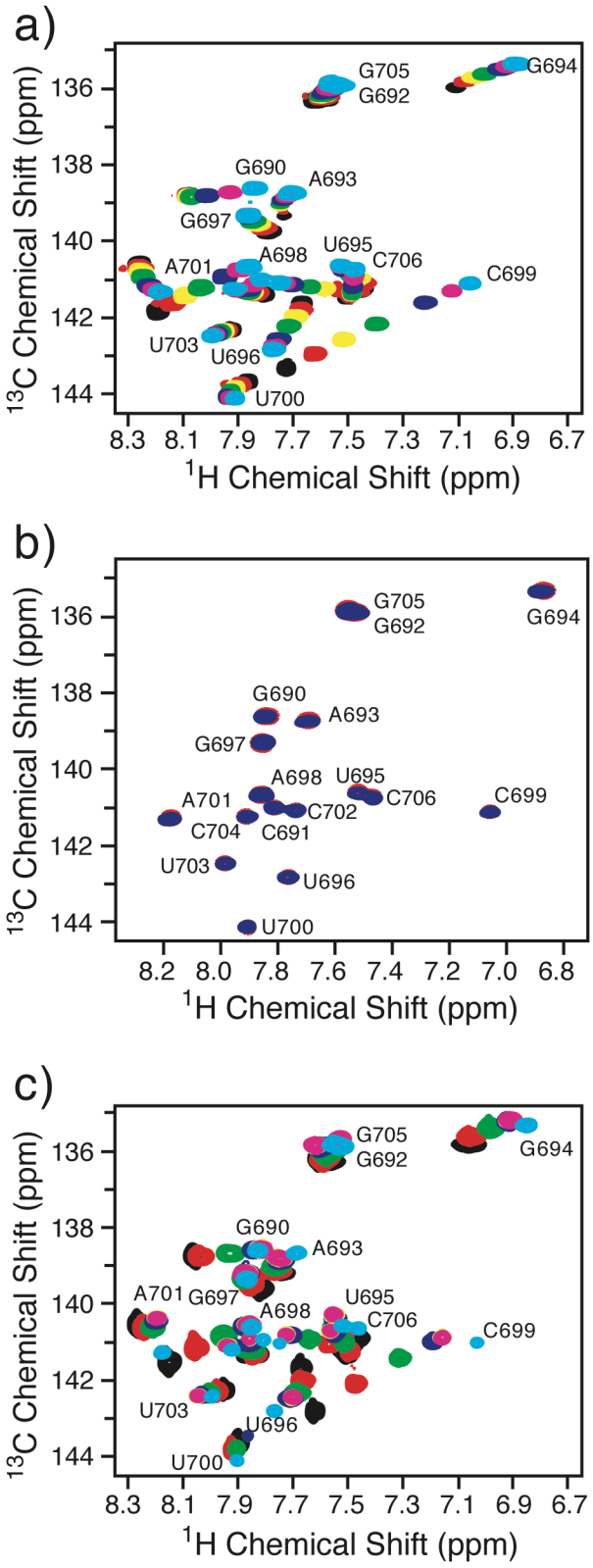
SUPPORTING INFORMATION

Figure S1. Effect of $MgCl_2$ and $Co(NH_3)_6^{3+}$ on SL5. **a)** Overlay of the H6-C6/H8-C8 region of 2D ^{1}H-^{13}C CT-HSQC spectra of SL5 at selected points of the $MgCl_2$ titration in NMR buffer B. Spectra are shown for 0 mM (black), 1.0 mM (red), 2.0 mM (yellow), 3.0 mM (green), 5.0 mM (blue), 15 mM (magenta) and 46 mM (cyan). Peak labels are adjacent to the last point of the $MgCl_2$ titration. **b)** Overlay of H6-C6/C8-H8 region of 2D ^{1}H-^{13}C CT-HSQC spectra of $SL5^{Mg}$ recorded for two sets of buffer conditions: in NMR buffer A supplemented with 40 mM $MgCl_2$ (red) and in NMR buffer B supplemented with 20 mM $MgCl_2$ (blue). **c)** Overlay of the H6-C6/H8-C8 region of 2D ^{1}H-^{13}C CT-HSQC spectra of SL5 at selected points of the $Co(NH_3)_6^{3+}$ titration in NMR buffer A. Spectra are shown for 0 mM (black), 0.5 mM (red), 1.0 mM (green), 2.0 mM (blue), 5.0 mM (yellow) and 6.0 mM (magenta). Peak labels are adjacent to the last point of the $Co(NH_3)_6^{3+}$ titration. The position of the peaks from the $MgCl_2$ titration in NMR buffer A at 40 mM $MgCl_2$ are shown in cyan [Campbell, D. O. and Legault, P. (2005) "NMR structure of the VS ribozyme stem-loop V RNA and magnesium-ion binding from chemical-shift mapping" *Biochemistry* **44**; 4157-4170]. All spectra were collected in 100% D_2O at 25 ˚C on a 600 MHz NMR spectrometer.

Figure S1


a)
G705
G692
G694
G690
A693
G697
A698
U695
C706
A701
C699
U703
U696
U700
^{13}C Chemical Shift (ppm)
^{1}H Chemical Shift (ppm)
b)
G705
G692
G694
G690
A693
G697
A698
U695
C706
A701
C702
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U696
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^{13}C Chemical Shift (ppm)
^{1}H Chemical Shift (ppm)
c)
G705
G692
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G690
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A701
G697
A698
U695
C706
C699
U703
U696
U700
^{13}C Chemical Shift (ppm)
^{1}H Chemical Shift (ppm)

Figure S2. 1D ^{31}P spectra of SL5^{free} (lower panel) and SL5^{Mg} (upper panel). Spectra were collected under the same conditions at 25 ºC using 1 mM RNA samples. For SL5^{Mg}, upfield-shifted and downfield-shifted resonances from the loop are labeled with their respective assignment.

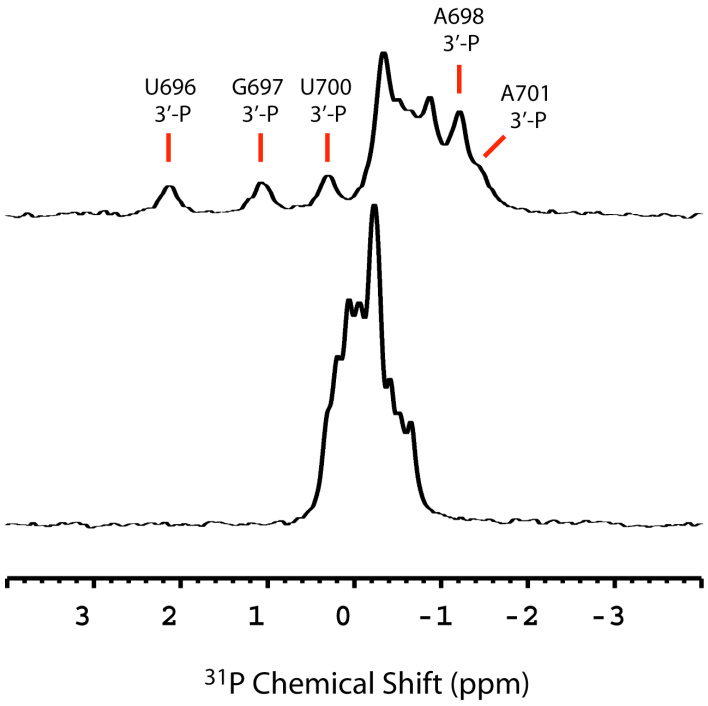

Figure S3. UV denaturation profiles of SL5 in NMR buffer B with various added salts and ionic strength (*I*) conditions. T_m values were derived from the second derivatives (see Experimental Procedures). At 50 mM NaCl (I = 0.05 M) the T_m is 72.8 ± 0.5 ºC, at 20 mM $MgCl_2$ (I = 0.06 M) the T_m is 82.1 ± 0.5 ºC, at 50 mM NaCl and 40 mM $MgCl_2$, (I = 0.17 M) the T_m is 81.5 ± 0.5 ºC and at 500 mM NaCl (I = 0.50 M) the T_m is 80.2 ± 0.5 ºC.

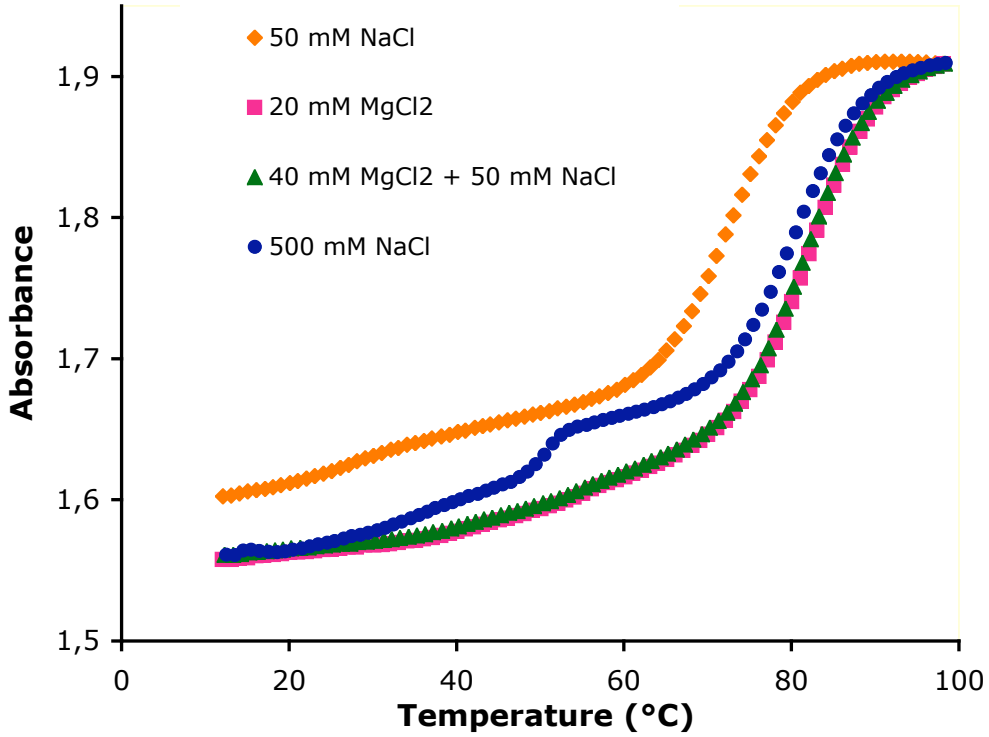

Table S1. Resonance assignment of $SL5^{Mg}$.

NON-EXCHANGEABLE PROTON CHEMICAL SHIFTS (ppm)[a] (ppm +/- 0.09)										
	H1'	**H2'**	**H3'**	**H4'**	**H5'**	**H5''**	**H2**	**H5**	**H6**	**H8**
G690	5.48	4.51	4.42	4.08	3.84	3.76				7.84
C691	5.56	4.46	4.56	4.42	4.55	4.10		5.13	7.81	
G692	5.70	4.55	4.65	4.46	4.54	4.11				7.54
A693	5.89	4.63	4.57	4.50	4.56	4.10	7.47			7.69
G694	5.52	4.47	4.19	4.42	4.45	4.00				6.87
U695	5.37	4.23	4.34	4.34	4.41	4.08		5.10	7.51	
U696	5.56	4.70	4.60	4.27	4.38	4.10		5.64	7.77	
G697	5.53	4.78	4.61	4.32	4.33	4.07				7.86
A698	5.85	4.30	4.91	4.37	4.35	4.04	7.96			7.86
C699	5.53	4.34	4.66	4.39	4.46	4.01		5.04	7.06	
U700	6.06	4.66	4.76	4.53	4.24	4.23		5.85	7.91	
A701	5.95	4.90	4.74	4.56	4.57	4.27	7.91			8.18
C702	5.41	4.20	4.55	4.46	4.56	4.11		5.31	7.74	
U703	5.55	4.46	4.60	4.45	4.57	4.13		5.33	7.98	
C704	5.61	4.45	4.57	4.47	4.55	4.12		5.62	7.91	
G705	5.65	4.39	4.56	4.42	4.56	4.07				7.55
C706	5.59	3.97	4.04	4.15	4.51	4.02		5.23	7.47	
CARBON AND PHOSPHORUS CHEMICAL SHIFTS (ppm) (ppm +/- 0.8)										
	C1'	**C2'**	**C3'**	**C4'**	**C5'**	**C2**	**C5**	**C6**	**C8**	**5'-P[b]**
G690	93.3	75.0	73.4	84.4	61.6				138.7	
C691	93.7	75.4	71.9	81.9	64.3		97.2	141.1		-1.12
G692	92.5	75.6	72.4	81.6	64.8				136.0	-0.46
A693	92.6	75.7	72.5	81.7	64.7	153.6			138.8	0.35
G694	92.8	75.4	72.7	81.9	65.3				135.4	-0.27
U695	93.8	75.9	72.0	82.5	64.1		103.5	140.7		
U696	94.1	74.9	71.9	82.3	64.6		103.5	142.9		-0.75
G697	93.6	75.1	72.2	82.9	64.1				139.4	2.18
A698	92.6	76.2	72.5	82.7	64.4	154.9			140.8	1.12
C699	93.3	75.8	75.0	83.3	66.5		98.0	141.2		-1.20
U700	90.5	75.0	77.4	86.1	67.4		105.5	144.2		-0.33
A701	93.9	75.3	72.9	83.7	65.2	154.5			141.4	0.52
C702	94.1	75.5	71.9	82.0	64.3		97.2	141.2		-1.43
U703	93.6	75.3	72.0	81.6	64.5		102.8	142.5		-0.85
C704	93.6	75.4	72.2	81.7	64.5		97.9	141.3		-0.62
G705	92.9	75.4	72.3	81.9	64.7				136.0	-0.27
C706	93.3	77.4	69.2	83.0	64.8		97.5	140.8		-0.48

BASE EXCHANGEABLE PROTON CHEMICAL SHIFTS (ppm)								
RESIDUES	H1[b]	H3[b]	H41[b]	H42[b]	H21[c]	H22[c]	H61[c]	H62[c]
G690								
C691			8.42	6.37				
G692	11.98							
A693								
G694	13.62							
U695								
U696								
G697					6.08	6.08		
A698							6.38	6.38
C699								
U700								
A701								
C702			8.23	6.92				
U703		14.07						
C704			8.22	6.47				
G705	12.98							
C706			8.19	6.67				
BASE NITROGEN CHEMICAL SHIFTS (ppm)								
RESIDUES	N1[d]	N2[d]	N3[e]	N4[d]	N6[d]	N7[d]	N9[d]	
G690						231.1	169.6	
C691				98.1				
G692	145.9						169.7	
A693	221.0		212.8			229.1	171.1	
G694	148.4					234.1	169.8	
U695			159.11					
U696								
G697		72.5				232.7	169.4	
A698	228.6		214.1		81.35	223.1	171.0	
C699								
U700								
A701	225.6		215.0			230.3	169.7	
C702				98.6				
U703			161.6					
C704				97.8				
G705	147.5					234.3	169.4	
C706				97.7				

a. The assignment of non-exchangeable ^{1}H, ^{13}C and ^{31}P was obtained in NMR buffer A supplemented with 40 mM $MgCl_2$ and the assignment of exchangeable ^{1}H and ^{15}N was obtained in NMR buffer B supplemented with 20 mM $MgCl_2$;
b. +/- 0.04 ppm;
c. +/- 0.02 ppm;
d. +/- 0.2 ppm;
e. +/- 0.2 ppm for adenines, 0.4 ppm for uridines.

Table S2. Resolved SL5 protons that gave an NOE crosspeak to $Co(NH_3)_6^{3+}$ in ^{13}C-edited HMQC-NOESY spectra.

NOE observed at a mixing time of 90 ms	NOE observed only at a mixing time of 180 ms
690 H8	690 H4'
691 H41, H42, H5	692.H8
693 H8	694 H1'
694 H1, H3', H8	695 H6
695 H5	702 H1'
696 H5	703 H1', H5
702 H41, H42	706 H5
703 H3	
704 H41, H42	
706 H41, H42	

Table S3. Results of the $MnCl_2$ titrations.

Nuclei and nuclei pairs that give rise to peaks broadened to baseline at 20 μM $MnCl_2$	Nuclei and nuclei pairs that give rise to peaks with little or no line broadening at 80 μM $MnCl_2$
690 C8-H8, H8-N7, H8-N9	692 C1'-H1'
691 N4-H41, N4-H42, C5-H5	693 C1'-H1', C2-H2
692 H8-N7, H8-N9	694 C1'-H1'
693 C8-H8, H8-N7, H8-N9	695 C1'-H1'
694 C8-H8, H8-N7, H8-N9	698 C1'-H1', C2'-H2', C2-H2, C4'-H4', C8-H8
695 C5-H5	699 C5-H5
696 C5-H5, C6-H6	700 C5-H5, C6-H6
697 C8-H8, H8-N7, H8-N9, 5'-P	701 C1'-H1' C2-H2
698 H8-N7, H8-N9, 5'-P	702 C1'-H1', C2'-H2'
699 5'-P	703 C6-H6
701 H8-N7, H8-N9	
702 N4-H41, N4-H42	
704 N4-H41, N4-H42	
705 H8-N7, H8-N9	
706 N4-H41, N4-H42	

Table S4. Statistics from the structure calculation of $SL5^{Mg}$ with modeling of the $Mn(H_2O)_6^{2+}$.

Distance Restraints	464
Number of NOE-derived distance restraints	379
From standard NOESY spectra	340
Inter-nucleotide	124
Intra-nucleotide	216
From 2D ^{1}H-^{15}N CMPG-NOESY spectra	39
Hydrogen-bond restraints	29
Distance restraints to Mn^{2+}	20
Repulsive distance restraints to Mn^{2+}	36
Dihedral Angle Restraints	24
Sugar Pucker (δ)	12
Backbone (γ)	12
Total Number of Restraints	488
RMSD from Experimental Restraints	
NOE (Å) (none > 0.1 Å)	0.004 +/- 0.002
Dihedral (°) (none > 5°)	0.037 +/- 0.010
RMSD from Idealized Geometry	
Bonds (Å)	0.003884 +/- 0.000024
Angles (°)	0.934310 +/- 0.000808
Impropers (°)	0.360510 +/- 0.001717
Heavy-Atom RMSDs to the Minimized Average Structure (Å)	
Overall (residues 691-705)	0.91 +/- 0.34
Stem (residues 691-695 and 701-705)	0.63 +/- 0.19
Loop (residues 696-700)	0.64 +/- 0.01
Heavy-Atom RMSDs between the Minimized Average Structures of $SL5^{Mg}$ and $SL5^{Mn}$ (Å)	
Overall (residues 691-705)	0.98
Stem (residues 691-695 and 701-705)	0.63
Loop (residues 696-700)	0.54