The NMR Structure of an Internal Loop from 23S Ribosomal RNA Differs from its Structure in Crystals of 50S Ribosomal Subunits

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Supporting Information

Table S1. List of Distance and Dihedral Angle Restraints Used for Modeling.

Hydrogen bonds for the stem region

Atom 1	*Atom 2*	*Lower limit*	*Upper limit*
G1H1	C18N3	1.800	2.500
G1O6	C18H42	1.800	2.500
G1H22	C18O2	1.800	2.500
G2H1	C17N3	1.800	2.500
G2O6	C17H42	1.800	2.500
G2H22	C17O2	1.800	2.500
C3N3	G16H1	1.800	2.500
C3H42	G16O6	1.800	2.500
C3O2	G16H22	1.800	2.500
G7H1	C12N3	1.800	2.500
G7O6	C12H42	1.800	2.500
G7H22	C12O2	1.800	2.500
A8H62	U11O4	1.800	2.500
A8N1	U11H3	1.800	2.500
C9N3	G10H1	1.800	2.500
C9H42	G10O6	1.800	2.500
C9O2	G10H22	1.800	2.500

NOESY restraints from 100 ms spectrum at 25 ºC

Proton 1	*Proton 2*	*Lower limit*	*Upper limit*	*NMR Distance*
G1H2'	G1H1'	2.30	4.27	3.29
G1H2'	G1H8	3.24	6.02	4.63
G1H2'	G2H8	2.05	3.81	2.93
G1H8	G1H3'	2.11	3.92	3.02
G2H8	G2H3'	2.23	4.14	3.19
G2H1'	G2H2'	1.82	3.38	2.60

G2H8	G1H1'	2.58	4.79	3.68
G2H8	G1H3'	1.95	3.62	2.78
G2H8	G2H1'	2.97	5.51	4.24
G2H8	G2H2'	2.18	4.05	3.11
C3H1'	C3H3'	2.33	4.32	3.33
C3H5	G2H2'	2.44	4.53	3.49
C3H6	G2H1'	2.54	4.71	3.62
C3H6	G2H2'	1.77	3.29	2.53
C3H6	C3H5	1.71	3.18	2.45
C3H6	C3H1'	2.46	4.56	3.51
C3H6	C3H2'	2.54	4.72	3.63
C3H6	C3H3'	2.09	3.88	2.98
C3H6	U4H5	3.36	6.24	4.80
U4H1'	U4H3'	2.04	3.79	2.91
U4H1'	U4H4'	2.18	4.06	3.12
U4H6	C3H3'	2.20	4.09	3.15
U4H1'	U4H2'	1.73	3.22	2.48
U4H6	C3H1'	2.54	4.72	3.63
U4H6	C3H2'	1.80	3.35	2.57
U4H6	U4H4'	2.98	5.53	4.25
U4H6	U4H1'	2.21	4.11	3.16
U4H6	U4H2'	2.70	5.01	3.86
U4H6	U4H3'	1.81	3.36	2.59
U4H6	U4H5	1.70	3.17	2.44
A5H1'	A5H4'	2.19	4.07	3.13
A5H1'	A5H2'	2.00	3.71	2.85
A5H3'	A5H1'	2.36	4.38	3.37
A5H3'	A5H8	2.20	4.09	3.14
A5H3'	A6H8	4.14	7.70	5.92
A5H8	U4H3'	2.15	3.99	3.07
A5H8	U4H1'	2.76	5.13	3.94

A5H8	U4H2'	1.88	3.49	2.69
A5H8	A5H4'	2.42	4.49	3.46
A5H8	A5H1'	2.57	4.78	3.67
A5H8	A5H2'	2.08	3.87	2.98
A6H1'	A6H3'	2.47	4.58	3.52
A6H1'	A6H4'	2.94	5.46	4.20
A6H8	A6H3'	2.65	4.93	3.79
A6H1'	A6H2'	2.28	4.24	3.26
A6H8	A5H1'	2.38	4.44	3.41
A6H8	A5H2'	2.02	3.76	2.89
A6H8	A6H1'	2.50	4.65	3.57
A6H8	A6H4'	3.48	6.46	4.97
A6H8	G7H8	2.35	4.36	3.35
G7H2'	G7H1'	1.69	3.14	2.41
G7H8	A6H3'	1.81	3.36	2.58
G7H8	G7H1'	2.69	5.00	3.84
G7H8	A6H1'	3.09	5.73	4.41
G7H8	G7H2'	2.41	4.47	3.44
A8H2	C9H1'	2.22	4.13	3.18
A8H8	A8H3'	2.09	3.89	2.99
A8H8	C9H5	3.02	5.60	4.31
A8H1'	G7H2'	2.80	5.21	4.01
A8H1'	A8H3'	2.27	4.22	3.25
A8H1'	A8H2'	1.68	3.11	2.39
A8H2	C12H1'	1.92	3.57	2.75
A8H8	G7H2'	1.80	3.34	2.57
A8H8	G7H8	3.58	6.65	5.11
A8H8	A8H1'	2.44	4.54	3.49
A8H8	A8H2'	2.35	4.37	3.36
C9H1'	A8H2'	2.24	4.16	3.20
C9H1'	C9H3'	2.03	3.77	2.90

C9H1'	C9H2'	1.68	3.11	2.39
C9H1'	C9H5	2.95	5.48	4.22
C9H5	A8H3'	2.65	4.92	3.79
C9H5	A8H2'	2.16	4.01	3.09
C9H5	C9H2'	3.07	5.70	4.39
C9H6	A8H3'	2.30	4.27	3.28
C9H6	A8H1'	2.59	4.81	3.70
C9H6	A8H2'	1.75	3.26	2.51
C9H6	C9H1'	2.38	4.41	3.39
C9H6	C9H2'	2.50	4.64	3.57
C9H6	C9H5	1.70	3.16	2.43
G10H1'	G10H2'	1.77	3.29	2.53
G10H1'	U11H5	3.05	5.66	4.35
U11H6	U11H2'	2.11	3.92	3.01
U11H6	U11H3'	1.88	3.50	2.69
U11H1'	U11H3'	2.42	4.49	3.46
U11H1'	U11H2'	1.80	3.35	2.57
U11H1'	C12H5	3.46	6.43	4.94
U11H5	G10H2'	2.09	3.88	2.99
U11H6	G10H1'	2.56	4.76	3.66
U11H6	G10H2'	1.62	3.00	2.31
U11H6	U11H1'	2.16	4.02	3.09
U11H6	U11H5	1.73	3.21	2.47
C12H1'	C12H3'	2.52	4.67	3.60
C12H6	U11H2'	1.71	3.17	2.44
C12H6	C12H3'	2.10	3.90	3.00
C12H6	C12H5	1.72	3.19	2.45
C12H1'	C12H2'	1.50	2.79	2.14
C12H5	U11H2'	2.33	4.32	3.33
C12H5	U11H5	2.39	4.44	3.42
C12H6	U11H1'	2.44	4.54	3.49

C12H6	C12H1'	2.34	4.34	3.34	
G13H1'	C12H2'	2.21	4.11	3.16	
G13H1'	G13H3'	2.22	4.12	3.17	
G13H3'	G13H2'	1.85	3.43	2.64	
G13H3'	G13H4'	1.75	3.25	2.50	
G13H8	A8H2	4.58	8.50	6.54	
G13H8	C12H2'	1.84	3.41	2.63	
G13H8	C12H3'	1.98	3.68	2.83	
G13H8	G13H2'	2.14	3.97	3.06	
G13H8	G13H3'	1.93	3.58	2.75	
G13H8	G13H4'	2.27	4.22	3.25	
G13H1'	G13H2'	1.71	3.17	2.44	
G13H1'	G13H4'	2.11	3.92	3.01	
G13H8	C12H1'	2.70	5.01	3.85	
G13H8	G13H1'	2.44	4.53	3.48	
A14H1'	A14H3'	2.96	5.49	4.22	
A14H2'	A14H3'	1.80	3.35	2.58	
A14H2'	A14H4'	2.24	4.15	3.20	
A14H8	G13H3'	2.94	5.46	4.20	
A14H8	A14H4'	2.35	4.36	3.36	
A14H1'	A14H4'	2.36	4.38	3.37	
A14H1'	A14H2'	1.79	3.32	2.55	
A14H2	A6H1'	2.05	3.81	2.93	
A14H2	A15H1'	1.88	3.48	2.68	
A14H2	A15H8	2.49	4.62	3.55	
A14H2'	A15H3'	2.37	5.52	3.94	± 40%
A14H8	G13H1'	2.73	5.08	3.91	
A14H8	G13H2'	1.95	3.62	2.79	
A14H8	A14H3'	2.00	3.72	2.86	
A14H8	A14H1'	2.38	4.42	3.40	
A14H8	A14H2'	2.35	4.37	3.36	

A14H8	A15H8	2.68	4.97	3.83	
A15H1'	A15H2'	1.89	3.51	2.70	
A15H8	A14H3'	2.12	3.94	3.03	
A15H8	A15H2'	1.90	4.44	3.17	± 40%
A15H1'	A15H3'	2.30	4.27	3.28	
A15H2	A5H1'	2.28	4.23	3.26	
A15H2	G16H1'	1.91	3.55	2.73	
A15H8	A15H1'	3.12	5.79	4.45	
A15H8	A15H3'	1.80	3.34	2.57	
G16H1'	A15H2'	3.08	5.73	4.40	
G16H1'	A15H3'	3.40	6.31	4.85	
G16H1'	G16H2'	1.71	3.18	2.44	
G16H1'	C17H5	3.06	5.68	4.37	
G16H8	A15H1'	2.73	5.08	3.91	
G16H8	A15H2'	1.73	3.21	2.47	
G16H8	A15H3'	2.05	3.80	2.93	
G16H8	G16H1'	2.62	4.87	3.74	
G16H8	C17H5	3.52	6.54	5.03	
C17H1'	C17H3'	1.63	3.81	2.72	± 40%
C17H1'	C17H2'	1.62	3.00	2.31	
C17H5	G16H2'	2.07	3.84	2.95	
C17H6	G16H1'	2.63	4.89	3.76	
C17H6	G16H2'	1.63	3.03	2.33	
C17H6	C17H1'	1.95	3.62	2.79	
C17H6	C17H2'	2.09	3.88	2.98	
C17H6	C17H5	1.74	3.22	2.48	
C18H6	C17H2'	1.73	3.21	2.47	
C18H1'	C17H2'	2.91	5.40	4.15	
C18H1'	C18H2'	1.67	3.10	2.38	
C18H6	C18H1'	2.43	4.52	3.48	
C18H6	C18H2'	1.83	4.27	3.05	± 40%

NOESY distance restraints from 200 ms 30 ºC spectrum

A5H2	G13H2'	2.47	5.76	4.11	± 40%
A5H2	A14H8	2.64	6.17	4.41	± 40%
A6H2	G7H1'	1.77	4.14	2.96	± 40%
A6H2	A6H1'	2.49	5.72	4.08	± 40%
A6H2	A14H1'	1.54	3.60	2.57	± 40%
A8H8	G7H1'	2.05	4.78	3.42	± 40%
A8H2	A8H1'	2.61	6.09	4.36	± 40%
A14H2	A6H2	3.02	7.06	5.04	± 40%
A14H8	A6H2	3.49	8.15	5.82	± 40%
A15H8	A14H1'	2.03	4.74	3.38	± 40%
A15H8	A14H2'	1.68	3.92	2.79	± 40%

SNOESY distance restraints from 150 ms 15 ºC spectrum

C3H41	G2H1	1.93	4.50	3.22	± 40%
U4H1'	G16H1	1.55	3.63	2.59	± 40%
G7H1	U11H3	2.67	6.23	4.45	± 40%
A8H2	G7H1	1.94	4.53	3.23	± 40%
A8H2	U11H3	1.74	4.06	2.90	± 40%
A8H1'	G7H1	1.79	4.18	2.98	± 40%
C12H41	U11H3	2.22	5.19	3.71	± 40%
C12H42	U11H3	2.39	5.58	3.98	± 40%
G13H1'	G7H1	1.74	4.05	2.89	± 40%
G16H1	G2H1	2.18	5.09	3.64	± 40%
C17H41	G16H1	2.14	5.01	3.57	± 40%
C18H1'	G2H1	2.13	4.99	3.56	± 40%

Dihedral angle restraints

Alpha O3'-P-O5'-C5'; A-form -68

Atom 1	*Atom 2*	*Atom 3*	*Atom 4*	*Lower*	*Upper*
G1O3'	G2P	G2O5'	G2C5	-120	120
G2O3'	C3P	C3O5'	C3C5'	-120	120
C3O3'	U4P	U4O5'	U4C5'	-120	120
G7O3'	A8P	A8O5'	A8C5'	-120	120
A8O3'	C9P	C9O5'	C9C5'	-120	120
G10O3'	U11P	U11O5'	U11C5'	-120	120
U11O3'	C12P	C12O5'	C12C5'	-120	120
C12O3'	G13P	G13O5'	G13C5'	-120	120
G16O3'	C17P	C17O5'	C17C5'	-120	120
C17O3'	C18P	C18O5'	C18C5'	-120	120

Beta P-O5'-C5'-C4'; A-form 178

G2P	G2O5'	G2C5'	G2C4'	150	-150
C3P	C3O5'	C3C5'	C3C4'	150	-150
G7P	G7O5'	G7C5'	G7C4'	150	-150
A8P	A8O5'	A8C5'	A8C4'	150	-150
C9P	C9O5'	C9C5'	C9C4'	150	-150
U11P	U11O5'	U11C5'	U11C4'	150	-150
C12P	C12O5'	C12C5'	C12C4'	150	-150
G16P	G16O5'	G16C5'	G16C4'	150	-150
C17P	C17O5'	C17C5'	C17C4'	150	-150
C18P	C18O5'	C18C5'	C18C4'	150	-150

Gamma O5'-C5'-C4'-C3'; A-form 54

G1O5'	G1C5'	G1C4'	G1C3'	30	90
G2O5'	G2C5'	G2C4'	G2C3'	30	90
C3O5'	C3C5'	C3C4'	C3C3'	30	90
G7O5'	G7C5'	G7C4'	G7C3'	30	90

A8O5'	A8C5'	A8C4'	A8C3'	30	90
C9O5'	C9C5'	C9C4'	C9C3'	30	90
G10O5'	G10C5’	G10C4'	G10C3'	30	90
U11O5'	U11C5'	U11C4'	U11C3'	30	90
C12O5'	C12C5'	C12C4'	C12C3'	30	90
G16O5'	G16C5'	G16C4'	G16C3'	30	90
C17O5'	C17C5'	C17C4'	C17C3'	30	90
C18O5'	C18C5'	C18C4'	C18C3'	30	90

Delta C5'-C4'-C3'-O3'; A-form 82

G1C5'	G1C4'	G1C3'	G1O3'	55	115
G2C5'	G2C4'	G2C3'	G2O3'	55	115
C3C5'	C3C4'	C3C3'	C3O3'	55	115
U4C5'	U4C4'	U4C3'	U4O3'	55	115
A5C5'	A5C4'	A5C3'	A5O3'	55	190
A6C5'	A6C4'	A6C3'	A6O3'	55	115
G7C5'	G7C4'	G7C3'	G7O3'	55	115
A8C5'	A8C4'	A8C3'	A8O3'	55	115
C9C5'	C9C4'	C9C3'	C9O3'	55	190
G10C5'	G10C4’	G10C3'	G10O3'	55	115
U11C5'	U11C4'	U11C3'	U11O3'	55	115
C12C5'	C12C4'	C12C3'	C12O3'	55	115
G13C5'	G13C4'	G13C3'	G13O3'	55	190
A14C5'	A14C4'	A14C3'	A14O3'	55	115
A15C5'	A15C4'	A15C3'	A15O3'	55	115
G16C5'	G16C4'	G16C3'	G16O3'	55	115
C17C5'	C17C4'	C17C3'	C17O3'	55	115
C18C5'	C18C4	C18C3'	C18O3'	55	190

Epsilon C4'-C3'-O3'-P; A-form -153

G1C4'	G1C3'	G1O3'	G2P	-180	-100

G2C4'	G2C3'	G2O3'	C3P	-180	-100
C3C4'	C3C3'	C3O3'	U4P	-180	-100
G7C4'	G7C3'	G7O3'	A8P	-180	-100
A8C4'	A8C3'	A8O3'	C9P	-180	-100
G10C4'	G10C3'	G10O3'	U11P	-180	-100
U11C4'	U11C3'	U11O3'	C12P	-180	-100
C12C4'	C12C3'	C12O3'	G13P	-180	-100
G16C4'	G16C3'	G16O3'	C17P	-180	-100
C17C4'	C17C3'	C17O3'	C18P	-180	-100

Zeta C3'-O3'-P-O5'; A-form -71

G1C3'	G1O3'	G2P	G2O5'	-120	120
G2C3'	G2O3'	C3P	C3O5'	-120	120
C3C3'	C3O3'	U4P	U4O5'	-120	120
G7C3'	G7O3'	A8P	A8O5'	-120	120
A8C3'	A8O3'	C9P	C9O5'	-120	120
G10C3'	G10O3'	U11P	U11O5'	-120	120
U11C3'	U11O3'	C12P	C12O5'	-120	120
C12C3'	C12O3'	G13P	G13O5'	-120	120
G16C3'	G16O3'	C17P	C17O5'	-120	120
C17C3'	C17O3'	C18P	C18O5'	-120	120

Chi Glycosidic torsions; A -form -158

G1O4'	G1C1'	G1N9	G1C4	150	-130
G2O4'	G2C1'	G2N9	G2C4	150	-130
C3O4'	C3C1'	C3N1	C3C2	150	-130
U4O4'	U4C1'	U4N1	U4C2	150	-30
A5O4'	A5C1'	A5N9	A5C4	150	-30
A6O4'	A6C1'	A6N9	A6C4	150	-30
G7O4'	G7C1'	G7N9	G7C4	150	-130
A8O4'	A8C1'	A8N9	A8C4	150	-130

C9O4'	C9C1'	C9N1	C9C2	150	-130
G10O4'	G10C1’	G10N9	G10C4	150	-130
U11O4'	U11C1'	U11N1	U11C2	150	-130
C12O4'	C12C1	C12N1	C12C2	150	-130
G13O4'	G13C1’	G13N9	G13C4	150	-30
A14O4'	A14C1'	A14N9	A14C4	150	-30
A15O4'	A15C1'	A15N9	A15C4	150	-30
G16O4'	G16C1’	G16N9	G16C4	150	-130
C17O4'	C17C1'	C17N1	C17C2	150	-130
C18O4'	C18C1'	C18N1	C18C2	150	-130

Table S2. Summary of NOE distance restraints.

Residue	Total	Intra residue	Inter residue
G1	9	3	6
G2	16	4	12
C3	16	5	11
U4	16	8	8
A5	16	7	9
A6	18	7	11
G7	18	3	15
A8	25	6	19
C9	18	7	11
G10	8	1	7
U11	21	6	15
C12	20	5	15
G13	19	9	10
A14	26	11	15
A15	19	5	14
G16	19	2	17
C17	16	5	11
C18	9	3	6

All restraints: 203

Intra residue: 97

Inter residue: 106

Table S3. List of the Chemical Shift assignments at 25°C (exchangeable protons were assigned at 10°C).

	H8/H6	H2/H5	H1’	H2'	H3'	H4'	H5'	H1/H3	H4/5/6Am
G1	7.912	-	5.640	4.808	4.507		3.938	12.57	
G2	7.477	-	5.873	4.579	4.532			13.37	
C3	7.394	5.109	5.465	4.623	4.273				8.247/ 6.817
U4	7.841	5.519	5.489	4.012	4.518	4.376		11.60	
A5	8.405	8.671	5.731	4.629	4.862	4.676			
A6	7.793	7.692	5.784	4.587	4.412	4.285			
G7	7.414	-	3.822	4.272	-	4.293		12.35	
A8	7.668	7.780	5.918	4.437	4.568				
C9	7.307	5.152	5.617	3.933	4.230				
G10	-	-	5.618	4.672	-			12.95	
U11	7.926	5.005	5.639	4.614	4.472			14.46	
C12	7.577	5.395	5.474	4.621	4.321				8.012/ 6.449
G13	8.067	-	5.557	4.423	5.010	4.536		10.20	
A14	8.128	7.844	5.849	4.949	4.681	4.601			
A15	7.690	7.678	5.038	4.473	4.443				
G16	6.842	-	5.254	4.373	4.328			13.04	
C17	7.645	5.099	5.510	4.292	4.444				8.513/ 6.855
C18	7.662	5.512	5.720	4.056	-	4.163			

Table S4. List of the H8 and H1' chemical shifts at 25 °C in presence and absence of $MgCl_2$. The differences in chemical shifts are shown.

	H8 + $MgCl_2$	H8 - $MgCl_2$	Difference in ppm	H1' + $MgCl_2$	H1' - $MgCl_2$	Difference in ppm
G1		7.91		5.58	5.64	0.06
G2	7.48	7.48	0.00	5.85	5.87	0.02
C3	7.40	7.39	0.01	5.46	5.46	0.00
U4	7.85	7.84	0.01	5.48	5.49	0.01
A5	8.40	8.38	0.01	5.73	5.73	0.00
A6	7.80	7.79	0.01	5.75	5.78	0.03
G7	7.49	7.43	0.06	3.81	3.82	0.01
A8	7.65	7.67	0.02	5.91	5.92	0.01
C9	7.31	7.30	0.01	5.59	5.62	0.03
G10				5.57	5.62	0.05
U11	7.94	7.93	0.01	5.61	5.64	0.03
C12	7.59	7.58	0.01	5.49	5.47	0.02
G13	8.12	8.07	0.05	5.55	5.56	0.01
A14	8.12	8.13	0.01	5.83	5.85	0.02
A15	7.68	7.69	0.01	5.09	5.04	0.05
G16	6.86	6.85	0.01	5.26	5.28	0.02
C17	7.65	7.64	0.01	5.50	5.51	0.01
C18	7.66	7.66	0.00	5.69	5.71	0.02

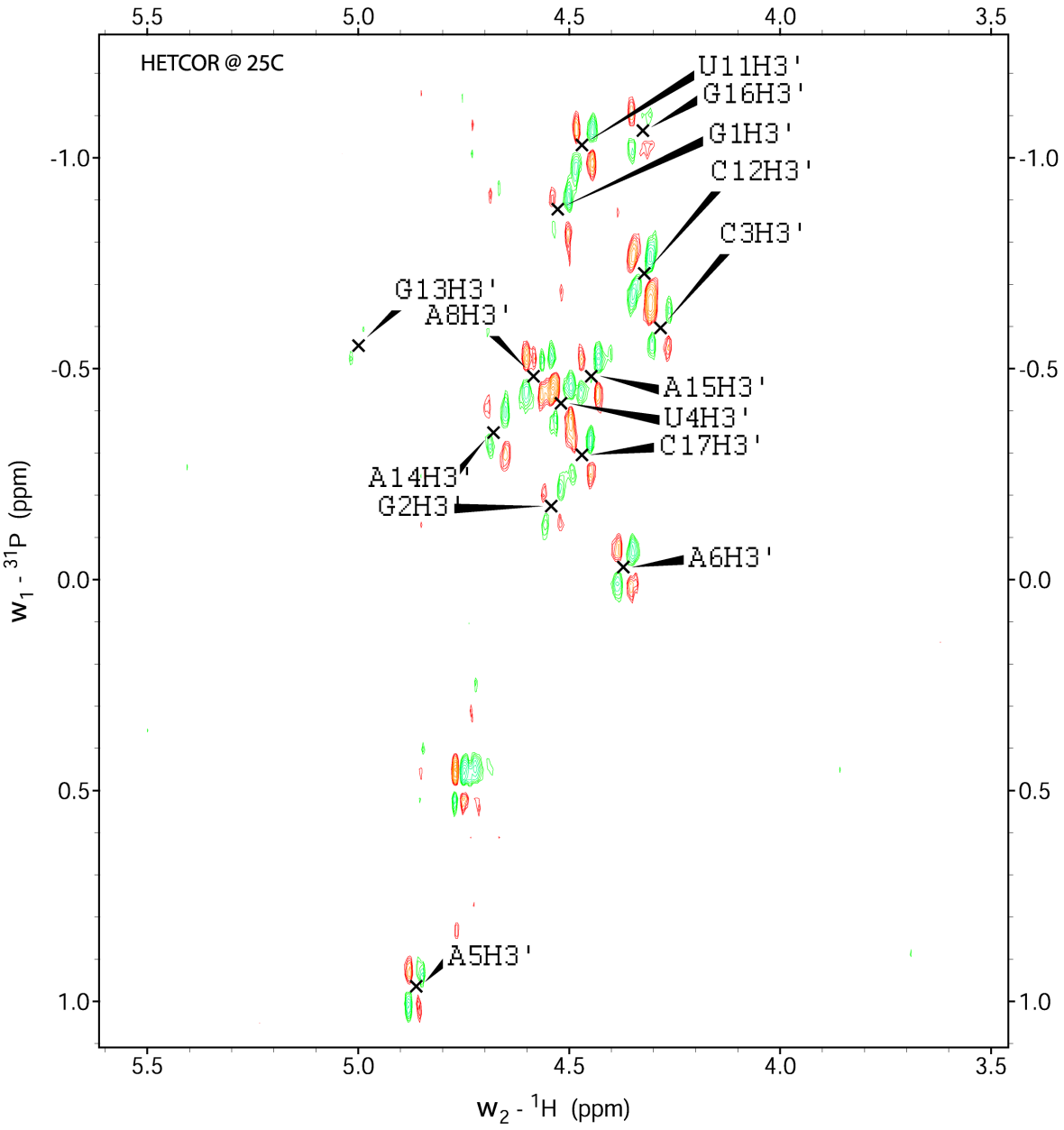

Figure S1. 31 P HETCOR spectrum at 25 °C.

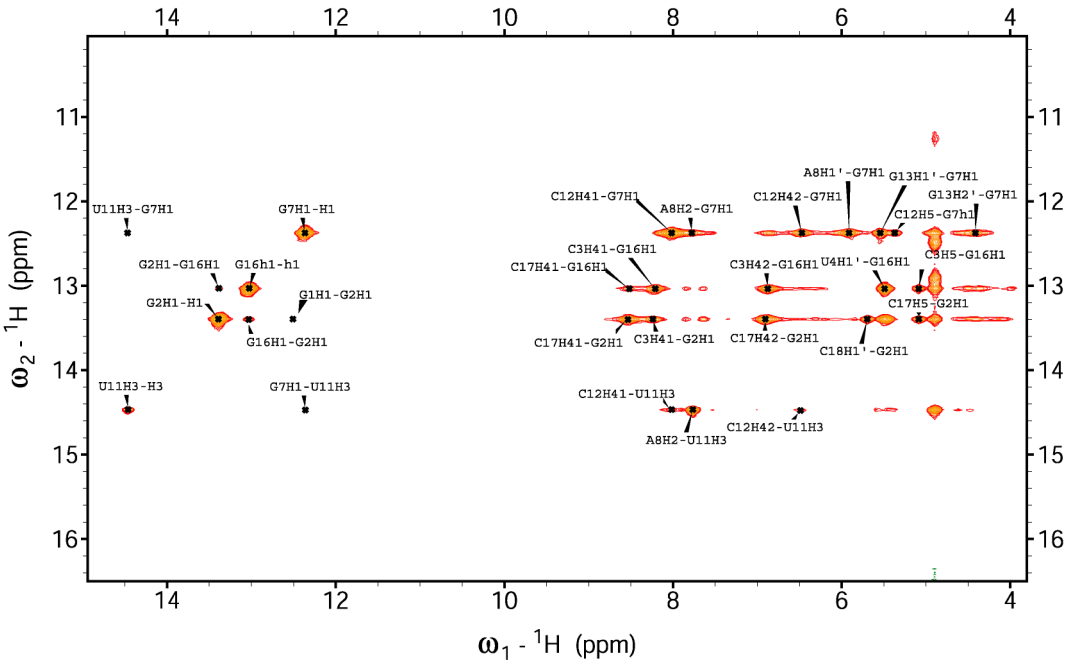

Figure S2. 150 ms SNOESY spectrum at 15 °C.

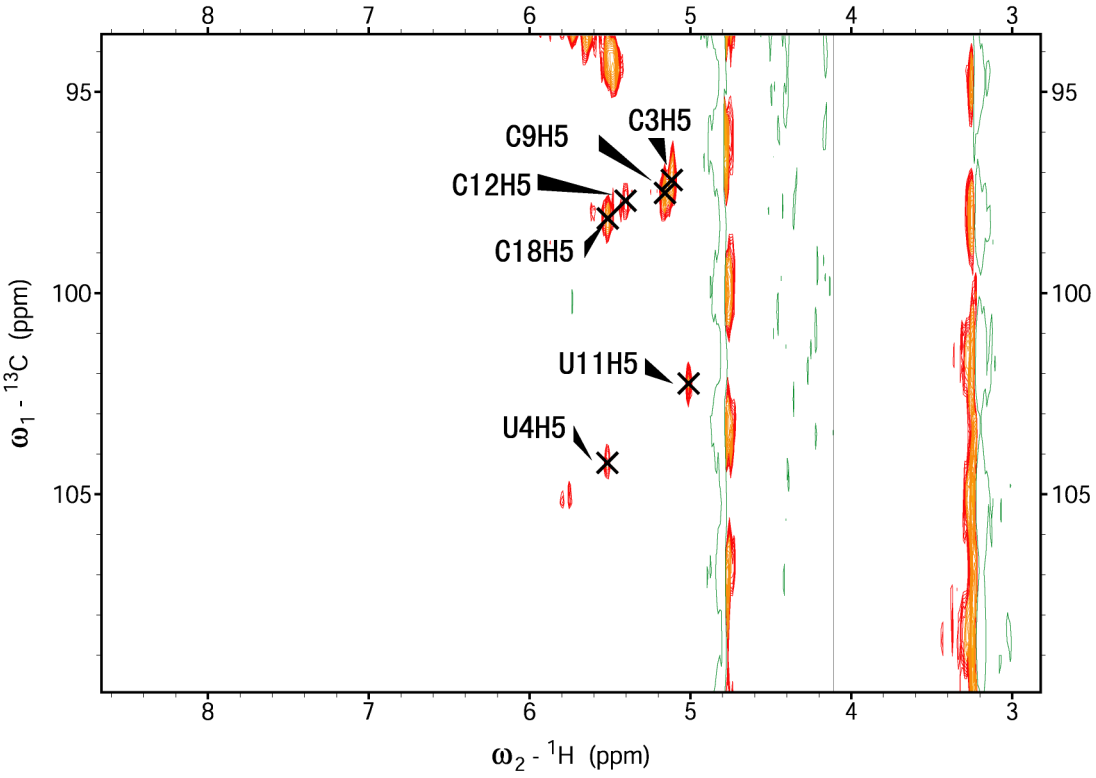

Figure S3. The region showing H5-C5 peaks of Cytosines and Uracils in the HSQC spectrum at 25 °C.

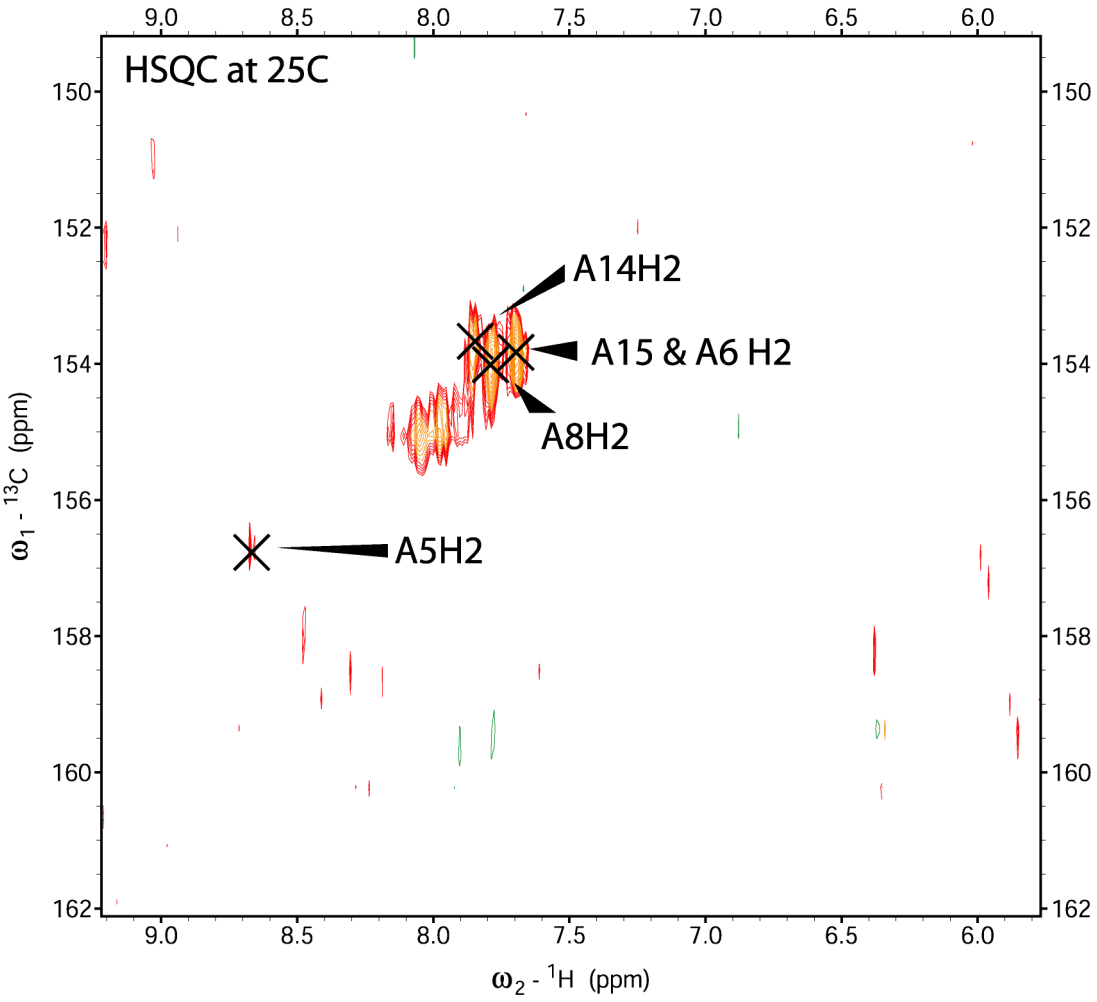

Figure S4. Natural abundance HSQC at 25 °C, showing adenine H2-C2.

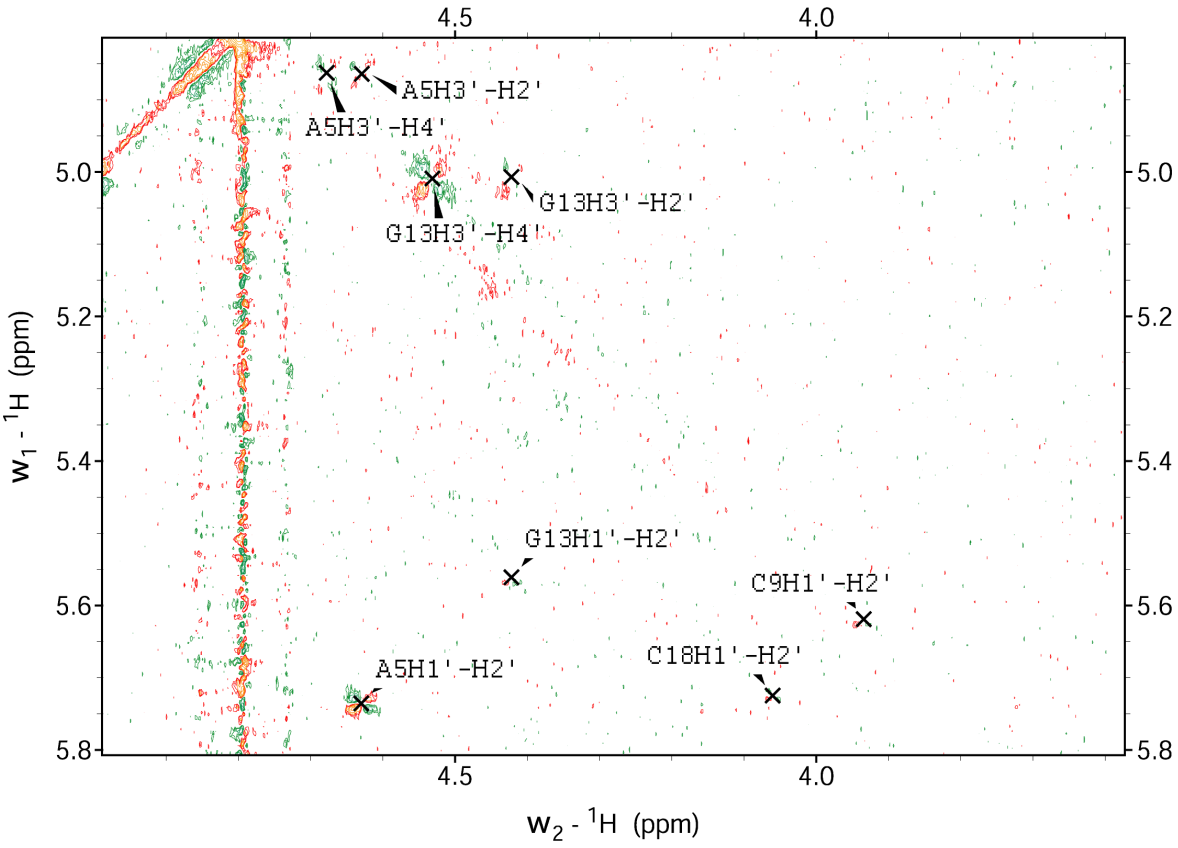

Figure S5. The H1'-H2', H3'- H2' and H3'- H4' region of DQ-COSY spectrum at 25 °C.

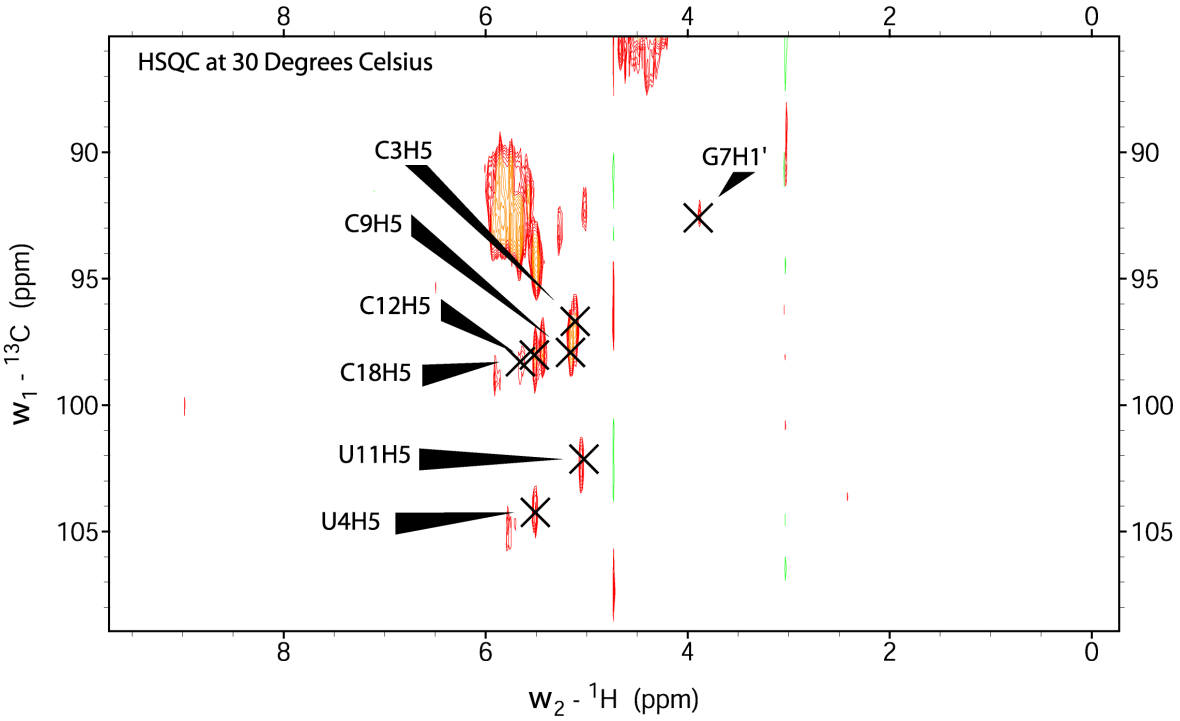

Figure S6. Natural abundance HSQC at 30 °C, showing G7H1'.

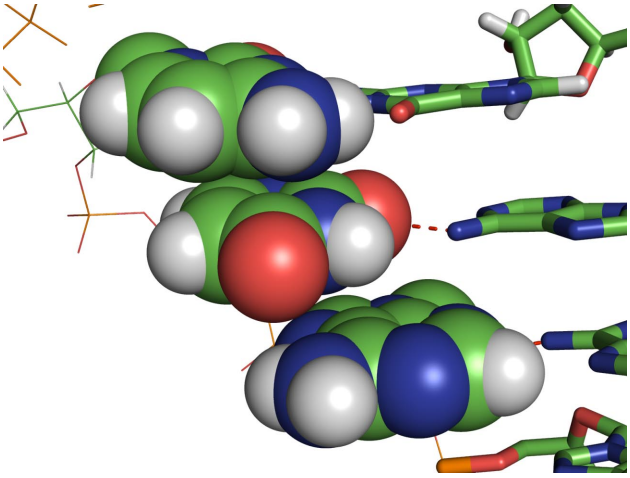

Figure S7. Stacking of U4 carbonyl-4 (red) between C3-amino (blue - top) and A5-amino (blue - bottom).

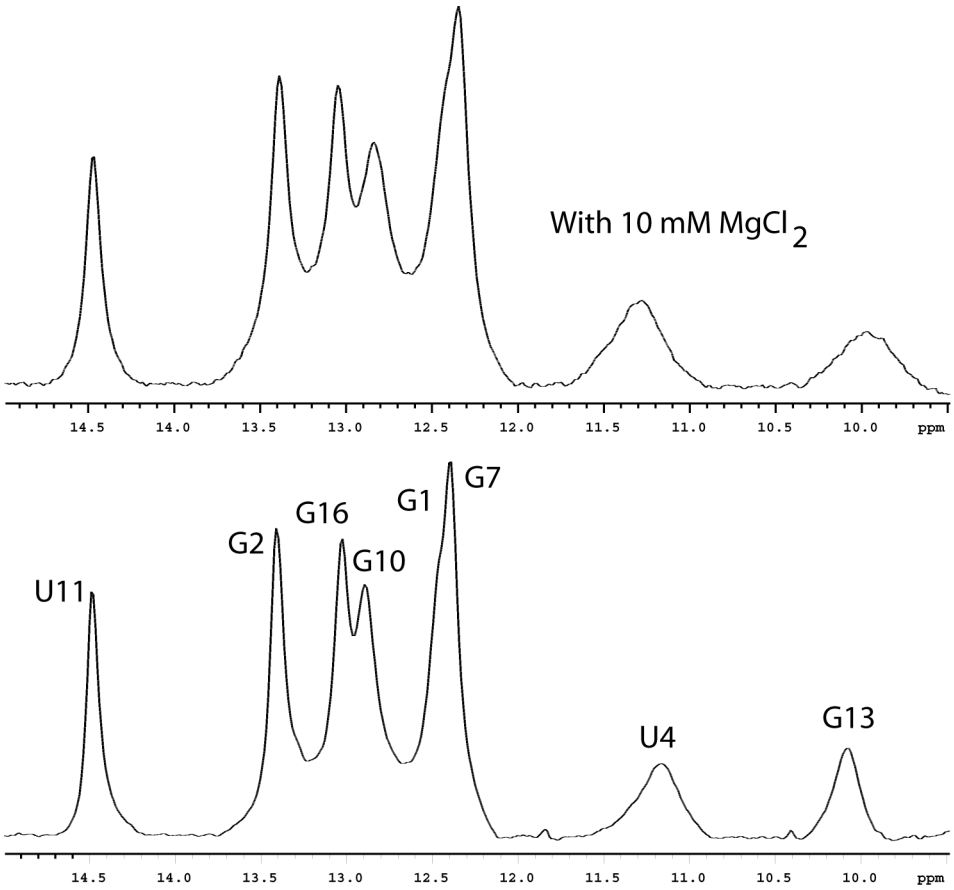

Figure S8. One-dimensional imino proton spectra of 5'GGCUAAGAC/3'CCGAAGCUG at 10 °C (pH 6.5), with and without 10 mM $MgCl_2$. Assignments are shown in the bottom spectrum.

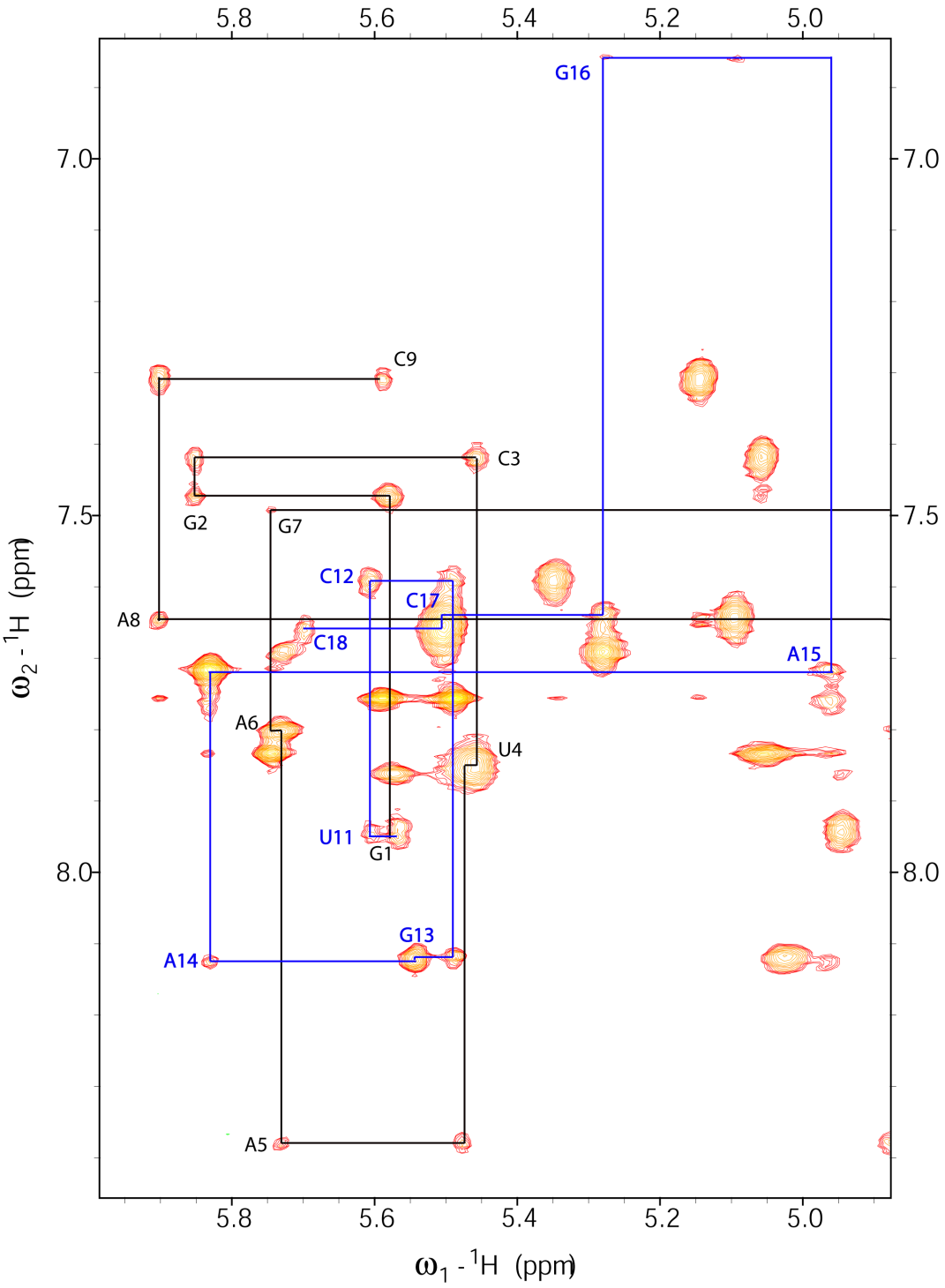

Figure S9. NOESY walk at 25 °C, 200 ms mixing time, for 5'GGCUAAGAC/3'CCGAAGCUG in the presence of 10 mM $MgCl_2$.