Supporting Information for

α-2'-Deoxyguanosine Can Switch DNA G-Quadruplex Topologies from Antiparallel to Parallel

Jana Filitcheva,[1] Patrick J. B. Edwards,[1] Gillian E. Norris, [1,2] Vyacheslav V. Filichev[1,2]*

[1] School of Fundamental Sciences, Massey University, Private Bag 11 222, Palmerston North, New Zealand; [2] Maurice Wilkins Centre for Molecular Biodiscovery, Auckland 1142, New Zealand;

Fax: (+) 64 6 3505682

E-mail: v.filichev@massey.ac.nz

Table of Contents

Materials and Methods

Oligonucleotide Synthesis

Unmodified DNAs were purchased from Integrated DNA Technology (IDT, Singapore). Modified oligonucleotides (ONs) were synthesised with an Mer-Maid 4 automated DNA synthesiser from BioAutomation Corporation using 4,5-dicyanoimidazole (DCI) as an activator. Oxidation and deprotection times were set to 40 s and the activation time at 60 s for 1.0 µmol synthesis scale. For coupling of modified nucleotides, automated DNA synthesis was paused after the deblocking step. Modified phosphoramidites (α-2'-deoxyguanosine and α-thymidine from ChemGenes Corporation, Wilmington, USA) were dissolved in anhydrous acetonitrile (0.1 M solutions). Coupling time was extended for modified nucleosides to 5 min. All oligonucleotides were synthesised in DMT-on mode. Modified ONs were cleaved from the support using 32% NH_4OH (for standard supports) or using ammonia/LiCl (15 mg LiCl in 1 mL concentrated aqueous ammonia, ca 0.1N LiCl) for DMT-ribose universal supports (from Bioautomation) at 65 °C overnight. ONs were precipitated from acetone/$LiClO_4$ solutions (- 20 °C, 2 hours), then centrifuged for 20 min at 13000 rpm and supernatant was removed. Pellets were washed with acetone (2 × 500 µL), centrifuged and the supernatant was removed. Then, the rest of the solvent was evaporated at 50 °C for 15 min. The pellets were dissolved in H_2O (1 mL) and then purified using reverse-phase HPLC instrument (Dionex) using a C-18 column from Alltech with 250 mm length and 10 mm internal diameter. Buffer A [0.05 M TEAA in H_2O (pH = 7.0)] and buffer B (75 % acetonitrile, 25 % H_2O). Flow 2.5 mL min^{-1}. Gradients: 2 min 100 % buffer A, linear gradient to 100 % buffer B in 48 min, linear gradient to 100 % buffer A in 2 min, 100 % buffer A for 10 min. The corresponding UV-active fractions were freeze-dried. After purification, DMT-on-ONs were treated with 80 % aqueous AcOH (100 µL) for 20 min at +4 °C to remove 5′-*O*-DMT group. NaOAc (3 M, 50 µL) followed by EtOH (1 mL) were added and vortexed. Solutions were cooled to -18 °C for two hours in order to precipitate the ONs. Samples were centrifuged for 20 min at 13000 rpm and supernatant was removed. Pellets were washed with EtOH (2 × 500 µL), centrifuged and the supernatant was removed. Then, the rest of the solvent was evaporated at 50 °C for 15 min. 100 µL H_2O was added to dissolve the ONs. Purities of ONs were confirmed by denaturing gel electrophoresis using 20 % polyacrylamide gel (0.75 mm thickness, 19:1 acrylamide/bisacrylamide ratio) and found to be more than 90 % pure. Gels were prepared in 1× TBE buffer (100 mM Tris, 90 mM boric acid, and 10 mM EDTA) under denaturing conditions (7 M urea). ONs were loaded onto gels after pre-incubation at 90 °C for 10 min. ESI-MS data (Table S1) confirmed composition of modified DNA.

Table S1. ESI-MS data of modified sequences synthesised.

DNA sequence, 5'-3'	Monomer molecular weight, Da	Calculated species	Calculated, m/z	Found, m/z (charge)
α-G_4β-T_4	2471.4	$[(\alpha\text{-}G_4\beta\text{-}T_4) - 5H]^{5-}$	822.0	822.5 (3-)
β-G_4α-T_4β-G_4	3787.6	$[(\beta\text{-}G_4\alpha\text{-}T_4\beta\text{-}G_4)_2 - 5H]^{5-}$	1261.5	1261.5 (3-)
α-G_4β-T_4α-G_4	3787.6	$[(\alpha\text{-}G_4\beta\text{-}T_4\alpha\text{-}G_4)_2 + 4NH_4 - 9H]^{5-}$	1527.6	1528.9 (5-)
α-($G_4T_4G_4$)	3787.6	$[(\alpha\text{-}G_4\beta\text{-}T_4\alpha\text{-}G_4)_1 - 4H]^{4-}$	945.9	945.9 (4-)
α-G_3T[a)]	5408.9	$[(G4\text{-}DNA)_1 + 2NH_4 - 6H]^{4-}$	1359.7	1359.7 (4-)
α-G_3T_2	6321.0	$[(G4\text{-}DNA)_1 + 2NH_4 - 7H]^{5-}$	1270.0	1270.0 (5-)
α-G_3T_3	7234.1	$[(G4\text{-}DNA)_1 + 2NH_4 - 7H]^{5-}$	1452.6	1452.6 (5-)
α-G_3T_4	7234.1	$[(G4\text{-}DNA)_1 + 3NH_4 - 8H]^{5-}$	1638.5	1639.5 (5-)

a) DNA sequences for α-G_3T_n (n=1-4) are provided in Table 2 in the main text. ESI-MS spectra were recorded and analysed as described in the section below.

Polyacrylamide Gel Electrophoresis

For non-denaturing 20 % PAGE, gels were prepared in 20 mM Tris (pH 7.2), 5 mM $MgCl_2$, 1 mM KCl and 99 mM NaCl, the same buffer was used as a running buffer with 0.75 mm thickness, 19:1 acrylamide/bisacrylamide ratio. ONs were prepared at 100 µM strand concentration in the running buffer, heated at 90 °C for 10 min before cooling down and incubating at 4 °C overnight. All gel electrophoresis was performed at room temperature. After the electrophoresis, gels were stained with 5 % Stains-All® in 50 % water/formamide for 5–10 min and then destained in H_2O until complete washing of the dye from the gel background occurred.

UV-Vis Spectroscopy

UV-Vis spectroscopy was performed using Cary 100Bio UV-Vis spectrometer using quartz cuvettes with 1 cm pathlength and a 2 × 6 multicell block with a Peltier temperature controller.

Circular Dichroism (CD) Spectroscopy

CD spectra were recorded using a Chirascan CD spectrophotometer (150 W Xe arc) from Applied Photophysics with a Quantum Northwest TC125 temperature controller. CD spectra (average of at least 3 scans) were recorded between 210 and 350 nm with 1 nm intervals, 120 nm/min scan rate followed by subtraction of a background spectrum (buffer only). CD spectra of G-quadruplexes were recorded at 5 µM strand concentration in 20 mM Tris (pH 7.2), 5 mM $MgCl_2$, 1 mM KCl and 99 mM NaCl (CD buffer, 1 cm pathlength cuvette). CD spectra of undiluted ESI-MS and NMR samples were recorded in 0.1 mm pathlength cuvettes. The absorbance of DNA samples at 260 nm was kept below 1.0 a.u.. For CD melting experiments samples in CD buffer were heated from 5 to 90 °C in a 5 ° step with equilibration time 5 min for each temperature. CD signals at 265 or 295 nm were converted to *f*, fraction folded. At 5 °C samples were assumed to be fully folded (*f*, fraction folded = 1) and at 90 °C fully unfolded ($f = 0$). The melting temperatures (T_m [°C]) were determined upon plotting *f* versus temperature and at $f = 0.5$, $T_m = T$.

ESI-mass Spectrometry

G4-DNA samples were annealed at 100 µM concentration in 150 mM ammonium acetate buffer (40 µL, pH 7.0). Samples were left at +4 °C for 5 days. Each sample was diluted to 10-20 µM concentration in HPLC-grade water or 150 mM ammonium acetate buffer (α-G_4 β-T_4 α-G_4 sample) and just before an injection samples were further diluted with 20% MeOH. Each diluted sample (5 µL) was injected into a Dionex Ultimate 3000 analytical liquid chromatography system delivering methanol at a flow rate of 100 µL min^{-1} in bypass mode. The LC eluent was directly infused into a Thermo Scientific Q Exactive Focus Hybrid Quadrupole-Orbitrap Mass Spectrometer. Ions generated by ESI operated at a sheath flow of 15 (arbitrary units), auxiliary gas flow rate of 8, auxiliary gas

temperature of 250 °C, and a capillary temperature of 320 °C were analysed in negative ion mode with a capillary voltage of 3,800 V.

Total ion count (TIC) was recorded in profile mode over the m/z range of 200-2,000 or 500 – 3,000 and analysed using Thermo Xcalibur (4.0.27.19) Qual Browser.

NMR Spectroscopy of Oligonucleotides

NMR spectroscopy of G-quadruplexes were performed on 700 MHz Bruker Avance spectrometer equipped with a cryoprobe, and processed with Topspin software (v. 2.1.8). ONs were heated at 90 °C for 5 min in 10 % D_2O, 10 mM Na^+ phosphate buffer, pH 7.0 and then slowly cooled down to room temperature and incubated overnight at 20 °C prior to NMR spectroscopy. Trimethylsilyl propionate (TSP, 25 µM) was used as internal standard in ^1H NMR (δ = 0.003 ppm). ONs concentrations were 1.2 mM for β-($G_4T_4G_4$) (Fig. 3A), 500 µM for α- G_4 β-T_4 (Fig. 3B), 750 µM for α- G_4 β-T_4 α- G_4 (Fig. 3C), 250 µM for α-G_3 T (Fig. 5A), 150 µM for α-G_3 T_2 (Fig. 5B) and 100 µM for α-G_3 T_3 (Fig. 5C).

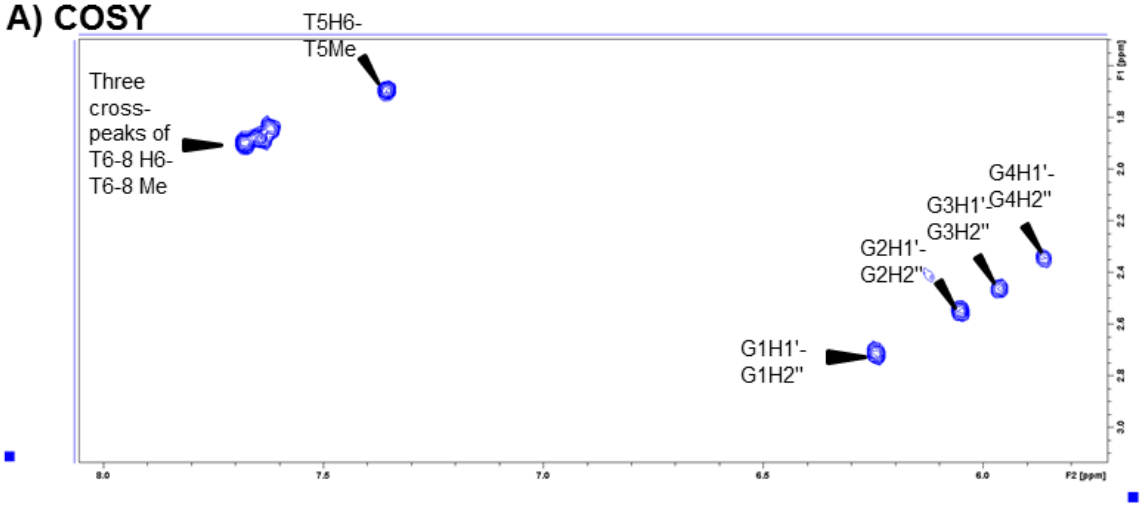
2D ^1H-^1H TOCSY, NOESY and ROESY NMR spectra were recorded at 308 K using standard, gradient-enhanced pulse sequences. Spectra were recorded using 2048 acquisition points and 400 increments using a spectral width of 10.5 kHz in both dimensions. Mixing times were typically 90 ms for TOCSY, 100 ms for NOESY and 200 ms for ROESY. Water suppression was performed using excitation sculpting. Each acquisition was followed with a 1.5 s recycle delay. Spectra were processed using standard parameters, including forward linear prediction.

Selected data for complexes studied

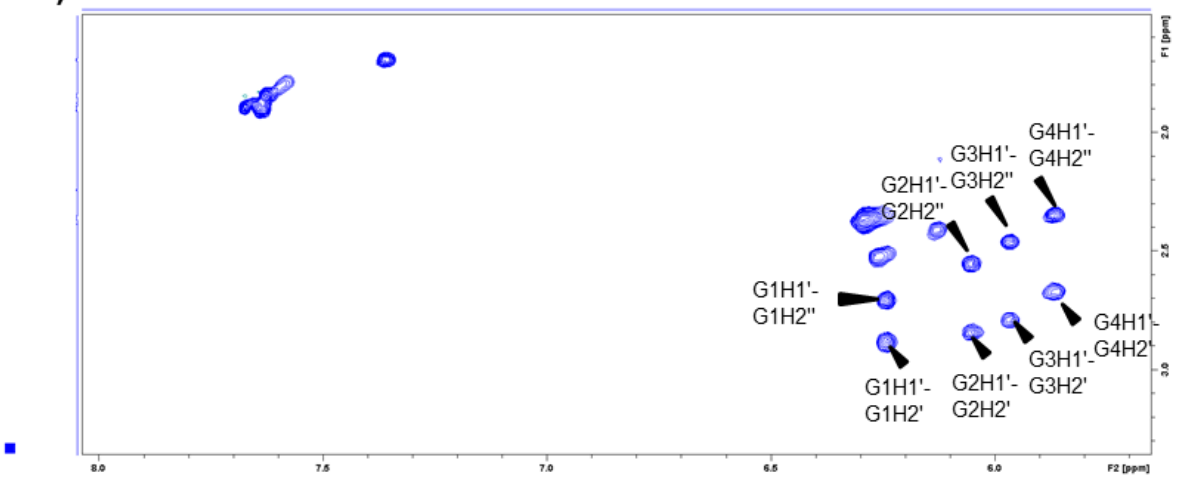
Description of NMR assignment and selected data for a tetramolecular complex formed by α-G_4β-T_4

Assignment of ^{1}H NMR spectrum of α-2'-deoxyguanosine was reported in the past using 2D NMR spectra.[1] For G4 DNA formed by α-G_4β-T_4, the number of thymine spin systems (four) observed in COSY (Figure S1A) is consistent with the formation of a single symmetrical complex. Four H1'-H2" cross-peaks were detected for each α-dG in COSY (see Figure 3C in the main text for numbering). Analysis of TOCSY spectrum (Figure S1B) revealed pairs of H1'-H2' and H1'-H2" for each α-dG. Additional peaks marked with an asterisk appeared in ROESY spectrum (Figure S1C), which serve as a starting point in establishment of the 'sequential walk' between α-2'-deoxyguanosines reported in Figure 3D in the main text. These cross-peaks were assigned as H1'(n)–H2"(n)–H1'(n+1), where n is a number of the nucleotides in the sequence counting from 5' to 3'-end. The ROE cross-peaks that correspond to H1'-H2' are more intense than the H1'-H2" cross-peaks, which is expected for α-dG and similar trend for NOE cross-peaks was reported in the past.[1] Moreover, ROE cross-peaks between H8 and H2" were detected for each α-2'-deoxyguanosine, which was previously observed for a free nucleoside in solution.[1] This supports a notion that α-2'-deoxyguanosines adopt *anti*- and not *syn*-configuration in this G4-DNA. The sequential assignment is supported by the fact that the H2" of 4th α-dG has a cross-peak in ROESY with H6 of the 5th dT (which is a β-nucleotide). Assignment of H3' and H4' signals was performed based on ROESY spectrum and reported in Table S2. Assignment of some signals in ^{13}C-NMR spectrum was performed with the help of ^{1}H-^{13}C HSQC (Figure S1D).

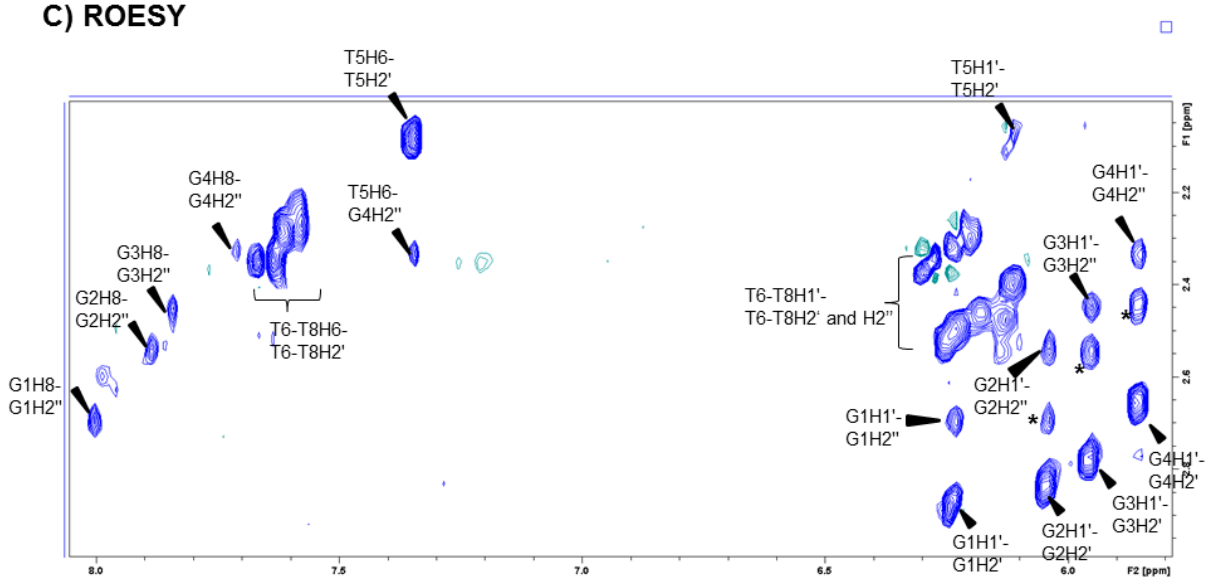
A) COSY



B) TOCSY



C) ROESY



D) HSQC

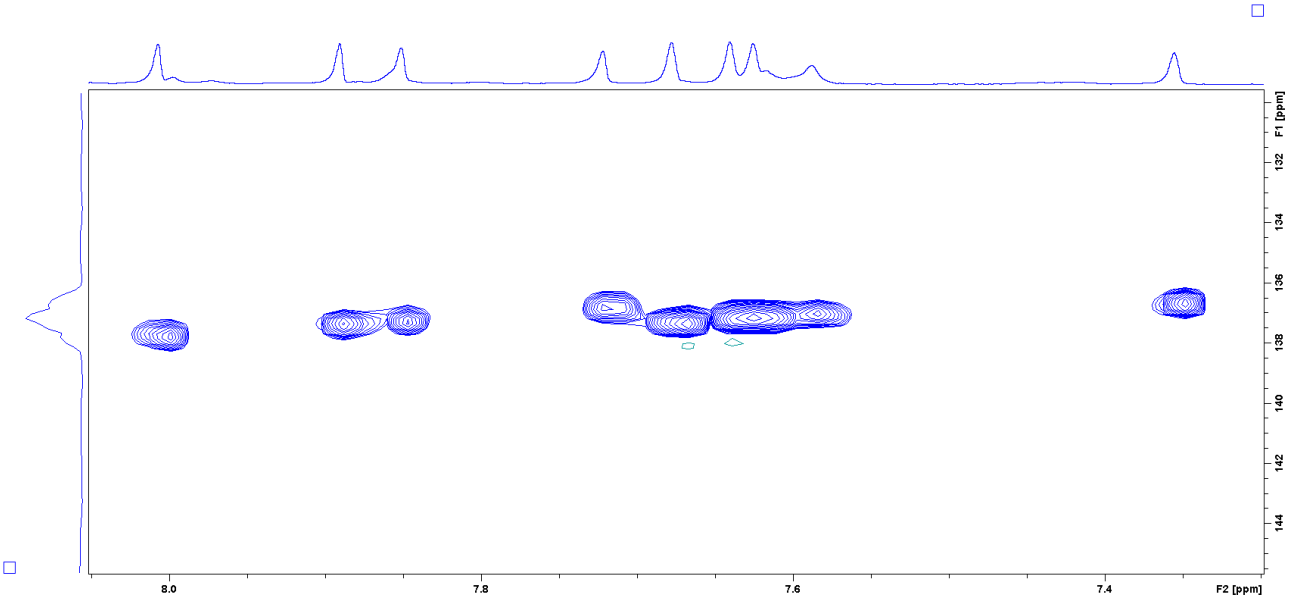


Figure S1. 2D NMR spectra of G4-DNA formed by α-G_4 β-T_4: A) Long-range COSY NMR spectrum showing correlations between CH_3 and H6 of thymines (four cross peaks) as well as four H1'-H2" cross-peaks each α-dG; B) TOCSY spectrum showing pairs of H1'-H2' and H1'-H2" for each α-dG; C) ROESY spectrum showing cross-peaks marked with an asteric used for establishment of the 'sequential walk' between α-dGs; D) ^{1}H–^{13}C HSQC NMR spectrum showing H6/H8-C6/C8 correlations. Note that all ^{13}C signals of C6/C8 are at around 137 ppm (see Table S2).

Table S2. ^{1}H and ^{13}C chemical shifts δ (in ppm) of protons and carbons of a tetramolecular complex formed by α-G_4 β-T_4.[a]

	H1'	H2'	H2"	H3'	H4'	H8/H6	H5'/H5"[b]	CH_3[c]	C8/C6	C1'	C2'
α-G1	6.24	2.88	2.70	4.83	4.62	8.01	3.74/3.9		137.73	84.77	38.67
α-G2	6.05	2.83	2.55	4.88	4.66	7.89	3.86/3.95		137.36	85.10	39.13
α-G3	5.96	2.79	2.46	4.88	4.62	7.86	4.08/4.25		137.28	85.28	39.50
α-G4	5.87	2.66	2.34	4.88	4.55	7.72	3.63/3.86		136.82	85.77	40.25
β-T5	6.12	2.09	2.33	4.79	n.a.	7.36	n.a.	1.69	136.68	84.46	37.89
β-T6	6.21	2.30	n.a.	n.a.	n.a.	7.62	n.a.	1.83	137.15	84.77	38.80
β-T7-T8	6.25 6.29	2.35 2.36	n.a.	n.a.	n.a.	7.64 7.68	n.a.	1.87 1.89	137.15 137.33	84.88 84.88	n.a.

[a] At 35 °C in 90% H_2O/10% D_2O, 10 mM sodium phosphate buffer, pH 7.0; H2' and H2" are *trans* and *cis* to the 3'-O-P, respectively; n.a. stands for not assigned.

[b] no stereospecific assignments.

[c] All CH_3 carbons have chemical shift of 11.61 ppm in ^{13}C NMR.

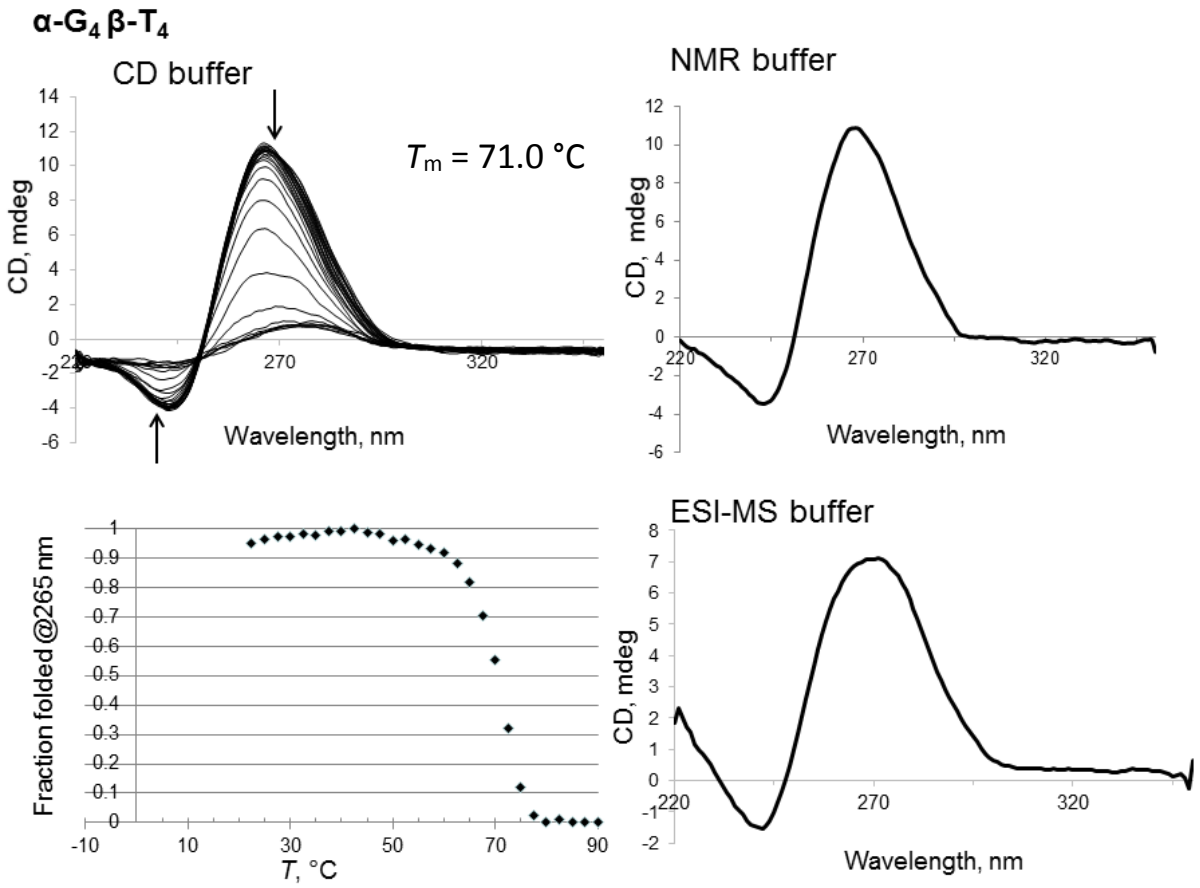


Figure S2. CD spectra of α-G_4 β-T_4 complex recorded in different buffers and its temperature denaturation profile (bottom left). CD thermal denaturation was performed from 20 to 90 °C. The arrow indicates direction of changes in the peak intensity from low to high temperatures. CD spectra in NMR and ESI-MS buffers were recorded at 20 °C.

Data for G4-DNAs formed by β- and α-modified $G_4T_4G_4$

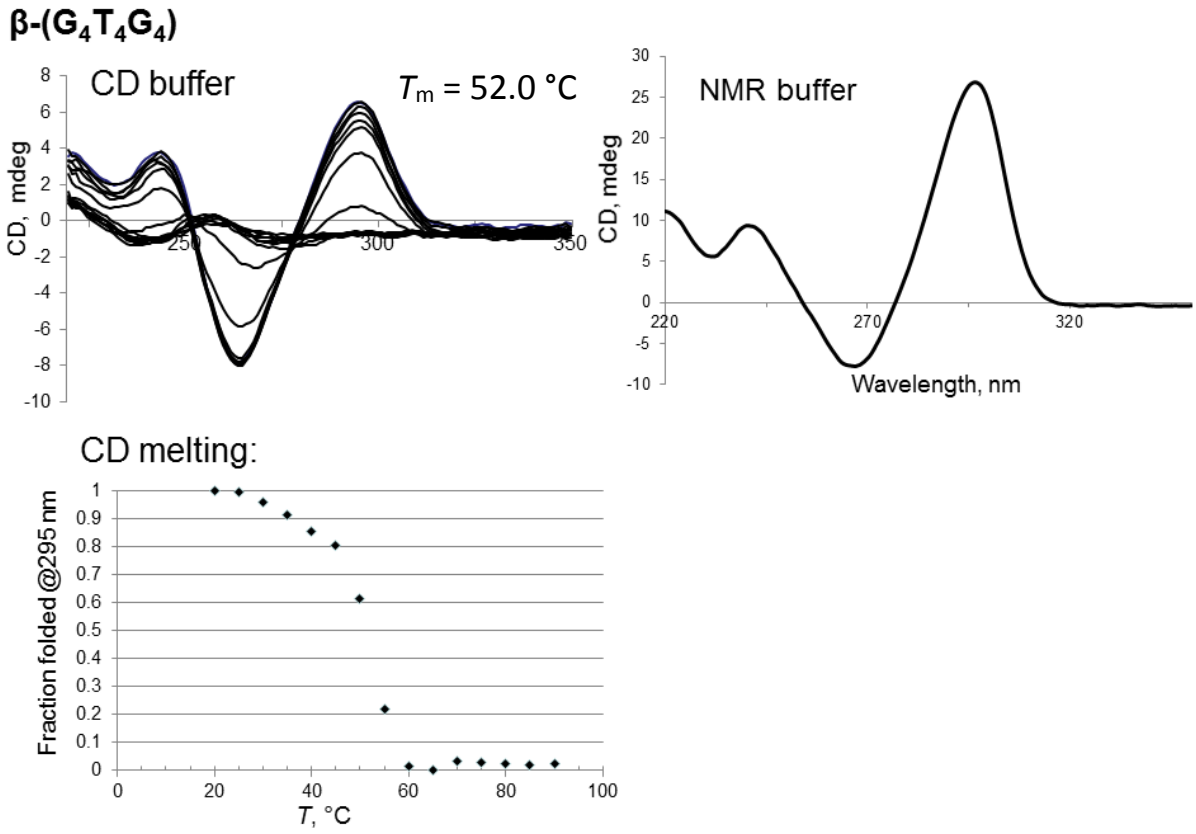


Figure S3. CD spectra of β-G_4T_4 G_4 complex recorded in different buffers and its temperature denaturation profile (bottom left). CD thermal denaturation was performed from 20 to 90 °C. The arrow indicates direction of changes in the peak intensity from low to high temperatures. CD spectra in NMR and ESI-MS buffers were recorded at 20 °C.

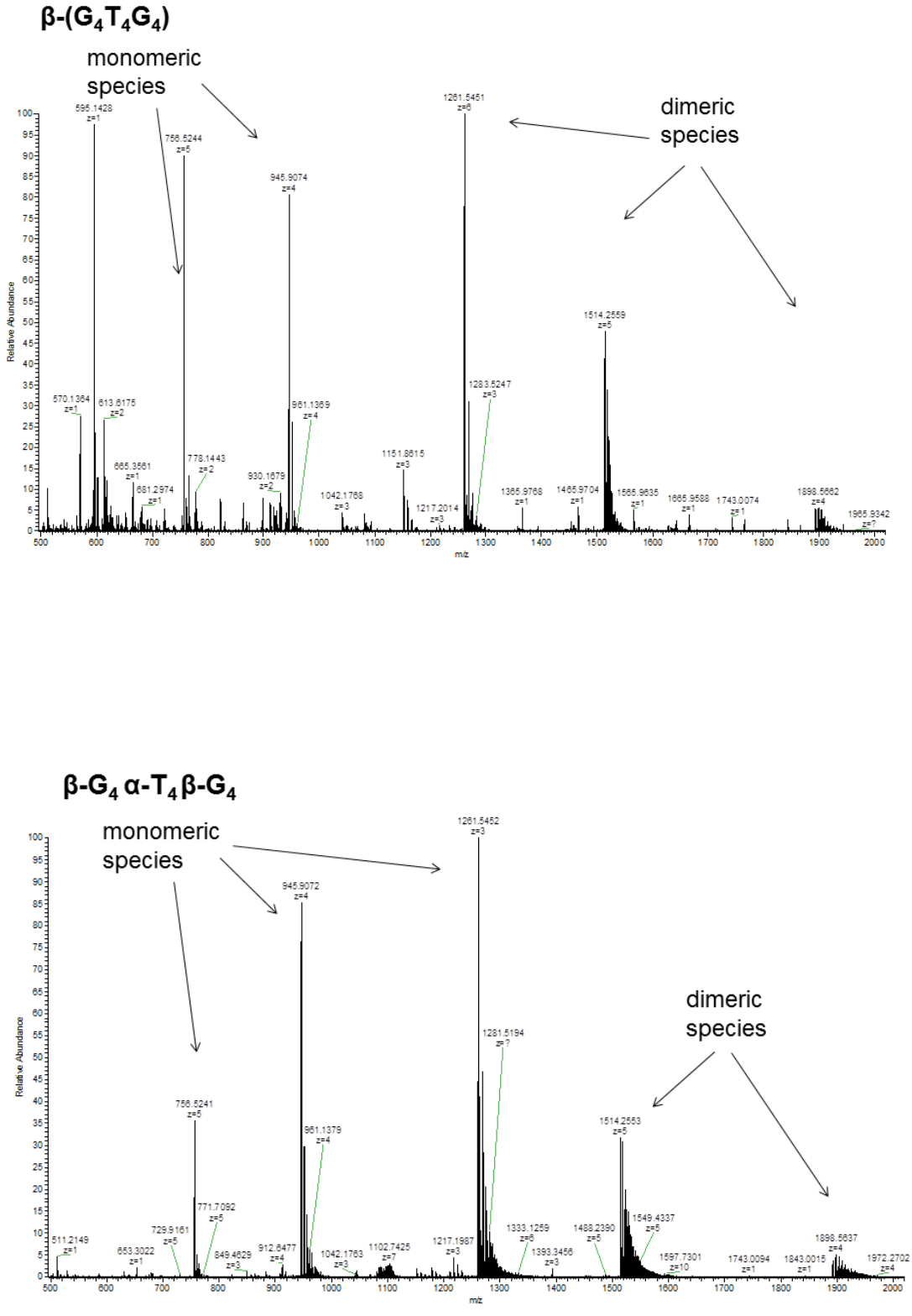


Figure S4. ESI-MS of β-($G_4T_4G_4$) and β-G_4 α-T_4 β-G_4.

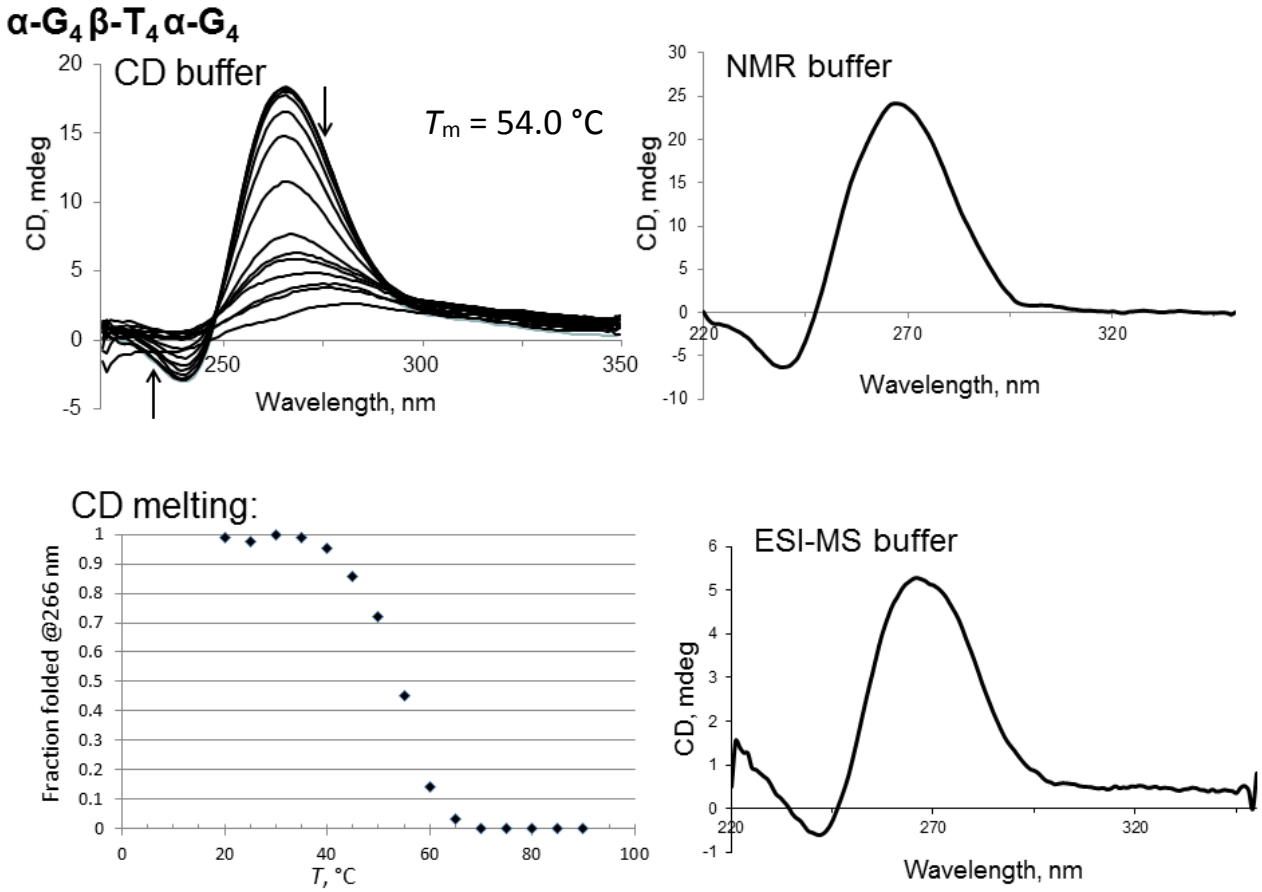


Figure S5. CD spectra of α-G_4 β-T_4 α-G_4 complex recorded in different buffers and its temperature denaturation profile (bottom left). CD thermal denaturation was performed from 20 to 90 °C. The arrow indicates direction of changes in the peak intensity from low to high temperatures. CD spectra in NMR and ESI-MS buffers were recorded at 20 °C.

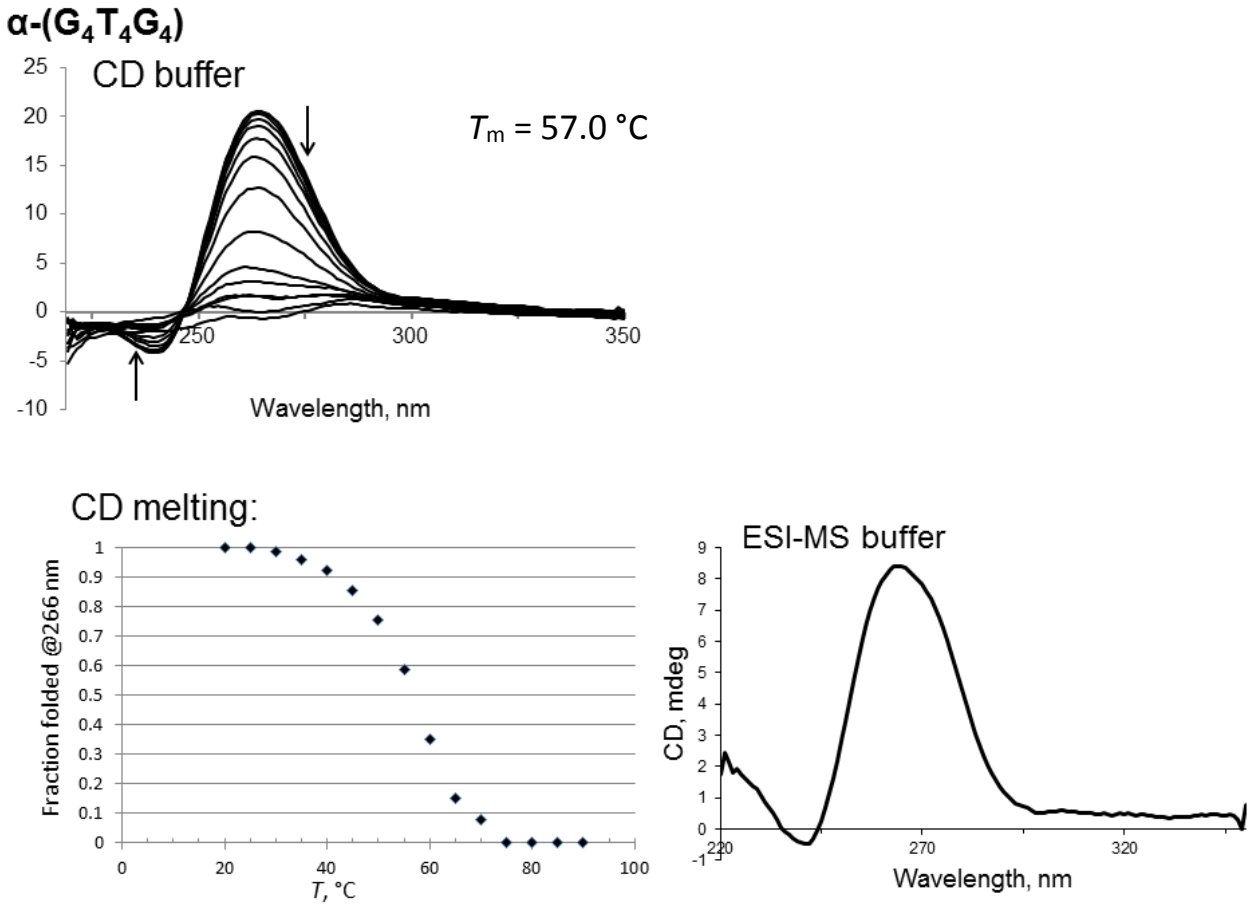
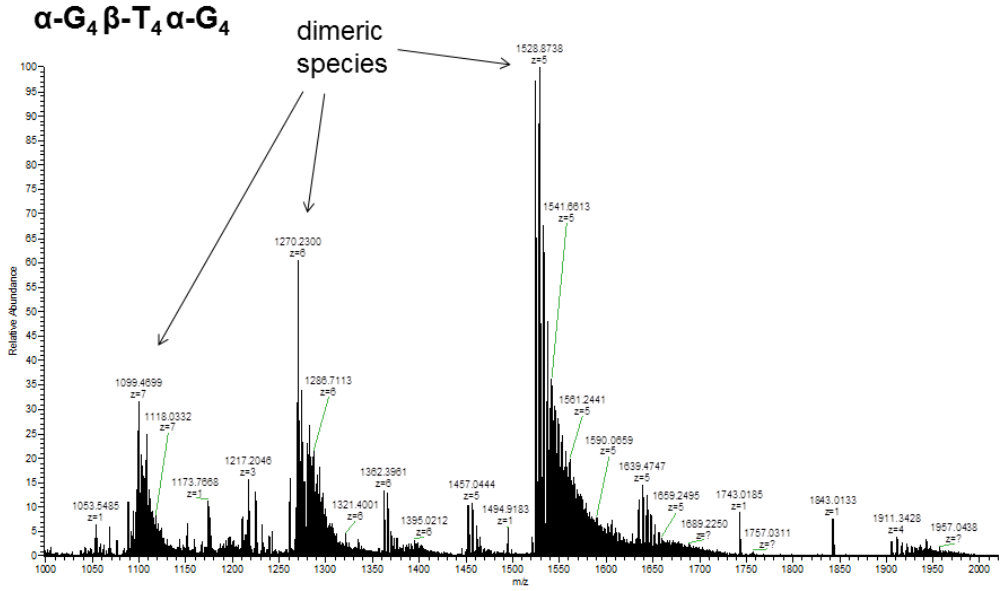


Figure S6. CD spectra of α-(G_4 T_4 G_4) complex recorded in different buffers and its temperature denaturation profile (bottom left). CD thermal denaturation was performed from 20 to 90 °C. The arrow indicates direction of changes in the peak intensity from low to high temperatures. CD spectra in NMR and ESI-MS buffers were recorded at 20 °C.



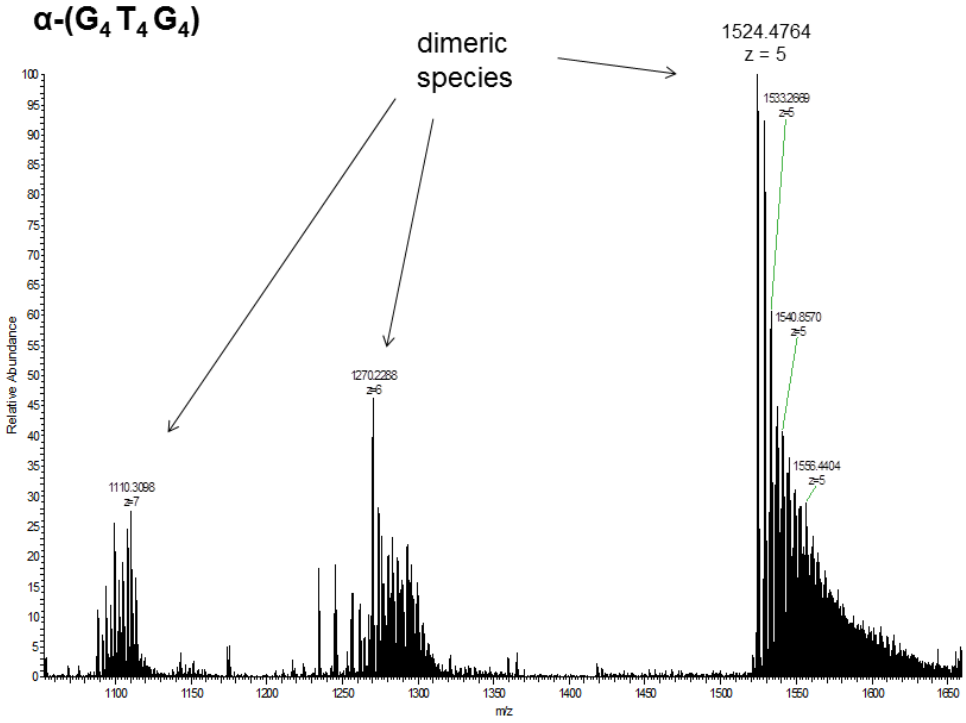


Figure S7. ESI-MS spectra for α-G_4 β-T_4 α-G_4 and α-($G_4T_4G_4$).

Data for G4-DNAs formed by β-modified (native) $dT(G_3T_n)_3G_3T$ sequences with various number of β-T in the loops (n = 1-4)

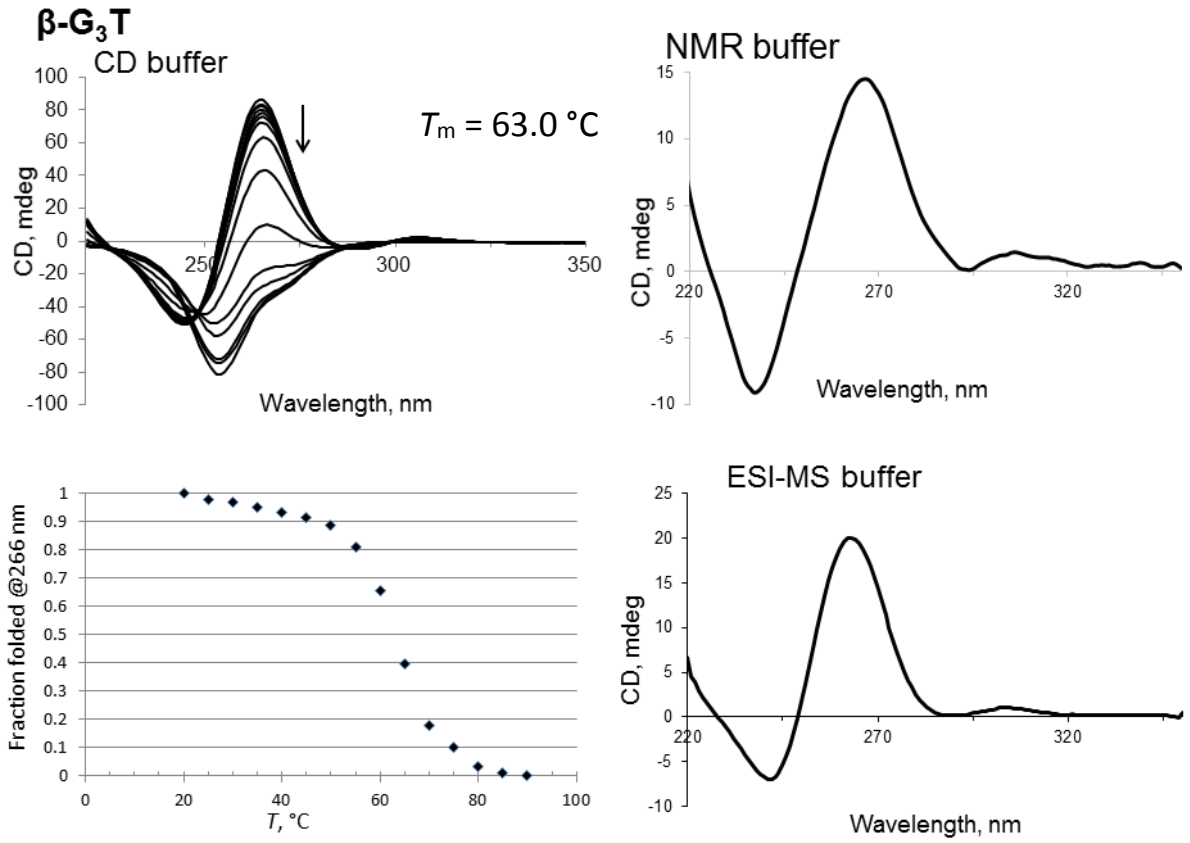


Figure S8. CD spectra of β-G_3 T complex recorded in different buffers and its temperature denaturation profile (bottom left). CD thermal denaturation was performed from 20 to 90 °C. The arrow indicates direction of changes in the peak intensity from low to high temperatures. CD spectra in NMR and ESI-MS buffers were recorded at 20 °C.

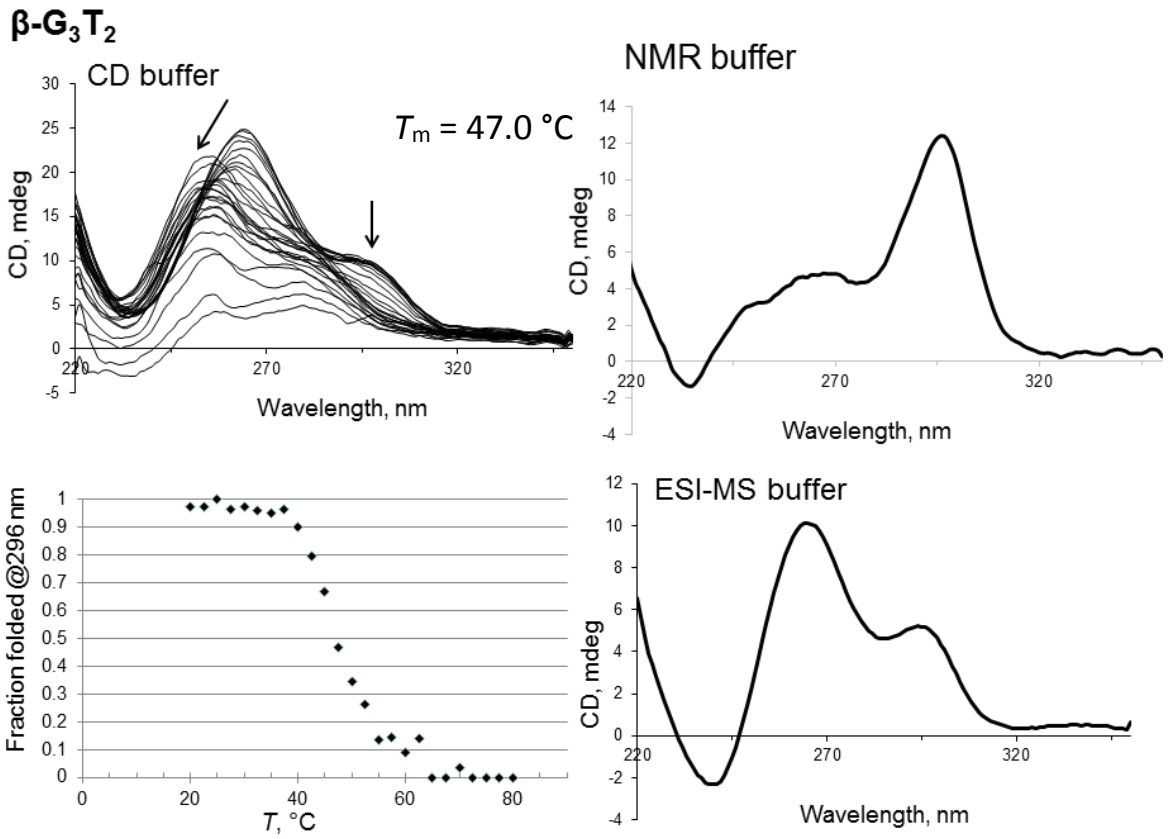


Figure S9. CD spectra of β-G_3 T_2 complex recorded in different buffers and its temperature denaturation profile (bottom left). CD thermal denaturation was performed from 20 to 90 °C. The arrow indicates direction of changes in the peak intensity from low to high temperatures. CD spectra in NMR and ESI-MS buffers were recorded at 20 °C.

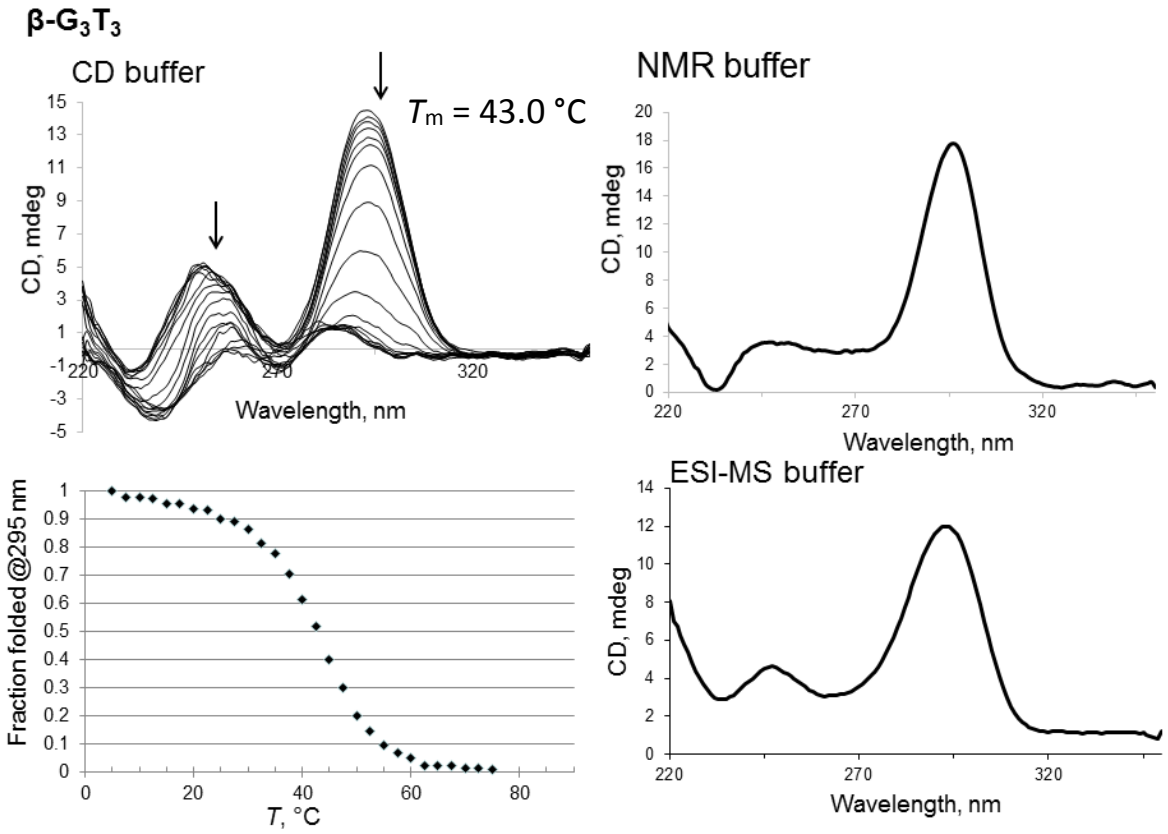


Figure S10. CD spectra of β-G_3 T_3 complex recorded in different buffers and its temperature denaturation profile (bottom left). CD thermal denaturation was performed from 5 to 75 °C. The arrow indicates direction of changes in the peak intensity from low to high temperatures. CD spectra in NMR and ESI-MS buffers were recorded at 20 °C.

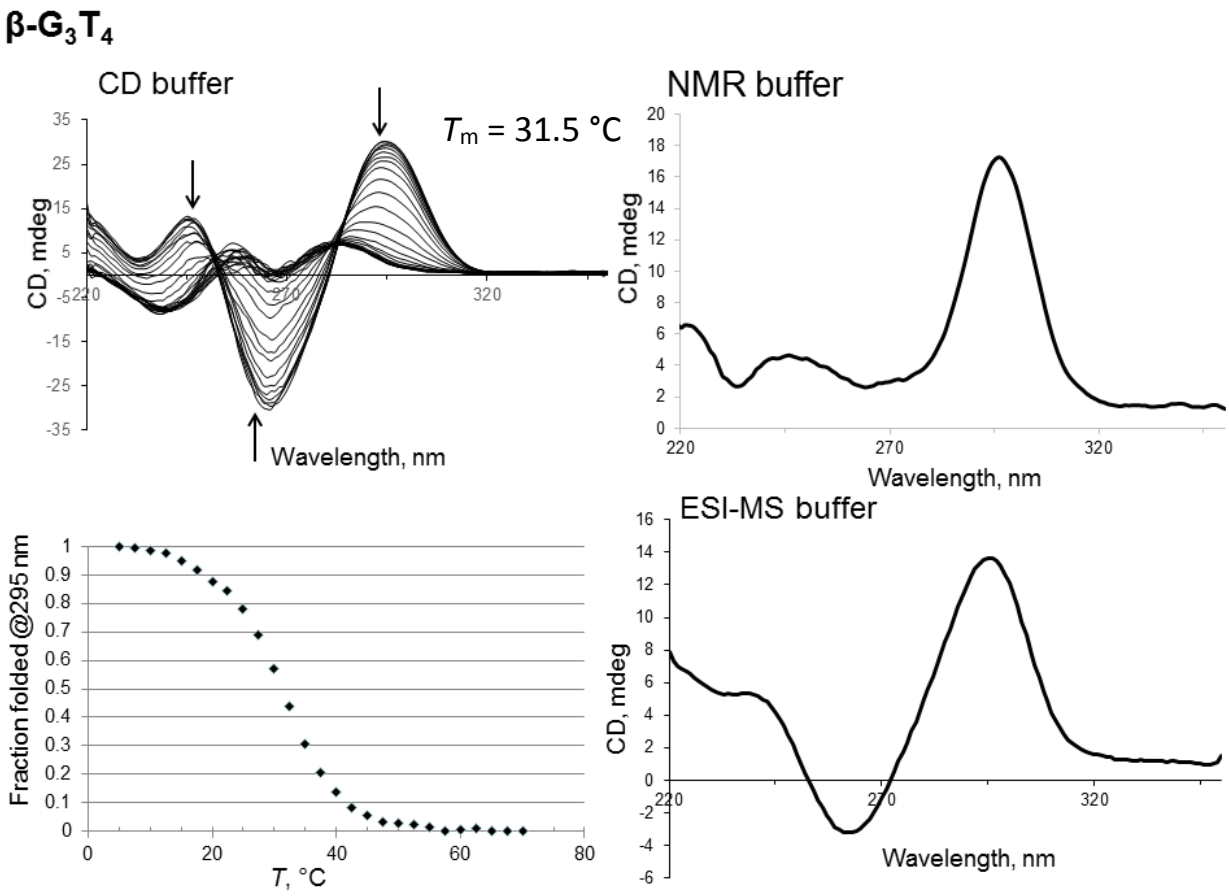
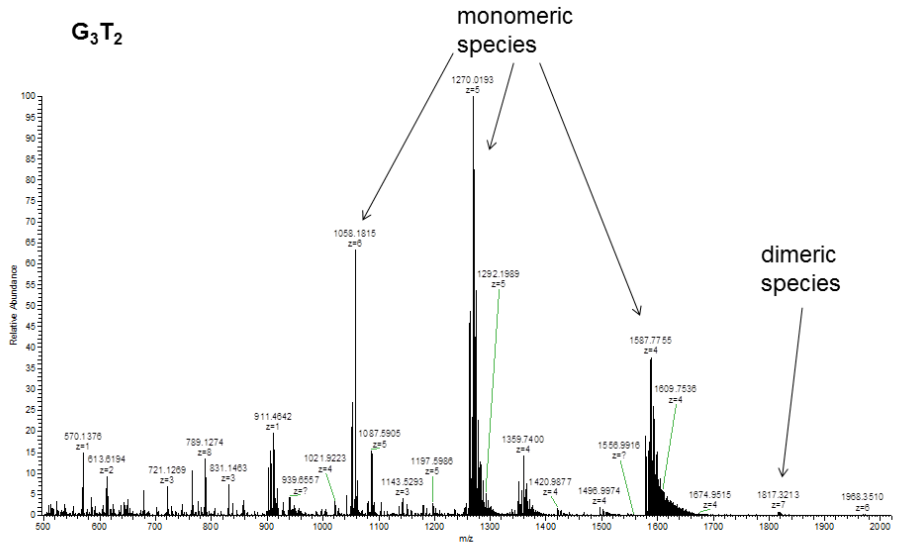


Figure S11. CD spectra of β-G_3 T_4 complex recorded in different buffers and its temperature denaturation profile (bottom left). CD thermal denaturation was performed from 5 to 70 °C. The arrow indicates direction of changes in the peak intensity from low to high temperatures. CD spectra in NMR and ESI-MS buffers were recorded at 20 °C.



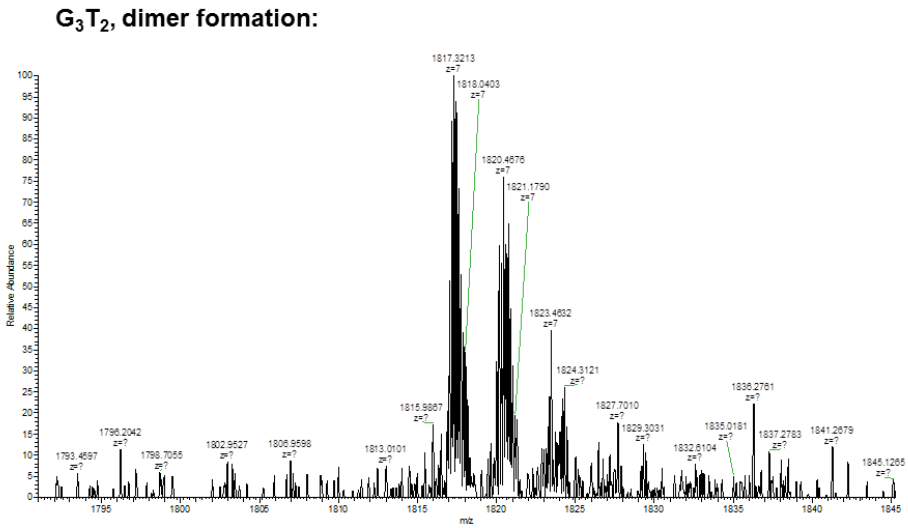


Figure S12. ESI-MS spectra for β- G_3T_2.

Data for G4-DNAs formed by α-modified $dT(G_3T_n)_3G_3T$ sequences with various number of β-T in the loops (n = 1-4)

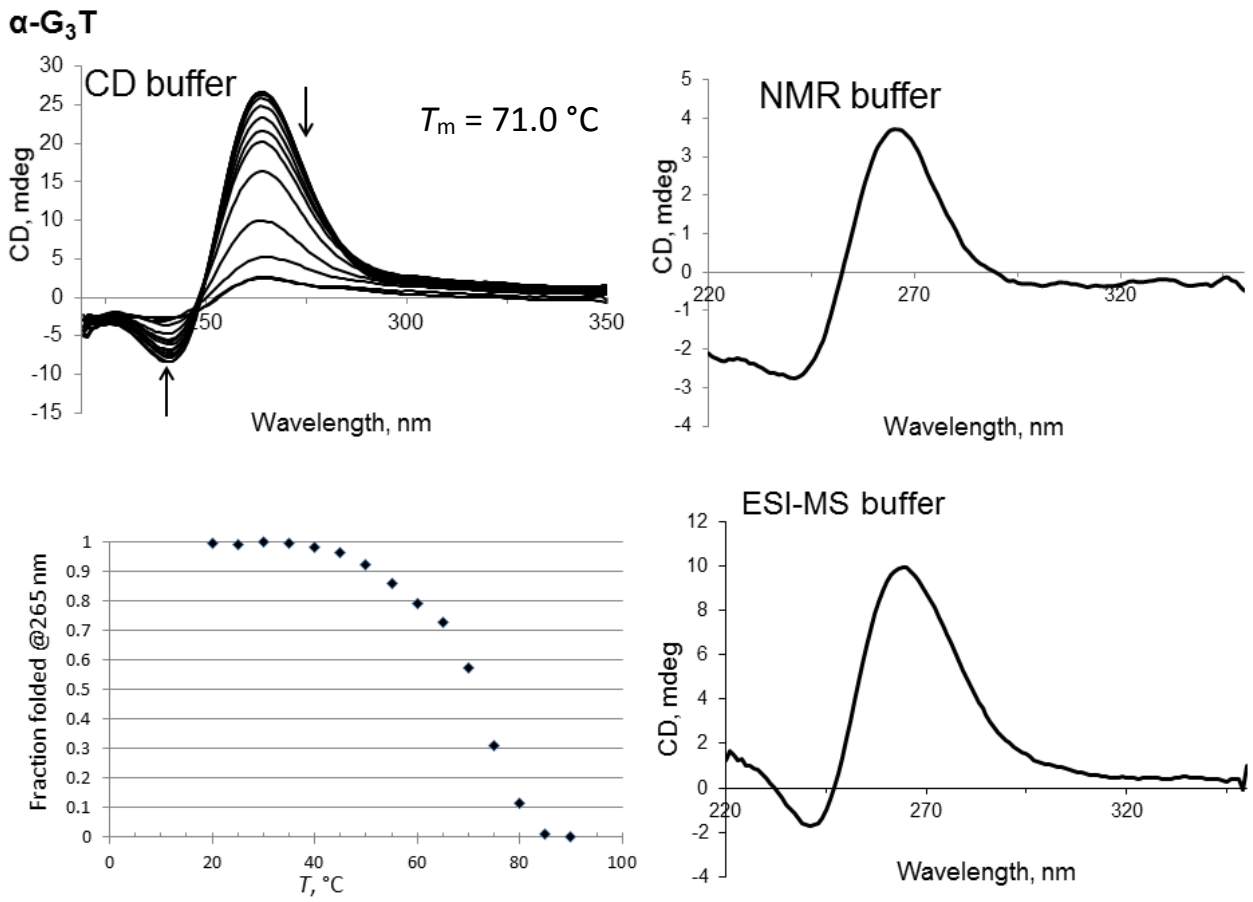


Figure S13. CD spectra of α-G_3 T complex recorded in different buffers and its temperature denaturation profile (bottom left). CD thermal denaturation was performed from 20 to 90 °C. The arrow indicates direction of changes in the peak intensity from low to high temperatures. CD spectra in NMR and ESI-MS buffers were recorded at 20 °C.

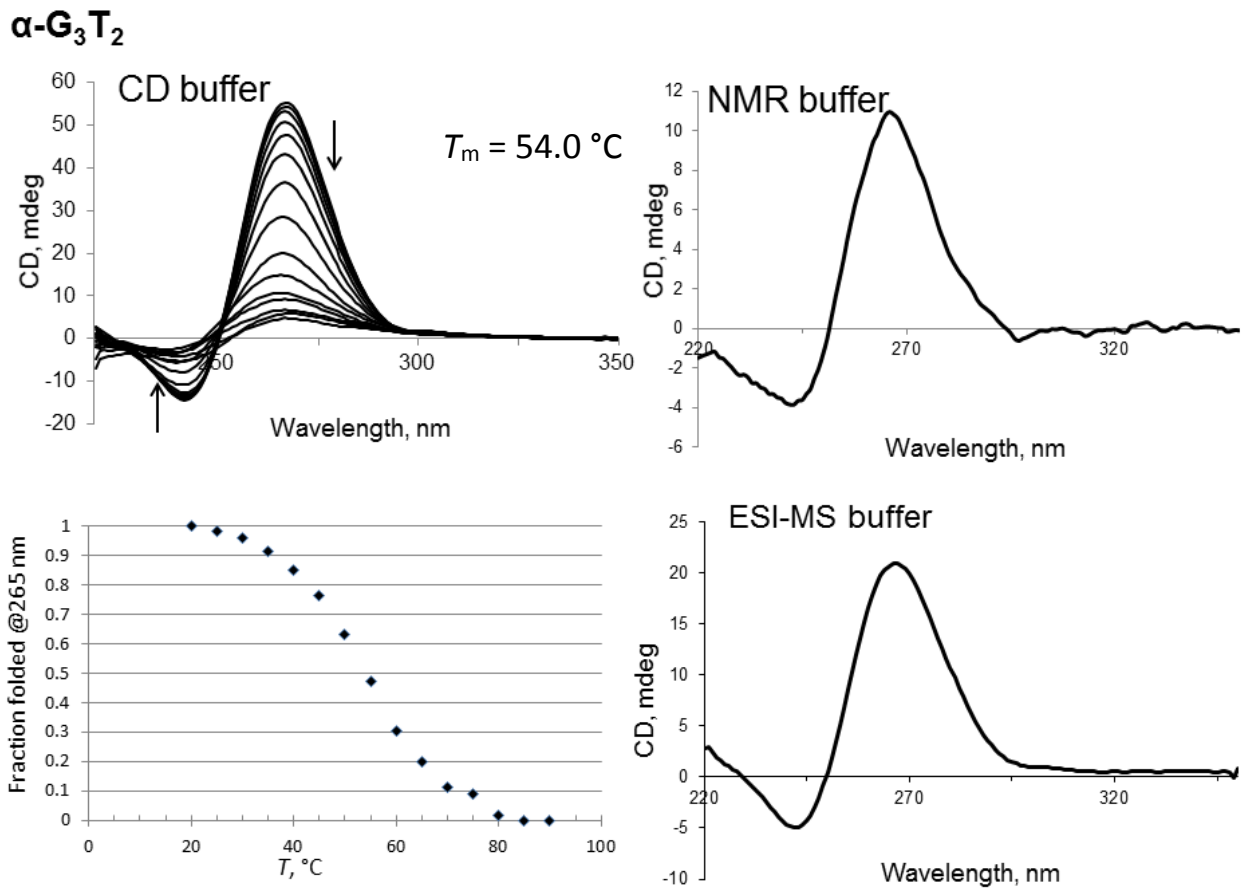


Figure S14. CD spectra of α-G_3 T_2 complex recorded in different buffers and its temperature denaturation profile (bottom left). CD thermal denaturation was performed from 20 to 90 °C. The arrow indicates direction of changes in the peak intensity from low to high temperatures. CD spectra in NMR and ESI-MS buffers were recorded at 20 °C.

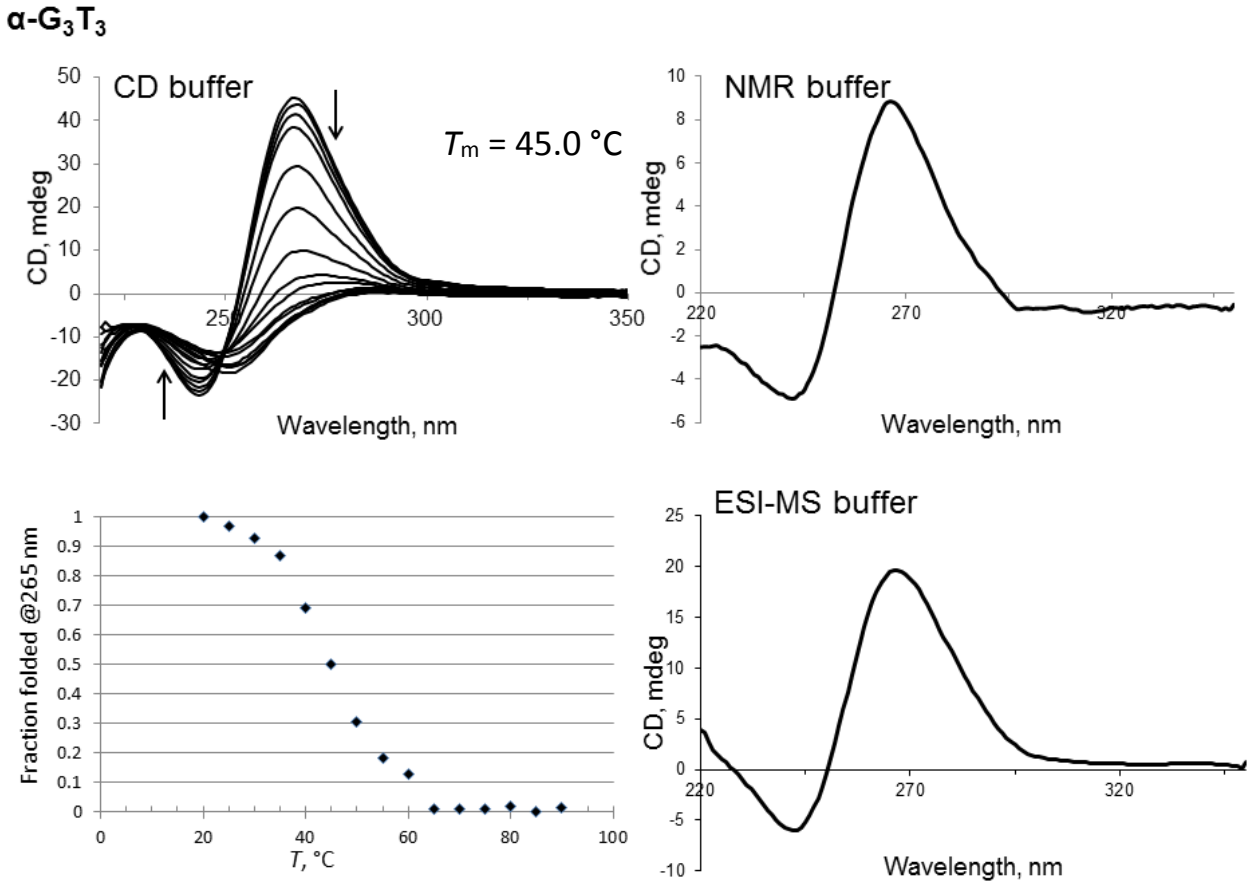


Figure S15. CD spectra of α-G_3 T_3 complex recorded in different buffers and its temperature denaturation profile (bottom left). CD thermal denaturation was performed from 20 to 90 °C. The arrow indicates direction of changes in the peak intensity from low to high temperatures. CD spectra in NMR and ESI-MS buffers were recorded at 20 °C.

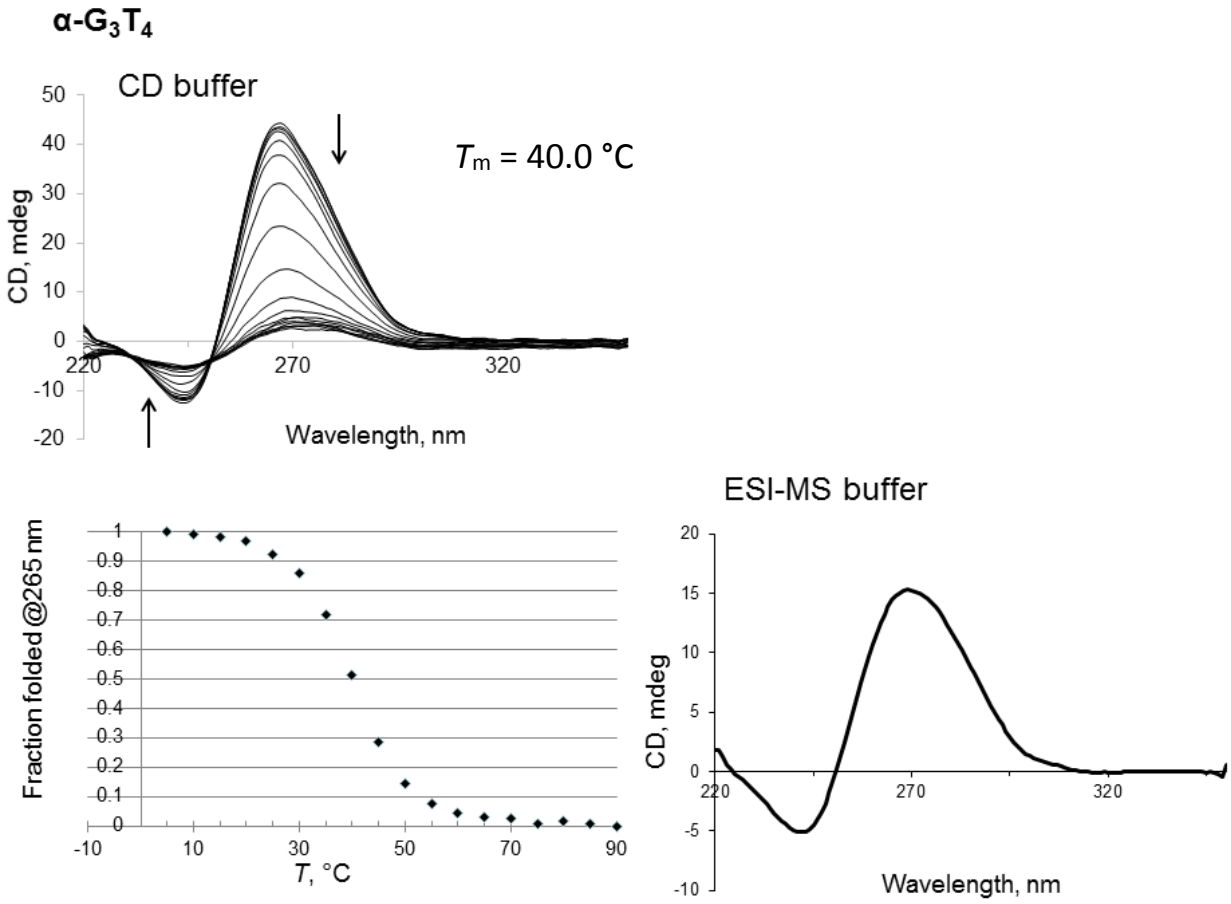


Figure S16. CD spectra of α-G_3 T_4 complex recorded in different buffers and its temperature denaturation profile (bottom left). CD thermal denaturation was performed from 5 to 90 °C. The arrow indicates direction of changes in the peak intensity from low to high temperatures. CD spectrum in ESI-MS buffer was recorded at 20 °C.

α-G_3T_2

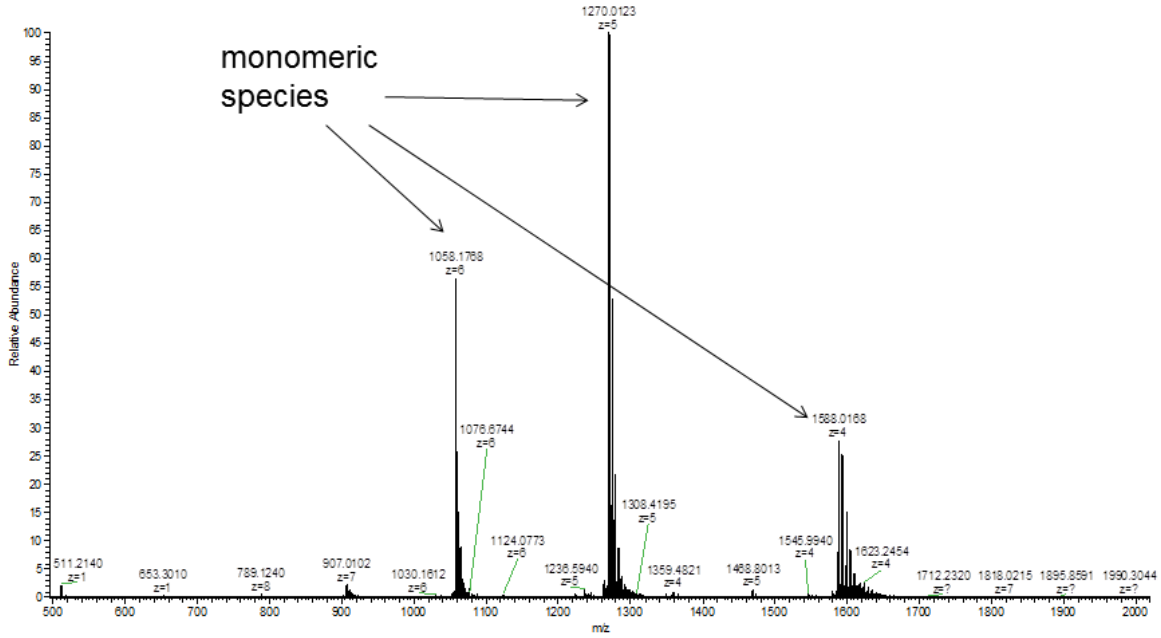


Figure S17. ESI-MS spectrum for α-G_3T_2

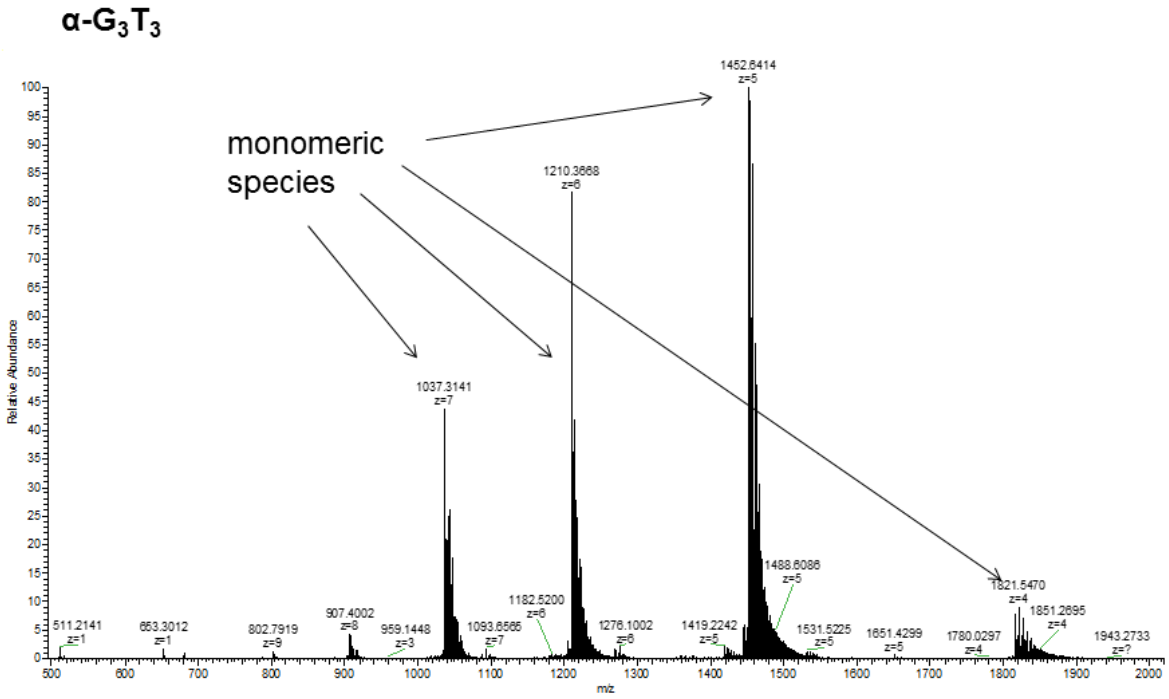


Figure S18. ESI-MS spectrum for α-G_3T_3

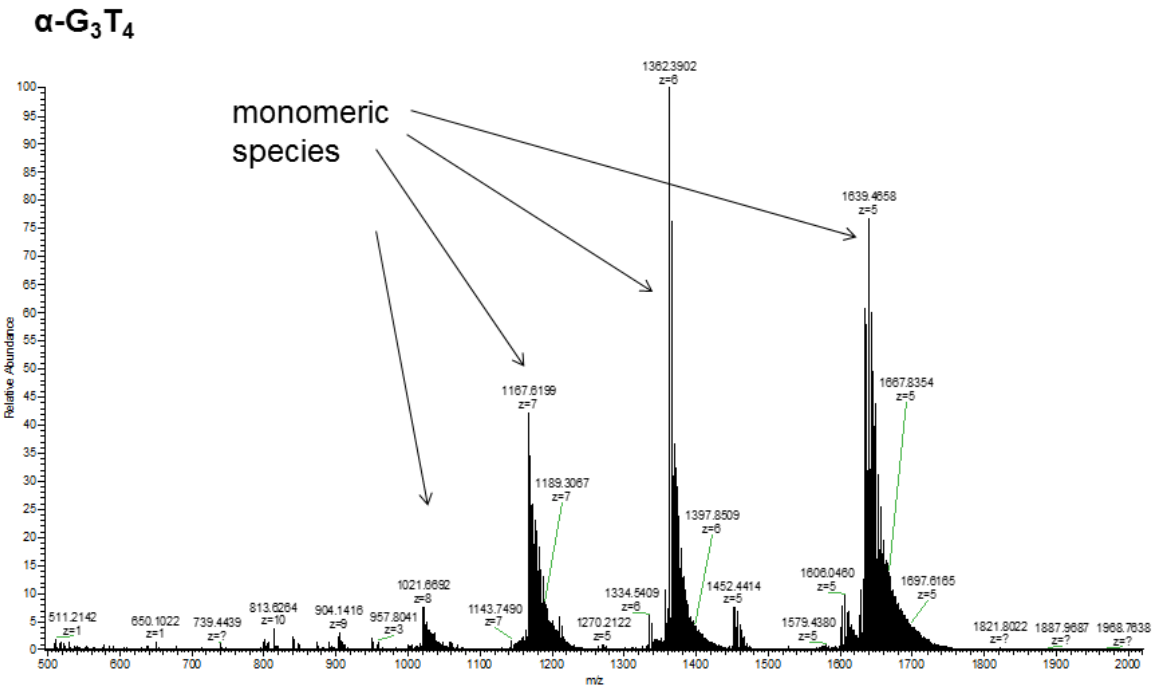


Figure S19. ESI-MS spectrum for α-G_3T_4

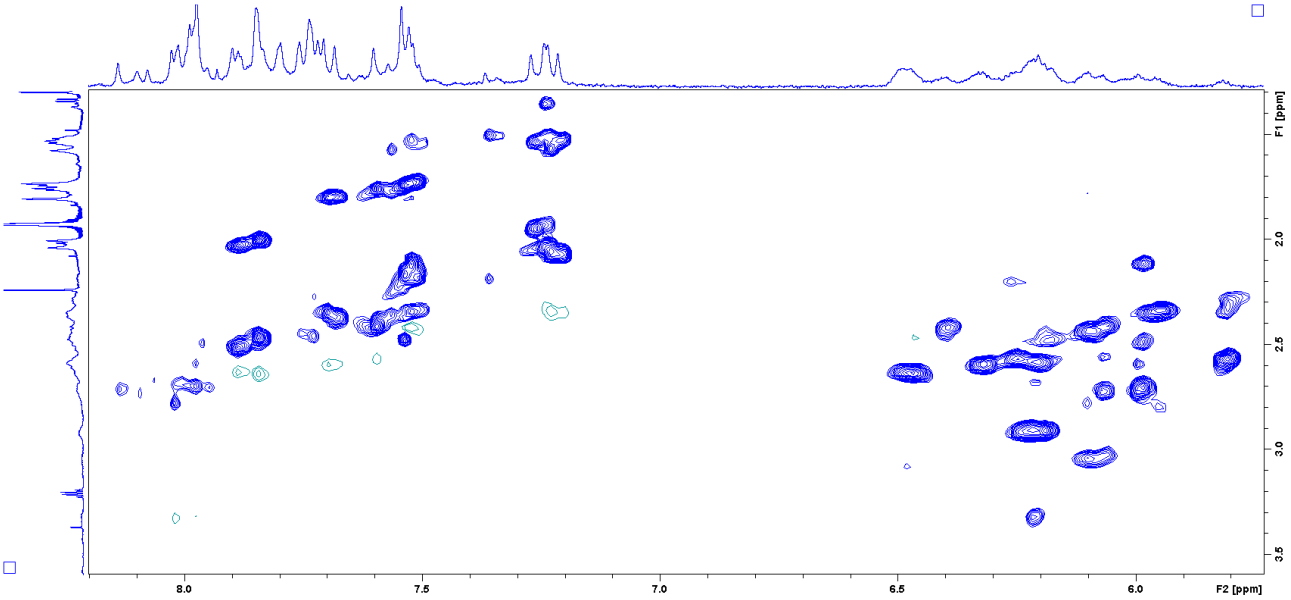


Figure S20. ROESY NMR spectrum for α-G_3T_3 in the region of H1'-H2'/H2" as well as CH_3-H6 and H8/H6- H2'/H2" cross-peaks.

References:

1. J. Gambino, T.-F. Yang and G. E. Wright, *Tetrahedron*, 1994, **50**, 11363-11368.