

Stacking Effects on Local Structure in RNA: The Structure of Tandem GA Pairs Changes When Flanking GC Pairs are Replaced by isoG-isoC Pairs

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Table S1. List of NMR distance restraints for the model of the RNA duplex (GCiGGAiCGCA)₂ with only one set of the 2-fold symmetric restraints shown. See text for dihedral angle restraints.

Hydrogen bond restraints:

1:G5'_1:H1	1:C_8*:N3	1.800	2.500
1:G5'_1:O6	1:C_8*:N4	1.800	3.500
1:G5'_1:N2	1:C_8*:O2	1.800	3.500
1:C_2:N3	1:G_7*:H1	1.800	2.500
1:C_2:N4	1:G_7*:O6	1.800	3.500
1:C_2:O2	1:G_7*:N2	1.800	3.500

Intrastrand restraints from SNOESY

1:G_4:H8	1:IG_3:H8	2.231	5.205
1:IG_3:H1	1:G_4:H1'	2.092	4.880
1:IG_3:H1	1:G_4:N2	2.350	5.500
1:G_4:H1	1:IG_3:N6	2.130	5.500

Cross-strand restraints from SNOESY

1:A_5:H8	1:G_4*:N2	1.800	5.000
1:G_4:H1	1:G_4*:H2'	2.398	5.596
1:A_5:H8	1:A_5*:N6	1.800	5.000
1:G_4:N2	1:G_4*:H2'	1.862	5.000

Cross-strand restraints from d2o noesy

1:A_5:H2	1:A_5*:H1'	1.800	3.751
1:G5'_1:H1'	1:A3'_9*:H2	2.000	5.000

Intra-strand restraints from d2o noesy

1:G5'_1:H8	1:G5'_1:H1'	2.516	4.672
1:G5'_1:H8	1:G5'_1:H2'	2.822	5.242
1:G5'_1:H8	1:G5'_1:H3'	2.317	4.303
1:G5'_1:H1'	1:G5'_1:H2'	1.896	3.522
1:G5'_1:H1'	1:G5'_1:H3'	2.428	4.509
1:G5'_1:H1'	1:G5'_1:H4'	2.345	4.355
1:G5'_1:H1'	1:C_2:H6	3.023	5.615
1:G5'_1:H3'	1:C_2:H6	2.136	3.966
1:C_2:H5	1:G5'_1:H2'	2.615	4.857

1:C_2:H5	1:G5'_1:H3'	2.352	4.368
1:C_2:H5	1:C_2:H6	1.800	3.072
1:C_2:H6	1:G5'_1:H2'	1.800	3.219
1:C_2:H6	1:C_2:H3'	1.820	5.000
1:C_2:H1'	1:G5'_1:H2'	3.244	5.000
1:C_2:H1'	1:C_2:H6	2.431	4.514
1:C_2:H1'	1:C_2:H2'	2.068	3.841
1:C_2:H1'	1:C_2:H3'	2.376	4.412
1:C_2:H1'	1:C_2:H4'	2.339	4.344
1:C_2:H1'	1:IG_3:H8	2.890	5.000
1:IG_3:H8	1:C_2:H2'	2.114	3.925
1:IG_3:H8	1:C_2:H3'	2.242	4.164
1:IG_3:H1'	1:IG_3:H8	2.669	4.957
1:IG_3:H8	1:IG_3:H2'	2.811	5.000
1:IG_3:H8	1:IG_3:H3'	2.489	4.623
1:IG_3:H1'	1:C_2:H2'	2.713	5.039
1:IG_3:H1'	1:IG_3:H2'	2.362	4.387
1:IG_3:H1'	1:IG_3:H3'	2.560	4.755
1:IG_3:H1'	1:IG_3:H4'	2.539	4.716
1:G_4:H8	1:IG_3:H2'	2.101	3.901
1:G_4:H8	1:G_4:H1'	2.867	5.000
1:G_4:H8	1:G_4:H3'	1.971	3.661
1:G_4:H8	1:IG_3:H1'	3.048	5.000
1:G_4:H8	1:IG_3:H3'	1.800	5.000
1:G_4:H8	1:G_4:H2'	1.800	5.000
1:G_4:H1'	1:G_4:H2'	2.254	4.186
1:G_4:H1'	1:G_4:H4'	2.572	4.777
1:G_4:H2'	1:A_5:H8	2.274	4.222
1:A_5:H2	1:IC_6:H1'	1.836	4.284
1:A_5:H8	1:A_5:H1'	2.697	5.000
1:A_5:H8	1:A_5:H2'	3.026	5.000
1:A_5:H8	1:A_5:H3'	2.249	4.177
1:A_5:H1'	1:A_5:H2'	2.004	3.721
1:A_5:H1'	1:A_5:H3'	2.564	4.762
1:A_5:H1'	1:A_5:H4'	2.552	4.739
1:A_5:H3'	1:IC_6:H6	1.800	5.000
1:IC_6:H5	1:A_5:H3'	2.649	4.919
1:IC_6:H5	1:IC_6:H6	1.945	3.613
1:IC_6:H6	1:A_5:H2'	2.305	4.282
1:IC_6:H6	1:IC_6:H2'	1.800	5.000
1:IC_6:H6	1:IC_6:H3'	1.800	5.000
1:IC_6:H6	1:IC_6:H1'	1.800	5.000
1:IC_6:H2'	1:G_7:H8	1.800	5.000
1:G_7:H8	1:IC_6:H3'	1.800	5.000
1:G_7:H8	1:IC_6:H1'	1.800	5.000
1:G_7:H8	1:G_7:H1'	2.592	4.814
1:G_7:H8	1:G_7:H2'	2.202	5.000

1:G_7:H8	1:G_7:H3'	1.840	3.417
1:G_7:H8	1:C_8:H5	3.005	5.000
1:G_7:H1'	1:G_7:H2'	1.800	3.304
1:G_7:H1'	1:C_8:H6	2.796	5.000
1:G_7:H2'	1:C_8:H6	1.800	3.039
1:G_7:H3'	1:G_7:H1'	2.613	4.853
1:G_7:H3'	1:C_8:H6	2.149	3.991
1:G_7:H4'	1:G_7:H1'	2.354	4.372
1:C_8:H5	1:G_7:H2'	2.235	4.151
1:C_8:H5	1:C_8:H6	1.800	3.162
1:C_8:H6	1:C_8:H3'	1.800	3.264
1:C_8:H6	1:C_8:H2'	1.966	5.000
1:C_8:H1'	1:G_7:H2'	2.412	4.480
1:C_8:H1'	1:C_8:H6	2.523	4.685
1:C_8:H2'	1:C_8:H1'	1.800	3.300
1:C_8:H3'	1:C_8:H1'	2.463	4.574
1:C_8:H3'	1:C_8:H5	2.581	4.792
1:C_8:H2'	1:A3'_9:H8	1.800	3.307
1:C_8:H3'	1:A3'_9:H8	2.080	3.863
1:A3'_9:H8	1:C_8:H1'	3.099	5.000
1:A3'_9:H8	1:A3'_9:H1'	2.496	4.635
1:A3'_9:H8	1:A3'_9:H2'	2.185	4.058
1:A3'_9:H8	1:A3'_9:H3'	1.895	3.518
1:A3'_9:H1'	1:A3'_9:H2'	1.931	3.587
1:A3'_9:H1'	1:A3'_9:H3'	2.567	4.768
1:A3'_9:H1'	1:A3'_9:H4'	2.241	4.162

Table S2. Proton chemical shifts assignments of (GCiGGAiCGCA)₂. The exchangeable protons were assigned at –5 °C by SNOESY and an exchangeable proton TOCSY. The nonexchangeable protons were assigned at 30 °C by combined analysis of NOESY, TOCSY, DQF-COSY, ¹H–³¹P HETCOR, and natural abundance ¹H–¹³C HMQC. Values in parentheses are for the major/minor conformation at 5°C for nonexchangeable protons and the minor conformation at –5°C for exchangeable protons.

	imino	amino	2'-OH	H6/H8	H2/H5	H1'	H2'	H3'	H4'	H5'/ H5''
G1	12.55	8.33/6.30	7.13	8.13	N/A	5.91	4.8	4.55	4.45	4.14/3.97
C2	N/A	8.43/6.59	7.52	7.77	5.22	5.59	4.69	4.59	4.46	4.60/4.18
iG3	12.90 (12.45)	9.75/6.50	7.43	7.41	N/A	5.62	4.52	4.36	4.45	4.45/4.17
G4	9.94 (12.0)	6.88	5.84	7.83	N/A	5.46 (5.35/5.9)	4.37	4.84	4.55	4.49/4.21
A5	N/A	5.85	7.11	8.08	7.95	5.87	4.78	4.43	4.57	4.58/4.25
iC6	N/A	8.75	6.75	7.52	5.66 (5.71/5.14)	4.39 (3.61/5.28)	4.40	4.37	4.74	4.35/4.12
G7	12.63 (13.15)	8.14/6.02	6.93	7.58	N/A	5.76	4.53	4.50	4.41	4.44/4.07
C8	N/A	8.01/6.97	6.79	7.48	5.16	5.48	4.39	4.43	4.38	4.52/4.07
A9	N/A	6.47	6.52	8.04	7.44	6.01	4.07	4.31	4.26	4.45/4.40

N/A: not applicable

Table S3. List of NMR distance restraints for the model of RNA duplex (GGiCGAiGCCA)₂ with only one set of the 2-fold symmetric restraints shown. See text for dihedral angle restraints.

Hydrogen-bond restraints

1:G5'_1:H1	1:C_8*:N3	1.800	2.500
1:G5'_1:O6	1:C_8*:N4	1.800	3.500
1:G5'_1:N2	1:C_8*:O2	1.800	3.500
1:C_7:N3	1:G_2*:H1	1.800	2.500
1:C_7:N4	1:G_2*:O6	1.800	3.500
1:C_7:O2	1:G_2*:N2	1.800	3.500

Intrastrand restraints from SNOESY

1:G_2:H1'	1:G5'_1:N2	2.01	4.74
1:IC_3:H1'	1:G_2:N2	1.77	4.30
1:IG_6:N6	1:C_7:N4	1.80	4.20
1:C_7:H5	1:C_8:N4	2.80	6.00

Cross-strand restraints from SNOESY

1:A_5:H2	1:G_4*:H1	1.76	3.26
1:G_2:H1	1:IG_6*:H1	1.80	4.20
1:G_2:H1	1:IG_6*:N6	2.80	6.00
1:C_8:H1'	1:G_2*:N2	2.50	4.30
1:A3'_9:H1'	1:G5'_1*:H1	2.80	6.00

Cross-strand restraints from d2o noesy

1:A_5*:H2	1:A_5:H1'	2.80	6.00
1:G5'_1*:H8	1:A3'_9:H2	2.80	6.00
1:A3'_9:H2	1:G5'_1*:H1'	3.15	5.85

Intra-strand restraints from d2o noesy

1:G5'_1:H1'	1:G5'_1:H2'	1.99	3.69
1:G5'_1:H1'	1:G5'_1:H3'	2.51	4.67
1:G5'_1:H1'	1:G5'_1:H4'	2.23	4.14
1:G5'_1:H8	1:G5'_1:H1'	2.50	4.63
1:G5'_1:H8	1:G5'_1:H3'	2.19	4.06
1:G5'_1:H8	1:G5'_1:H4'	2.65	4.93
1:G5'_1:H8	1:G_2:H8	2.80	6.00
1:G_2:H1'	1:G5'_1:H2'	2.90	5.38
1:G_2:H8	1:G5'_1:H2'	1.92	3.57
1:G5'_1:H2'	1:G5'_1:H4'	2.57	4.77
1:G_2:H8	1:G_2:H1'	2.74	5.09
1:G_2:H1'	1:G_2:H2'	1.97	3.66
1:G_2:H8	1:G_2:H2'	2.47	4.60
1:IC_3:H5	1:G_2:H2'	2.40	4.46
1:IC_3:H6	1:G_2:H2'	1.76	3.27

1:IC_3:H6	1:G_2:H3'	2.08	3.86
1:IC_3:H6	1:G_2:H3'	2.50	4.50
1:IC_3:H6	1:IC_3:H1'	2.73	5.08
1:G_2:H1'	1:G_2:H3'	2.23	4.15
1:G_2:H8	1:G_2:H3'	2.45	4.55
1:G_4:H8	1:IC_3:H2'	1.93	3.59
1:IC_3:H6	1:IC_3:H3'	1.98	3.67
1:G_4:H8	1:IC_3:H3'	2.20	4.09
1:IC_3:H6	1:IC_3:H5	1.89	3.52
1:G_4:H8	1:G_4:H1'	2.83	5.25
1:G_4:H8	1:G_4:H2'	2.80	6.00
1:A_5:H8	1:G_4:H2'	1.80	4.20
1:A_5:H2	1:IG_6:H1'	2.35	4.36
1:G_4:H8	1:G_4:H3'	2.18	4.04
1:A_5:H8	1:G_4:H3'	2.80	6.00
1:A_5:H8	1:A_5:H1'	2.92	5.42
1:A_5:H1'	1:A_5:H2'	1.89	3.50
1:IG_6:H1'	1:IG_6:H2'	1.98	3.68
1:IG_6:H1'	1:IG_6:H3'	2.07	3.85
1:IG_6:H8	1:A_5:H2'	1.94	3.61
1:IG_6:H8	1:A_5:H3'	2.29	4.25
1:IG_6:H8	1:IG_6:H1'	2.80	6.00
1:IG_6:H8	1:IG_6:H3'	2.11	3.91
1:IG_6:H8	1:IG_6:H3'	2.00	4.00
1:A_5:H2'	1:A_5:H3'	1.65	3.07
1:A_5:H8	1:A_5:H3'	1.80	4.20
1:C_7:H5	1:IG_6:H8	3.16	5.86
1:C_7:H5	1:C_8:H5	2.50	4.56
1:C_7:H5	1:IG_6:H2'	2.60	4.82
1:C_7:H6	1:IG_6:H2'	1.62	3.01
1:C_7:H5	1:IG_6:H3'	2.80	6.00
1:C_7:H6	1:IG_6:H3'	2.80	6.00
1:C_7:H6	1:C_7:H1'	2.45	4.55
1:C_7:H1'	1:C_7:H2'	1.84	3.42
1:C_7:H6	1:C_7:H2'	2.41	4.48
1:C_8:H1'	1:C_7:H2'	2.70	5.02
1:C_8:H5	1:C_7:H2'	2.62	4.87
1:C_8:H6	1:C_7:H2'	1.67	3.10
1:C_7:H1'	1:C_7:H3'	1.80	4.20
1:C_7:H5	1:C_7:H3'	2.80	6.00
1:C_7:H6	1:C_7:H3'	1.80	4.20
1:C_8:H5	1:C_7:H3'	1.80	4.20
1:C_7:H6	1:IG_6:H8	2.80	6.00
1:C_7:H6	1:C_7:H5	1.74	3.22
1:C_8:H6	1:C_8:H1'	2.39	4.44
1:C_8:H1'	1:C_8:H2'	1.80	3.34

1:C_8:H5	1:C_8:H2'	3.49	6.48
1:A3'_9:H8	1:C_8:H2'	1.88	3.49
1:C_8:H1'	1:C_8:H3'	2.47	4.58
1:C_8:H6	1:C_8:H3'	1.64	3.05
1:A3'_9:H8	1:C_8:H3'	2.03	3.76
1:C_8:H1'	1:C_8:H4'	2.43	4.51
1:A3'_9:H8	1:C_8:H6	2.80	6.00
1:A3'_9:H8	1:A3'_9:H1'	2.82	5.25
1:A3'_9:H1'	1:A3'_9:H2'	1.96	3.65
1:A3'_9:H1'	1:A3'_9:H3'	2.42	4.49
1:A3'_9:H1'	1:A3'_9:H4'	2.10	3.90
1:A3'_9:H8	1:A3'_9:H2'	2.31	4.30
1:A3'_9:H8	1:A3'_9:H3'	1.97	3.66
1:A3'_9:H8	1:A3'_9:H4'	2.83	5.25

Table S4. Proton chemical shifts assignments of the major conformation of (GGiCGAiGCCA)₂. The exchangeable protons were assigned at 5 °C by watergateNOESY. The nonexchangeable protons were assigned at 5 °C by combined analysis of NOESY, TOCSY, DQF-COSY, ¹H-³¹P HETCOR, and natural abundance ¹H-¹³C HMQC. Values in parentheses are for the minor conformation. Shifts labeled with * were determined at 42 °C because they could not be definitely assigned at 5 °C.

	imino	amino	H6/H8	H2/H5	H1'	H2'	H3'	H4'	H5'/H5''
G1	12.26 (12.20)	8.15/6.28	8.14	N/A	5.95	5.02	4.57	4.46	4.13/3.98
G2	13.19 (13.11)	8.51/5.92	7.35 (7.39)	N/A	5.81	4.49	4.55		4.64*/4.13
iC3	N/A		7.74 (7.34)	5.15 (4.79)	5.43 (5.52)	4.15	4.69	4.46	4.54*/4.17*
G4	11.57 (9.77)	5.27	8.02	N/A	5.95 (5.66)	4.93	4.85	4.53	4.51*/4.23*
A5	N/A		7.92	7.59 (7.80)	6.06 (5.88)	4.75	4.37	4.66	4.48*/4.11*
iG6	13.02 (13.40)	10.29/7.43	6.92 (7.65)	N/A	5.50 (4.23)	4.37	4.43		
C7	N/A	8.32/7.00	7.72 (7.60)	5.31	5.40	4.19	4.43	4.36	4.52/4.04
C8	N/A	8.18/6.94	7.61	5.41	5.45	4.42	4.46	4.36	4.50/4.04
A9	N/A		7.99	7.29	5.98	4.03	4.30	4.23	4.46/4.05

N/A: not applicable

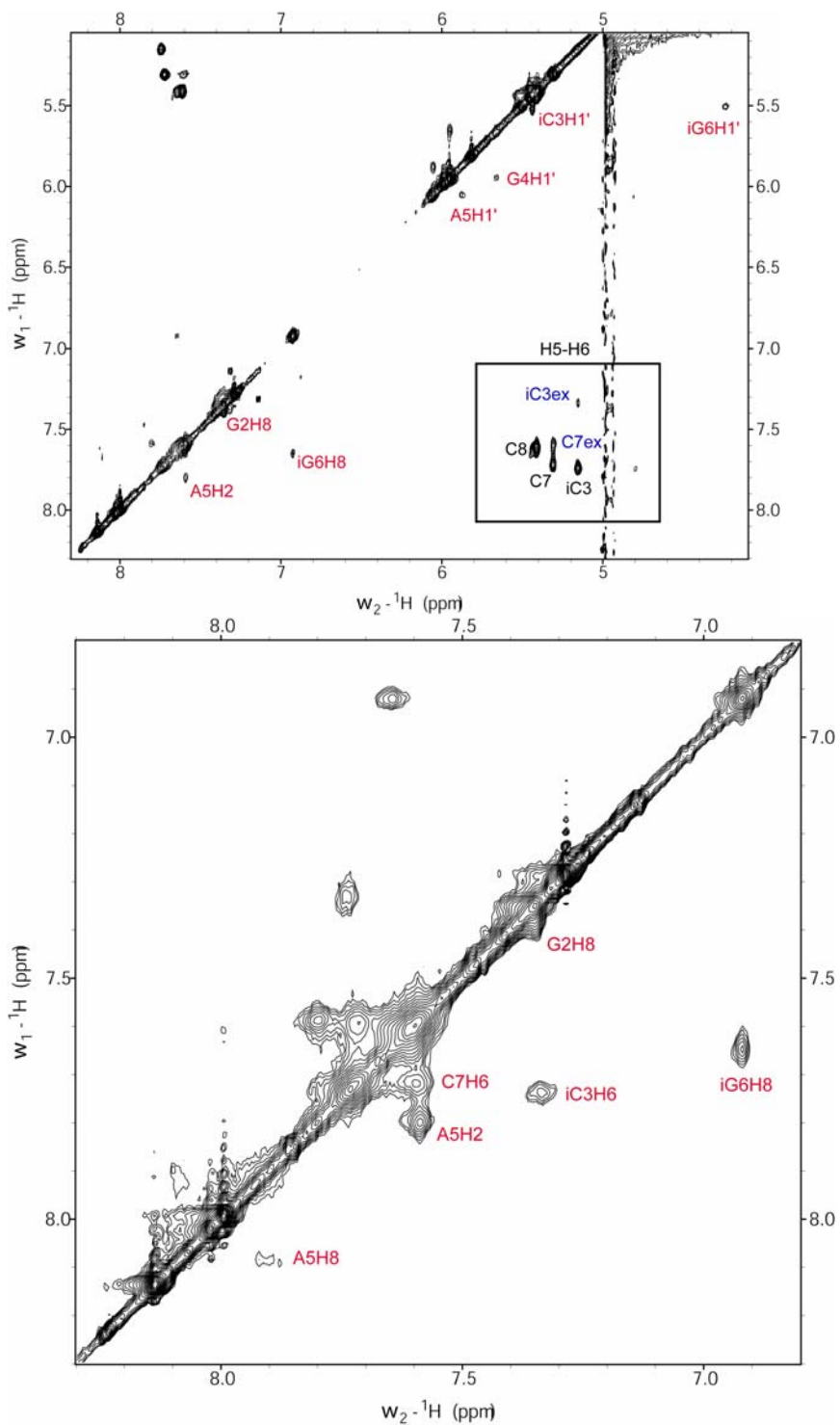


Figure S1) Top: 2D TOCSY of $(GGiCGAiGCCA)_2$ at 5°C with mixing time of 40 ms. Peaks with black labels are pyrimidine H5-H6 proton cross peaks from the major conformation. Peaks with blue labels are indirect TOCSY cross peaks due to exchange. Peaks with red labels are direct exchange cross peaks. Bottom: Aromatic region of 2D NOESY with mixing time of 100 ms at 5°C indicating direct exchange cross peaks.

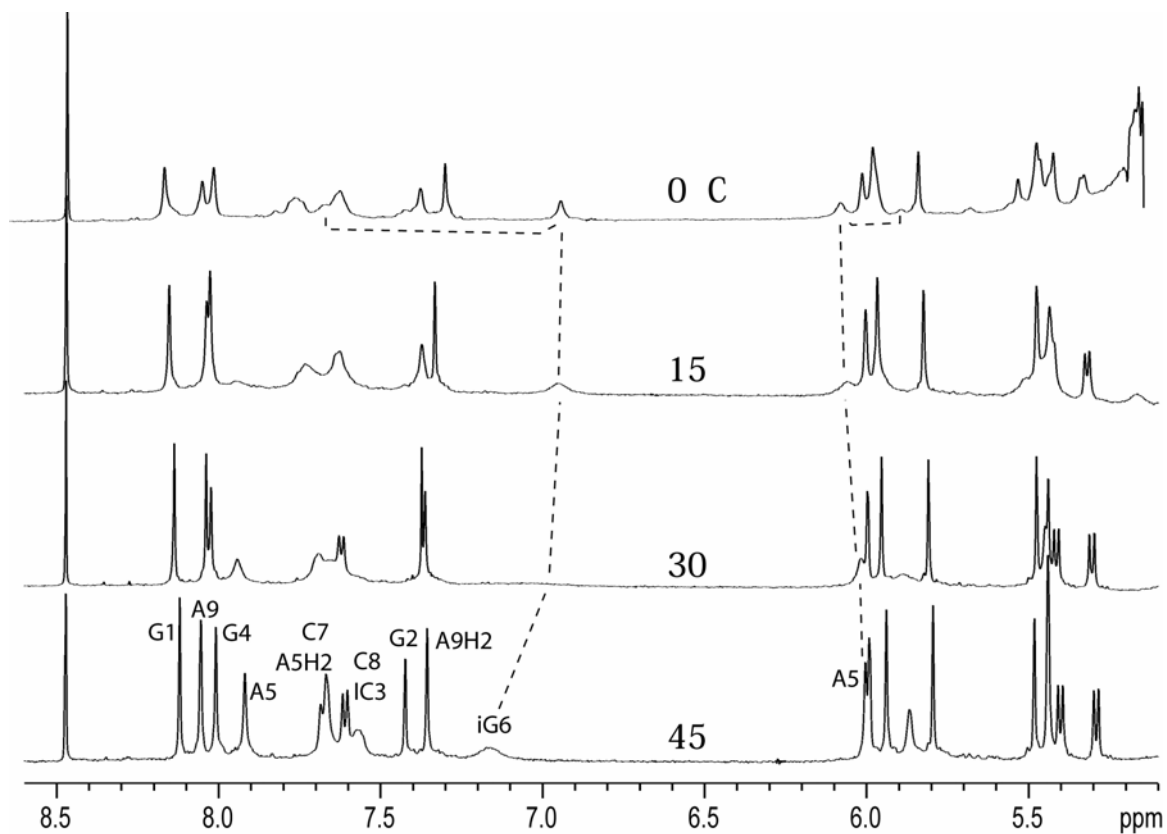


Figure S2) Aromatic and H1'/H5 region of proton spectra of (GGiCGAiGCCA)₂ in D₂O at the indicated temperatures. At low temperature major and minor conformations are observed for some resonances, indicated here for iG6H8 and A5H1', which become one average resonance at high temperature.

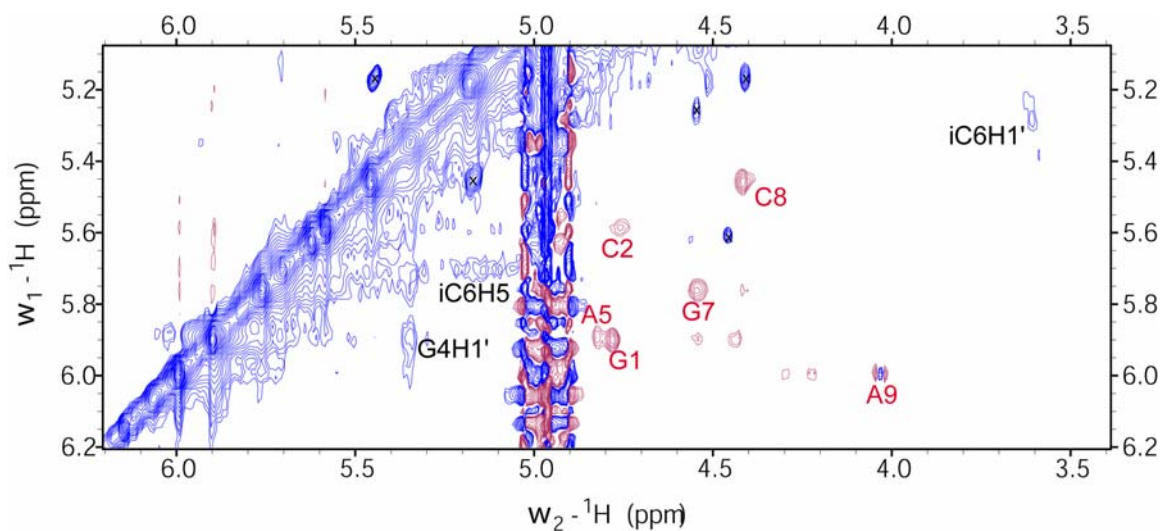


Figure S3) 2D ROESY of $(GCiGGAiCGCA)_2$ at 5°C . The mixing time is 25 ms. Maroon colored peaks have opposite sign with respect to blue peaks. Through-space interactions in ROESY spectra result in cross-peaks with opposite sign relative to peaks on the diagonal. Blue cross-peaks with black labels are direct exchange cross peaks. Maroon cross-peaks with red labels are $\text{H1}'\text{-H2}'$ ROE cross-peaks from the major conformation. The ROE cross-peak for $iC6H1'$ is outside the plotted region and the cross-peaks for $iG3H1'\text{-H2}'$ and $G4H1'\text{-H2}'$ are below the lowest contour. Narrow, blue cross-peaks marked with an 'x' involve scalar coupling within an impurity.

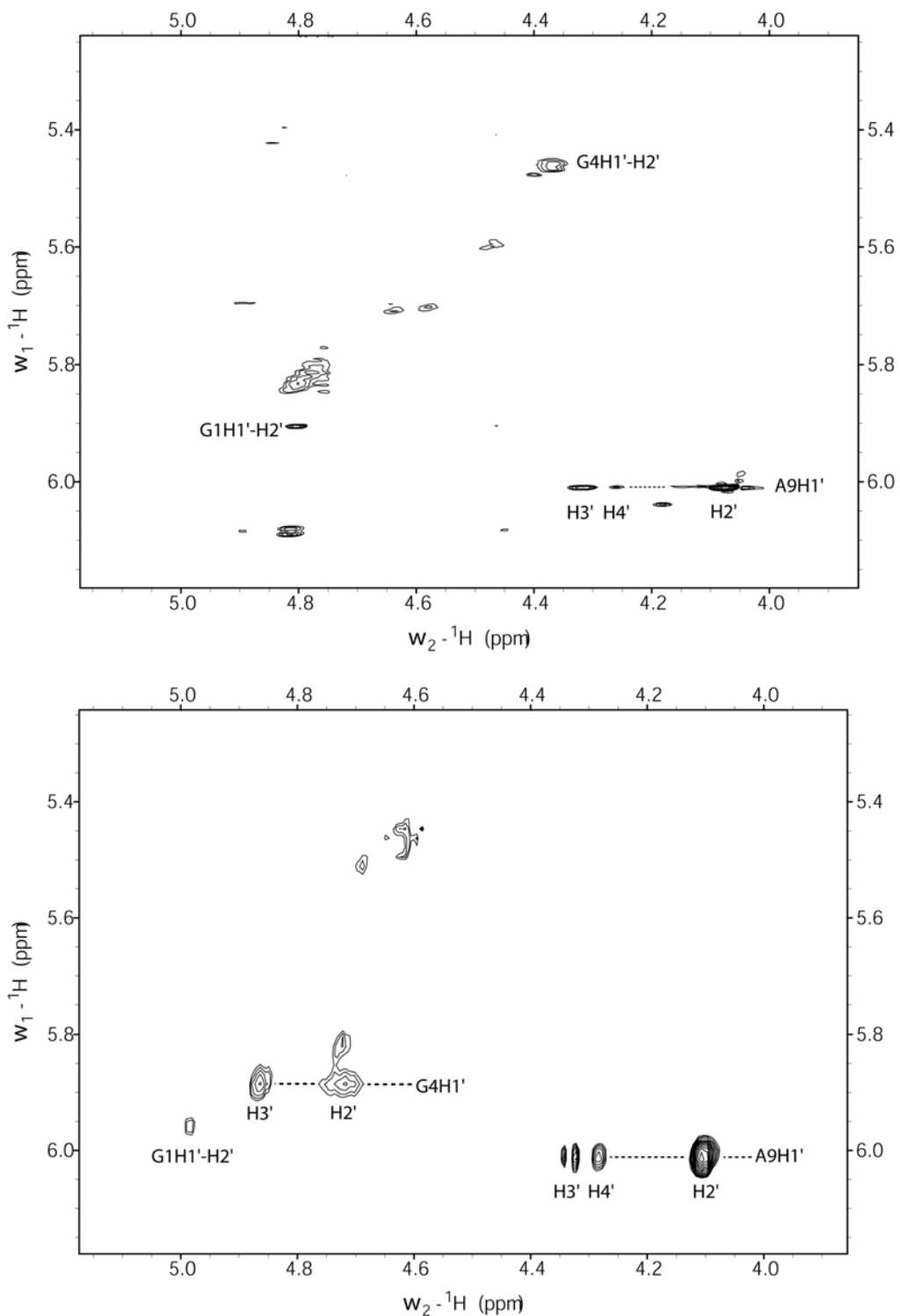


Figure S4) 2D ^1H - ^1H TOCSY of $(\text{GCiGGAiCGCA})_2$ at 30°C with 40 ms mixing time (top) and $(\text{GGiCGAiGCCA})_2$ at 42°C with 100 ms mixing time (bottom) showing weak scalar coupling between $\text{G4H1}'$ and $\text{G4H2}'$. Unlabeled cross-peaks are not associated with the duplexes and are most likely due to single-strand RNA present at temperatures approaching the melting temperature.