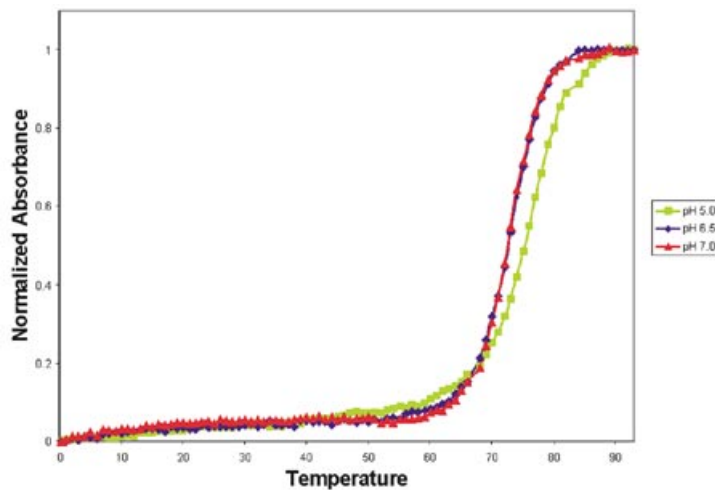
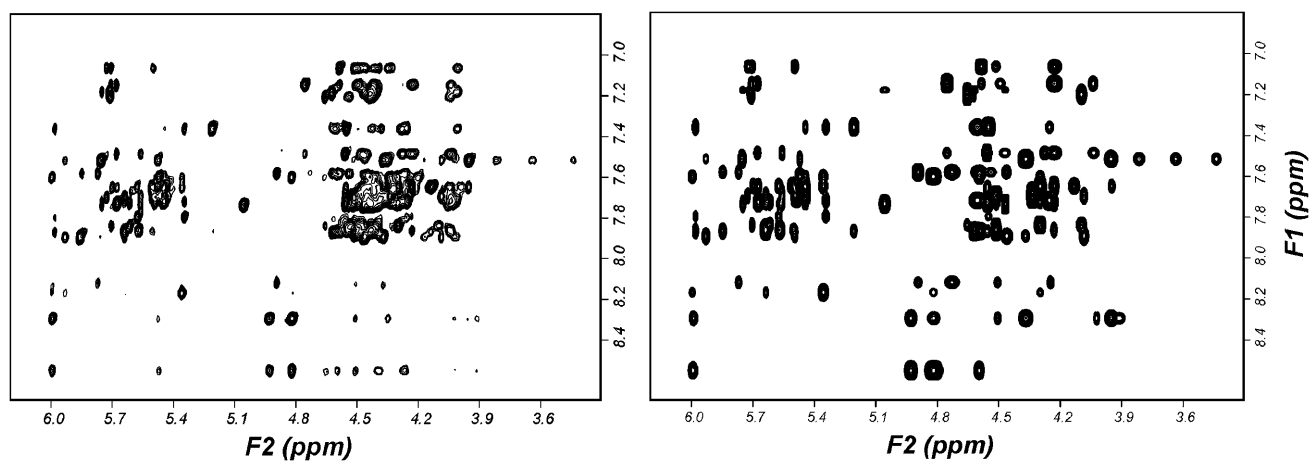


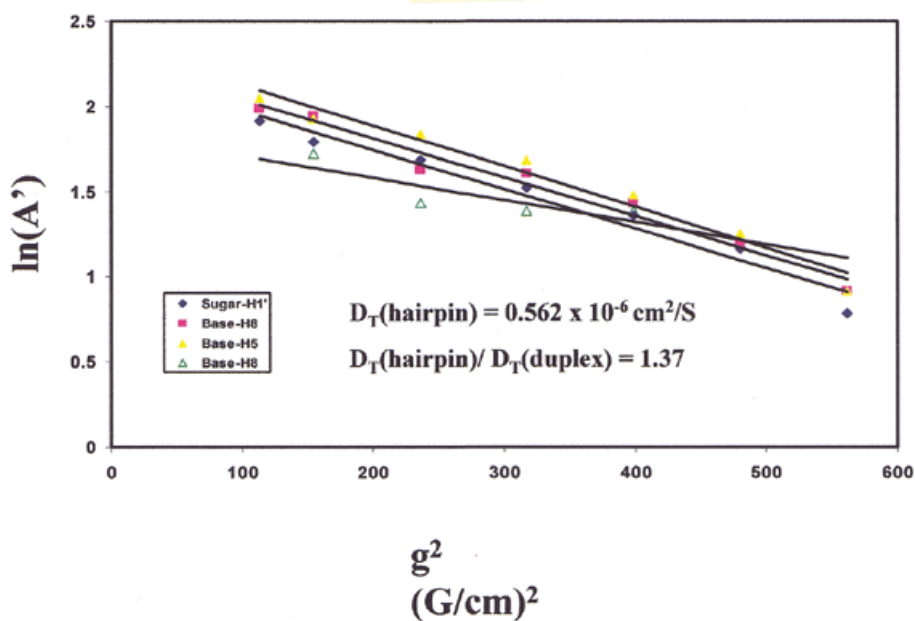
## SUPPLEMENTARY MATERIAL



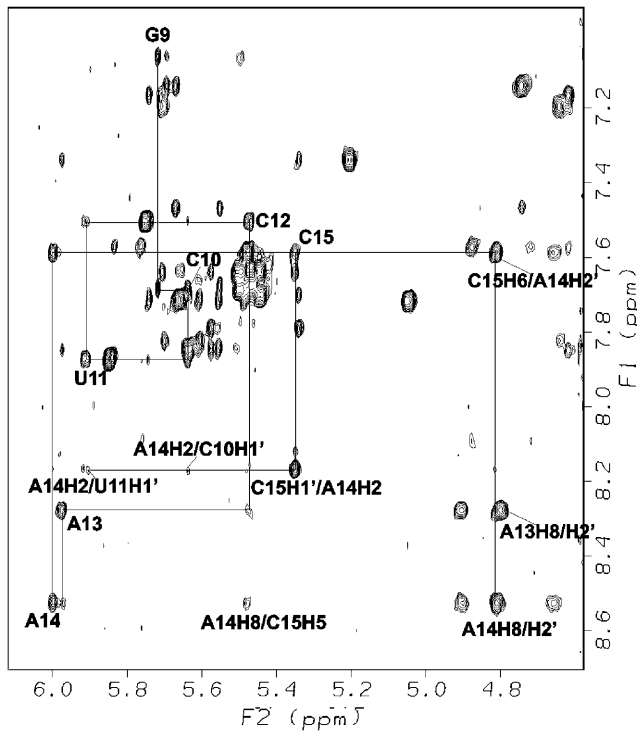
**Figure S1.** UV melting profiles of the pentaloop at 260 nm. The buffer included 10 mM  $\text{NaH}_2\text{PO}_4$  and 0.1 mM EDTA under different pH conditions. The  $T_m$  at pH 5.0 is 6°C higher than that at pH 6.5 and 7.0. Analysis of the pattern of the melting profiles at three pH values indicates a hairpin to duplex conformational exchange at the lower pH. These results are also supported by the NMR line broadening of the loop residues at pH 5.0.



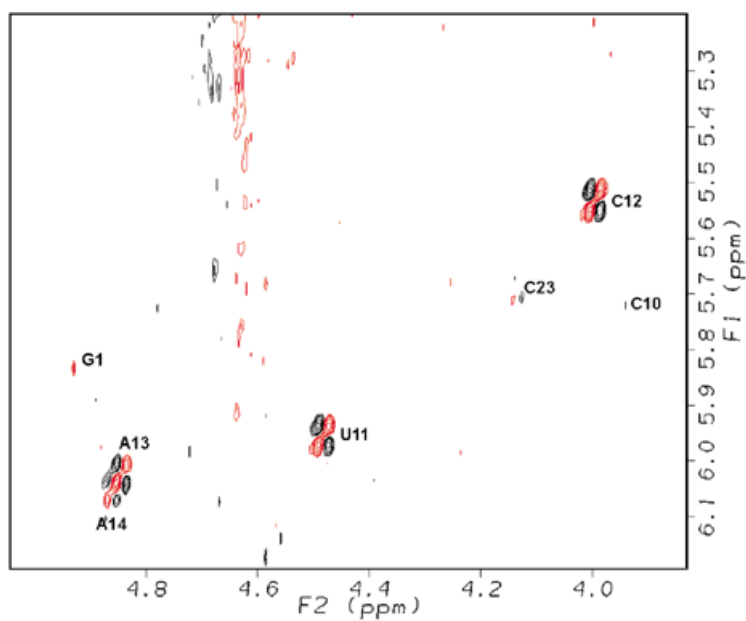
**Figure S2.** Comparison of the experimental and back-calculated (500 ms mixing time) 2D NOESY spectra. NOE connectivities between the base (F2) and the sugar (F1) proton resonances are displayed.



**Figure S3.** Representative  $^1\text{H}$  signal intensity  $[\ln(A')]$  as a function of gradient field strength  $g^2$  ( $\text{G}/\text{cm}$ ) $^2$  recorded at  $25^\circ\text{C}$ . The closed legend keys represent the proton resonances of the RNA and the open triangle represents the base-H8 resonance of the reference DNA oligomer.  $D_T$  of the RNA oligomer was  $0.562 \times 10^{-6} \text{ cm}^2/\text{S}$  and the ratio of  $D_T$  of hairpin and duplex was 1.37.



**Figure S4.** Expanded 2D NOESY spectra (500 ms) recorded at  $35^\circ\text{C}$ . NOE connectivities from base (F1) to sugar (F2) protons are displayed. Sequential connectivities of the loop residues are traced.



**Figure S5.** Expanded DQF-COSY spectra recorded at 35°C representing the H1' (F1) and H2' (F2) correlations. Residues U11, C12 and A13 in the loop region have large scalar couplings of 7–10 Hz. Residue C10 has a very small scalar coupling and residue A14 has a moderate scalar coupling. The terminal residues G1 and C23 also have small scalar couplings. Other stem residues do not exhibit detectable cross-peaks.

**Table S1.** Thermodynamic parameters of the pentaloop under different pH conditions<sup>a</sup>

pH	$T_m$ (°C)	$\Delta H^\circ$ (kcal/mol)	$\Delta S^\circ$ (eu)	$\Delta G^\circ$ (kcal/mol)
5.0	79	-91.67	-234.35	-19.02
6.5	73	-120.93	-323.69	-20.58
7.0	73	-128.36	-344.66	-21.52

<sup>a</sup>The values reported here are the averages of three melting transition experiments.