Ball with Hair: Modular functionalization of highly stable G-quadruplex DNA nano-scaffolds through N2-guanine modification

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Supporting Information

Keywords: G-quadruplex, nanoparticle, stability, N2-modifications, functionalization

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Supporting Material Contents:

Table S1. DNA Sequences explored in systematic study of N2-modification of (3+1) G-quadruplex nano-scaffold

Name	Sequence								
	1 2	3 4 5	6 7 8	9 10 11	12 13 14	15 16 17	18 19 20	21 22 23	24
Native	TT	GGG	TTA	GGG	TTA	GGG	TTA	GGG	A
Met-3	TT	**M**GG	TTA	GGG	TTA	GGG	TTA	GGG	A
Met-4	TT	G**M**G	TTA	GGG	TTA	GGG	TTA	GGG	A
Met-5	TT	GG**M**	TTA	GGG	TTA	GGG	TTA	GGG	A
Met-9	TT	GGG	TTA	**M**GG	TTA	GGG	TTA	GGG	A
Met-10	TT	GGG	TTA	G**M**G	TTA	GGG	TTA	GGG	A
Met-11	TT	GGG	TTA	GG**M**	TTA	GGG	TTA	GGG	A
Met-15	TT	GGG	TTA	GGG	TTA	**M**GG	TTA	GGG	A
Met-16	TT	GGG	TTA	GGG	TTA	G**M**G	TTA	GGG	A
Met-17	TT	GGG	TTA	GGG	TTA	GG**M**	TTA	GGG	A
Met-21	TT	GGG	TTA	GGG	TTA	GGG	TTA	**M**GG	A
Met-22	TT	GGG	TTA	GGG	TTA	GGG	TTA	G**M**G	A
Met-23	TT	GGG	TTA	GGG	TTA	GGG	TTA	GG**M**	A
Ben-3	TT	**B**GG	TTA	GGG	TTA	GGG	TTA	GGG	A
Ben-4	TT	G**B**G	TTA	GGG	TTA	GGG	TTA	GGG	A
Ben-5	TT	GG**B**	TTA	GGG	TTA	GGG	TTA	GGG	A
Ben-9	TT	GGG	TTA	**B**GG	TTA	GGG	TTA	GGG	A
Ben-10	TT	GGG	TTA	G**B**G	TTA	GGG	TTA	GGG	A
Ben-11	TT	GGG	TTA	GG**B**	TTA	GGG	TTA	GGG	A
Ben-15	TT	GGG	TTA	GGG	TTA	**B**GG	TTA	GGG	A
Ben-16	TT	GGG	TTA	GGG	TTA	G**B**G	TTA	GGG	A
Ben-17	TT	GGG	TTA	GGG	TTA	GG**B**	TTA	GGG	A
Ben-21	TT	GGG	TTA	GGG	TTA	GGG	TTA	**B**GG	A
Ben-22	TT	GGG	TTA	GGG	TTA	GGG	TTA	G**B**G	A
Ben-23	TT	GGG	TTA	GGG	TTA	GGG	TTA	GG**B**	A
Hex-3	TT	**H**GG	TTA	GGG	TTA	GGG	TTA	GGG	A
Hex -4	TT	G**H**G	TTA	GGG	TTA	GGG	TTA	GGG	A
Hex -5	TT	GG**H**	TTA	GGG	TTA	GGG	TTA	GGG	A
Hex -9	TT	GGG	TTA	**H**GG	TTA	GGG	TTA	GGG	A
Hex -10	TT	GGG	TTA	G**H**G	TTA	GGG	TTA	GGG	A
Hex -11	TT	GGG	TTA	GG**H**	TTA	GGG	TTA	GGG	A
Hex -15	TT	GGG	TTA	GGG	TTA	**H**GG	TTA	GGG	A
Hex -16	TT	GGG	TTA	GGG	TTA	G**H**G	TTA	GGG	A
Hex -17	TT	GGG	TTA	GGG	TTA	GG**H**	TTA	GGG	A
Hex -21	TT	GGG	TTA	GGG	TTA	GGG	TTA	**H**GG	A
Hex -22	TT	GGG	TTA	GGG	TTA	GGG	TTA	G**H**G	A
Hex -23	TT	GGG	TTA	GGG	TTA	GGG	TTA	GG**H**	A

Position of N2-modified-guanines are indicated as such: N2-Methyl-Guanine (**M**), N2-benzyl-guanine (**B**), N2-6-amino-hexyl-guanine (**H**),

Table S2. Classification of G-quadruplex conformation and stability

Sequence	NMR [a]			UV (°C)	
	Single Major Species	Minor Population	Multiple Major Species	Tm [b]	Δ Tm
Native	•			54.4 ± 0.4	-
Met-3				59.7 ± 0.2	5.3
Met-4	•			60.1 ± 0.7	5.7
Met-5	•			58.4 ± 0.5	4.0
Met-9		•		62.7 ± 0.8	8.3
Met-10	•			58.5 ± 0.4	4.1
Met-11	•			57.8 ± 0.6	3.5
Met-15	•			59.9 ± 0.4	5.6
Met-16	•			57.5 ± 0.1	3.1
Met-17		•		57.2 ± 0.3	2.8
Met-21	•			59.6 ± 0.3	5.2
Met-22		•		59.9 ± 0.5	5.5
Met-23	•			56.9 ± 0.2	2.5
Ben-3		•		60.7 ± 0.1	6.3
Ben-4	•			64.0 ± 0.5	9.6
Ben-5			•	-	-
Ben-9	•			67.7 ± 0.5	13.3
Ben-10	•			61.3 ± 0.4	6.9
Ben-11	•			61.9 ± 0.3	7.5
Ben-15	•			57.4 ± 0.1	3.0
Ben-16			•	-	-
Ben-17		•		58.9 ± 0.5	4.5
Ben-21	•			62.3 ± 0.7	8.0
Ben-22	•			59.6 ± 0.3	5.2
Ben-23		•		57.2 ± 0.3	2.8
Hex-3	•			63.6 ± 0.4	9.2
Hex-4	•			62.7 ± 0.4	8.3
Hex-5			•	-	-
Hex-9	•			63.5 ± 0.5	9.1
Hex-10			•	-	-
Hex-11	•			59.0 ± 0.4	4.6
Hex-15	•			59.7 ± 0.1	5.3
Hex-16			•	-	-
Hex-17	•			56.4 ± 0.3	2.0
Hex-21			•	-	-
Hex-22	•			62.3 ± 0.1	7.9
Hex-23	•			57.5 ± 0.0	3.1

[a] Determination of the same (3+1) conformation being adopted is based off a qualitative analysis of the amount of minor conformation present in NMR spectra and the similarity of NMR and CD spectra between the modified and unmodified sequences.

[b] The ± values indicated the hysteresis observed between heating and cooling curved in UV melting experiments

Table S3. Single N2-substitutions into a parallel G-quadruplex nano-scaffold construct

Sequence
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
TT GGG T GGG T GGG T GGG T
TT GGG T **M**GG T GGG T GGG T
TT GGG T G**M**G T GGG T GGG T
TT GGG T GG**M** T GGG T GGG T
TT GGG T **B**GG T GGG T GGG T
TT GGG T G**B**G T GGG T GGG T
TT GGG T GG**B** T GGG T GGG T
TT GGG T **H**GG T GGG T GGG T
TT GGG T G**H**G T GGG T GGG T
TT GGG T GG**H** T GGG T GGG T

The position of N2-modified-guanines are indicated as such: N2-Methyl-Guanine (**M**), N2-benzyl-guanine (**B**), N2-6-amino-hexyl-guanine (**H**)

Table S4. Nano-scaffold sequences containing multiple N2-modivitaions and their stabilities

Name	Sequence [a]	T_m [b]	ΔT_m
Native	TT GGG TTA GGG TTA GGG TTA GGG A	45.5 ± 0.2	-
N2-One-Groove	TT GGG TTA G**MM** TTA GGG TTA GGG A	52.4 ± 1.0	6.9
N2-Two-Groove	TT **H**GG TTA **H**GG TTA GGG TTA GGG A	63.8 ± 0.3	18.2
N2-All-Groove	TT **H**GG TTA **H**G**M** TTA **M**GG TTA GGG A	72.1 ± 0.2	26.6
N2-Ball-with-Hair	TT **HH**G TTA **H**G**H** TTA **H**G**H** TTA G**HH** A	91.7 ± 0.1 [c]	46.2

Samples were examined in "Low Salt" conditions (1 mM KPi + 1 mM KCl).
[a] The position of N2-modified guanines is given by the following notation: "**M**" for ^{Met}G; "**H**" for ^{Hex}G
[b] ± indicate the hysteresis between heating and cooling curves in CD melting experiments
[c] Incomplete melting transition observed. Tm determined by single base line correction with high temperature baseline set to a constant of ≈0 absorbance

Table S5. Nano-scaffold sequences containing functional N2-modifications

Name	Sequence [a]	T_m [b]	ΔT_m
	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24		
Native	TT GGG TTA GGG TTA GGG TTA GGG A	54.4 ± 0.4	
Bio-22	TT GGG TTA GGG TTA GGG TTA G**O**G A	56.0 ± 0.6	1.7
Bio-11-22	TT GGG TTA GG**O** TTA GGG TTA G**O**G A	57.8 ± 0.2	3.4

Samples were examined in "Moderate Salt" conditions (10 mM KPi + 10 mM KCl).
[a] The position of N2-modified-guanines are indicated as such: N2-Biotin-Guanine (**O**)
[b] ± indicate the hysteresis between heating and cooling curves in UV melting experiments

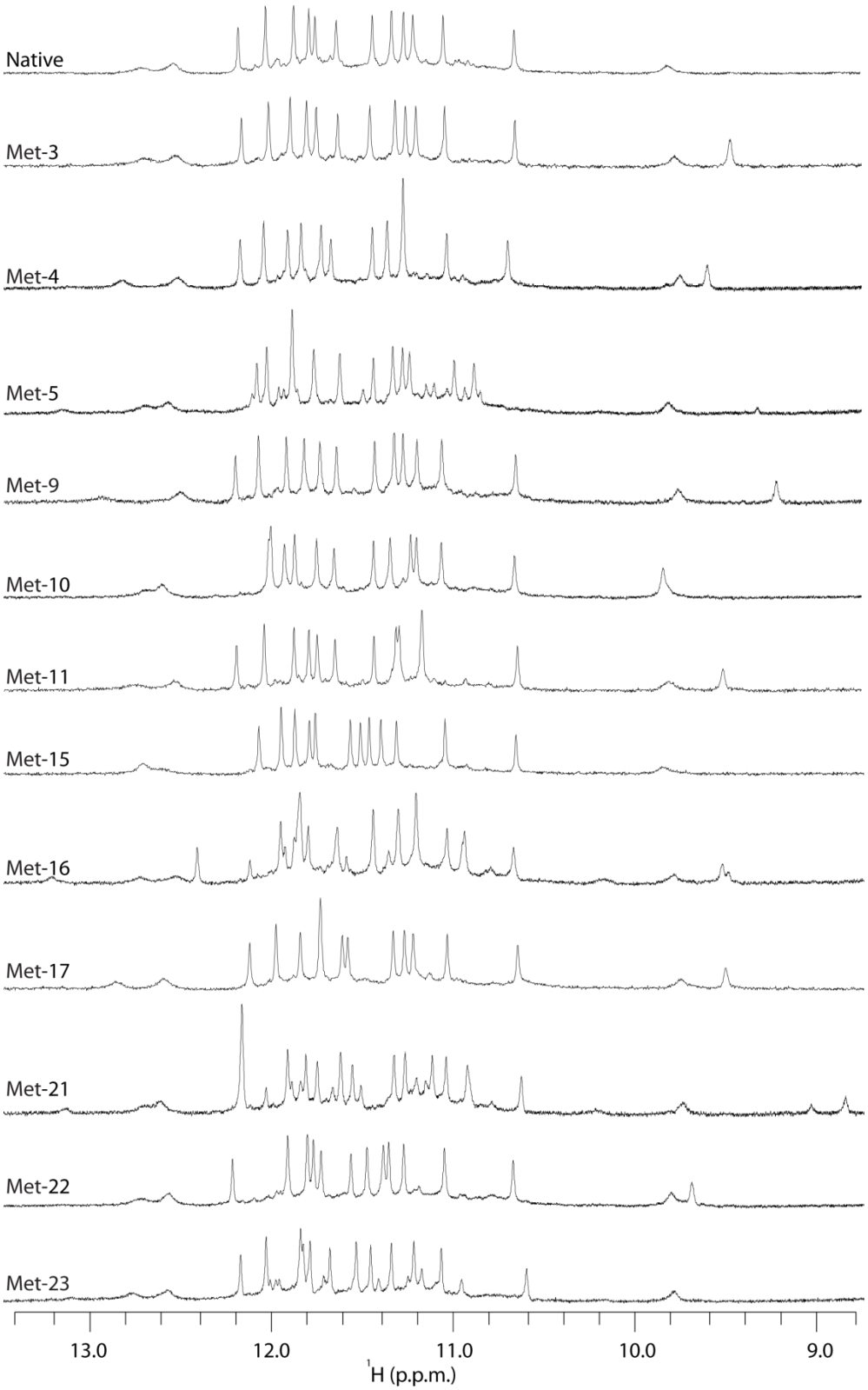


Figure S1. NMR spectra of single N2-methyl-guanine modifications (*MET*) to the (3+1) G-quadruplex nano-scaffold

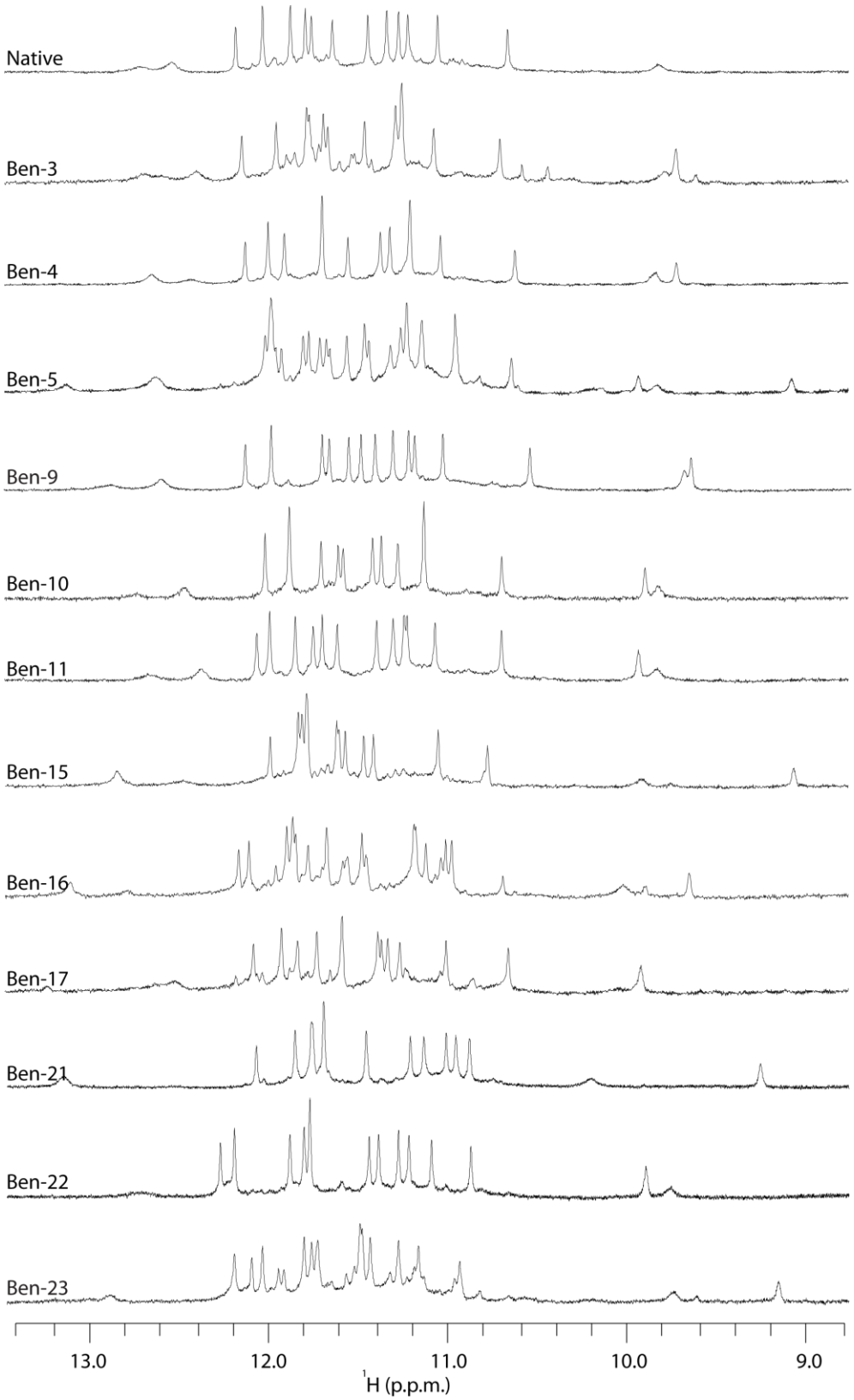


Figure S2. NMR spectra of single N2-benzyl-guanine modifications (*BEN*) to the (3+1) G-quadruplex nano-scaffold

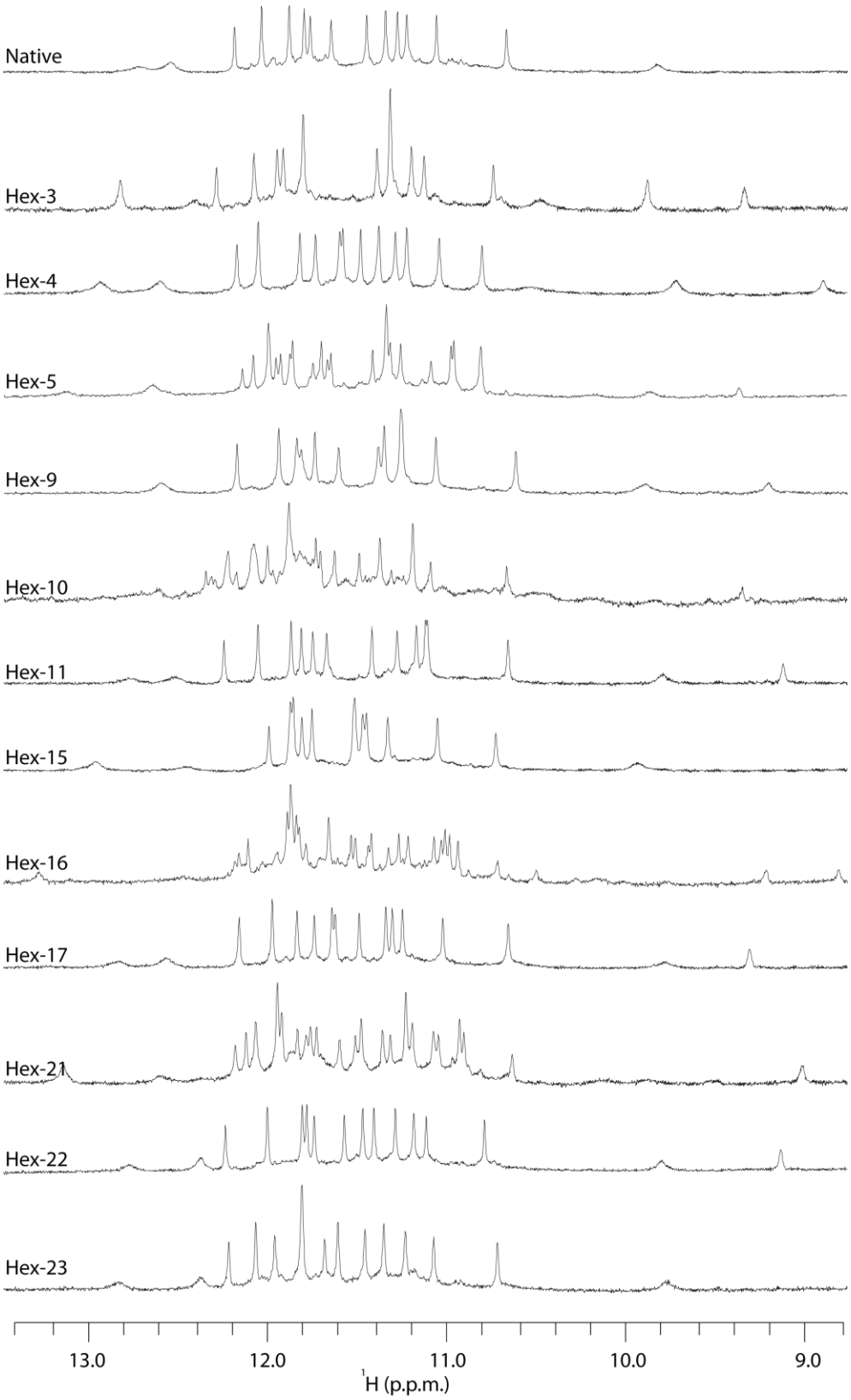


Figure S3. NMR spectra of single N2-6-amino-hexyl-guanine modifications (*HEX*) to the (3+1) G-quadruplex nano-scaffold

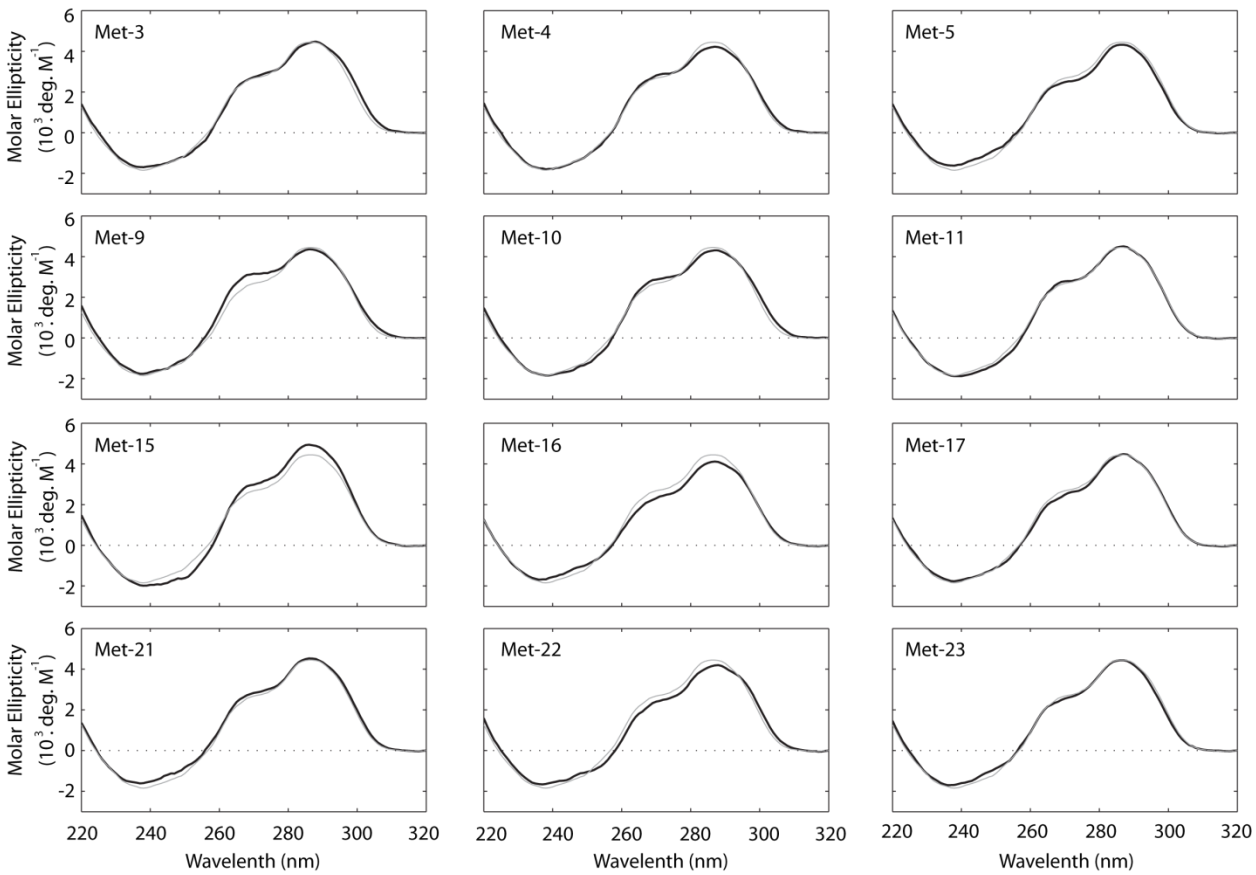


Figure S4. CD spectra of single N2-methyl-guanine (*MET*) modifications to the (3+1) G-quadruplex nano-scaffold. Modified sequences are shown in black. The *Native* reference sequence is shown in grey.

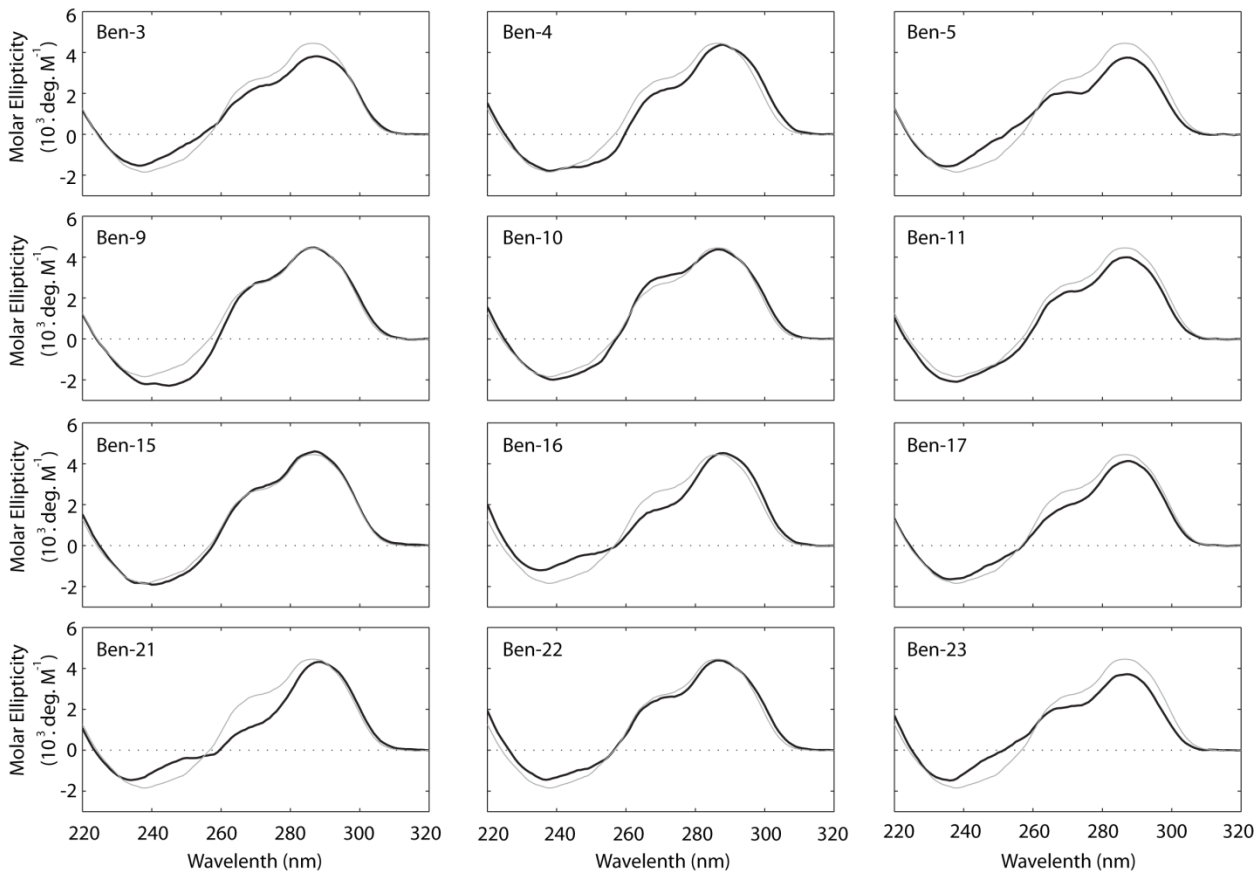


Figure S5. CD spectra of single N2-benzyl-guanine (*BEN*) modifications to the (3+1) G-quadruplex nano-scaffold. Modified sequences are shown in black. The *Native* reference sequence is shown in grey

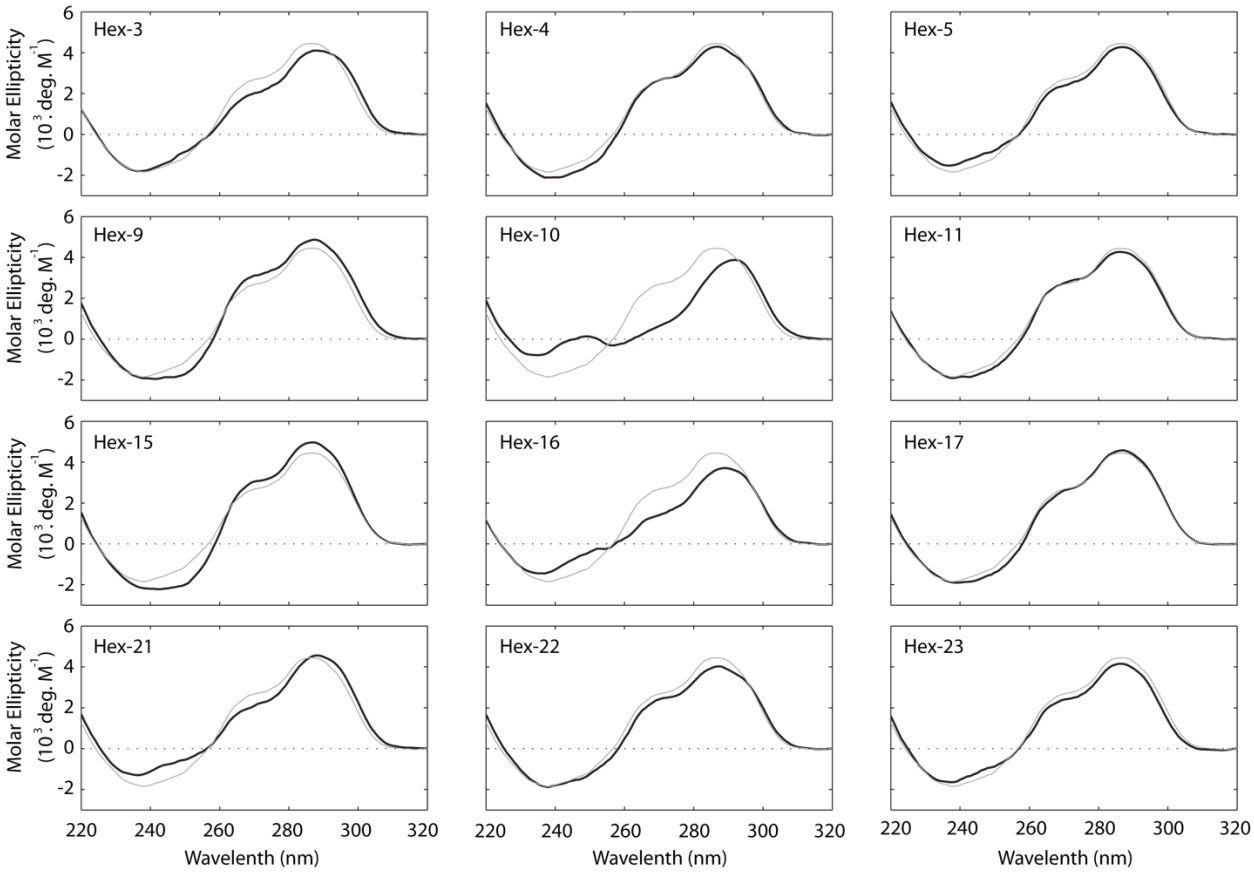
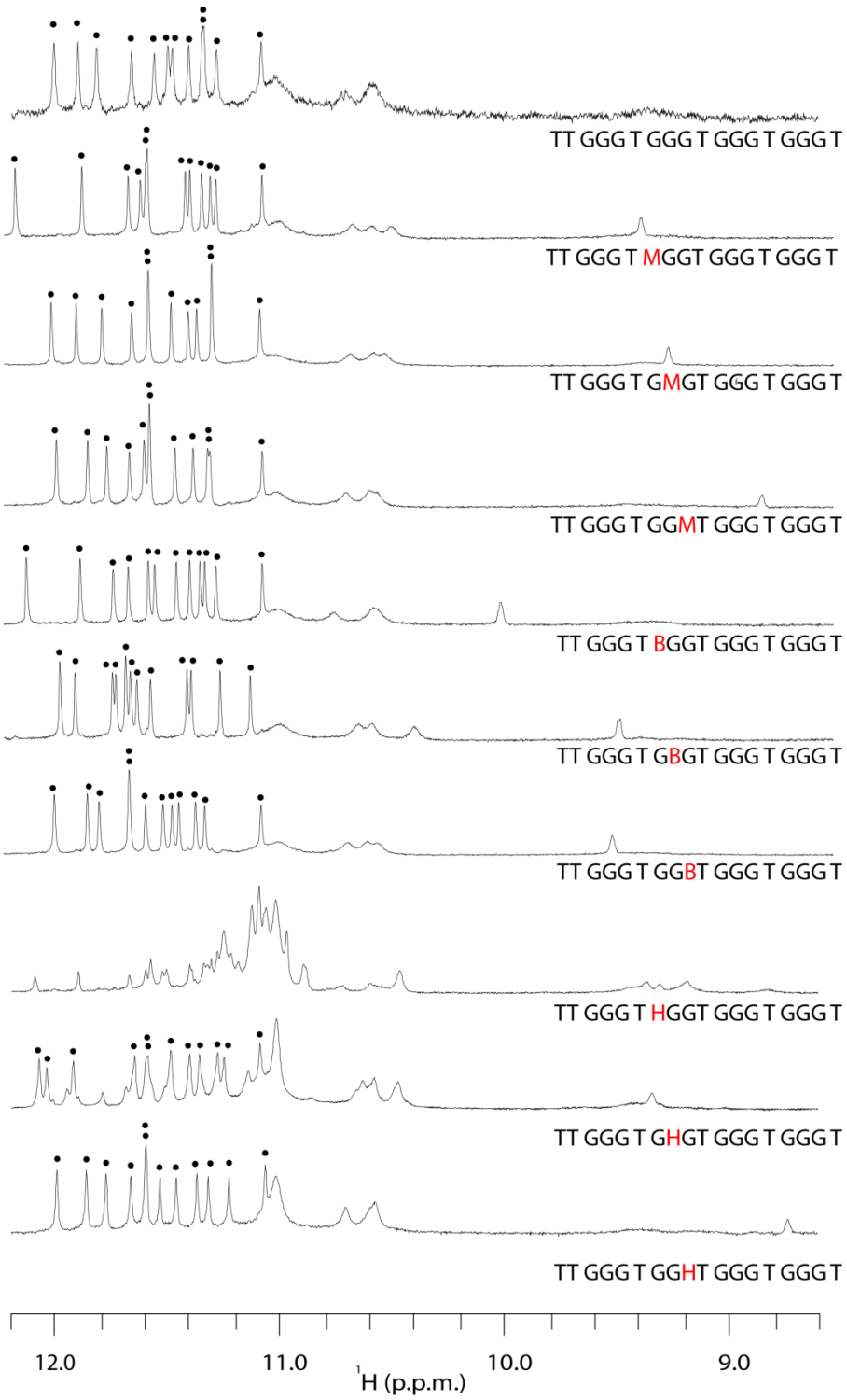


Figure S6. CD spectra of single N2-6-amino-hexyl-guanine (*HEX*) modifications to the (3+1) G-quadruplex nano-scaffold. Modified sequences are shown in black. The *Native* reference sequence is shown in grey



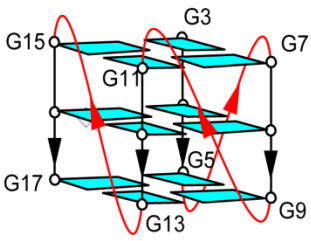


Figure S7. NMR spectra of single substations of N2-methyl-guanine (M), N2-benzyl-guanine (B), or N2-6-amino-hexyl-guanine (H) into the parallel G-quadruplex nano-scaffold formed by the d[$T_2(G_3T)_4$] sequence. Substitution position indicated in red. Black dots indicate imino proton resonances of the monomer G-quadruplex.

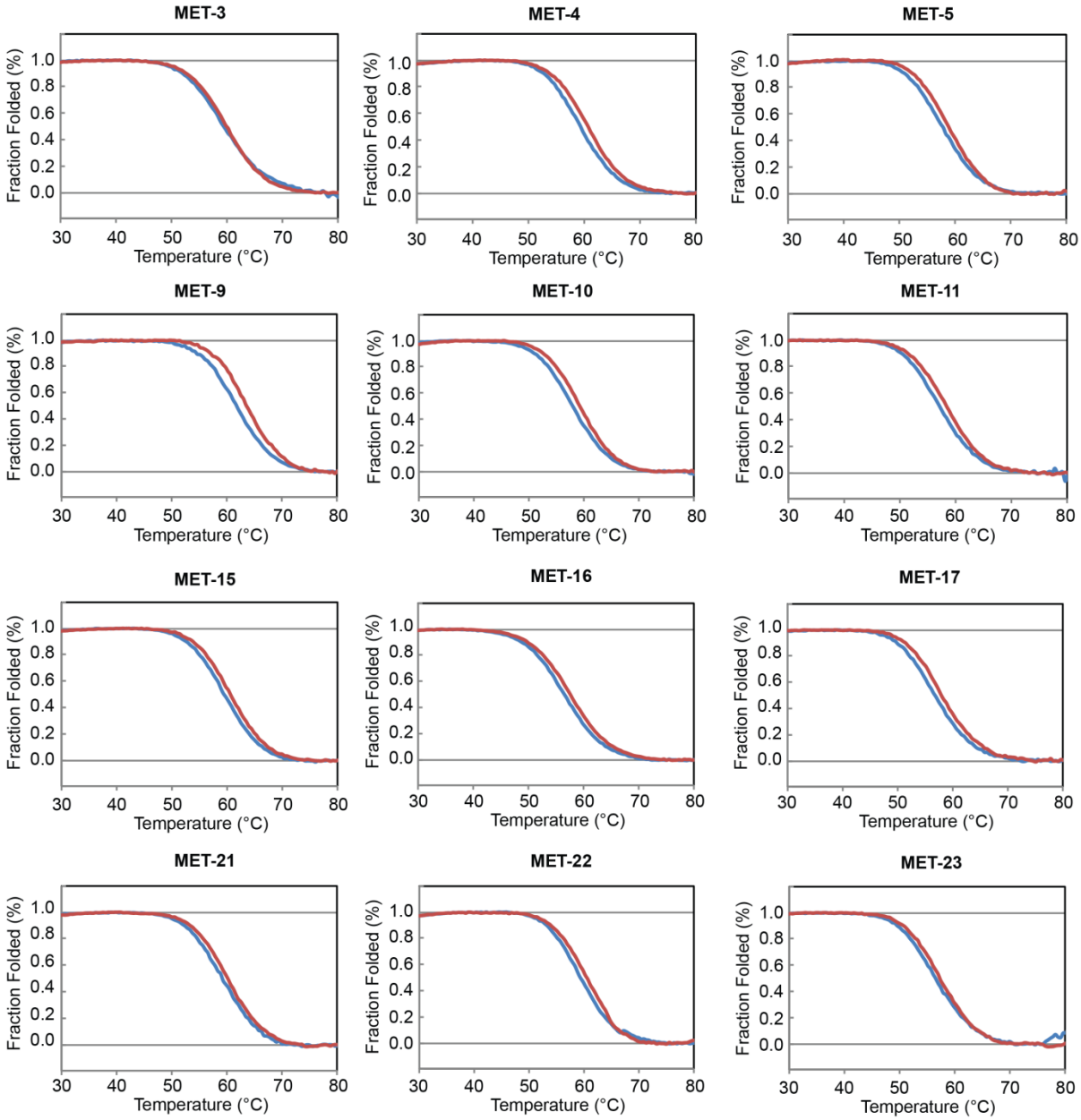


Figure S8. Fraction folded curves from UV melting experiments of modified (3+1) G-quadruplex forming sequences containing single MET substitutions

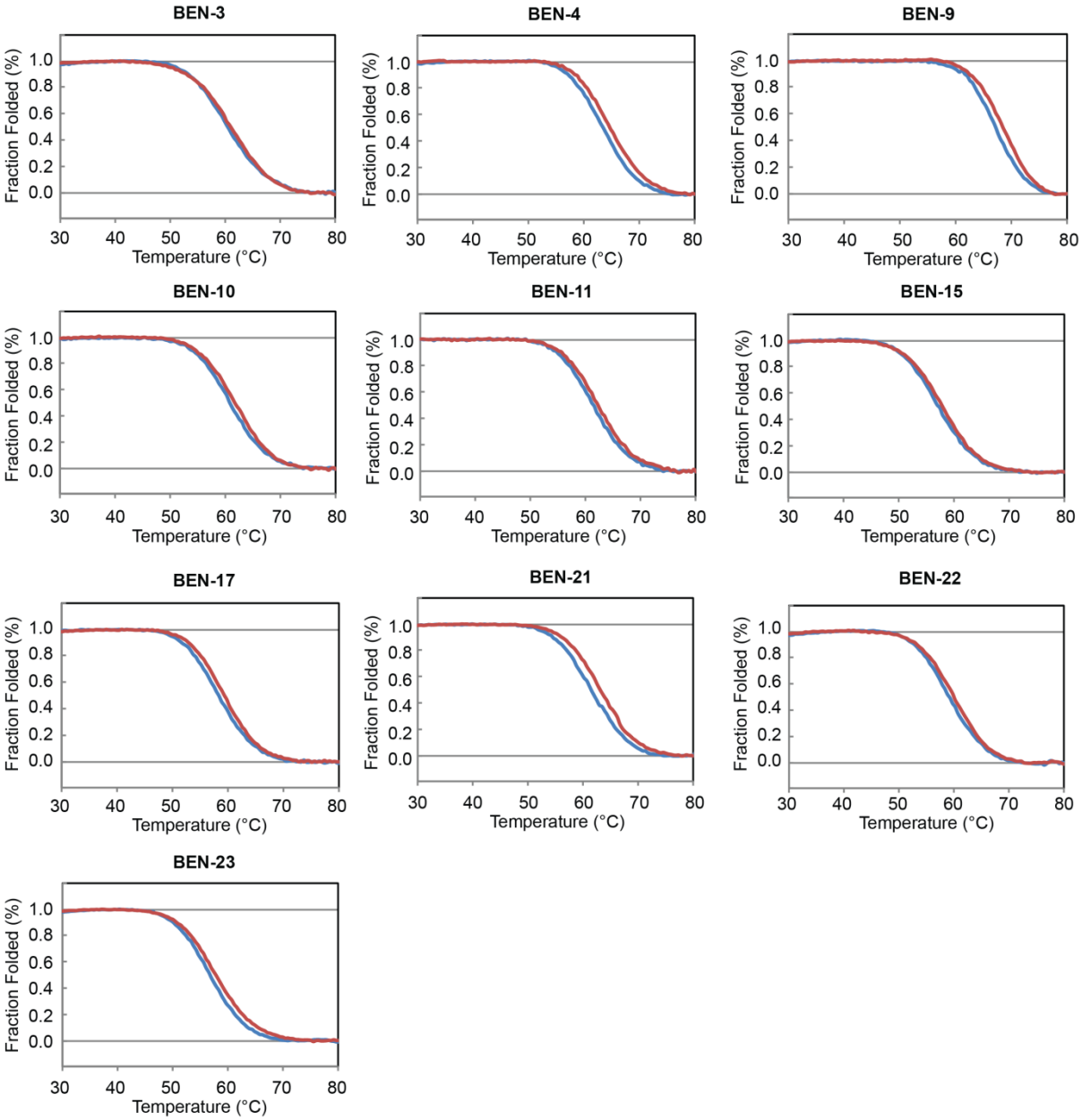


Figure S9. Fraction folded curves from UV melting experiments of modified (3+1) G-quadruplex forming sequences containing single BEN substitutions

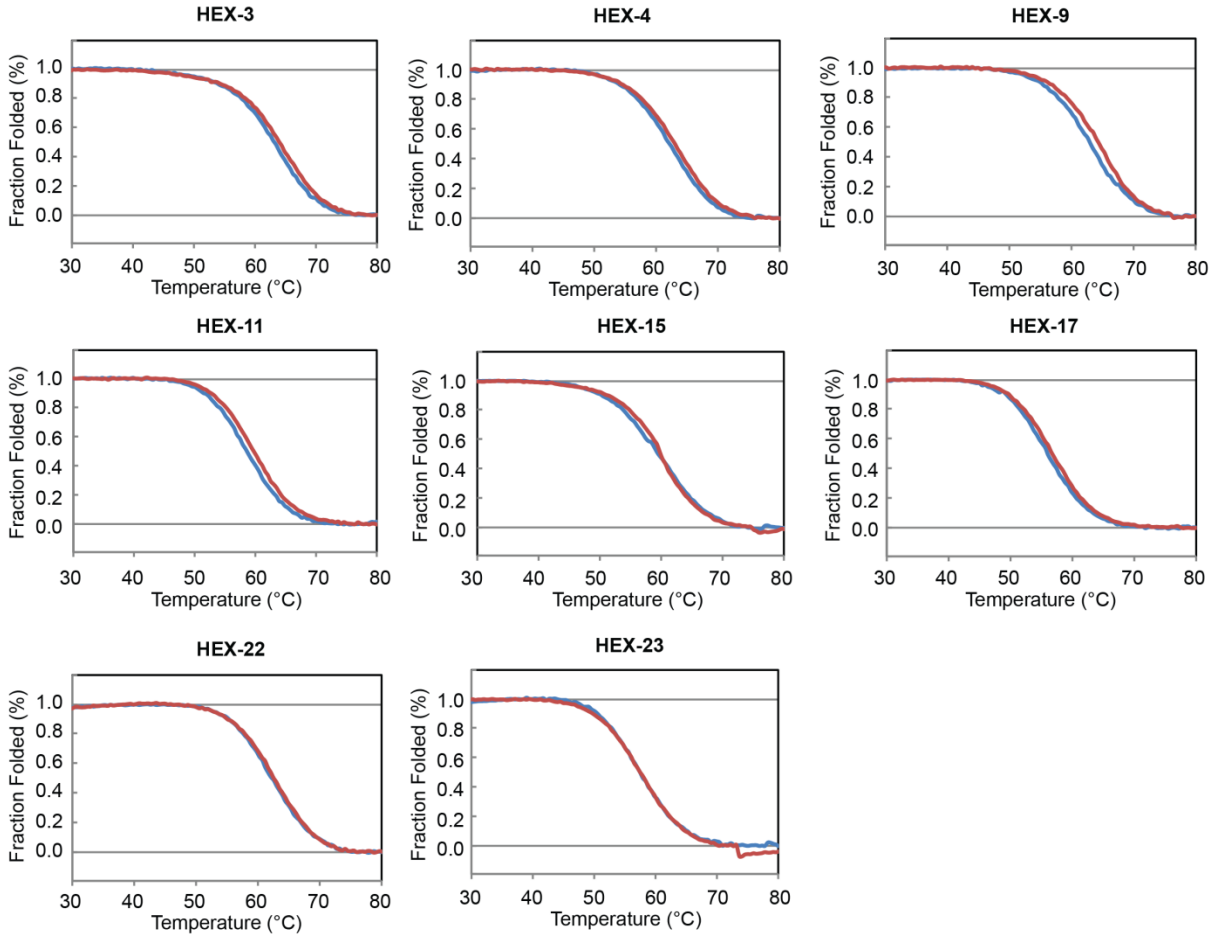


Figure S10. Fraction folded curves from UV melting experiments of modified (3+1) G-quadruplex forming sequences containing single HEX substitutions

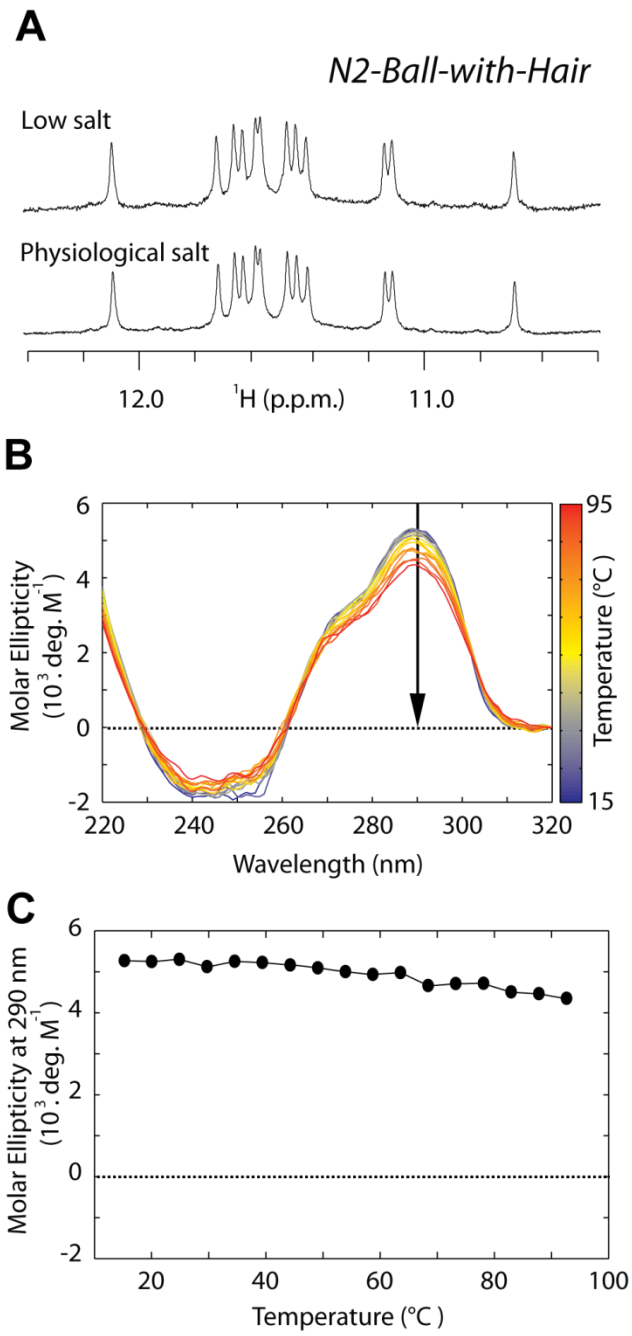


Figure S11. Design of a ^{Hex}G modified "Ball-with-hair" G-quadruplex nano-scaffold: **(A)** NMR spectra of N2-Ball-with-Hair sequence in "low" and "physiological" salt conditions shows the same structure is formed. **(B)** Temperature dependent CD spectra demonstrate the incredible stability of the "Ball-with-hair" nano-scaffold in physiological salt conditions. **(C)** Projection of CD intensity at 290 nm over varying temperatures.

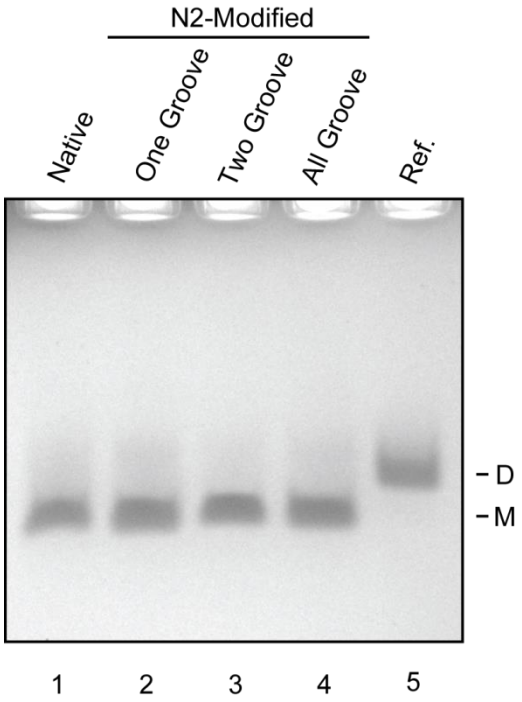


Figure S12. Non-denaturing PAGE investigating G-quadruplex nano-scaffold stoichiometry: (lane 1) *Native* sequence and known monomer, (lane 2) *N2-One groove*, (lane 3) *N2-Two Groove*, (lane 4) *N2-All Groove*, (lane 5) Reference sequence $d(G_3T)_4$ known to form a dimer G-quadruplex. Monomer (M) and Dimer (D) migration rates are indicated at right