molecules

MDPI

Supplementary Materials

Structural diversity of sense and antisense RNA hexanucleotide repeats associated with ALS and FTLD

Tim Božič [1], Matja Zalar [1], Boris Rogelj [2,3], Janez Plavec [1,4,5,*] and Primož Šket [1,*]

[1] Slovenian NMR Centre, National Institute of Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia.
[2] Department of Biotechnology, Jožef Stefan Institute, Jamova 39, SI-1000 Ljubljana, Slovenia.
[3] Biomedical Research Institute BRIS, Puhova 10, SI-1000 Ljubljana, Slovenia.
[4] EN-FIST Center of Excellence, Trg OF 13, SI-1000 Ljubljana, Slovenia.
[5] Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, SI-1000 Ljubljana, Slovenia.
* Correspondence and requests for materials should be addressed to P. Šket (email: primoz.sket@ki.si) and J. Plavec (email: janez.plavec@ki.si)

Table of contents

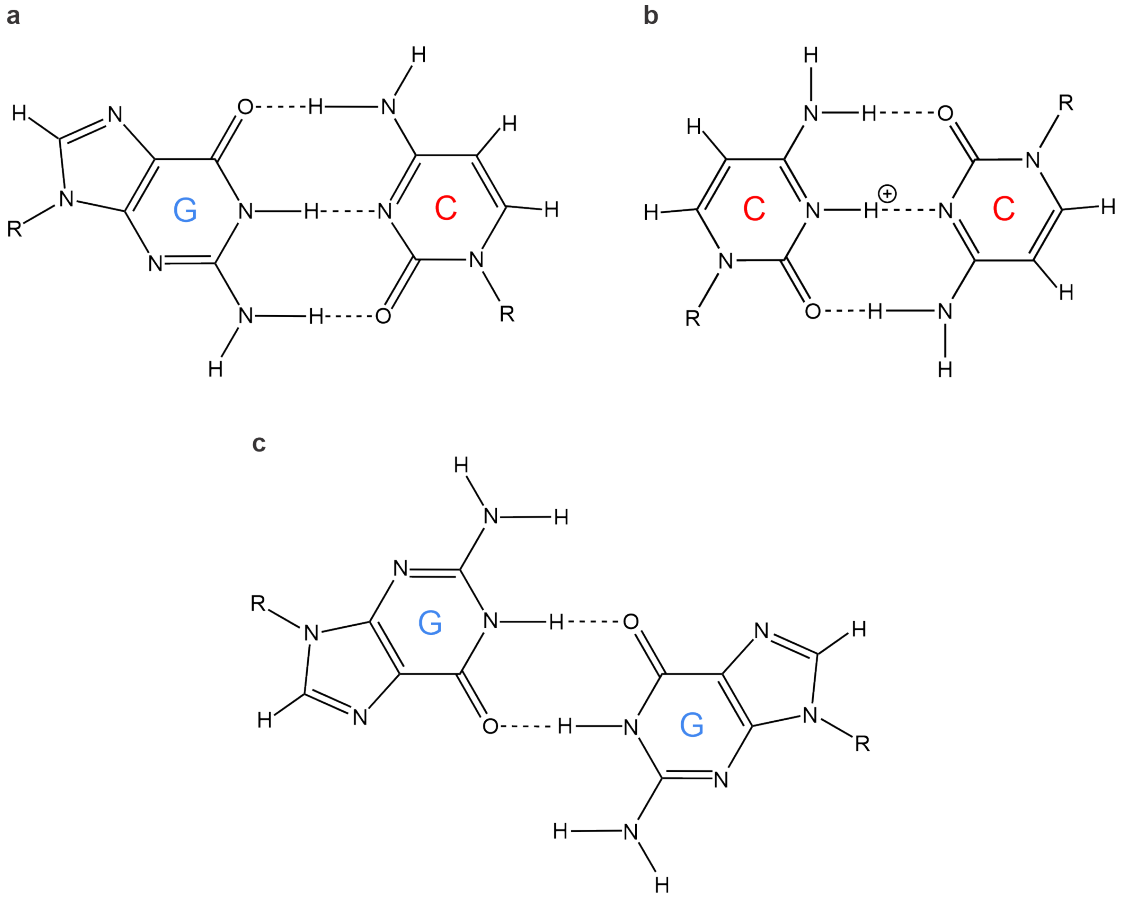


Figure S1. Canonical and non-canonical base pairs. Schematic presentation of **a**) Watson-Crick G-C base pair, **b**) hemi-protonated C^+-C base pair and **c**) G-G base pair in N1-carbonyl symmetric geometry.

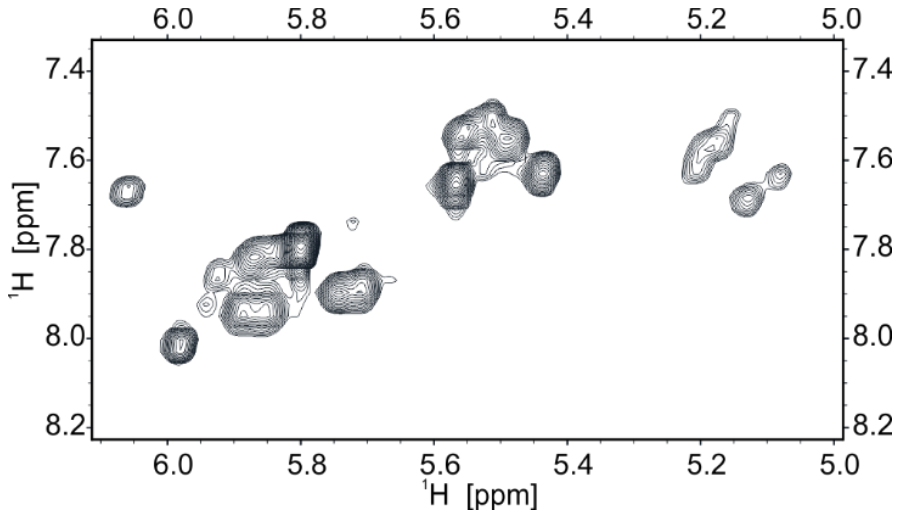


Figure S2. Cytosine H5/H6 correlation region of 2D TOCSY NMR spectrum of r(G_2C_4)$_4$. Spectrum was recorded at pH 7.0 and 25 °C at mixing time of 80 ms and concentration of 0.9 mM per strand.

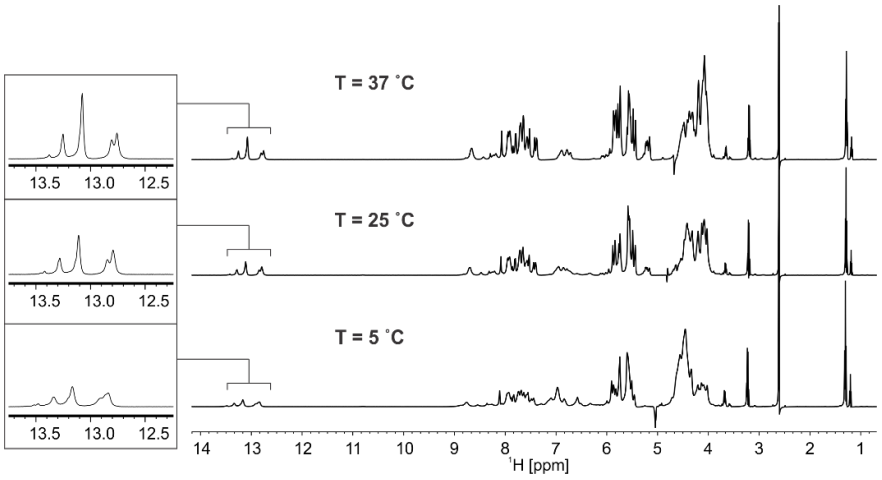
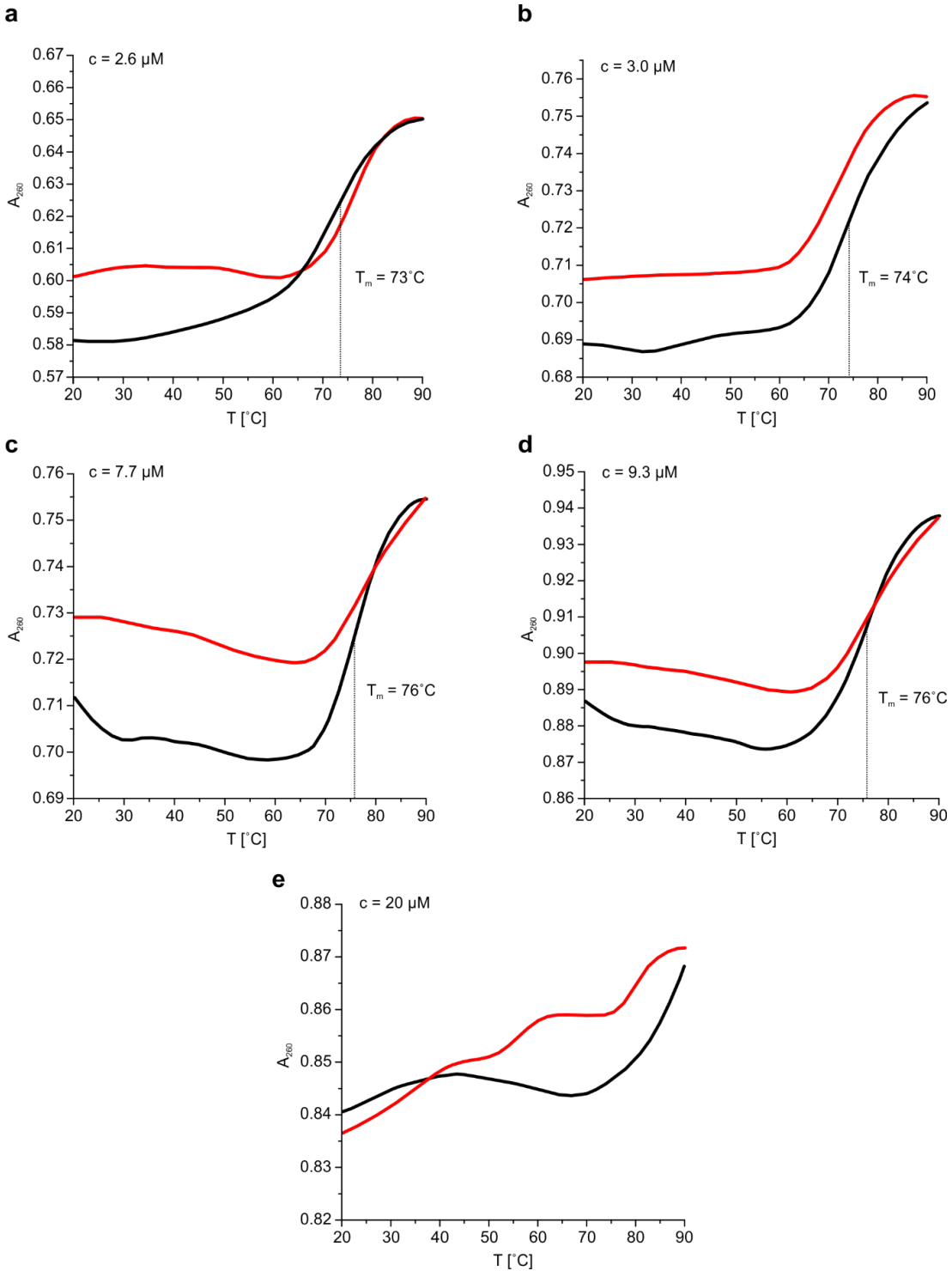


Figure S3. ^{1}H NMR spectra of r(G_2C_4)$_4$ in 10% ^2H_2O recorded at 5, 25 and 37 °C. At 25 ˚C the pH was 7.0. All NMR spectra were acquired at concentration of 0.9 mM per strand.



.

Figure S4. UV melting experiment of homodimer adopted by $r(G_2C_4)_4$. The absorbance at 260 nm was measured during one cycle of heating (black) and cooling (red) the sample in the temperature range between 20 and 90 °C. Temperature of half transition for homodimer adopted by $r(G_2C_4)_4$ was determined for all four oligonucleotide concentrations from melting curve obtained during heating. All samples were prepared in 20 mM Li^+ cacodylate buffer with pH 7.0. Concentrations of $r(G_2C_4)_4$ were **a**) 2.6, **b**) 3.0, **c**) 7.7, **d**) 9.3 and **e**) 20 μM per strand.

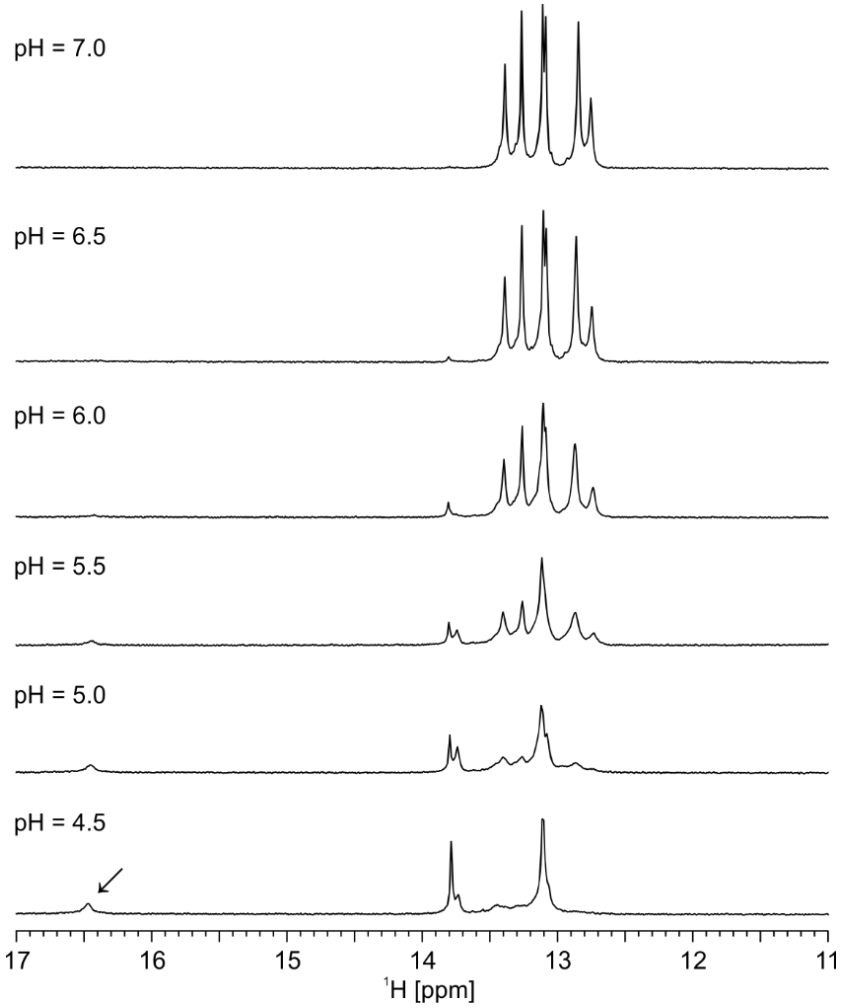


Figure S5. Imino region of ^{1}H NMR spectra of r(G$_2$C$_4$)$_4$ in 10% ^{2}H$_2$O at different pH. All NMR spectra were acquired at 25 °C and 0.3 mM oligonucleotide concentration per strand. Signal corresponding to hemi-protonated C$^+$-C base pairs is marked with an arrow.

Table 1. Translational diffusion coefficients (D_t) of r(G$_2$C$_4$)$_4$ at different pH values. All 2D DOSY spectra were acquired at 25 °C and concentration of 0.3 mM per strand. Standard deviation of the data set was ± 0.05.

pH	D_t [* 10^(−10) m^{2}s^{-1}]
7.0	0.91
6.5	0.94
6.0	1.25
5.5	1.30
5.0	1.35
4.5	1.40

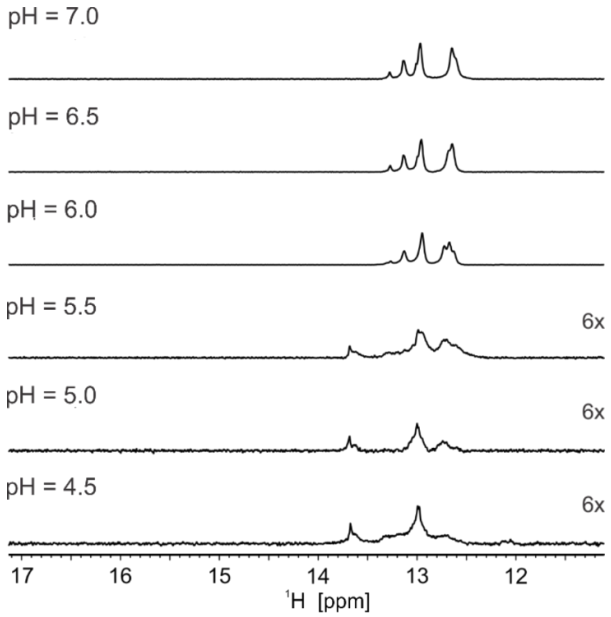


Figure S6. Imino region of ^{1}H NMR spectra of r(G$_2$C$_4$)$_4$ in 10% ^{2}H$_2$O at different pH and in the presence of 10% *w*/*v* PEG. All NMR spectra were acquired at 25 °C and concentration of 0.3 mM per strand. The vertical scales of the spectra with pH ranging from 5.5 to 4.5 have been increased six-fold (6×).

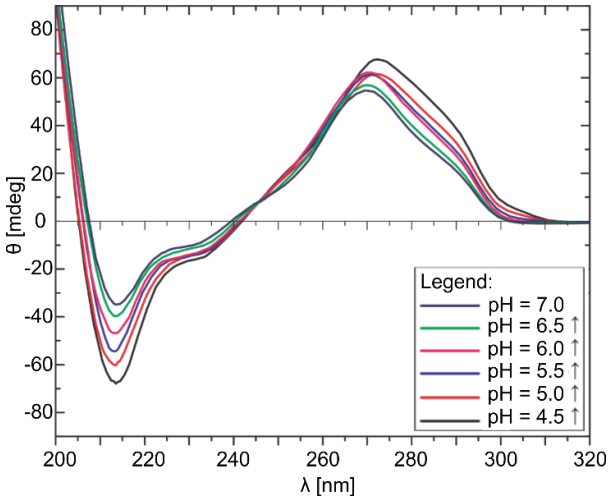


Figure S7. CD spectra of r(G$_2$C$_4$)$_4$ with pH ranging from 4.5 to 7.0. Arrows represent the direction of change in pH. All CD spectra were acquired at 25 °C and concentration of 100 μM per strand.

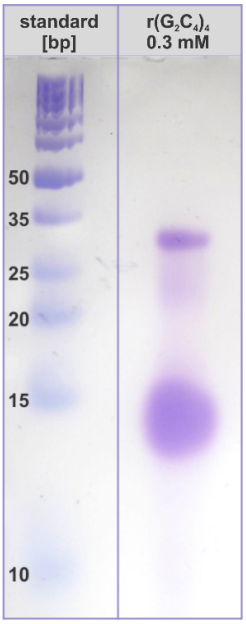


Figure S8. 20% native PAGE of r(G2C4)4 at pH 6.0 and 5 °C. Concentration of r(G2C4)4 was 0.3 mM per strand. DNA ladder was used as a standard.

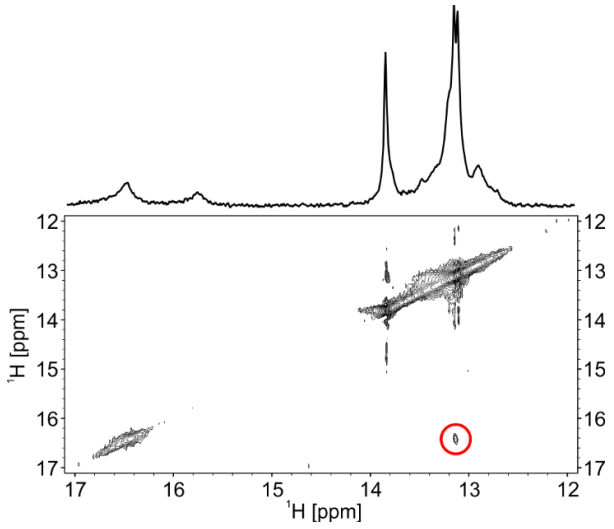


Figure S9. Imino-imino spectral region of 2D NOESY spectrum of r(G2C4)4. Spectrum was recorded at pH 4.5 and 5 °C at mixing time of 100 ms and concentration of 0.7 mM per strand. Above 2D NOESY spectrum is 1D ^{1}H NMR spectrum of r(G2C4)4 recorded at pH 4.5, 5 °C and oligonucleotide concentration of 0.7 mM per strand. NOE contact (marked with red circle) confirms the presence of hemi-protonated C^+-C base pairs within the hairpin.

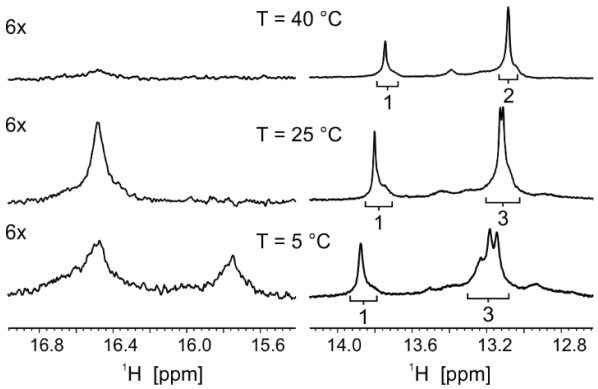


Figure S10. Imino regions of ^{1}H NMR spectra of r(G_2C_4)$_4$ in 10% $^{2}H_2O$ at 5, 25 and 40 °C. All NMR spectra were acquired at concentration of 0.3 mM per strand. The pH was 4.5 at 25 °C. Numbers under signals represent integral values. The vertical scales of the spectral regions between δ 15.6 to 16.8 ppm have been increased six-fold (6×).

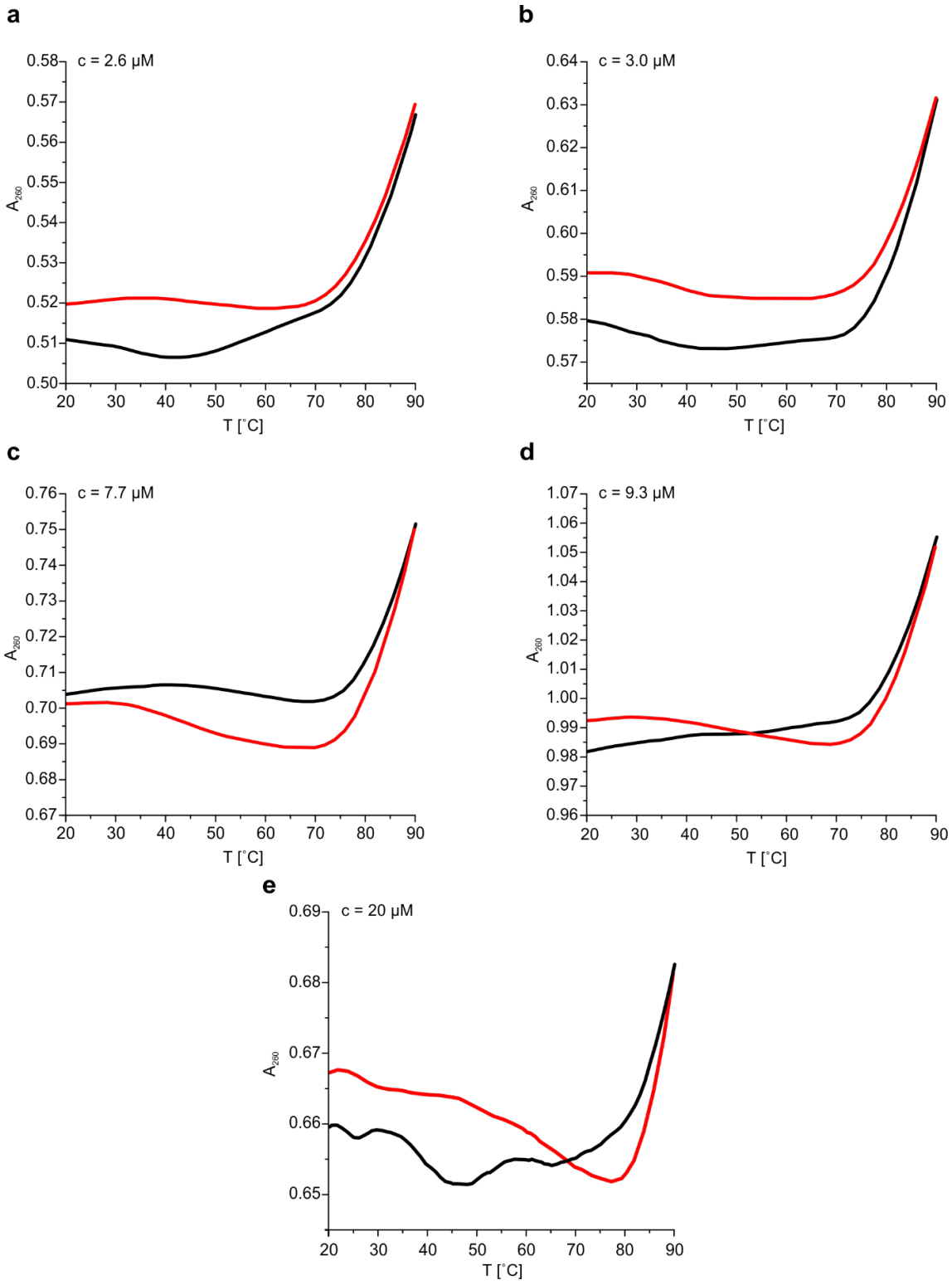


Figure S11. UV melting experiment of hairpin adopted by r(G2C4)4. The absorbance at 260 nm was measured during one cycle of heating (black) and cooling (red) the sample in the temperature range between 20 and 90 °C. All samples were prepared in 20 mM Li^+ cacodylate buffer with pH 5.0. Concentrations of r(G2C4)4 were **a**) 2.6, **b**) 3.0, **c**) 7.7, **d**) 9.3 and **e**) 20 μM per strand.

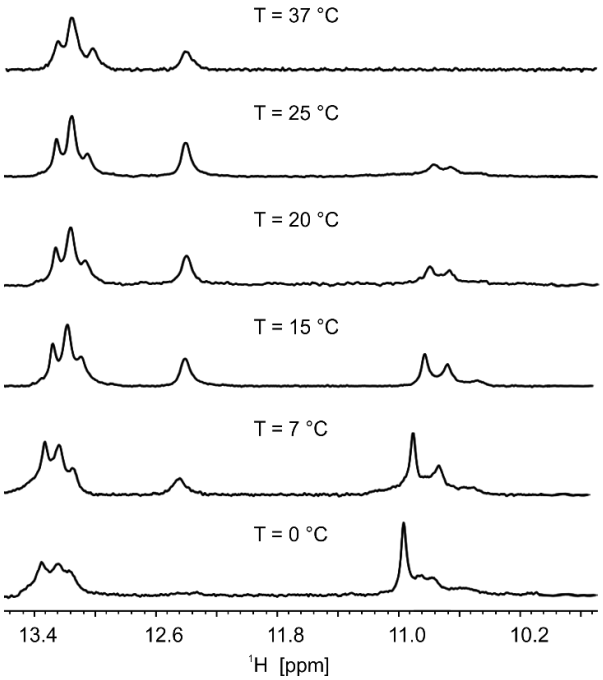


Figure S12. ^{1}H NMR spectra of r(G_4C_2)$_4$ in 10% $^{2}H_2O$ at 0, 7, 15, 20, 25 and 37 °C. All NMR spectra were acquired at concentration of 0.3 mM per strand. The pH was 6.0 at 25 °C.

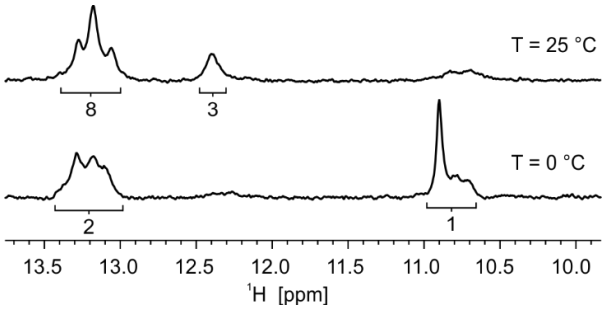


Figure S13. Imino regions of ^{1}H NMR spectra of r(G_4C_2)$_4$ in 10% $^{2}H_2O$ at 0 and 25 °C. Both NMR spectra were acquired at pH 6.0 and concentration of 0.3 mM per strand. Numbers under signals represent integral values.

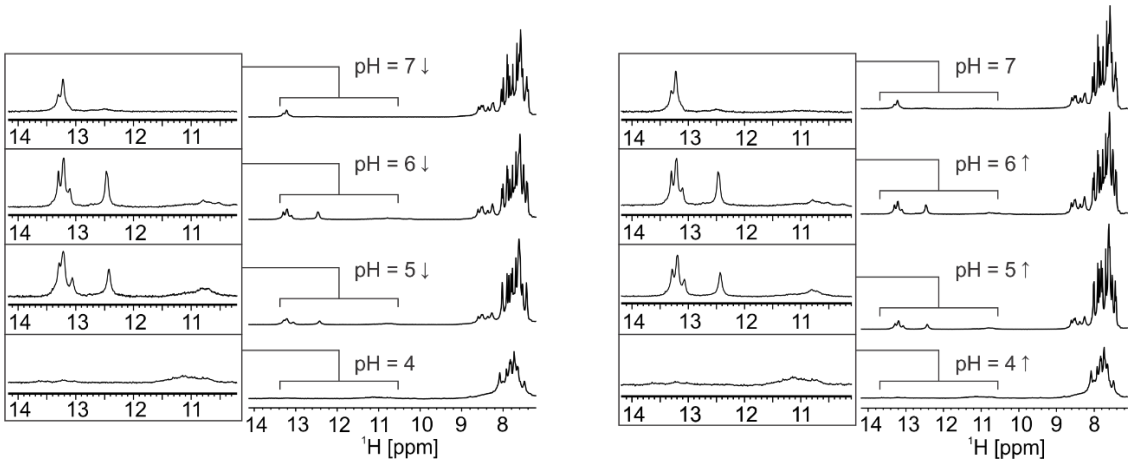


Figure S14. Imino and aromatic region of ^{1}H NMR spectra of r(G$_4$C$_2$)$_4$ in 10% ^{2}H$_2$O at different pH. Arrows represent the direction of change in pH. All NMR spectra were acquired on 800 MHz spectrometer at 25 °C and concentration of 0.3 mM per strand.

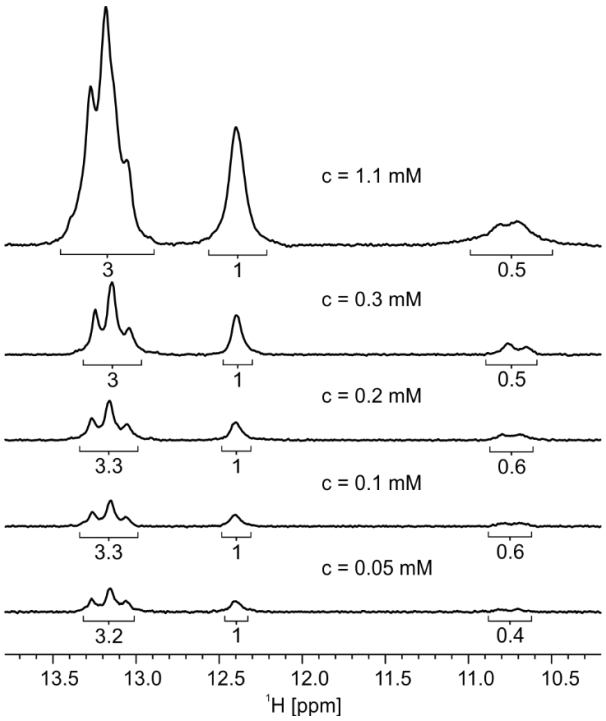


Figure S15. Imino regions of ^{1}H NMR spectra of r(G$_4$C$_2$)$_4$ in 10% ^{2}H$_2$O at concentration of 0.05, 0.1, 0.2, 0.3, and 1.1 mM per strand. All NMR spectra were acquired at pH 6.0 and 25 °C. Numbers under signals represent integral values.

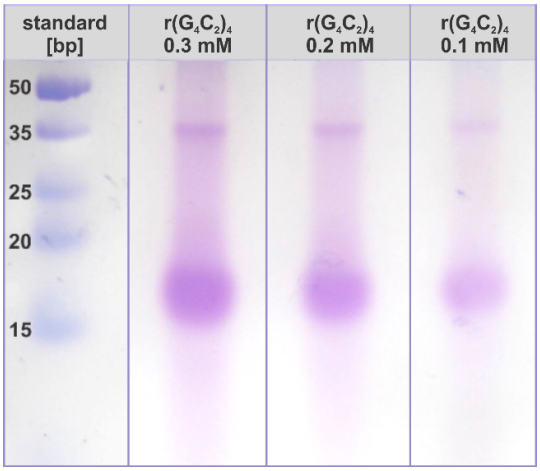


Figure S16. 20% native PAGE of r(G$_4$C$_2$)$_4$ at pH 6.0 and 5 °C. Concentration of r(G$_4$C$_2$)$_4$ was 0.3, 0.2 and 0.1 mM per strand. DNA ladder was used as a standard.

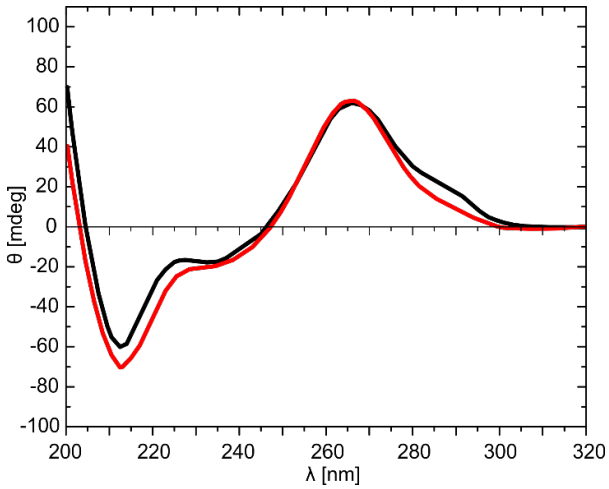


Figure S17. CD spectra of equimolar mixture of r(G2C4)4 and r(G4C2)4 before (black) and after (red) annealing. Both CD spectra were acquired at pH 6.0, 25 °C and concentration of 100 μM per sense and antisense strand.

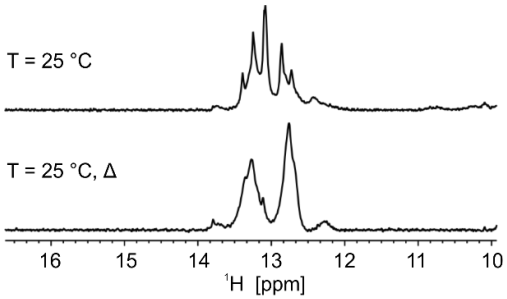


Figure S18. ^{1}H NMR spectra of equimolar mixture of r(G2C4)4 and r(G2C4)4 before and after annealing. Δ represents NMR spectrum after annealing. Both NMR spectra were acquired at 25 °C, pH 6.0 and concentration of 0.2 mM per sense and antisense strand.

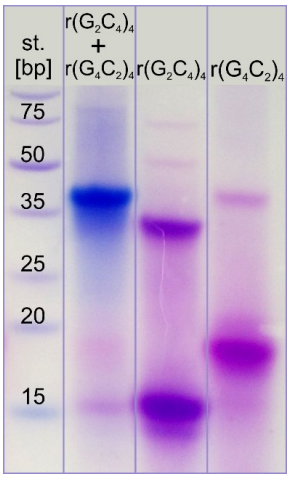


Figure S19. 20% native PAGE of r(G4C2)4, r(G2C4)4 and r(G4C2)4 + r(G2C4)4 at pH 6.0 and 5 °C. In all samples concentration of r(G4C2)4 and r(G2C4)4 was 0.1 mM per strand. DNA ladder was used as a standard.