Supporting Information

Contents

1. NMR experiments

Oligonucleotides were purified by RP-HPLC using a TEAA/acetonitrile buffer mixture and were lyophilized several times from aqueous ammonium solution and water to remove any trace of volatile HPLC material prior to preparation of the NMR sample. Oligonucleotides were dissolved in 180 μL of D_2O containing 150 mM NaCl and 10 mM phosphate buffer (Na_2HPO_4/KH_2PO_4) at pH 7.4 (uncorrected for deuterium effect) for NMR analysis. The concentration of the 2'-F RNA sample was 1.4 mM and that of the native RNA was 1.75 mM. For the acquisition of spectra containing the exchangeable protons, the sample was dried and the residue immediately taken up in 180 μL of H_2O/D_2O (9:1; v/v).

NMR experiments were performed either on Bruker AVANCE 800 MHz or 600 MHz spectrometers. Different sets of two-dimensional spectra were recorded at different temperatures varying from 5 to 40°C. NOESY experiments in D_2O buffer were acquired with mixing times of 400, 250, 125, and 62.5 ms. NOESY spectra detecting exchangeable protons were recorded with mixing times of 400, 250, and 100 ms. DQF-COSY and TOCSY spectra were recorded to aid the assignment, the latter with a mixing time of 80 ms. All two-dimensional spectra were acquired with 2k complex data points in *t*2 and 512 real points in *t*1 with a relaxation delay of 2 s.

^{1}H, ^{13}C HMQC spectra were recorded on a Bruker AVANCE 14.1 T (600 MHz, ^{1}H nuclei) spectrometer at 25°C in 50% D_2O/H_2O. Two-dimensional spectra were acquired with 2k complex data points in *t*2 and 128 real points in *t*1 with a relaxation delay of either 2 s or 1.5 s.

Sequential assignment of exchangeable and non-exchangeable protons was conducted following standard methods for duplex RNA. The DQF-COSY spectrum showed four pairs of cross peaks, characteristic of J-coupled spins of the H_5 and H_6 protons in cytosine and uracil rings. Due to their labile nature H_8 protons from guanine bases in 2'-F RNA were difficult to assign and were therefore identified from leftover cross peaks and from $H_8/H_{2'}$ cross peaks.

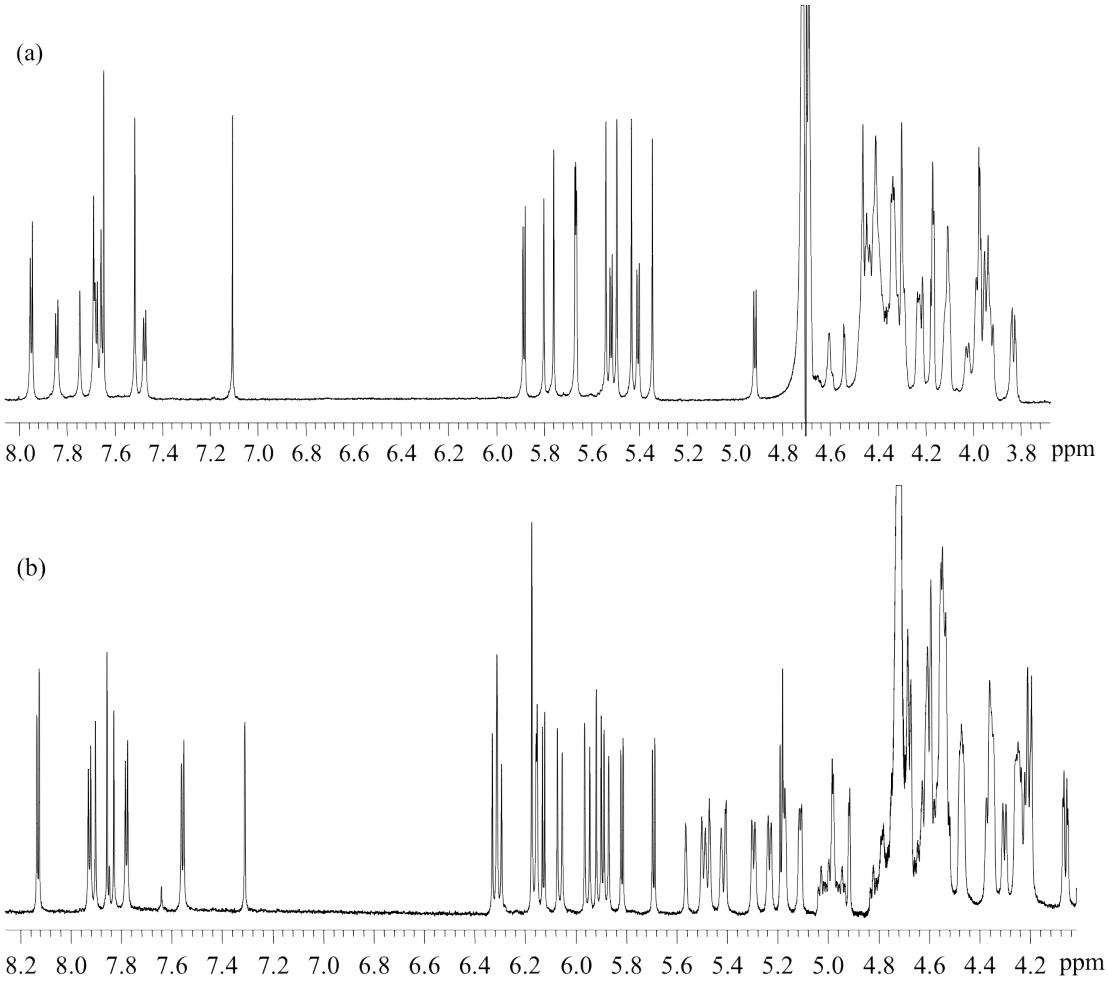
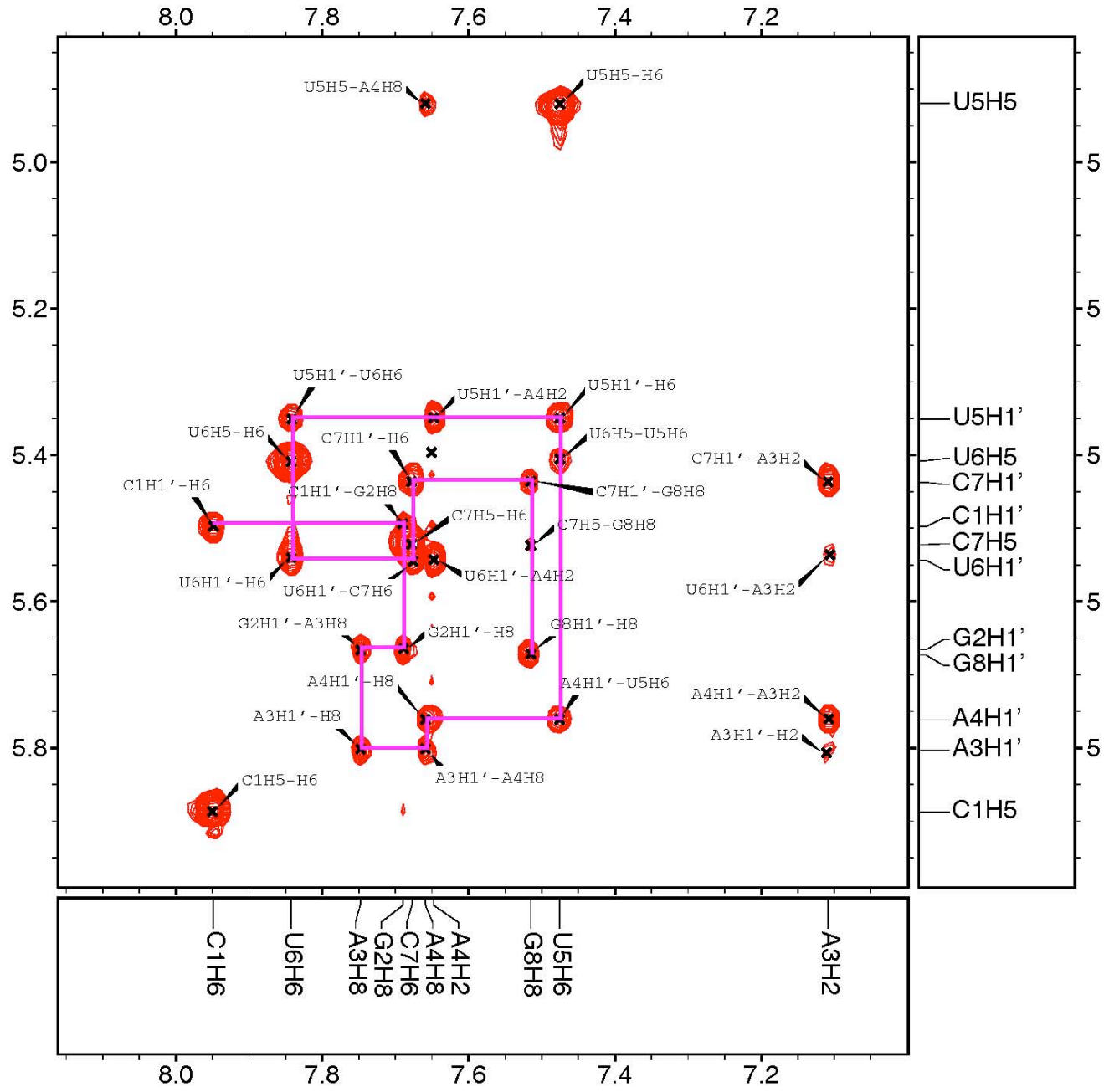


Figure S1. One dimensional ^{1}H NMR spectrum of (a) $[r(CGAAUUCG)]_2$ and (b) $[f(CGAAUUCG)]_2$ in phosphate-buffered D_2O at 25°C.

(a)


8.0
7.8
7.6
7.4
7.2
5.0
5.2
5.4
5.6
5.8
U5H5-A4H8
U5H5-H6
U5H1'-U6H6
U5H1'-A4H2
U5H1'-H6
U6H5-H6
C7H1'-H6
U6H5-U5H6
C7H1'-A3H2
C1H1'-H6
C1H1'-G2H8
C7H1'-G8H8
C7H5-H6
C7H5-G8H8
U6H1'-H6
U6H1'-C7H6
U6H1'-A4H2
U6H1'-A3H2
G2H1'-A3H8
G2H1'-H8
G8H1'-H8
A4H1'-H8
A4H1'-U5H6
A4H1'-A3H2
A3H1'-H8
A3H1'-H2
A3H1'-A4H8
C1H5-H6
U5H5
U5H1'
U6H5
C7H1'
C1H1'
C7H5
U6H1'
G2H1'
G8H1'
A4H1'
A3H1'
C1H5
C1H6
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A4H2
G8H8
U5H6
A3H2

(b)

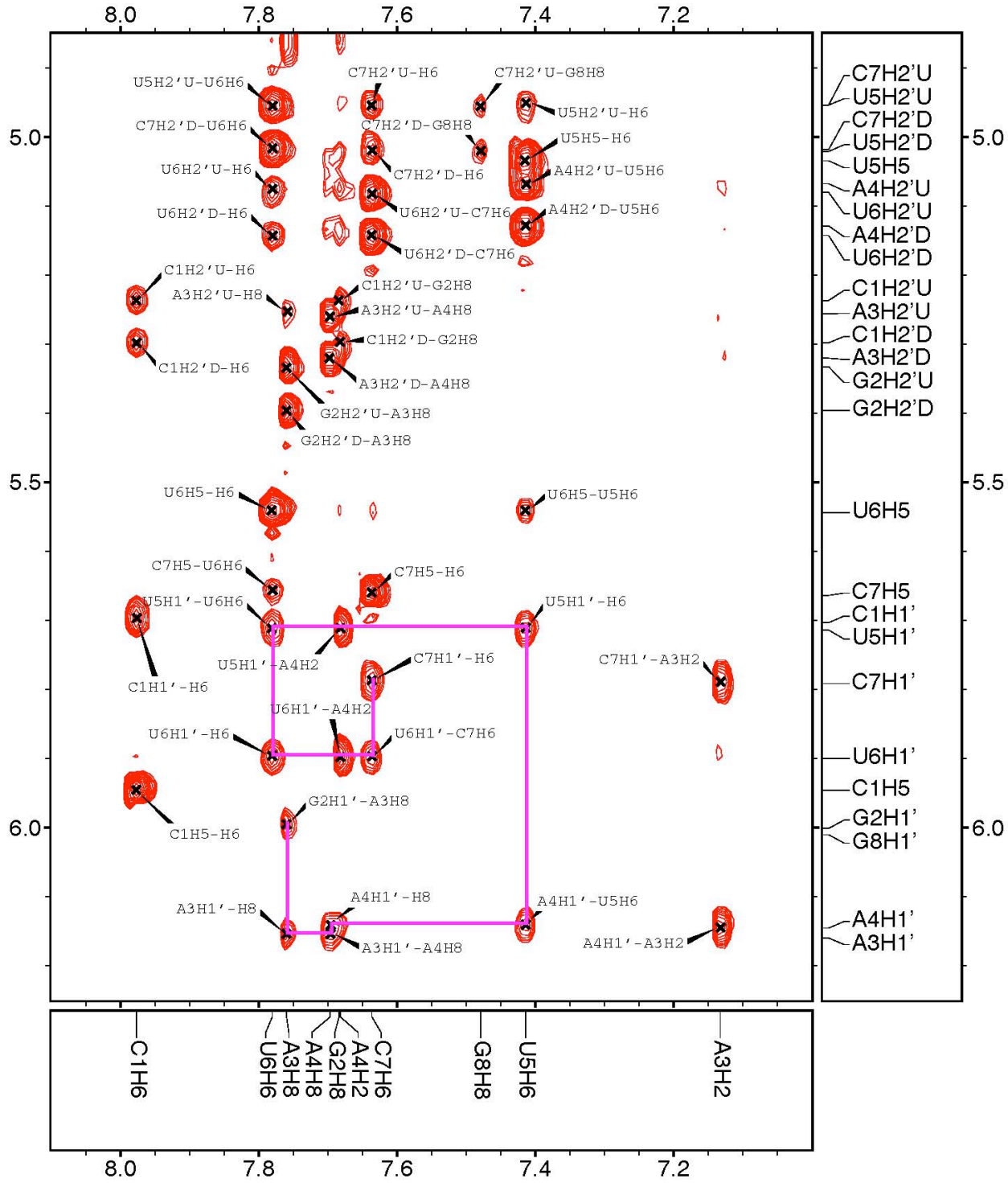

Figure S2. Expansion of the NOESY spectrum of (a) $[r(CGAAUUCG)]_2$ and (b) $[f(CGAAUUCG)]_2$ in buffered D_2O at 800 MHz, 298 K and a mixing time of 250 ms, showing NOE connectivities between H1′ resonances of riboses and protons of nucleobases.

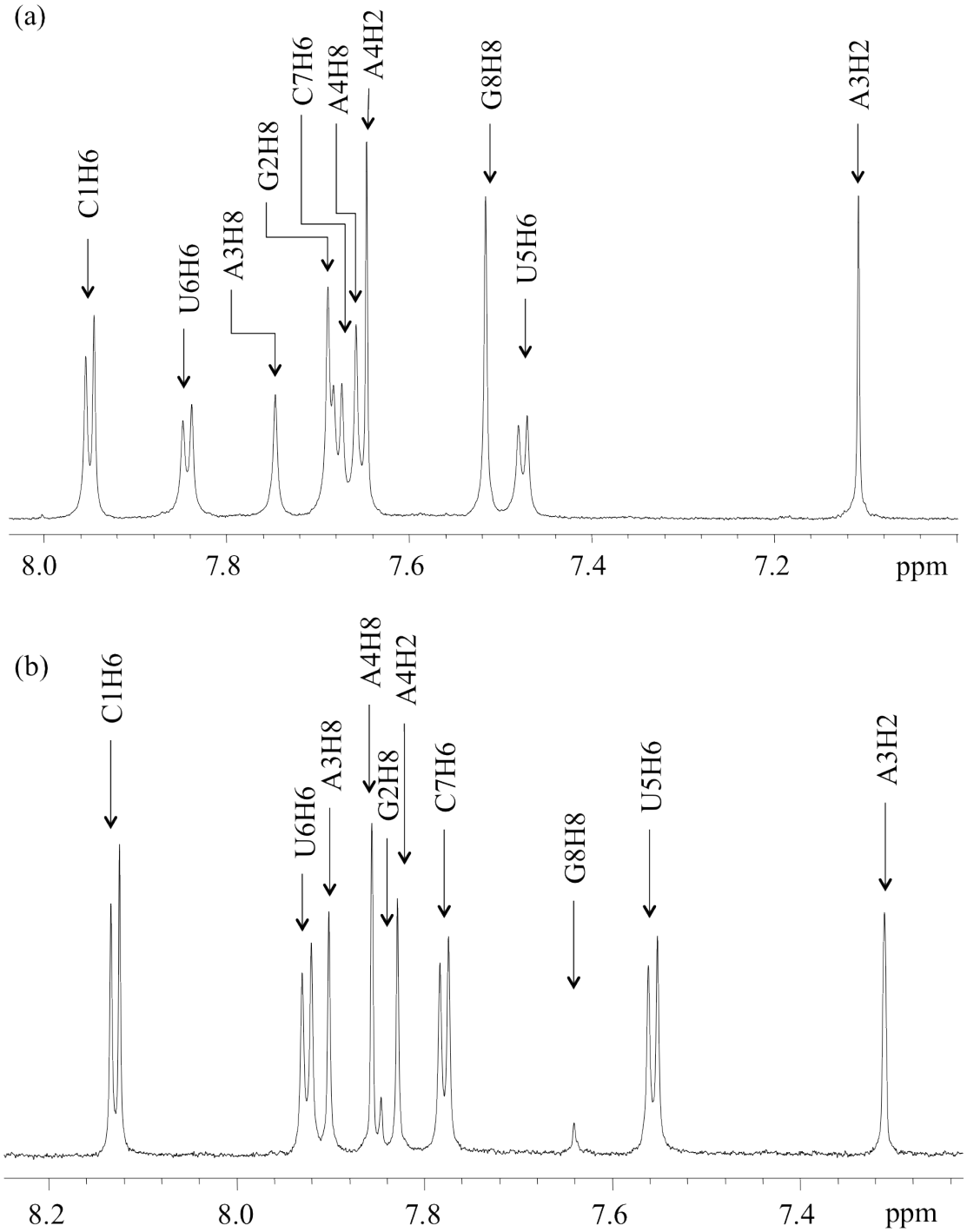

Figure S3. Low field region of ^{1}H-NMR spectrum of (a) [r(CGAAUUCG)]$_2$ at 298 K and (b) [f(CGAAUUCG)]$_2$ at 313 K in phosphate-buffered D_2O at 800 MHz. Resonances of individual protons are labeled.

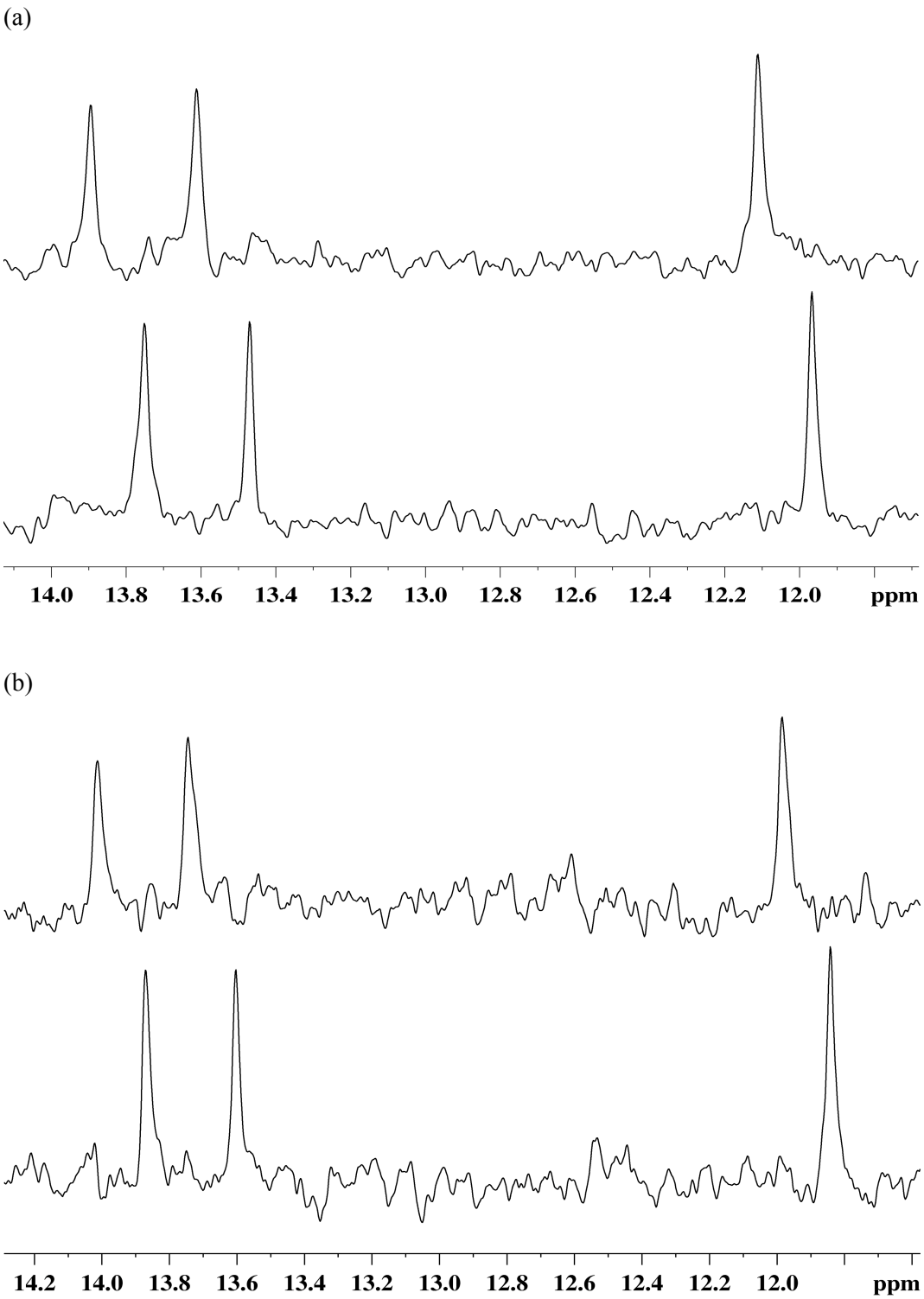

Figure S4. One-dimensional ^{15}N-coupled ^{1}H IPAP spectra in the imino region of (a) 5'-[r(CGAAUUCG)]$_2$ and (b) [f(CGAAUUCG)]$_2$ in 10% D_2O/H_2O at 5°C. The specta at the top in (a) and (b) were obtained from adding the IP and AP spectra, whereas the lower spectra were obtained by subtraction, IP – AP.

Table S1. Deuterium isotope effect (DIE) measurements for RNA and 2'-F RNA[a]

Base Pair	RNA (δ^{13}C2{^{1}H3})[b]	δ_{H3} (RNA)	2'-F RNA (δ^{13}C2{^{1}H3})[b]	δ_{H3} (2'-F RNA)
A3:U14	-55.3±0.6	7.17	-63.6±0.1	7.15
A4:U13	-58.1±0.5	7.70	-62.8±0.2	7.70

[a] Units of $^{n}\Delta A = \delta_A\{^{1}H3\} - \delta_A\{^{2}H3\}$ are in ppb, and units of δ_{H3} are in ppm. [b] Shown here are values from two separate data sets.

2. UV thermal melting experiments

Melting of each oligonucleotide hairpin (16 mM) was performed in 10 mM sodium cacodylate (pH = 7.4), 0.1 mM EDTA, and 300 mM NaCl in the presence of 0, 5, 10, 15, and 20 % weight/volume of either ethylene glycol or acetamide (**Table 1**). Absorbance vs. temperature profiles were measured at 280 nm on a Shimadzu 800 UV-Visible spectrometer equipped with an eight-position Peltier temperature controller. The temperature was increased at 0.5 °C per minute. The melting temperatures were obtained using the Shimadzu LabSolution TmAnalysis software. The experimental absorbance vs. temperature curves were converted into a fraction of strands remaining hybridized (α) vs. temperature curves by fitting the melting profile to a two-state transition model, with linearly sloping lower and upper base lines. The melting temperatures (T_m, **Table 1**, column 2) were obtained directly from the temperature at $\alpha = 0.5$. The final T_m was an approximation of usually five to eight measurements.

The thermodynamic parameters (ΔH, ΔS and ΔG) were obtained by van't Hoff analysis of melting curves using Varian Cary software (**Table 1**, columns 4-6). In addition, the enthalpy of the melting was determined from the width at the half-height of differentiated melting curves (**Table 1**, column 3). The fraction of strands remaining hybridized (α) vs. temperature curves were converted into differentiated melting curves [$\delta\alpha/\delta(T_m^{-1})$ vs. T_m] using Varian Cary software. The width of the differentiated melting curve at the half-height is inversely proportional to the van't Hoff transition enthalpy; for a monomolecular transition $\Delta H = 7.0/(T_1^{-1} - T_2^{-1})$ where T_1 is the lower temperature and T_2 is the upper temperature (both in K) at one-half of [$\delta\alpha/\delta(T_m^{-1})$].[1]

Osmotic stress experiments were performed as described in previous publications.[2] A detailed experimental manual has been also published.[3]

[1] K. J. Breslauer, *Methods Enzymol.* **1995**, *259*, 221-242.

[2] (a) E. Rozners, J. Moulder, *Nucleic Acids Res.* **2004**, *32*, 248-254. (b) P. S. Pallan, E. M. Greene, P. A. Jicman, R. K. Pandey, M. Manoharan, E. Rozners, M. Egli, *Nucleic Acids Res.* **2011**, *39*, 3482–3495.

[3] E. Rozners, *Curr. Protoc. Nucleic Acid Chem.* **2010**, 7.14.1-7.4.13.

Table S2. Experimental t_m and thermodynamic data for melting of r[GCGUUUCGC]

Trial #	0%	5%	10%	15%	20%	T1	T2	-ΔH (cal/mol) δα/δTm	Van't Hoff -ΔH (kcal/mol)	Van't Hoff -ΔS (kcal/mol*K)	ΔG(37°C) (kcal/mo)
Ethylene Glycol at 280nm											
	52.5	51.7	50.5	49.9	49.0	39.34	66.58	27256	29.99	0.09193	-1.5
	51.5	51.6	50.2	50.1	48.7	38.73	64.85	28225	31.72	0.09772	-1.4
	51	51.9	50.3	50.0	48.3	38.17	64.01	28408	31.87	0.09832	-1.4
	52.7	51.1	50.3	48.3	48.4	38.88	65.26	27994	30.91	0.09497	-1.5
	52.1	51.4	50.5	49.8	48.6	41.07	69.05	26876	31.02	0.09449	-1.7
	51.6	50.9	50.2	49.2	48.2	38.81	64.95	28219	30.18	0.09275	-1.4
		51.9	50.3	49.4	47.3						
		51.8	50.5	49.9	48.8						
		52.6	50.0	49.0	48.9						
			50.5		48.5						
			50.8		48.7						
Average	51.9	**51.7**	**50.4**	**49.5**	**48.5**	39.2	65.8	27829.5	30.9	0.09503	-1.5
Standard Deviation	0.6	**0.5**	**0.2**	**0.6**	**0.5**	1.0	1.8	617.7	0.8	0.0025754	0.122359
Acetamide at 280nm											
		49.8	48.2	46.3	45.0						
		49.7	48.0	46.5	44.8						
		49.7	48.3	46.5	44.8						
		49.4	48.7	46	44.4						
		49.4	48.5	46.3	44.6						
		49.5	48.2	46.9	44.8						
		50.2	47.5	46.8	44.8						
		50.4	48.1	46.4	44.4						
		50.4		47	44.6						

		49.8									
Average		**49.8**	**48.2**	**46.5**	**44.7**						
Standard Deviation		**0.4**	**0.4**	**0.3**	**0.2**						

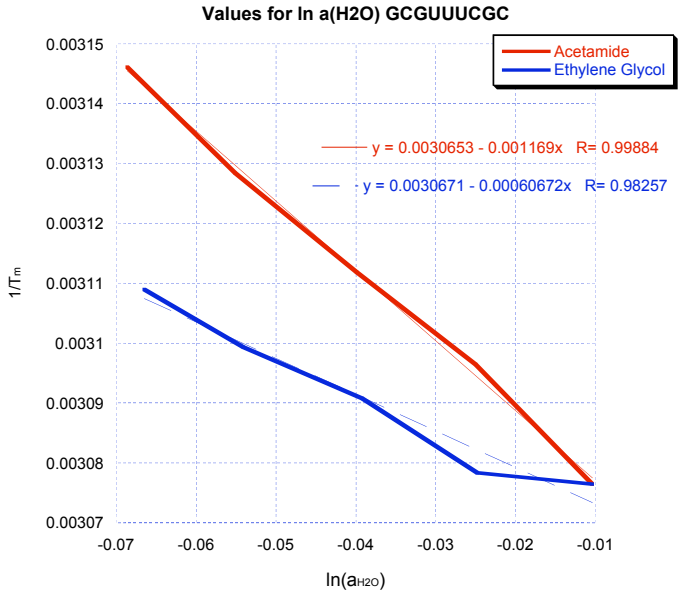

Figure S5. Plot of the inverse of the melting temperature versus the natural log of the water activity for both acetamide and ethylene glycol.

Table S3. Experimental t_m and thermodynamic data for melting of r(GCGUUUCGCA)

Trial #	0%	5%	10%	15%	20%	T1	T2	ΔH (cal/mol) δα/δTm	Van't Hoff ΔH (kcal/mol)	Van't Hoff ΔS (eu)	ΔG(37°C) (kcal/mol)
Ethylene Glycol at 280nm											
	64.1	63	61.3	56.7	55.5	53.1	76.9	33514	33.45	0.09905	-2.7
	63.4	62.6	61.0	57.2	55.9	52.8	76.4	33732	34.4	0.1	-2.8
	63.6	62.3	60.6	57.7	56.7	53.25	77.57	32920	33.93	0.1004	-2.8
	63	62.4	61.5	57.9	55.3	52.39	75.69	34087	33.69	0.1001	-2.7
	63.7	62.9	61.1	57.5	55.6	54.57	76.26	36923	37.56	0.1106	-3.3
	63.3	62.1	61.2	57.2	55.1	52.68	75.97	34159	34.1	0.1012	-2.7
	63.3				55.3	52.2	75.55	33980	34.09	0.1013	-2.7
						52.3	75.34	34427	33.84	0.1004	-2.7
						52.28	77.1	32118	36.34	0.1076	-3.0
						53.83	75.96	36076	36.58	0.1085	-2.9
						53.73	77.72	33436	36.57	0.108	-3.1
						53.14	75.8	35141	38.03	0.1127	-3.1
						52.72	75.75	34527	34.93	0.1034	-2.9
Average	63.5	**62.6**	**61.1**	**57.4**	**55.6**	53.0	76.3	34233.9	35.2	0.10426	-2.87396
Standard Deviation	0.4	**0.4**	**0.3**	**0.4**	**0.5**	0.7	0.8	1267.2	1.6	0.00459	0.19014
Acetamide at 280nm											
		61.1	57.4	55.7	52.9						
		60.1	57.5	55.2	53.4						
		61.7	58.0	55.0	52.8						
		61.4	57.4	55.6	53.2						
		60.2	57.2	55.3	52.0						
		61.1	57.6	55.1	52.5						
		60.9	58.3	55.1	52.3						

		60.4	57.6	56.2							
		61.0	57.9	56.2							
		60.9	58.0	55.6							
		60.3	58.2	56.2							
		60.1		55.8							
		60.7		55.8							
		60.2		56.0							
		59.9		56.3							
				55.7							
Average		**60.7**	**57.7**	**55.7**	**52.7**						
Standard Deviation		**0.5**	**0.4**	**0.4**	**0.5**						

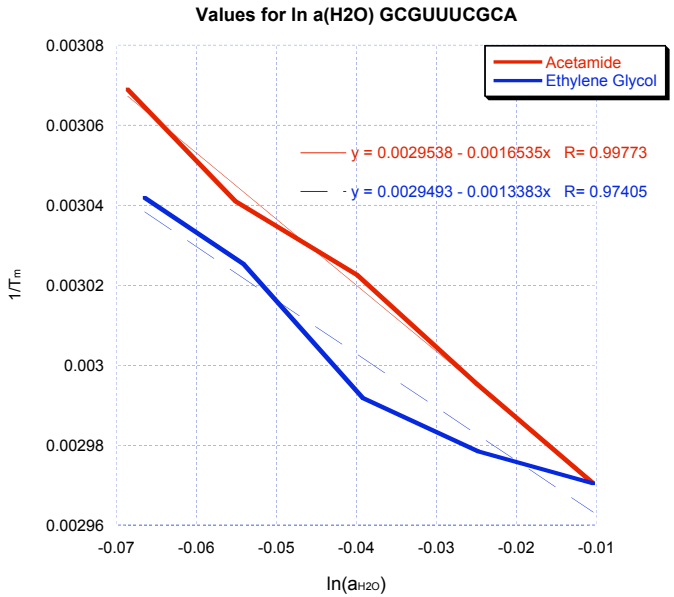

Figure S6. Plot of the inverse of the melting temperature versus the natural log of the water activity for both acetamide and ethylene glycol.

Table S4. Experimental t_m and thermodynamic data for melting of r(GCGUUUCGCAA)

Trial #	0%	5%	10%	15%	20%	T1	T2	ΔH (cal/mol) δα/δTm	Van't Hoff ΔH (kcal/mol)	Van't Hoff ΔS (eu)	ΔG(37°C) (kcal/mol)
Ethylene Glycol at 280nm											
	67.7	65.9	63.9	62	60.8	57.7	80.6	35770	38.56	0.1127	-3.623
	67.4	65.2	64	62.4	60.8	56.7	79.8	35270	39.8	0.1	-3.633
	68.1	66.4	64	62.7	60.8	55.4	78.34	35208	34.43	0.1014	-2.996
	67.6	65.7	63.8	62.4	60.5	55.4	79.08	34179	34.44	0.1013	-3.037
	68.4	65	63.8		60.9	56.6	81.31	33082	35.7	0.1043	-3.367
	67.9	65.2	63.8		60.6	56.4	80.09	34367	37.27	0.1092	-3.418
		65	63.8		60.4	57.6	79.41	37393	39.62	0.116	-3.66
		66.4	63.6		60.7	56.5	79	36084	40.03	0.1174	-3.636
		65.8	64		60.7	57.6	78.68	38608	42.24	0.1238	-3.862
		65.7	63.2		60.5	56.9	78.66	37320	40.1	0.1177	-3.613
Average	67.9	**65.6**	**63.8**	**62.4**	**60.7**	56.7	79.5	35728.1	38.2	0.11205	-3.4845
Standard Deviation	0.4	**0.5**	**0.2**	**0.3**	**0.2**	0.8	0.9	1682.0	2.7	0.007699	0.281094
Acetamide at 280nm											
		65.7	61.4	58.8	55.8						
		65.2	61.7	59.1	55.5						
		64.5	61.9	59	54.7						
		65.6	61.8	59	55.1						
		64.6		59.4	55.6						
		64.3		59.3	55.7						
					55.2						
					55.0						
					54.7						

Average		**65.0**	**61.7**	**59.1**	**55.3**						
Standard Deviation		**0.6**	**0.2**	**0.2**	**0.4**						

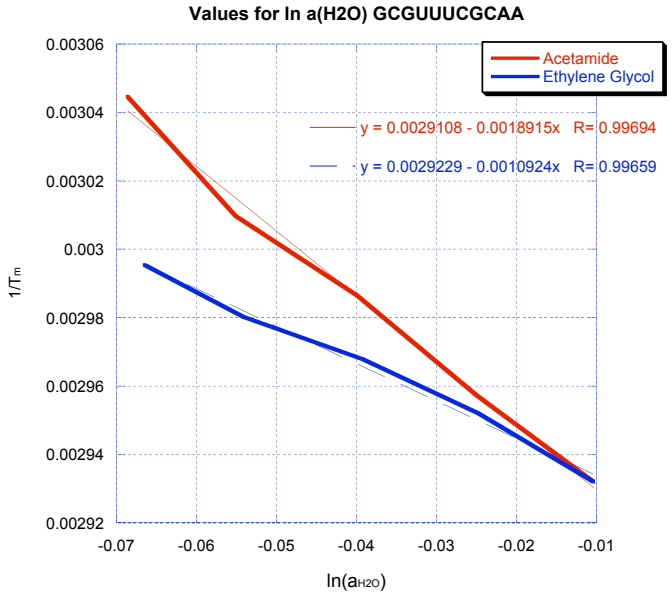

Figure S7. Plot of the inverse of the melting temperature versus the natural log of the water activity for both acetamide and ethylene glycol.

Table S5. Experimental t_m and thermodynamic data for melting of $G_fCGUUUCGC_f$

Trial #	0%	5%	10%	15%	20%	T1	T2	ΔH (cal/mol) $\delta\alpha/\delta T_m$	Van't Hoff ΔH (kcal/mol)	Van't Hoff ΔS (eu)	ΔG(37°C) (kcal/mol)
Ethylene Glycol at 280nm											
	57.3	56.4	55.6	54	53.9	42.94	72.22	26075.19	29.59	0.08929	-1.9101
	57.1	56.2	56.1	54.7	53.4	43.12	71.36	26983.48	30.46	0.09215	-1.8935
	57.6	56.6	55.9	54.8	53.6	43.25	69.7	28682.5	31.17	0.09444	-1.8936
	57.6	56	55.4	55.1	52.9	43.55	72.14	26749.86	28.84	0.08711	-1.8359
	57.5	56.7	55.4	54.9	52.9	43.23	72.97	25751.27	29.65	0.08937	-1.9453
	57	55.8		54.3	53.8	42.32	73.31	24665.68	27.09	0.08153	-1.8157
		56.7		54.1	53.1						
		56		54.9	53.4						
				54.6							
Average	57.4	**56.3**	**55.7**	**54.6**	**53.4**	43.1	72.0	26484.7	29.5	0.088982	-1.88235
Standard Deviation	0.3	**0.4**	**0.3**	**0.4**	**0.4**	0.4	1.3	1353.8	1.4	0.004452	0.048132
Acetamide at 280nm											
		55.7	53.8	51.9	50.7						
		56.0	53.6	52.2	50.3						
		55.8	52.9	52.0	51.1						
		56.1	53.7		49.8						
		56.7									
Average		**56.1**	**53.5**	**52.0**	**50.5**						
Standard Deviation		**0.4**	**0.4**	**0.2**	**0.6**						

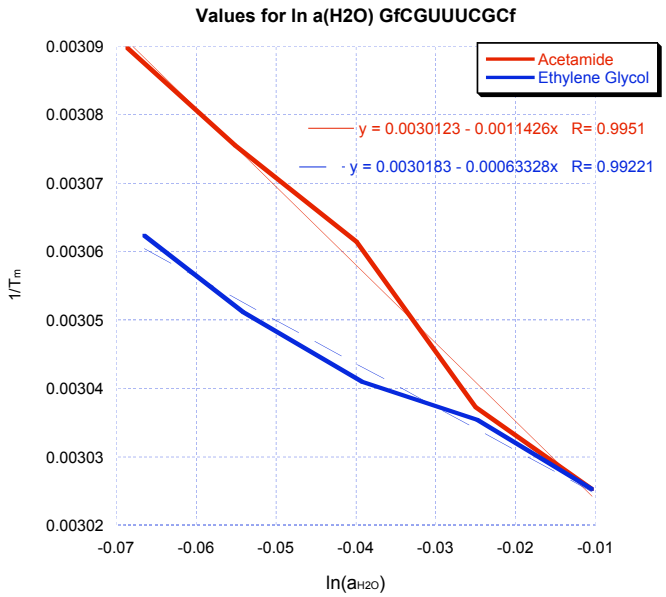

Figure S8. Plot of the inverse of the melting temperature versus the natural log of the water activity for both acetamide and ethylene glycol.

Table S6. Experimental t_m and thermodynamic data for melting of $G_fCGUUUCGC_fA_f$

Trial #	0%	5%	10%	15%	20%	T1	T2	ΔH (cal/mol) δα/δTm	Van't Hoff ΔH (kcal/mol)	Van't Hoff ΔS (eu)	ΔG(37°C) (kcal/mol)
Ethylene Glycol at 280nm											
	75	72.6	69.2	69.3	68.4	62.53	87.06	34475.19	37.64	0.1082	-4.098
	74.6	72.7	70.2	69.9	68.7	60.79	84.11	35780.37	39.75	0.1151	-4.069
	74.5	72.4	69.8	68.7	67.9	62.03	87.1	33686.08	37.16	0.1069	-4.021
	73.3	72.5	69.4	69.2	67.6	61.92	86.34	34498.41	37.38	0.1076	-4.024
	74	72.1	69.7	69.5	67.5	61.81	85.24	35834.24	37.64	0.1086	-3.974
	74.5	71.1	69.6	69	68	63.92	84.02	41891.06	39.74	0.1144	-4.276
	74.5	71.7	70.8	68.7	67.1						
		71.6	70.6	68.6	67.2						
		72.3	70.1	68.7	67						
		71.3	69.9	68.1	67.1						
		72		68.8	67.3						
		72.5		68.6	67.3						
		71.5		69	67.4						
				69.2	67.7						
Average	74.3	**72.0**	**69.9**	**69.0**	**67.6**	62.2	85.6	36027.6	38.2	0.110133	-4.077
Standard Deviation	0.5	**0.5**	**0.5**	**0.5**	**0.5**	1.0	1.4	2990.3	1.2	0.003629	0.106452
Acetamide at 280nm											
		72.0	67.9	66.0	64.6						
		70.9	67.7	65.5	63.7						
		71.8	67.5	65.2	63.9						
		70.0	67.8	66.3	63.7						

		69.9	68.2	66.0	64.2						
		70.0	67.4	65.3	64.0						
		71.7	68.5	65.8	63.9						
		70.6	67.9	65.8	63.8						
		70.3	67.4	66.1	64.1						
		70.0		66.8	63.4						
		71.5		65.0	63.7						
		69.9		65.4	63.5						
Average		**70.7**	**67.8**	**65.8**	**63.9**						
Standard Deviation		**0.8**	**0.4**	**0.5**	**0.3**						

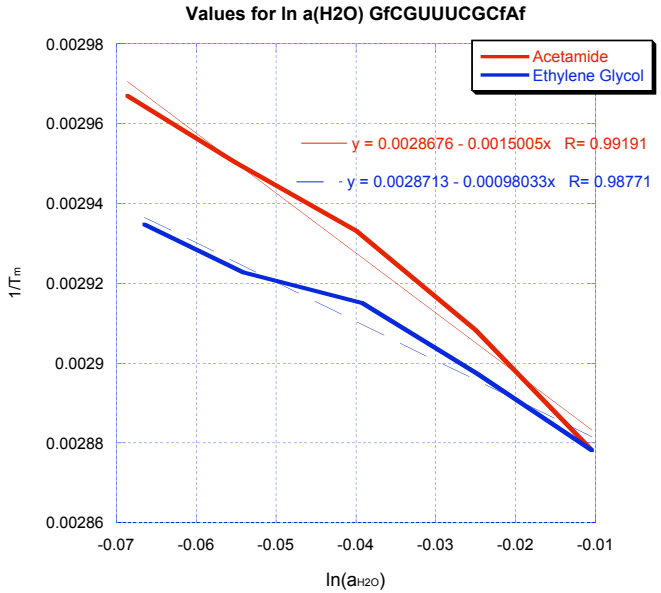

Figure S9. Plot of the inverse of the melting temperature versus the natural log of the water activity for both acetamide and ethylene glycol.

Table S7. Experimental t_m and thermodynamic data for melting of $G_fCGUUUCGC_fA_fA_f$

Trial #	0%	5%	10%	15%	20%	T1	T2	ΔH (cal/mol) δα/δTm	Van't Hoff ΔH (kcal/mol)	Van't Hoff ΔS (eu)	ΔG(37°C) (kcal/mol)
Ethylene Glycol at 280nm											
	77.2	74.9	72.7	71.3	69.3	65.69	88.91	36952.07	38.59	0.1102	-4.428
	77	73.9	72.1	71.6	69.3	64.89	89.02	35485.31	39.05	0.1117	-4.423
	76.8	74.7	72.5	71	69.3	64.5	88.16	36062.57	39.83	0.1139	-4.521
	76.5	74.5	71.9	71.1	69.8	67.02	89.94	37689.7	40.85	0.1161	-4.859
	77.4	74	72.6	71.2	68.8	64.33	87.69	36459.8	39.69	0.1138	-4.412
	77.2	74.9	72.4	71	68.8	65.73	90.34	35006.87	36.95	0.1053	-4.307
	77.3	73.8	72.2	71.6	68.9						
		74.7	73	71.1							
		75	73	71.4							
		74.0	73.2	71.3							
		74.9	72.7	70.6							
				70.0							
Average	77.1	**74.5**	**72.6**	**71.1**	**69.2**	65.4	89.0	36276.1	39.2	0.111833	-4.49167
Standard Deviation	0.3	**0.5**	**0.4**	**0.4**	**0.4**	1.0	1.0	976.8	1.3	0.003787	0.19236
Acetamide at 280nm											
		74.3	70.6	69.2	66.0						
		73.7	71.2	68.4	66.1						
		73.5	72.0	68.1	65.4						
		73.3	70.9	68.7	66.9						
		73.8	71.0	69.5	65.7						
		74.4	70.5	69.4							
		72.7	70.8								

		73.5									
Average		**73.7**	**71.0**	**68.9**	**66.0**						
Standard Deviation		**0.5**	**0.5**	**0.6**	**0.6**						

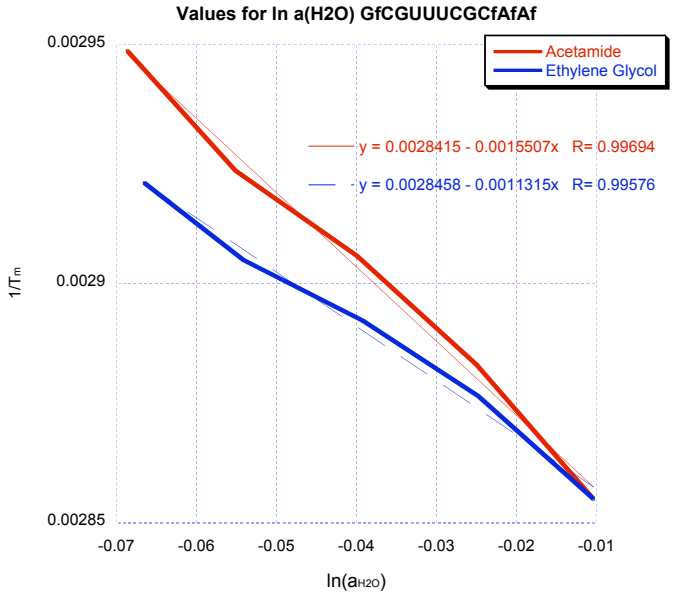

Figure S10. Plot of the inverse of the melting temperature versus the natural log of the water activity for both acetamide and ethylene glycol.

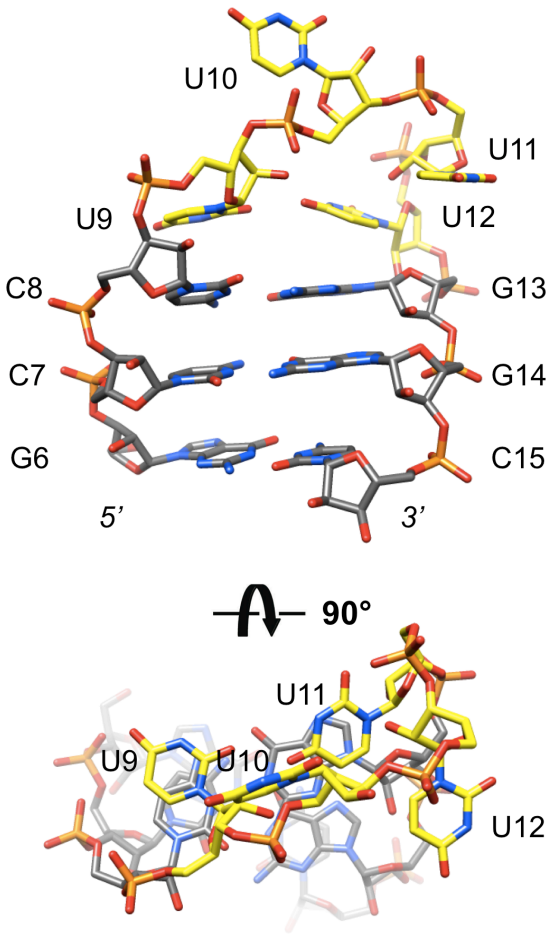

Figure S11. NMR solution structure of a portion of the U2 snRNA stem I from *S. cerevisiae* (PDB ID 2o33)[4] viewed into the minor groove (top) and rotated around the horizontal by 90 degrees (bottom). Only the (GCC):(GGC) stem and the U4 loop are depicted, with carbon atoms colored in gray and yellow, respectively. The two projections illustrate the absence of stacking among loop uracils and very limited stacking between U9 and C8 and between U12 and G13. Similarly, no hydrogen bonds are established between nucleobase moieties from loop residues.

[4] D. G. Sashital, V. Venditti, C.G. Angers, G. Cornilescu, S. E. Butcher, *RNA* **2007**, *13*, 328–338.