Supporting Information for

NMR Structure of Prohead RNA E-loop Hairpin

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NMR Data Collection

Exchangeable proton spectra were taken at 1 ^{o}C using Watergate or SSnoesy pulse sequences. NOESY spectra were recorded at 50, 100, and 200 ms at 1 ^{o}C for exchangeable protons with a delay time of 1 s, 256 increments in the T1dimension with 1024 data points and 128 scans per FID. Zero filling was used to finish the T1 matrix and had a 20 ppm spectral sweep width. Apodization was done using sine bell shift weighting. The non-exchangeable proton spectra were recorded at 20 and 25 ^{o}C using RNA tnnoesy pulse sequence with presaturation water suppression. NOESY spectra were collected at 50, 100, 150 and 400 ms at 25 ^{o}C with a delay time of 1 s, 300 increments in the T1 dimension with 2048 data points and 30 scans per FID. Zero filling was used to finish the T1 matrix and the spectral sweep width was 8.5 ppm. Apodization was done using sine bell shift weighting. DQF-COSY experiments were performed at 25 ^{o}C with 300 increments in the T1 dimension with 2048 data points and 48 scans per FID. The spectral sweep width was set to 8.5 ppm and the delay time was 1 s. Apodization was done using gaussian functions.

The ^{1}H-^{13}C HSQC data were recorded using a spectral sweep width of 12 ppm for the ^{1}H and 45 ppm for the ^{13}C dimension with a 1 s delay time. There were 32 T1 increments and 500 scans with 2048 points. The data were apodized with a sine bell shifted function in both directions.

The ^{1}H-^{31}P HETCOR spectra were collected using a Varian 600 MHz spectrometer with a 5 ppm spectral width in both the ^{1}H and the ^{31}P dimension Data was collected in 512 T1 increments, 128 scans, and 2048 data points. Apodization was done using sine bell shift weighting.

Structure Calculation Methods

In the high temperature annealing stage, the temperature was raised to 20000 K with bond, angle, and improper restraints on and NOE and dihedral angle restraints set to 150 kcal/mol $Å^2$ and 5 kcal/mol rad ^{2}, respectively. The first slow-cool annealing stage was done using torsion dynamics with the electrostatics off except between hydrogens and other atoms, which were set to 10%. The temperature was cooled in 125 K temperature steps with the NOE restraints held at 150 kcal/mol $Å^2$ and the dihedral angle restraints set to 200 kcal/mol rad^2. The system was allowed to develop for 25 ps using 6000 MD steps at each temperature. The second slow cooled annealing stage was done in Cartesian space and allowed the system to continue from 2000 K to 0 K. The temperature steps were set to 15 K and allowed to develop for 15 ps while the van der Waals scale factor increased linearly from 1 to 4. All other parameters were unchanged during the second annealing stage. The final energy minimization was done by applying Powell energy minimization with the van der Waals scale factor at 1 and the electrostatic terms at 100% using 30 cycles of minimization each with 2000 MD steps. The scale factor for the Powell NOE and dihedral angles were 75 kcal/mol $Å^2$ and 400 kcal/mol rad^2, respectively. Planarity (150 kcal/mol $Å^2$) as well as hydrogen bond distance restraints were applied to the stem region of the hairpin.

SI Table 1: Proton Resonance Assignments

	H6/H8	H2/H5	H1'	H2'	H3'	H4'	H1/H3 imino	H2/H4/H6 amino
G1	8.215	-	5.945	4.953	4.612		11.74	7.473/5.883
G2	7.554	-	5.847	4.501	4.185	4.185	12.36	8.034/6.254
U3	7.791	5.214	5.568	4.527	4.659		13.86	-
G4	7.739	-	5.831	4.591	4.687	4.166	13.16	8.419
A5	7.639	7.888	5.859	4.566	4.286		-	7.294/6.855
U6	7.495	5.271	5.115	4.007	4.413	4.116	11.23	-
U7	7.54	5.291	5.506	4.271	4.501		10.72	-
G8	7.919	-	5.55	4.603	4.283	4.030	Not Assigned	7.884/7.124
A9	8.067	8.409	6.188	4.603	5.000		-	Not Assigned
G10	8.203	-	6.194	5.028	3.91	3.548	Not Assigned	Not Assigned
U11	7.39	5.822	4.8	5.009	4.349	3.978	11.64	-
U12	7.788	4.978	5.677	4.502	4.363	4.133	14.42	-
C13	7.77	5.785	5.569	4.482	4.230		-	8.419/7.027
A14	8.203	7.389	5.907	4.468	4.137	3.992	-	7.953/6.596
C15	7.623	5.242	5.427	4.22	4.38	4.068	-	8.272/7.002
C16	7.623	5.428	5.478	4.396	4.509	4.075	-	8.616/6.665
A17	8.098	7.450	5.990	4.066	4.308	4.260	-	Not Assigned

SI Table 1: Proton chemical shift assignments. The protons were referenced to water, which was referenced to 3-trimethylsilyl propionic acid in 10 mM phosphate buffer pH 6. The non exchangeable protons were assigned in experiments at 20 ^{o}C and the exchangeable protons were assigned in experiments conducted at 1 ^{o}C. G10 H5' and G10 H5'' resonate at 4.285 and 4.358 ppm, respectively, and no other H5' protons were specifically assigned.

SI Table 2: Carbon and Phosphorus Resonance Assignments

	A C2	C5	C6/C8	C1'	C2'	C3'	P
G1	-	-	143.3	93.67	71.9	74.7	-0.3534
G2	-	-	146	93.38	71.8	74.6	-0.5613
U3	-	103.3	144.9	93.63	71.7	75	-0.9328
G4	-	-	139.9	92.36	71.4	74.1	-0.4403
A5	157.6	-	142.6	93.25	71.3	74.2	-0.4206
U6	-	103	143.6	93.37	71.4	75.4	-0.672
U7	-	104.1	140.3	94.27	74.1	74.5	-0.8072
G8	-	-	146	93.86	71.7	77.2	-1.189
A9	157.7	-	143.2	87.9	73.1	74.5	-1.536
G10	-	-	143.3	93.31	71.8	78.5	-0.4017
U11	-	105.6	146.7			74.8	-0.3003
U12	-	103.2	144.9	92.97	71.1	73.5	0.8143
C13	-	98.93	145	94.59	71.3	73.7	-0.9186
A14	156.6	-	142.3	92.95	71.5	74.9	-0.3293
C15	-	97.24	144.4	93.65	71.2	75.3	-0.3391
C16	-	98.18	144.3	94.25	72.1	74.9	-0.6962
A17	156.8/144	-	144.2	92.10	69	76.8	-0.6742

SI Table 2: Carbon and phosphorus chemical shift assignments. The carbon resonances were referenced to an external standard and the phosphorus resonances were referenced to phosphate buffer at pH 6.

SI Table 3: NOE assignments

(G1 H22)	(G1 H1)	3.0, 1.0, 1.5
(G1 H22)	(G1 H21)	2.1, 0.6, 0.9
(G1 H21)	(G1 H1)	3.0, 1.0, 1.5
(G1 H1')	(G1 H2')	2.1, 0.6, 0.9
(G1 H1')	(G1 H3')	4.0, 1.5, 2.0
(G1 H2')	(G1 H3')	2.1, 0.6, 0.9
(G1 H8)	(G1 H1')	4.0, 1.5, 2.0
(G1 H8)	(G1 H2')	3.0, 1.0, 1.5
(G1 H8)	(G1 H3')	4.0, 1.5, 2.0
(G1 H8)	(G2 H8)	4.0, 1.5, 2.0
(G1 H1')	(G2 H8)	4.0, 1.5, 2.0
(G1 H2')	(G2 H8)	3.0, 1.0, 1.5
(G1 H3')	(G2 H8)	4.0, 1.5, 2.0
(G1 H1)	(C16 H42)	3.0, 1.0, 1.5
(G1 H1)	(C16 H41)	3.0, 1.0, 1.5
(G1 H1')	(A17 H2)	4.0, 1.5, 2.0
(G2 H22)	(G2 H1)	3.0, 1.0, 1.5
(G2 H22)	(G2 H21)	2.1, 0.6, 0.9
(G2 H21)	(G2 H1)	3.0, 1.0, 1.5
(G2 H1')	(G2 H2')	2.1, 0.6, 0.9
(G2 H2')	(G2 H3')	2.1, 0.6, 0.9
(G2 H8)	(G2 H1')	4.0, 1.5, 2.0
(G2 H8)	(G2 H2')	3.0, 1.0, 1.5
(G2 H8)	(G2 H3')	4.0, 1.5, 2.0
(G2 H2')	(U3 H5)	4.0, 1.5, 2.0
(G2 H1')	(U3 H6)	4.0, 1.5, 2.0
(G2 H2')	(U3 H6)	3.0, 1.0, 1.5
(G2 H1)	(C15 H42)	3.0, 1.0, 1.5
(G2 H1)	(C15 H41)	3.0, 1.0, 1.5
(U3 H1')	(U3 H2')	2.1, 0.6, 0.9
(U3 H6)	(U3 H1')	4.0, 1.5, 2.0
(U3 H6)	(U3 H3')	4.0, 1.5, 2.0
(U3 H6)	(U3 H5)	2.1, 0.6, 0.9
(U3 H6)	(U3 H2')	2.1, 0.6, 0.9
(U3 H3)	(G4 H1)	3.0, 1.0, 1.5
(U3 H1')	(G4 H8)	4.0, 1.5, 2.0
(U3 H2')	(G4 H8)	3.0, 1.0, 1.5
(U3 H3)	(A14 H2)	3.0, 1.0, 1.5
(G4 H1')	(G4 H2')	2.1, 0.6, 0.9
(G4 H2')	(G4 H3')	2.1, 0.6, 0.9

(G4 H8)	(G4 H1')	4.0, 1.5, 2.0
(G4 H8)	(G4 H2')	3.0, 1.0, 1.5
(G4 H8)	(G4 H3')	4.0, 1.5, 2.0
(G4 H8)	(G4 H4')	4.0, 1.5, 2.0
(G4 H1')	(A5 H8)	4.0, 1.5, 2.0
(G4 H2')	(A5 H8)	3.0, 1.0, 1.5
(G4 H3')	(A5 H8)	4.0, 1.5, 2.0
(G4 H1)	(C13 H41)	3.0, 1.0, 1.5
(G4 H1)	(C13 H42)	3.0, 1.0, 1.5
(A5 H1')	(A5 H2')	2.1, 0.6, 0.9
(A5 H1')	(A5 H3')	3.0, 1.0, 1.5
(A5 H62)	(A5 H61)	2.1, 0.6, 0.9
(A5 H2)	(A5 H1')	4.0, 1.5, 2.0
(A5 H8)	(A5 H1')	4.0, 1.5, 2.0
(A5 H8)	(A5 H3')	3.0, 1.0, 1.5
(A5 H2')	(U6 H5)	4.0, 1.5, 2.0
(A5 H3')	(U6 H5)	4.0, 1.5, 2.0
(A5 H2)	(U6 H1')	4.0, 1.5, 2.0
(A5 H1')	(U6 H6)	4.0, 1.5, 2.0
(A5 H2')	(U6 H6)	3.0, 1.0, 1.5
(A5 H3')	(U6 H6)	4.0, 1.5, 2.0
(A5 H2)	(U12 H3)	3.0, 1.0, 1.5
(A5 H62)	(U12 H3)	3.0, 1.0, 1.5
(A5 H61)	(U12 H3)	3.0, 1.0, 1.5
(A5 H2)	(C13 H1')	4.0, 1.5, 2.0
(U6 H1')	(U6 H2')	2.1, 0.6, 0.9
(U6 H1')	(U6 H3')	4.0, 1.5, 2.0
(U6 H6)	(U6 H1')	4.0, 1.5, 2.0
(U6 H6)	(U6 H2')	3.0, 1.0, 1.5
(U6 H6)	(U6 H3')	4.0, 1.5, 2.0
(U6 H6)	(U6 H4')	4.0, 1.5, 2.0
(U6 H6)	(U6 H5)	2.1, 0.6, 0.9
(U6 H2')	(U7 H2')	4.0, 1.0, 1.5
(U6 H2')	(U7 H5)	4.0, 1.0, 1.5
(U6 H1')	(U7 H6)	4.0, 1.5, 2.0
(U6 H2')	(U7 H6)	3.0, 1.0, 1.5
(U6 H3)	(U11 H3)	2.5, 0.7, 1.2
(U6 H3)	(U12 H3)	4.0, 1.0, 1.5
(U7 H6)	(U7 H1')	4.0, 1.5, 2.0
(U7 H6)	(U7 H2')	3.0, 1.0, 1.5

SI Table 3: NOE assignments (cont.)

(U7 H6)	(U7 H3')	4.0, 1.5, 2.0
(U7 H6)	(U7 H5)	2.1, 0.6, 0.9
(U7 H6)	(U7 H51)	4.0, 1.5, 2.0
(U7 H1')	(A9 H8)	4.0, 1.5, 2.0
(U7 H2')	(A9 H8)	4.0, 1.5, 2.0
(U7 H3')	(A9 H8)	4.0, 1.5, 2.0
(U7 H51)	(A9 H8)	4.0, 1.5, 2.0
(U7 H3)	(U11 H3)	3.5, 0.8, 2.0
(G8 H21)	(G8 H22)	2.1, 0.6, 0.9
(G8 H1')	(G8 H2')	2.1, 0.6, 0.9
(G8 H1')	(G8 H3')	4.0, 1.5, 2.0
(G8 H3')	(G8 H2')	2.1, 0.6, 0.9
(G8 H4')	(G8 H3')	3.0, 1.0, 1.5
(G8 H51)	(G8 H4')	3.0, 1.0, 1.5
(G8 H8)	(G8 H1')	3.0, 1.0, 1.0
(G8 H8)	(G8 H2')	3.5, 1.0, 1.5
(G8 H8)	(G8 H3')	3.0, 1.0, 1.2
(G8 H8)	(G8 H4')	4.0, 1.0, 1.0
(G8 H8)	(G8 H5')	3.0, 1.0, 1.0
(G8 H1')	(A9 H8)	4.0, 1.5, 2.0
(G8 H2')	(A9 H5')	2.2, 1.0, 1.0
(G8 H2')	(A9 H8)	3.0, 1.0, 1.5
(G8 H4')	(A9 H8)	4.0, 1.5, 2.0
(A9 H1)	(A9 H2')	2.1, 0.6, 0.9
(A9 H3')	(A9 H2')	3.0, 1.0, 1.5
(A9 H8)	(A9 H1')	4.0, 1.5, 2.0
(A9 H8)	(A9 H2')	3.0, 1.0, 1.5
(A9 H8)	(A9 H3')	2.1, 0.6, 0.6
(A9 H2)	(U11 H4')	4.0, 1.0, 2.0
(G10 H1')	(G10 H2')	2.1, 0.6, 0.9
(G10 H1')	(G10 H3')	4.0, 1.5, 2.0
(G10 H1')	(G10 H52)	4.0, 1.5, 2.0
(G10 H1')	(G10 H51)	4.0, 1.5, 2.0
(G10 H3')	(G10 H4')	3.0, 1.0, 1.5
(G10 H51)	(G10 H3')	4.0, 1.5, 2.0
(G10 H51)	(G10 H4')	3.0, 1.0, 1.5
(G10 H8)	(G10 H1')	4.0, 1.5, 2.0
(G10 H8)	(G10 H2')	3.0, 1.0, 1.5
(G10 H8)	(G10 H51)	3.0, 1.5, 1.5
(G10 H8)	(U11 H5)	4.0, 1.5, 2.0

(G10 H1')	(U11 H6)	4.0, 1.5, 2.0
(G10 H2')	(U11 H5)	3.0, 1.5, 2.0
(G10 H2')	(U11 H6)	3.0, 1.5, 1.0
(G10 H3')	(U11 H6)	4.0, 1.5, 2.0
(G10 H52)	(U11 H6)	4.0, 1.5, 2.0
(G10 H51)	(U11 H6)	4.0, 1.5, 2.0
(G10 H52)	(U11 H5)	4.0, 1.5, 2.0
(G10 H51)	(U11 H5)	4.0, 1.5, 2.0
(G10 H8)	(U11 H4')	4.0, 1.5, 2.0,
(G10 H8)	(U11 H5)	4.0, 1.5, 2.0
(U11 H2')	(U11 H3')	3.0, 1.0, 1.5
(U11 H6)	(U11 H2')	3.0, 1.0, 1.5
(U11 H6)	(U11 H3')	4.0, 1.5, 2.0
(U11 H6)	(U11 H4')	4.0, 1.5, 2.0
(U11 H6)	(U11 H5)	2.1, 0.6, 0.9
(U11 H3)	(U12 H3)	3.0, 1.0, 1.5
(U11 H4')	(U12 H6)	4.0, 1.5, 2.0
(U11 H2')	(U12 H4')	4.0, 1.5, 2.0
(U12 H1')	(U12 H2')	2.1, 0.6, 0.9
(U12 H1')	(U12 H3')	4.0, 1.5, 2.0
(U12 H6)	(U12 H1')	4.0, 1.5, 2.0
(U12 H6)	(U12 H2')	3.0, 1.0, 1.5
(U12 H6)	(U12 H3')	4.0, 1.5, 2.0
(U12 H6)	(U12 H4')	4.0, 1.5, 2.0
(U12 H6)	(U12 H5)	2.1, 0.6, 0.9
(C13 H42)	(C13 H41)	2.1, 0.6, 0.9
(C13 H1')	(C13 H2')	2.1, 0.6, 0.9
(C13 H2')	(C13 H3')	2.1, 0.6, 0.9
(C13 H6)	(C13 H1')	4.0, 1.5, 2.0
(C13 H6)	(C13 H2')	3.0, 1.0, 1.5
(C13 H6)	(C13 H3')	4.0, 1.5, 2.0
(C13 H6)	(C13 H5)	2.1, 0.6, 0.9
(C13 H1')	(A14 H8)	4.0, 1.5, 2.0
(C13 H2')	(A14 H8)	3.0, 1.0, 1.5
(A14 H1')	(A14 H2')	2.1, 0.6, 0.9
(A14 H2)	(A14 H1')	4.0, 1.5, 2.0
(A14 H3')	(A14 H4')	3.0, 1.0, 1.5
(A14 H8)	(A14 H1')	4.0, 1.5, 2.0
(A14 H8)	(A14 H3')	3.0, 1.0, 1.5
(A14 H8)	(A14 H4')	4.0, 1.5, 2.0

SI Table 3: NOE assignments (cont.)

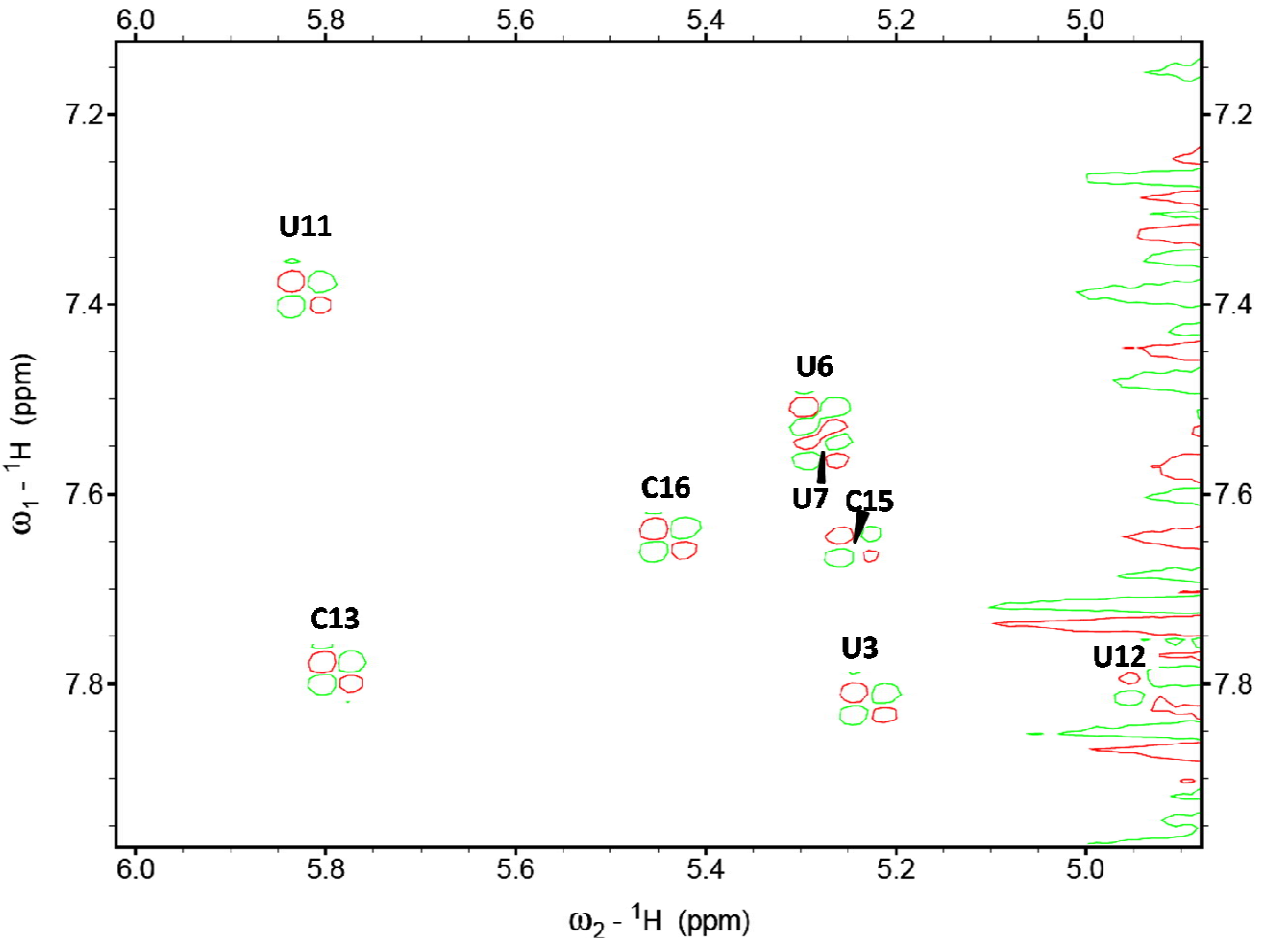
(A14 H2')	(C15 H6)	3.0, 1.0, 1.5
(A14 H1')	(C15 H6)	4.0, 1.5, 2.0
(A14 H2)	(C15 H1')	4.0, 1.5, 2.0
(A14 H8)	(C15 H6)	4.0, 1.5, 2.0
(C15 H1')	(C15 H2')	2.1, 0.6, 0.9
(C15 H42)	(C15 H41)	2.1, 0.6, 0.9
(C15 H1')	(C15 H3')	4.0, 1.5, 2.0
(C15 H3')	(C15 H2')	3.0, 1.0, 1.5
(C15 H3')	(C15 H4')	3.0, 1.0, 1.5
(C15 H6)	(C15 H1')	4.0, 1.5, 2.0
(C15 H6)	(C15 H3')	3.0, 1.0, 1.5
(C15 H6)	(C15 H5)	3.0, 1.0, 1.5
(C15 H3')	(C16 H3')	4.0, 1.5, 2.0
(C15 H2')	(C16 H6)	3.0, 1.0, 1.5
(C16 H1')	(C16 H2')	2.1, 0.6, 0.9
(C16 H3')	(C16 H4')	3.0, 1.0, 1.5
(C16 H6)	(C16 H1')	3.0, 1.0, 1.5
(C16 H6)	(C16 H3')	3.0, 1.0, 1.5
(C16 H6)	(C16 H4')	3.0, 1.0, 1.5
(C16 H6)	(C16 H5)	2.1, 0.6, 0.9
(C16 H5)	(C16 H6)	2.1, 0.6, 0.9
(C16 H5)	(C16 H42)	3.0, 1.0, 1.5
(C16 H5)	(C16 H41)	3.0, 1.0, 1.5
(C16 H42)	(C16 H41)	2.1, 0.6, 0.9
(C16 H1')	(A17 H8)	4.0, 1.5, 2.0
(C16 H2')	(A17 H8)	3.0, 1.0, 1.5
(C16 H6)	(A17 H8)	4.0, 1.5, 2.0
(A17 H1')	(A17 H2')	2.1, 0.6, 0.9
(A17 H1')	(A17 H3')	4.0, 1.5, 2.0
(A17 H1')	(A17 H4')	4.0, 1.5, 2.0
(A17 H3')	(A17 H2')	3.0, 1.0, 1.5
(A17 H8)	(A17 H1')	4.0, 1.5, 2.0
(A17 H8)	(A17 H2')	3.0, 1.0, 1.5
(A17 H8)	(A17 H3')	4.0, 1.5, 2.0
(A17 H8)	(A17 H4')	4.0, 1.5, 2.0

SI Table 3: NOEs used for molecular modeling with distance bins for restraints. The first number is the median distance. The second number is the maximum distance below the median, and the third number is the maximum above the median distance.

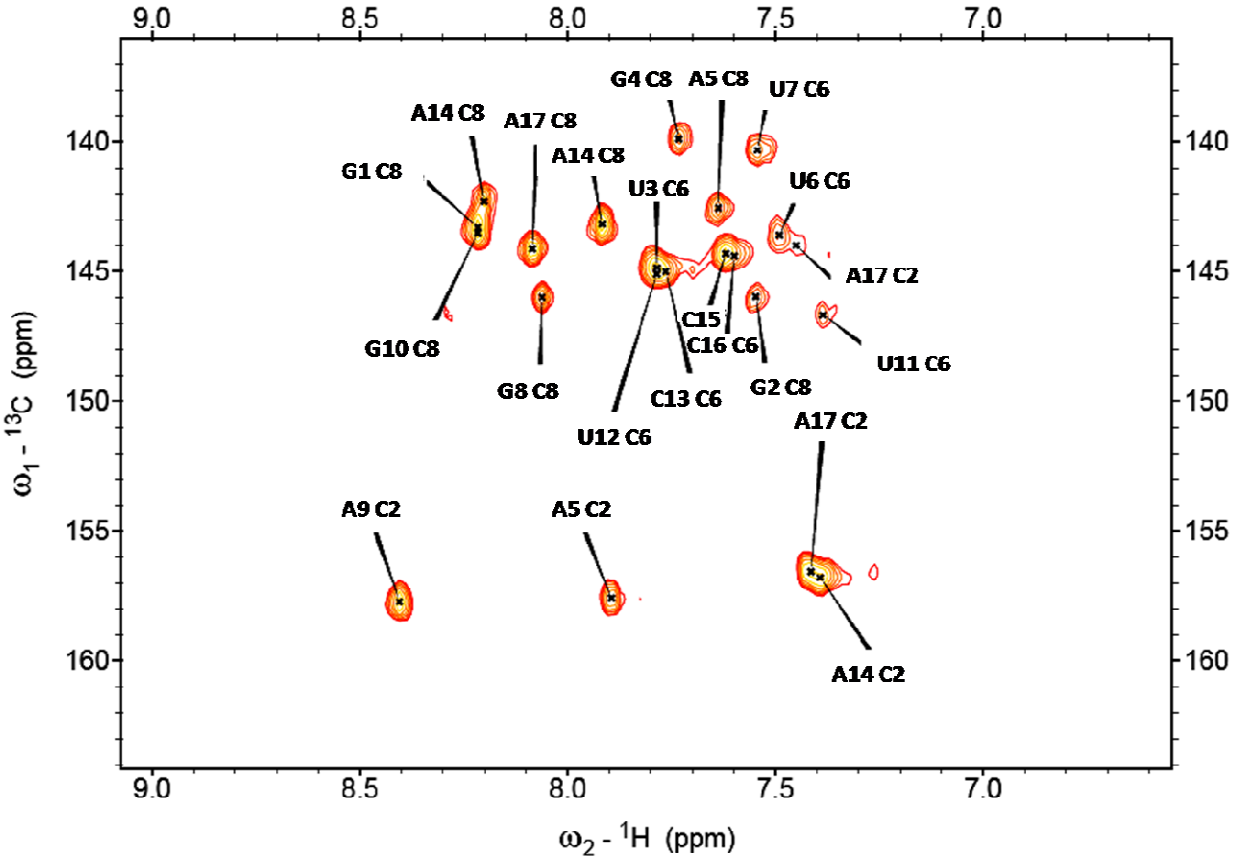
SI Table 4: Restraints for Phi29 E-Loop Hairpin

	Preliminary Refinement			Additional Hairpin Restraints		
Base	**inter**	**Dihedral**	**RMSD (Å)**	**inter**	**Dihedral**	**RMSD (Å)**
G1	7	3	1.71	7	3	1.76
G2	9	5	1.22	9	5	1.38
U3	7	5	1.08	7	5	1.05
G4	8	5	1.06	8	5	1.00
A5	13	3	1.28	13	3	1.08
U6	12	0	1.41	12	0	1.23
U7	9	0	1.63	9	0	1.33
G8	3	0	4.05	**4**	0	3.49
A9	8	0	2.21	**11**	0	1.83
G10	9	0	2.98	9	**2**	2.34
U11	15	0	1.63	**17**	0	1.45
U12	7	3	1.44	7	3	1.35
C13	5	5	0.99	5	5	1.08
A14	7	5	0.85	7	5	0.92
C15	8	5	0.88	8	5	0.90
C16	7	5	1.20	7	5	1.02
A17	4	3	2.03	4	3	2.23
Total	69	47	1.62	72	47	1.50

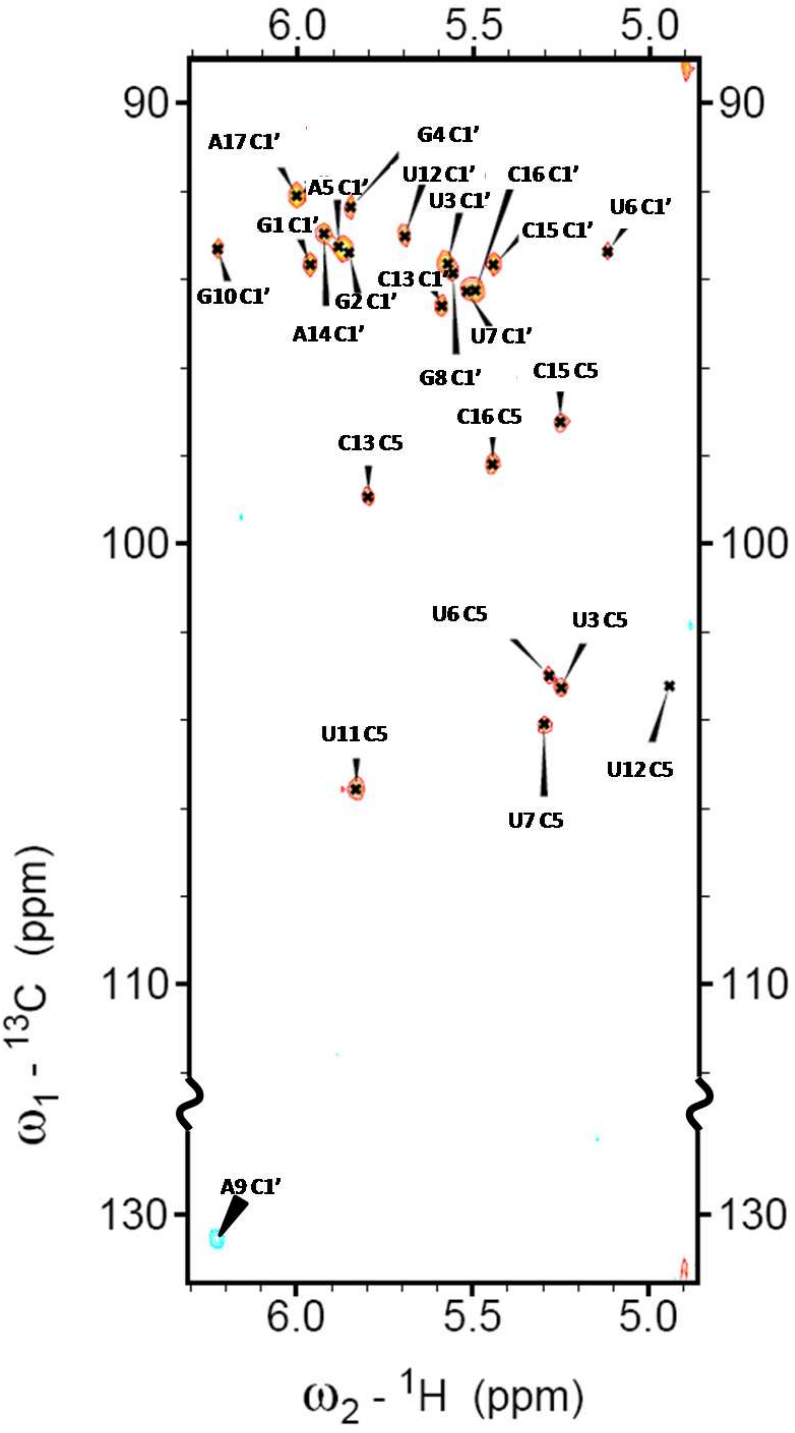
SI Table 4: The conservative preliminary refinement did not restrain any of the hairpin nucleotide backbone angles in order to fully explore conformational space. The additional hairpin restraints include G10 syn, G10 C2'endo, and 3 ribose internucleotide NOEs. These additional restraints improve slightly the rmsd of the structure but do not significantly change the main structural features of the hairpin. Bases are numbered from the 5' to 3' direction. All intranucleotide NOEs are listed in Table 1 of the main text. "Inter" refers to NOES between protons on different nucleotides. Note that each NOE occurs between two protons, so the total internucleotide NOEs are the sum of the internucleotide NOEs for each nucleotide divided by 2. RMSD is an abbreviation for the all-atom root mean square deviation of the structures in the final ensemble.



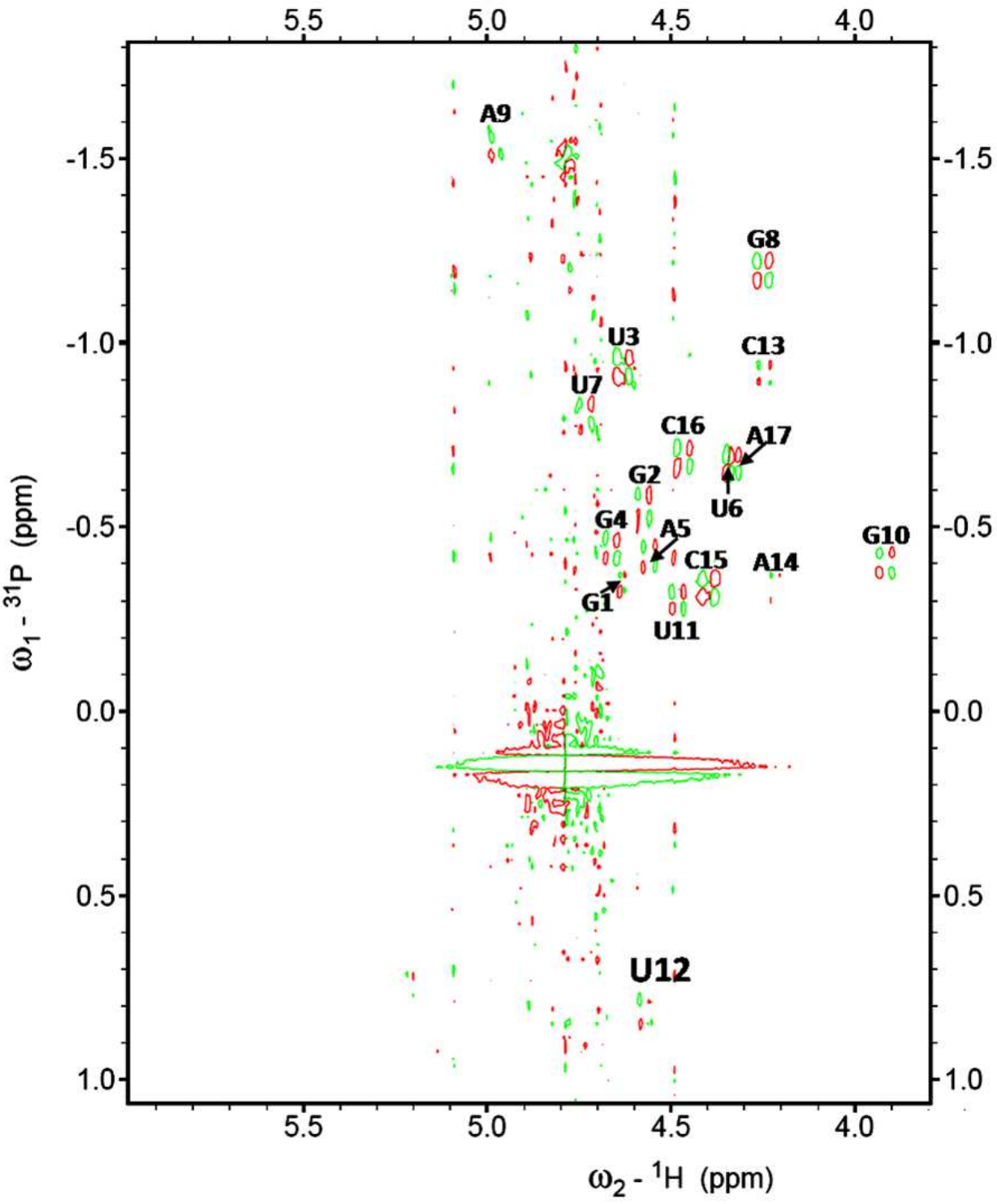
SI Figure 1: COSY spectrum showing H5-H6 pyrimidine crosspeaks. Spectrum was taken at 25 °C in 10mM NaCl, 10 mM $NaPO_4$, and 0.5mM Na_2EDTA in H_2O with 10% D_2O.



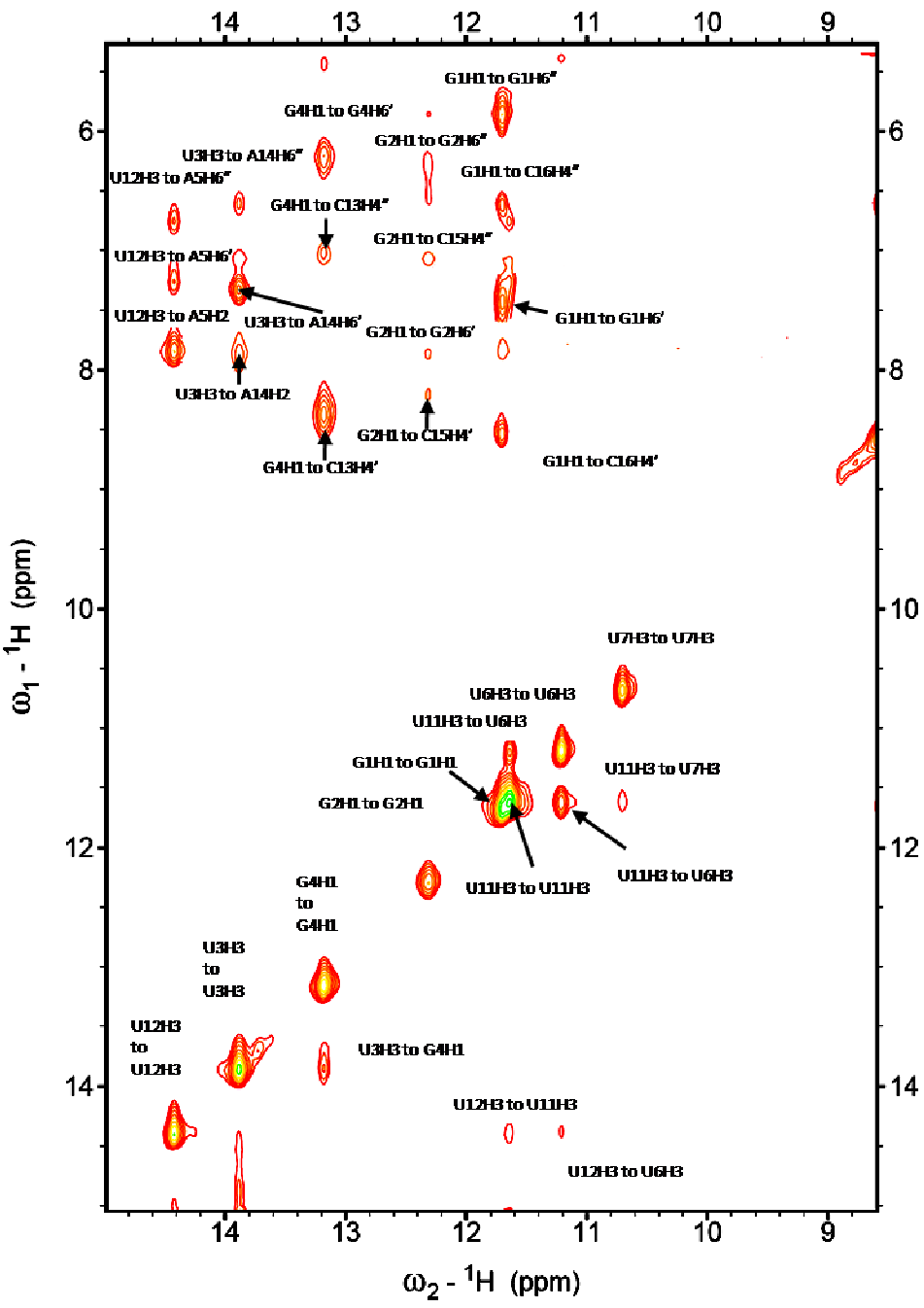
SI Figure 2: Aromatic ^{13}C HSQC showing C2, C6 and C8 carbon resonances. Spectrum was taken at 20 ^{o}C in 10mM NaCl, 10 mM $NaPO_4$, and 0.5mM Na_2EDTA in 100% D_2O.



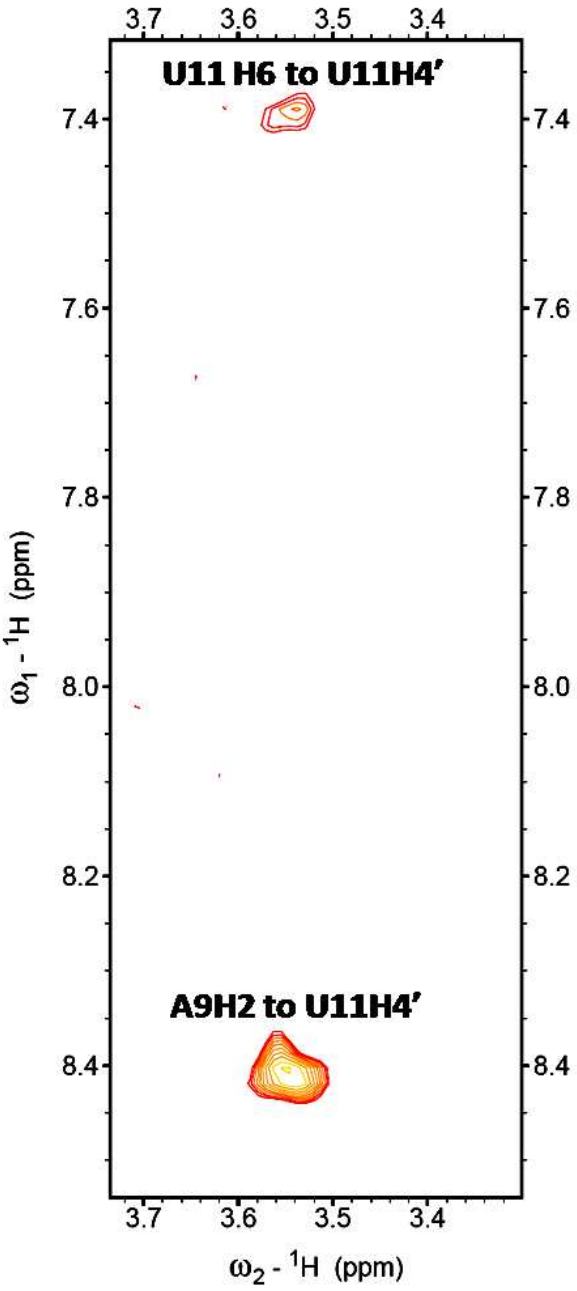
SI Figure 3: Ribose ^{13}C HSQC showing the C1' and C5 region of the spectra. The A9 C1' resonance has been folded in and resonating at ~130 ppm. Spectrum was taken at 20 ^{o}C in 10mM NaCl, 10 mM $NaPO_4$, and 0.5mM Na_2EDTA in 100% D_2O.



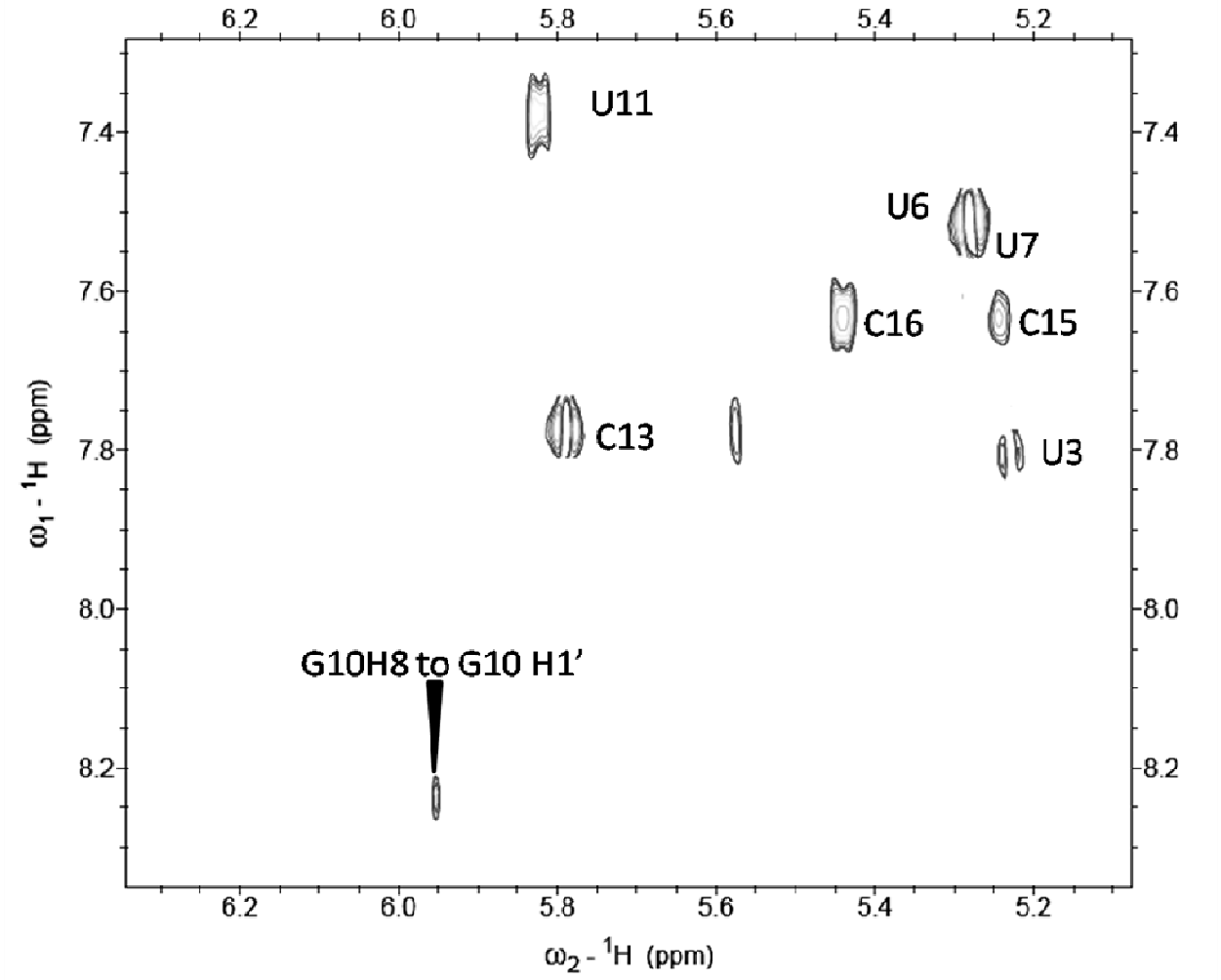
SI Figure 4: ^{31}P HETCOR showing C3' resonances. Spectrum was taken at 20 ^{o}C in 10mM NaCl, 10 mM $NaPO_4$, 0.5mM Na_2EDTA pH 6 in 100% D_2O.



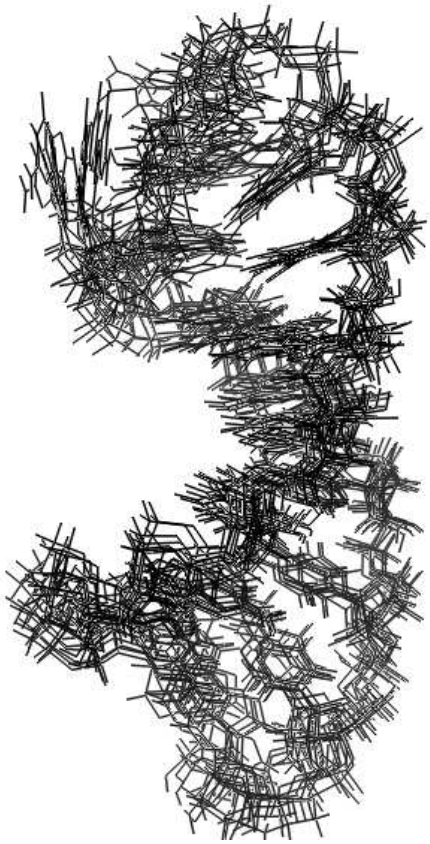
SI Figure 5: H_2O NOESY showing exchangeable proton NOEs. Spectrum was taken at 1 ^{o}C in 10mM NaCl, 10 mM $NaPO_4$, and 0.5mM Na_2EDTA in H_2O with 10% D_2O.



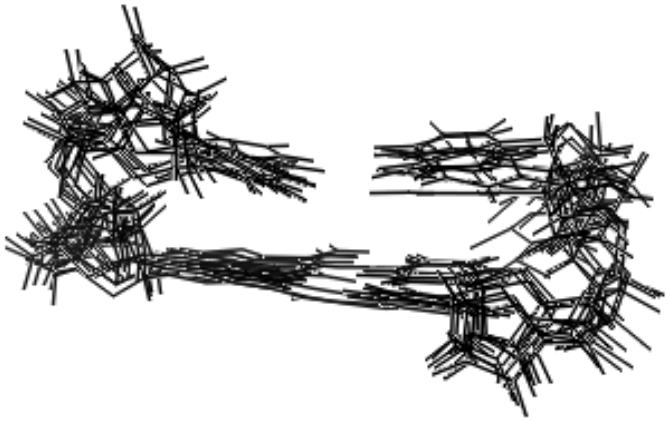
SI Figure 6: Section of the 400 ms NOESY at 20 ^{o}C showing the NOE to A9 H2- U11 H4'.



SI Figure 7: 50 ms D_2O NOESY taken at 20 ^{o} C showing the pyrimidine H5-H6 and the strong G10H8 to G10H1' crosspeaks.

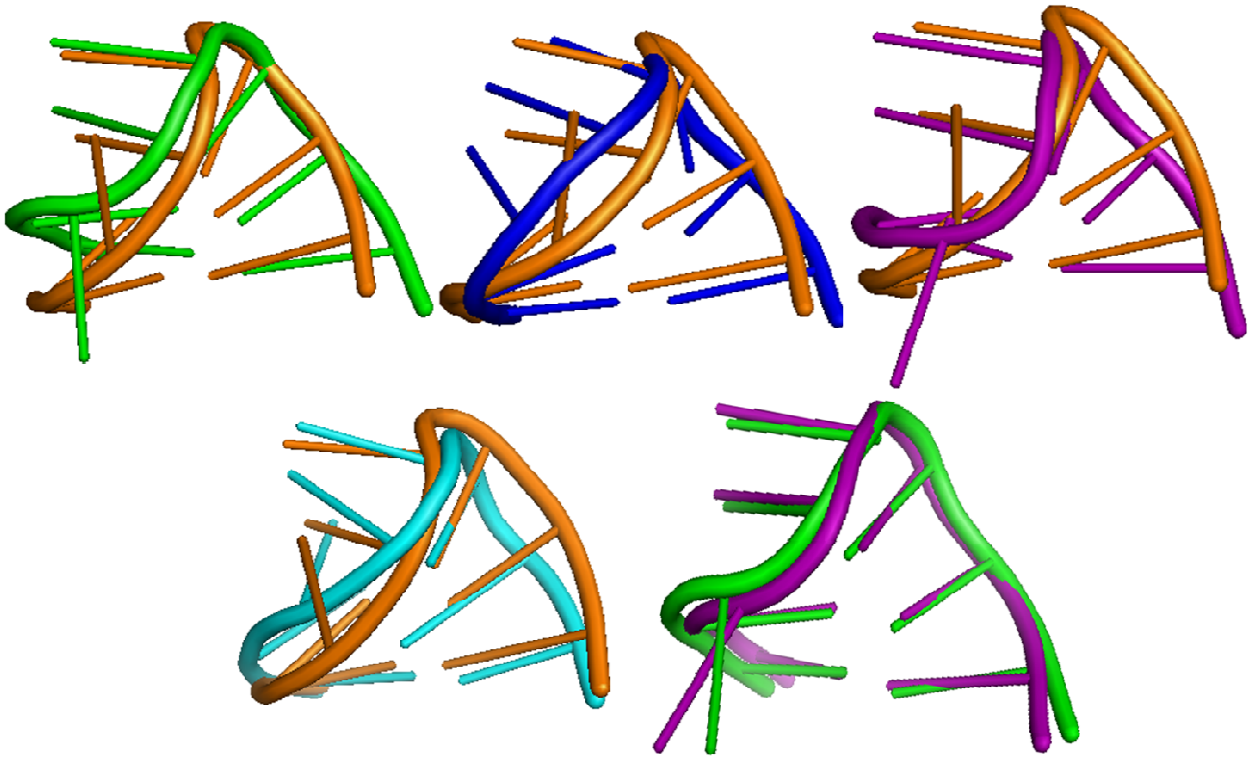


A.



B.

SI Figure 8: A. Overlay of the 10 lowest energy conformers from the ensemble of 31 structures. Note that the ribose backbone for nucleotides G8-U11 comes across the front of the hairpin. B. The U6-U11 base pair stacked on the A5-U12 pair in the overlay of the 10 lowest energy structures.



SI Figure 9: Comparison of the backbones of the different hairpin structures. The orange hairpin is the phi29 NMR structure. The green hairpin is the S2 NMR structure. The blue hairpin is the phi29 MC-SYM structure predicted with no NOEs. The purple hairpin is the phi29 MC-SYM structure predicted with the 2 NOEs that were violated in the predicted structure, A9H2-U11H4' and U7H3-U11H3. The teal hairpin is the GA1 hairpin. Note that the position of G10 in the phi29 hairpin and its equivalent purine nucleotide is less well-defined by NMR data than other nucleotides; and thus the differences in the average position of this dynamic nucleotide that is projecting into solution reflect a lack of NMR restraints rather than a significant structural difference.