Supporting Information

PDGFR-β Promoter Forms a Vacancy G-Quadruplex that Can be Filled-in by dGMP: Solution Structure and Molecular Recognition of Guanine Metabolites and Drugs

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Table S1. Chemical structures of tested guanine metabolites, drugs, and derivatives.

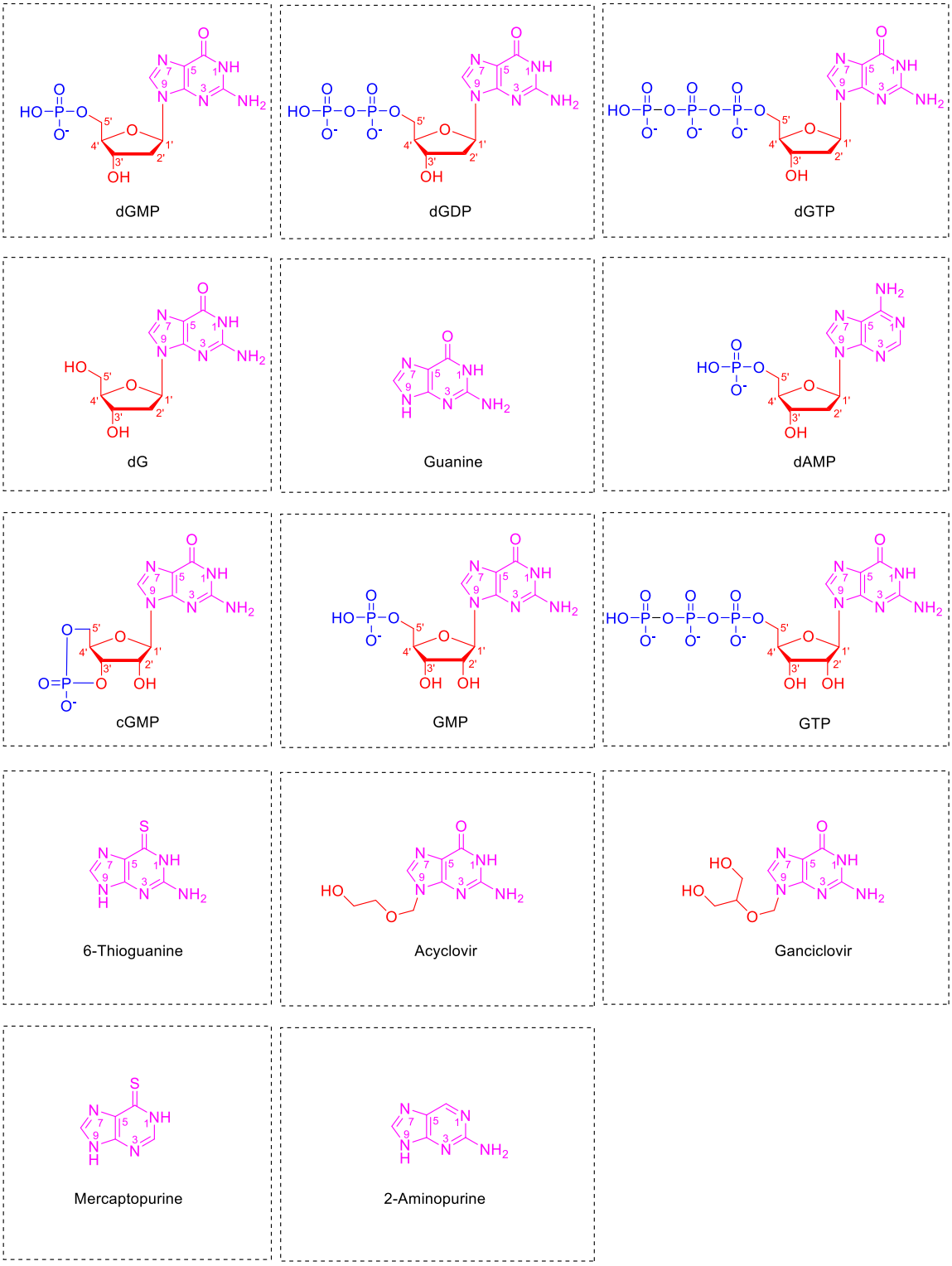

Table S2. The DNA sequences used in this study.

Sequence Name	**DNA Sequence** (5′ to 3′)
Pu19	AAGGGGGGGCGGCGGGGCA
Pu19m1	AAGGGAGGGCGGCGGGGCA
Pu19m2	AAGGGAGGGCGGCGGGACA
Pu19m4	TTGGGAGGGCGGCGGGACA
Pu18m2	AGGGAGGGCGGCGGGACA
Pu18m3	TGGGAGGGCGGCGGGACA
Pu20m1	AAGGGAGGGCGGCGGGGCAG
Pu22	AAGGGGGGGCGGCGGGGCAGGG
Pu22m1	AAGGGAGGGCGGCGGGGCAGGG
Pu23m1	AAGGGAGGGCGGCGGGGCAGGGA
Pu24m1	AAGGGAGGGCGGCGGGGCAGGGAG
Pu25m1	AAGGGAGGGCGGCGGGGCAGGGAGG

Table S3. Proton chemical shifts of 1:1 dGMP-Pu19m2 complex at 15 °C in pH 7, 50 mM K^+-containing solution.

Base	H1/H2/H5	H6/H8	H1′	H2′, H2″	H3′	H4′	H5′, H5″*
dGMP	11.90	8.10	6.25	2.97, 2.88	4.72	4.27	3.92, 3.92
A1	7.82	8.03	6.14	2.36, 2.42	4.66	4.02	3.51, 3.51
A2	7.80	7.91	5.93	2.19, 2.55	4.81	4.05	3.46, 3.82
G3	11.89	8.11	6.14	2.86, 3.09	4.97	4.47	3.98, 4.12
G4	11.31	7.68	6.13	2.62, 2.89	5.02	4.55	4.29, 4.33
G5	11.15	7.67	6.40	2.70, 2.59	5.13	4.64	4.30, 4.40
A6	8.29	8.52	6.67	2.92, 2.91	5.22	4.69	4.30, 4.38
G7	11.27	7.97	6.06	2.37, 2.89	5.16	4.50	4.24, 4.38
G8	11.50	7.97	6.14	2.69, 2.83	5.09	4.58	4.26, 4.40
G9	11.38	7.78	6.43	2.62, 2.56	5.03	4.63	4.29, 4.31
C10	6.17	8.00	6.47	2.43, 2.77	5.00	4.57	4.30, 4.38
G11	11.24	7.65	6.27	2.95, 2.98	4.90	4.58	4.36, 4.45
G12	11.18	7.92	6.48	2.68, 2.62	5.10	4.68	4.27, 4.37
C13	6.18	8.00	6.49	2.40, 2.74	5.10	4.63	4.29, 4.38
G14	11.24	7.98	6.04	2.31, 2.82	5.16	4.50	4.29, 4.29
G15	11.44	7.96	5.93	2.66, 2.64	5.07	4.52	4.19, 4.26
G16	10.98	7.40	6.06	2.31, 2.73	4.96	4.51	4.18, 4.26
A17	7.70	8.14	6.01	2.48, 2.49	4.85	4.34	4.18, 4.23
C18	5.47	7.26	5.65	1.51, 1.95	4.42	3.62	3.76, 3.76
A19	7.76	8.02	6.05	2.58, 2.40	4.48	3.98	3.74, 3.77

Note: *Assignments are not stereospecific

Table S4. Proton chemical shifts of free-dGMP (dGMPb) and bound-dGMP (dGMPa) at 15 °C in pH 7, 50 mM K^+-containing solution.

Proton	Free-dGMP	Bound-dGMP
H1	Not determined	11.90
H8	8.10	8.10
H1′	6.24	6.25
H2′	2.75	2.97
H2″	2.47	2.88
H3′	4.67	4.72
H4′	4.18	4.27
H5′, H5″	3.92, 3.92	3.92, 3.92

Table S5. Intermolecular NOEs between the Pu19m2 DNA and bound-dGMP (dGMPa).

	G11		G7	G8
dGMPa	**H1**	**H8**	**H1**	**H1**
H1	M			
H8		M	W	VW
H1′		M		
H2′		M		
H2″		M		
H3′		VW		
H5′		VW		
H5″		VW		

Note: M = Medium intensity, W = weak intensity, VW = very weak intensity.

Table S6. Inter-residue NOEs of the 5′-end capping structure.

	G14	G7			G3	A2			
A1	**H1**	**H1**	**H8**	**H1′**	**H1**	**H8**	**H4′**	**H5′**	**H5″**
H2				W					
H8	M	M			W	M			
H1′					M	S	VW	VW	VW
H2′					W	M			
H2″					W	S			
H3′						M			
H4′			W		M	M			
H5′			M		W	W			
H5″			M		W	W			

	G14		G3			
A2	**H1**	**H1′**	**H1**	**H8**	**H5′**	**H5″**
H2	W	M				
H8	M		W			
H1′	M			M	VW	W
H2′	VW			VW		
H2″	VW			W		
H3′				W		
H4′				M		
H5′				VW		
H5″				W		

Note: S = Strong intensity, M = Medium intensity, W = weak intensity, VW = very weak intensity.

Table S7. Inter-residue NOEs of the 3′-end capping structure.

	C18		G5	G12	G16						A19	
A17	**H5**	**H6**	**H1**	**H1**	**H1**	**H8**	**H1′**	**H2′**	**H2″**	**H3′**	**H8**	**H2**
H2			W	M	M						W	
H8	M	M				M	M	M	M	M		
H1′		M										W
H2′	W	M										
H2″	W	M										
H3′		W										
H4′												M

	A19
C18	**H8**
H2′	W
H2″	W

	G5			G16
A19	**H1**	**H2′**	**H2″**	**H1**
H2		W	W	VW
H8	W			VW
H2′	W			
H2″	VW			

Note: S = Strong intensity, M = Medium intensity, W = weak intensity, VW = very weak intensity.

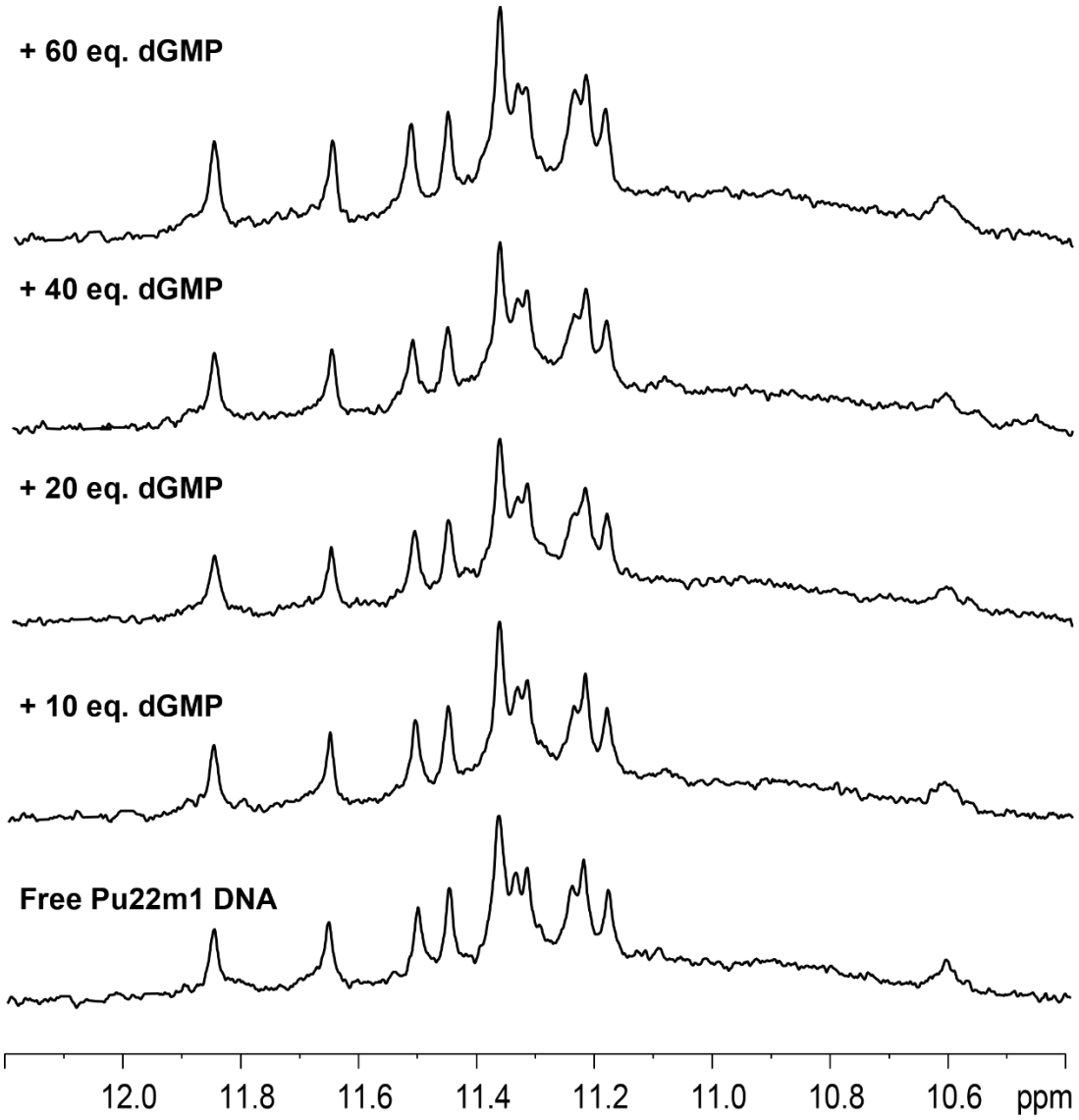

Figure S1. 1D ^{1}H NMR titration of Pu22m1 DNA with dGMP at 25 °C.

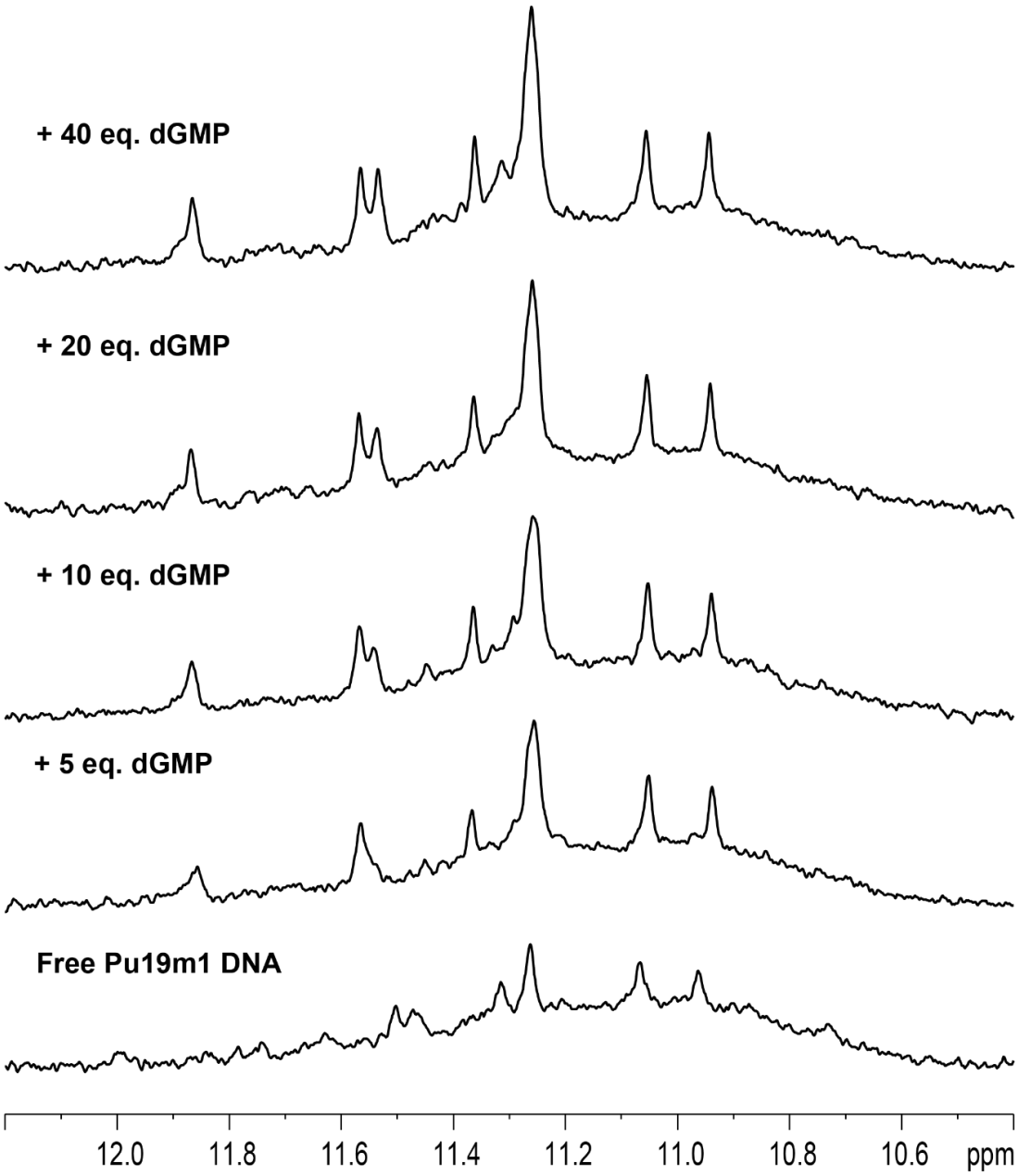

Figure S2. 1D ^{1}H NMR titration of Pu19m1 DNA with dGMP at 25 °C.

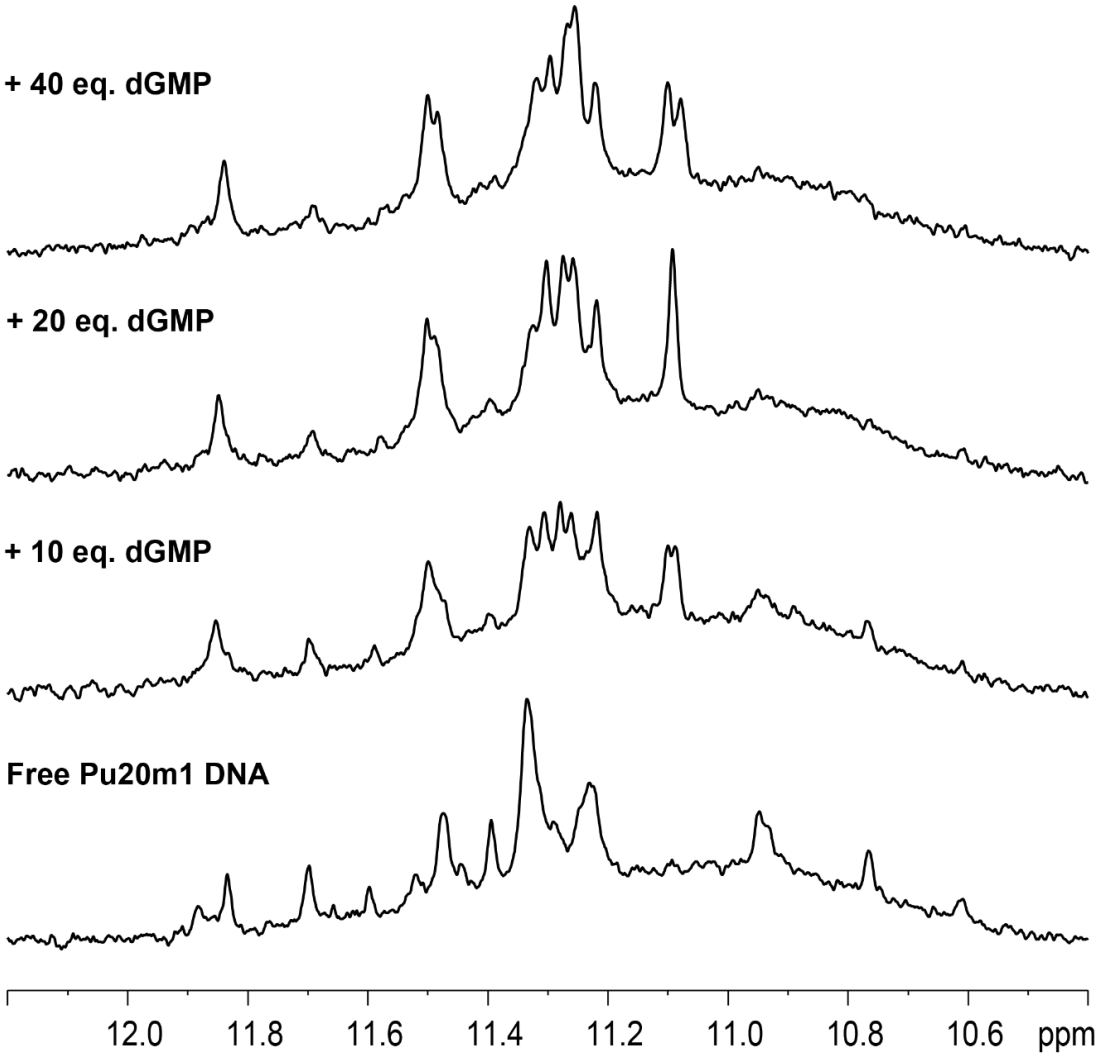

Figure S3. 1D ^{1}H NMR titration of Pu20m1 DNA with dGMP at 25 °C.

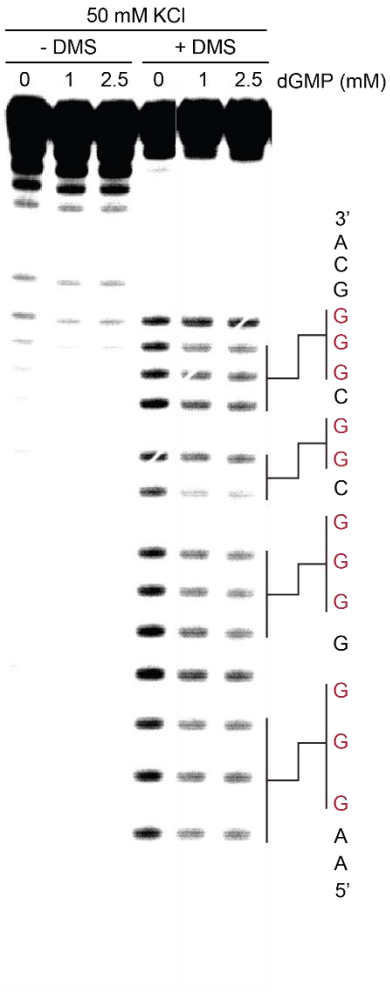

Figure S4. DMS footprinting of the wild-type Pu19 DNA in the presence of various concentration of dGMP.

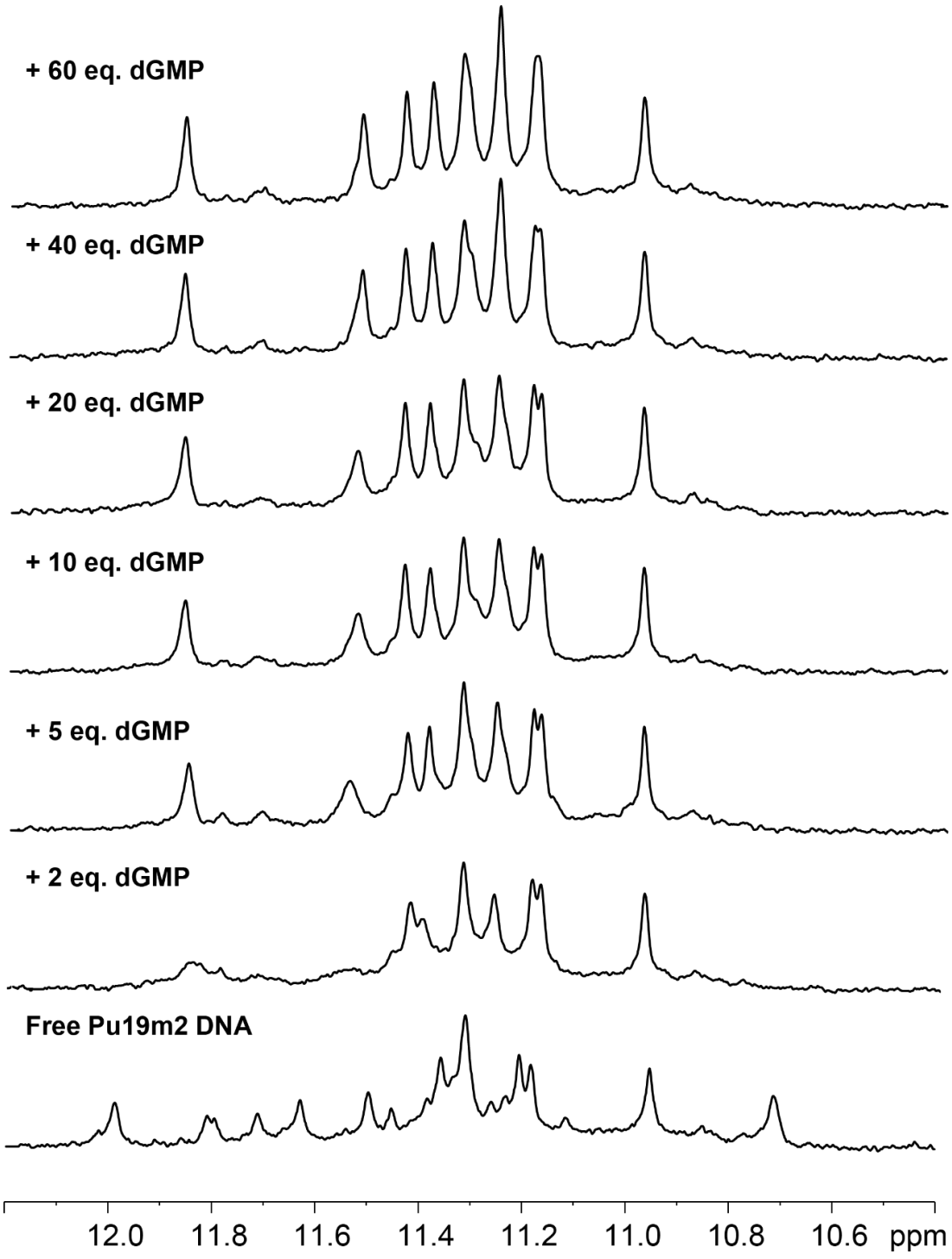

Figure S5. 1D ^{1}H NMR titration of Pu19m2 DNA with dGMP at 25 °C.

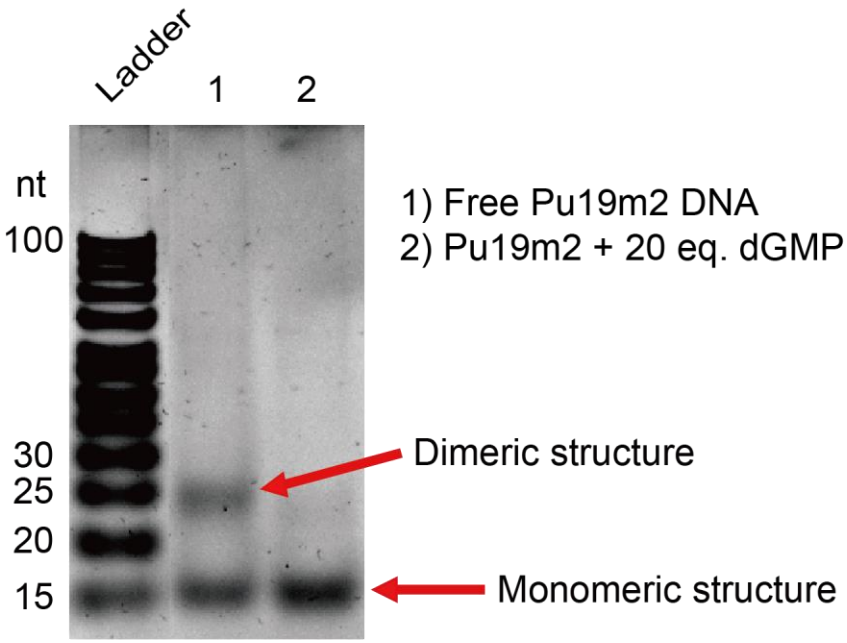

Figure S6. Native EMSA gel of free Pu19m2 DNA and dGMP-Pu19m2 complex. Each sample contained 5 μL of 50 µM DNA. DNA bands were visualized using UV light at 260 nm.

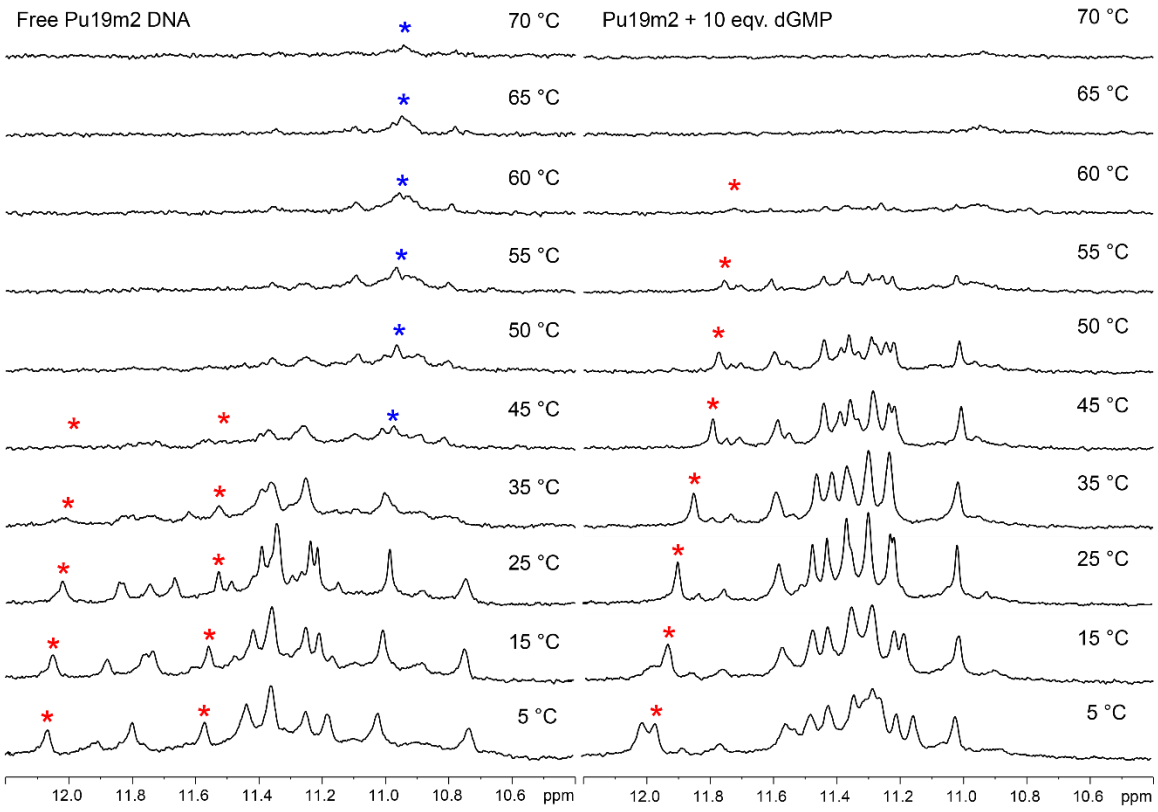

Figure S7. The imino regions of 1D ^{1}HNMR spectra of Free Pu19m2 DNA (left) and dGMP-Pu19m2 complex (right) at various temperatures. Imino protons corresponding to momemeric and dimeric structures are labeled with red and blue asterisks, respectively. Conditions: 150 μM DNA, pH 7, 50 mM K^{+} solution.

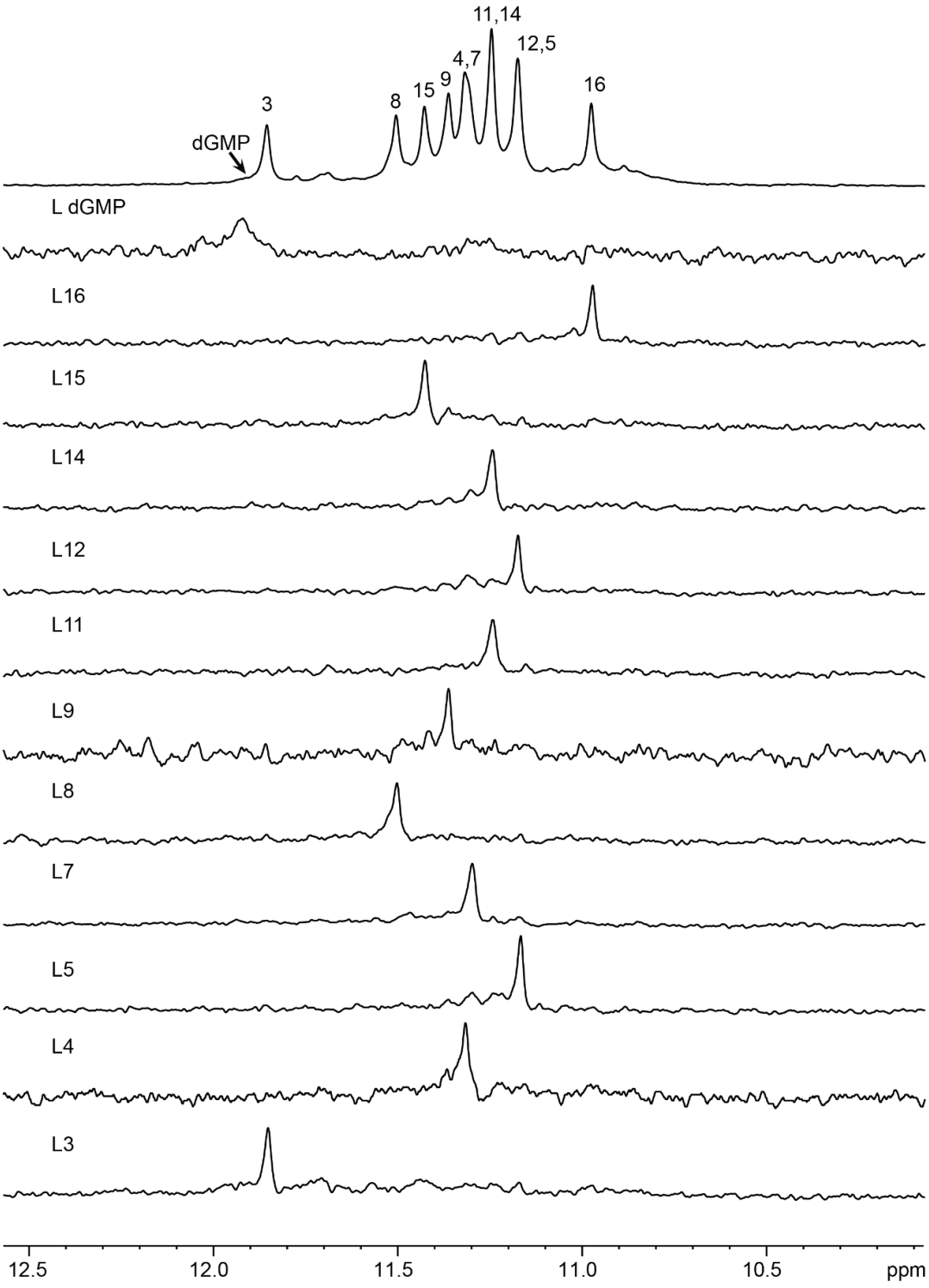

Figure S8. Imino H1 proton assignments of dGMP-Pu19m2 complex by 1D ^{15}N-edited experiments using site-specific labeled oligonucleotides. Conditions: 600 μM Pu19m2 DNA, dGMP:Pu19m2 = 20:1, pH 7, 50 mM K^+ solution, 25 °C.

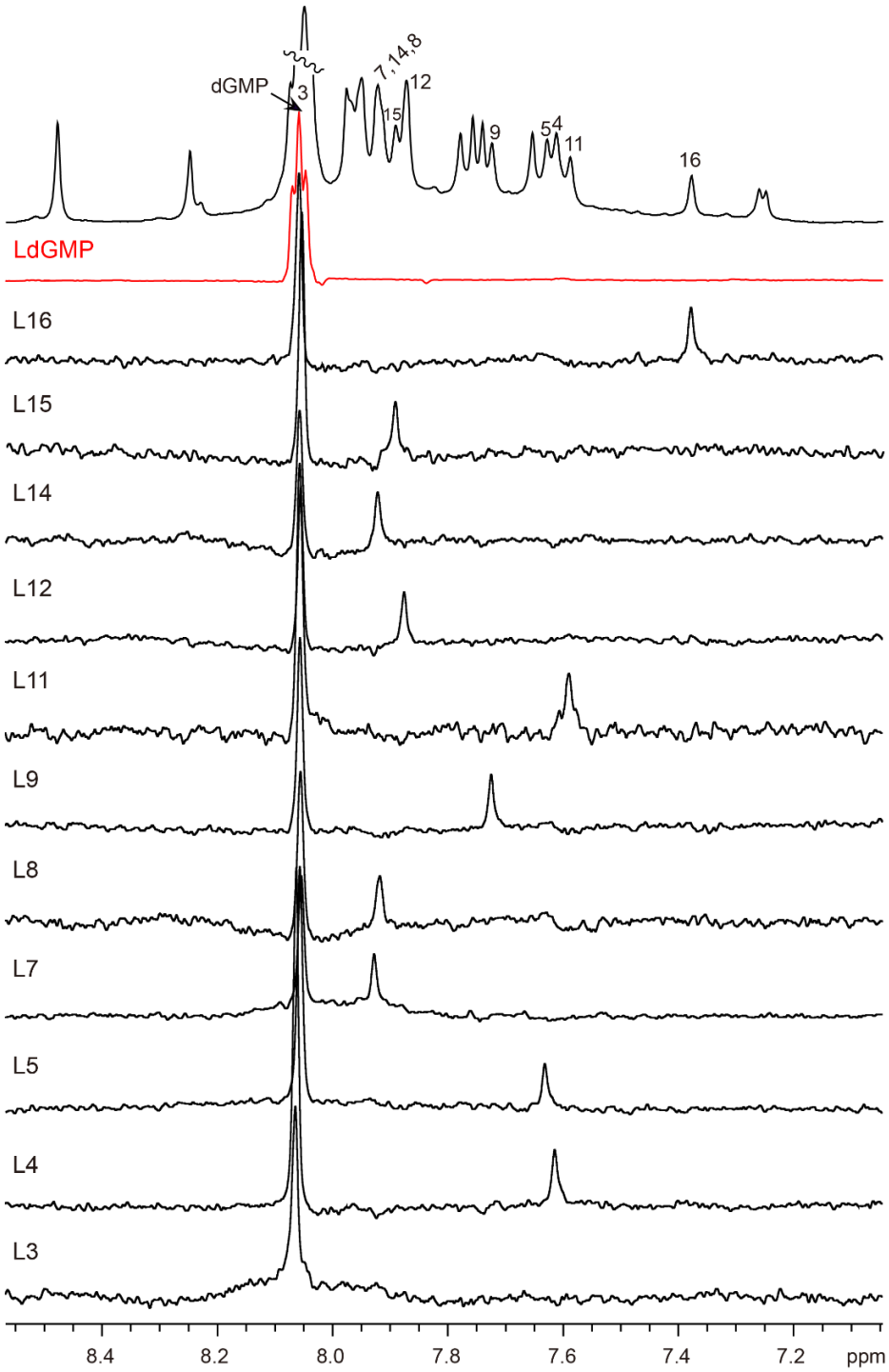

Figure S9. Aromatic H8 proton assignments of dGMP-Pu19m2 complex by 1D ^{15}N- edited (for labelled Pu19m2) or ^{13}C-edited (for labelled dGMP) experiments using site-specific labeled oligonucleotides. The peak around 8.12 ppm is the unlabeled dGMP H8. Conditions: 600 μM Pu19m2 DNA, dGMP:Pu19m2 = 20:1, pH 7, 50 mM K^+ solution, 25 °C.

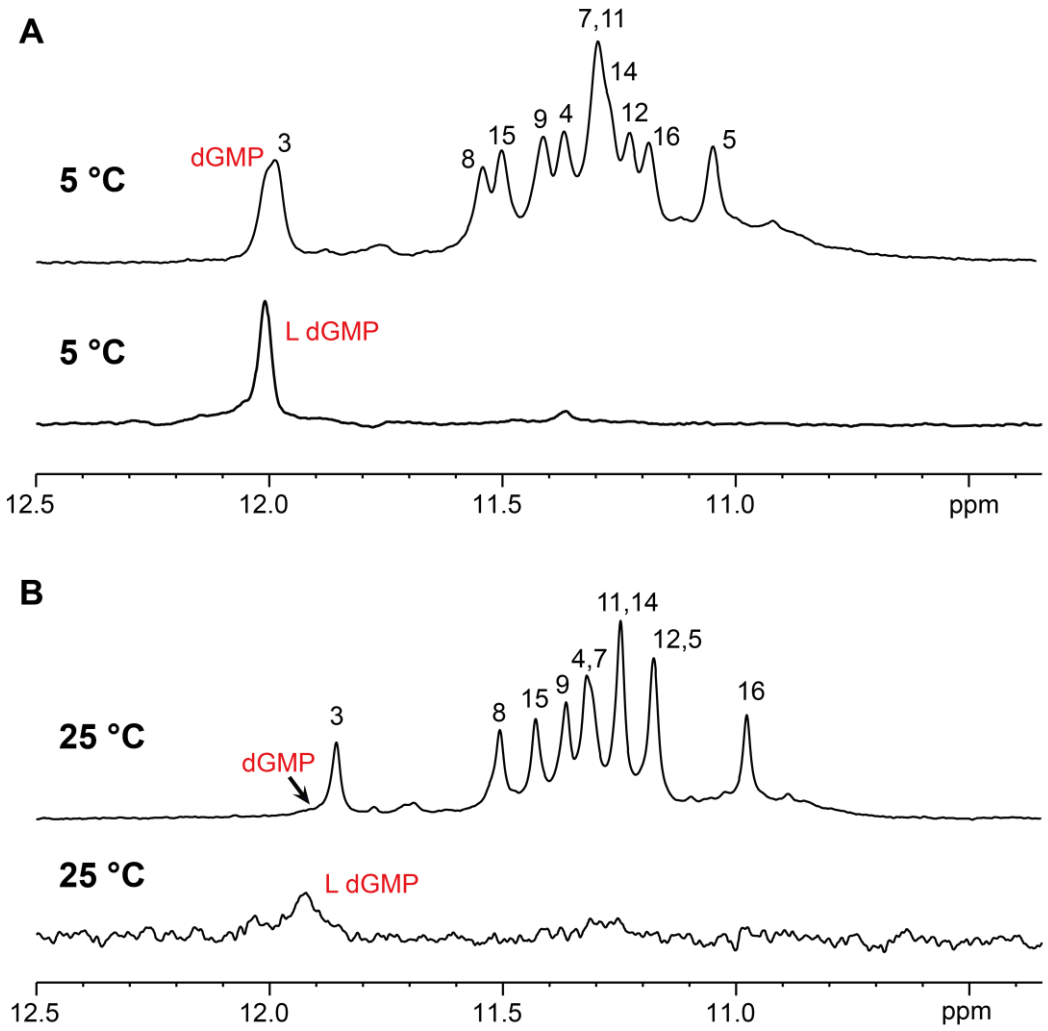

Figure S10. Imino H1 proton assignment for dGMP in dGMP-Pu19m2 complex by 1D ^{15}N-edited experiment using site-specific labeled ^{13}C-^{15}N-labeled dGMP at 5 °C (A) and 25 °C (B). Conditions: 600 μM Pu19m2 DNA, dGMP:Pu19m2 = 20:1, pH 7, 50 mM K^+ solution.

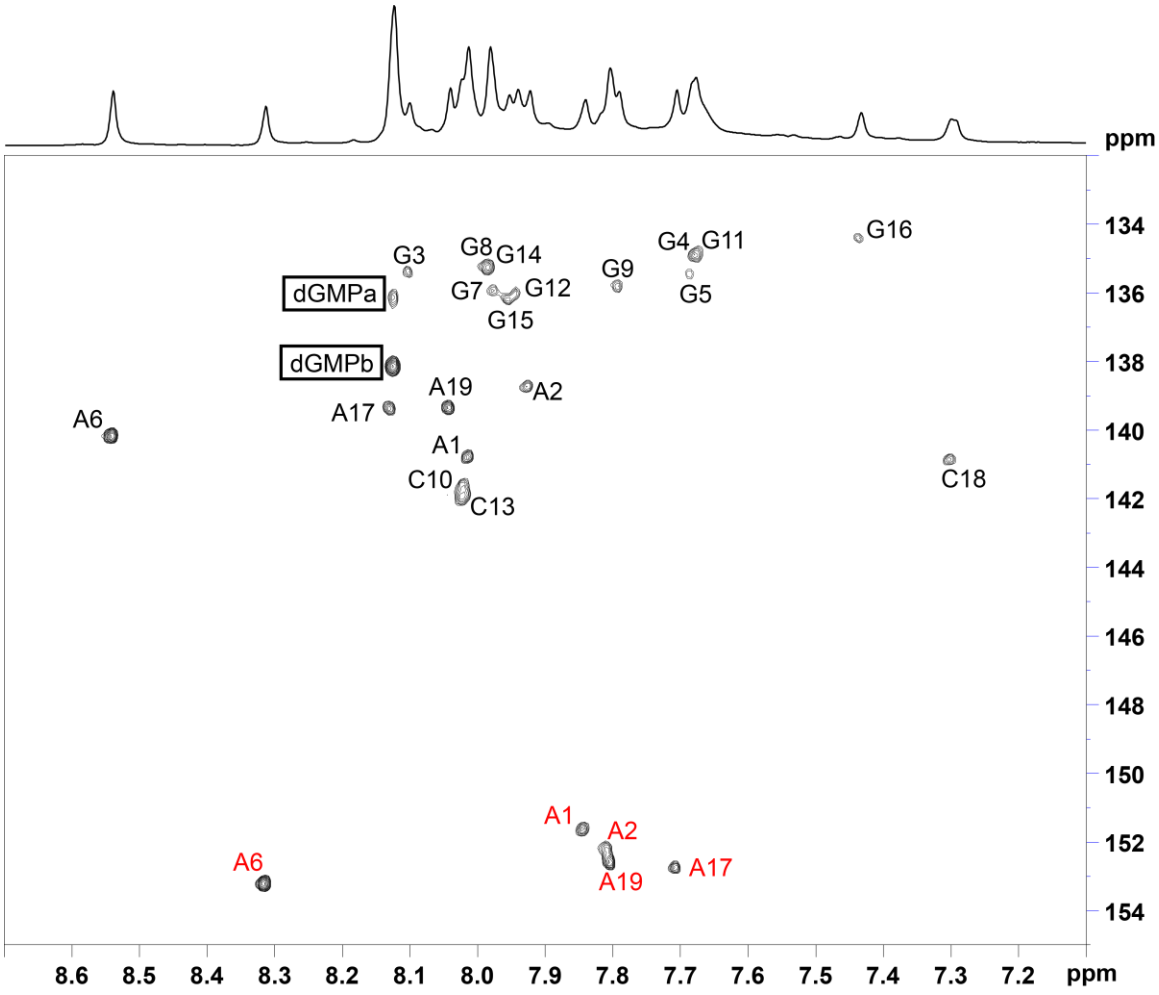

Figure S11. H6−C6/H8−C8 cross-peaks for all bases (black label) and the adenine H2-C2 contacts (red label) assignments for dGMP-Pu19m2 complex by HSQC experiments. The H8−C8 cross-peaks of free-dGMP (dGMPb) and bound-dGMP (dGMPa) are marked by black boxes. Conditions: 1.4 mM Pu19m2 DNA, dGMP:Pu19m2 ≈ 3:1, pH 7, 25 °C, 50 mM K^+ solution.

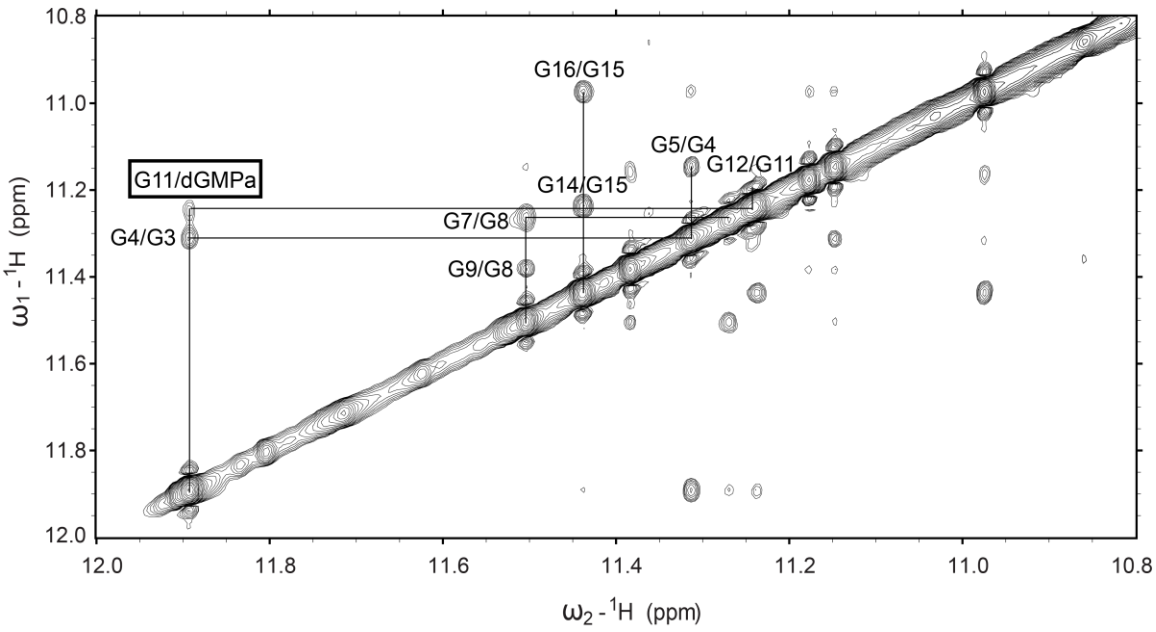

Figure S12. The H1–H1 region of the 2D-NOESY spectrum of dGMP-Pu19m2 complex in H_2O with sequential assignment pathway. The G11H1/dGMPaH1 cross-peak is marked by black box. Conditions: 2.8 mM Pu19m2 DNA, dGMP:Pu19m2 ≈ 3:1, pH 7, 15 °C, 50 mM K^+ solution, mixing time of 250 ms.

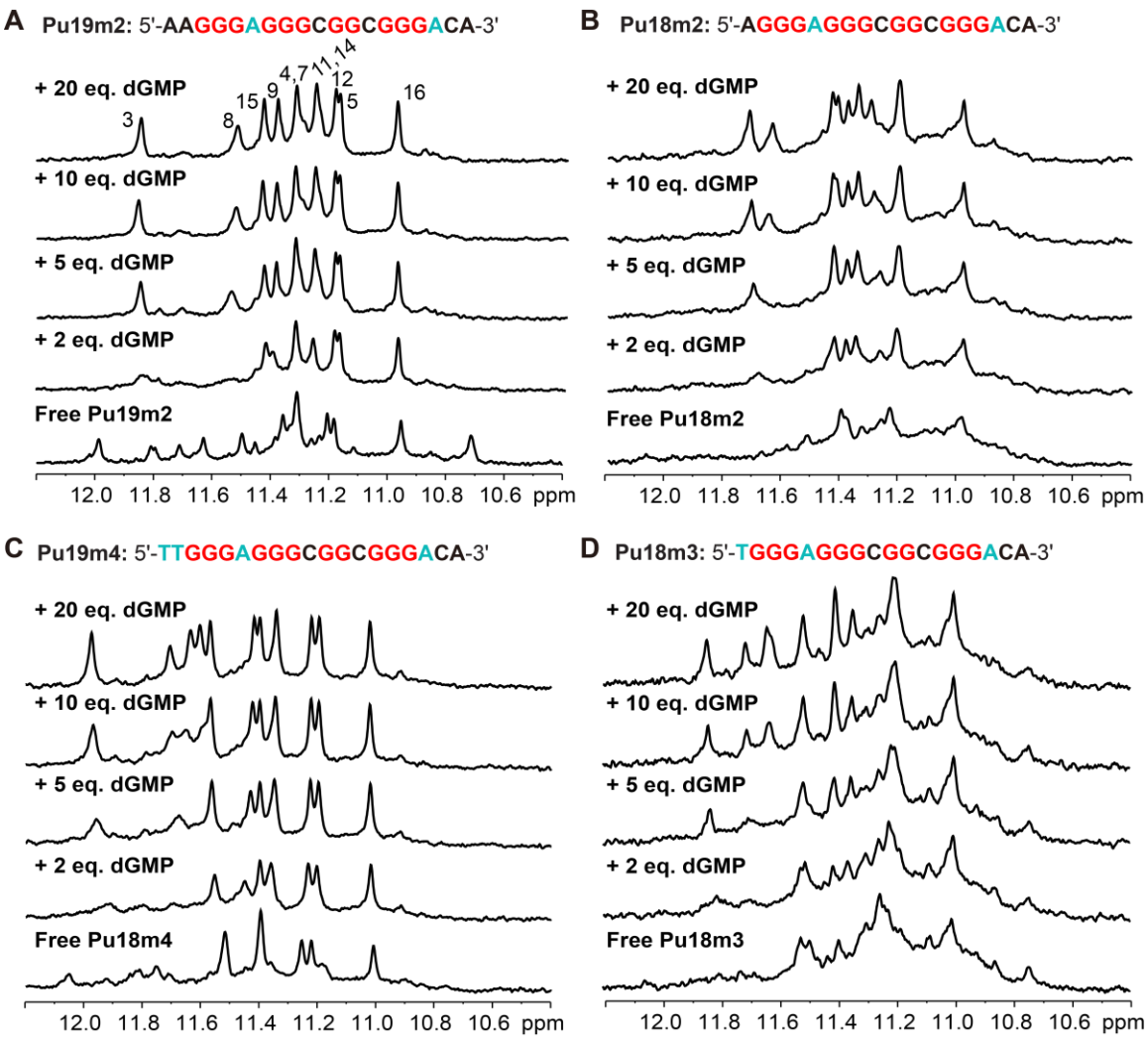

Figure S13. 1D ^{1}H NMR titration of Pu19m2, Pu18m2, Pu19m4, and Pu18m3 DNA sequences with dGMP, respectively, at 25 °C.

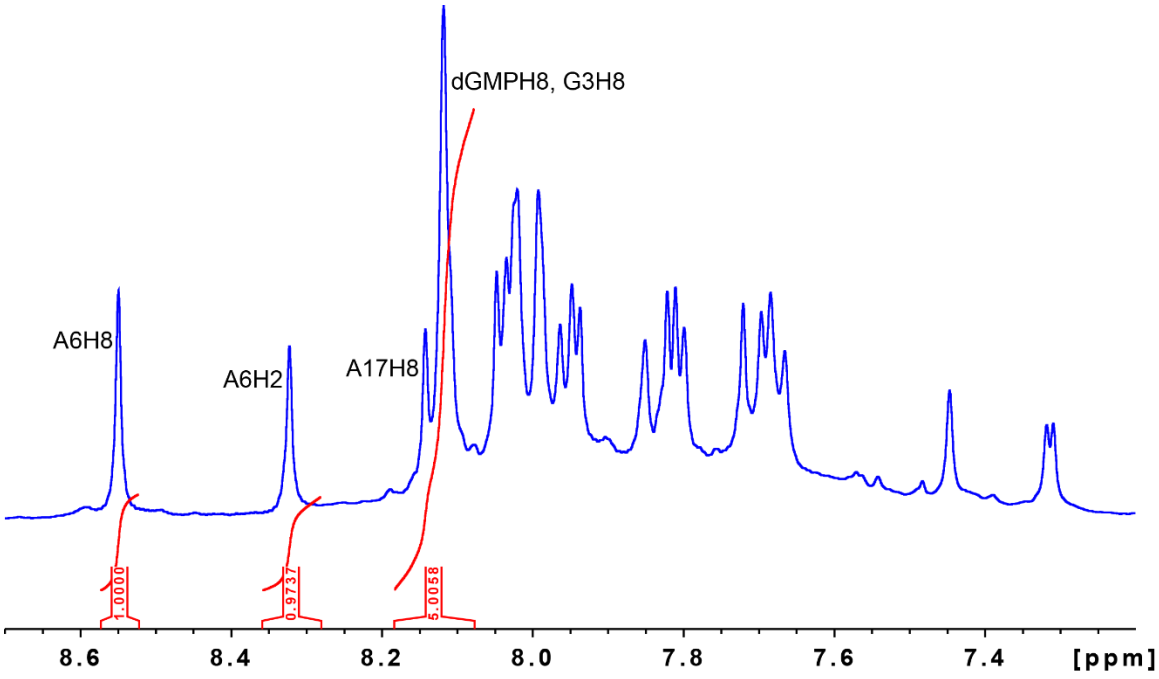

Figure S14. Aromatic region of dGMP-Pu19m2 complex by 1D NMR spectra. The integration values show the relative molar ratio of the dGMP and Pu19m2 DNA of about 3:1. Conditions: 2.8 mM Pu19m2 DNA, pH 7, 25 °C, 50 mM K^+ solution.

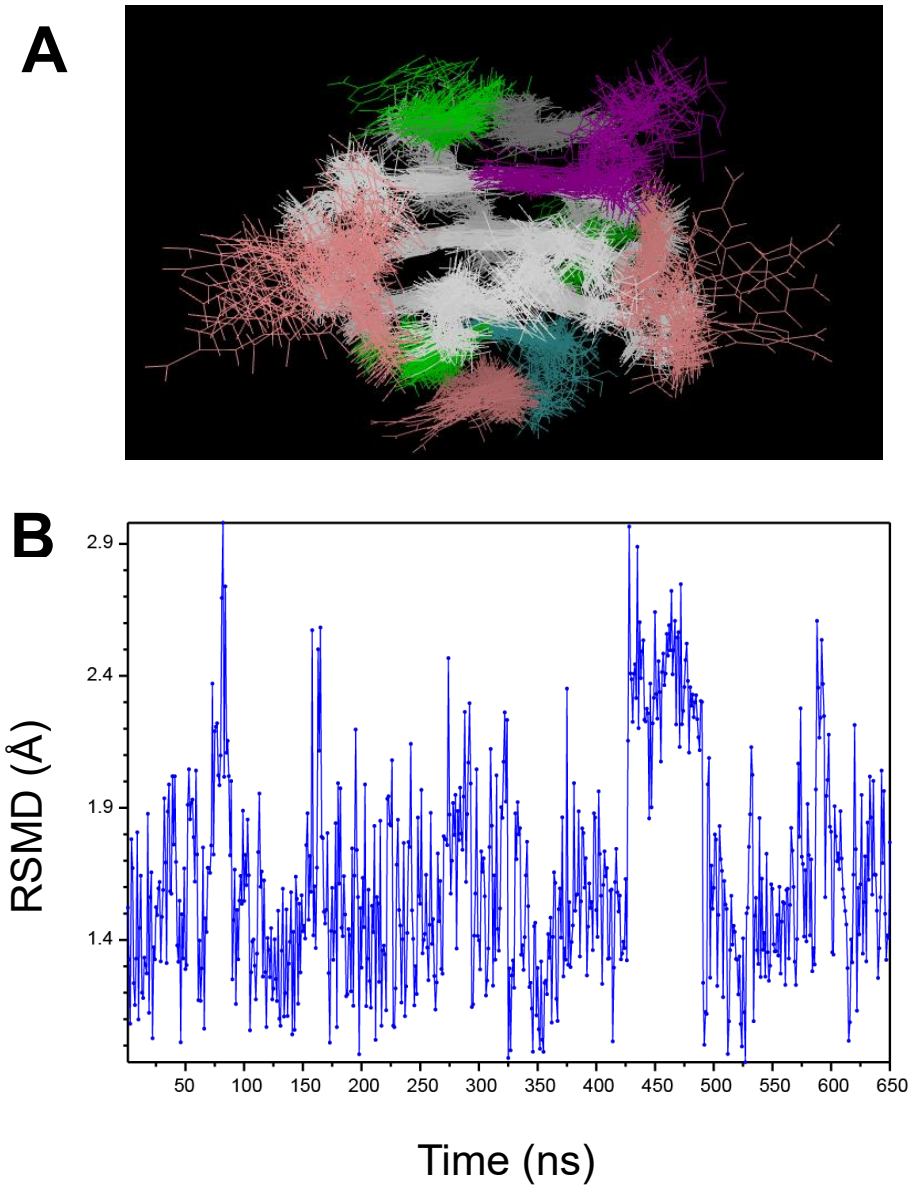

Figure S15. 650 ns long MD simulation of the dGMP-Pu19m2 complex. (A) Superposition of 32 conformations that are sampled at a time step of 20 ns. dGMPs are colored in purple. (B) Tracing of RSMD values for all atoms over 650 ns.

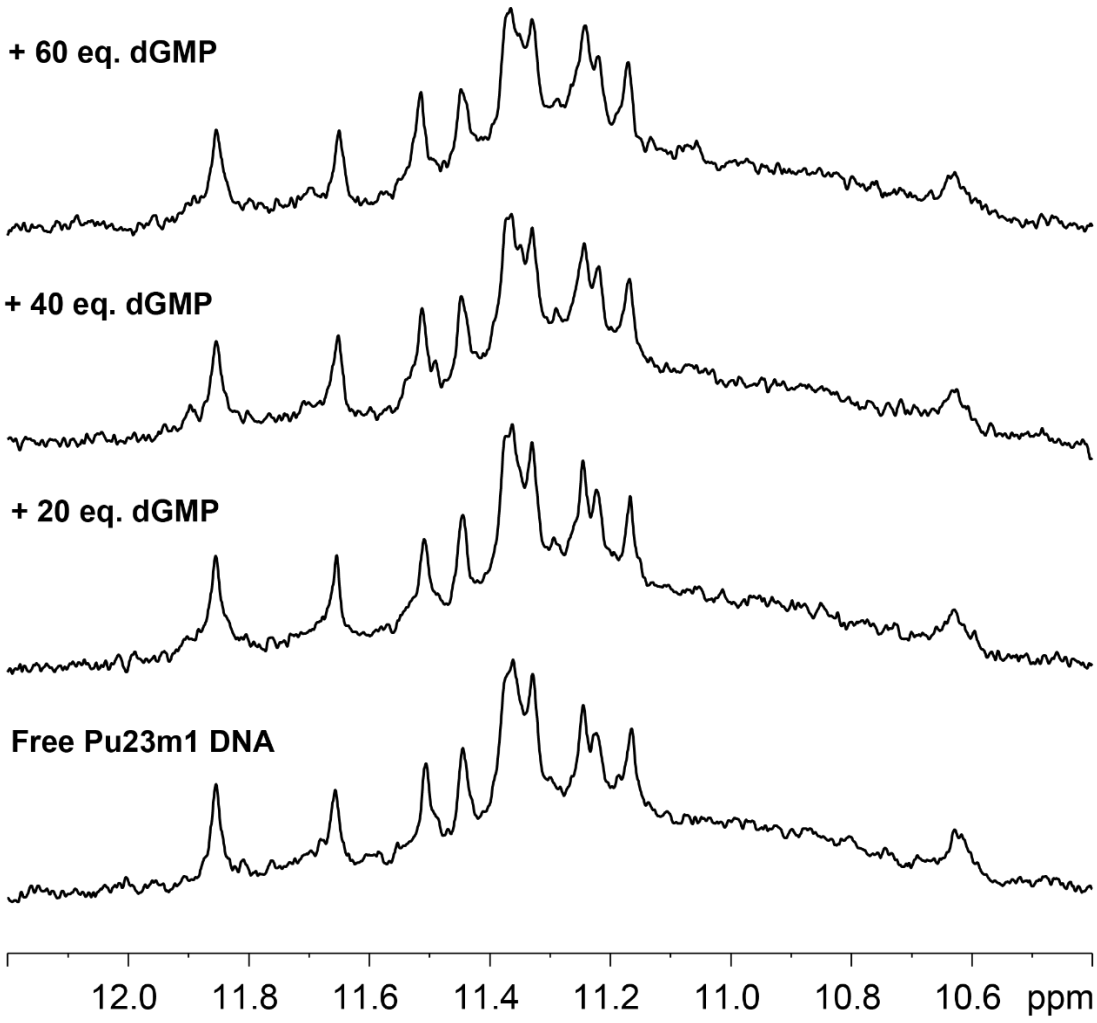

Figure S16. 1D ^{1}H NMR titration of Pu23m1 DNA with dGMP at 25 °C.

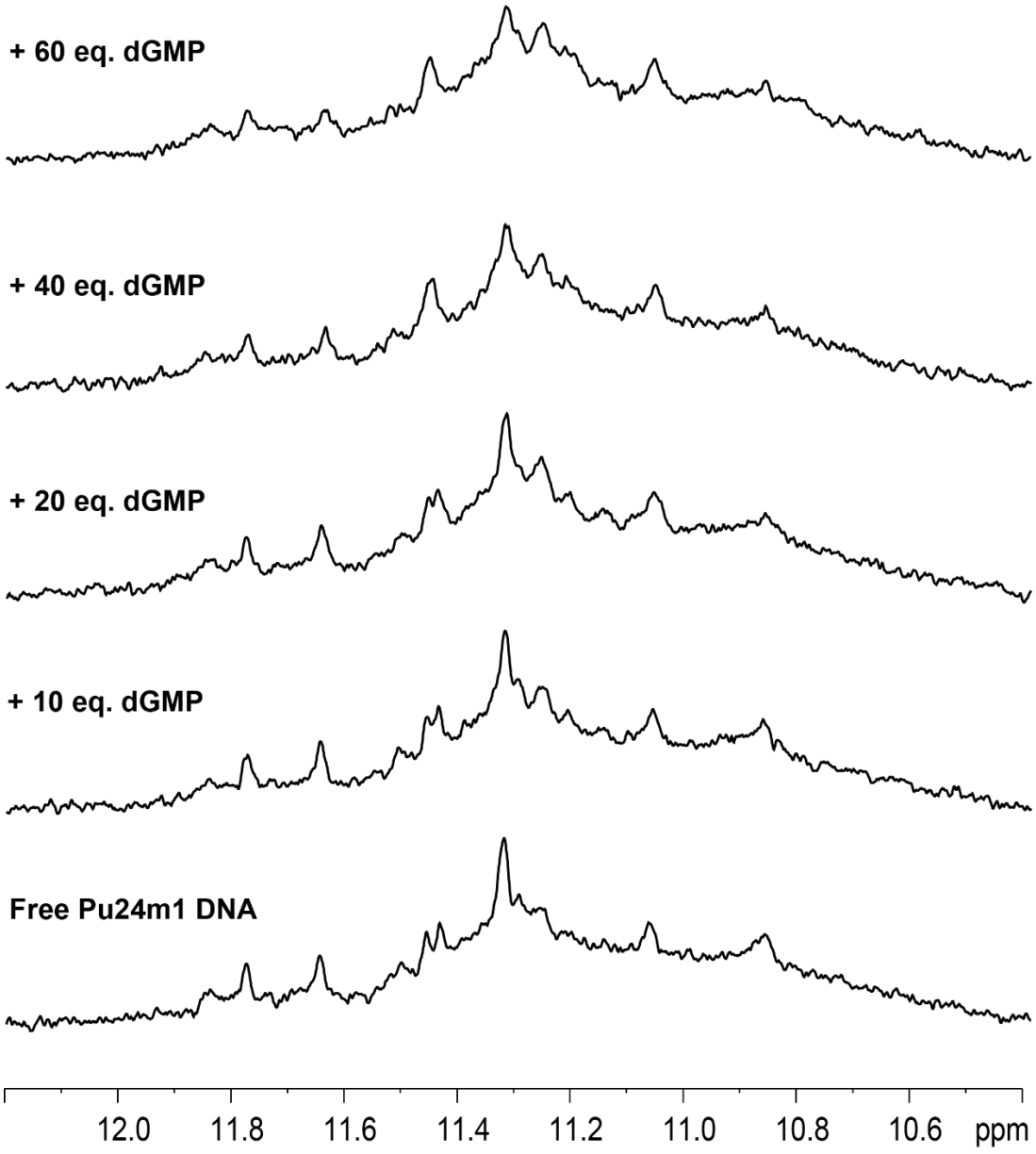

Figure S17. 1D ^{1}H NMR titration of Pu24m1 DNA with dGMP at 25 °C.

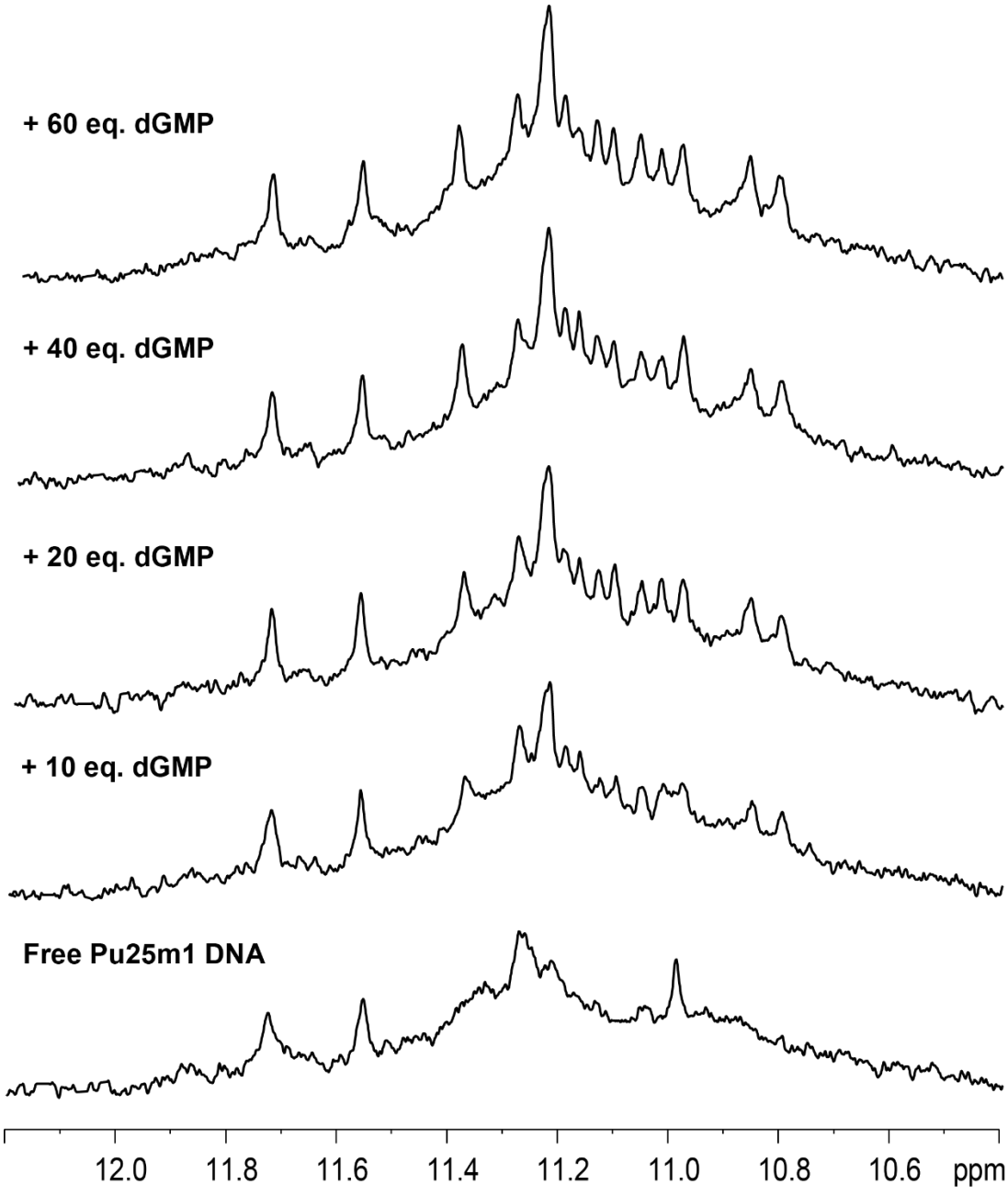

Figure S18. 1D ^{1}H NMR titration of Pu25m1 DNA with dGMP at 25 °C.

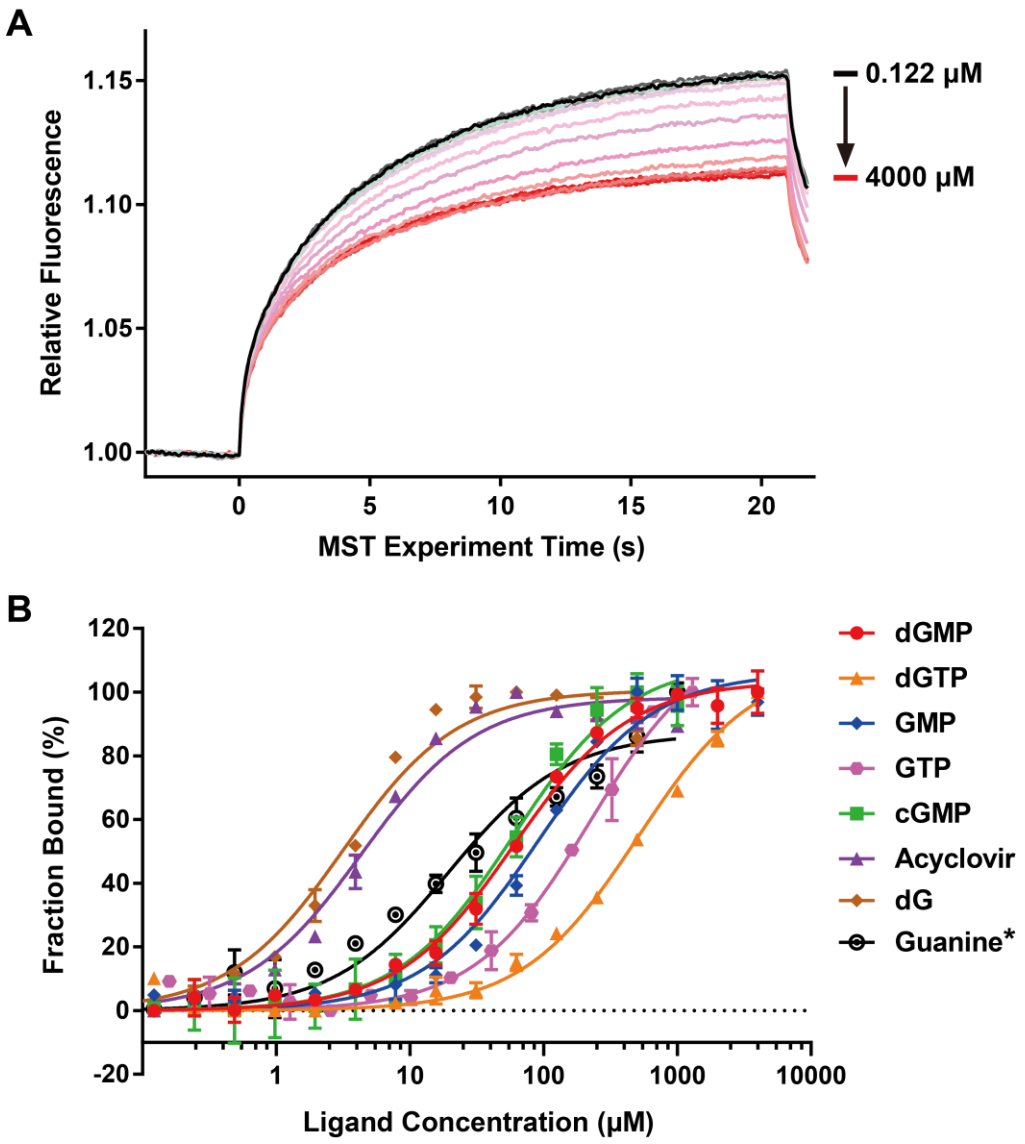

Figure S19. (A) An example of raw Microscale Thermophoresis (MST) traces of 3'-FAM-labeled Pu19m2 DNA in complex with dGMP in a temperature gradient. (B) The fraction bound activity (the normalized fluorescence difference between ligand bound-state and unbound-state from MTS experiments) is plotted against the concentration of each guanine derivatives. A curve fit yields the dissociation constant (K_d) value of each ligand as described in Material and Methods section. Experiments were run in duplicate. For some points, the error bars are shorter than the height of the symbol and are not shown.

*Guanine appears to have more than one binding events to the labeled Pu19m2 DNA.

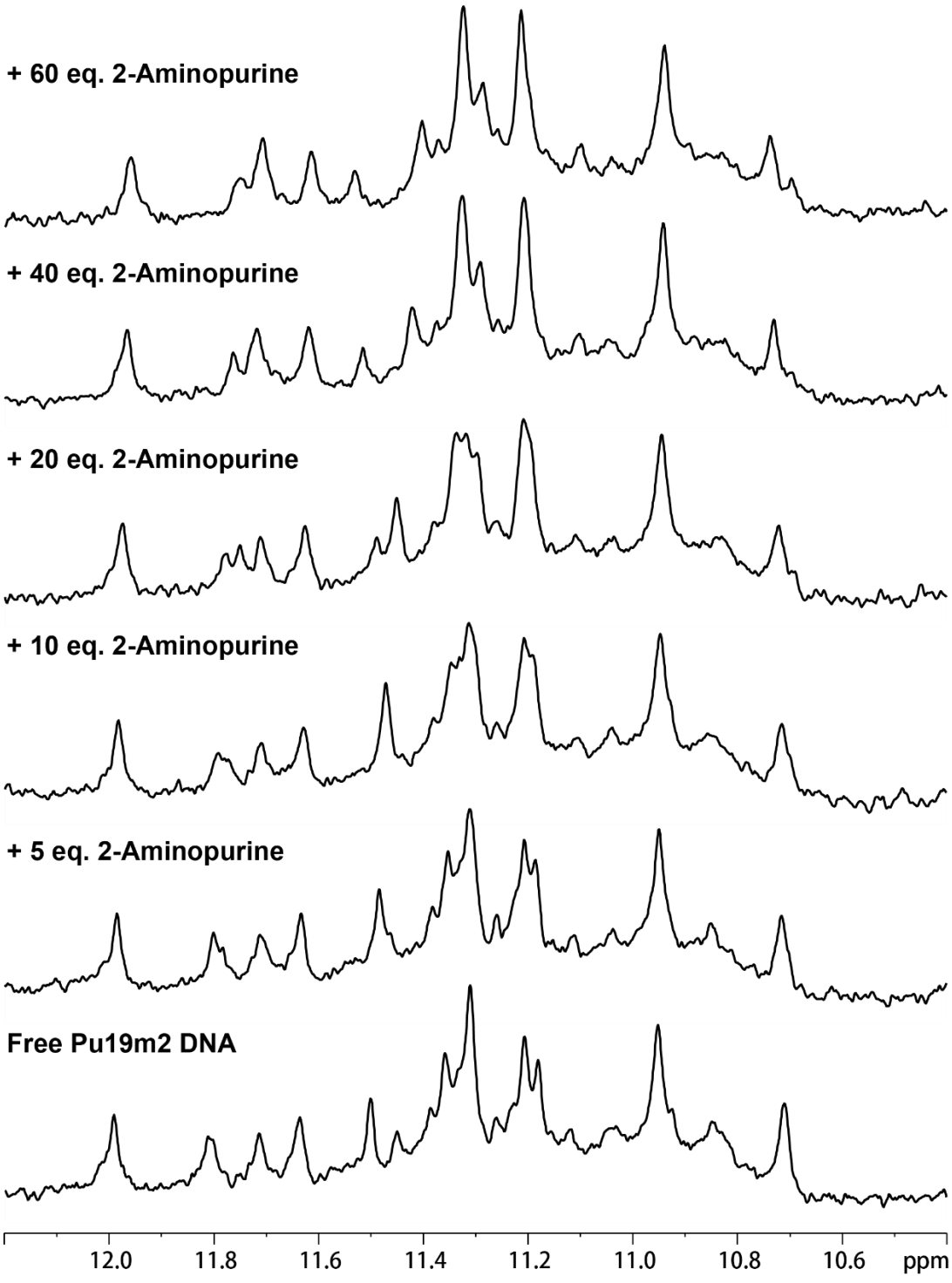

Figure S20. 1D ^{1}H NMR titration of Pu19m2 DNA with 2-aminopurine at 25 °C.

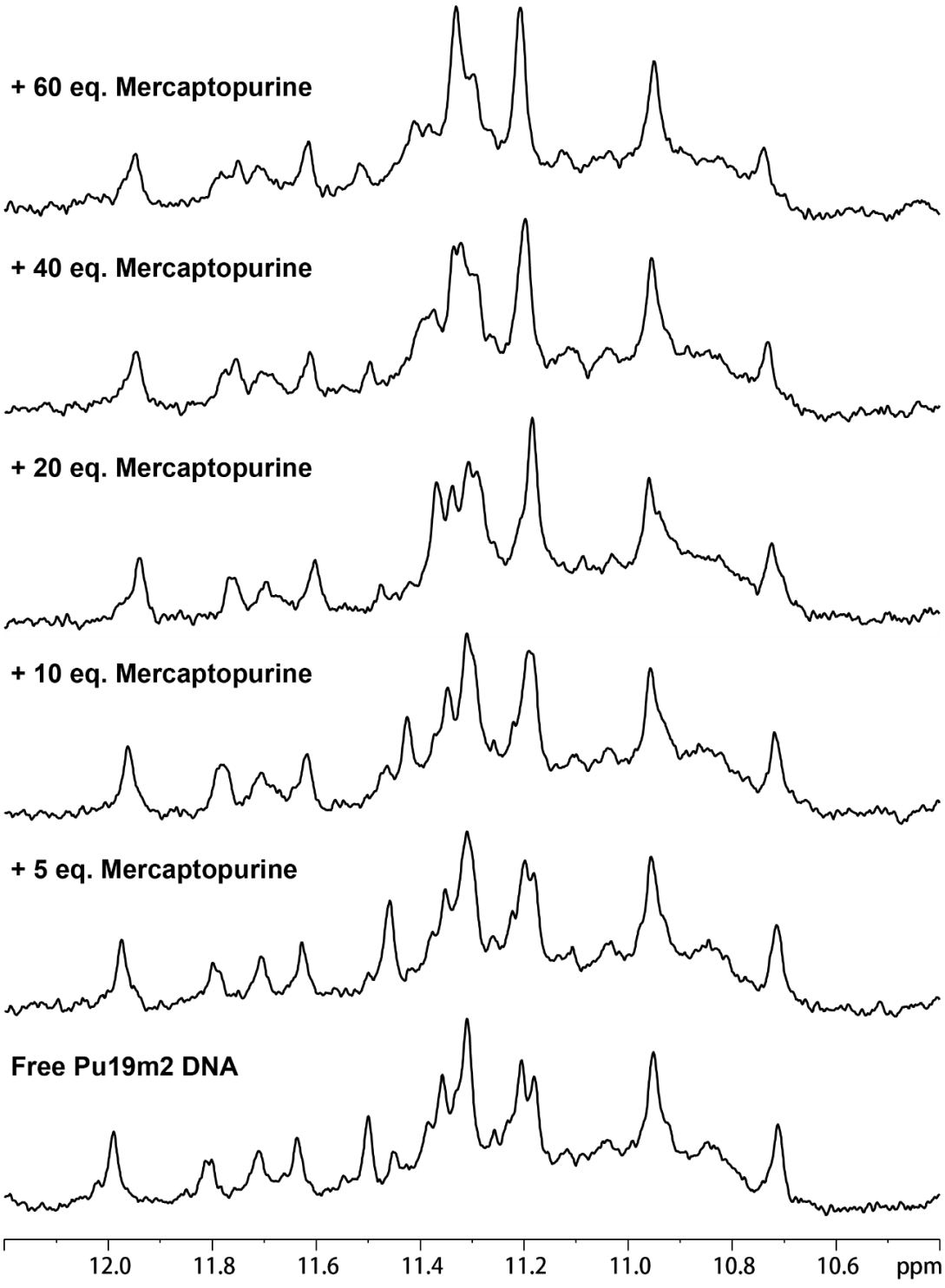

Figure S21. 1D ^{1}H NMR titration of Pu19m2 DNA with mercaptopurine at 25 °C.

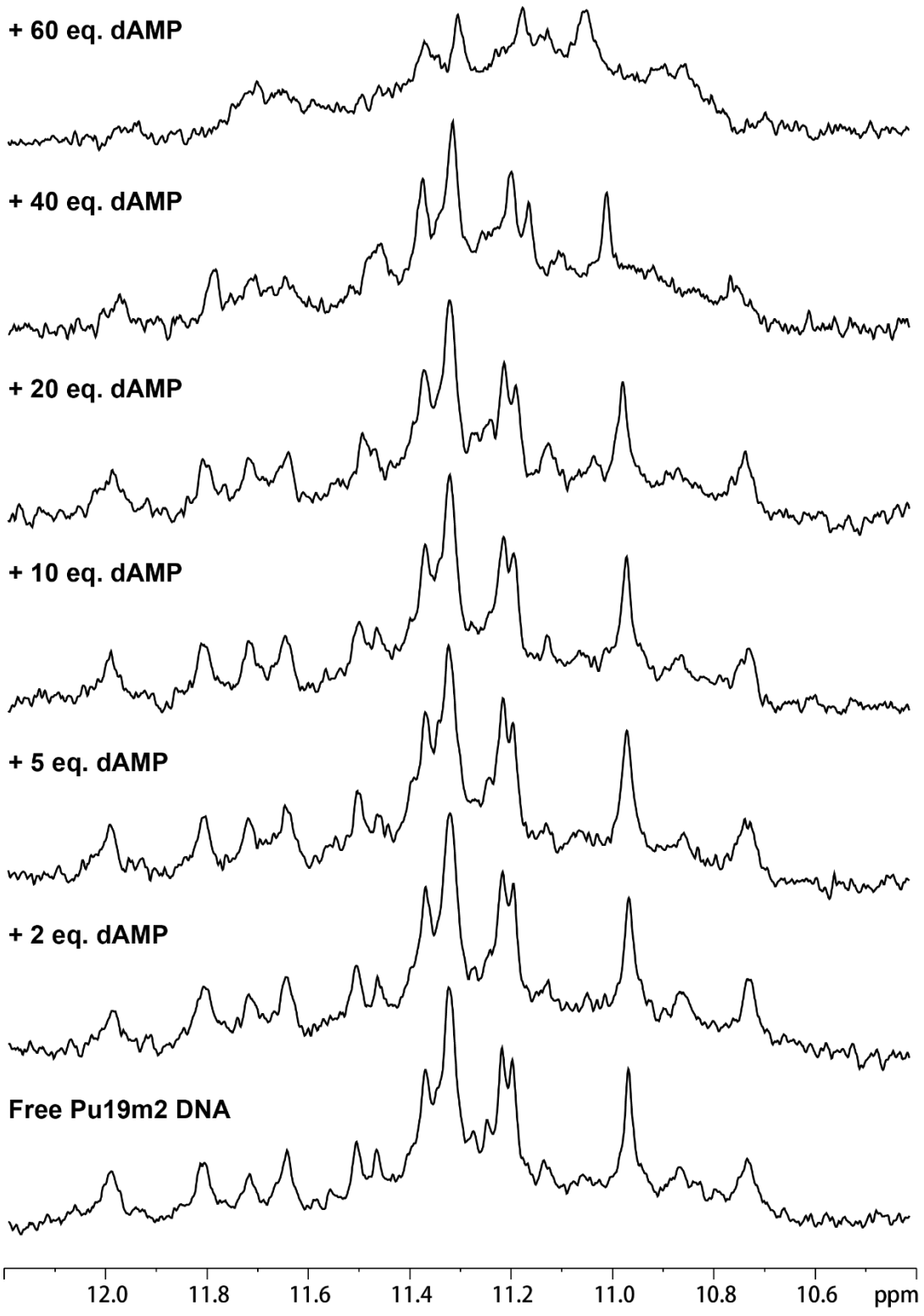

Figure S22. 1D ^{1}H NMR titration of Pu19m2 DNA with dAMP at 25 °C.

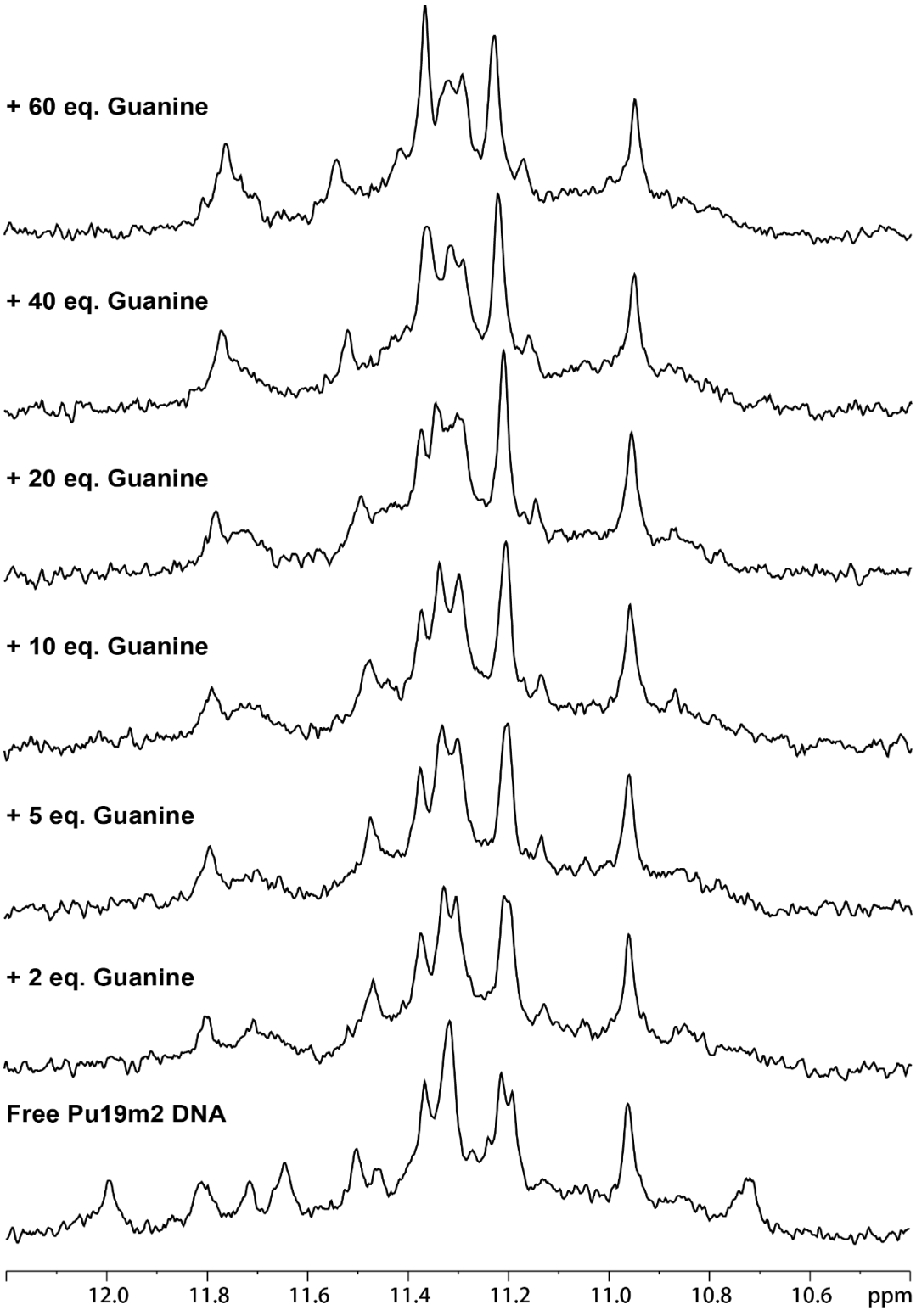

Figure S23. 1D 1H NMR titration of Pu19m2 DNA with guanine at 25 °C.

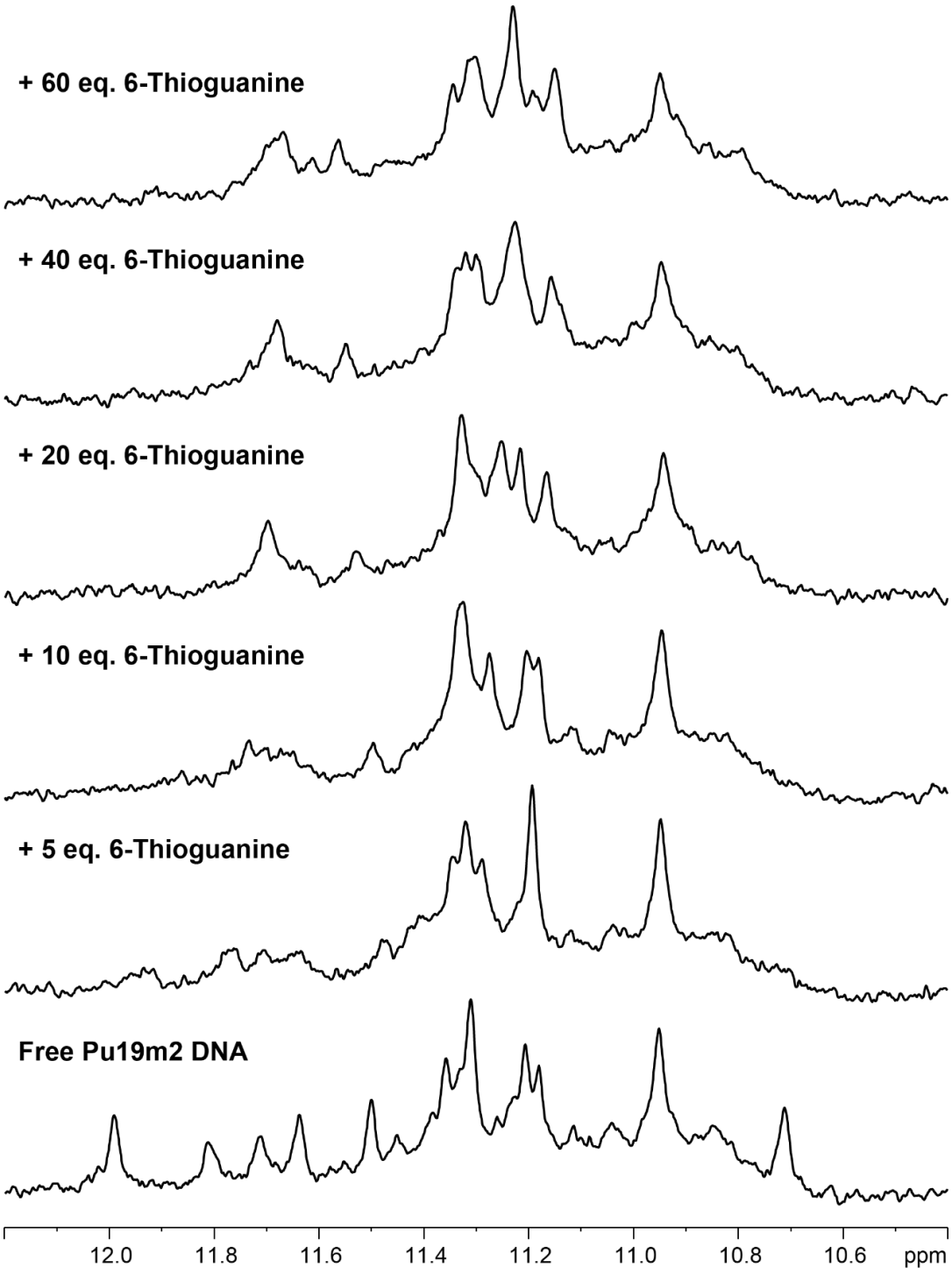

Figure S24. 1D ^{1}H NMR titration of Pu19m2 DNA with 6-thioguanine at 25 °C.

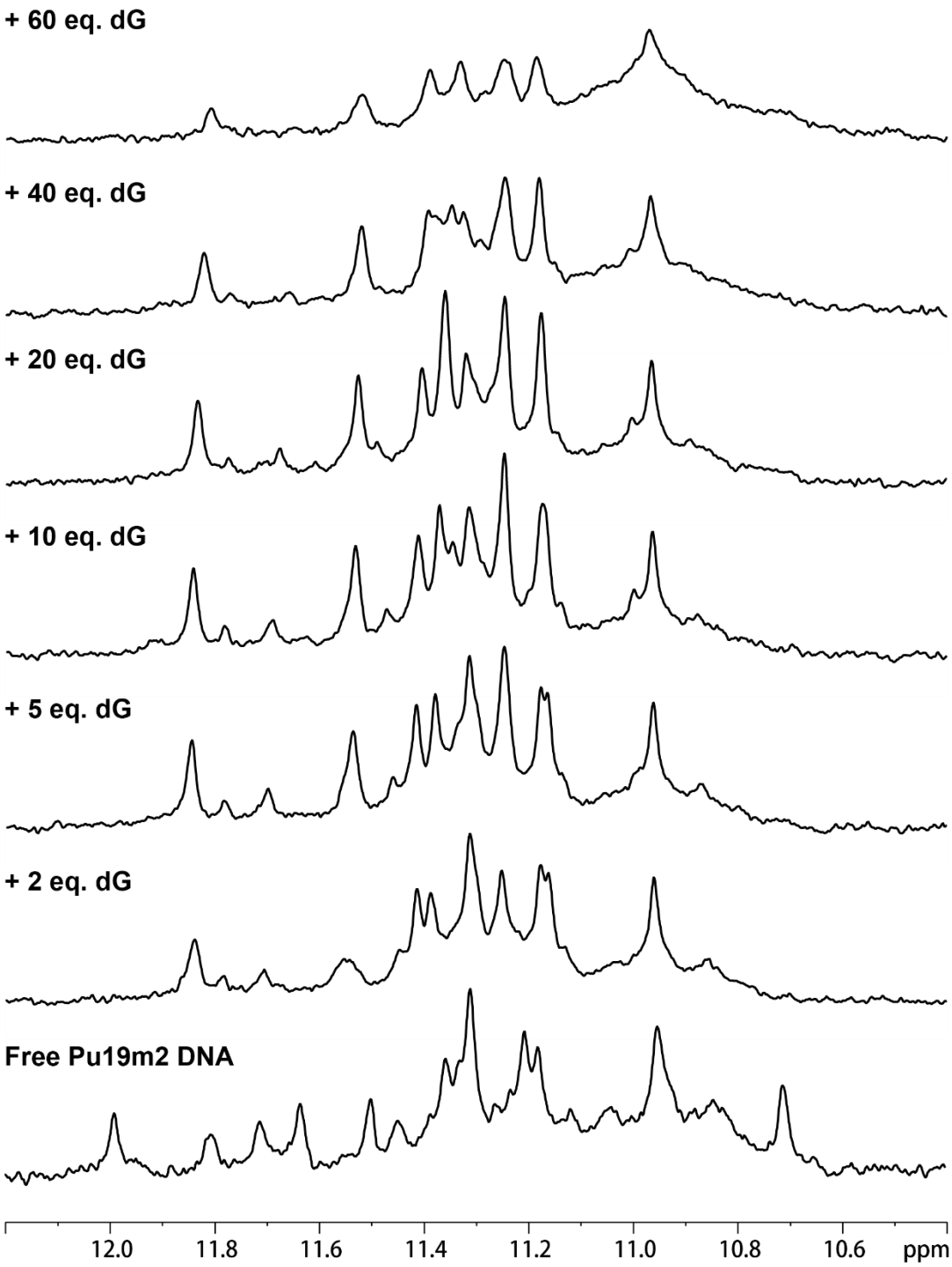

Figure S25. 1D ^{1}H NMR titration of Pu19m2 DNA with dG at 25 °C. The concentration of dG higher than 3 mM (20 eq) caused seriously aggregation as shown by reduced intensity of imino proton signals.

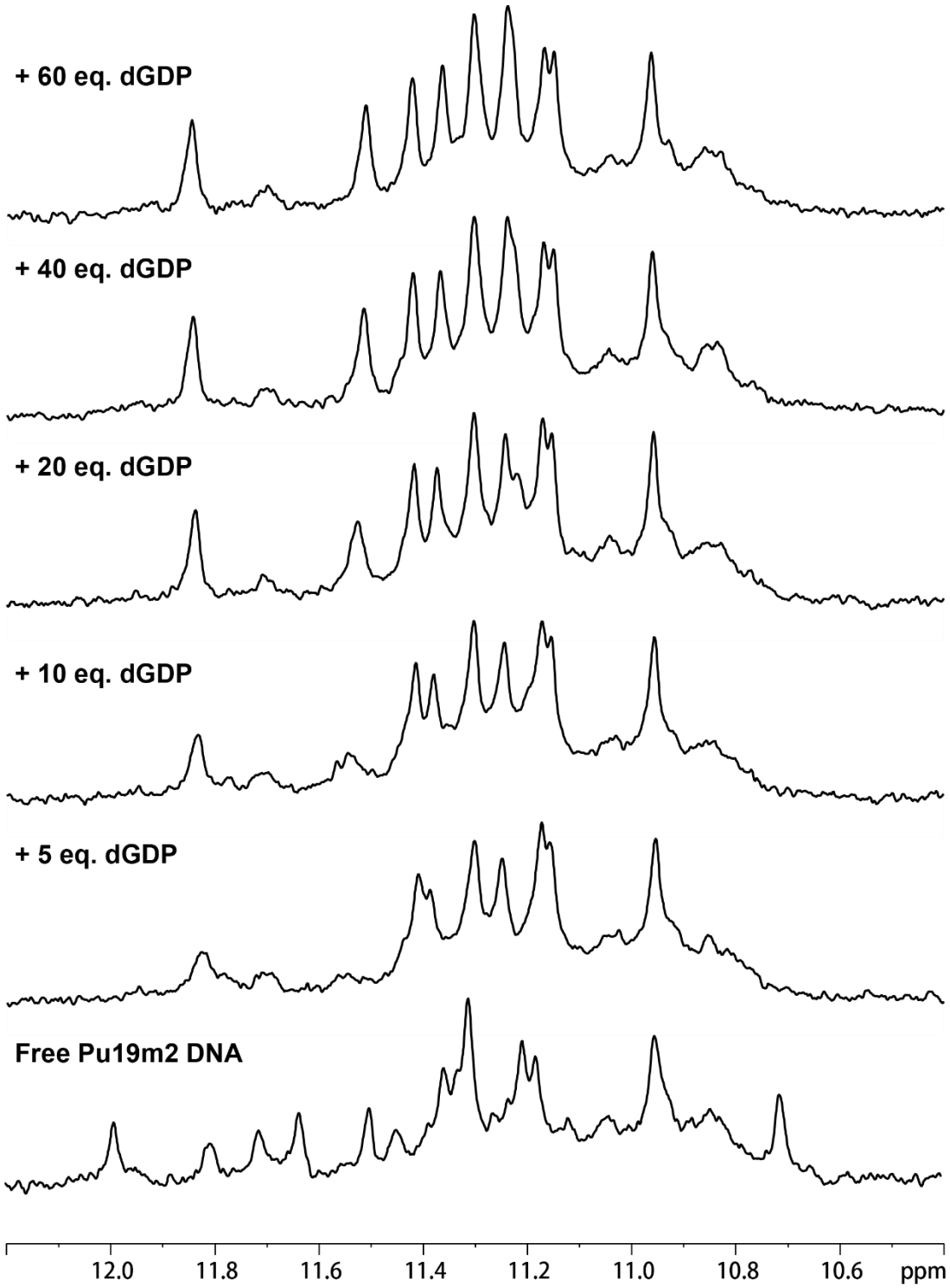

Figure S26. 1D ^{1}H NMR titration of Pu19m2 DNA with dGDP at 25 °C.

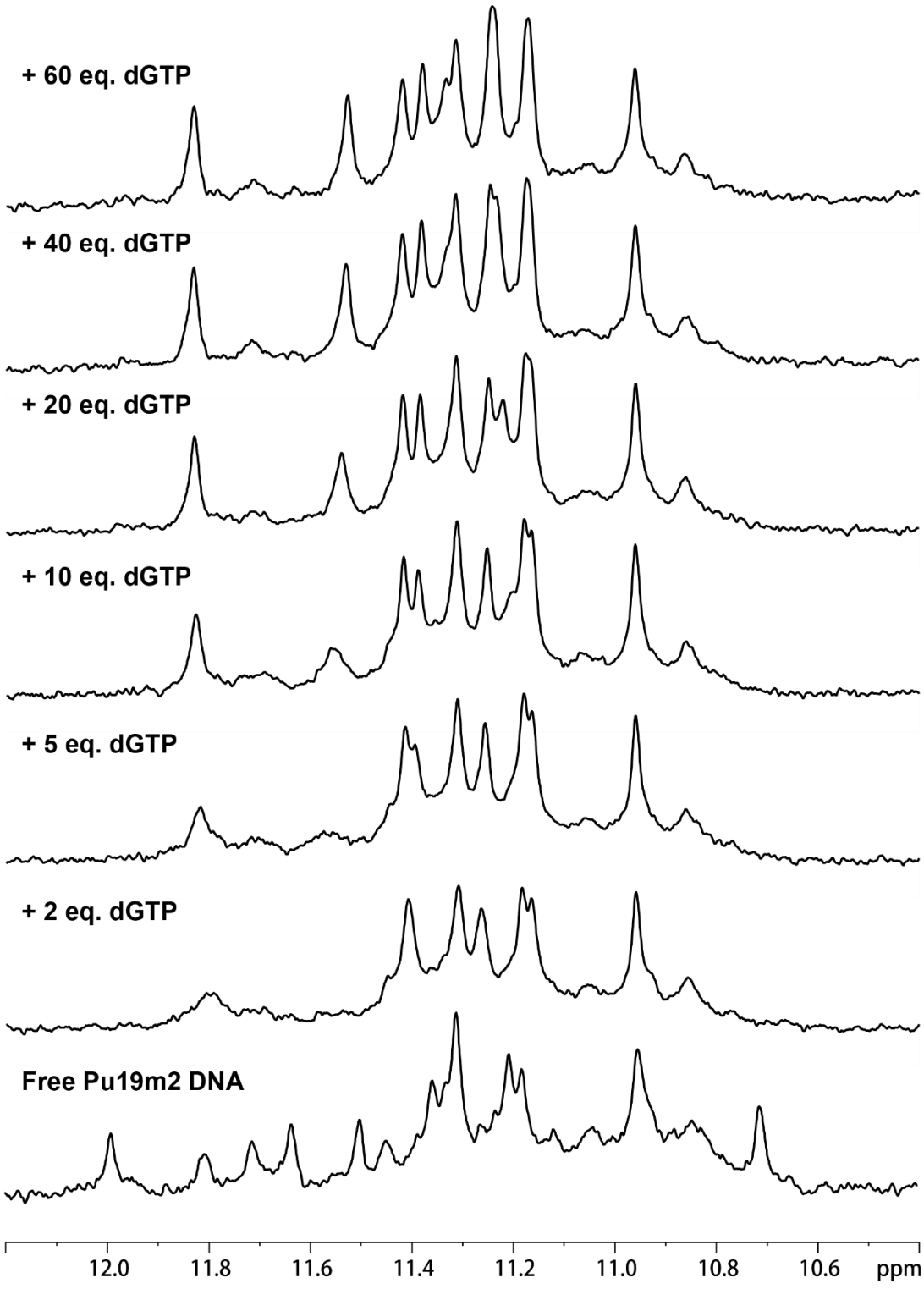

Figure S27. 1D ^{1}H NMR titration of Pu19m2 DNA with dGTP at 25 °C.

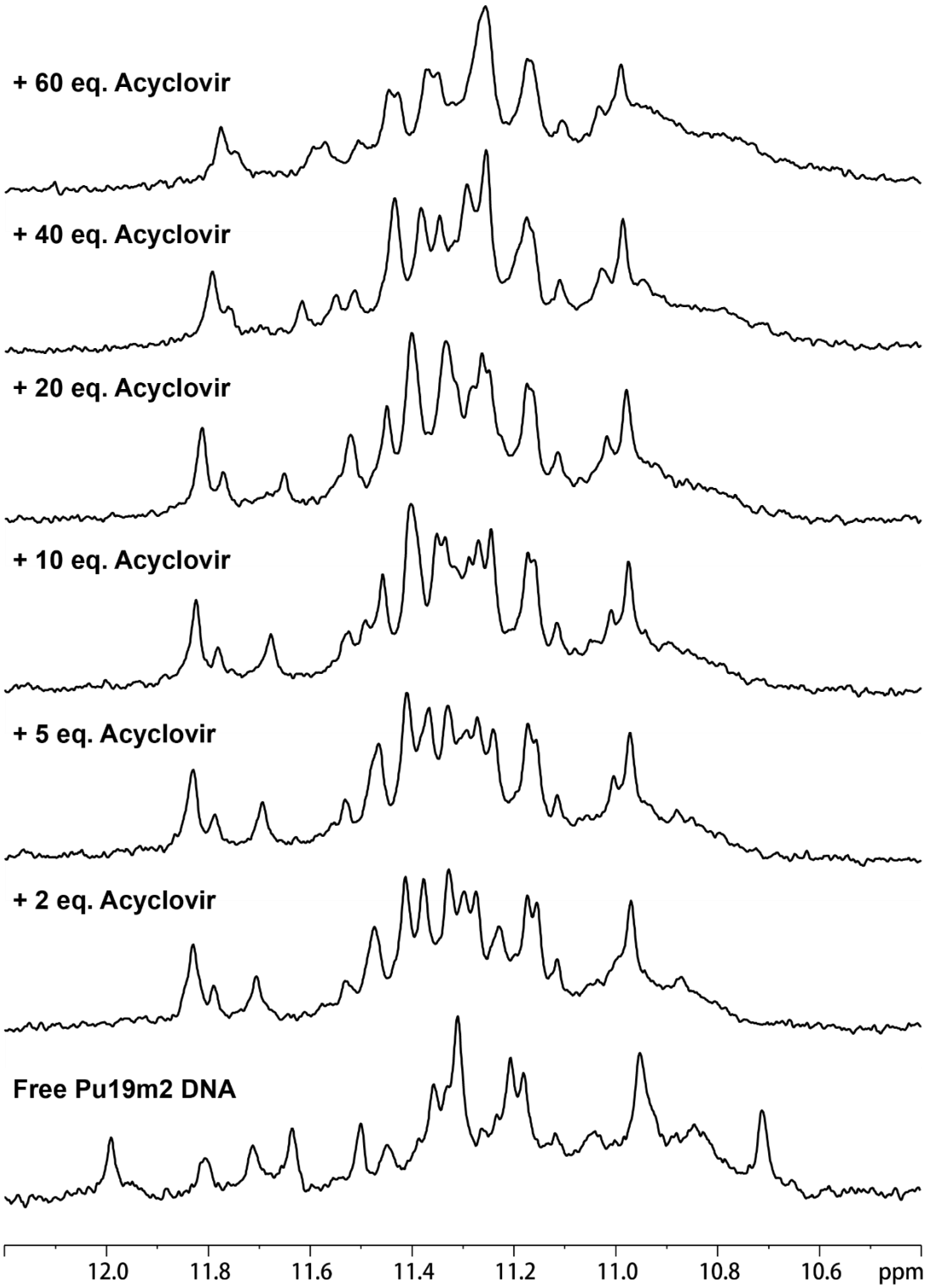

Figure S28. 1D ^{1}H NMR titration of Pu19m2 DNA with acyclovir at 25 °C.

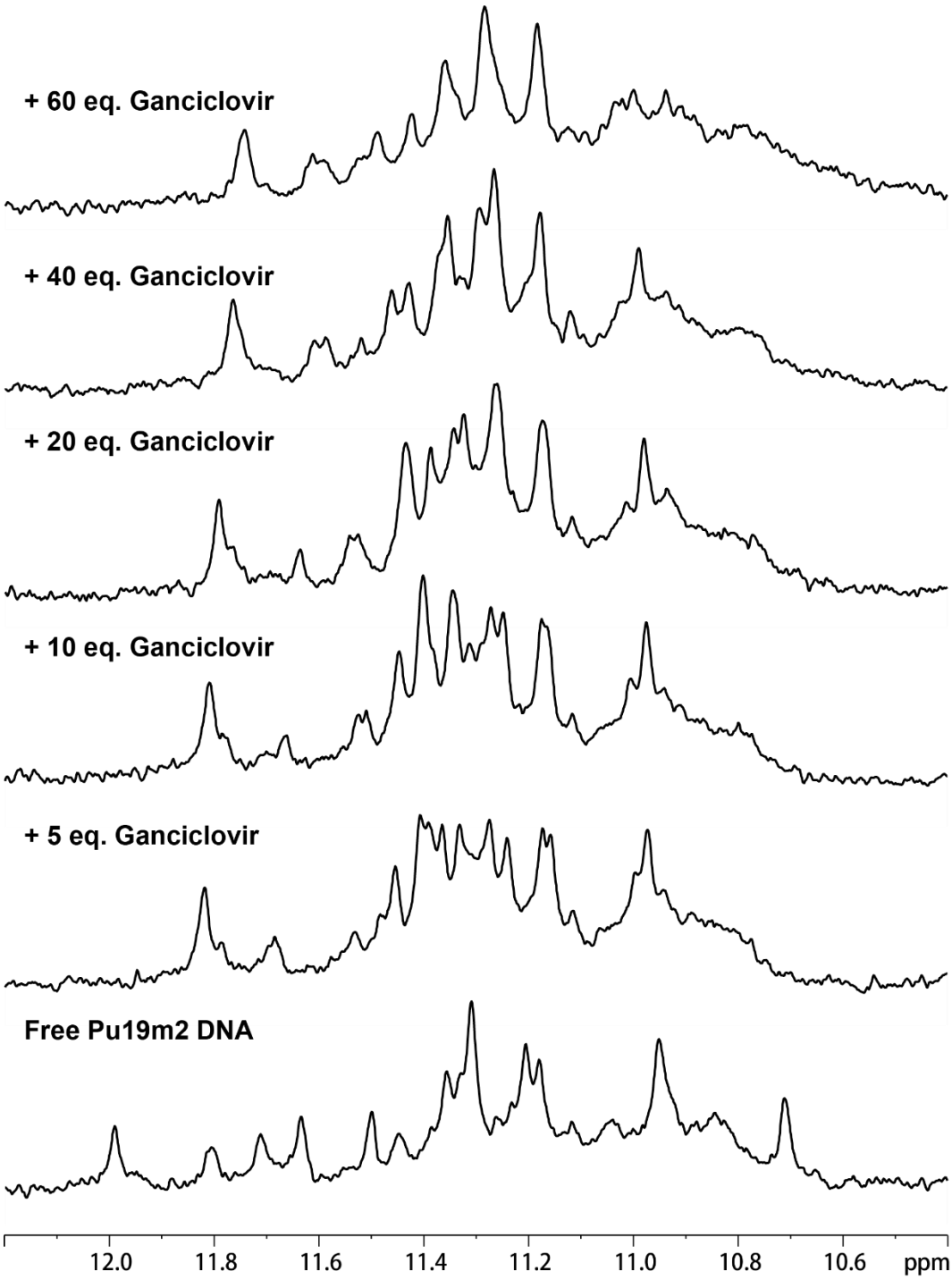

Figure S29. 1D ^{1}H NMR titration of Pu19m2 DNA with ganciclovir at 25 °C.

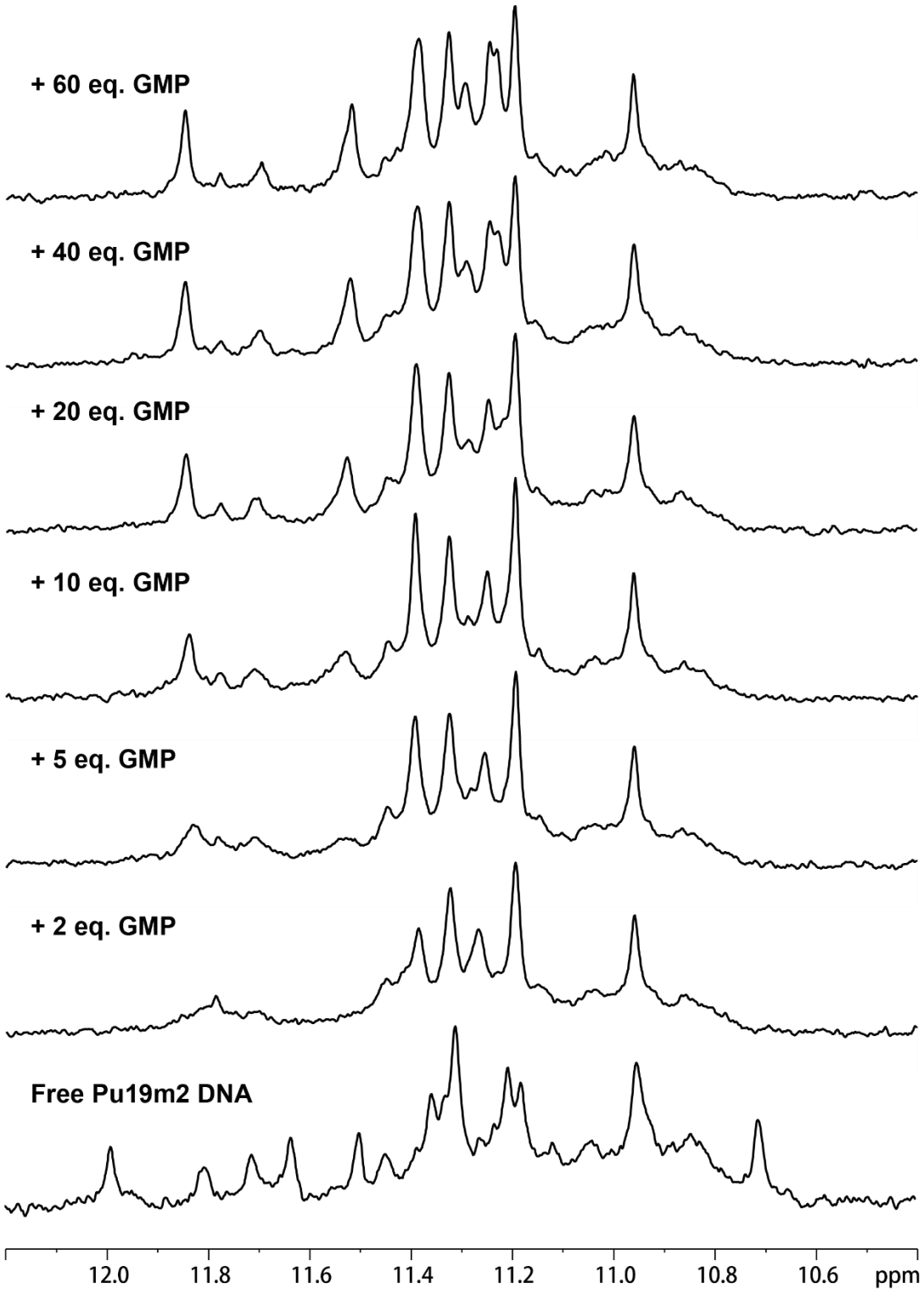

Figure S30. 1D ^{1}H NMR titration of Pu19m2 DNA with GMP at 25 °C.

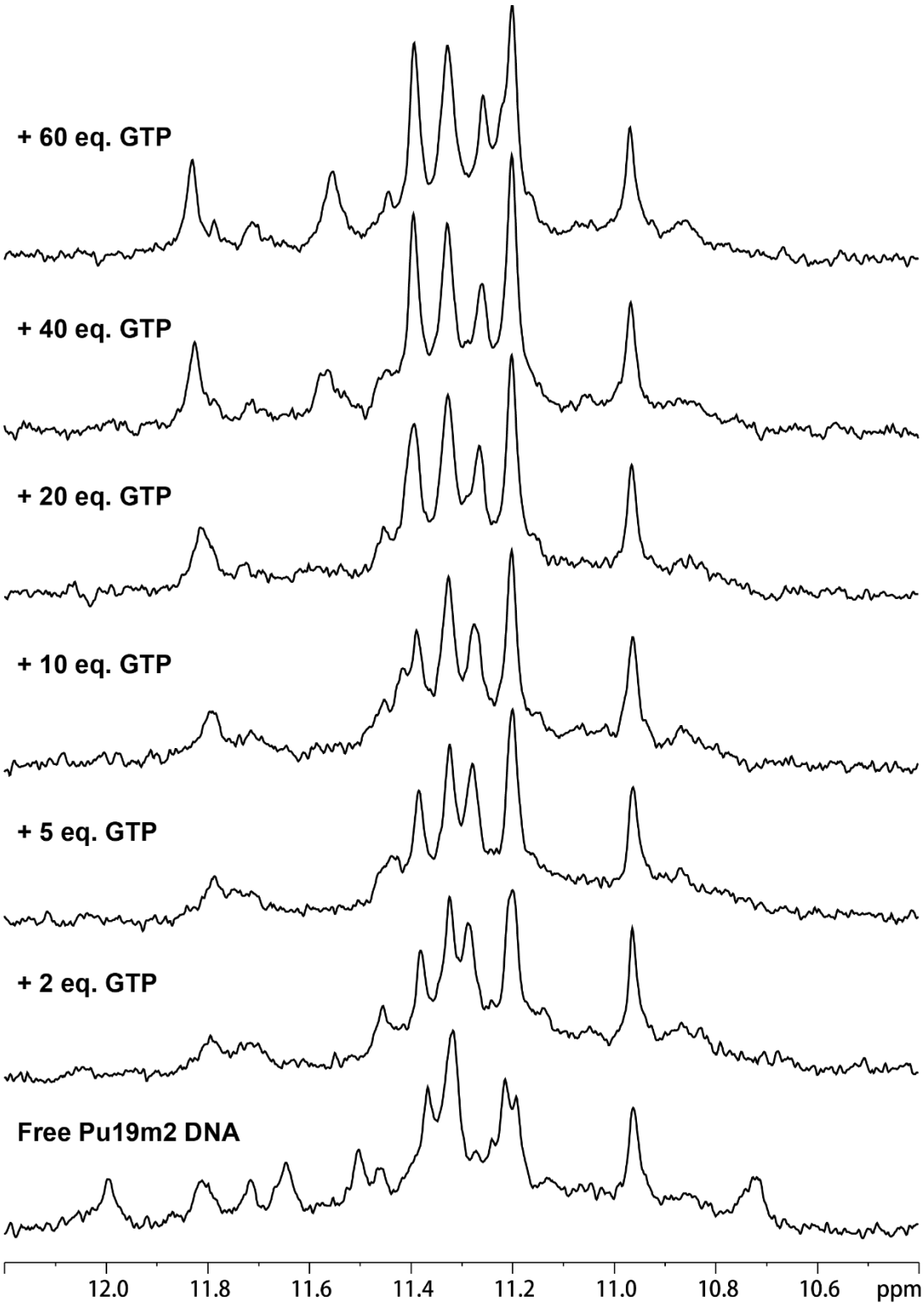

Figure S31. 1D ^{1}H NMR titration of Pu19m2 DNA with GTP at 25 °C.

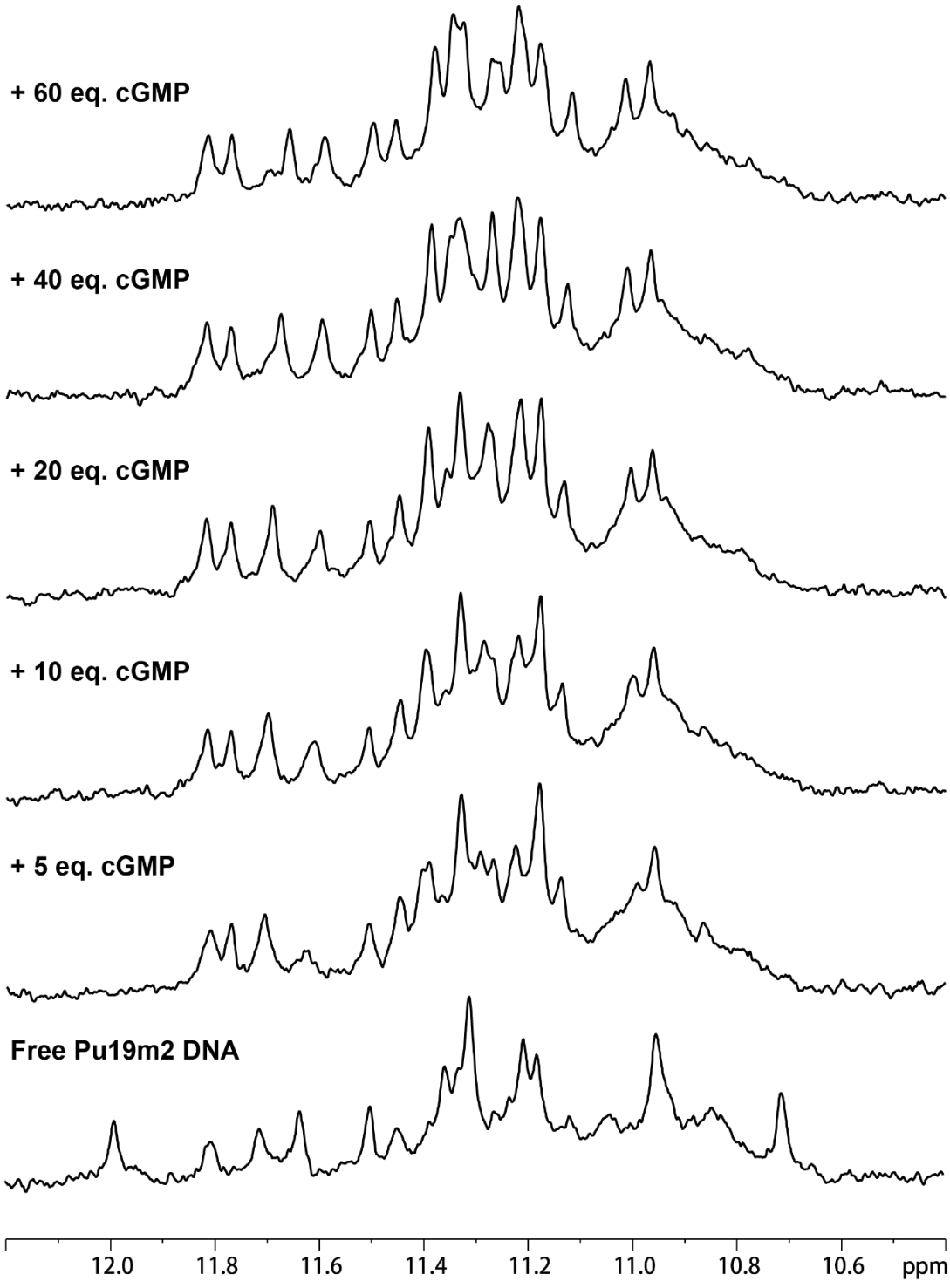

Figure S32. 1D ^{1}H NMR titration of Pu19m2 DNA with cGMP at 25 °C.

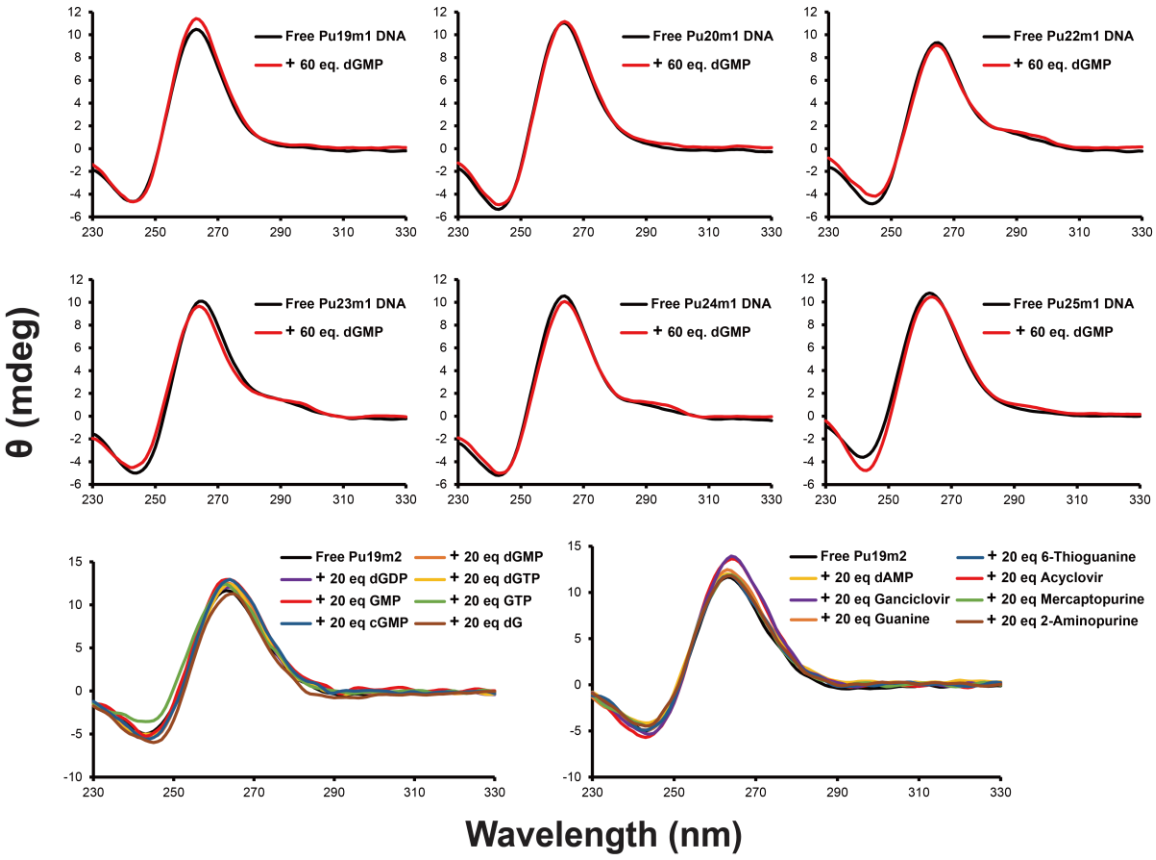

Figure S33. CD spectra of tested DNA samples in the presence and absence of various guanine derivatives. Conditions: 15 μM DNA, pH 7, 50 mM K^+ solution.