Supporting Information for:

NMR Evidence for Dynamic Stacking of the Expected Branch Point Adenosine in Duplexes containing Pseudouridine-Modified or Unmodified U2 snRNA Sites

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Table S1.

NOE distances obtained for pseudouridylated or unmodified duplexes.

		NOE-derived Distance	
H6/8-H1'		**Ψ**	**U**
	1H8-H1'	3.6	3.6
	2H6-H1'	3.5	3.6
	3H6-H1'		
	4H8-H1'	4.0	3.9
	5H6-H1'		3.8
	6H6-H1'	3.9	
	9H6-H1'	3.5	3.4
	10H8-H1'	3.7	3.7
	33H6-H1'	3.4	3.5
	34H8-H1'	3.8	3.7
	36H8-H1'	4.1	4.0
	37H8-H1'	4.0	3.5
	38H6-H1'	3.6	3.6
	40H8-H1'	3.7	3.9
	41H6-H1'	3.5	3.6
H6/8-(n-1)H2'			
	2H6-1H2'	2.7	2.7
	4H8-3H2'	2.5	2.4
	5H6-4H2'	2.6	2.5
	6H6-5H2'	2.5	2.5
	10H8-9H2'		
	34H8-33H2'	2.4	2.4
	37H8-36H2'	2.6	2.6
	38H6-37H2'	2.3	2.3
	39H8-38H2'	2.4	2.4
	40H8-39H2'	2.4	2.5
H2-H1'			
	39H2-4H1'	3.7	3.7
	39H2-40H1'	3.4	3.5
	4H2-39H1'	3.6	3.6
	4H2-5H1'	3.3	3.4

Table S1 (continued).

Loop			
	8H2-36H1'	3.6	3.5
	8H2-9H1'	3.6	3.4
	8H8-6H2'	4.1	4.6
	8H8-6H1'	4.5	4.6
	8H8-7H1'	4.8	5.2
	8H8-7H2’	>4	>4
	7H8-6H2'	3.4	3.3
	36H2-8H1'	4.6	3.9
	36H2-7H1'	5.4	4.3
	9H6-8H2'	2.6	2.5
	35H6-34H2'	2.6	2.7
	36H8-35H2'	2.6	2.5
	35H6-H1'	3.6	3.6
	8H8-H1'	3.8	3.9
	7H8-7H1'	3.0	2.8
	36H8-35H3'	3.1	
	36H1'-35H2'	4.7	
H1'-(n-1)H2'			
	5H1-4H2'	4.1	
	10H1'-9H2'	4.1	
	34H1'-33H2'	4.5	
	37H1'-36H2'	4.6	
	38H1'-37H2'	4.9	
	40H1'-39H2'	3.9	

Table S2. Comparison of pseudouridine-containing (Ψ) and unmodified (U) duplexes. Difference is given and shown in bold if greater than 0.05 ppm. Branch site and neighboring nucleotides are shaded. "NA" is not applicable; "-" is not assigned.
(a) Chemical shifts of non-exchangeable protons of samples at 25 °C in 100% D_2O (10 mM K_2PO_4, 0.25 mM EDTA pH 6.8).

	H6/H8			H5/H2			H1'			H2'			H3'			H4'			H5"			H5'		
Residue	**U**	**Ψ**	**|U-Ψ|**	**U**	**Ψ**	**|U-Ψ|**	**U**	**Ψ**	**|U-Ψ|**	**U**	**Ψ**	**|U-Ψ|**	**U**	**Ψ**	**|U-Ψ|**	**U**	**Ψ**	**|U-Ψ|**	**U**	**Ψ**	**|U-Ψ|**	**U**	**Ψ**	**|U-Ψ|**
G1	8.14	8.13	0.01	NA	NA	NA	5.89	5.89	0.01	4.82	4.82	0.00	4.57	4.56	0.01	4.45	4.44	0.01	3.96	3.96	0.00	4.12	4.12	0.01
C2	7.85	7.83	0.02	5.26	5.26	0.00	5.58	5.58	0.00	4.45	4.45	0.00	4.54	4.53	0.01	4.49	4.49	0.00	4.17	4.17	0.00	-	4.59	-
U3	7.90	7.89	0.01	5.51	5.50	0.01	5.53	5.53	0.00	4.60	4.60	0.01	4.64	4.63	0.01	4.46	4.46	0.00	4.15	4.15	0.00	-	-	-
A4	8.17	8.17	0.00	7.04	7.05	0.01	5.97	5.97	0.00	4.56	4.56	0.00	4.65	4.65	0.00	4.53	4.52	0.00	4.19	4.19	0.00	-	4.57	-
C5	7.43	7.43	0.00	5.23	5.24	0.01	5.27	5.27	0.00	4.05	4.04	0.01	4.31	4.32	0.00	4.36	4.35	0.01	4.03	4.03	0.00	-	4.48	-
U6	7.64	7.65	0.00	5.31	5.31	0.01	5.76	5.78	0.02	4.28	4.28	0.01	4.60	4.62	0.02	4.24	4.26	0.02	4.01	4.01	0.00	4.27	4.28	0.01
G7	7.89	7.92	0.03	NA	NA	NA	5.84	5.89	**0.05**	4.69	4.75	**0.06**	5.02	5.03	0.01	4.48	4.51	0.03	4.19	4.20	0.01	4.33	4.33	0.00
A8	8.29	8.33	0.03	7.62	7.64	0.01	5.97	6.02	**0.05**	4.68	4.71	0.03	4.62	4.64	0.02	4.58	4.59	0.01	4.42	4.42	0.01	4.32	4.33	0.00
C9	7.47	7.47	0.00	5.14	5.08	**0.06**	5.49	5.48	0.01	4.38	4.37	0.00	4.42	4.43	0.00	4.43	4.45	0.03	4.15	4.15	0.01	-	-	-
G10	7.49	7.49	0.00	NA	NA	NA	5.69	5.70	0.01	4.50	4.49	0.01	4.54	4.55	0.00	4.41	4.41	0.00	4.10	4.10	0.00	4.43	-	-
A11	7.87	7.87	0.00	7.86	7.86	0.00	5.97	5.96	0.00	4.13	4.12	0.00	4.28	4.28	0.01	4.26	4.26	0.00	4.06	4.06	0.01	-	4.42	-
C33	8.08	8.09	0.01	5.99	5.98	0.01	5.65	5.63	0.01	4.67	4.68	0.01	4.53	4.52	0.01	4.38	4.39	0.01	3.96	3.96	0.00	4.08	4.09	0.01
G34	7.67	7.72	**0.06**	NA	NA	NA	5.75	5.78	0.02	4.57	4.75	**0.18**	4.57	4.63	**0.06**	4.50	4.49	0.01	4.18	4.17	0.01	-	-	-
U/Ψ35	7.56	7.02	**0.54**	5.22	NA	NA	5.51	4.64	**0.87**	4.47	4.40	**0.07**	4.53	4.48	0.05	4.44	4.23	**0.21**	4.13	4.04	**0.09**	4.52	4.41	**0.11**
A36	8.22	8.14	**0.08**	7.03	6.97	**0.06**	5.89	5.86	0.03	4.74	4.71	0.03	4.69	4.70	0.00	4.50	4.48	0.02	4.20	4.16	0.04	-	-	-
G37	7.17	7.14	0.04	NA	NA	NA	5.34	5.37	0.03	4.30	4.30	0.00	4.32	4.32	0.00	4.42	4.42	0.01	4.07	4.05	0.03	-	4.39	-
U38	7.68	7.66	0.02	5.07	5.06	0.01	5.48	5.48	0.00	4.45	4.46	0.00	4.57	4.56	0.01	4.39	4.39	0.00	4.07	4.06	0.00	4.52	4.52	0.00
A39	8.07	8.07	0.01	6.79	6.79	0.00	5.98	5.98	0.00	4.62	4.62	0.00	4.73	4.72	0.01	4.51	4.51	0.00	4.16	4.16	0.00	-	-	-
G40	7.30	7.29	0.01	NA	NA	NA	5.59	5.58	0.00	4.38	4.38	0.00	4.39	4.38	0.00	4.43	4.43	0.00	4.09	4.08	0.00	4.46	-	-
C41	7.37	7.37	0.00	5.09	5.09	0.01	5.42	5.43	0.00	4.36	4.35	0.01	4.36	4.35	0.01	4.38	-	-	4.03	4.03	0.00	4.48	4.48	0.01
A42	8.03	8.03	0.00	7.45	7.46	0.01	6.01	6.00	0.00	4.05	4.05	0.00	4.30	4.29	0.00	4.23	4.23	0.00	4.03	4.04	0.00	-	4.41	-

Table S2 (continued).

(b) Chemical shifts of exchangeable protons of samples at 0 °C and ^{31}P at 25 °C. ^{31}P shifts are relative to trimethylphosphate. Amino 1 is GH21, CH42, or AH62. Amino 2 is GH22, CH41, or AH61.

	H1/H3			Amino 1			Amino 2			P		
Residue	**U**	**Ψ**	**|U-Ψ|**	**U**	**Ψ**	**|U-Ψ|**	**U**	**Ψ**	**|U-Ψ|**	**U**	**Ψ**	**|U-Ψ|**
G1	12.71	12.71	0.00	6.33	6.35	0.02	8.40	8.41	0.01	NA	NA	NA
C2	NA	NA	NA	6.86	6.87	0.01	8.62	8.62	0.00	-4.24	-4.22	0.02
U3	13.29	13.30	0.01	NA	NA	NA	NA	NA	NA	-3.94	-3.94	0.01
A4	NA	NA	NA	6.48	6.50	0.01	7.87	7.90	0.02	-3.84	-3.83	0.01
C5	NA	NA	NA	7.13	7.15	0.02	8.08	8.10	0.03	-4.07	-4.06	0.01
U6	13.33	13.57	**0.24**	NA	NA	NA	NA	NA	NA	-4.39	-4.39	0.01
G7	10.49	10.67	**0.18**	-	-	-	-	-	-	-3.25	-3.28	0.02
A8	NA	NA	NA	-	-	-	-	-		-3.60	-3.50	**0.10**
C9	NA	NA	NA	6.69	6.65	0.04	8.08	8.00	**0.07**	-3.88	-3.92	0.04
G10	12.35	12.33	0.02	-	-	-	-	-	-	-3.76	-3.79	0.02
A11	NA	NA	NA	-	-	-	-	-	-	-3.85	-3.85	0.00
				0.00			0.00	0.00				
C33	NA	NA	NA	6.87	6.90	0.02	8.38	8.42	0.03	NA	NA	NA
G34	12.90	12.90	0.00	5.96	5.88	0.08	8.18	8.13	0.04	-3.60	-3.65	**0.05**
U/Ψ35H3	13.38	12.82	**0.56**	NA	NA	NA	NA	NA	-	-3.52	-3.36	**0.16**
A36	NA	NA	NA	-	-	-	-	-	-	-3.59	-3.63	**0.05**
G37	13.38	13.40	0.02	6.20	6.18	0.02	8.40	8.42	0.02	-3.76	-3.71	**0.05**
U38	13.53	13.52	0.01	NA	NA	NA	NA	NA	NA	-4.45	-4.43	0.02
A39	NA	NA	NA	6.35	6.37	0.01	7.82	7.83	0.01	-3.71	-3.70	0.01
G40	13.44	13.44	0.00	6.15	6.13	0.02	8.52	8.58	**0.06**	-3.87	-3.86	0.01
C41	NA	NA	NA	6.93	6.95	0.01	8.12	8.13	0.00	-4.13	-4.14	0.01
A42	NA	NA	NA	-	-	-	-	-	-	-3.72	-3.74	0.01
Ψ35H1		10.33										

Table S3. Effects of sequential NaCl (80 mM, Ψ+Na) and $MgCl_2$ (1.75 mM, Ψ+NaMg) additions on the chemical shifts of non-exchangeable protons in the pseudouridine-containing U2 snRNA – BPS duplex in 95% H_2O/5% D_2O (10 mM K_2PO_4, 0.25 mM EDTA pH 6.8). Chemical shift differences between Ψ and Ψ+NaMg are given and shown in bold if greater than 0.05 ppm.
(a) H6/H8, H5/H2, H1′, H2′ protons at 25°C.

	H6/H8				H5/H2				H1'				H2'			
Residue	**Ψ+NaMg**	**Ψ+Na**	**Ψ**	**diff.**	**Ψ+NaMg**	**Ψ+Na**	**Ψ**	**diff.**	**Ψ+NaMg**	**Ψ+Na**	**Ψ**	**diff.**	**Ψ+NaMg**	**Ψ+Na**	**Ψ**	**diff.**
G1	8.14	8.14	8.14	0.002	NA	NA	NA	NA	5.89	5.89	5.89	0.000	4.81	4.81	4.82	0.012
C2	7.86	7.84	7.83	0.033	5.25	5.26	5.27	0.013	5.58	5.58	5.58	0.002	4.44	4.45	4.46	0.014
U3	7.91	7.90	7.89	0.027	5.51	5.51	5.51	0.001	5.53	5.53	5.53	0.002	4.59	4.59	4.60	0.013
A4	8.18	8.17	8.17	0.007	7.05	7.06	7.06	0.010	5.98	5.98	5.98	0.003	4.55	4.56	4.57	0.016
C5	7.46	7.44	7.42	0.036	5.26	5.26	5.25	0.002	5.28	5.28	5.27	0.010	4.04	4.03	4.04	0.002
U6	7.66	7.64	7.64	0.028	5.32	5.31	5.31	0.015	5.81	5.80	5.79	0.014	4.29	4.29	4.28	0.012
G7	7.95	7.94	7.93	0.019	NA	NA	NA	NA	5.91	5.91	5.90	0.016	4.78	4.77	4.75	0.031
A8	8.32	8.33	8.34	0.025	7.57	7.60	7.62	**0.047**	6.03	6.03	6.03	0.004	4.71	4.71	4.72	0.005
C9	7.48	7.48	7.47	0.004	5.05	5.07	5.09	0.031	5.48	5.49	5.49	0.011	4.38	4.38	4.38	0.003
G10	7.47	7.47	7.48	0.011	NA	NA	NA	NA	5.70	5.70	5.70	0.000	4.48	4.48	4.49	0.005
A11	7.86	7.86	7.87	0.010	7.86	7.86	7.86	0.005	5.96	5.96	5.97	0.006	4.12	4.12	4.13	0.019
C33	8.10	8.09	8.09	0.002	5.99	5.99	5.99	0.000	5.64	5.63	5.64	0.002	4.69	4.69	4.69	0.000
G34	7.73	7.72	7.72	0.015	NA	NA	NA	NA	5.78	5.78	5.78	0.003	4.74	4.74	4.75	0.005
Ψ35	7.01	7.00	7.01	0.009	NA	NA	NA	NA	4.66	4.65	4.65	0.007	4.43	4.43	4.42	0.012
A36	8.12	8.13	8.14	0.019	6.92	6.95	6.97	**0.055**	5.85	5.86	5.87	0.018	4.69	4.71	4.72	0.032
G37	7.17	7.14	7.13	0.048	NA	NA	NA	NA	5.39	5.37	5.37	0.028	4.28	4.30	4.31	0.027
U38	7.68	7.66	7.65	0.023	5.07	5.06	5.06	NA	5.47	5.48	5.48	0.011	4.45	4.46	4.47	0.019
A39	8.06	8.07	8.07	0.012	6.80	6.79	6.79	0.003	5.99	5.99	5.99	0.001	4.60	4.62	4.63	0.025
G40	7.28	7.29	7.29	0.009	NA	NA	NA	NA	5.58	5.58	5.59	0.007	4.37	4.38	4.39	0.013
C41	7.35	7.35	7.36	0.012	5.10	5.09	5.09	0.001	5.42	5.42	5.43	0.010	4.35	4.36	4.36	0.004
A42	8.03	8.03	8.03	0.004	7.46	7.46	7.46	0.002	6.00	6.01	6.01	0.002	4.05	4.06	4.06	0.006

Table S3 (continued).
(b) H1/H3 protons at 0 °C.

			H1/H3	
Residue	**Ψ+NaMg**	**Ψ+Na**	**Ψ**	**diff.**
G1	12.72	12.72	12.72	0.000
C2	NA	NA	NA	NA
U3	13.34	13.34	13.31	0.030
A4	NA	NA	NA	NA
C5	NA	NA	NA	NA
U6	13.48	13.51	13.58	**0.100**
G7	-	-	10.70	NA
A8	NA	NA	NA	NA
C9	NA	NA	NA	NA
G10	12.34	12.35	12.34	0.000
A11	NA	NA	NA	NA
C33	NA	NA	NA	NA
G34	12.94	12.94	12.93	0.010
Ψ35H3	12.88	12.87	12.84	0.040
A36	NA	NA	NA	NA
G37	13.45	13.42	13.41	0.040
U38	13.56	13.55	13.54	0.020
A39	NA	NA	NA	NA
G40	13.49	13.47	13.46	0.030
C41	NA	NA	NA	NA
A42	NA	NA	NA	NA
Ψ35H1	10.31	10.29	10.33	0.020

Table S4. Effect of NaCl (80 mM, U+Na) and and then $MgCl_2$ (1.75 mM, U+NaMg) on chemical shifts of non-exchangeable protons of **un**modified U2 snRNA – BPS duplex in 95% H_2O/5% D_2O (10 mM K_2PO_4, 0.25 mM EDTA pH 6.8). Chemical shift differences between U and U+NaMg are given and shown in bold if greater than 0.05 ppm.
(a) H6/H8, H5/H2, H1′, H2′ protons at 25°C.

	H6/H8				H5/H2				H1'				H2'			
Residue	**U+NaMg**	**U+Na**	**U**	**diff.**	**U+NaMg**	**U+Na**	**U**	**diff.**	**U+NaMg**	**U+Na**	**U**	**diff.**	**U+NaMg**	**U+Na**	**U**	**diff.**
G1	8.15	8.14	8.15	0.000	NA	NA	NA	NA	5.90	5.90	5.90	0.001	4.81	4.81	4.83	0.023
C2	7.87	7.86	7.83	0.044	5.26	5.26	5.28	0.019	5.58	5.58	5.59	0.002	4.44	4.45	4.46	0.022
U3	7.92	7.91	7.89	0.038	5.51	5.51	5.52	0.007	5.53	5.53	5.53	0.007	4.59	4.60	4.61	0.022
A4	8.18	8.17	8.17	0.009	7.05	7.05	7.06	0.014	5.98	5.98	5.98	0.002	4.55	4.56	4.57	0.026
C5	7.46	7.44	7.41	0.047	5.24	5.24	5.24	0.001	5.29	5.28	5.28	0.009	4.04	4.04	4.06	0.021
U6	7.67	7.65	7.64	0.029	5.33	5.32	5.32	0.010	5.79	5.78	5.76	0.029	4.30	4.29	4.29	0.011
G7	7.93	7.91	7.89	0.037	NA	NA	NA	NA	5.87	5.85	5.83	0.042	4.74	4.71	4.68	**0.063**
A8	8.28	8.29	8.29	0.018	7.54	7.59	7.65	**0.109**	5.98	5.97	5.97	0.005	4.68	4.68	4.69	0.012
C9	7.48	7.47	7.47	0.006	5.12	5.14	5.16	0.043	5.49	5.49	5.51	0.022	4.38	4.38	4.38	0.003
G10	7.47	7.48	7.51	0.031	NA	NA	NA	NA	5.69	5.69	5.70	0.010	4.49	4.49	4.51	0.018
A11	7.86	7.87	7.90	0.033	7.87	7.87	7.89	0.020	5.97	5.97	5.98	0.013	4.13	4.14	4.17	0.039
C33	8.08	8.08	8.07	0.012	6.00	6.00	5.99	0.011	5.65	5.65	5.66	0.012	4.68	4.67	4.67	0.008
G34	7.68	7.67	7.67	0.009	NA	NA	NA	NA	5.76	5.76	5.76	0.001	4.58	4.58	4.59	0.015
U35	7.57	7.56	7.56	0.015	5.20	5.21	5.235	0.031	5.51	5.51	5.53	0.021	4.50	4.49	4.48	0.022
A36	8.20	8.20	8.23	0.030	6.97	7.01	7.06	**0.088**	5.88	5.89	5.90	0.023	4.71	4.73	4.75	0.046
G37	7.21	7.18	7.17	0.042	NA	NA	NA	NA	5.38	5.35	5.35	0.022	4.28	4.29	4.32	0.047
U38	7.69	7.68	7.66	0.028	5.08	5.07	5.07	NA	5.47	5.48	5.49	0.018	4.44	4.45	4.46	0.025
A39	8.06	8.07	8.08	0.021	6.80	6.80	6.80	0.001	5.99	5.99	5.99	0.002	4.59	4.61	4.63	0.040
G40	7.29	7.29	7.30	0.016	NA	NA	NA	NA	5.58	5.59	5.59	0.012	4.37	4.38	4.39	0.019
C41	7.35	7.35	7.37	0.020	5.10	5.10	5.10	0.002	5.42	5.42	5.44	0.017	4.35	4.36	4.36	0.011
A42	8.03	8.03	8.04	0.011	7.46	7.47	7.47	0.010	6.01	6.01	6.01	0.008	4.06	4.06	4.07	0.010

(b) H1/H3 protons at 0 °C.

			H1/H3	
Residue	**U+NaMg**	**U+Na**	**U**	**diff.**
G1	12.72	12.72	12.71	0.010
C2	NA	NA	NA	NA
U3	13.34	13.33	13.29	**0.050**
A4	NA	NA	NA	NA
C5	NA	NA	NA	NA
U6	13.28	13.29	13.33	**0.050**
G7	-	-	10.49	NA
A8	NA	NA	NA	NA
C9	NA	NA	NA	NA
G10	12.37	12.37	12.35	0.020
A11	NA	NA	NA	NA
C33	NA	NA	NA	NA
G34	12.93	12.93	12.9	0.030
U35H3	13.4	13.36	13.38	0.020
A36	NA	NA	NA	NA
G37	13.44	13.41	13.38	**0.060**
U38	13.56	13.56	13.53	0.030
A39	NA	NA	NA	NA
G40	13.5	13.48	13.44	**0.060**
C41	NA	NA	NA	NA
A42	NA	NA	NA	NA

Fig. S1. 2D NOESY spectrum of pseudouridine-containing duplex in 95/5% H_2O/D_2O at 0 °C with 200 ms mixing time. Cross-peaks from imino protons to intra-strand and cross-strand H1' protons are labeled. The G7 imino proton only exhibits cross-peaks to water and a very broad cross-peak near 6.1 ppm, which probably results from the G7 amino protons. The Watson-Crick pair peak assignments (blue box) are shown in Fig. 1.

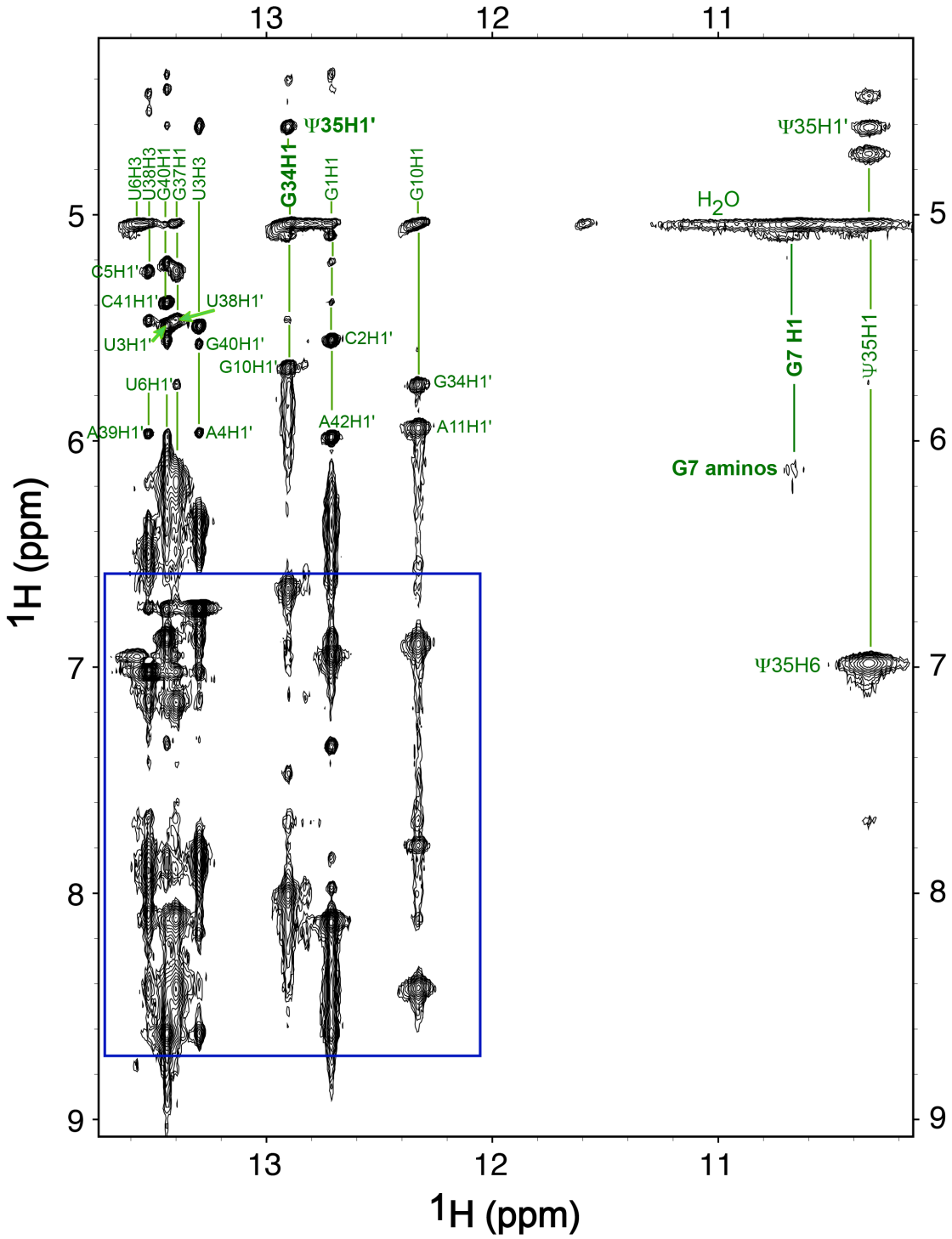

Fig. S2. Imino proton region of 1D slices taken from 2D NOESY spectra at the chemical shift of water protons at 0 °C in phosphate buffer. (a) pseudouridine-containing and (b) unmodified duplexes. Relative peak intensities reflect fraction of indicated imino protons that exchanged with water protons during NOESY mix time: (a) 50 msec, (b) 200 msec. Hydrogen-bonded protons that are not solvent-exposed have the lowest peak intensity.

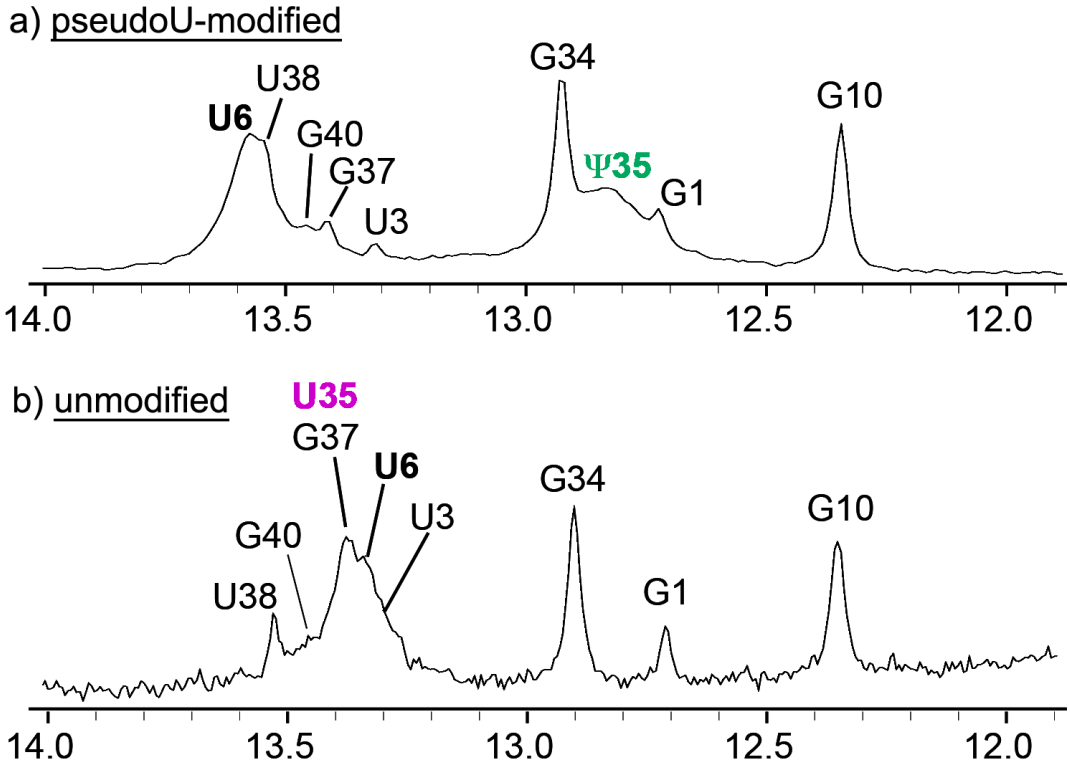

Fig. S3. 1D NMR spectra of the imino region of pseudouridine-modified (left) and unmodified (right) U2 snRNA – BPS duplexes at 0 °C in the indicated buffer conditions.

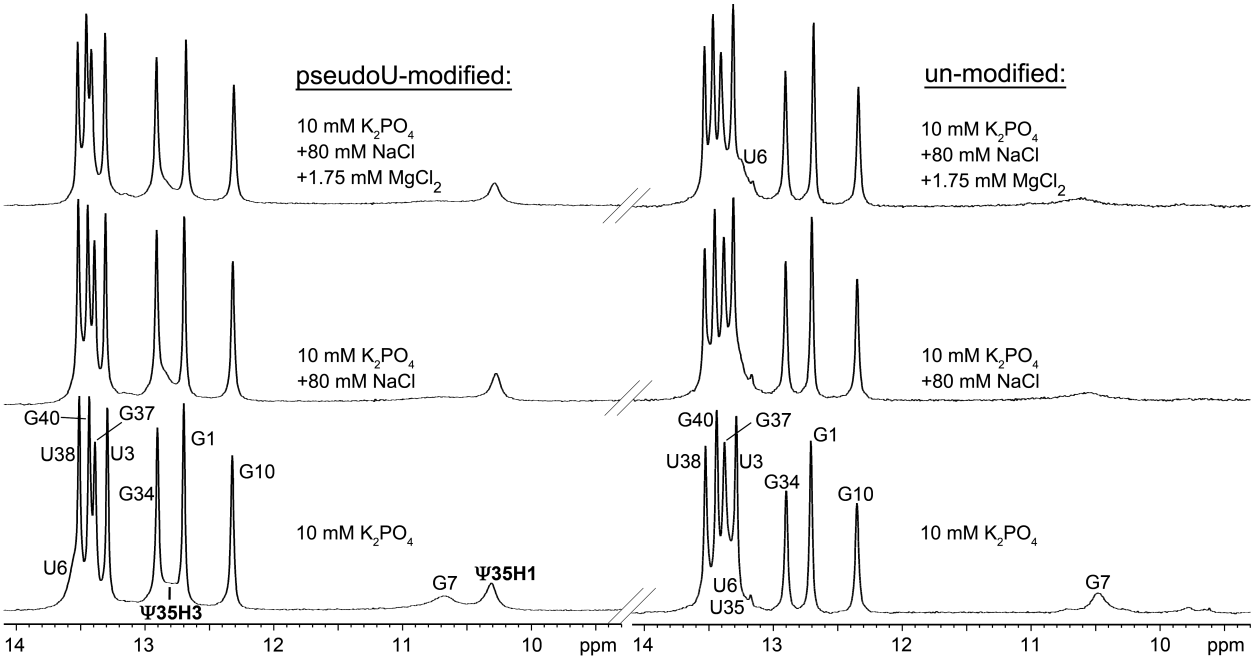

Fig. S4. 2D NMR spectra of the imino-aromatic/amino region of pseudouridine-modified U2 snRNA – BPS duplex at 0 °C in either (a) 10 mM K_2PO_4 or (b) 10 mM K_2PO_4 pH 6.8, 80 mM NaCl, and $MgCl_2$ added in a 1:1 molar ratio with the RNA strands. Contours in the boxed region (green) are drawn five-fold lower than in the rest of the spectrum to show Ψ35-A8.

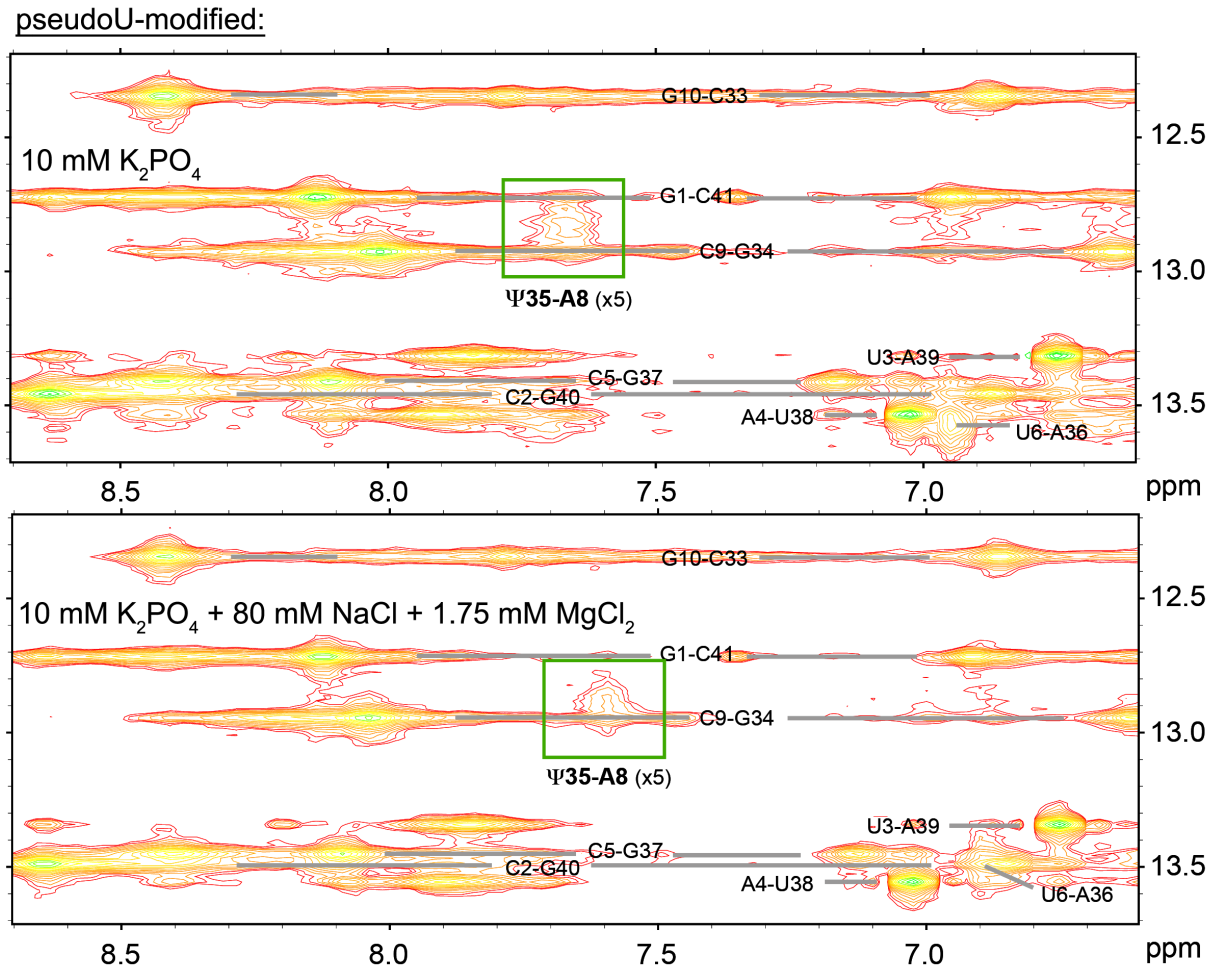

Fig. S5. Effect of NaCl and $MgCl_2$ on representative NMR spectra of the pseudouridine-modified and unmodified RNA duplexes. (a) NOESY cross-peak volumes from the branch site region of the pseudouridine-modified (green) and unmodified (purple) U2 snRNA/BPS-containing duplexes in either phosphate buffer (dark) or higher ionic strength buffer with NaCl and $MgCl_2$ added (light). The average and standard deviation of the H1′ – H6/H8 and H2′– (n+1)H8/6 NOE volumes of the base paired stem regions are given for comparison. (b)-(c) Proton shift correlations in low salt *versus* higher ionic strength buffer of branch site nucleotides U6-C9 (filled, red) and other nucleotides 1-5, 10-11, 33, 37-42 (hollow) for (b) pseudouridine-modified or (c) unmodified RNA duplexes. Shifts of H6/H8/H2. The H5/H1′, and H2′/H3′/H4′ protons are expressed relative to 7.5, 5.5, and 4.5 ppm, respectively. See Table S3 for assigned chemical shifts. Both samples were in 95% H_2O/5% D_2O.