**Supporting Information**

# Structural features of a 3′ splice site in influenza A

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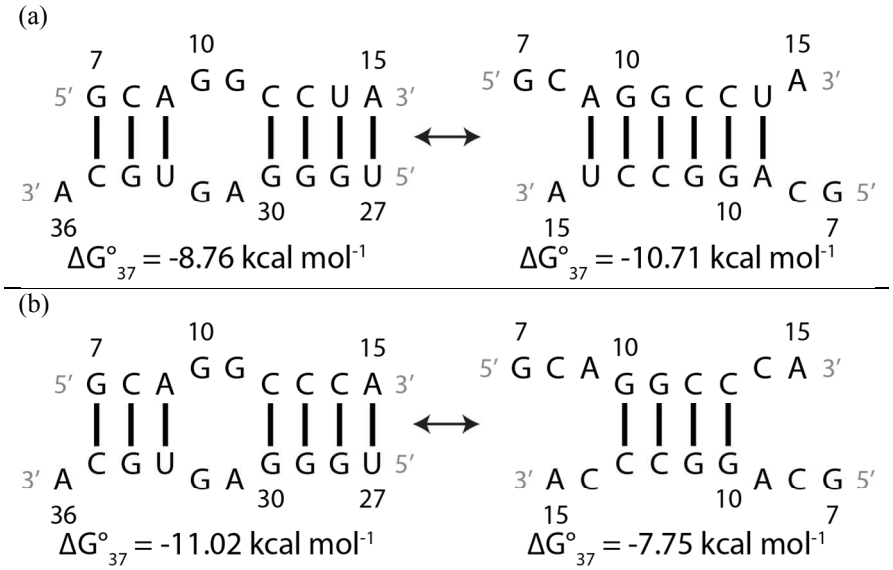



Figure S1: Equilibria of 19-nt duplex mimics of a portion of the 39-nt hairpin with homodimers of the top strands of their duplexes. (a) If residue 14 of a duplex is a uridine, as in the 39-mer, then a homodimer may form that is more stable than the target heterodimer. (b) If residue 14 of a duplex is a cytidine, then the analogous homodimer is less stable than the target heterodimer.

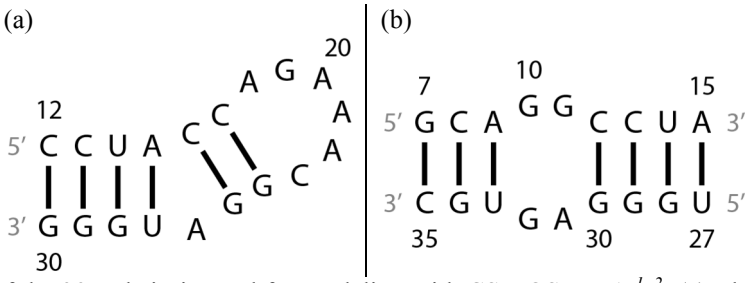



Figure S2: Fragments of the 39-nt hairpin used for modeling with CS-ROSETTA.[1, 2] (a) The 19-nt hairpin with the A26 bulge and hairpin loop. (b) The 18-nt duplex with the 2 × 2 nt internal loop.

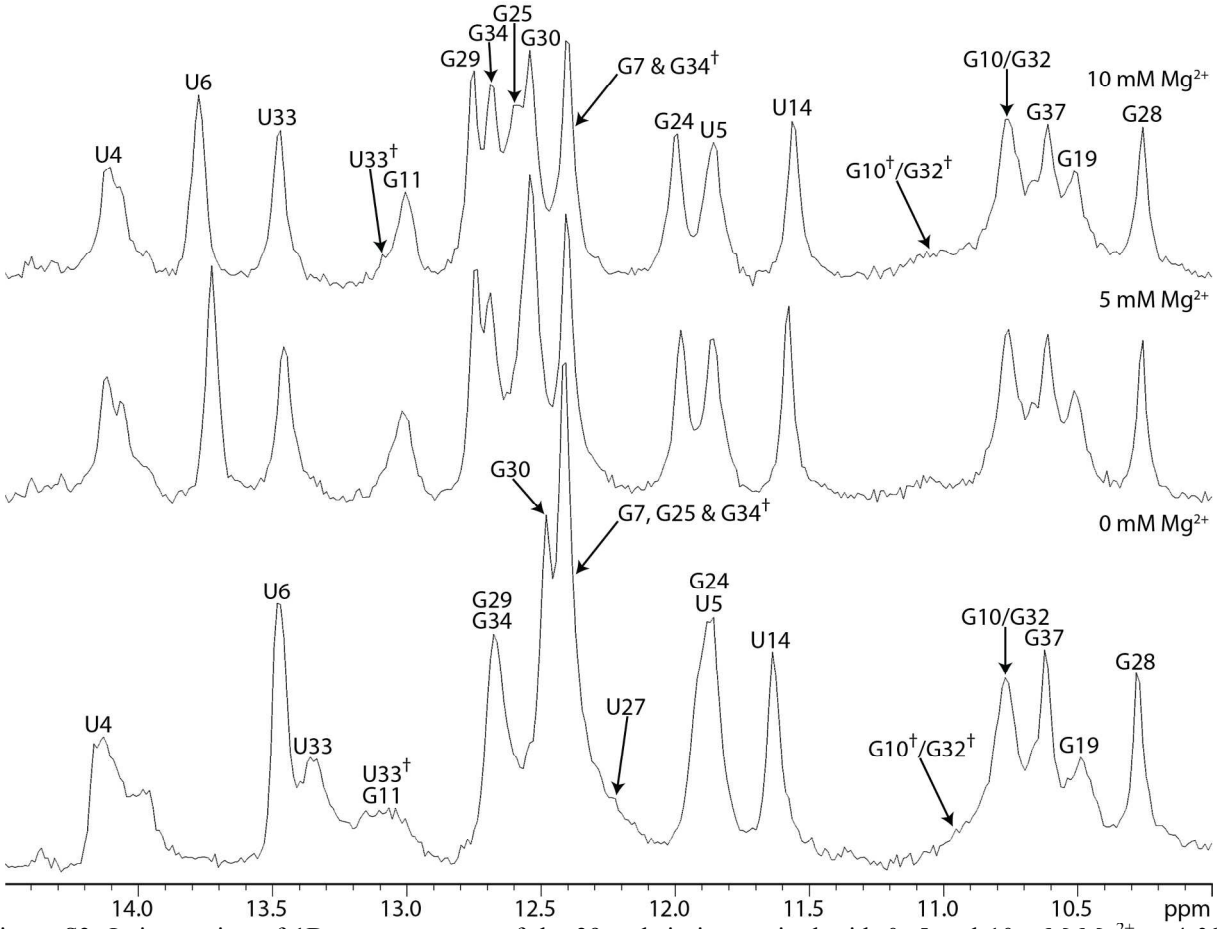



Figure S3: Imino region of 1D proton spectra of the 39-nt hairpin acquired with 0, 5 and 10 mM $Mg^{2+}$ at 4 °C. These spectra were acquired on the 39-nt hairpin synthesized with commercial transcription kits and do not have a signal for U39. † Chemical exchange peak.

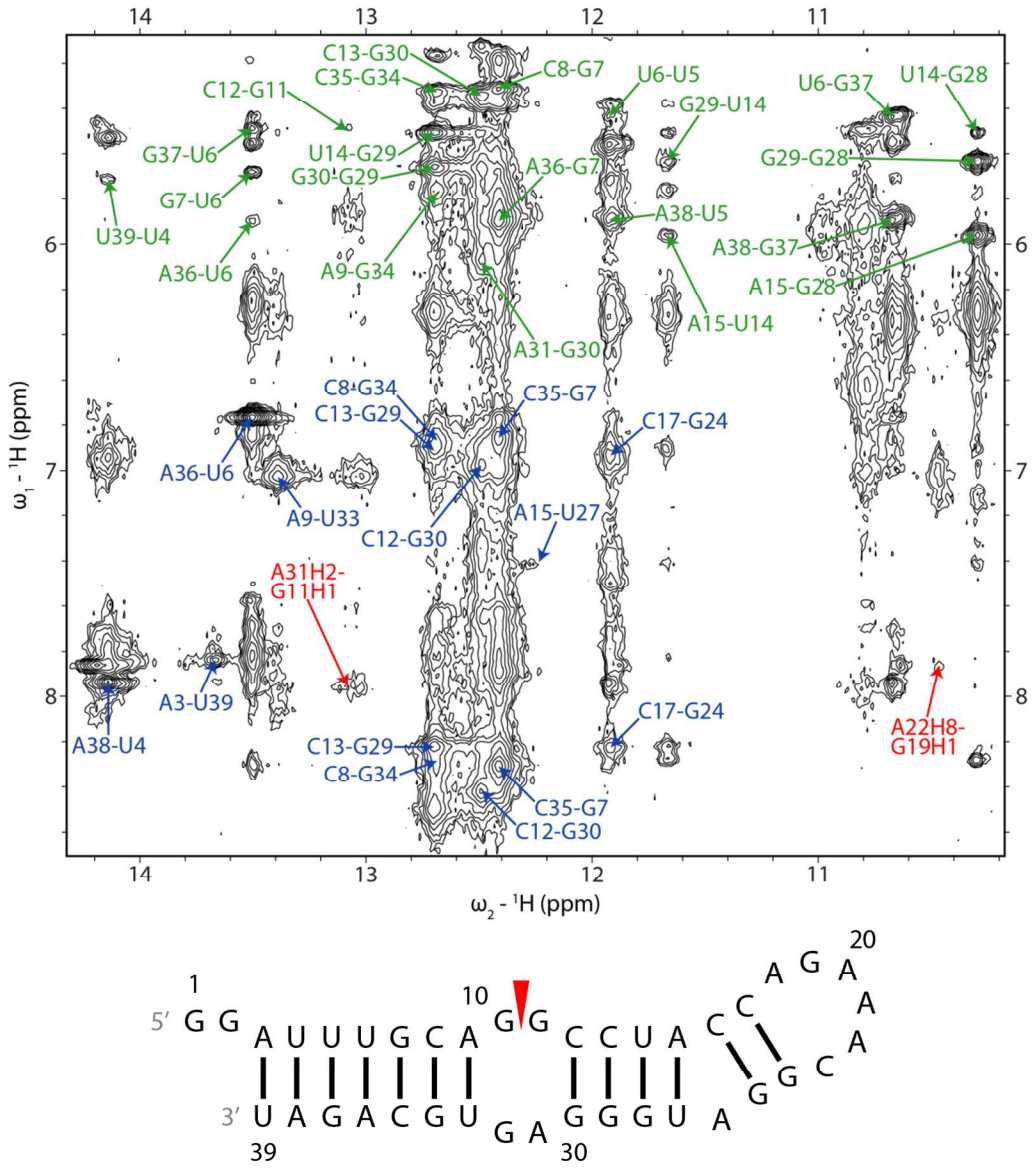



Figure S4: Imino to amino/non-exchangeable proton region of a 2D NOESY spectrum of the 39-nt hairpin. Cross-peaks within base pairs from C amino proton(s) to GH1 and AH2 to UH3 are labeled blue, where the C amino proton or AH2 and GH1 or UH3 corresponds to the first and second residues, respectively. Interresidue H1′ to H1/H3 cross-peaks are labeled green, where H1′ and H1/H3 correspond to the first and second residues, respectively. The A22H8-G19H1 and A31H2-G11H1 cross-peaks are labeled red. The water signal was suppressed with an S-pulse. The spectrum was acquired at −2 °C with 125 ms mixing time.

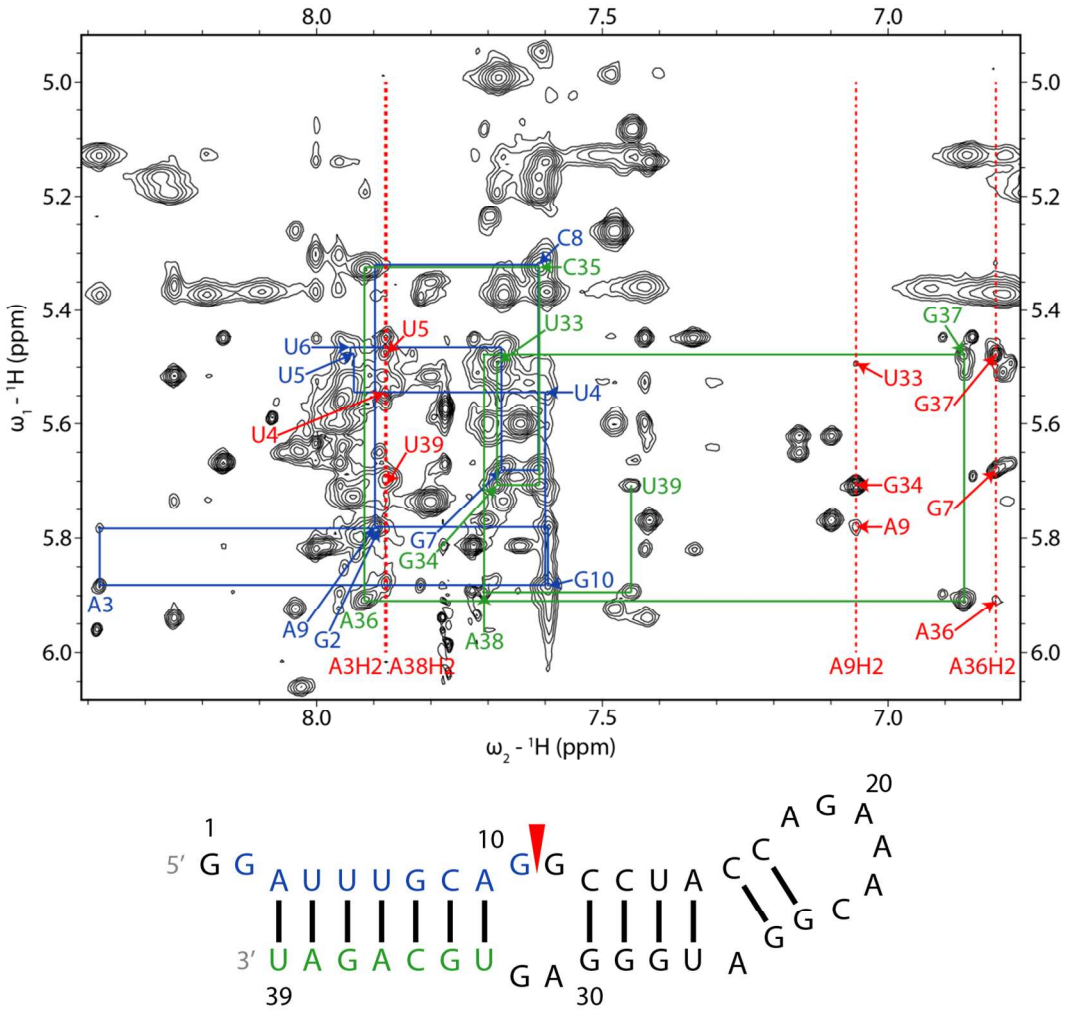
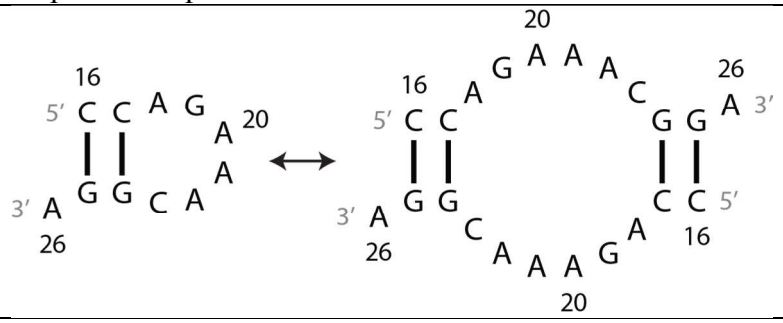
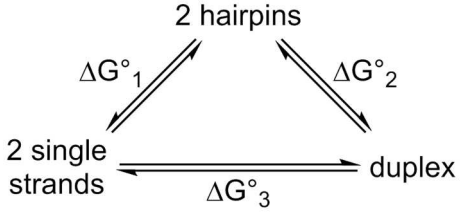



Figure S5: H1′ to H6/H8 region of a 2D proton NOESY spectrum of the 39-nt hairpin showing sequential proton walks for residues G2 to G10 and U33 to U39. H1′ to H6/H8 walk NOEs are labeled blue for residues 2 to 10 and green for residues 33 to 39. Adenine H2 signals are labeled with red dashed lines. H1′ to adenine H2 NOEs are labeled red with only the label of the residue for the H1′. Spectrum was acquired at 20 °C and 350 ms mixing time with a WATERGATE pulse to suppress the water signal. In the secondary structure of the 39-nt hairpin., residues whose intraresidue H1′ to H6/H8 NOEs were identified in the NOESY walks are labeled blue for residues 2 to 10 and green for residues 33 to 39.

Equilibrium of hairpin and duplex conformations.




Equilibria between single strands and hairpin and duplex conformations.




Calculate concentration of RNA in hairpin at 2 °C.

$\Delta H^\circ_{hairpin} = -29.29$ kcal mol$^{-1}$ and $\Delta S^\circ_{hairpin} = -92.99$ cal mol$^{-1}$ · K$^{-1}$.

$\Delta H^\circ_{duplex} = -39.27$ kcal mol$^{-1}$ and $\Delta S^\circ_{duplex} = -121.03$ cal mol$^{-1}$ · K$^{-1}$.

$\Delta G^\circ_1 = \Delta G^\circ_{hairpin}$ and $\Delta G^\circ_3 = -\Delta G^\circ_{duplex}$.

The free energy change from hairpin to duplex at 2 °C follows. This calculation assumes that all single strands form hairpin or duplex.

$$\Delta G^\circ_1 + \Delta G^\circ_2 + \Delta G^\circ_3 = 0$$

$$\Delta G^\circ_2 = -2\Delta G^\circ_1 - \Delta G^\circ_3$$

$$\Delta G^\circ_2 = 1436.26 \text{ cal mol}^{-1}$$

$$\Delta G^\circ_2 = -RT\ln\left(\frac{[duplex]}{[hairpin]^2}\right)$$

$$\Delta G^\circ_2 = -RT\ln\left(\frac{x/2}{(C_T - x)^2}\right)$$

Where $x$ is the concentration of single-stranded RNA in duplex and $C_T$ is the total concentration of strands. The concentration of duplex and hairpin are $x/2$ and $C_T - x$, respectively. The value of $x$ at 2 °C, with $C_T$ = 4 mM, is 2.31 × 10$^{-6}$ M. Therefore, the concentration of hairpin is approximately 4 mM. At $C_T$ = 0.2 mM, $x$ is 5.78 × 10$^{-9}$ M. Therefore, the concentration of hairpin is approximately 0.2 mM.

Figure S6: Thermodynamics calculations with the nearest neighbor model showing that the 11-nt hairpin conformation is preferred to a duplex formed by self-dimerization of the RNA sequence.

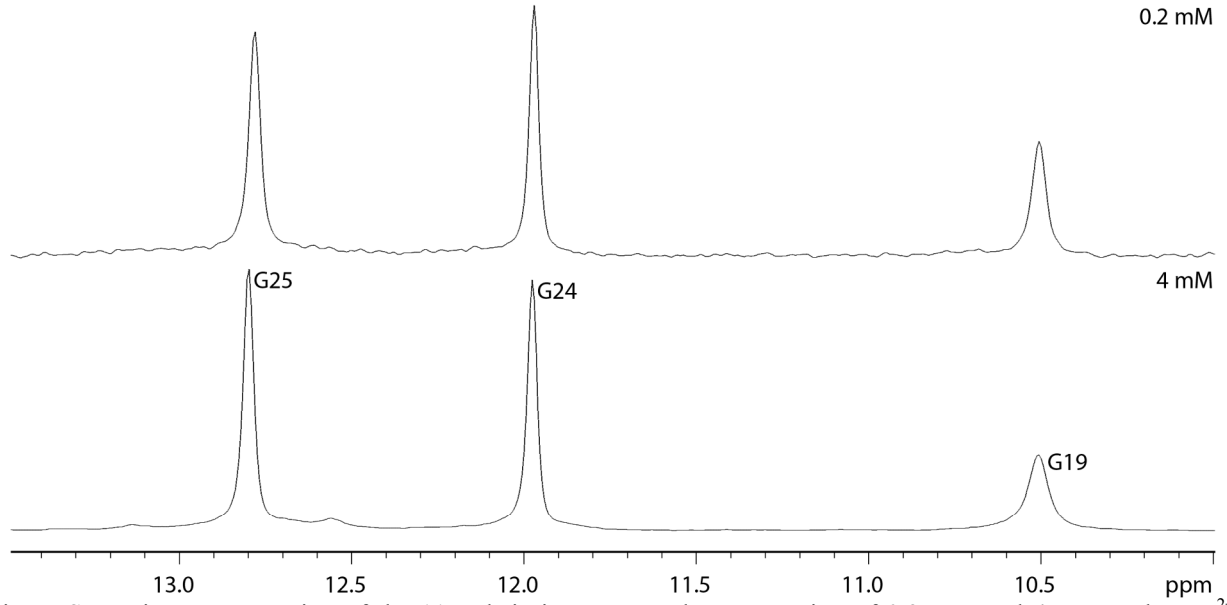


Figure S7: Imino proton region of the 11-nt hairpin at a strand concentration of 0.2 mM and 4 mM. The $Mg^{2+}$ concentration was 0.25 mM and 5 mM for the 0.2 mM and 4 mM samples, respectively. The phosphates concentration was the same in the two samples. Spectra were acquired with a 1-1-echo pulse at 2 °C.

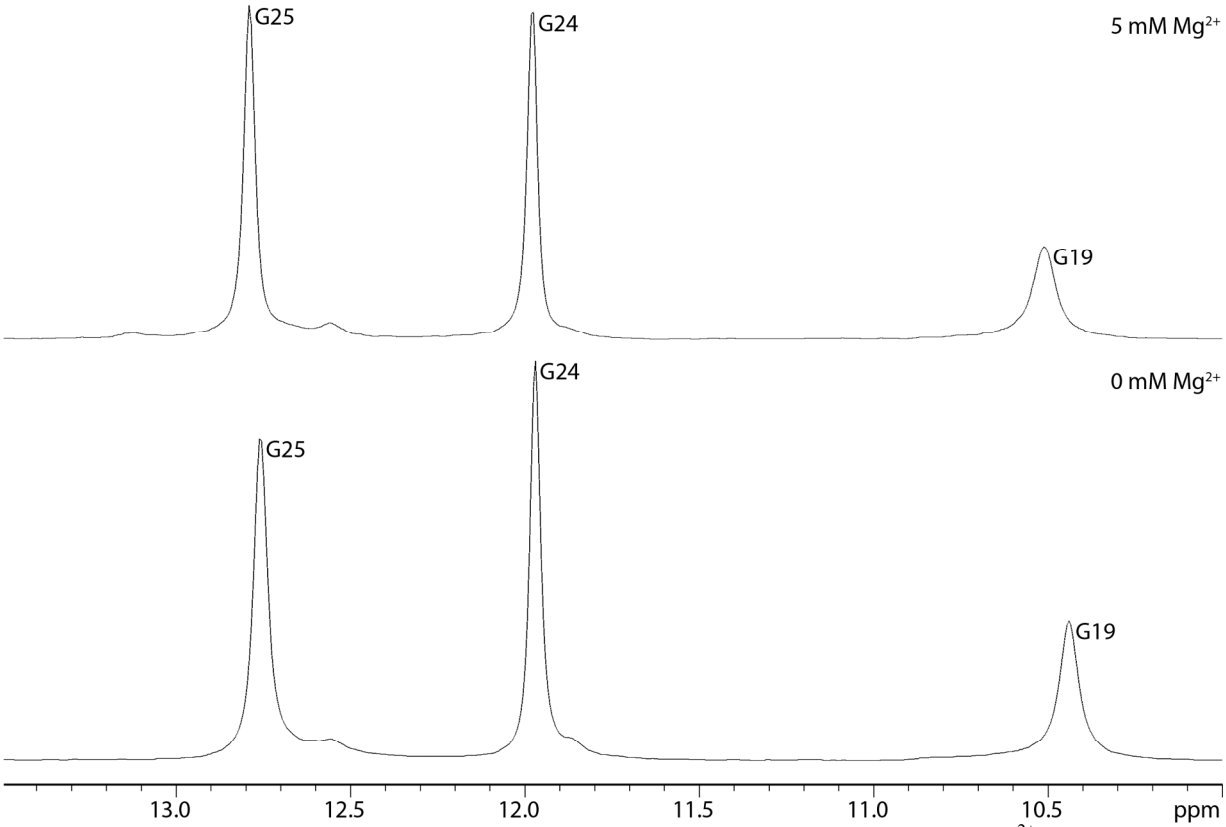


Figure S8: Imino region of 1D proton spectra of the 11-nt hairpin acquired with 0 and 5 mM $Mg^{2+}$ at 5 °C.

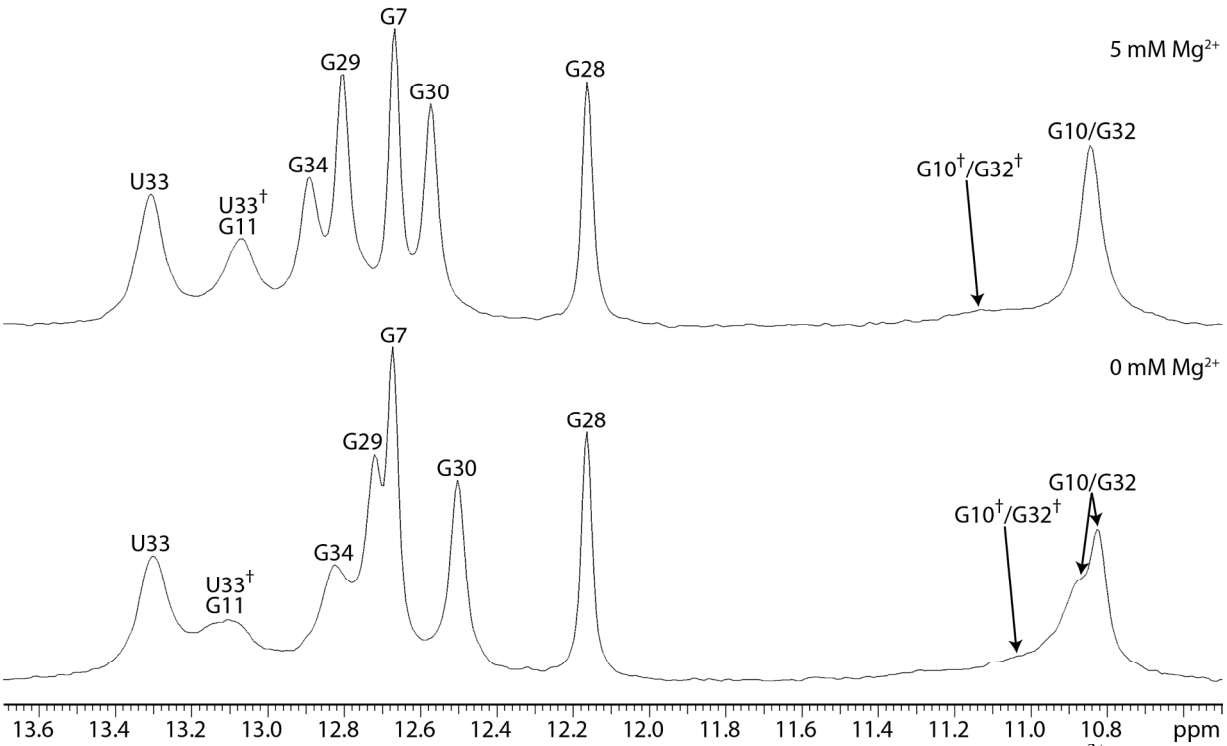



Figure S9: Imino region of 1D proton spectra of the 19-nt duplex acquired with 0 and 5 mM $Mg^{2+}$ at 5 °C. † Chemical exchange peak.

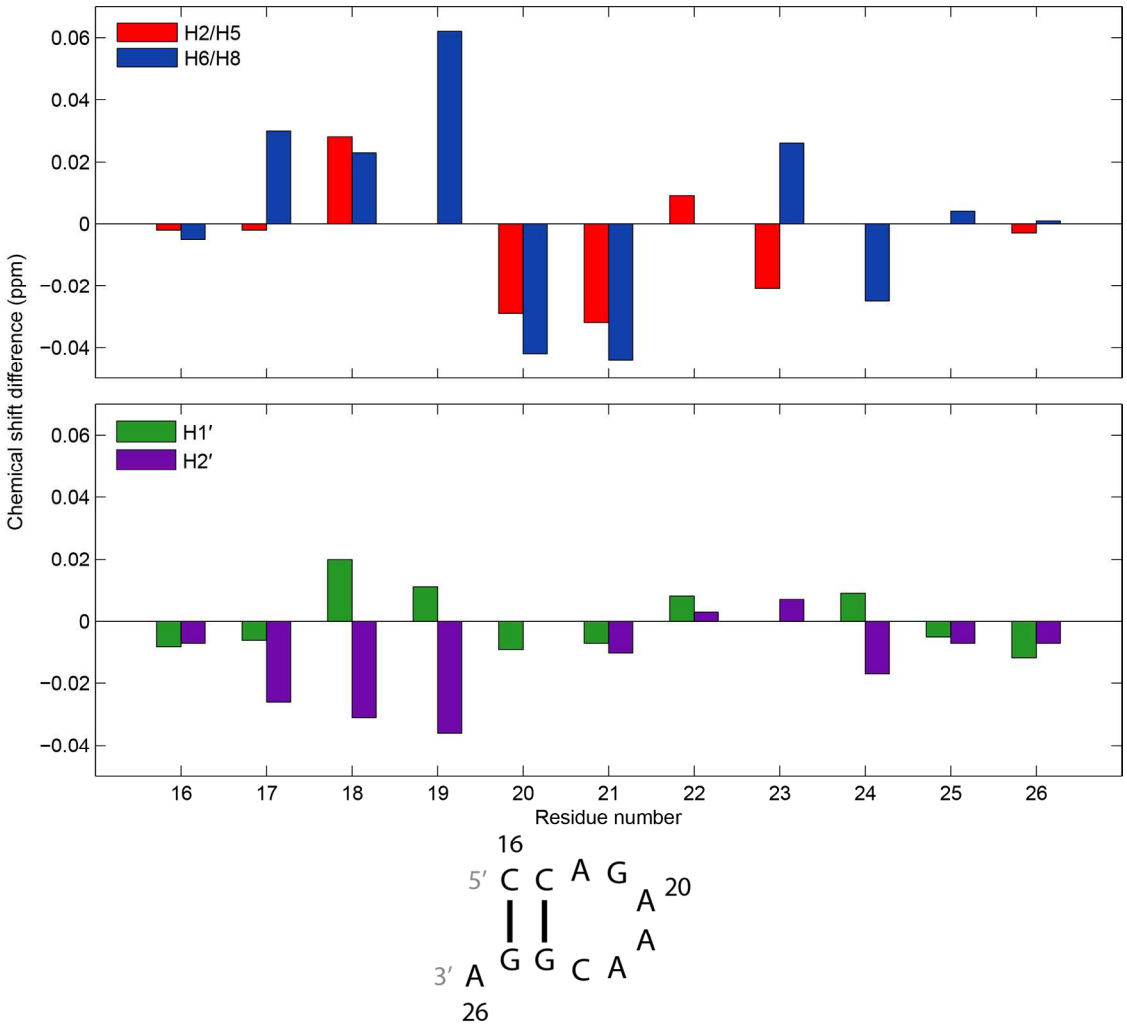



Figure S10: Differences in chemical shifts of select non-exchangeable hydrogens of the 11-nt hairpin with and without 5 mM $Mg^{2+}$ at −2 °C. Residue numbers on the x-axis align with the middle of each set of two bars in each plot.

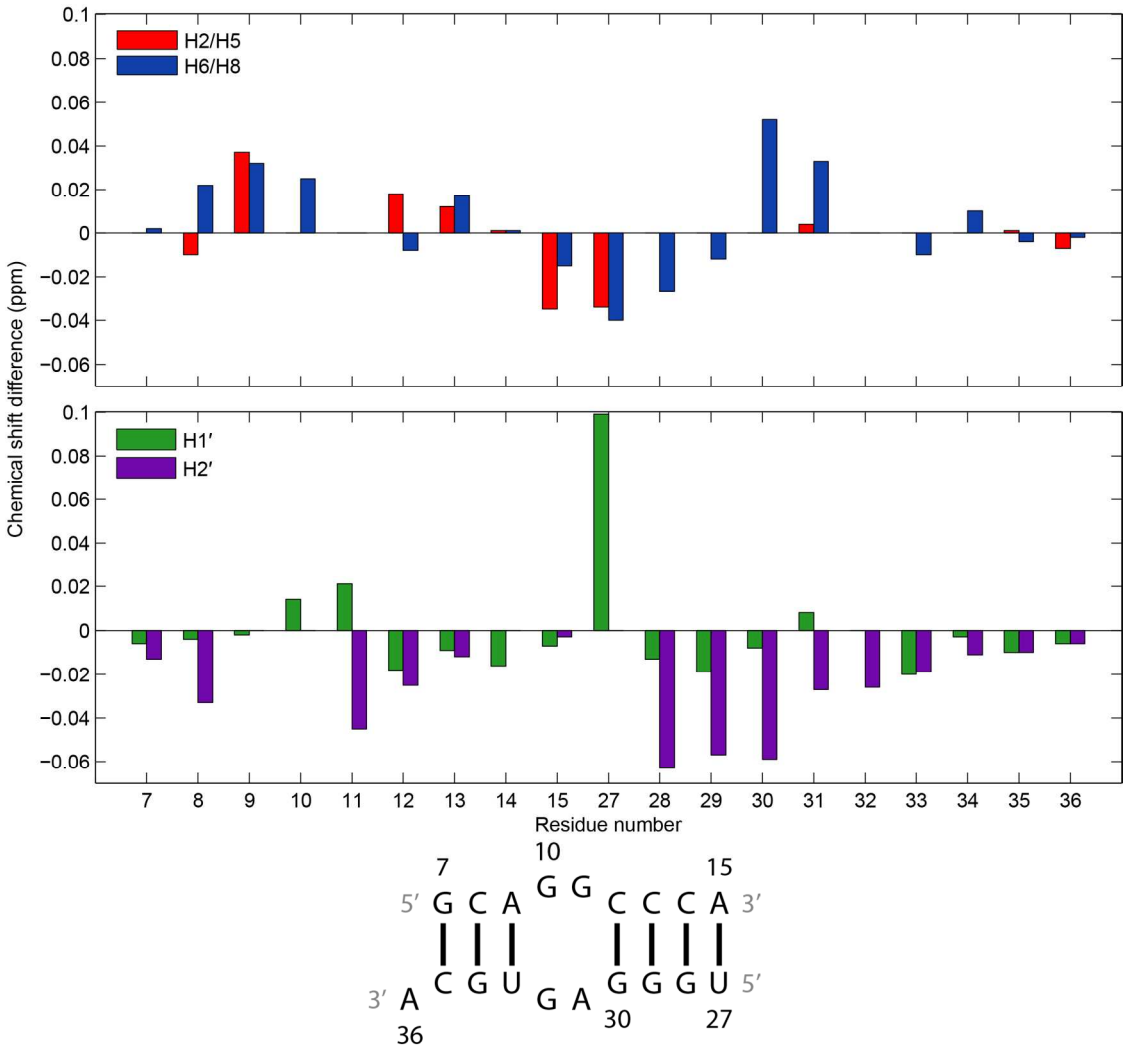



Figure S11: Differences in chemical shifts of select non-exchangeable hydrogens of the 19-nt duplex with and without 5 mM $Mg^{2+}$ at −2 °C. Residue numbers on the x-axis align with the middle of each set of two bars in each plot.

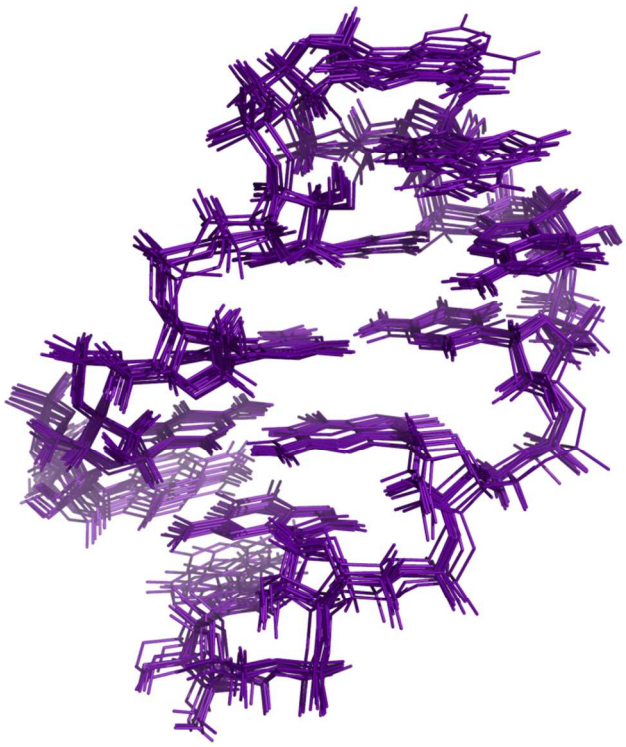


Figure S12: The ensemble of the 10 structures of the 11-nt hairpin modeled in AMBER with the lowest distance restraint violation energies and which agreed with NMR restraints.

Figure S13: The ensemble of the 10 structures of the 19-nt duplex modeled in AMBER with the lowest distance restraint violation energies and which agreed with NMR restraints.

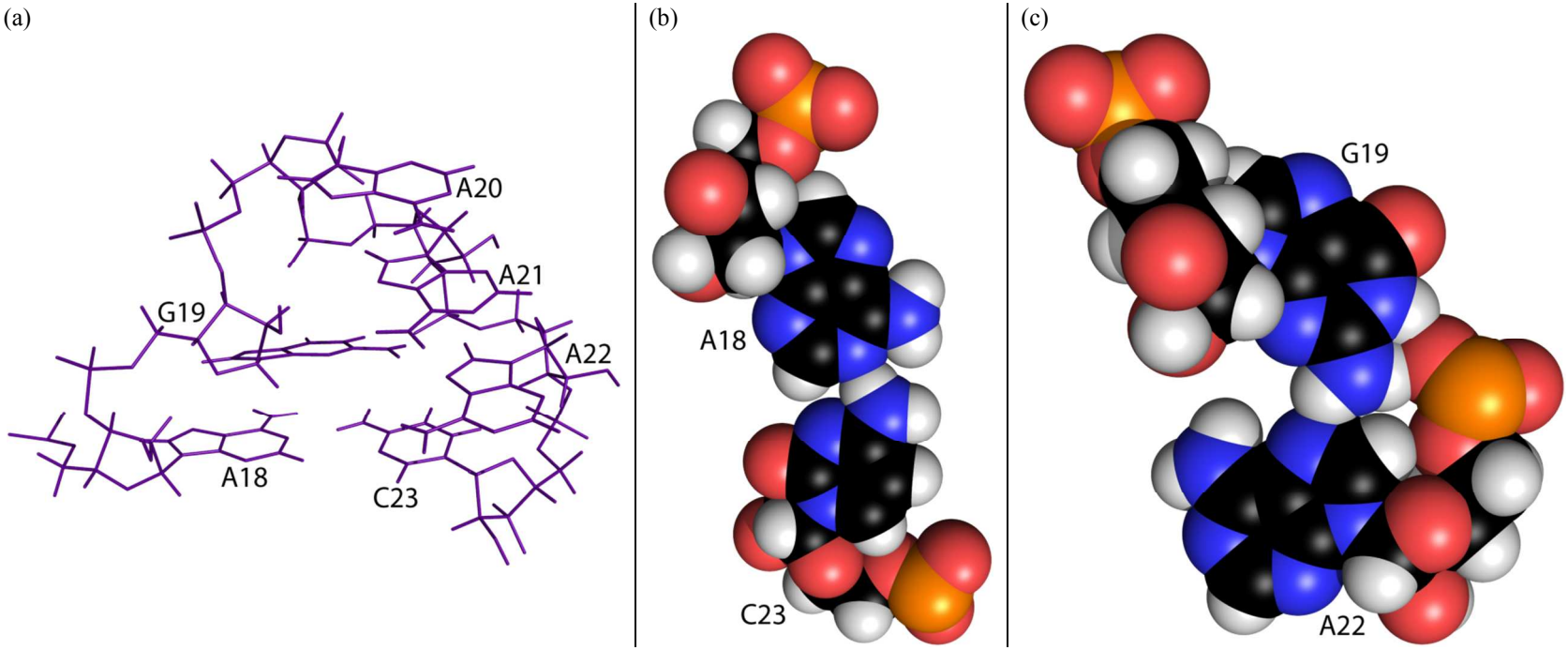



Figure S14: Calculated structure of the hairpin loop of the 11-nt hairpin construct. (a) Model of the GAAA loop and closing AC pair of the 11-nt hairpin construct calculated by AMBER, showing the 3′ $A_3$ stack and an AC pair with a hydrogen bond from the C23 amino group to A18N1. (b) Space-filling model of the A18-C23 pair. (c) Space-filling model of the G19-A22 pair.

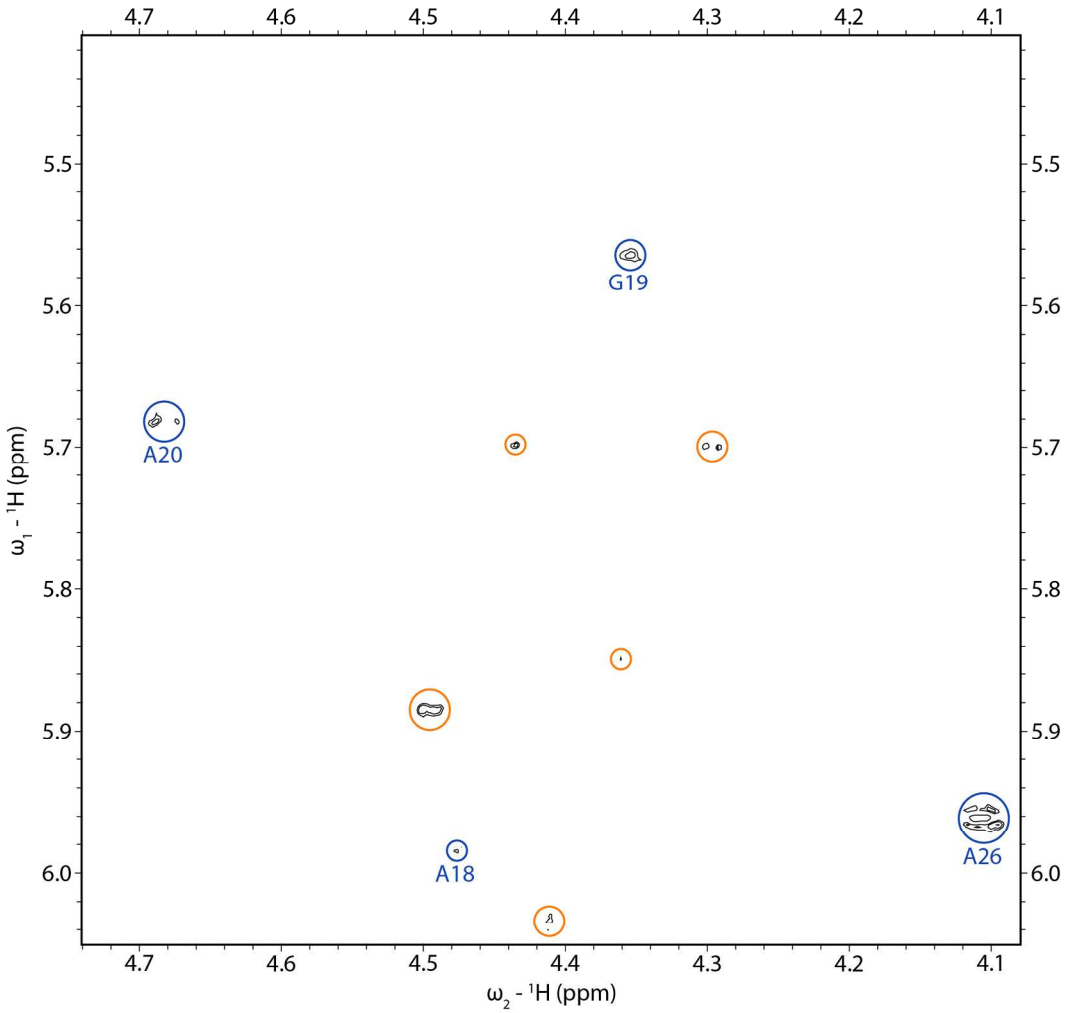



Figure S15: H1′ to H2′ region of a 2D TOCSY spectrum of the 11-nt hairpin. All visible cross-peaks are circled. Cross-peaks circled blue correspond to H1′ to H2′ cross-peaks for residues labeled with the same color. Cross-peaks circled orange are unassigned artifacts. As expected, the 3′ terminal A26 residue has a relatively large H1′ to H2′ cross-peak. All H1′ to H2′ cross-peaks are small compared to C or U H5 to H6 cross-peaks in the same spectrum (not shown), indicating weak coupling between these protons and that all residues primarily have a C3′ endo sugar pucker. The spectrum was acquired at 20 °C in $D_2O$ and 5 mM $Mg^{2+}$.

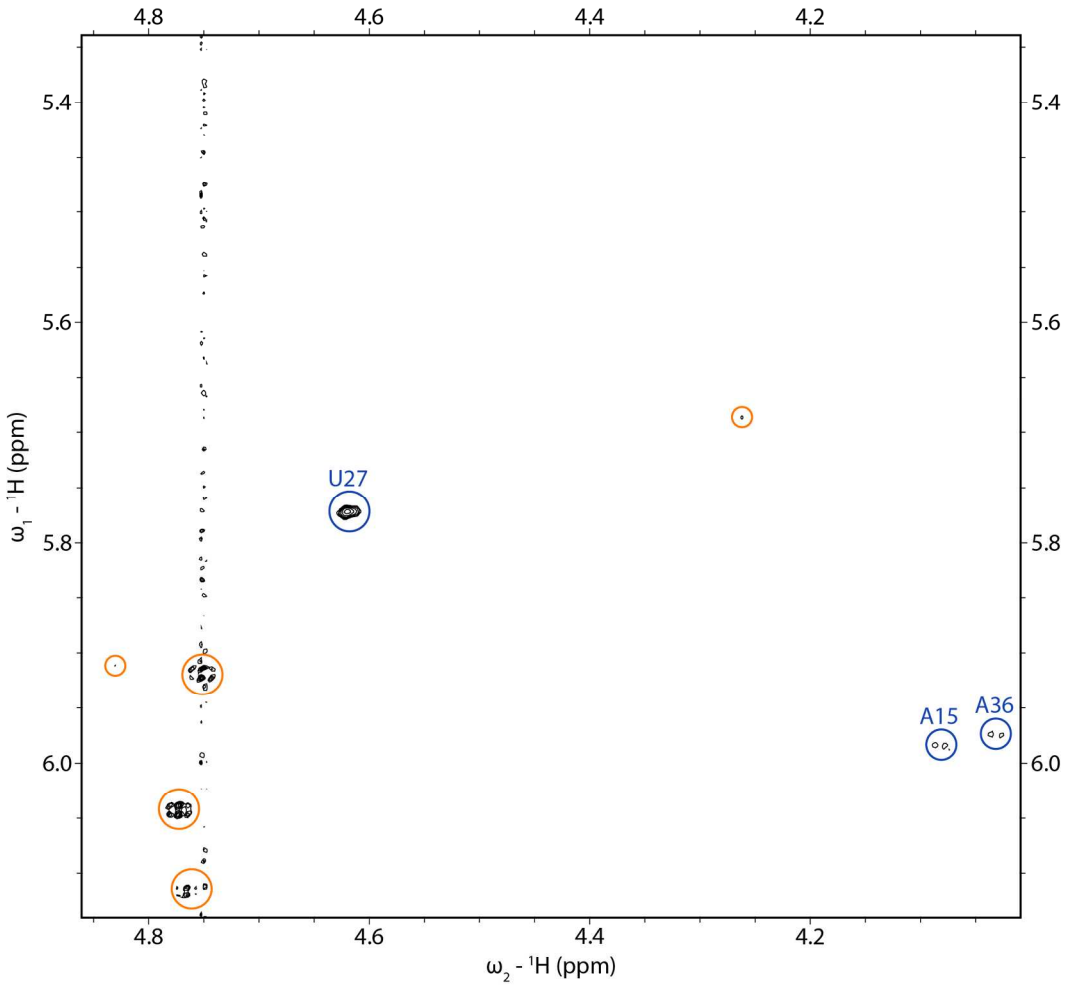



Figure S16: H1′ to H2′ region of a 2D TOCSY spectrum of the 19-nt duplex. All visible cross-peaks are circled. Cross-peaks circled blue correspond to H1′ to H2′ cross-peaks for residues labeled with the same color. Cross-peaks circled orange are unassigned artifacts. As expected, terminal residues have a relatively large H1′ to H2′ cross-peak. All H1′ to H2′ cross-peaks are small compared to C or U H5 to H6 cross-peaks in the same spectrum (not shown), indicating weak coupling between these protons and that all residues primarily have a C3′ endo sugar pucker. The noise at 4.75 ppm is the residual water peak. The spectrum was acquired at 25 °C in $D_2O$ and 5 mM $Mg^{2+}$.

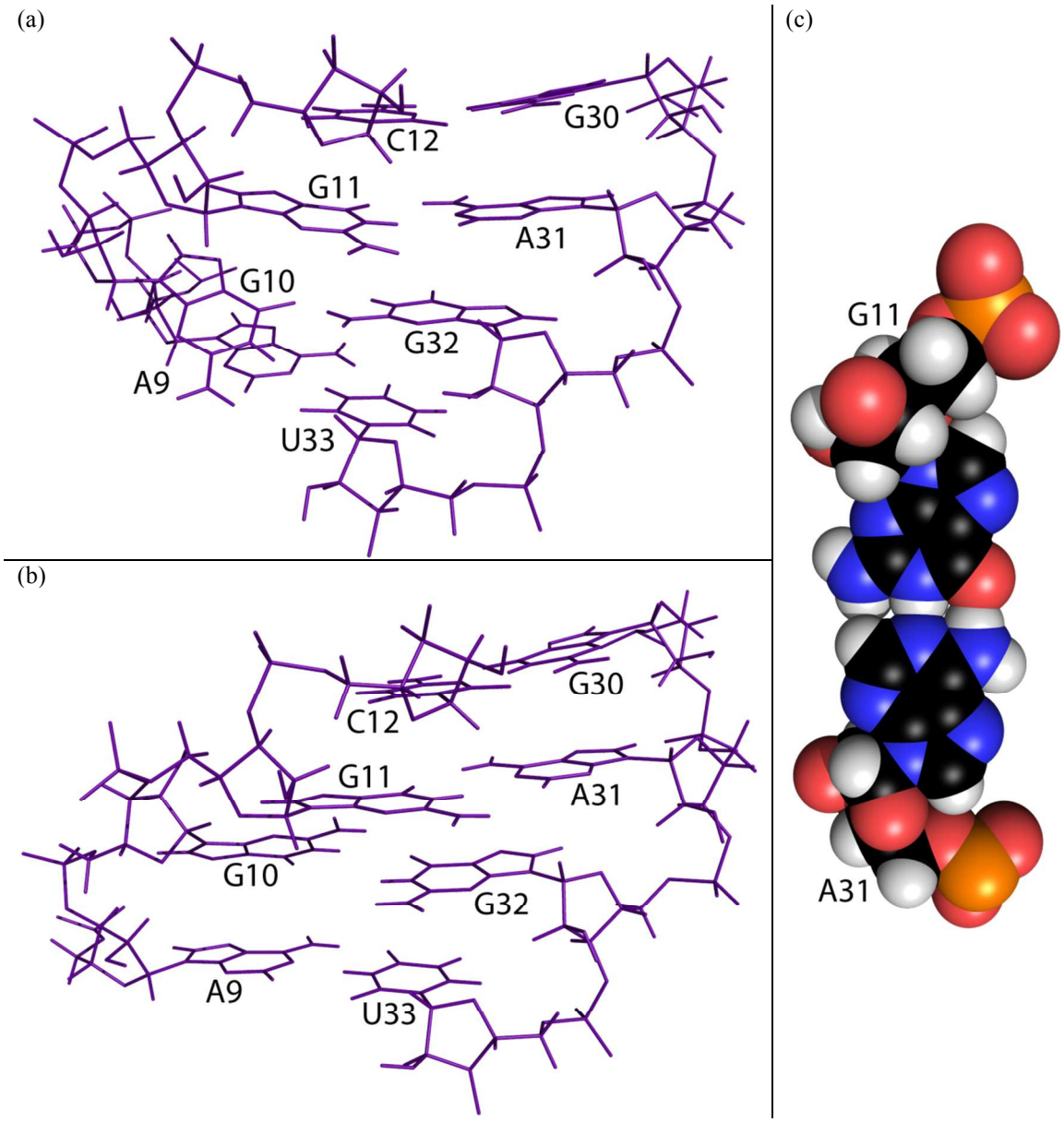



Figure S17: Calculated structure of the internal loop of the 19-nt duplex construct. (a) G10 is stacked below G11 with an *anti* conformation. (b) G10 flipped out of the helix with an *anti* conformation. (c) Space-filling model of the G11-A31 pair.

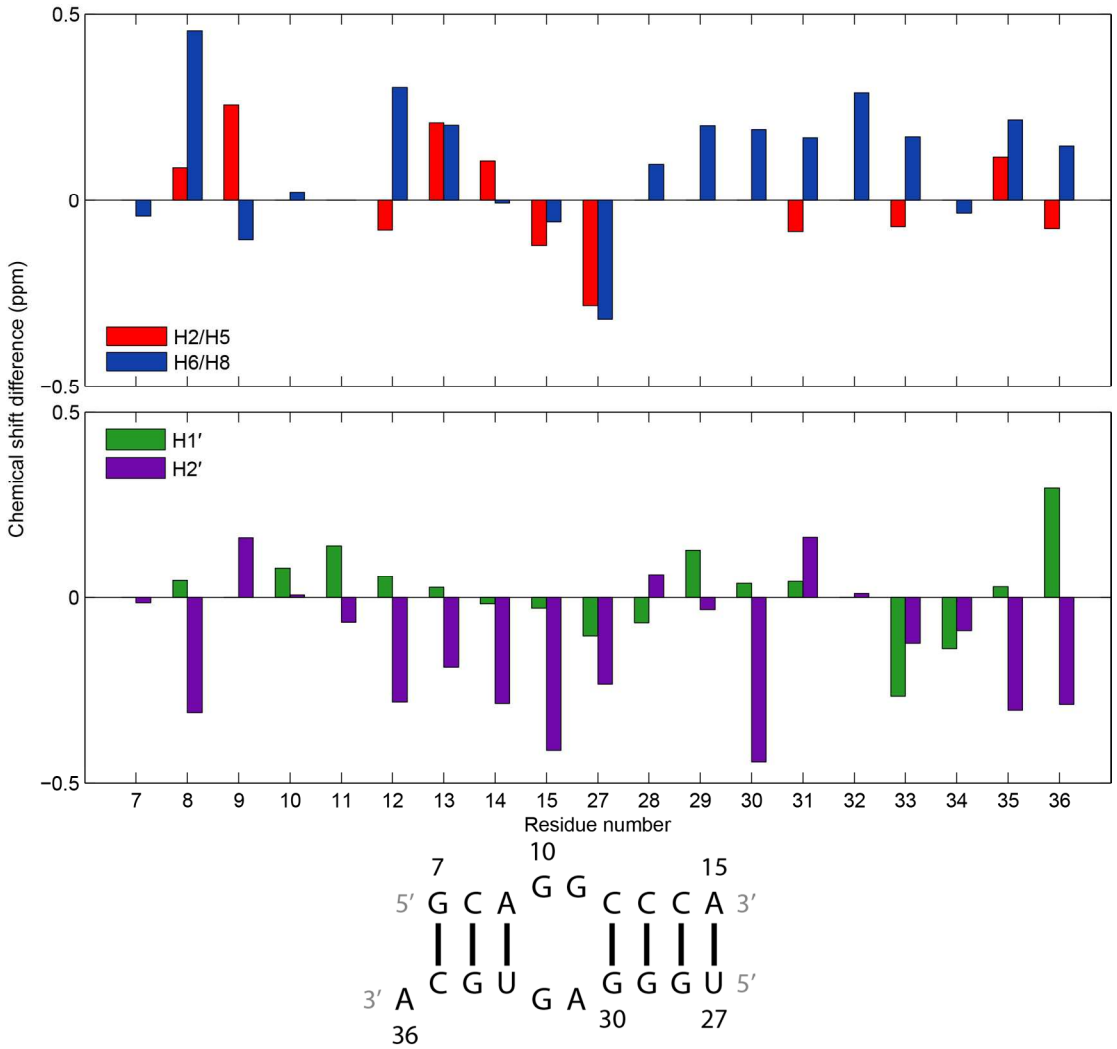



Figure S18: Chemical shift differences of the 19-nt duplex between experiment, assigned at 25 °C in the presence of 5 mM $Mg^{2+}$, and those predicted by NUCHEMICS for H2/H5, H6/H8, H1′ and H2′ in an ensemble of 20 structures generated with NMR restraints. Residue numbers on the x-axis align with the middle of each set of two bars in each plot.

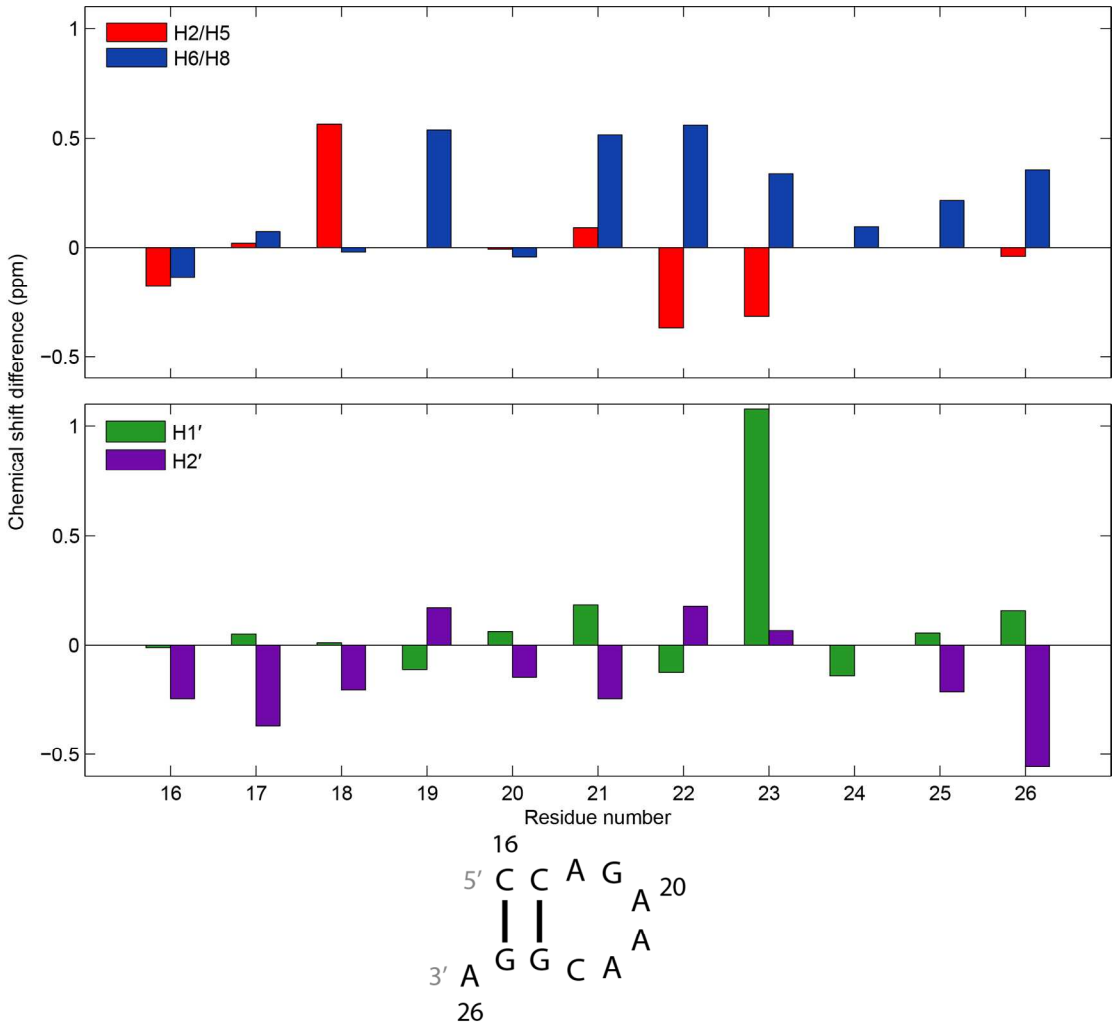



Figure S19: Chemical shift differences of the 11-nt hairpin between experiment, assigned at 20 °C in the presence of 5 mM $Mg^{2+}$, and those predicted by NUCHEMICS for H2/H5, H6/H8, H1′ and H2′ in an ensemble of 20 structures generated with NMR restraints. Residue numbers on the x-axis align with the middle of each set of two bars in each plot.

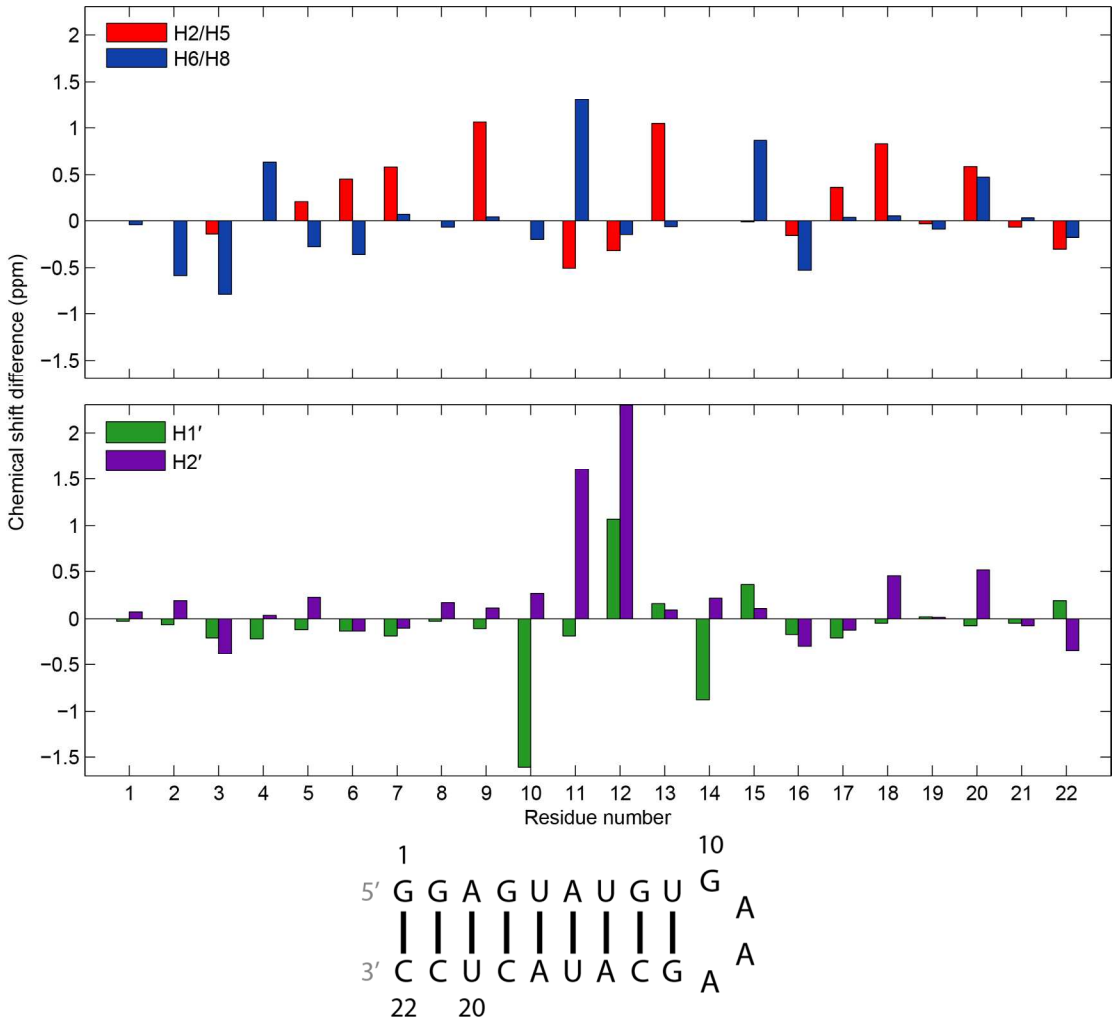



Figure S20: Differences between experimental and NUCHEMICS-predicted chemical shifts of a GAAA hairpin, closed by a GU pair, which consists of a helical region of a group II intron. Residue numbers on the x-axis align with the middle of each set of two bars in each plot. Experimental chemical shifts were obtained from BMRB entry 15859 and 3D structures were obtained from PDB entry 2K66.[3]

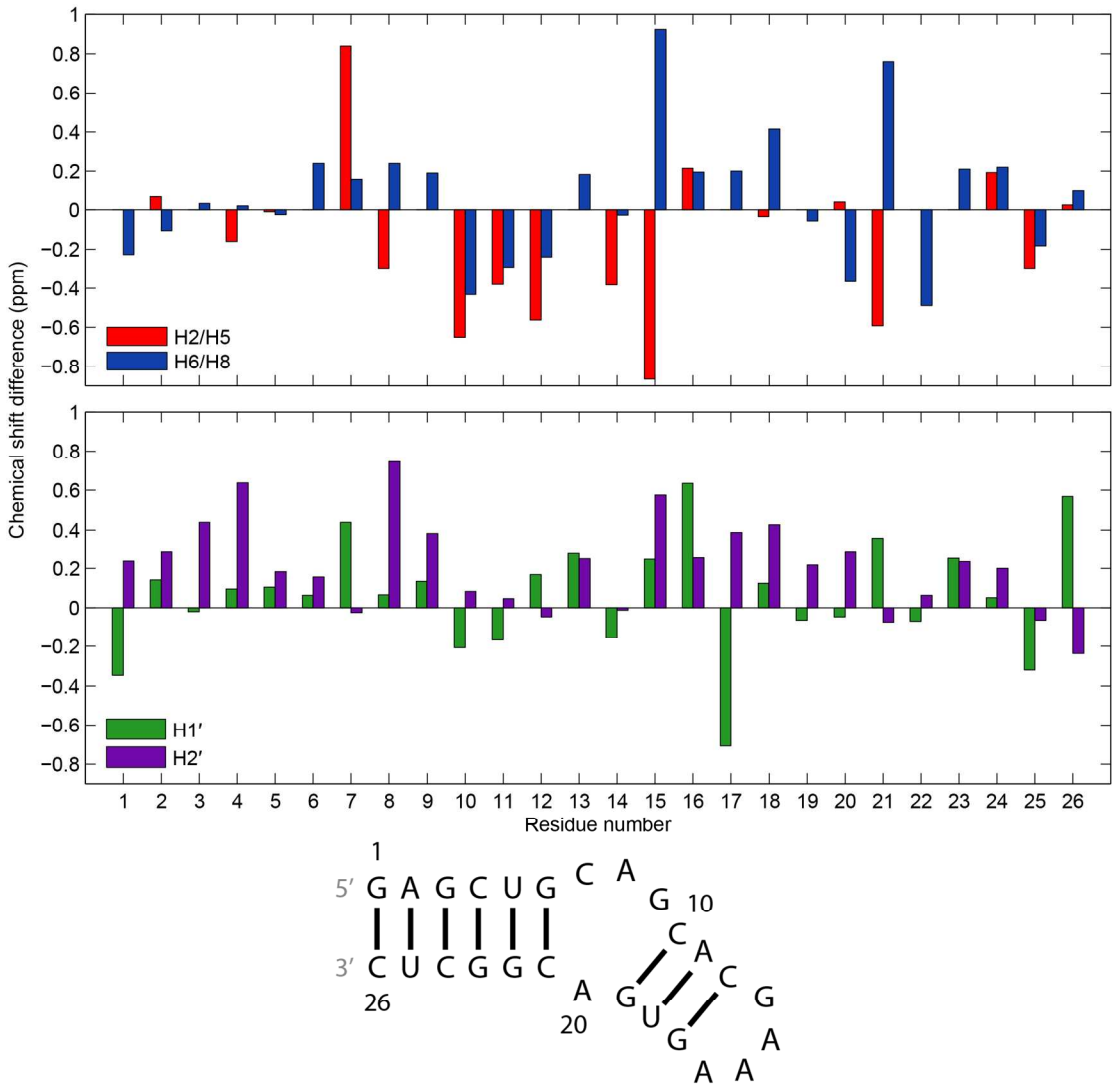



Figure S21: Differences between experimental and NUCHEMICS-predicted chemical shifts of a GAAA hairpin, closed by a GC pair, which contains an internal loop from a ribozyme. Residue numbers on the x-axis align with the middle of each set of two bars in each plot. Experimental chemical shifts were obtained from BMRB entry 17292 and 3D structures were obtained from PDB entry 2L5Z.[4]

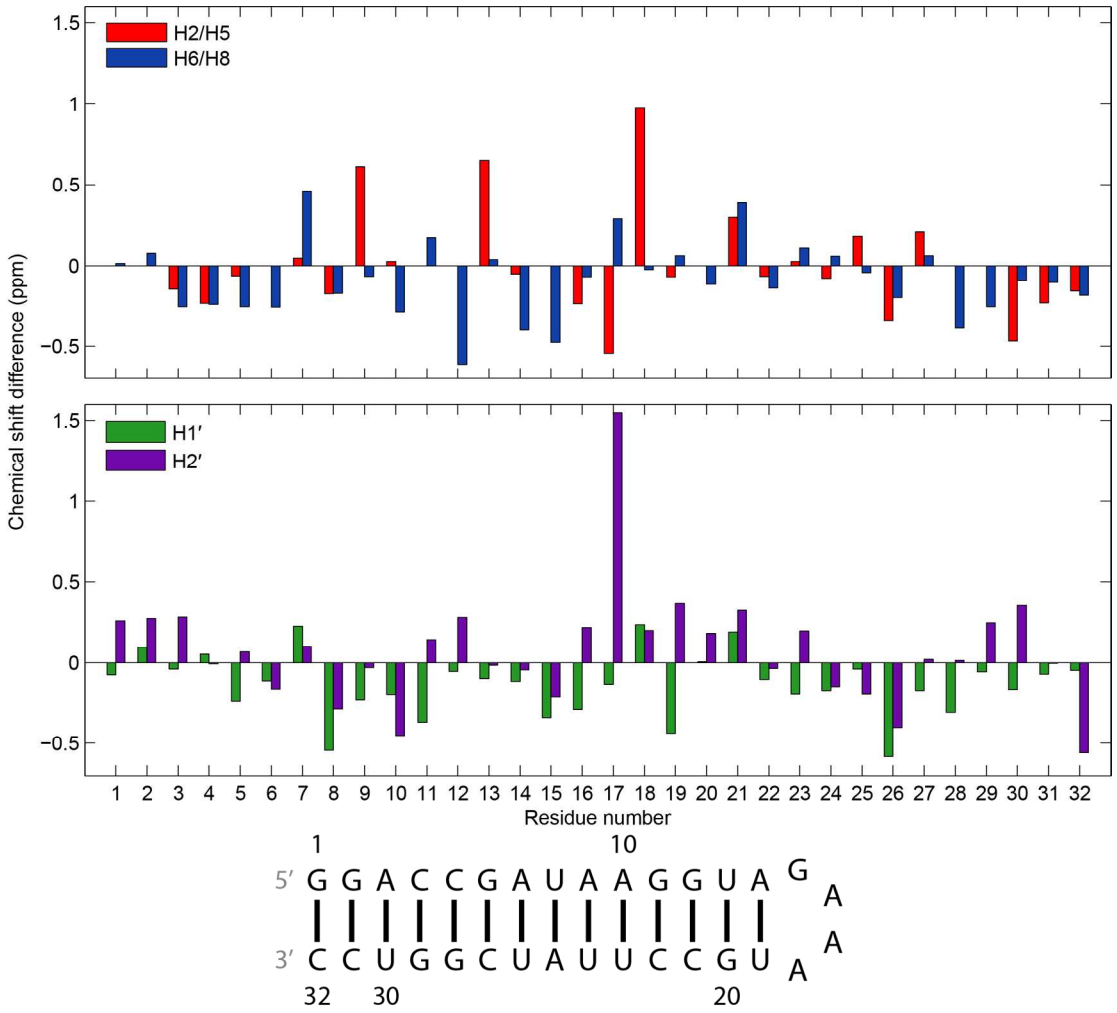



Figure S22: Differences between experimental and NUCHEMICS-predicted chemical shifts of a GAAA hairpin, closed by an AU pair, which consists of a helical region of a ribozyme. Residue numbers on the x-axis align with the middle of each set of two bars in each plot. Experimental chemical shifts were obtained from BMRB entry 17877 and 3D structures were obtained from PDB entry 2LI4.[5]

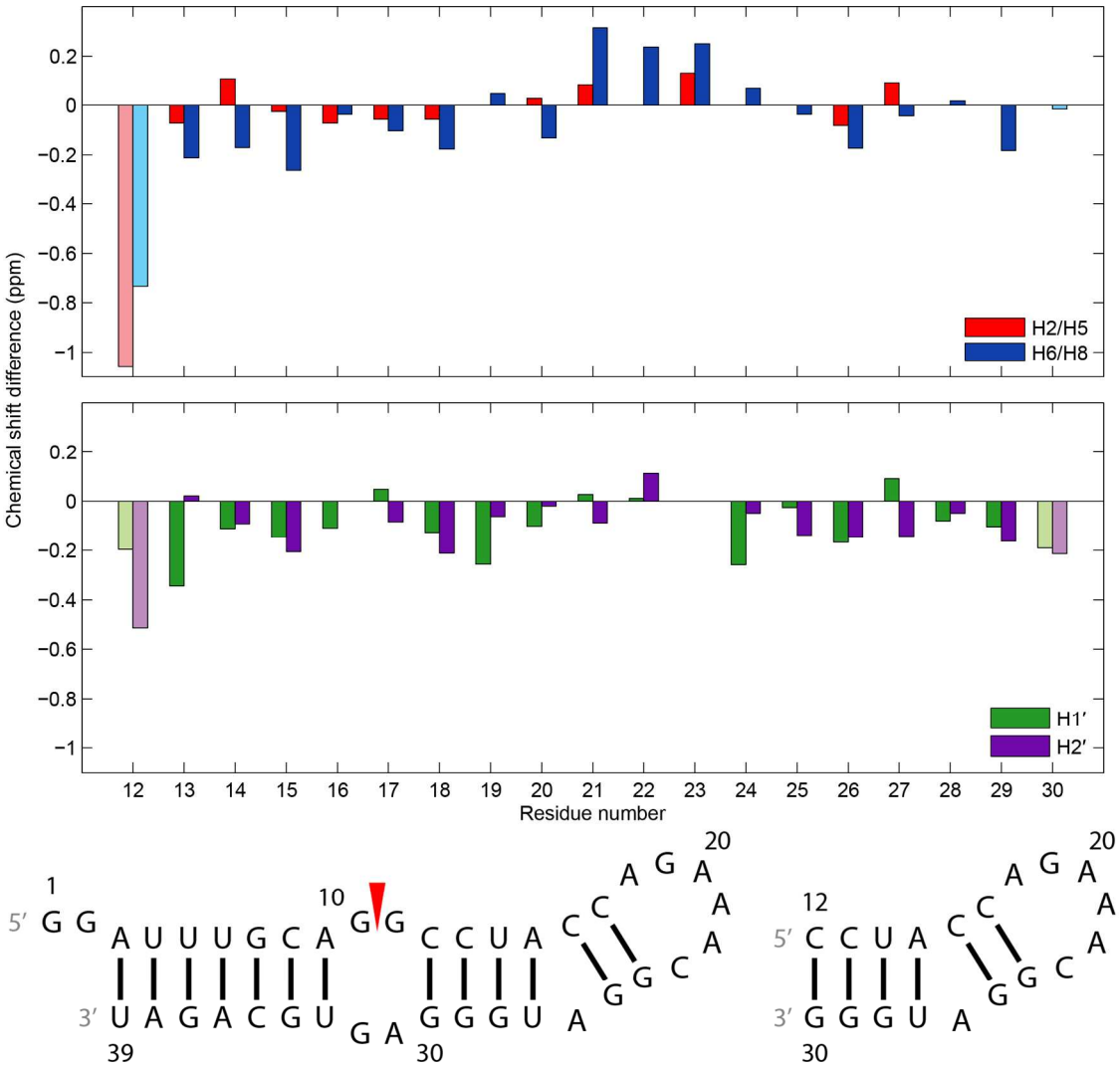



Figure S23: Differences between experimental chemical shifts of the 39-nt hairpin, assigned at 20 °C, and those predicted by NUCHEMICS for H2/H5, H6/H8, H1′ and H2′ in an ensemble of 20 structures of the 19-nt hairpin generated by CS-ROSETTA with chemical shift restraints. Bars colored with light shades belong to terminal helix residues 12 and 30 of the 19-mer that are not at the termini of any helixes of the 39-mer, and thus are in structurally inequivalent regions among the constructs. Residue numbers on the x-axis align with the middle of each set of two bars in each plot.

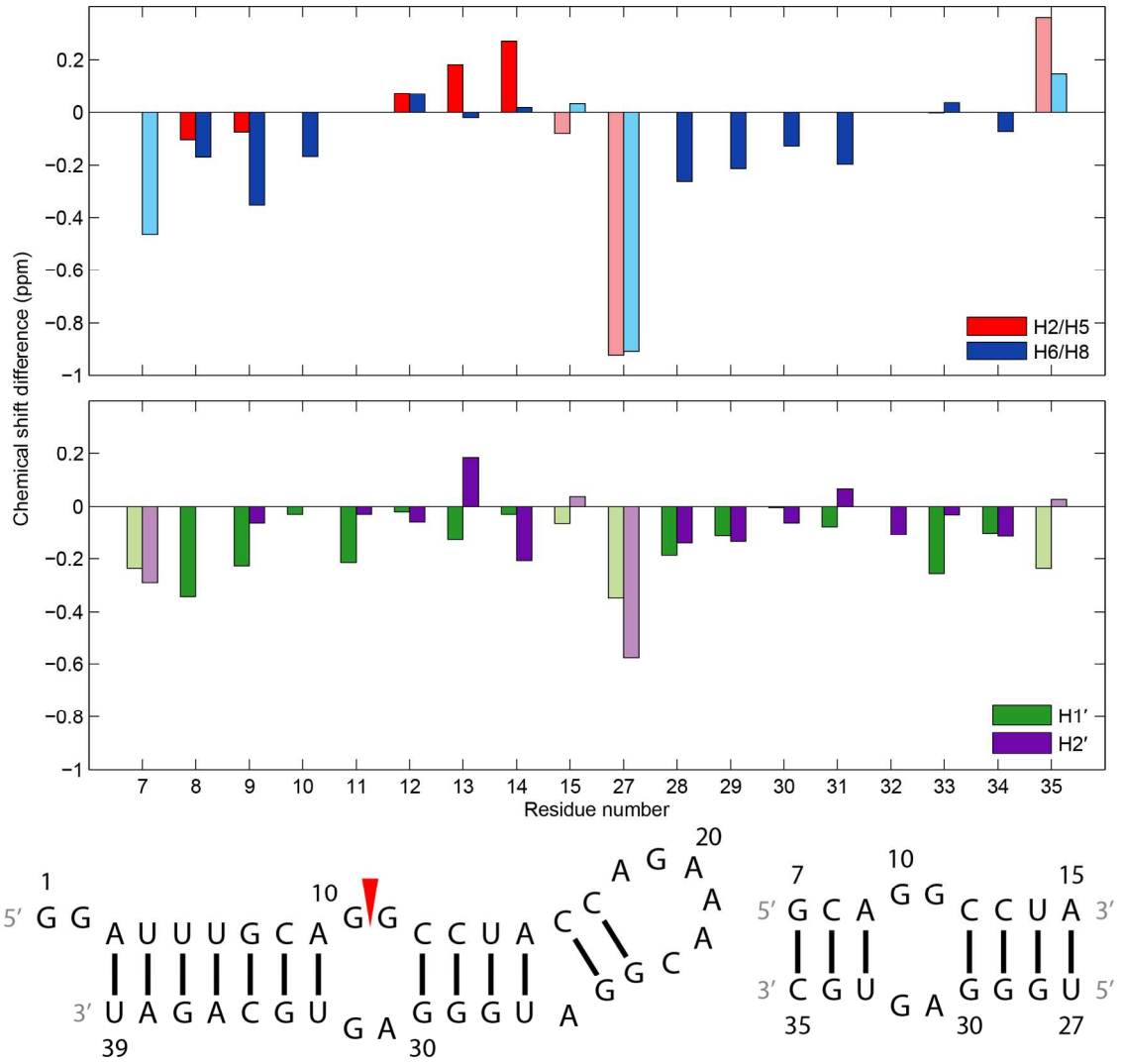



Figure S24: Differences between experimental chemical shifts of the 39-nt hairpin, assigned at 20 °C, and those predicted by NUCHEMICS for H2/H5, H6/H8, H1′ and H2′ in an ensemble of 20 structures of the 18-nt duplex generated by CS-ROSETTA with chemical shift restraints. Bars colored with light shades belong to terminal helix residues 7, 15, 27 and 35 of the 18-mer that are not at the termini of any helixes of the 39-mer, and thus are in structurally inequivalent regions among the constructs. Residue numbers on the x-axis align with the middle of each set of two bars in each plot.

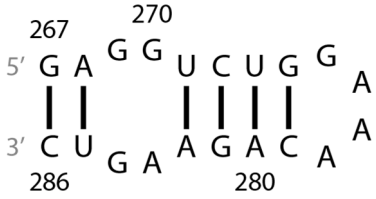



Figure S25: Secondary structure of a hairpin with a 2 × 2 internal loop from a group II intron of *Oceanobacillus iheyensis*.[6]

Table S1: Chemical shifts used to model fragments of the segment 7 hairpin with CS-ROSETTA. Chemical shifts were taken from spectra of the 39-nt hairpin (red), 19-nt duplex (blue) and 11-nt hairpin (green) at 20 °C (39-nt hairpin and 11-nt hairpin) or 25 °C (19-nt duplex) unless otherwise noted. The spectra for the 39-nt hairpin were acquired with no $Mg^{2+}$ and those for the 19-nt duplex and 11-nt hairpin were acquired with 5 mM $Mg^{2+}$.

| Residue | Atom | Chemical shift (ppm) | Residue | Atom | Chemical shift (ppm) | Residue | Atom | Chemical Shift (ppm) |
|---|---|---|---|---|---|---|---|---|
| G7 | H1′ | 5.681 | C17 | H2′ | 4.178 | U27 | H2′ | 4.401 |
| G7 | H2′ | 4.415 | C17 | H5 | 5.365 | U27 | H5 | 5.262 |
| G7 | H8 | 7.677 | C17 | H6 | 7.596 | U27 | H6 | 7.479 |
| C8 | H1′ | 5.504 | A18 | H1′ | 5.980 | G28 | H1′ | 5.769 |
| C8 | H2′ | 4.316 | A18 | H2 | 7.670 | G28 | H2′ | 4.719 |
| C8 | H3′ | 4.605 | A18 | H2′ | 4.474 | G28 | H8 | 7.700 |
| C8 | H4′ | 4.461 | A18 | H3′ | 4.848 | G29 | H1′ | 5.745 |
| C8 | H5 | 5.273 | A18 | H8 | 8.184 | G29 | H2′ | 4.560 |
| C8 | H6 | 7.835 | G19 | H1′ | 5.563 | G29 | H3′ | 4.504 |
| A9 | H1′ | 5.878 | G19 | H2′ | 4.352 | G29 | H4′ | 4.507 |
| A9 | H2 | 7.198 | G19 | H3′ | 4.607 | G29 | H8 | 7.329 |
| A9 | H2′ | 4.491 | G19 | H4′ | 4.366 | G30 | H1′ | 5.660 |
| A9 | H3′ | 4.852 | G19 | H8 | 7.335 | G30 | H2′ | 4.169 |
| A9 | H4′ | 4.391 | A20 | H1′ | 5.681 | G30 | H3′ | 4.647 |
| A9 | H8 | 8.045 | A20 | H2 | 7.726 | G30 | H4′ | 4.420 |
| G10 | H1′ | 6.038 | A20 | H2′ | 4.680 | G30 | H8 | 7.264 |
| G10 | H2′ | 4.500 | A20 | H3′ | 4.475 | A31 | H1′ | 6.104 |
| G10 | H3′ | 4.716 | A20 | H4′ | 4.250 | A31 | H2 | 7.948 |
| G10 | H4′ | 4.491 | A20 | H8 | 8.210 | A31 | H2′ | 4.842 |
| G10 | H8 | 7.679 | A21 | H1′ | 5.515 | A31 | H3′ | 4.678 |
| G11 | H1′ | 5.567 | A21 | H2 | 7.610 | A31 | H4′ | 4.480 |
| G11 | H2′ | 4.583 | A21 | H2′ | 4.558 | A31 | H8 | 8.078 |
| G11 | H3′ | 4.353 | A21 | H3′ | 4.655 | G32 | H1′ | 5.370[a] |
| G11 | H8 | 7.369[a] | A21 | H4′ | 4.410 | G32 | H2′ | 4.358 |
| C12 | H1′ | 5.523 | A21 | H8 | 7.888 | G32 | H3′ | 4.229 |
| C12 | H2′ | 4.239 | A22 | H1′ | 5.850 | G32 | H8 | 7.100 |
| C12 | H3′ | 4.451 | A22 | H2 | 8.082 | U33 | H1′ | 5.518 |
| C12 | H5 | 5.208 | A22 | H2′ | 4.675 | U33 | H2′ | 4.600 |
| C12 | H6 | 7.512 | A22 | H3′ | 4.620 | U33 | H3′ | 4.533 |
| C13 | H1′ | 5.367 | A22 | H4′ | 4.468 | U33 | H4′ | 4.422 |
| C13 | H2′ | 4.290 | A22 | H8 | 7.930 | U33 | H5 | 5.040 |
| C13 | H3′ | 4.485 | C23 | H1′ | 4.850 | U33 | H6 | 7.732 |
| C13 | H4′ | 4.363 | C23 | H2′ | 4.414 | G34 | H1′ | 5.745 |
| C13 | H5 | 5.423 | C23 | H3′ | 4.338 | G34 | H2′ | 4.415 |
| C13 | H6 | 7.837 | C23 | H5 | 5.583 | G34 | H3′ | 4.558 |
| U14 | H1′ | 5.516 | C23 | H6 | 7.730 | G34 | H4′ | 4.435 |
| U14 | H2′ | 4.252 | G24 | H1′ | 5.528 | G34 | H8 | 7.693 |
| U14 | H5 | 5.736 | G24 | H2′ | 4.698 | C35 | H1′ | 5.357 |
| U14 | H6 | 7.802 | G24 | H8 | 7.596 | C35 | H2′ | 4.303 |
| A15 | H1′ | 5.939 | G25 | H1′ | 5.640 | C35 | H3′ | 4.370 |
| A15 | H2 | 7.417 | G25 | H2′ | 4.391 | C35 | H4′ | 4.330 |
| A15 | H2′ | 4.519 | G25 | H3′ | 4.564 | C35 | H5 | 5.170 |
| A15 | H3′ | 4.613 | G25 | H8 | 7.307 | C35 | H6 | 7.438 |
| A15 | H8 | 8.250 | A26 | H1′ | 5.924 | | | |
| C16 | H1′ | 5.139 | A26 | H2 | 7.960 | | | |
| C16 | H5 | 5.360 | A26 | H2′ | 4.387 | | | |
| C16 | H6 | 7.421 | A26 | H8 | 8.036 | | | |
| C17 | H1′ | 5.302 | U27 | H1′ | 5.598 | | | |

[a] Assigned at −2 °C.

Table S2: Assigned chemical shifts of the 39-nt 3′ splice site segment 7 hairpin construct.

| Residue | H1′ | H2′ | H2/H5 | H6/H8 | H1/H3 | H21/41/61, H22/42/62 amino |
|---|---|---|---|---|---|---|
| G2 | 5.78 | 4.76 | N/A | 7.89 | - | - |
| A3 | 5.88 | 4.71 | 7.88 | 8.38 | N/A | - |
| U4 | 5.54 | 4.56 | 5.14 | 7.60 | 14.14 | N/A |
| U5 | 5.48 | - | 5.51 | 7.93 | 11.92 | N/A |
| U6 | 5.47 | 4.56 | 5.53 | 7.94 | 13.51 | N/A |
| G7 | 5.68 | 4.42 | N/A | 7.68 | 12.41 | 6.25 |
| C8 | 5.32 | | 5.17 | 7.61 | N/A | 8.28, 6.87 |
| A9 | 5.78 | 4.47 | 7.06 | 7.90 | N/A | - |
| G10 | 5.88 | - | N/A | 7.60 | 10.79[a] | - |
| G11 | 5.51 | 4.58 | N/A | - | 13.08 | 5.88 |
| C12 | 5.52 | 4.26 | 5.13 | 7.50 | N/A | 8.42, 6.98 |
| C13 | 5.35 | 4.51 | 5.37 | 7.67 | N/A | 8.22, 6.91 |
| U14 | 5.52 | 4.25 | 5.74 | 7.80 | 11.66 | N/A |
| A15 | 5.94 | 4.52 | 7.42 | 8.25 | N/A | - |
| C16 | 5.14 | - | 5.36 | 7.42 | N/A | 8.10, 6.92[b] |
| C17 | 5.30 | 4.18 | 5.37 | 7.60 | N/A | 8.22, 6.93 |
| A18 | 5.82 | 4.36 | 7.43 | 8.00 | N/A | - |
| G19 | 5.45 | 4.36 | N/A | 7.34 | 10.46 | 7.02 |
| A20 | 5.67 | 4.62 | 7.78 | 8.16 | N/A | - |
| A21 | 5.57 | 4.55 | 7.73 | 7.96 | N/A | - |
| A22 | 5.81 | 4.64 | - | 7.98 | N/A | - |
| C23 | - | - | 5.60 | 7.64 | N/A | - |
| G24 | 5.53 | 4.70 | N/A | 7.60 | 11.92 | - |
| G25 | 5.64 | 4.39 | N/A | 7.31 | 12.41[b] | - |
| A26 | 5.92 | 4.39 | 7.96 | 8.04 | N/A | - |
| U27 | 5.60 | 4.40 | 5.26 | 7.48 | 12.23 | N/A |
| G28 | 5.77 | 4.72 | N/A | 7.70 | 10.30 | 6.30 |
| G29 | 5.62 | 4.51 | N/A | 7.10 | 12.70 | 6.17[b] |
| G30 | 5.65 | 4.23 | N/A | 7.16 | 12.49 | - |
| A31 | 6.06 | 4.86 | - | 8.03 | N/A | - |
| G32 | - | 4.37 | N/A | - | 10.79[a] | - |
| U33 | 5.49 | 4.60 | 4.99 | 7.68 | 13.39 | N/A |
| G34 | 5.71 | 4.37 | N/A | 7.68 | 12.69/12.41[c] | - |
| C35 | 5.32 | 4.36 | 5.19 | 7.61 | N/A | 8.31, 6.86 |
| A36 | 5.91 | 4.59 | 6.81 | 7.92 | N/A | - |
| G37 | 5.48 | 4.61 | N/A | 6.87 | 10.67 | 6.34 |
| A38 | 5.89 | 4.34 | 7.88 | 7.71 | N/A | - |
| U39 | 5.71 | 4.10 | 5.08 | 7.45 | 13.66 | N/A |

Exchangeable proton chemical shifts were assigned in a −2 °C spectrum. Non-exchangeable proton chemical shifts were assigned in a 20 °C spectrum. Exceptions are noted. [a] Ambiguous. [b] Assigned in a 20 °C spectrum. [c] Minor conformation.

Table S3: Assigned chemical shifts of the 11-nt hairpin construct.

| Residue | H1′ | H2′ | H3′ | H4′ | H5′ | H5″ | H2/H5 | H6/H8 | H1/H3 | H21/41/61, H22/42/62 amino | P[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C16 | 5.62 | 4.50 | 4.46 | 4.40 | 4.12 | 3.94 | 6.04 | 8.13 | N/A | 8.55, 7.10 | |
| C17 | 5.43 | 4.28 | 4.68 | 4.41 | 4.58 | 4.16 | 5.53 | 7.82 | N/A | 8.49, 6.93 | −4.10 |
| A18 | 5.98 | 4.47 | 4.85 | - | 4.52 | 4.18 | 7.67 | 8.18 | N/A | - | −3.98 |
| G19 | 5.56 | 4.35 | 4.61 | 4.37 | 4.42 | 4.11 | N/A | 7.34 | 10.49 | 7.16 | −3.47 |
| A20 | 5.68 | 4.68 | 4.48 | 4.25 | 4.26 | 3.95 | 7.73 | 8.21 | N/A | - | −2.12 |
| A21 | 5.52 | 4.56 | 4.66 | 4.41 | 4.30 | 4.02 | 7.61 | 7.89 | N/A | - | −3.21 |
| A22 | 5.85 | 4.68 | 4.62 | 4.47 | 4.58 | 4.21 | 8.08 | 7.93 | N/A | - | −4.16 |
| C23 | 4.85 | 4.41 | 4.34 | 4.30[a] | 4.36 | 4.13 | 5.58 | 7.73 | N/A | 7.72, 6.58 | −3.95 |
| G24 | 5.59 | 4.72 | 4.33 | - | 4.25 | 4.10 | N/A | 7.58 | 11.97 | 5.87, 7.39 | −3.56 |
| G25 | 5.69 | 4.52 | 4.57 | 4.42 | 4.50 | 4.07 | N/A | 7.30 | 12.80 | 8.18 | −4.07 |
| A26 | 5.96 | 4.10 | 4.29 | 4.26 | 4.43 | 4.06 | 7.91 | 7.85 | N/A | - | −3.86 |

Exchangeable proton chemical shifts were assigned in a −1 °C spectrum in $H_2O$. Non-exchangeable proton chemical shifts were assigned in a 20 °C spectrum in $D_2O$. All chemical shifts were assigned in spectra obtained with 5 mM $Mg^{2+}$. Exceptions are noted. [a] Assigned in a 20 °C spectrum without $Mg^{2+}$.

Table S4: Assigned chemical shifts of the 19-nt duplex construct.

| Residue | H1′ | H2′ | H3′ | H4′ | H5′ | H5″ | H2/H5 | H6/H8 | H1/H3 | H21/41/61, H22/42/62 amino | P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| G7 | 5.87 | 4.79 | 4.56 | 4.44 | 4.13 | 3.95 | N/A | 8.13 | 12.67 | - | |
| C8 | 5.50 | 4.32 | 4.61 | 4.46 | - | 4.17 | 5.27 | 7.84 | N/A | 8.54, 6.82 | −4.34 |
| A9 | 5.88 | 4.49 | 4.85 | 4.39 | - | 4.19 | 7.20 | 8.05 | N/A | - | −4.02 |
| G10 | 6.04 | 4.50 | 4.72 | 4.49 | - | - | N/A | 7.68 | 10.86[a] | - | |
| G11 | 5.57 | 4.58 | 4.35 | - | - | - | N/A | 7.37[b] | 13.08 | 5.91 | −3.93 |
| C12 | 5.52 | 4.24 | 4.45 | - | - | 4.10 | 5.21 | 7.51 | N/A | 8.47, 7.06 | −4.49 |
| C13 | 5.37 | 4.29 | 4.49 | 4.36 | - | 4.05 | 5.42 | 7.84 | N/A | 8.42, 6.96 | −4.54 |
| C14 | 5.41 | 4.32 | 4.47 | 4.34 | - | 4.05 | 5.43 | 7.64 | N/A | 8.25, 6.97 | −4.07 |
| A15 | 5.98 | 4.08 | 4.31 | 4.24 | - | 4.09 | 7.46 | 8.03 | N/A | - | −3.94 |
| U27 | 5.77 | 4.62 | 4.63 | 4.37 | 4.00 | 3.90 | 5.85 | 8.01 | - | N/A | |
| G28 | 5.91 | 4.79 | 4.69 | 4.56 | - | - | N/A | 8.02 | 12.16 | - | −3.74 |
| G29 | 5.75 | 4.56 | 4.50 | 4.51 | 4.51 | 4.14 | N/A | 7.33 | 12.81 | - | −3.69 |
| G30 | 5.66 | 4.17 | 4.65 | 4.42 | 4.54 | 4.09 | N/A | 7.26 | 12.57 | - | |
| A31 | 6.10 | 4.84 | 4.68 | 4.48 | - | - | 7.95 | 8.08 | N/A | - | |
| G32 | 5.37[b] | 4.36 | 4.23 | - | - | - | N/A | 7.10 | 10.86[a] | - | −4.00 |
| U33 | 5.52 | 4.60 | 4.53 | 4.42 | - | 4.09 | 5.04 | 7.73 | 13.31 | N/A | −4.85 |
| G34 | 5.75 | 4.42 | 4.56 | 4.44 | - | 4.11 | N/A | 7.69 | 12.91 | - | −4.05 |
| C35 | 5.36 | 4.30 | 4.37 | 4.33 | - | 4.04 | 5.17 | 7.44 | N/A | 8.07, 6.91 | −4.23 |
| A36 | 5.97 | 4.03 | 4.27 | 4.21 | 4.40 | 4.03 | 7.41 | 8.01 | N/A | - | −3.85 |

Exchangeable proton chemical shifts were assigned in a 0 °C spectrum in $H_2O$. Non-exchangeable proton and phosphorus chemical shifts were assigned in a 25 °C spectrum in $D_2O$. All chemical shifts were assigned in spectra obtained with 5 mM $Mg^{2+}$. Exceptions are noted. [a] Ambiguous. [b] Assigned in a −2 °C spectrum.

**Plasmid insert design for *in vitro* transcription of the 39-nt 3′ segment 7 mRNA hairpin by T7 RNA polymerase**

Cloning vector: pUC18 (Fermentas)

Intended sequence of the 39-nt RNA hairpin:
5′GGAUUUGCAGGCCUACCAGAAACGGAUGGGAGUGCAGAU3′

Sequence of the insert:
5′GACGAAGCTTTAATACGACTCACTATAGGATTTGCAGGCCTACCAGAAACGGATGGGAGTGC
AGATATCGAATTCGAGC3′

Reverse complement of the insert:
5′GCTCGAATTCGATATCTGCACTCCCATCCGTTTCTGGTAGGCCTGCAAATCCTATAGTGAGT
CGTATTAAAGCTTCGTC3′

Notation:
EcoR1 recognition sequence: 5′GAATTC3′
EcoRV recognition sequence: 5′GATATC3′[a]
HindIII recognition sequence: 5′AAGCTT3′
RNA hairpin sequence
T7 RNA polymerase promoter

[a] Bases that are part of the RNA hairpin sequence are labeled red.

**Distance restraints for AMBER modeling of 39-nt hairpin**

```
assign (residue 17 and name H1'  ) (residue 17 and name  H6  )  4.00  0.84  1.04 !
assign (residue 17 and name H2'  ) (residue 17 and name  H6  )  3.69  0.77  0.96 !
assign (residue 17 and name H2'  ) (residue 18 and name H1'  )  4.70  0.99  1.22 !
assign (residue 17 and name H2'  ) (residue 18 and name  H8  )  2.47  0.52  0.64 !
assign (residue 17 and name H3'  ) (residue 17 and name H1'  )  3.69  0.78  0.96 !
assign (residue 17 and name H3'  ) (residue 18 and name  H8  )  3.11  0.65  0.81 !
assign (residue 18 and name H1'  ) (residue 18 and name  H8  )  4.27  0.90  1.11 !
assign (residue 18 and name H2'  ) (residue 18 and name  H8  )  3.46  0.73  0.90 !
assign (residue 18 and name H3'  ) (residue 18 and name H1'  )  4.46  0.94  1.16 !
assign (residue 18 and name H3'  ) (residue 18 and name  H8  )  2.64  0.55  0.69 !
assign (residue 19 and name H1'  ) (residue 18 and name  H2  )  5.03  1.06  1.31 !
assign (residue 19 and name H2'  ) (residue 19 and name H1'  )  2.82  0.59  0.73 !
assign (residue 19 and name H3'  ) (residue 19 and name  H8  )  2.72  0.57  0.71 !
assign (residue 19 and name H4'  ) (residue 19 and name H1'  )  3.15  0.66  0.82 !
assign (residue 20 and name H1'  ) (residue 20 and name  H8  )  3.85  0.81  1.00 !
assign (residue 20 and name H2'  ) (residue 20 and name  H8  )  3.79  0.80  0.98 !
assign (residue 20 and name H2'  ) (residue 21 and name H1'  )  3.45  0.72  0.90 !
assign (residue 20 and name H2'  ) (residue 21 and name  H8  )  2.77  0.58  0.72 !
assign (residue 20 and name H3'  ) (residue 20 and name  H8  )  3.28  0.69  0.85 !
assign (residue 20 and name H3'  ) (residue 21 and name  H8  )  2.59  0.54  0.67 !
assign (residue 21 and name H1'  ) (residue 20 and name  H2  )  3.25  0.68  0.85 !
assign (residue 21 and name H1'  ) (residue 21 and name  H8  )  4.47  0.94  1.16 !
assign (residue 21 and name H2'  ) (residue 22 and name H1'  )  5.01  1.05  1.30 !
assign (residue 21 and name H3'  ) (residue 21 and name  H8  )  2.90  0.61  0.75 !
assign (residue 22 and name H1'  ) (residue 21 and name  H2  )  4.02  0.84  1.05 !
assign (residue 22 and name H4'  ) (residue 22 and name H1'  )  3.72  0.78  0.97 !
assign (residue 23 and name H2'  ) (residue 23 and name  H6  )  3.60  0.76  0.94 !
assign (residue 25 and name H3'  ) (residue 25 and name  H8  )  3.10  0.65  0.81 !
assign (residue 17 and name H3'  ) (residue 17 and name  H6  )  2.60  0.55  0.68 !
assign (residue 18 and name H2'  ) (residue 18 and name H1'  )  2.60  0.55  0.68 !
assign (residue 18 and name H2'  ) (residue 19 and name H1'  )  3.69  0.78  0.96 !
assign (residue 18 and name H2'  ) (residue 19 and name  H8  )  3.02  0.63  0.79 !
assign (residue 19 and name H2'  ) (residue 21 and name  H8  )  3.90  0.82  1.01 !
assign (residue 19 and name H3'  ) (residue 19 and name H1'  )  4.09  0.86  1.06 !
assign (residue 20 and name H4'  ) (residue 20 and name H1'  )  3.47  0.73  0.90 !
assign (residue 21 and name H2'  ) (residue 21 and name H1'  )  2.72  0.57  0.71 !
assign (residue 21 and name H3'  ) (residue 21 and name H1'  )  3.66  0.77  0.95 !
assign (residue 21 and name H4'  ) (residue 21 and name H1'  )  3.62  0.76  0.94 !
assign (residue 22 and name H2'  ) (residue 22 and name H1'  )  2.87  0.60  0.75 !
assign (residue 22 and name H2'  ) (residue 23 and name  H6  )  2.80  0.59  0.73 !
assign (residue 22 and name H3'  ) (residue 23 and name  H6  )  2.63  0.55  0.68 !
assign (residue 23 and name H2'  ) (residue 24 and name H1'  )  3.13  0.66  0.82 !
assign (residue 17 and name H41  ) (residue 24 and name  H1  )  2.33  0.93  0.93 !
assign (residue 18 and name H1'  ) (residue 24 and name  N2  )  2.62  1.05  2.05 !
assign (residue 22 and name  H8  ) (residue 19 and name H21  )  2.88  1.15  1.15 !
assign (residue 23 and name  N4  ) (residue 18 and name  H2  )  2.70  1.08  2.08 !
assign (residue 25 and name H1'  ) (residue 24 and name  N2  )  2.77  1.11  2.11 !
assign (residue  8 and name H2'  ) (residue  8 and name H1'  )  2.60  0.55  0.68 !
assign (residue  8 and name H3'  ) (residue  8 and name  H6  )  2.42  0.51  0.63 !
assign (residue  8 and name H4'  ) (residue  8 and name H1'  )  2.75  0.58  0.71 !
assign (residue  9 and name H2'  ) (residue  9 and name H1'  )  2.62  0.55  0.68 !
assign (residue  9 and name H2'  ) (residue 10 and name  H8  )  1.80  0.00  3.20 !
assign (residue  9 and name H3'  ) (residue  9 and name  H8  )  3.05  0.64  0.79 !
```

**Distance restraints for AMBER modeling of 39-nt hairpin (continued)**

```
assign (residue  9 and name H4'  ) (residue  9 and name H1'  )  3.19  0.67  0.83 !
assign (residue 11 and name H2'  ) (residue 11 and name H1'  )  2.90  0.61  0.75 !
assign (residue 11 and name H2'  ) (residue 12 and name  H6  )  2.51  0.53  0.65 !
assign (residue 12 and name H2'  ) (residue 12 and name H1'  )  2.71  0.57  0.71 !
assign (residue 12 and name H2'  ) (residue 12 and name  H6  )  4.05  0.85  1.05 !
assign (residue 12 and name H2'  ) (residue 13 and name  H6  )  2.32  0.49  0.60 !
assign (residue 12 and name H3'  ) (residue 12 and name  H6  )  2.54  0.53  0.66 !
assign (residue 13 and name H3'  ) (residue 13 and name H1'  )  3.11  0.65  0.81 !
assign (residue 13 and name H3'  ) (residue 13 and name  H6  )  2.35  0.49  0.61 !
assign (residue 13 and name H4'  ) (residue 13 and name H1'  )  3.05  0.64  0.79 !
assign (residue 28 and name H2'  ) (residue 28 and name  H8  )  3.68  0.77  0.96 !
assign (residue 28 and name H2'  ) (residue 29 and name H1'  )  4.33  0.91  1.13 !
assign (residue 28 and name H2'  ) (residue 29 and name  H8  )  2.48  0.52  0.65 !
assign (residue 28 and name H3'  ) (residue 28 and name H1'  )  3.21  0.67  0.84 !
assign (residue 28 and name H3'  ) (residue 28 and name  H8  )  2.89  0.61  0.75 !
assign (residue 28 and name H3'  ) (residue 29 and name  H8  )  3.28  0.69  0.85 !
assign (residue 28 and name H4'  ) (residue 28 and name H1'  )  3.18  0.67  0.83 !
assign (residue 29 and name H2'  ) (residue 29 and name  H8  )  4.42  0.93  1.15 !
assign (residue 29 and name H2'  ) (residue 30 and name H1'  )  4.37  0.92  1.14 !
assign (residue 29 and name H2'  ) (residue 30 and name  H8  )  2.48  0.52  0.64 !
assign (residue 29 and name H3'  ) (residue 29 and name  H8  )  2.81  0.59  0.73 !
assign (residue 29 and name H3'  ) (residue 30 and name  H8  )  3.38  0.71  0.88 !
assign (residue 30 and name H2'  ) (residue 30 and name H1'  )  2.77  0.58  0.72 !
assign (residue 30 and name H2'  ) (residue 31 and name  H8  )  2.49  0.52  0.65 !
assign (residue 30 and name H3'  ) (residue 30 and name H1'  )  3.99  0.84  1.04 !
assign (residue 30 and name H3'  ) (residue 30 and name  H8  )  2.88  0.61  0.75 !
assign (residue 30 and name H3'  ) (residue 31 and name  H8  )  3.74  0.79  0.97 !
assign (residue 30 and name H4'  ) (residue 30 and name H1'  )  3.47  0.73  0.90 !
assign (residue 31 and name H2'  ) (residue 31 and name H1'  )  2.61  0.55  0.68 !
assign (residue 31 and name H3'  ) (residue 31 and name H1'  )  3.82  0.80  0.99 !
assign (residue 31 and name H3'  ) (residue 31 and name  H8  )  2.78  0.58  0.72 !
assign (residue 31 and name H4'  ) (residue 31 and name H1'  )  3.34  0.70  0.87 !
assign (residue 32 and name H2'  ) (residue 33 and name  H6  )  2.44  0.51  0.63 !
assign (residue 32 and name H3'  ) (residue 33 and name  H6  )  3.21  0.67  0.83 !
assign (residue 33 and name H2'  ) (residue 34 and name H1'  )  3.64  0.76  0.95 !
assign (residue 33 and name H2'  ) (residue 34 and name  H8  )  2.34  0.49  0.61 !
assign (residue 33 and name H3'  ) (residue 33 and name H1'  )  2.50  0.00  2.00 !
assign (residue 33 and name H3'  ) (residue 33 and name  H6  )  2.43  0.51  0.63 !
assign (residue 34 and name H2'  ) (residue 34 and name  H8  )  3.51  0.74  0.91 !
assign (residue 34 and name H2'  ) (residue 35 and name H1'  )  3.71  0.78  0.96 !
assign (residue 34 and name H2'  ) (residue 35 and name  H6  )  2.44  0.51  0.63 !
assign (residue 34 and name H3'  ) (residue 34 and name  H8  )  2.53  0.53  0.66 !
assign (residue 35 and name H3'  ) (residue 35 and name  H6  )  2.51  0.53  0.65 !
assign (residue  8 and name H1'  ) (residue  8 and name  H6  )  3.83  0.80  0.99 !
assign (residue  8 and name H3'  ) (residue  8 and name H1'  )  3.19  0.67  0.83 !
assign (residue 10 and name H1'  ) (residue 10 and name  H8  )  3.02  0.63  0.78 !
assign (residue 11 and name H1'  ) (residue 11 and name  H8  )  3.20  0.00  0.90 !
assign (residue 12 and name H1'  ) (residue 12 and name  H6  )  3.20  0.00  0.90 !
assign (residue 13 and name H1'  ) (residue 13 and name  H6  )  4.48  0.94  1.17 !
assign (residue 29 and name H2'  ) (residue 29 and name H1'  )  2.45  0.51  0.64 !
assign (residue 29 and name H3'  ) (residue 29 and name H1'  )  2.50  0.00  2.00 !
assign (residue 31 and name H2'  ) (residue 32 and name  H8  )  3.17  0.67  0.82 !
assign (residue 32 and name H1'  ) (residue 31 and name  H2  )  3.51  0.74  0.91 !
```

**Distance restraints for AMBER modeling of 39-nt hairpin (continued)**

```
assign (residue 34 and name H1'  ) (residue  9 and name  H2  )  4.10  0.86  1.07 !
assign (residue 34 and name H2'  ) (residue 34 and name H1'  )  2.39  0.50  0.62 !
assign (residue 34 and name H3'  ) (residue 34 and name H1'  )  3.10  0.65  0.81 !
assign (residue 34 and name H3'  ) (residue 35 and name  H6  )  3.00  0.63  0.78 !
assign (residue 35 and name H1'  ) (residue 35 and name  H6  )  3.84  0.81  1.00 !
assign (residue  9 and name  H2  ) (residue 33 and name  H3  )  2.57  1.03  1.03 !
assign (residue  9 and name  H2  ) (residue 34 and name  H1  )  4.19  1.68  1.68 !
assign (residue 12 and name H1'  ) (residue 11 and name  H1  )  3.55  1.42  1.42 !
assign (residue 12 and name H41  ) (residue 30 and name  H1  )  2.15  0.86  0.86 !
assign (residue 13 and name H1'  ) (residue 30 and name  H1  )  4.41  1.76  1.76 !
assign (residue 30 and name H1'  ) (residue 29 and name  H1  )  3.42  1.37  1.37 !
assign (residue 31 and name  H2  ) (residue 11 and name  H1  )  2.77  1.11  1.11 !
assign (residue 11 and name H22  ) (residue 31 and name  H2  )  1.80  0.00  1.50 !
assign (residue  5 and name H1'  ) (residue  4 and name  H3  )  5.25  2.10  2.10 !
assign (residue  6 and name  H5  ) (residue  5 and name  H3  )  5.66  2.27  2.27 !
assign (residue 28 and name  H1  ) (residue 14 and name  H3  )  2.29  0.92  0.92 !
assign (residue 37 and name  H1  ) (residue  5 and name  H3  )  3.10  1.24  1.24 !
assign (residue 37 and name  N2  ) (residue  5 and name  H3  )  3.00  0.00  3.00 !
assign (residue  3 and name  H2  ) (residue 39 and name  H3  )  4.01  1.60  1.60 !
assign (residue  5 and name  H3  ) (residue  4 and name  H3  )  4.07  1.63  1.63 !
assign (residue  5 and name  H3  ) (residue  6 and name  H3  )  4.54  1.81  1.81 !
assign (residue  6 and name H1'  ) (residue 37 and name  H1  )  3.81  1.52  1.52 !
assign (residue  7 and name  H1  ) (residue  6 and name  H3  )  5.07  2.03  2.03 !
assign (residue  7 and name H1'  ) (residue  6 and name  H3  )  3.00  0.00  5.00 !
assign (residue  7 and name  N2  ) (residue  6 and name  H3  )  3.00  0.00  4.00 !
assign (residue 14 and name H1'  ) (residue 14 and name  H3  )  4.84  1.94  1.94 !
assign (residue 14 and name H1'  ) (residue 28 and name  H1  )  4.86  1.94  1.94 !
assign (residue 14 and name H1'  ) (residue 29 and name  H1  )  3.49  1.40  1.40 !
assign (residue 14 and name  H3  ) (residue 29 and name  H1  )  4.08  1.63  1.63 !
assign (residue 15 and name H1'  ) (residue 14 and name  H3  )  4.73  1.89  1.89 !
assign (residue 15 and name H1'  ) (residue 28 and name  H1  )  3.59  1.44  1.44 !
assign (residue 15 and name  H2  ) (residue 14 and name  H3  )  5.65  2.26  2.26 !
assign (residue 15 and name  H2  ) (residue 28 and name  H1  )  5.16  2.06  2.06 !
assign (residue 28 and name  H1  ) (residue 14 and name  H3  )  2.76  1.10  1.10 !
assign (residue 28 and name  H1  ) (residue 29 and name  H1  )  4.58  1.83  1.83 !
assign (residue 28 and name  N2  ) (residue 14 and name  H3  )  3.00  0.00  3.00 !
assign (residue 28 and name  N2  ) (residue 29 and name  H1  )  3.00  0.00  4.00 !
assign (residue 29 and name H1'  ) (residue 14 and name  H3  )  3.00  0.00  5.00 !
assign (residue 35 and name  N4  ) (residue  6 and name  H3  )  4.31  1.72  2.72 !
assign (residue 36 and name H1'  ) (residue  6 and name  H3  )  3.00  0.00  5.00 !
assign (residue 37 and name  H1  ) (residue  4 and name  H3  )  5.52  2.21  2.21 !
assign (residue 37 and name  H1  ) (residue  6 and name  H3  )  4.37  1.75  1.75 !
assign (residue 37 and name H1'  ) (residue  6 and name  H3  )  4.51  1.80  1.80 !
assign (residue 39 and name H1'  ) (residue  4 and name  H3  )  5.08  2.03  2.03 !
assign (residue  3 and name H1'  ) (residue  3 and name  H8  )  5.00  2.00  2.00 !
assign (residue  3 and name H2'  ) (residue  3 and name H1'  )  3.26  1.31  1.31 !
assign (residue  3 and name H2'  ) (residue  3 and name  H8  )  2.00  0.00  2.50 !
assign (residue  4 and name H1'  ) (residue  3 and name  H2  )  2.00  0.00  3.00 !
assign (residue  4 and name H1'  ) (residue  4 and name  H6  )  2.00  0.00  3.00 !
assign (residue  4 and name H2'  ) (residue  4 and name H1'  )  3.23  1.29  1.29 !
assign (residue  5 and name H1'  ) (residue 38 and name  H2  )  3.43  1.37  1.37 !
assign (residue  7 and name H1'  ) (residue 36 and name  H2  )  3.58  1.43  1.43 !
assign (residue  7 and name H2'  ) (residue  8 and name  H6  )  1.80  0.00  2.70 !
```

**Distance restraints for AMBER modeling of 39-nt hairpin (continued)**

```
assign (residue 13 and name H2'  ) (residue 14 and name  H6  )  2.29  0.92  0.92 !
assign (residue 14 and name H1'  ) (residue 14 and name  H6  )  3.33  1.33  1.33 !
assign (residue 14 and name H2'  ) (residue 14 and name  H6  )  3.05  1.22  1.22 !
assign (residue 14 and name H2'  ) (residue 15 and name  H8  )  2.18  0.87  0.87 !
assign (residue 14 and name  H5  ) (residue 13 and name  H6  )  3.74  1.50  1.50 !
assign (residue 15 and name  H8  ) (residue 14 and name  H6  )  4.52  1.81  1.81 !
assign (residue 15 and name H1'  ) (residue 15 and name  H8  )  3.87  1.55  1.55 !
assign (residue 15 and name H1'  ) (residue 28 and name  N2  )  2.00  0.00  2.00 !
assign (residue 15 and name H2'  ) (residue 15 and name H1'  )  2.00  0.00  1.50 !
assign (residue 15 and name H2'  ) (residue 15 and name  H8  )  2.00  0.00  2.50 !
assign (residue 15 and name H2'  ) (residue 16 and name  H6  )  2.00  0.00  2.00 !
assign (residue 16 and name H1'  ) (residue 15 and name  H2  )  3.01  1.20  1.20 !
assign (residue 17 and name H1'  ) (residue 26 and name  H2  )  3.00  0.00  2.50 !
assign (residue 23 and name  H5  ) (residue 18 and name  H2  )  3.66  1.46  2.46 !
assign (residue 25 and name H2'  ) (residue 27 and name  H6  )  4.00  0.00 99.00 !
assign (residue 26 and name H1'  ) (residue 26 and name  H8  )  4.38  1.75  1.75 !
assign (residue 26 and name H2'  ) (residue 26 and name H1'  )  2.71  1.08  1.08 !
assign (residue 27 and name H1'  ) (residue 27 and name  H6  )  3.37  1.35  1.35 !
assign (residue 27 and name H2'  ) (residue 27 and name H1'  )  2.00  0.00  1.50 !
assign (residue 27 and name  H5  ) (residue 26 and name  H8  )  4.07  1.63  1.63 !
assign (residue 26 and name  H8  ) (residue 27 and name  H6  )  4.07  1.63  1.63 !
assign (residue 28 and name H1'  ) (residue 15 and name  H2  )  3.19  1.28  1.28 !
assign (residue 29 and name H1'  ) (residue 28 and name  N2  )  2.00  0.00  2.00 !
assign (residue 35 and name H2'  ) (residue 36 and name  H8  )  1.80  0.00  2.70 !
assign (residue 36 and name H1'  ) (residue 36 and name  H8  )  4.24  1.70  1.70 !
assign (residue 36 and name H2'  ) (residue 36 and name H1'  )  2.45  0.98  0.98 !
assign (residue 36 and name H2'  ) (residue 37 and name  H8  )  2.53  1.01  1.01 !
assign (residue 37 and name H1'  ) (residue 36 and name  H2  )  4.62  1.85  1.85 !
assign (residue 38 and name H1'  ) (residue 37 and name  N2  )  2.00  0.00  2.00 !
assign (residue 38 and name H2'  ) (residue 39 and name  H6  )  2.00  0.00  2.50 !
assign (residue 39 and name H1'  ) (residue 39 and name  H6  )  4.02  1.61  1.61 !
assign (residue 39 and name H2'  ) (residue 39 and name H1'  )  3.26  1.30  1.30 !
assign (residue 39 and name H2'  ) (residue 39 and name  H6  )  2.00  0.00  2.50 !

assign (resid 3 and name N1  ) (resid 39 and name H3  )  2.1 0.3 0.3
assign (resid 3 and name H61  ) (resid 39 and name O4  ) 2.1 0.3 0.3
assign (resid 4 and name H3  ) (resid 38 and name N1  )  2.1 0.3 0.3
assign (resid 4 and name O4  ) (resid 38 and name H61  ) 2.1 0.3 0.3
assign (resid 5 and name H3  ) (resid 37 and name O6  )  2.1 0.3 0.3
assign (resid 5 and name O2  ) (resid 37 and name H1  ) 2.1 0.3 0.3
assign (resid 6 and name H3  ) (resid 36 and name N1  )  2.1 0.3 0.3
assign (resid 6 and name O4  ) (resid 36 and name H61  ) 2.1 0.3 0.3
assign (resid 7 and name H1  ) (resid 35 and name N3  )  2.1 0.3 0.3
assign (resid 7 and name O6  ) (resid 35 and name H41  ) 2.1 0.3 0.3
assign (resid 7 and name H22  ) (resid 35 and name O2  ) 2.1 0.3 0.3
assign (resid 8 and name N3  ) (resid 34 and name H1  )  2.1 0.3 0.3
assign (resid 8 and name H41  ) (resid 34 and name O6  ) 2.1 0.3 0.3
assign (resid 8 and name O2  ) (resid 34 and name H22  ) 2.1 0.3 0.3
assign (resid 9 and name N1  ) (resid 33 and name H3  )  2.1 0.3 0.3
assign (resid 9 and name H61  ) (resid 33 and name O4  ) 2.1 0.3 0.3
assign (resid 12 and name N3  ) (resid 30 and name H1  )  2.1 0.3 0.3
assign (resid 12 and name H41  ) (resid 30 and name O6  ) 2.1 0.3 0.3
assign (resid 12 and name O2  ) (resid 30 and name H22  ) 2.1 0.3 0.3
```

**Distance restraints for AMBER modeling of 39-nt hairpin (continued)**

```
assign (resid 13 and name N3  ) (resid 29 and name H1  )  2.1 0.3 0.3
assign (resid 13 and name H41  ) (resid 29 and name O6  ) 2.1 0.3 0.3
assign (resid 13 and name O2  ) (resid 29 and name H22  ) 2.1 0.3 0.3
assign (resid 14 and name H3  ) (resid 28 and name O6  )  2.1 0.3 0.3
assign (resid 14 and name O2  ) (resid 28 and name H1  ) 2.1 0.3 0.3
assign (resid 15 and name N1  ) (resid 27 and name H3  )  2.1 0.3 0.3
assign (resid 15 and name H61  ) (resid 27 and name O4  ) 2.1 0.3 0.3
assign (resid 16 and name N3  ) (resid 25 and name H1  )  2.1 0.3 0.3
assign (resid 16 and name H41  ) (resid 25 and name O6  ) 2.1 0.3 0.3
assign (resid 16 and name O2  ) (resid 25 and name H22  ) 2.1 0.3 0.3
assign (resid 17 and name N3  ) (resid 24 and name H1  )  2.1 0.3 0.3
assign (resid 17 and name H41  ) (resid 24 and name O6  ) 2.1 0.3 0.3
assign (resid 17 and name O2  ) (resid 24 and name H22  ) 2.1 0.3 0.3
```

## Dihedral restraints for AMBER modeling of 39-nt hairpin

```
 2 GUA   ALPHA -155.0   25.0
 3 ADE   ALPHA -155.0   25.0
 4 URA   ALPHA -155.0   25.0
 5 URA   ALPHA -155.0   25.0
 6 URA   ALPHA -155.0   25.0
 7 GUA   ALPHA -155.0   25.0
 8 CYT   ALPHA -155.0   25.0
 9 ADE   ALPHA -155.0   25.0
13 CYT   ALPHA -155.0   25.0
14 URA   ALPHA -155.0   25.0
17 CYT   ALPHA -155.0   25.0
18 ADE   ALPHA -155.0   25.0
28 GUA   ALPHA -155.0   25.0
29 GUA   ALPHA -155.0   25.0
30 GUA   ALPHA -155.0   25.0
34 GUA   ALPHA -155.0   25.0
35 CYT   ALPHA -155.0   25.0
36 ADE   ALPHA -155.0   25.0
37 GUA   ALPHA -155.0   25.0
38 ADE   ALPHA -155.0   25.0
39 URA   ALPHA -155.0   25.0
 2 GUA    BETA   90.0  240.0
 3 ADE    BETA   90.0  240.0
 4 URA    BETA   90.0  240.0
 5 URA    BETA   90.0  240.0
 6 URA    BETA   90.0  240.0
 7 GUA    BETA   90.0  240.0
 8 CYT    BETA   90.0  240.0
 9 ADE    BETA   90.0  240.0
13 CYT    BETA   90.0  240.0
14 URA    BETA   90.0  240.0
17 CYT    BETA   90.0  240.0
18 ADE    BETA   90.0  240.0
28 GUA    BETA   90.0  240.0
29 GUA    BETA   90.0  240.0
30 GUA    BETA   90.0  240.0
34 GUA    BETA   90.0  240.0
35 CYT    BETA   90.0  240.0
36 ADE    BETA   90.0  240.0
37 GUA    BETA   90.0  240.0
38 ADE    BETA   90.0  240.0
39 URA    BETA   90.0  240.0
 1 GUA   GAMMA    0.0  120.0
 2 GUA   GAMMA    0.0  120.0
 3 ADE   GAMMA    0.0  120.0
 4 URA   GAMMA    0.0  120.0
 5 URA   GAMMA    0.0  120.0
 6 URA   GAMMA    0.0  120.0
 7 GUA   GAMMA    0.0  120.0
 8 CYT   GAMMA    0.0  120.0
 9 ADE   GAMMA    0.0  120.0
13 CYT   GAMMA    0.0  120.0
14 URA   GAMMA    0.0  120.0
17 CYT   GAMMA    0.0  120.0
18 ADE   GAMMA    0.0  120.0
28 GUA   GAMMA    0.0  120.0
29 GUA   GAMMA    0.0  120.0
30 GUA   GAMMA    0.0  120.0
34 GUA   GAMMA    0.0  120.0
35 CYT   GAMMA    0.0  120.0
36 ADE   GAMMA    0.0  120.0
37 GUA   GAMMA    0.0  120.0
38 ADE   GAMMA    0.0  120.0
39 URA   GAMMA    0.0  120.0
 1 GUA   DELTA   45.0  115.0
 2 GUA   DELTA   45.0  115.0
 3 ADE   DELTA   45.0  115.0
 4 URA   DELTA   45.0  115.0
 5 URA   DELTA   45.0  115.0
 6 URA   DELTA   45.0  115.0
 7 GUA   DELTA   45.0  115.0
 8 CYT   DELTA   45.0  115.0
 9 ADE   DELTA   45.0  115.0
12 CYT   DELTA   45.0  115.0
13 CYT   DELTA   45.0  115.0
14 URA   DELTA   45.0  115.0
15 ADE   DELTA   45.0  115.0
16 CYT   DELTA   45.0  115.0
17 CYT   DELTA   45.0  115.0
18 ADE   DELTA   45.0  115.0
23 CYT   DELTA   45.0  115.0
26 ADE   DELTA   45.0  115.0
27 URA   DELTA   45.0  115.0
28 GUA   DELTA   45.0  115.0
29 GUA   DELTA   45.0  115.0
30 GUA   DELTA   45.0  115.0
31 ADE   DELTA   45.0  115.0
33 URA   DELTA   45.0  115.0
34 GUA   DELTA   45.0  115.0
35 CYT   DELTA   45.0  115.0
36 ADE   DELTA   45.0  115.0
37 GUA   DELTA   45.0  115.0
38 ADE   DELTA   45.0  115.0
39 URA   DELTA   45.0  115.0
 1 GUA  EPSILN -240.0   10.0
 2 GUA  EPSILN -240.0   10.0
 3 ADE  EPSILN -240.0   10.0
 4 URA  EPSILN -240.0   10.0
 5 URA  EPSILN -240.0   10.0
 6 URA  EPSILN -240.0   10.0
 7 GUA  EPSILN -240.0   10.0
 8 CYT  EPSILN -240.0   10.0
12 CYT  EPSILN -240.0   10.0
13 CYT  EPSILN -240.0   10.0
14 URA  EPSILN -240.0   10.0
16 CYT  EPSILN -240.0   10.0
17 CYT  EPSILN -240.0   10.0
27 URA  EPSILN -240.0   10.0
```

**Dihedral restraints for AMBER modeling of 39-nt hairpin (continued)**

```
28 GUA  EPSILN -240.0   10.0
29 GUA  EPSILN -240.0   10.0
33 URA  EPSILN -240.0   10.0
34 GUA  EPSILN -240.0   10.0
35 CYT  EPSILN -240.0   10.0
36 ADE  EPSILN -240.0   10.0
37 GUA  EPSILN -240.0   10.0
38 ADE  EPSILN -240.0   10.0
 1 GUA    ZETA -160.0   20.0
 2 GUA    ZETA -160.0   20.0
 3 ADE    ZETA -160.0   20.0
 4 URA    ZETA -160.0   20.0
 5 URA    ZETA -160.0   20.0
 6 URA    ZETA -160.0   20.0
 7 GUA    ZETA -160.0   20.0
 8 CYT    ZETA -160.0   20.0
12 CYT    ZETA -160.0   20.0
13 CYT    ZETA -160.0   20.0
14 URA    ZETA -160.0   20.0
16 CYT    ZETA -160.0   20.0
17 CYT    ZETA -160.0   20.0
27 URA    ZETA -160.0   20.0
28 GUA    ZETA -160.0   20.0
29 GUA    ZETA -160.0   20.0
33 URA    ZETA -160.0   20.0
34 GUA    ZETA -160.0   20.0
35 CYT    ZETA -160.0   20.0
36 ADE    ZETA -160.0   20.0
37 GUA    ZETA -160.0   20.0
38 ADE    ZETA -160.0   20.0
 1 GUA     CHI  170.0  340.0
 2 GUA     CHI  170.0  340.0
 3 ADE     CHI  170.0  340.0
 4 URA     CHI  170.0  340.0
 5 URA     CHI  170.0  340.0
 6 URA     CHI  170.0  340.0
 7 GUA     CHI  170.0  340.0
 8 CYT     CHI  170.0  340.0
 9 ADE     CHI  170.0  340.0
11 GUA     CHI  170.0  340.0
12 CYT     CHI  170.0  340.0
13 CYT     CHI  170.0  340.0
14 URA     CHI  170.0  340.0
15 ADE     CHI  170.0  340.0
16 CYT     CHI  170.0  340.0
17 CYT     CHI  170.0  340.0
18 ADE     CHI  170.0  340.0
19 GUA     CHI  170.0  340.0
20 ADE     CHI  170.0  340.0
21 ADE     CHI  170.0  340.0
22 ADE     CHI  170.0  340.0
23 CYT     CHI  170.0  340.0
24 GUA     CHI  170.0  340.0
25 GUA     CHI  170.0  340.0
26 ADE     CHI  170.0  340.0
27 URA     CHI  170.0  340.0
28 GUA     CHI  170.0  340.0
29 GUA     CHI  170.0  340.0
30 GUA     CHI  170.0  340.0
31 ADE     CHI  170.0  340.0
32 GUA     CHI  170.0  340.0
33 URA     CHI  170.0  340.0
34 GUA     CHI  170.0  340.0
35 CYT     CHI  170.0  340.0
36 ADE     CHI  170.0  340.0
37 GUA     CHI  170.0  340.0
38 ADE     CHI  170.0  340.0
39 URA     CHI  170.0  340.0
```

**Distance restraints for AMBER modeling of 11-nt hairpin**

```
assign (residue 16 and name H1'  ) (residue 16 and name  H6  )  3.68  0.77  0.96
assign (residue 16 and name H1'  ) (residue 26 and name  H2  )  3.86  0.81  1.00
assign (residue 17 and name H1'  ) (residue 17 and name  H6  )  4.00  0.84  1.04
assign (residue 17 and name H2'  ) (residue 17 and name  H6  )  3.69  0.77  0.96
assign (residue 17 and name H2'  ) (residue 18 and name H1'  )  4.70  0.99  1.22
assign (residue 17 and name H2'  ) (residue 18 and name  H8  )  2.47  0.52  0.64
assign (residue 17 and name H3'  ) (residue 17 and name H1'  )  3.69  0.78  0.96
assign (residue 17 and name H3'  ) (residue 18 and name  H8  )  3.11  0.65  0.81
assign (residue 18 and name H1'  ) (residue 18 and name  H8  )  4.27  0.90  1.11
assign (residue 18 and name H2'  ) (residue 18 and name  H8  )  3.46  0.73  0.90
assign (residue 18 and name H3'  ) (residue 18 and name H1'  )  4.46  0.94  1.16
assign (residue 18 and name H3'  ) (residue 18 and name  H8  )  2.64  0.55  0.69
assign (residue 19 and name H1'  ) (residue 18 and name  H2  )  5.03  1.06  1.31
assign (residue 19 and name H2'  ) (residue 19 and name H1'  )  2.82  0.59  0.73
assign (residue 19 and name H3'  ) (residue 19 and name  H8  )  2.72  0.57  0.71
assign (residue 19 and name H4'  ) (residue 19 and name H1'  )  3.15  0.66  0.82
assign (residue 20 and name H1'  ) (residue 20 and name  H8  )  3.85  0.81  1.00
assign (residue 20 and name H2'  ) (residue 20 and name  H8  )  3.79  0.80  0.98
assign (residue 20 and name H2'  ) (residue 21 and name H1'  )  3.45  0.72  0.90
assign (residue 20 and name H2'  ) (residue 21 and name  H8  )  2.77  0.58  0.72
assign (residue 20 and name H3'  ) (residue 20 and name  H8  )  3.28  0.69  0.85
assign (residue 20 and name H3'  ) (residue 21 and name  H8  )  2.59  0.54  0.67
assign (residue 21 and name H1'  ) (residue 20 and name  H2  )  3.25  0.68  0.85
assign (residue 21 and name H1'  ) (residue 21 and name  H8  )  4.47  0.94  1.16
assign (residue 21 and name H2'  ) (residue 22 and name H1'  )  5.01  1.05  1.30
assign (residue 21 and name H3'  ) (residue 21 and name  H8  )  2.90  0.61  0.75
assign (residue 22 and name H1'  ) (residue 21 and name  H2  )  4.02  0.84  1.05
assign (residue 22 and name H4'  ) (residue 22 and name H1'  )  3.72  0.78  0.97
assign (residue 23 and name H2'  ) (residue 23 and name  H6  )  3.60  0.76  0.94
assign (residue 23 and name H2'  ) (residue 24 and name  H8  )  3.17  0.67  0.83
assign (residue 24 and name H2'  ) (residue 25 and name  H8  )  2.52  0.53  0.65
assign (residue 24 and name H3'  ) (residue 24 and name H1'  )  3.09  0.65  0.80
assign (residue 24 and name H3'  ) (residue 24 and name  H8  )  2.90  0.61  0.75
assign (residue 24 and name H3'  ) (residue 25 and name  H8  )  3.03  0.64  0.79
assign (residue 25 and name H1'  ) (residue 25 and name  H8  )  4.47  0.94  1.16
assign (residue 25 and name H2'  ) (residue 25 and name H1'  )  2.66  0.56  0.69
assign (residue 25 and name H2'  ) (residue 26 and name H1'  )  4.54  0.95  1.18
assign (residue 25 and name H3'  ) (residue 25 and name  H8  )  3.10  0.65  0.81
assign (residue 26 and name H1'  ) (residue 26 and name  H2  )  5.58  1.17  1.45
assign (residue 26 and name H1'  ) (residue 26 and name  H8  )  4.29  0.90  1.12
assign (residue 17 and name H3'  ) (residue 17 and name  H6  )  2.60  0.55  0.68
assign (residue 18 and name H2'  ) (residue 18 and name H1'  )  2.60  0.55  0.68
assign (residue 18 and name H2'  ) (residue 19 and name H1'  )  3.69  0.78  0.96
assign (residue 18 and name H2'  ) (residue 19 and name  H8  )  3.02  0.63  0.79
assign (residue 19 and name H2'  ) (residue 21 and name  H8  )  3.90  0.82  1.01
assign (residue 19 and name H3'  ) (residue 19 and name H1'  )  4.09  0.86  1.06
assign (residue 20 and name H4'  ) (residue 20 and name H1'  )  3.47  0.73  0.90
assign (residue 21 and name H2'  ) (residue 21 and name H1'  )  2.72  0.57  0.71
assign (residue 21 and name H3'  ) (residue 21 and name H1'  )  3.66  0.77  0.95
assign (residue 21 and name H4'  ) (residue 21 and name H1'  )  3.62  0.76  0.94
assign (residue 22 and name H2'  ) (residue 22 and name H1'  )  2.87  0.60  0.75
assign (residue 22 and name H2'  ) (residue 23 and name  H6  )  2.80  0.59  0.73
assign (residue 22 and name H3'  ) (residue 23 and name  H6  )  2.63  0.55  0.68
```

**Distance restraints for AMBER modeling of 11-nt hairpin (continued)**

```
assign (residue 23 and name H2'  ) (residue 24 and name H1'  )  3.13  0.66  0.82
assign (residue 17 and name H41  ) (residue 24 and name  H1  )  2.33  0.93  0.93
assign (residue 18 and name H1'  ) (residue 24 and name  N2  )  2.62  1.05  2.05
assign (residue 22 and name  H8  ) (residue 19 and name H21  )  2.88  1.15  1.15
assign (residue 23 and name  N4  ) (residue 18 and name  H2  )  2.70  1.08  2.08
assign (residue 25 and name H1'  ) (residue 24 and name  N2  )  2.77  1.11  2.11

assign (resid 16 and name N3  ) (resid 25 and name H1  )  2.1 0.3 0.3
assign (resid 16 and name H41  ) (resid 25 and name O6  ) 2.1 0.3 0.3
assign (resid 16 and name O2  ) (resid 25 and name H22  ) 2.1 0.3 0.3
assign (resid 17 and name N3  ) (resid 24 and name H1  )  2.1 0.3 0.3
assign (resid 17 and name H41  ) (resid 24 and name O6  ) 2.1 0.3 0.3
assign (resid 17 and name O2  ) (resid 24 and name H22  ) 2.1 0.3 0.3
assign (resid 19 and name H1  ) (resid 19 and name H22  ) 1.8 0 0.7
assign (resid 24 and name H1  ) (resid 24 and name H22  ) 1.8 0 0.7
assign (resid 25 and name H1  ) (resid 25 and name H22  ) 1.8 0 0.7
```

**Dihedral restraints for AMBER modeling of 11-nt hairpin**

```
17 CYT   ALPHA -155.0   25.0
18 ADE   ALPHA -155.0   25.0
17 CYT    BETA   90.0  240.0
18 ADE    BETA   90.0  240.0
17 CYT   GAMMA    0.0  120.0
18 ADE   GAMMA    0.0  120.0
16 CYT   DELTA   45.0  115.0
17 CYT   DELTA   45.0  115.0
18 ADE   DELTA   45.0  115.0
23 CYT   DELTA   45.0  115.0
26 ADE   DELTA   45.0  115.0
16 CYT  EPSILN -240.0   10.0
17 CYT  EPSILN -240.0   10.0
16 CYT     CHI  170.0  340.0
17 CYT     CHI  170.0  340.0
18 ADE     CHI  170.0  340.0
19 GUA     CHI  170.0  340.0
20 ADE     CHI  170.0  340.0
21 ADE     CHI  170.0  340.0
22 ADE     CHI  170.0  340.0
23 CYT     CHI  170.0  340.0
24 GUA     CHI  170.0  340.0
25 GUA     CHI  170.0  340.0
26 ADE     CHI  170.0  340.0
```

**Distance restraints for AMBER modeling of 19-nt duplex**

```
assign (residue  8 and name H1'  ) (residue  7 and name  H1  )  3.93  1.57  1.57
assign (residue  9 and name  H2  ) (residue 33 and name  H3  )  2.57  1.03  1.03
assign (residue  9 and name  H2  ) (residue 34 and name  H1  )  4.19  1.68  1.68
assign (residue 12 and name H1'  ) (residue 11 and name  H1  )  3.55  1.42  1.42
assign (residue 12 and name H41  ) (residue 30 and name  H1  )  2.15  0.86  0.86
assign (residue 13 and name H1'  ) (residue 30 and name  H1  )  4.41  1.76  1.76
assign (residue 14 and name H1'  ) (residue 29 and name  H1  )  3.93  1.57  1.57
assign (residue 14 and name H41  ) (residue 28 and name  H1  )  2.28  0.91  0.91
assign (residue 30 and name H1'  ) (residue 29 and name  H1  )  3.42  1.37  1.37
assign (residue 31 and name  H2  ) (residue 11 and name  H1  )  2.77  1.11  1.11
assign (residue 35 and name H41  ) (residue  7 and name  H1  )  2.10  0.84  0.84
assign (residue 36 and name H1'  ) (residue  7 and name  H1  )  3.57  1.43  1.43
assign (residue 11 and name H22  ) (residue 31 and name  H2  )  1.80  0.00  1.50
assign (residue  7 and name H2'  ) (residue  8 and name  H6  )  2.44  0.51  0.64
assign (residue  7 and name H3'  ) (residue  7 and name  H8  )  3.45  0.72  0.90
assign (residue  7 and name H4'  ) (residue  7 and name H1'  )  3.18  0.67  0.83
assign (residue  8 and name H2'  ) (residue  8 and name H1'  )  2.60  0.55  0.68
assign (residue  8 and name H3'  ) (residue  8 and name  H6  )  2.42  0.51  0.63
assign (residue  8 and name H4'  ) (residue  8 and name H1'  )  2.75  0.58  0.71
assign (residue  9 and name H2'  ) (residue  9 and name H1'  )  2.62  0.55  0.68
assign (residue  9 and name H2'  ) (residue 10 and name  H8  )  1.80  0.00  3.20
assign (residue  9 and name H3'  ) (residue  9 and name  H8  )  3.05  0.64  0.79
assign (residue  9 and name H4'  ) (residue  9 and name H1'  )  3.19  0.67  0.83
assign (residue 11 and name H2'  ) (residue 11 and name H1'  )  2.90  0.61  0.75
assign (residue 11 and name H2'  ) (residue 12 and name  H6  )  2.51  0.53  0.65
assign (residue 12 and name H2'  ) (residue 12 and name H1'  )  2.71  0.57  0.71
assign (residue 12 and name H2'  ) (residue 12 and name  H6  )  4.05  0.85  1.05
assign (residue 12 and name H2'  ) (residue 13 and name  H6  )  2.32  0.49  0.60
assign (residue 12 and name H3'  ) (residue 12 and name  H6  )  2.54  0.53  0.66
assign (residue 13 and name H2'  ) (residue 14 and name  H6  )  2.41  0.51  0.63
assign (residue 13 and name H3'  ) (residue 13 and name H1'  )  3.11  0.65  0.81
assign (residue 13 and name H3'  ) (residue 13 and name  H6  )  2.35  0.49  0.61
assign (residue 13 and name H4'  ) (residue 13 and name H1'  )  3.05  0.64  0.79
assign (residue 14 and name H2'  ) (residue 14 and name H1'  )  2.57  0.54  0.67
assign (residue 14 and name H3'  ) (residue 15 and name  H8  )  3.08  0.65  0.80
assign (residue 27 and name H4'  ) (residue 27 and name H1'  )  3.08  0.65  0.80
assign (residue 28 and name H2'  ) (residue 28 and name  H8  )  3.68  0.77  0.96
assign (residue 28 and name H2'  ) (residue 29 and name H1'  )  4.33  0.91  1.13
assign (residue 28 and name H2'  ) (residue 29 and name  H8  )  2.48  0.52  0.65
assign (residue 28 and name H3'  ) (residue 28 and name H1'  )  3.21  0.67  0.84
assign (residue 28 and name H3'  ) (residue 28 and name  H8  )  2.89  0.61  0.75
assign (residue 28 and name H3'  ) (residue 29 and name  H8  )  3.28  0.69  0.85
assign (residue 28 and name H4'  ) (residue 28 and name H1'  )  3.18  0.67  0.83
assign (residue 29 and name H2'  ) (residue 29 and name  H8  )  4.42  0.93  1.15
assign (residue 29 and name H2'  ) (residue 30 and name H1'  )  4.37  0.92  1.14
assign (residue 29 and name H2'  ) (residue 30 and name  H8  )  2.48  0.52  0.64
assign (residue 29 and name H3'  ) (residue 29 and name  H8  )  2.81  0.59  0.73
assign (residue 29 and name H3'  ) (residue 30 and name  H8  )  3.38  0.71  0.88
assign (residue 30 and name H2'  ) (residue 30 and name H1'  )  2.77  0.58  0.72
assign (residue 30 and name H2'  ) (residue 31 and name  H8  )  2.49  0.52  0.65
assign (residue 30 and name H3'  ) (residue 30 and name H1'  )  3.99  0.84  1.04
assign (residue 30 and name H3'  ) (residue 30 and name  H8  )  2.88  0.61  0.75
assign (residue 30 and name H3'  ) (residue 31 and name  H8  )  3.74  0.79  0.97
```

**Distance restraints for AMBER modeling of 19-nt duplex (continued)**

```
assign (residue 30 and name H4'  ) (residue 30 and name H1'  )  3.47  0.73  0.90
assign (residue 31 and name H2'  ) (residue 31 and name H1'  )  2.61  0.55  0.68
assign (residue 31 and name H3'  ) (residue 31 and name H1'  )  3.82  0.80  0.99
assign (residue 31 and name H3'  ) (residue 31 and name  H8  )  2.78  0.58  0.72
assign (residue 31 and name H4'  ) (residue 31 and name H1'  )  3.34  0.70  0.87
assign (residue 32 and name H2'  ) (residue 33 and name  H6  )  2.44  0.51  0.63
assign (residue 32 and name H3'  ) (residue 33 and name  H6  )  3.21  0.67  0.83
assign (residue 33 and name H2'  ) (residue 34 and name H1'  )  3.64  0.76  0.95
assign (residue 33 and name H2'  ) (residue 34 and name  H8  )  2.34  0.49  0.61
assign (residue 33 and name H3'  ) (residue 33 and name H1'  )  2.50  0.00  2.00
assign (residue 33 and name H3'  ) (residue 33 and name  H6  )  2.43  0.51  0.63
assign (residue 34 and name H2'  ) (residue 34 and name  H8  )  3.51  0.74  0.91
assign (residue 34 and name H2'  ) (residue 35 and name H1'  )  3.71  0.78  0.96
assign (residue 34 and name H2'  ) (residue 35 and name  H6  )  2.44  0.51  0.63
assign (residue 34 and name H3'  ) (residue 34 and name  H8  )  2.53  0.53  0.66
assign (residue 35 and name H3'  ) (residue 35 and name  H6  )  2.51  0.53  0.65
assign (residue  7 and name H1'  ) (residue  7 and name  H8  )  4.05  0.85  1.05
assign (residue  8 and name H1'  ) (residue  8 and name  H6  )  3.83  0.80  0.99
assign (residue  8 and name H3'  ) (residue  8 and name H1'  )  3.19  0.67  0.83
assign (residue 10 and name H1'  ) (residue 10 and name  H8  )  3.02  0.63  0.78
assign (residue 11 and name H1'  ) (residue 11 and name  H8  )  3.20  0.00  0.90
assign (residue 12 and name H1'  ) (residue 12 and name  H6  )  3.20  0.00  0.90
assign (residue 13 and name H1'  ) (residue 13 and name  H6  )  4.48  0.94  1.17
assign (residue 15 and name H1'  ) (residue 15 and name  H8  )  3.79  0.80  0.98
assign (residue 29 and name H2'  ) (residue 29 and name H1'  )  2.45  0.51  0.64
assign (residue 29 and name H3'  ) (residue 29 and name H1'  )  2.50  0.00  2.00
assign (residue 31 and name H2'  ) (residue 32 and name  H8  )  3.17  0.67  0.82
assign (residue 32 and name H1'  ) (residue 31 and name  H2  )  3.51  0.74  0.91
assign (residue 34 and name H1'  ) (residue  9 and name  H2  )  4.10  0.86  1.07
assign (residue 34 and name H2'  ) (residue 34 and name H1'  )  2.39  0.50  0.62
assign (residue 34 and name H3'  ) (residue 34 and name H1'  )  3.10  0.65  0.81
assign (residue 34 and name H3'  ) (residue 35 and name  H6  )  3.00  0.63  0.78
assign (residue 35 and name H1'  ) (residue 35 and name  H6  )  3.84  0.81  1.00
assign (residue 36 and name H1'  ) (residue 36 and name  H8  )  4.10  0.86  1.07

assign (resid 7 and name H1  ) (resid 35 and name N3  )  2.1 0.3 0.3
assign (resid 7 and name O6  ) (resid 35 and name H41  ) 2.1 0.3 0.3
assign (resid 7 and name H22  ) (resid 35 and name O2  ) 2.1 0.3 0.3
assign (resid 8 and name N3  ) (resid 34 and name H1  )  2.1 0.3 0.3
assign (resid 8 and name H41  ) (resid 34 and name O6  ) 2.1 0.3 0.3
assign (resid 8 and name O2  ) (resid 34 and name H22  ) 2.1 0.3 0.3
assign (resid 9 and name N1  ) (resid 33 and name H3  )  2.1 0.3 0.3
assign (resid 9 and name H61  ) (resid 33 and name O4  ) 2.1 0.3 0.3
assign (resid 12 and name N3  ) (resid 30 and name H1  )  2.1 0.3 0.3
assign (resid 12 and name H41  ) (resid 30 and name O6  ) 2.1 0.3 0.3
assign (resid 12 and name O2  ) (resid 30 and name H22  ) 2.1 0.3 0.3
assign (resid 13 and name N3  ) (resid 29 and name H1  )  2.1 0.3 0.3
assign (resid 13 and name H41  ) (resid 29 and name O6  ) 2.1 0.3 0.3
assign (resid 13 and name O2  ) (resid 29 and name H22  ) 2.1 0.3 0.3
assign (resid 14 and name N3  ) (resid 28 and name H1  )  2.1 0.3 0.3
assign (resid 14 and name H41  ) (resid 28 and name O6  ) 2.1 0.3 0.3
assign (resid 14 and name O2  ) (resid 28 and name H22  ) 2.1 0.3 0.3
assign (resid 15 and name N1  ) (resid 27 and name H3  )  2.1 0.3 0.3
```

**Distance restraints for AMBER modeling of 19-nt duplex (continued)**

```
assign (resid 15 and name H61  ) (resid 27 and name O4  ) 2.1 0.3 0.3
```

**Dihedral restraints for AMBER modeling of 19-nt duplex (continued)**

```
 8 CYT   ALPHA -155.0   25.0
 9 ADE   ALPHA -155.0   25.0
13 CYT   ALPHA -155.0   25.0
14 CYT   ALPHA -155.0   25.0
15 ADE   ALPHA -155.0   25.0
28 GUA   ALPHA -155.0   25.0
29 GUA   ALPHA -155.0   25.0
30 GUA   ALPHA -155.0   25.0
34 GUA   ALPHA -155.0   25.0
35 CYT   ALPHA -155.0   25.0
36 ADE   ALPHA -155.0   25.0
 8 CYT    BETA   90.0  240.0
 9 ADE    BETA   90.0  240.0
13 CYT    BETA   90.0  240.0
14 CYT    BETA   90.0  240.0
15 ADE    BETA   90.0  240.0
28 GUA    BETA   90.0  240.0
29 GUA    BETA   90.0  240.0
30 GUA    BETA   90.0  240.0
34 GUA    BETA   90.0  240.0
35 CYT    BETA   90.0  240.0
36 ADE    BETA   90.0  240.0
 7 GUA   GAMMA    0.0  120.0
 8 CYT   GAMMA    0.0  120.0
 9 ADE   GAMMA    0.0  120.0
13 CYT   GAMMA    0.0  120.0
14 CYT   GAMMA    0.0  120.0
15 ADE   GAMMA    0.0  120.0
27 URA   GAMMA    0.0  120.0
28 GUA   GAMMA    0.0  120.0
29 GUA   GAMMA    0.0  120.0
30 GUA   GAMMA    0.0  120.0
34 GUA   GAMMA    0.0  120.0
35 CYT   GAMMA    0.0  120.0
36 ADE   GAMMA    0.0  120.0
 7 GUA   DELTA   45.0  115.0
 8 CYT   DELTA   45.0  115.0
 9 ADE   DELTA   45.0  115.0
10 GUA   DELTA   45.0  115.0
11 GUA   DELTA   45.0  115.0
12 CYT   DELTA   45.0  115.0
13 CYT   DELTA   45.0  115.0
14 CYT   DELTA   45.0  115.0
15 ADE   DELTA   45.0  115.0
27 URA   DELTA   45.0  115.0
28 GUA   DELTA   45.0  115.0
29 GUA   DELTA   45.0  115.0
30 GUA   DELTA   45.0  115.0
31 ADE   DELTA   45.0  115.0
32 GUA   DELTA   45.0  115.0
33 URA   DELTA   45.0  115.0
34 GUA   DELTA   45.0  115.0
35 CYT   DELTA   45.0  115.0
36 ADE   DELTA   45.0  115.0
```

```
 7 GUA  EPSILN -240.0   10.0
 8 CYT  EPSILN -240.0   10.0
12 CYT  EPSILN -240.0   10.0
13 CYT  EPSILN -240.0   10.0
14 CYT  EPSILN -240.0   10.0
27 URA  EPSILN -240.0   10.0
28 GUA  EPSILN -240.0   10.0
29 GUA  EPSILN -240.0   10.0
33 URA  EPSILN -240.0   10.0
34 GUA  EPSILN -240.0   10.0
35 CYT  EPSILN -240.0   10.0
 7 GUA    ZETA -160.0   20.0
 8 CYT    ZETA -160.0   20.0
12 CYT    ZETA -160.0   20.0
13 CYT    ZETA -160.0   20.0
14 CYT    ZETA -160.0   20.0
27 URA    ZETA -160.0   20.0
28 GUA    ZETA -160.0   20.0
29 GUA    ZETA -160.0   20.0
33 URA    ZETA -160.0   20.0
34 GUA    ZETA -160.0   20.0
35 CYT    ZETA -160.0   20.0
 7 GUA     CHI  170.0  340.0
 8 CYT     CHI  170.0  340.0
 9 ADE     CHI  170.0  340.0
12 CYT     CHI  170.0  340.0
13 CYT     CHI  170.0  340.0
14 CYT     CHI  170.0  340.0
15 ADE     CHI  170.0  340.0
27 URA     CHI  170.0  340.0
28 GUA     CHI  170.0  340.0
29 GUA     CHI  170.0  340.0
30 GUA     CHI  170.0  340.0
31 ADE     CHI  170.0  340.0
32 GUA     CHI  170.0  340.0
33 URA     CHI  170.0  340.0
34 GUA     CHI  170.0  340.0
35 CYT     CHI  170.0  340.0
36 ADE     CHI  170.0  340.0
```

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