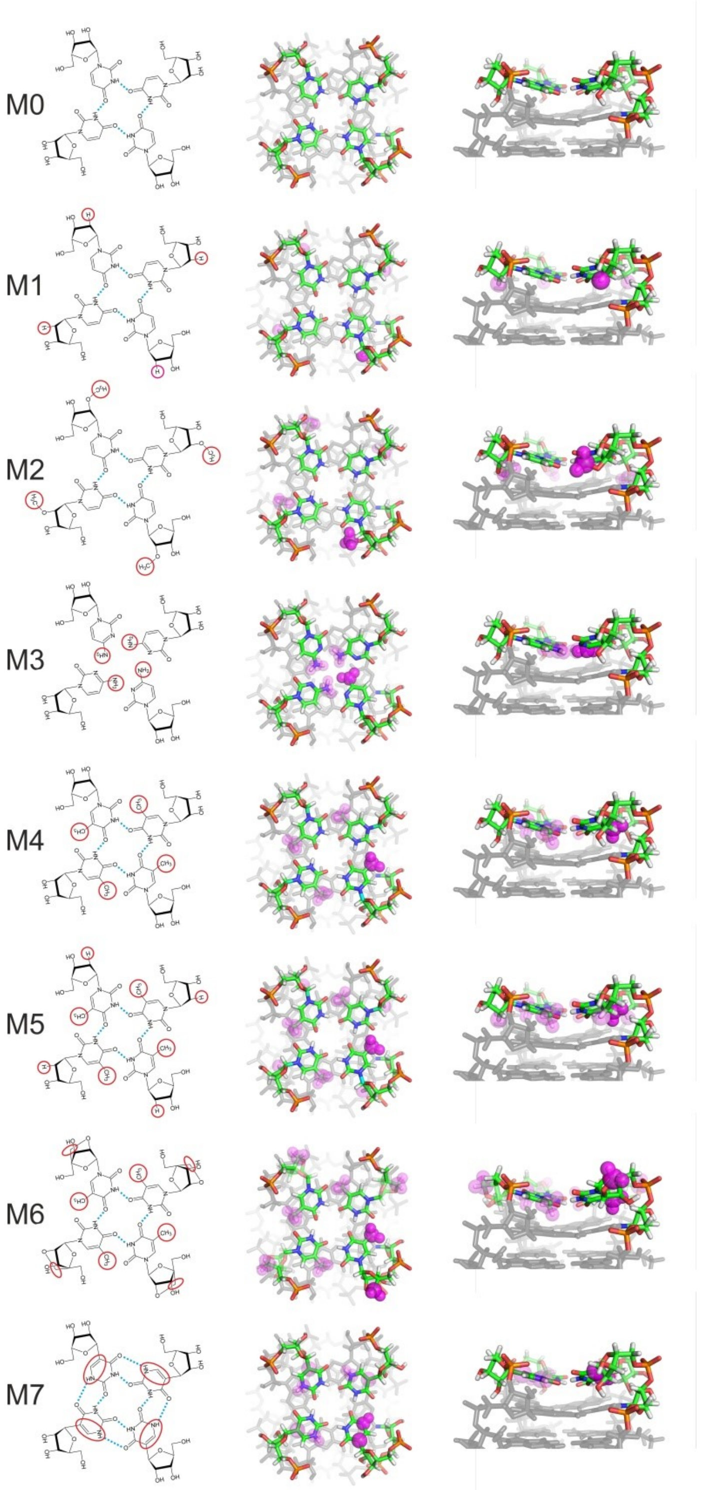
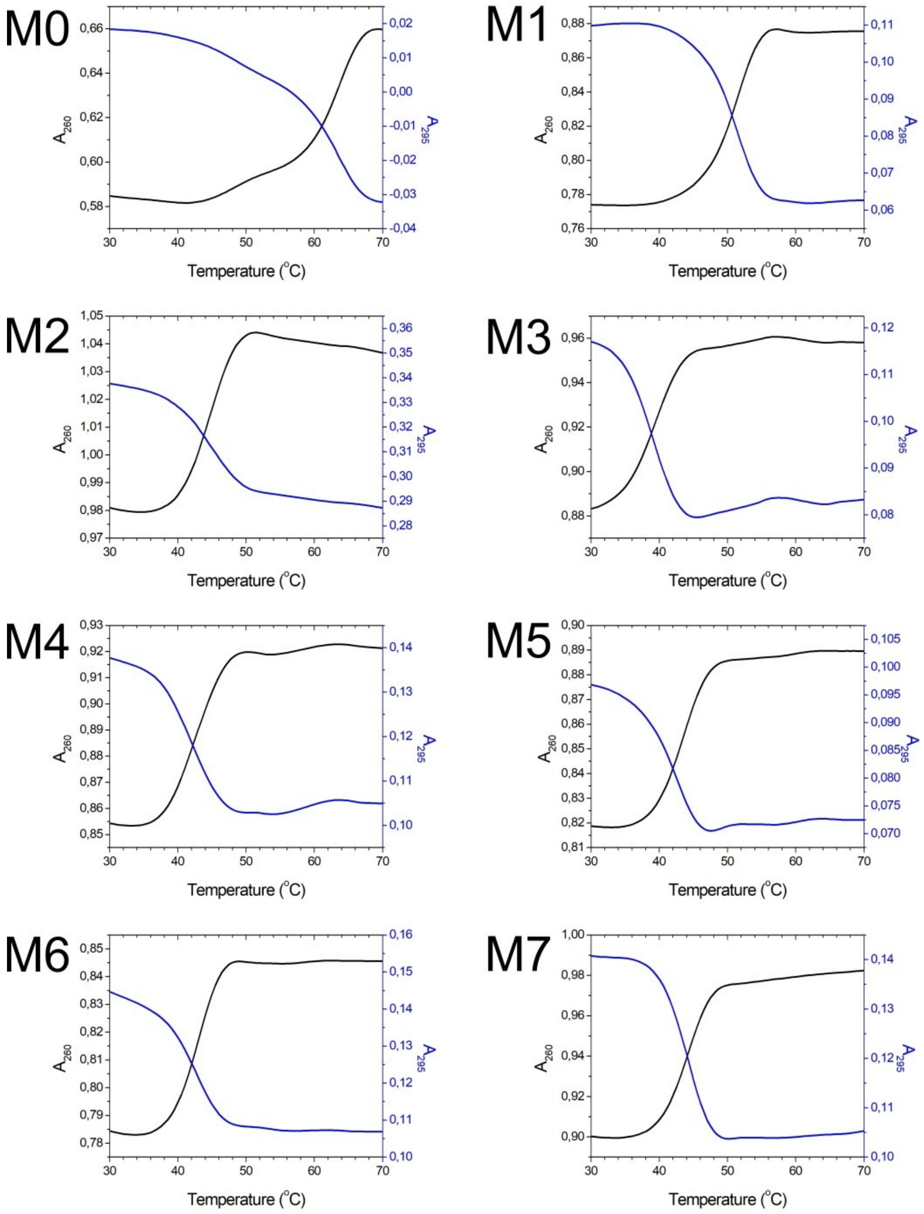
Supplemental Fig. S1. The chemical modifications of the 3’-terminal uridine residue (G-quadruplexes M0-M7). The left panel presents the scheme of a hypothetical tetrad formed by each modified 3’-terminal residue with predicted hydrogen bonds marked in blue and the modified sites in red. The central and right panels show this hypothetical tetrad in the context of the 3’-terminal part of the r(UGGUGGU)$_4$ G-quadruplex, assuming that the ‘reversed’ U-tetrad conformation is preserved.

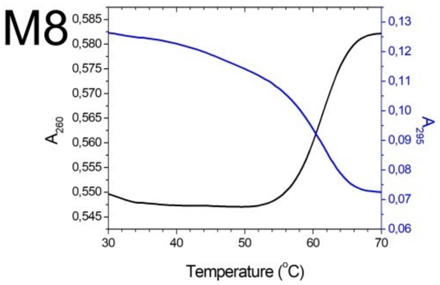


Supplemental Fig. S2. The UV melting curves recorded for the G-quadruplexes M0-M8 in the Na^+ containing buffer.

3'-terminal modifications

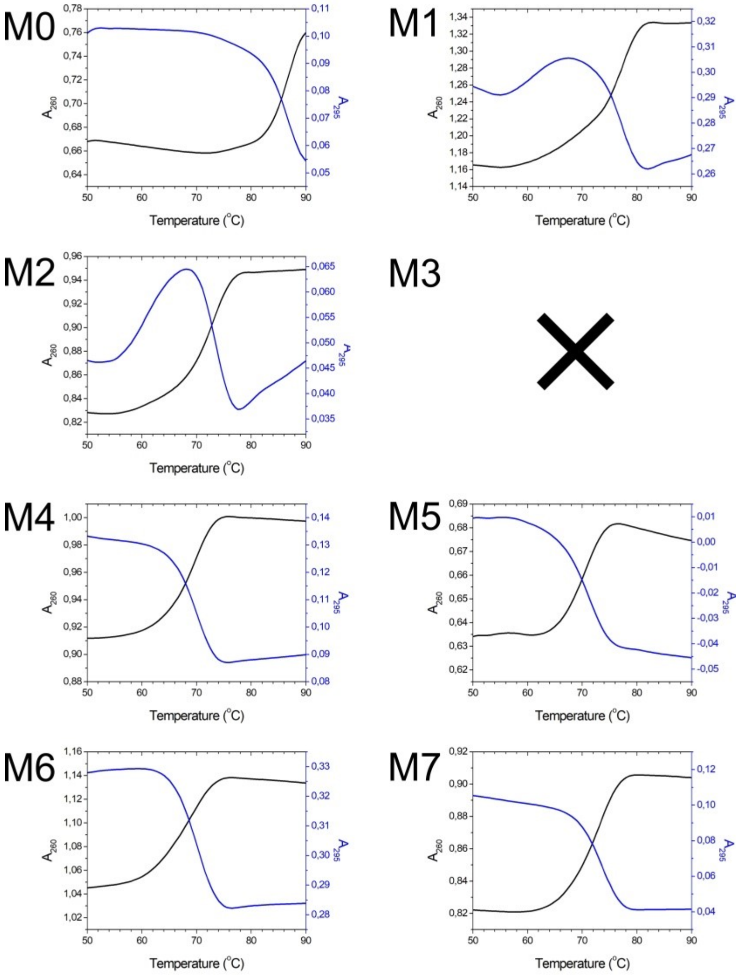


internal modifications

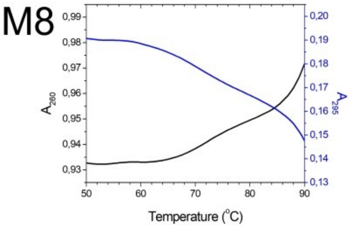


Supplemental Fig. S3. The UV melting curves recorded for the G-quadruplexes M0-M8 in the K^+ containing buffer.

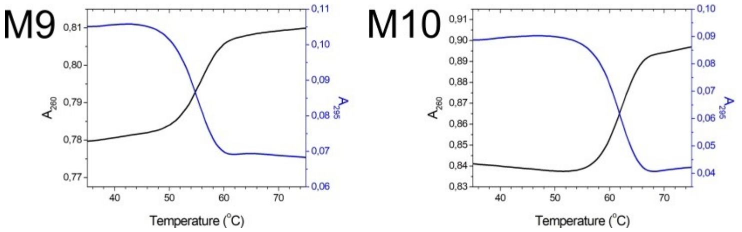
3'-terminal modifications



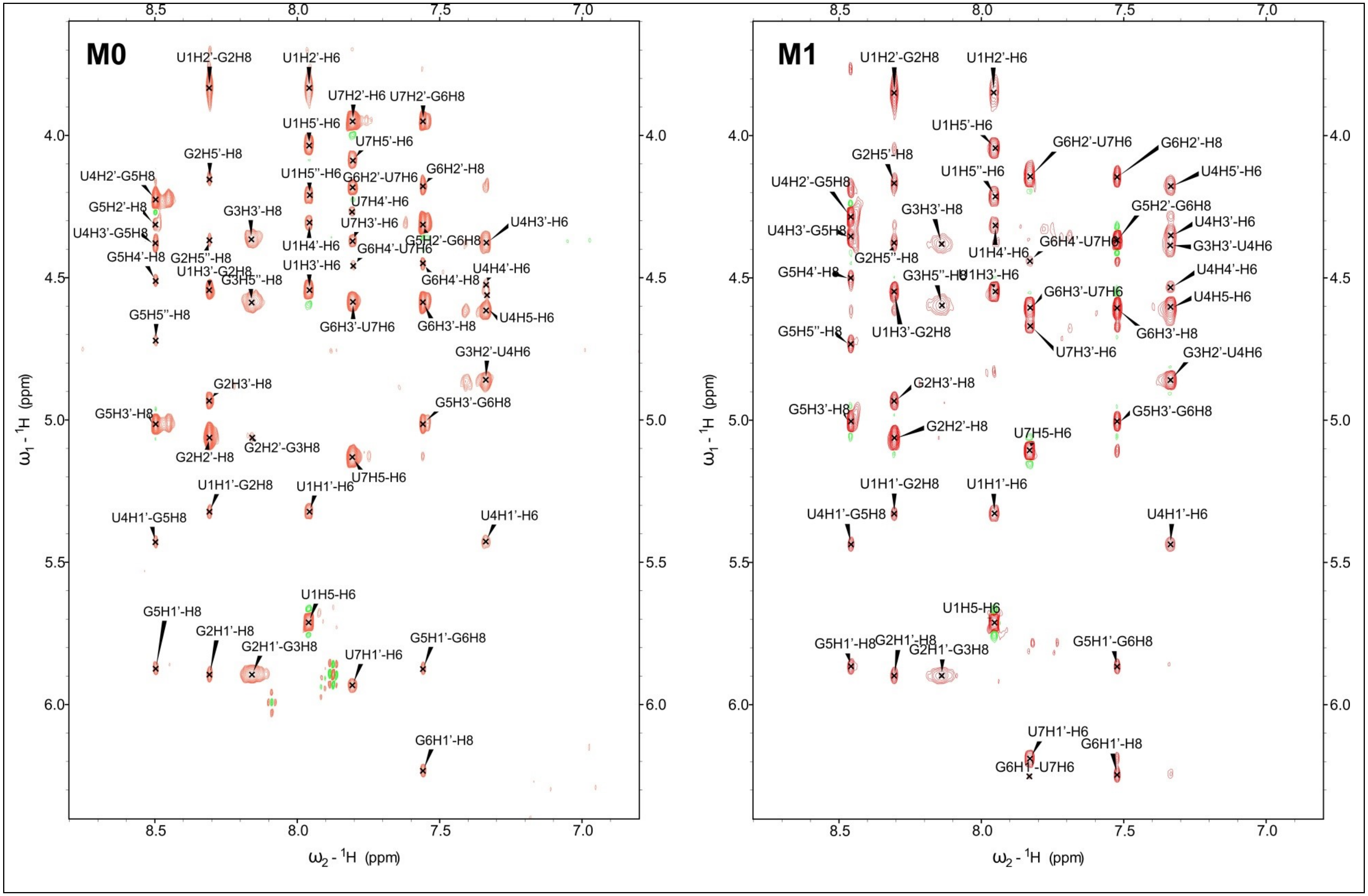
internal modifications

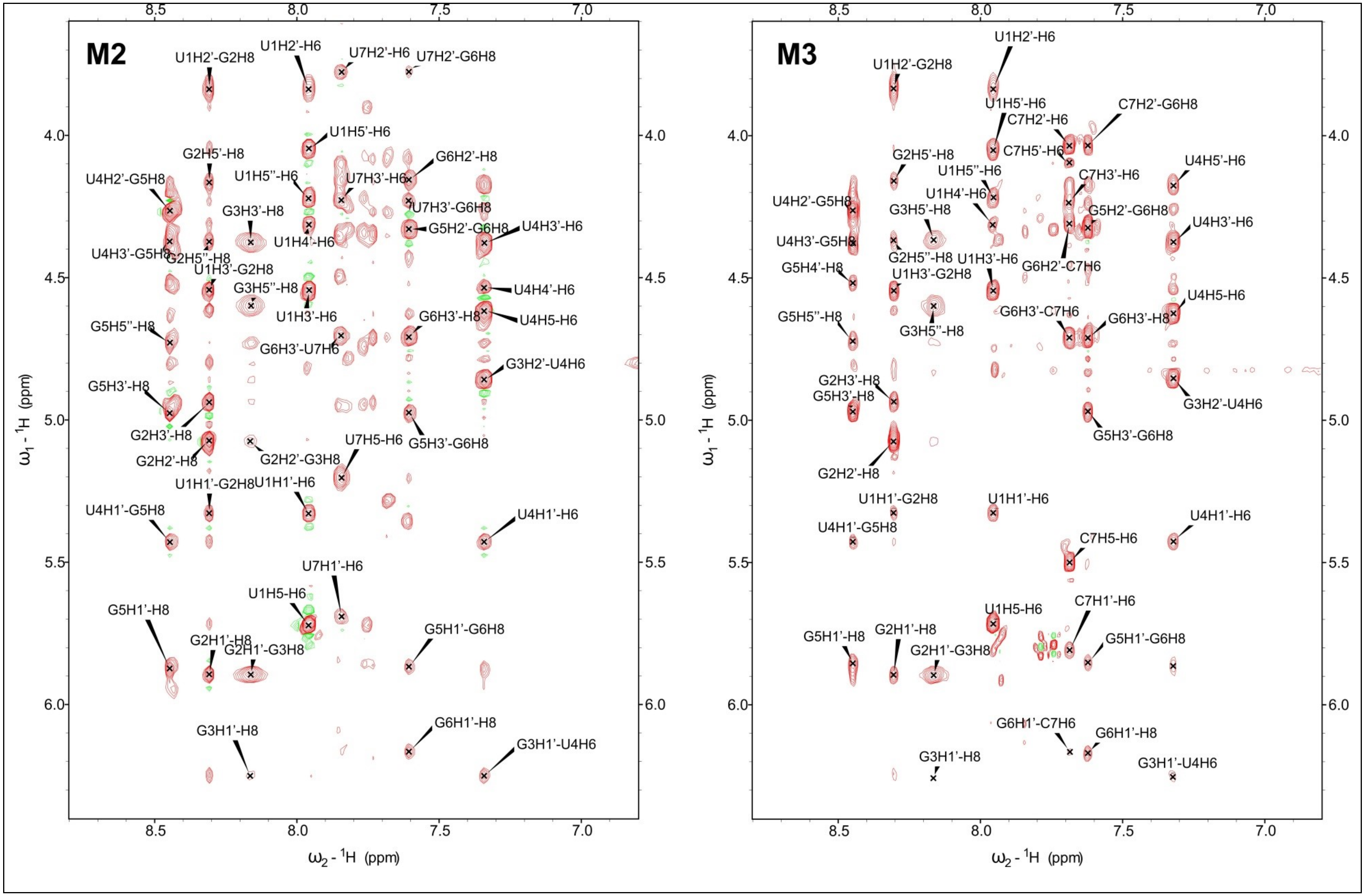


DNA series



Supplemental Fig. S4. The aromatic-to-sugar regions of the NOESY spectra of the M0-M7 G-quadruplexes in Na$^+$ containing conditions at 25 °C.

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U1H2'-H6
U7H2'-H6
U7H2'-G6H8
U1H5'-H6
G2H5'-H8
G6H2'-H8
U4H2'-G5H8
U1H5''-H6
U7H3'-H6
G3H3'-H8
U7H3'-G6H8
G5H2'-G6H8
U4H3'-H6
U1H4'-H6
U4H3'-G5H8
G2H5''-H8
U1H3'-G2H8
G3H5''-H8
U4H4'-H6
U1H3'-H6
U4H5-H6
G6H3'-H8
G5H5''-H8
G6H3'-U7H6
G3H2'-U4H6
G5H3'-H8
G2H3'-H8
U7H5-H6
G5H3'-G6H8
G2H2'-H8
G2H2'-G3H8
U1H1'-G2H8
U1H1'-H6
U4H1'-G5H8
U4H1'-H6
U7H1'-H6
U1H5-H6
G5H1'-H8
G5H1'-G6H8
G2H1'-H8
G2H1'-G3H8
G6H1'-H8
G3H1'-H8
G3H1'-U4H6
8.5
8.0
7.5
7.0
4.0
4.5
5.0
5.5
6.0
ω1 - 1H (ppm)
ω2 - 1H (ppm)
M3
U1H2'-H6
U1H2'-G2H8
U1H5'-H6
C7H2'-G6H8
C7H2'-H6
G2H5'-H8
C7H5'-H6
U4H5'-H6
U1H5''-H6
C7H3'-H6
U1H4'-H6
U4H2'-G5H8
G3H5'-H8
G5H2'-G6H8
U4H3'-H6
U4H3'-G5H8
G2H5''-H8
U1H3'-H6
G6H2'-C7H6
U1H3'-G2H8
G5H4'-H8
U4H5-H6
G6H3'-C7H6
G6H3'-H8
G5H5''-H8
G3H5''-H8
G2H3'-H8
G5H3'-H8
G3H2'-U4H6
G5H3'-G6H8
G2H2'-H8
U1H1'-G2H8
U1H1'-H6
U4H1'-H6
U4H1'-G5H8
C7H5-H6
C7H1'-H6
U1H5-H6
G5H1'-H8
G2H1'-H8
G5H1'-G6H8
G2H1'-G3H8
G6H1'-C7H6
G6H1'-H8
G3H1'-H8
G3H1'-U4H6
8.5
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7.5
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4.5
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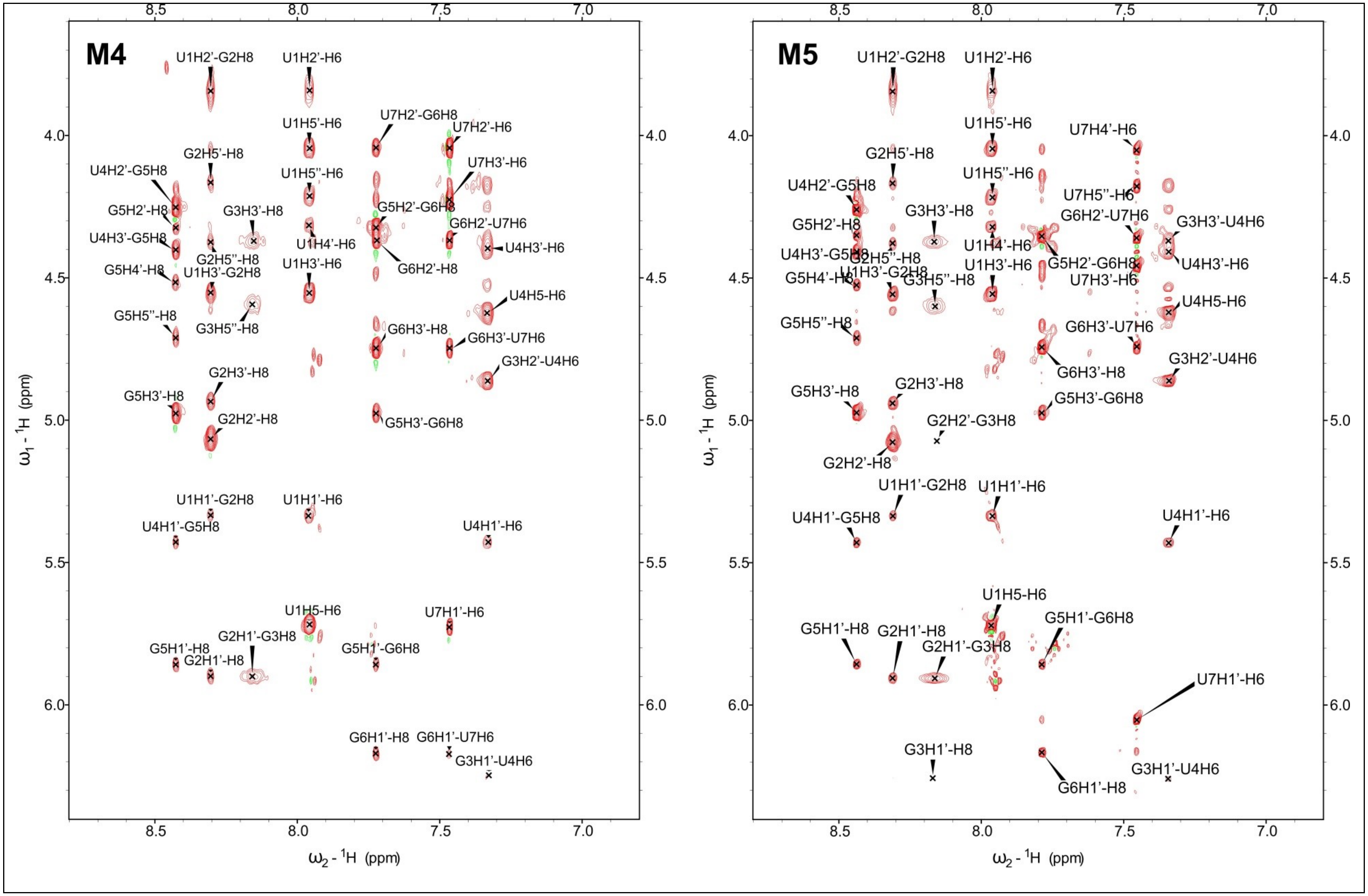
5.5
6.0
ω1 - 1H (ppm)
ω2 - 1H (ppm)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U1H5'-H6
U7H2'-G6H8
U7H2'-H6
G2H5'-H8
U4H2'-G5H8
U1H5''-H6
U7H3'-H6
G5H2'-H8
G3H3'-H8
G5H2'-G6H8
G6H2'-U7H6
U4H3'-G5H8
U1H4'-H6
U4H3'-H6
G2H5''-H8
G6H2'-H8
U1H3'-H6
G5H4'-H8
U1H3'-G2H8
U4H5-H6
G5H5''-H8
G3H5''-H8
G6H3'-H8
G6H3'-U7H6
G3H2'-U4H6
G2H3'-H8
G5H3'-H8
G2H2'-H8
G5H3'-G6H8
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U1H1'-H6
U4H1'-G5H8
U4H1'-H6
U1H5-H6
U7H1'-H6
G2H1'-G3H8
G5H1'-H8
G5H1'-G6H8
G2H1'-H8
G6H1'-H8
G6H1'-U7H6
G3H1'-U4H6
8.5
8.0
7.5
7.0
4.0
4.5
5.0
5.5
6.0
ω1 - 1H (ppm)
ω2 - 1H (ppm)
M5
U1H2'-G2H8
U1H2'-H6
U1H5'-H6
U7H4'-H6
G2H5'-H8
U4H2'-G5H8
U1H5''-H6
U7H5''-H6
G3H3'-H8
G6H2'-U7H6
G3H3'-U4H6
G5H2'-H8
U1H4'-H6
U4H3'-G5H8
G2H5''-H8
G5H2'-G6H8
U4H3'-H6
U1H3'-G2H8
U1H3'-H6
G5H4'-H8
G3H5''-H8
U7H3'-H6
U4H5-H6
G5H5''-H8
G6H3'-U7H6
G6H3'-H8
G3H2'-U4H6
G5H3'-H8
G2H3'-H8
G5H3'-G6H8
G2H2'-G3H8
G2H2'-H8
U1H1'-G2H8
U1H1'-H6
U4H1'-G5H8
U4H1'-H6
U1H5-H6
G5H1'-G6H8
G5H1'-H8
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G2H1'-G3H8
U7H1'-H6
G3H1'-H8
G3H1'-U4H6
G6H1'-H8
8.5
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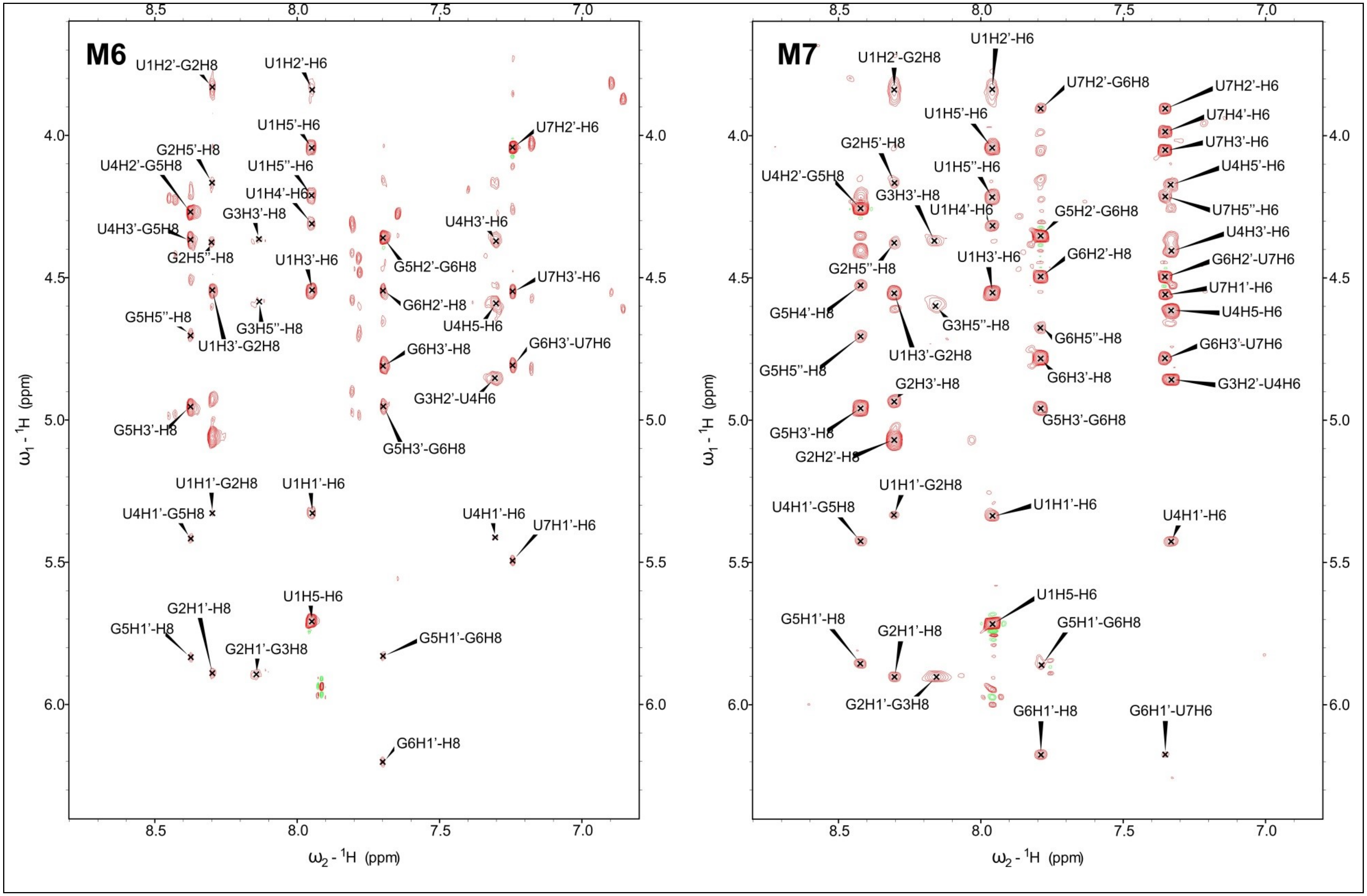
7.5
7.0
4.0
4.5
5.0
5.5
6.0
ω1 - 1H (ppm)
ω2 - 1H (ppm)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U1H2'-H6
U1H5'-H6
U7H2'-H6
G2H5'-H8
U4H2'-G5H8
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U1H4'-H6
G3H3'-H8
U4H3'-H6
U4H3'-G5H8
G2H5''-H8
U1H3'-H6
G5H2'-G6H8
U7H3'-H6
G6H2'-H8
G3H5''-H8
G5H5''-H8
U4H5-H6
U1H3'-G2H8
G6H3'-H8
G6H3'-U7H6
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U1H1'-H6
U4H1'-G5H8
U4H1'-H6
U7H1'-H6
U1H5-H6
G2H1'-H8
G5H1'-H8
G5H1'-G6H8
G2H1'-G3H8
G6H1'-H8
M7
U1H2'-H6
U1H2'-G2H8
U7H2'-G6H8
U7H2'-H6
U1H5'-H6
U7H4'-H6
G2H5'-H8
U7H3'-H6
U1H5''-H6
U4H5'-H6
U4H2'-G5H8
G3H3'-H8
U1H4'-H6
G5H2'-G6H8
U7H5''-H6
U4H3'-H6
U1H3'-H6
G6H2'-H8
G6H2'-U7H6
G2H5''-H8
U7H1'-H6
G5H4'-H8
G3H5''-H8
U4H5-H6
G6H5''-H8
G6H3'-U7H6
U1H3'-G2H8
G5H5''-H8
G6H3'-H8
G3H2'-U4H6
G2H3'-H8
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U4H1'-H6
U1H5-H6
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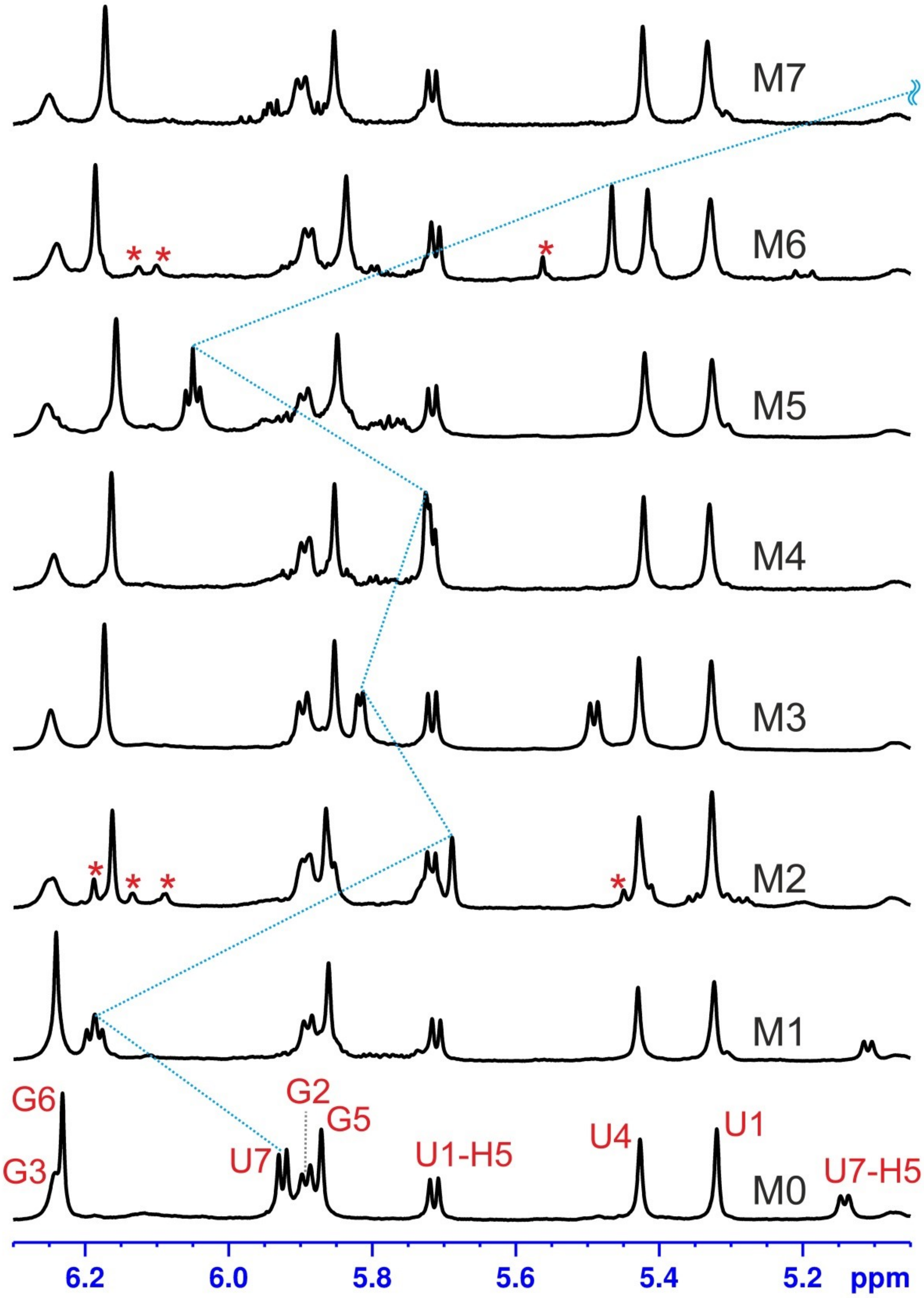
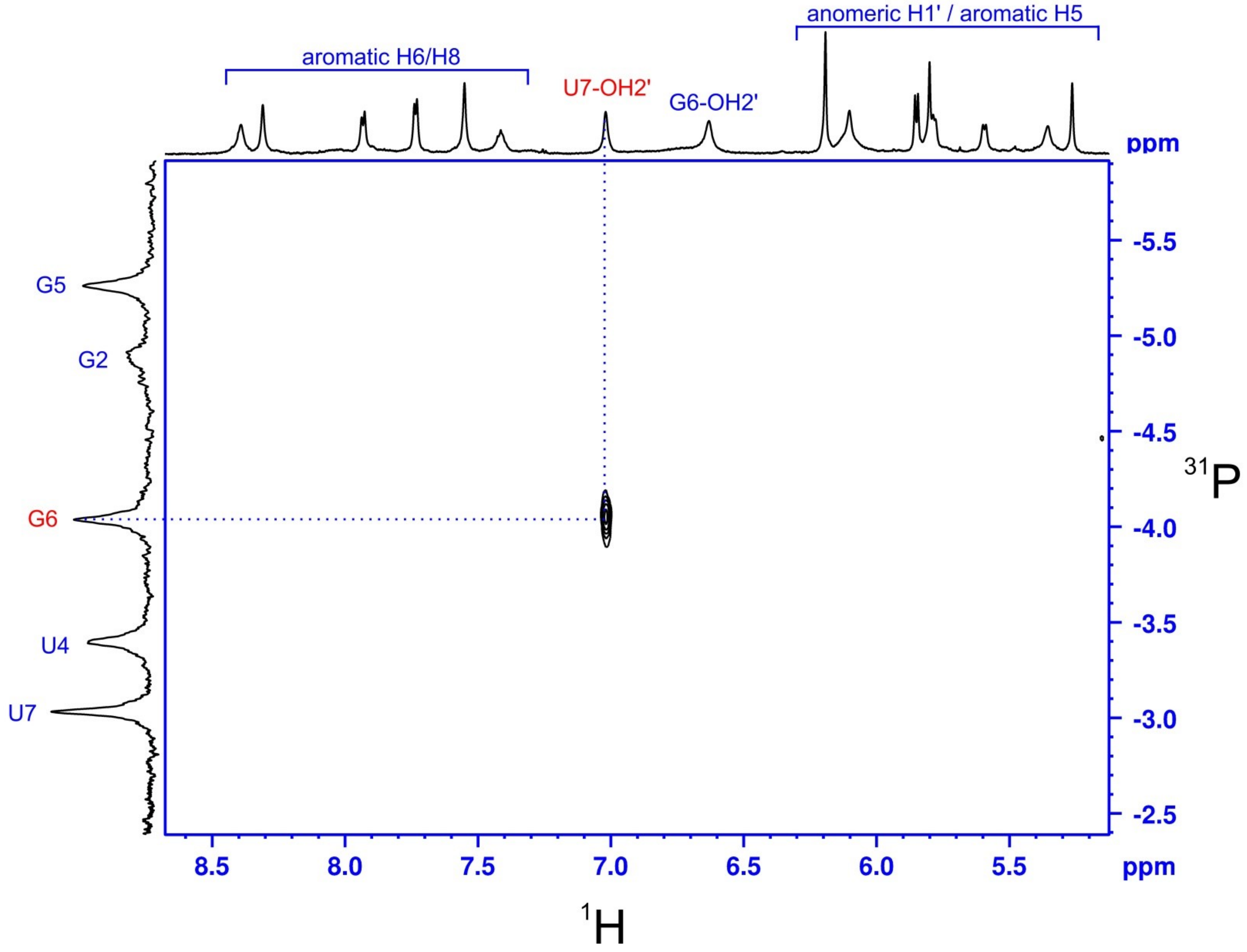
G5H1'-G6H8
G2H1'-H8
G2H1'-G3H8
G6H1'-H8
G6H1'-U7H6
8.5
8.0
7.5
7.0
4.0
4.5
5.0
5.5
6.0
ω_1 - ^{1}H (ppm)
ω_2 - ^{1}H (ppm)

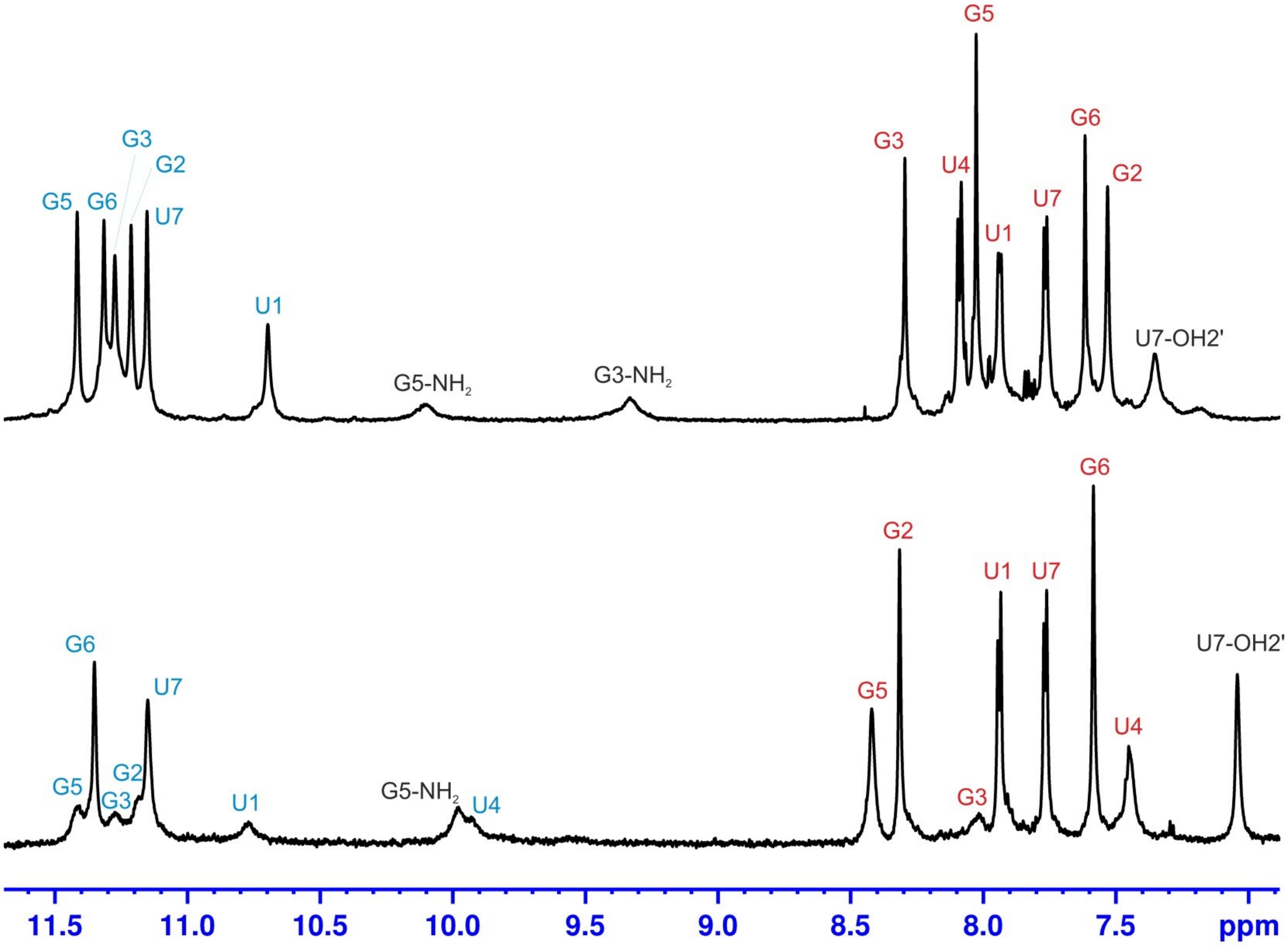
Supplemental Fig. S5. The ^{1}H-NMR spectra (anomeric region) of G-quadruplexes M0-M7 recorded in Na$^+$ containing conditions at 25 °C. The blue dotted lines follow the H1' resonance of the modified 3'-terminal residue. Additional NMR signals from minor species present in some of the samples are marked with asterisks.



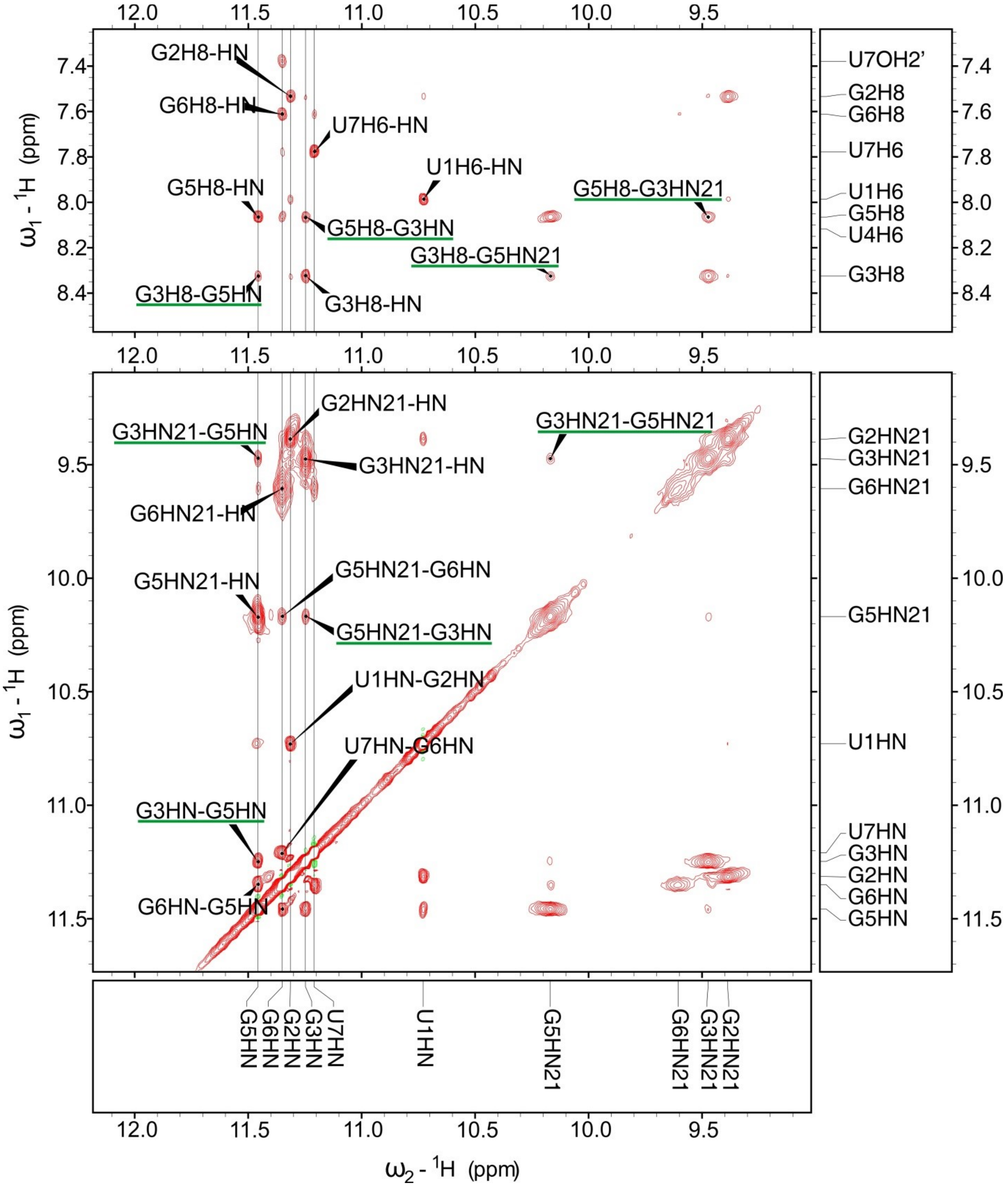
Supplemental Fig. S6. The ^{1}H-^{31}P-SOFAST-HMQC spectrum recorded for M0 at 15°C in K^{+} containing conditions. The full proton and phosphorus spectra recorded in the same conditions are presented as projections



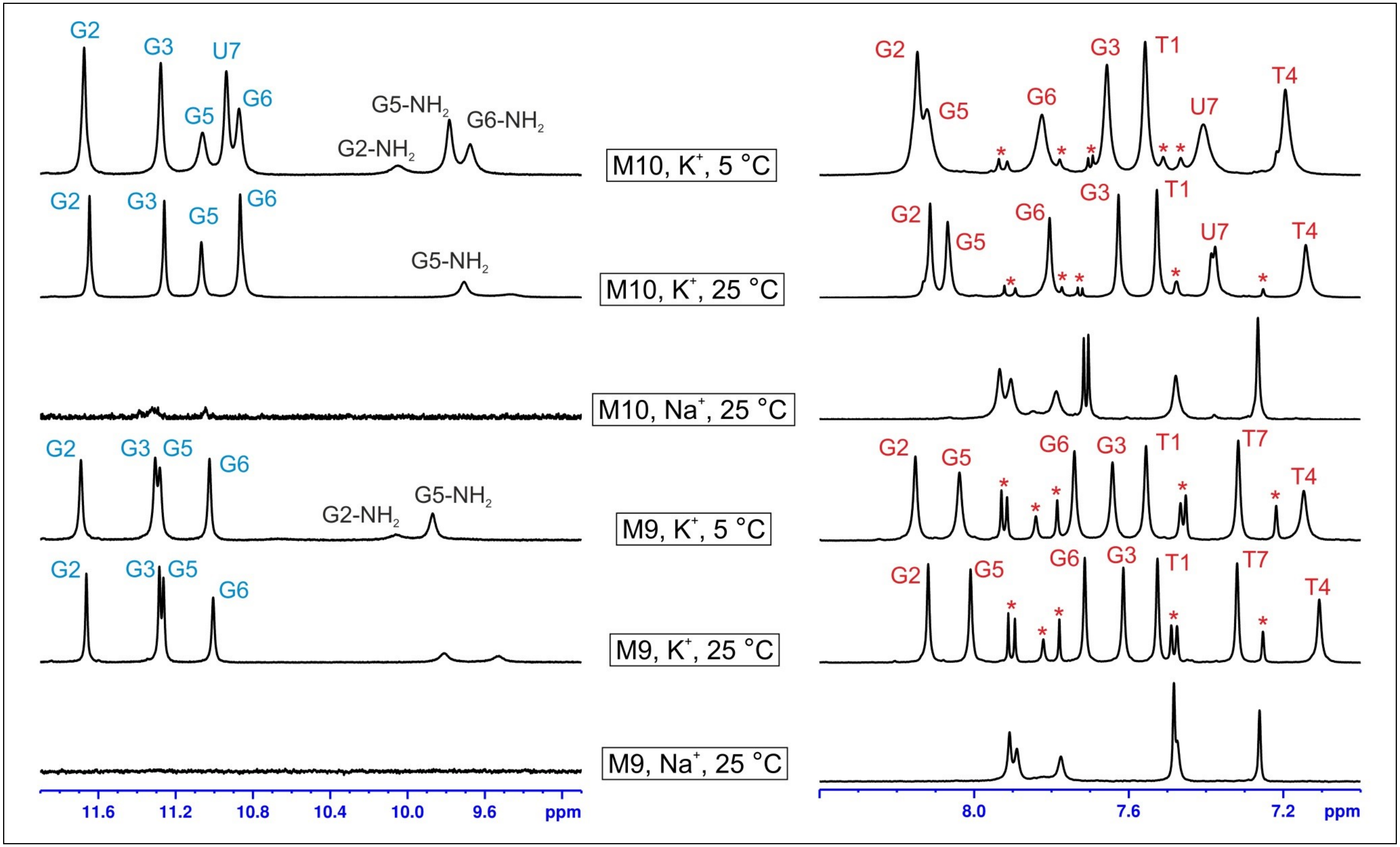
Supplemental Fig. S7. The ^{1}H-NMR spectra (imino and aromatic regions) of G-quadruplexes M8 (top) and M0 (bottom; for comparison) recorded in K$^+$ containing conditions at 25 °C.



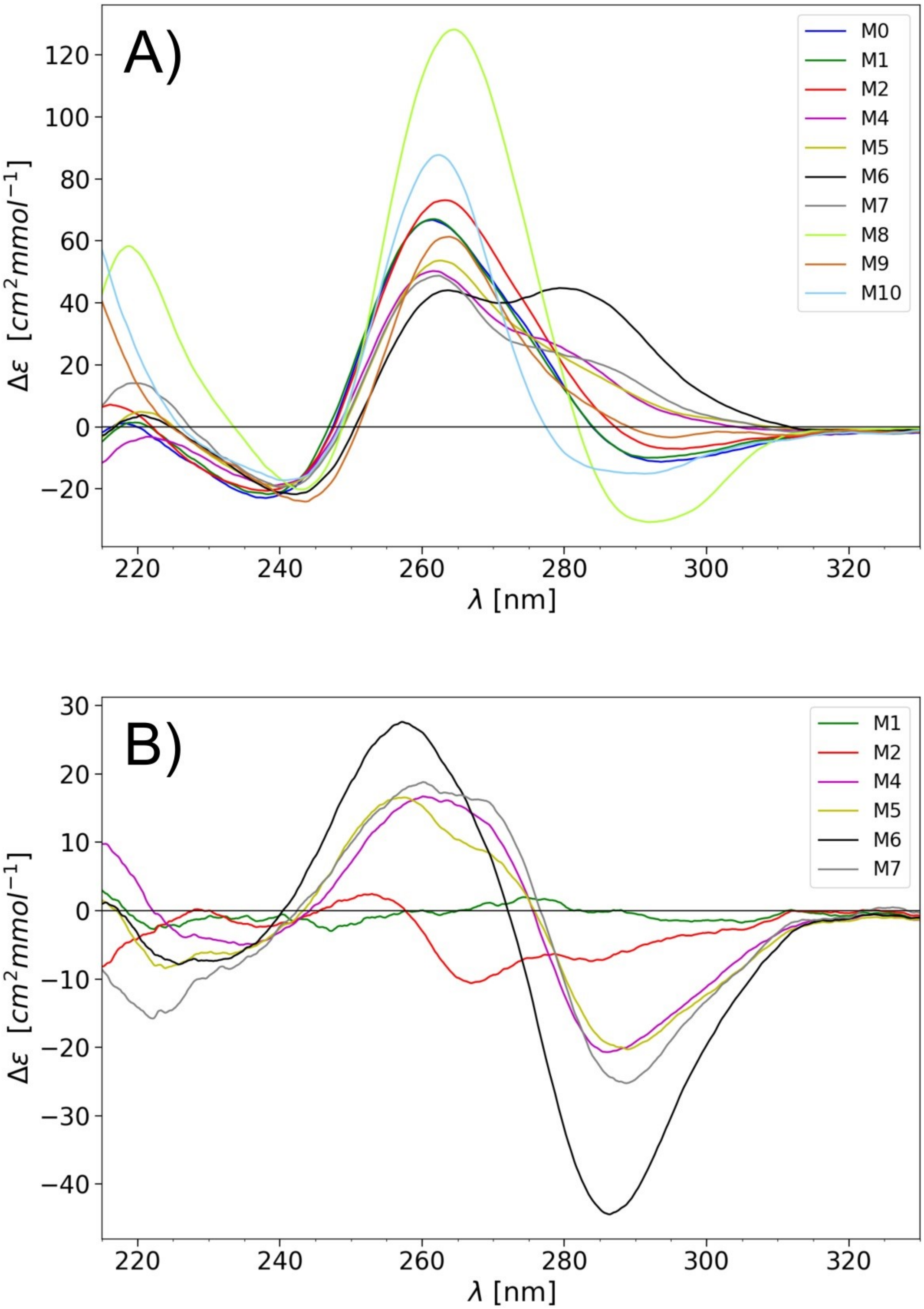
Supplemental Fig. S8. The imino-imino and imino-aromatic regions of the NOESY spectrum recorded for M8 in K^{+} containing conditions at 25 °C. The NOE cross-peaks indicating spatial proximity between residues G3 and G5 (and thus bulging-out of U4) are underlined in green.



Supplemental Fig. S9. The ^{1}H-NMR spectra (imino and aromatic regions) of G-quadruplexes M9 and M10. In the panels presenting the spectra recorded in K$^+$ containing conditions the asterisks mark the resonances due to residual amounts of single-stranded species.

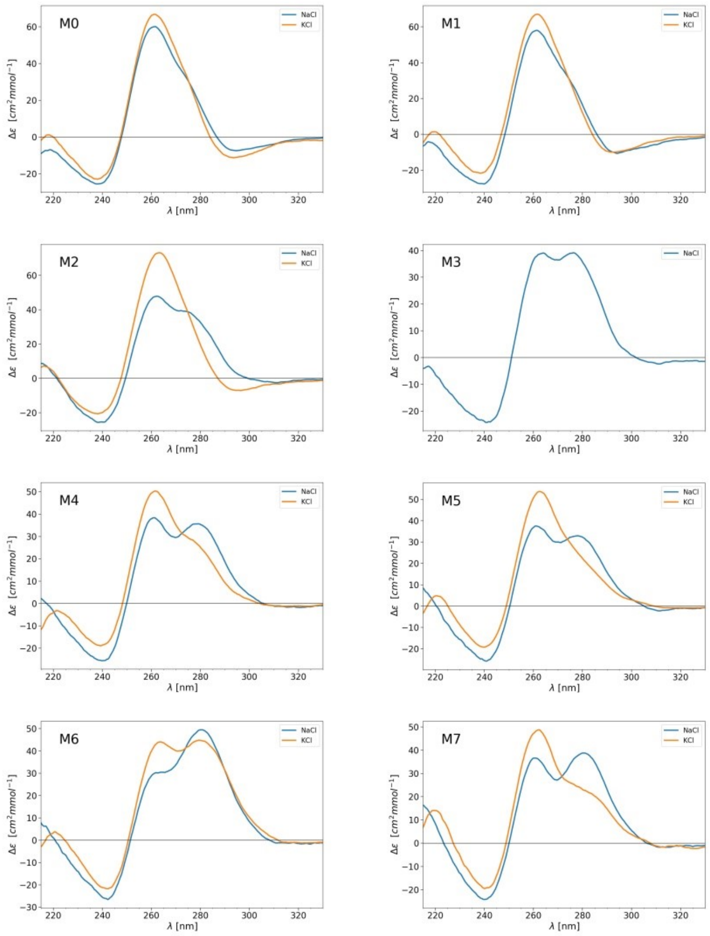


Supplemental Fig. S10. (A) The CD spectra recorded for G-quadruplexes M0-M10 in K^+ containing conditions at 25 °C; (B) the difference between the CD spectrum of each 3'-modified G-quadruplex (M1-M7) and that of the reference one (M0).

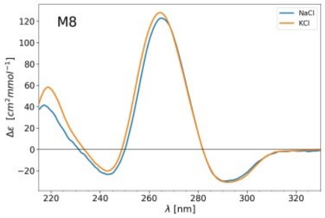


Supplemental Fig. S11. A direct comparison between the CD spectra recorded for G-quadruplexes M0-M10 in Na^+ and K^+ containing conditions.

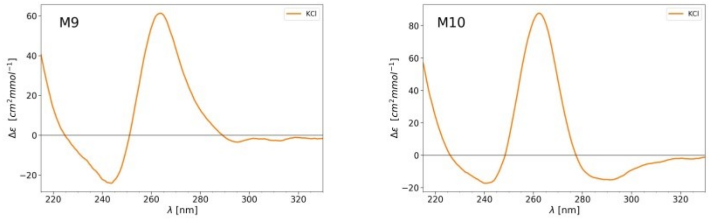
3'-terminal modifications



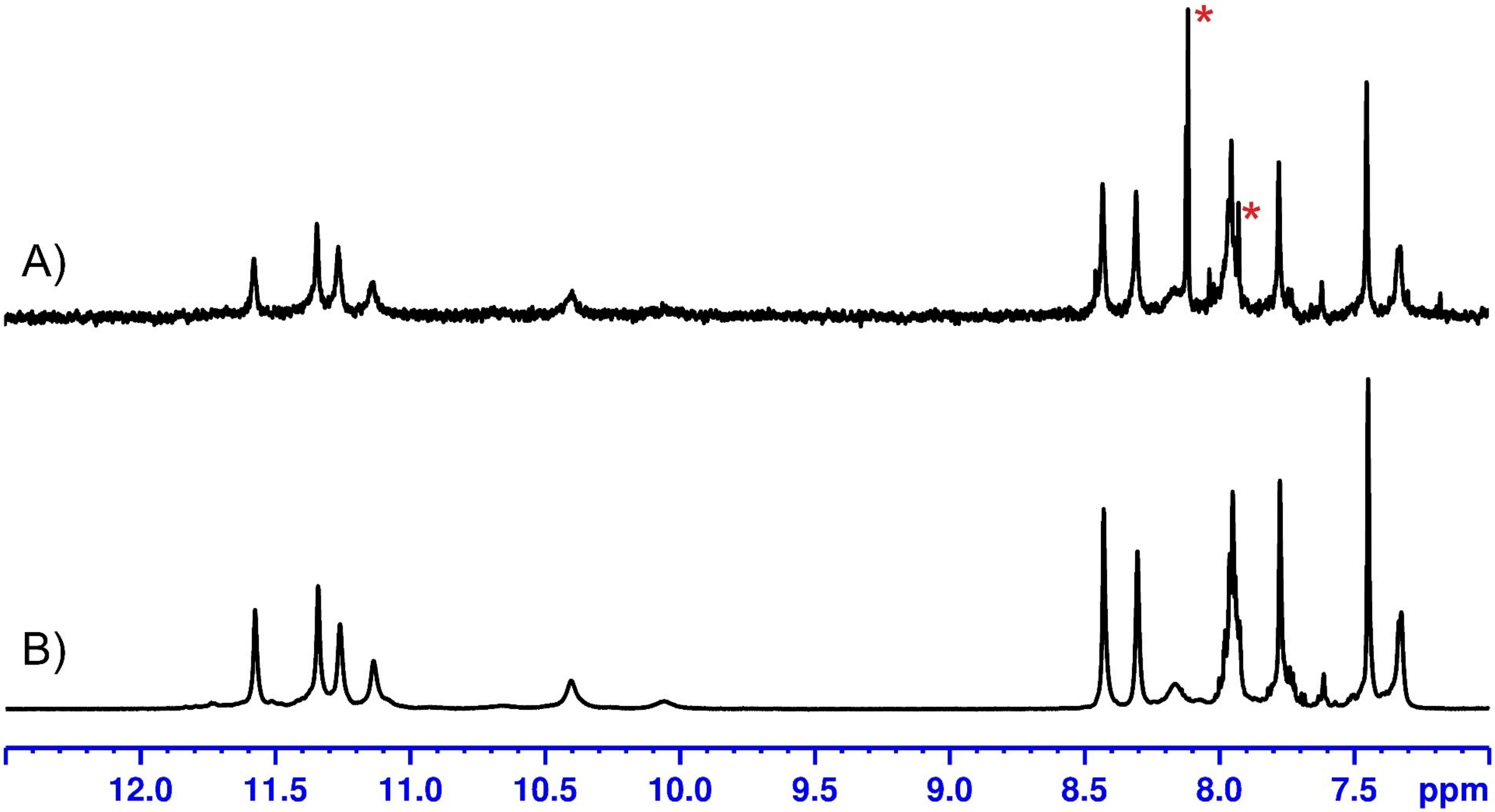
internal modifications



DNA series



Supplemental Fig. S12. The ^{1}H-NMR spectra (imino and aromatic regions) of G-quadruplex M4 recorded at 25 in Na+ containing conditions at A) CD sample concentration, B) NMR sample concentration.



Supplemental Table S1 The proton chemical shift (CS) values measured for the G-quadruplexes M0-M7. All resonances for which the chemical shift difference (ΔCS) with respect to M0 is higher than 0.1 ppm are marked in orange.

Residue	Atom	M0	M1	M2	M3	M4	M5	M6	M7
U1	H1'	5.323	5.329	5.329	5.325	5.335	5.336	5.328	5.333
U1	H2'	3.831	3.849	3.837	3.836	3.843	3.844	3.839	3.839
U1	H3'	4.546	4.547	4.545	4.547	4.553	4.558	4.545	4.553
U1	H4'	4.311	4.317	4.313	4.314	4.315	4.321	4.311	4.316
U1	H5'	4.043	4.044	4.046	4.045	4.045	4.045	4.044	4.042
U1	H5''	4.216	4.213	4.218	4.214	4.216	4.216	4.212	4.217
U1	H5	5.713	5.711	5.722	5.716	5.717	5.722	5.708	5.721
U1	H6	7.958	7.954	7.958	7.955	7.958	7.961	7.948	7.960
G2	H1'	5.893	5.896	5.895	5.896	5.898	5.904	5.890	5.901
G2	H2'	5.068	5.060	5.073	5.074	5.066	5.075	5.060	5.070
G2	H3'	4.934	4.933	4.935	4.934	4.934	4.941	4.926	4.934
G2	H4'	4.605	4.609	4.608	4.609	4.610	4.614	4.603	4.608
G2	H5'	4.159	4.168	4.165	4.159	4.165	4.167	4.164	4.166
G2	H5''	4.370	4.375	4.373	4.367	4.374	4.378	4.371	4.377
G2	H8	8.306	8.305	8.308	8.304	8.304	8.311	8.298	8.304
G3	H1'	6.244	6.239	6.251	6.252	6.247	6.259	6.238	6.252
G3	H2'	4.859	4.859	4.858	4.856	4.862	4.861	4.852	4.858
G3	H3'	4.368	4.393	4.376	4.366	4.368	4.373	4.364	4.367
G3	H4'	4.724	4.722	4.730	4.727	4.728	4.732	4.722	4.724
G3	H5'	4.375	4.364	---	4.368	4.372	---	---	---
G3	H5''	4.594	4.580	4.599	4.599	4.592	4.601	4.585	4.595
G3	H8	8.161	8.139	8.163	8.164	8.156	8.163	8.137	8.159
U4	H1'	5.430	5.436	5.428	5.427	5.428	5.430	5.418	5.426
U4	H2'	4.230	4.285	4.265	4.262	4.254	4.260	4.272	4.256
U4	H3'	4.380	4.347	4.377	4.382	4.399	4.410	4.371	4.404
U4	H4'	4.523	4.537	4.533	4.528	4.527	4.534	4.525	4.525
U4	H5'	---	4.174	---	4.176	---	---	---	4.172
U4	H5''	4.647	4.634	---	4.640	---	---	---	---
U4	H5	4.619	4.603	4.617	4.624	4.623	4.619	---	4.613
U4	H6	7.333	7.336	7.342	7.320	7.330	7.342	7.304	7.331
G5	H1'	5.875	5.865	5.868	5.853	5.858	5.856	5.835	5.856
G5	H2'	4.318	4.366	4.329	4.334	4.324	4.350	4.359	4.353
G5	H3'	5.015	5.004	4.977	4.976	4.976	4.974	4.954	4.958
G5	H4'	4.514	4.498	4.516	4.514	4.514	4.523	4.504	4.524
G5	H5'	4.181	4.188	4.200	4.191	4.199	4.210	4.199	4.210
G5	H5''	4.725	4.730	4.728	4.722	4.709	4.710	4.702	4.705
G5	H8	8.494	8.458	8.446	8.448	8.427	8.438	8.374	8.422

G6	H1'	6.234	6.246	6.163	6.173	6.172	6.167	6.199	6.176
G6	H2'	4.188	4.149	4.157	4.308	4.369	4.359	4.547	4.496
G6	H3'	4.587	4.604	4.702	4.710	4.747	4.742	4.810	4.782
G6	H4'	4.453	4.438	4.436	4.471	4.481	4.491	4.453	4.485
G6	H5'	4.172	4.139	4.157	4.161	4.151	4.139	4.161	4.161
G6	H5''	4.645	4.613	4.632	4.675	4.664	4.669	4.648	4.674
G6	H8	7.556	7.524	7.606	7.621	7.724	7.788	7.699	7.790
U7	H1'	5.931	6.188	5.692	5.808	5.728	6.051	5.493	4.559
U7	H2'	3.955	1.908	3.778	4.035	4.043	2.072	4.045	3.905
U7	H3'	4.373	4.667	4.228	4.239	4.225	4.456	4.547	4.049
U7	H4'	4.270	4.128	4.118	4.194	4.177	4.052	---	3.981
U7	H5'	4.088	4.120	4.102	4.091	4.058	4.046	4.106	3.986
U7	H5''	---[a]	---[a]	4.368	---[a]	4.240	4.174	4.252	4.213
U7	H5	5.137	5.107	5.203	5.500	na	5.131	na	na
U7	H6	7.802	7.830	7.843	7.686	7.466	7.455	7.244	7.352
U7	H2''	na	2.573	na	na	na	2.242	na	na
U7	2'OMe	na	na	3.585	na	na	na	na	na
U7	Me[b]	na	na	na	na	1.578	1.603	1.777	na
U7	H6'[c]	na	na	na	na	na	na	3.735	na
U7	H6''[c]	na	na	na	na	na	na	3.851	na

[a] most probably degenerate with U7H5'

[b] the thymine base CH_3 group

[c] the LNA sugar bridging CH_2 group

Supplemental Table S2 The ^{31}P chemical shift (CS) values measured for the G-quadruplexes M0-M7.

Residue	Atom	M0	M1	M2	M3	M4	M5	M6	M7
U1	P	-0.945	-0.966	-0.940	-0.799	-0.947	-0.814	-1.006	-0.896
G2	P	-5.008	---	-5.011	-5.028	-5.007	-5.069	-5.021	-5.028
G3	P	-2.032	-2.098	-2.038	-2.077	-2.050	-2.066	-2.071	-2.066
U4	P	-3.046	-2.931	-2.894	-2.939	-2.969	-2.942	-2.906	-2.952
G5	P	-4.906	-5.224	-5.354	-5.026	-5.074	-5.138	-5.237	-5.062
G6	P	-3.828	-4.024	-3.357	-4.001	-4.116	-4.305	-4.065	-4.107
U7	P	-2.966	-3.247	-4.216	-3.633	-3.799	-3.889	-3.931	-3.504

Supplemental Table S3 The results of the MM-GBSA analysis of the MD trajectories recorded for M0 in two distinct conformations – with reversed and standard U-tetrads at the 3'-terminus.

Energy Component [kcal/mol]	Reversed U-tetrad (system A)	σ M	Regular U-tetrad (system B)	σ M	Energy difference (A - B)
BOND	240.0	0.2	240.3	0.2	-0.3
ANGLE	460.8	0.3	461.9	0.3	-1.1
DIHED	692.0	0.2	684.3	0.2	7.7
VDW	-572.9	0.2	-561.7	0.2	-11.2
ELE	1031.4	0.6	1151.9	0.6	-120.5
1-4 VDW	233.2	0.1	233.8	0.1	-0.6
1-4 ELE	-2662.2	0.3	-2664.1	0.3	1.9
EGB	-4997.6	0.5	-5109.3	0.5	111.7
ESURF	29.4	0.0	31.3	0.0	-1.9
G gas	-577.7	0.6	-453.7	0.6	-124.0
G solv	-4968.2	0.5	-5078.0	0.5	109.8
E polar_total	-6628.4		-6621.6		-6.8
E np_total	-310.3		-296.6		-13.7
TOTAL	-5545.9	0.4	-5531.7	0.3	-14.2

BOND, ANGLE and DIHED denote internal (bond, angle and dihedral angle, respectively) potential energies. VDW represents non-bonded van der Waals energy, ELE - non-bonded electrostatic energy, 1-4 VDW and 1-4 ELE - energy contribution from the non-bonded interactions (VDW and ELE, respectively) between atoms separated by three consecutive bonds, EPB - electrostatic component to solvation free energy modelled with Generalized Born (GB) model, ESURF - nonelectrostatic contribution to solvation energy, G gas - total vacuum energy (G gas = BOND + ANGLE + DIHEDRAL + VDW + ELE + 1-4 VDW + 1-4 ELE), G solv - total solvation free energy,(G solv = EGB + ESURF), E polar_total = ELE + 1-4 ELE + EGB, E np_total = VDW + 1-4 VDW + ESURF, TOTAL = G gas + G sol = BOND + ANGLE + DIHEDRAL + E polar_total + E np_total, σ_M - standard error of the mean.