"The helical structure of disodium guanosine 5'-monophosphate self-assembly in neutral solution"

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Supporting Information

Materials and Methods

Hydrated disodium salt of guanosine 5´-monophosphate (purity > 99%) was obtained from Sigma-Aldrich (Ontario, Canada). All NMR spectra were acquired on Bruker Avance-500 (11.7 T) and Avance-600 (14.0 T) spectrometers. Standard solution NMR methods such as ^{1}H DOSY, DOSY-NOESY, ROESY, TOCSY, DQF-COSY, ^{1}H–^{13}C HSQC, ^{1}H–^{13}C HMBC, refocused ^{1}H–^{13}C HMBC, and ^{1}H–^{31}P COSY experiments were used for spectral assignment and structure determination. Experimental details are given in the Supplementary Information. Quantum chemical calculations of NMR parameters such as chemical shielding and indirect spin-spin coupling constants were performed on a Sun Fire cluster using the Gaussian 03 suite of programs[28]. The Sun Fire cluster is comprised of seven Sun Fire 25000 servers. Each of the Sun Fire 25000 servers is equipped with 72 × (2 MB on-chip L2 cache and 32 MB L3 cache) dual-core (CPU) UltraSPARC-IV+ processors and 576 GB of RAM.

Table S1. Experimental chemical shifts of proton, carbon, and phosphorus resonances (in ppm) and indirect spin-spin coupling constants (in Hz) for 1.0 M Na_2(5′-GMP). Chemical shifts for exchangeable protons were measured at 278 K; other data were determined at 298 K.

$\delta(^1H)$	C2′-endo (S)	C3′-endo (N)	$\delta(^{13}C)/(^1J_{CH})$	C2′-endo (S)	C3′-endo (N)
exchangeable protons			ribose carbons		
N_1H	10.83	10.78	C1′	83.80 (162.5)	90.70 (172.6)
N_2H^A	9.83	9.30	C2′	74.10 (144.2)	72.80 (153.8)
N_2H^B	5.12	4.29	C3′	73.49 (154.2)	67.92 (144.2)
2′-OH	5.09	7.44	C4′	85.30 (151.3)	82.10 (150.4)
3′-OH	9.30	9.20	C5′	64.48 (155.4/150.4)	65.04 (150.6/144.6)
Non-exchangeable protons			base carbons		
H8	8.26	6.96	C2	152.70	153.20
H1′	5.59	5.67	C4	152.48	148.85
H2′	4.93	4.50	C5	113.96	115.01
H3′	4.21	4.12	C6	159.70	159.70
H4′	4.52	4.29	C8	137.56 (209.1)	133.89 (214.1)
H5′/H5″	3.63/3.98	3.99/4.15			
$^3J_{HH}$			$^3J_{PH}$		
H1′/ H2′	9 ± 1	< 2	^{31}P/H5′	< 2	< 3
H2′/ H3′	6 ± 1	7 ± 1	^{31}P/H5″	7 ± 2	9 ± 2
H3′/ H4′	< 2	8 ± 1	^{31}P/H4′	2 ± 2	—
H4′/ H5′	2 ± 1	2 ± 1			
H4′/ H5″	3 ± 1	8 ± 1	$\delta(^{31}P)^a$	2.3	4.6

[a]Phosphorus-31 chemical shifts are referenced to 85% H_3PO_4 (aq).

Table S2. Torsion angles, pseudorotation phase angle (P), and puckering amplitude (ν_m) determined for the two 5′-GMP molecules. All quantities are in degrees.

Pucker	χ	β	δ	γ	ν_0	ν_1	ν_2	ν_3	ν_4	P	ν_m
C2′-*endo*	-60	-150	138	40	-18.8	30.2	-28.4	17.6	0.6	159.9	30.2
C3′-*endo*	-130	-150	83	-140	3.4	-25.7	37.3	-36.2	20.8	13.7	38.4

Table S3. Computed ^{1}H and ^{13}C chemical shifts (in ppm) and $^1J_{CH}$ coupling constants (in Hz) for 5′-GMP molecules in C2′-*endo* and C3′-*endo* sugar pucker conformation. The level of calculations was B3LYP/6-311++G(d,p).[a]

$\delta(^1H)$	C2′-*endo* (S)	C3′-*endo* (N)	$\delta(^{13}C)/(^1J_{CH})$	C2′-*endo* (S)	C3′-*endo* (N)
Non-exchangeable protons			ribose carbons		
H8	8.26	8.06	C1′	87.9 (171.6)	97.4 (173.8)
H1′	5.44	5.55	C2′	78.5 (155.3)	77.2 (158.6)
H2′	5.40	3.06	C3′	75.5 (170.3)	72.9 (143.3)
H3′	4.63	3.94	C4′	93.7 (147.0)	83.3 (153.6)
H4′	3.33	4.08	C5′	58.9 (139.7/133.3)	60.9 (138.6/131.3)
H5′/H5″	2.62/3.38	3.27/4.20	base carbons		
			C2	157.6	157.7
			C4	159.9	158.0
			C5	126.0	126.6
			C6	162.0	161.6
			C8	141.4 (194.7)	140.0 (195.2)

[a]Chemical shifts (δ) are converted from the calculated chemical shielding (σ) using $\delta = \sigma_{ref} - \sigma$ where $\sigma_{ref}(^{13}C) = 185.4$ ppm and $\sigma_{ref}(^1H) = 34.9$ ppm.

Table S4. Comparison of geometric parameters of common nucleic acid helices

	B-DNA	A-DNA A-RNA	Z-DNA	Poly G[a]	Na_2(5′-GMP)[b]	Na_2(5′-GMP) (this work)
Helix sense	Right	Right	Left	Right	Right	Right
Base per turn	10	11	12	11.5	12	12
Rise per base (Å)	3.4	2.6	3.7	3.36	3.4	3.4
Rotation per base (°)	36.0	32.7	–60 (per dimer)	31.2	30	30
Glycosyl bond	*anti*	*anti*	dC: *anti* dG: *syn*	*anti*	*anti*	*anti* (C3′-*endo*) high-*anti* (C2′-*endo*)
Sugar pucker	C2′-*endo*	C3′-*endo*	dC: C2′-*endo* dG: C3′-*endo*	C3′-*endo*	C3′-*endo*	C2′-*endo* C3′-*endo*
Exocyclic C4′–C5′ bond (°)	38	47	dC: 55 dG: –170	68	47	40 (C2′-*endo*) –140 (C3′-*endo*)
Distance of P from axis (Å)	9.0	8.7	d(CpG): 6.9 d(GpC): 8.0	9.98	9.6	9.3 (C2′-*endo*) 11.8 (C3′-*endo*)

[a]From: Zimmerman, S. B.; Gerson, H. C.; Davies, D. R., *J. Mol. Biol*. **1975**, *92*, 181-192.

[b]From: Zimmerman, S. B., *J. Mol. Biol*. **1976**, *106*, 663-672.

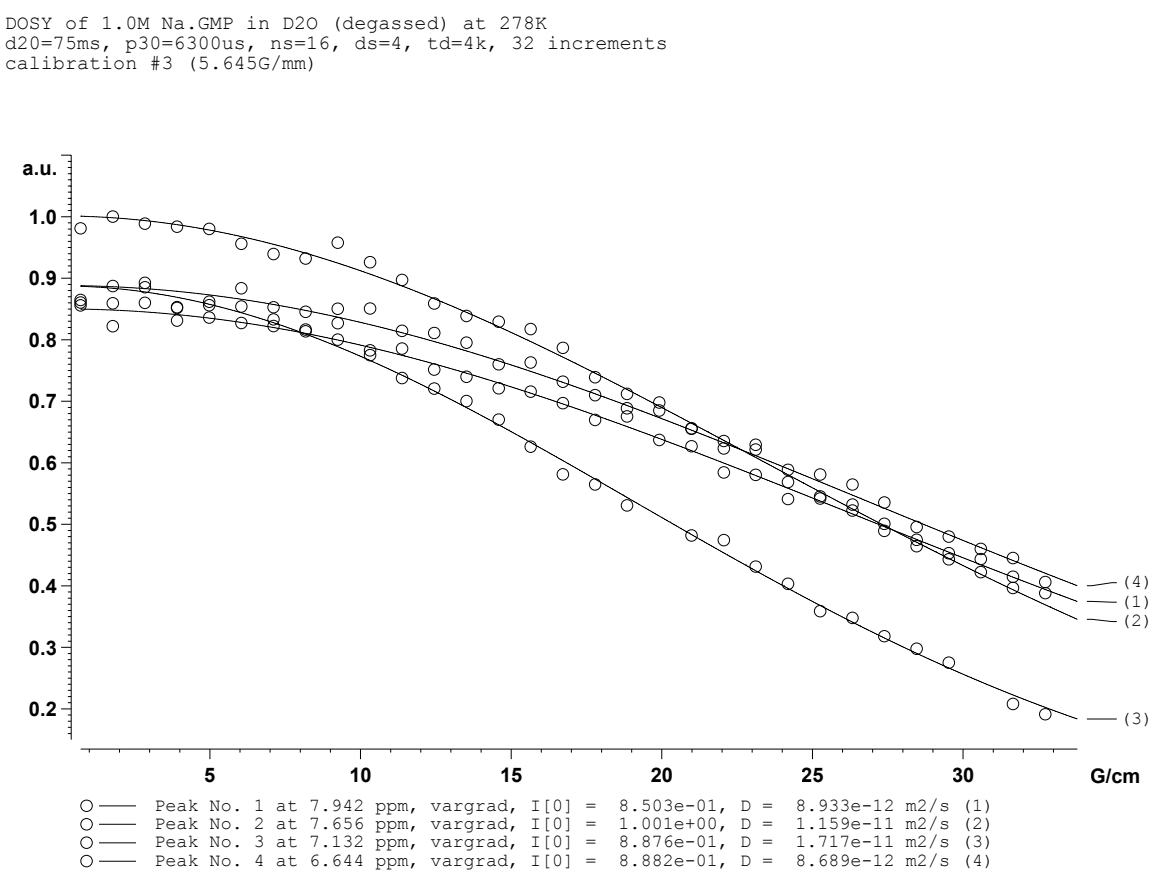

Figure S1. **Diffusion ^{1}H NMR results**. Diffusion NMR experiments were carried out on an Avance-600 Bruker spectrometer for 1.0 M Na_2(5'-GMP) in D_2O at 278.0 K. The pulse sequence of longitudinal eddy current delay with bipolar-gradient pulse (LEDBPGP2s) was employed. The pulse field gradient duration (δ) was 7.5 ms, and the variable gradient strength (G) was 5.645 mT/m. The diffusion period (Δ) was 75.0 ms, and a total of 4096 experiments with 16 scans were collected for each of the 32 increment steps with a recycle delay of 5.0 s. The eddy current delay (t_w) employed was 5 ms, and the gradient recovery delay was set at 0.2 ms. Calibration of the field gradient strength was performed by measuring the value of translational diffusion coefficient (D_t) for the residual ^{1}H signal in D_2O (99.99% ^{2}H atom), where $D = 1.90 \times 10^{-9}$ m^2/s [Van Geet, A.L. Anal. Chem. **42**, 679-680 (1970)]. The spectral width was 4194.6 Hz and a line-broadening of 10 Hz was employed in data processing (F2).

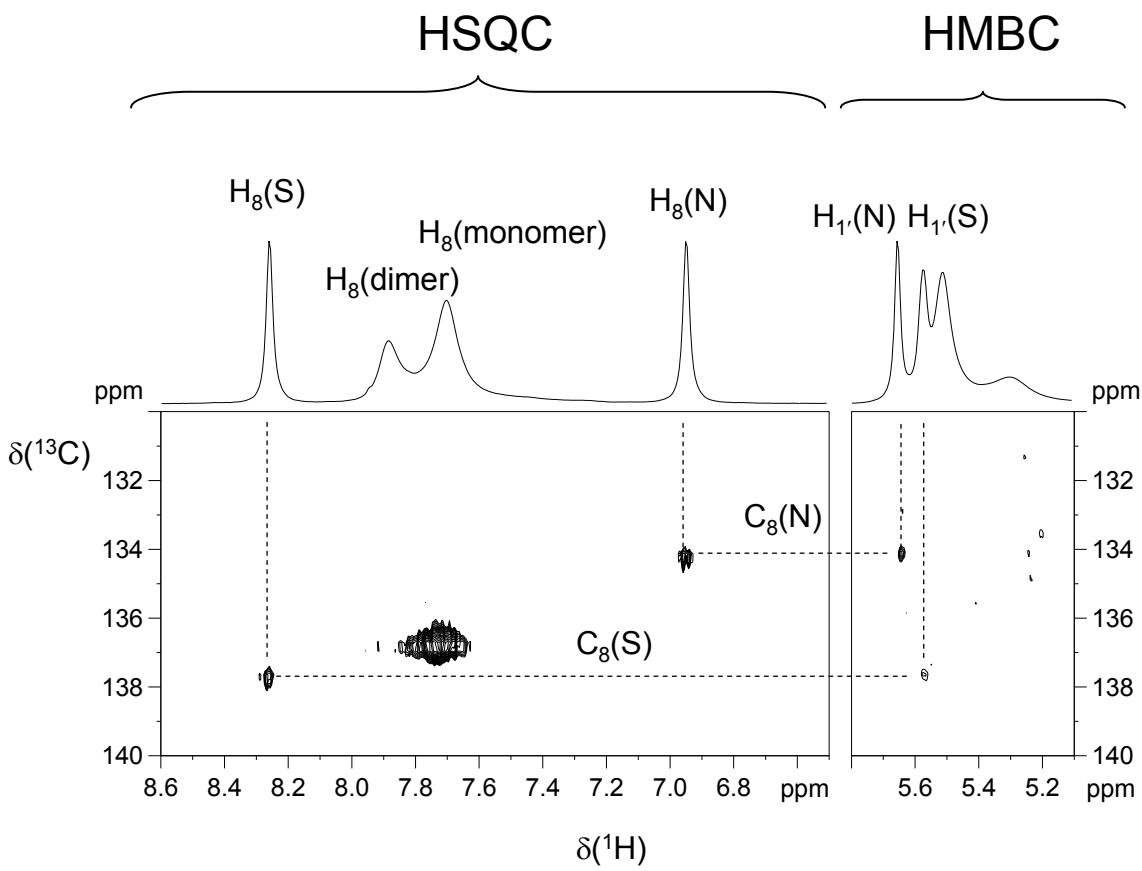

Figure S2. Connectivity between H8 and H1′ signals. (Right) 2D gradient-enhanced HMBC (^{1}H-^{13}C correlation through heteronuclear zero and double quantum coherence transfer) experiment was performed using the Bruker pulse program HMBCGPLPNDQF at 298.0 K on 1.0 M Na_2(5′-GMP) in H_2O/D_2O (1:1). A low-pass *J*-filter was employed to suppress one-bond correlations, and no ^{13}C decoupling was applied during acquisition. The long-range coupling delay was 62.5 ms (corresponding to a long-range J_{CH} coupling of 8 Hz) and the one-bond coupling delay was 3.45 ms (145 Hz). A total of 450 scans were collected with a relaxation delay of 1.0 s. The 2D spectral widths were 6009.6 Hz (F2) and 21,130.1 Hz (F1), and the final data matrix was 4096 (F2) x 256 (F1). (Left) 2D HSQC (^{1}H-^{13}C heteronuclear one-bond correlation via double INEPT transfer) without decoupling was performed on 1.0 M Na_2(5′-GMP) in D_2O at 298.0 K using the Bruker pulse program HSQCETGPSISPNP. A trim pulse (1 ms, F1 dimension) was employed in the INEPT transfer while shape pulses were employed on the F2 dimension. The 180° shape pulses for inversion and refocusing at –1.3 dB on the F2 dimension were 500 μs and 2000 μs, respectively. A correlation delay of 1.25 ms, which corresponds to a $^{1}J_{HC}$ of 200 Hz, was used to observe the ^{1}H-^{13}C correlation. The 2D spectral widths were 6613.7 Hz (F1) and 21,129.4 Hz (F2). A total of 30 scans were collected with a relaxation delay of 1.0 s, and the final data matrix was 2048 (F2) x 1024 (F1).

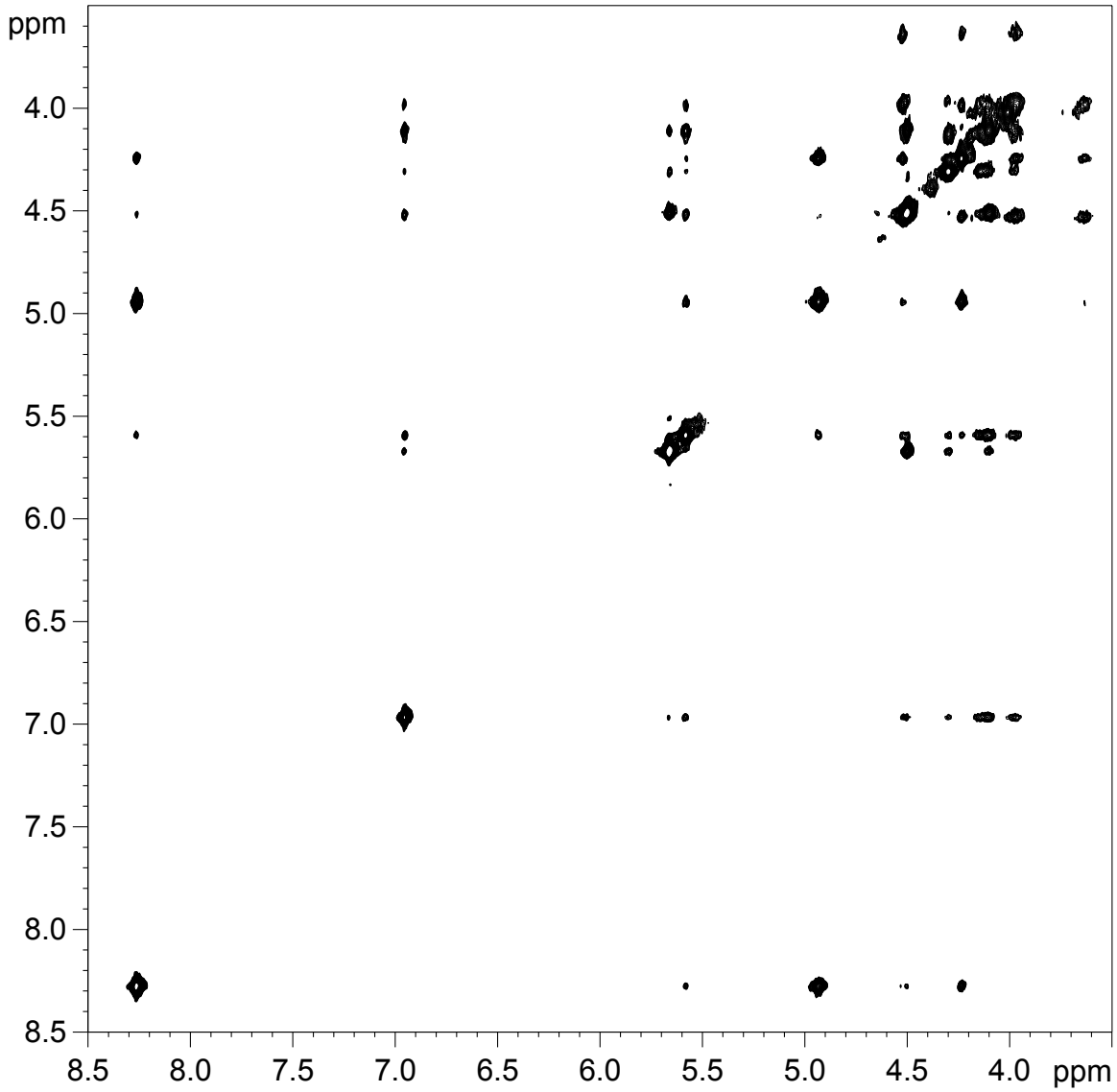

Figure S3. DOSY-NOESY A 2D DOSY-NOESY experiment was created by modifying the 3D DOSY-NOESY experiment (LEDBPGPNO3s3d) and was carried out on 1.0 M Na_2(5'-GMP) in D_2O at 278 K. The pulse field gradient duration (δ) was set to 1.0 ms, and the diffusion period (Δ) was 300.0 ms. A total of 8 scans were collected with a recycle delay of 3.0 s. The mixing time (τ_{mix}) for NOESY was varied between 50 and 100 ms, and the final data matrix was 2048 (F2) × 512 (F1).

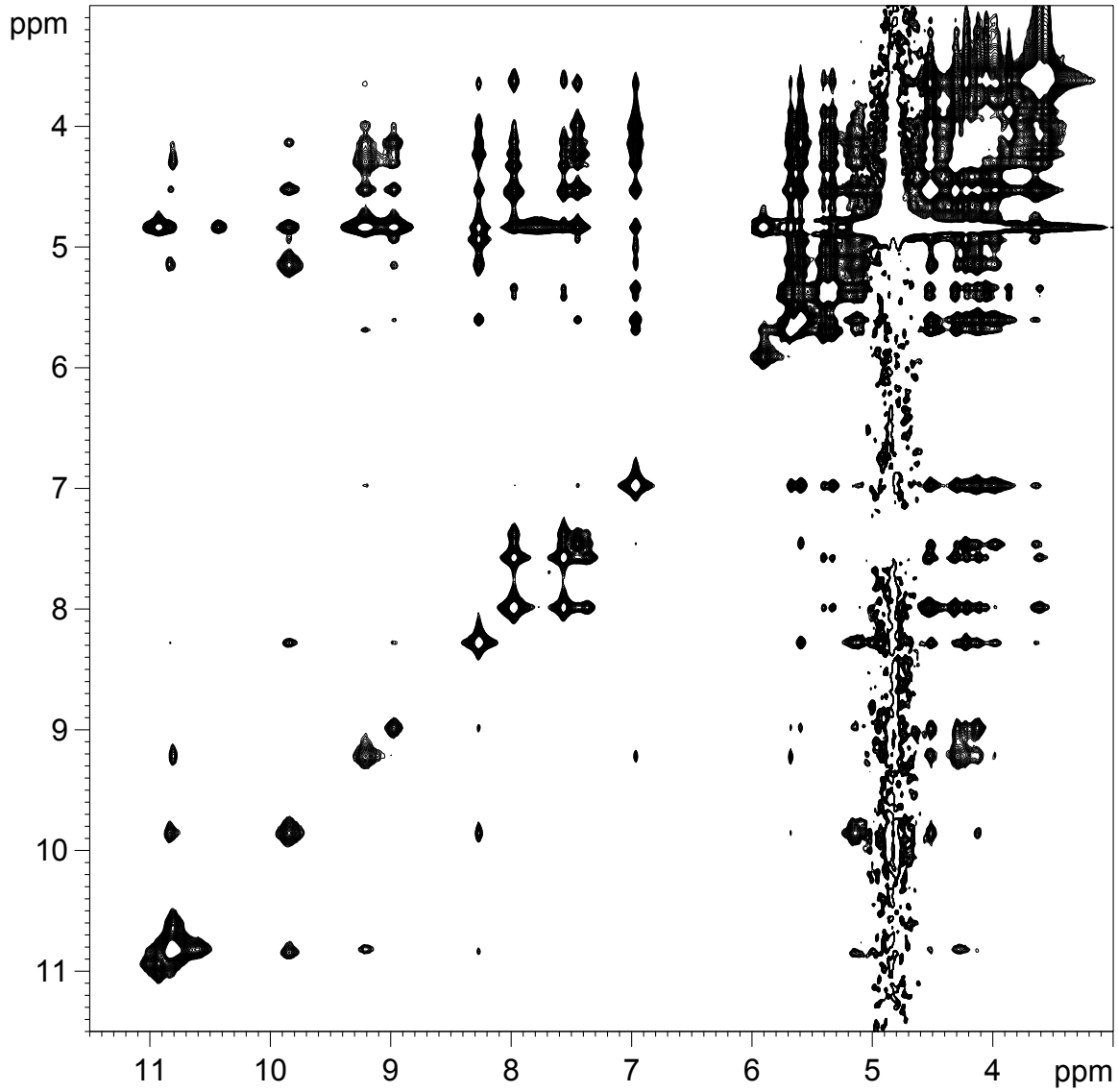

Figure S4. NOESY. The 2D NOESY (homonuclear correlation via dipolar coupling) experiment was performed 1.0 M Na_2(5'-GMP) in D_2O/H_2O (1:1) at 278.0 K using the Bruker pulse program NOESYGPPH with different mixing times (τ_{mix}): 50, 100, 200, 400 ms. The recycle delay was 4.0 s, and a spectral width of 7183.9 Hz in each dimension was employed. A total of 16 scans were collected for each experiment, and the final data matrix was 2048 (F2) × 512 (F1).

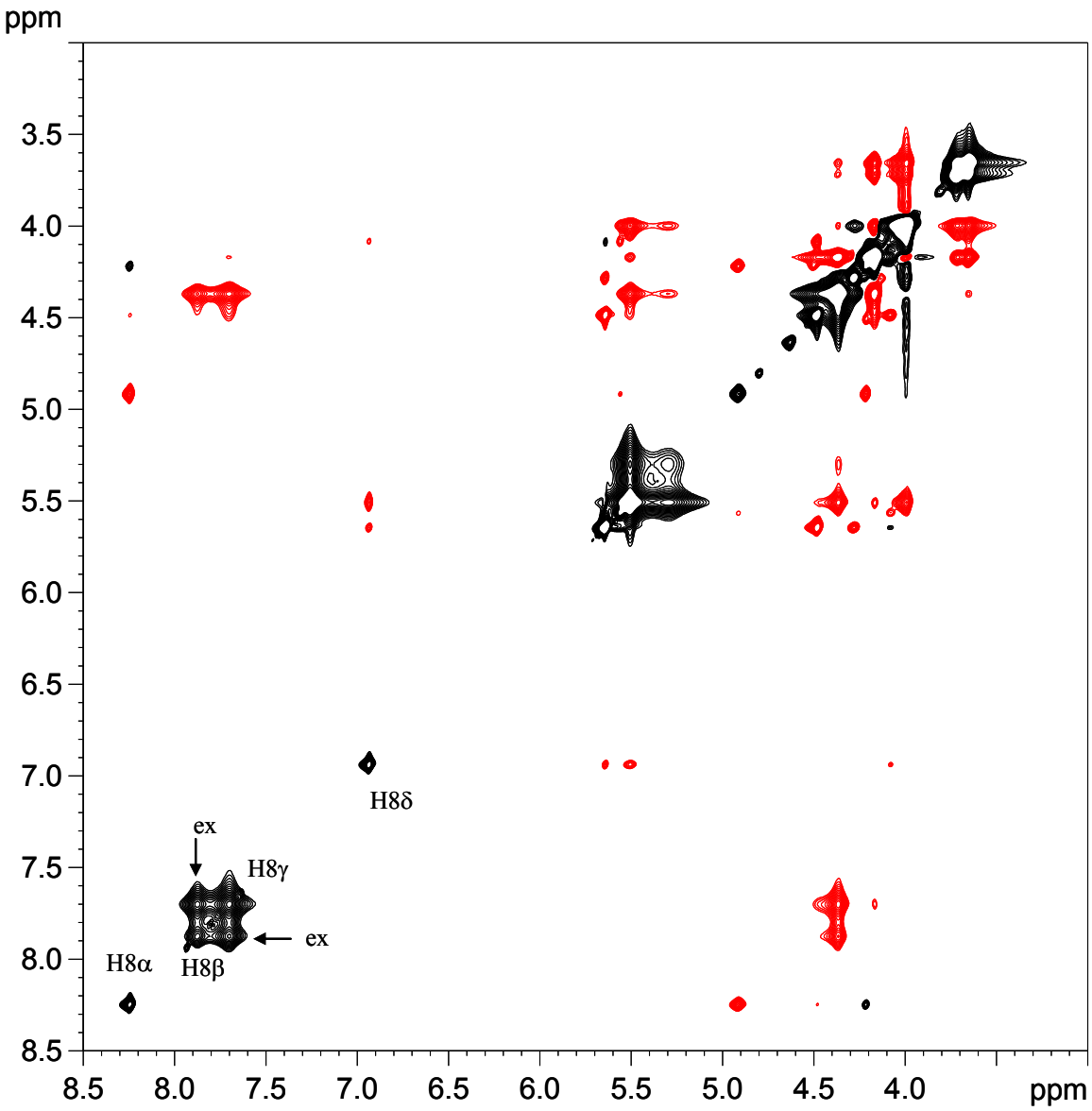

Figure S5. ROESY. 2D ROSEY (homonuclear NOE experiment measured under spin-locked condition) experiment was performed on 1.0 M Na_2(5′-GMP) in H_2O/D_2O (1:1) at 298.0 K using the Bruker pulse program ROESYETGP. A continuous wave (CW) spin-lock was used for mixing with an echo/antiecho-TPPI gradient selection. A series of experiments were performed with different spin-lock mixing times (5 – 40 ms). The spectral width was 4194.6 Hz on either dimension, and a total of 16 scans were collected with a relaxation delay of 3.0 s. The final data matrix was 2048 (F2) × 512 (F1). The cross peaks due to chemical exchange are labeled by "ex".

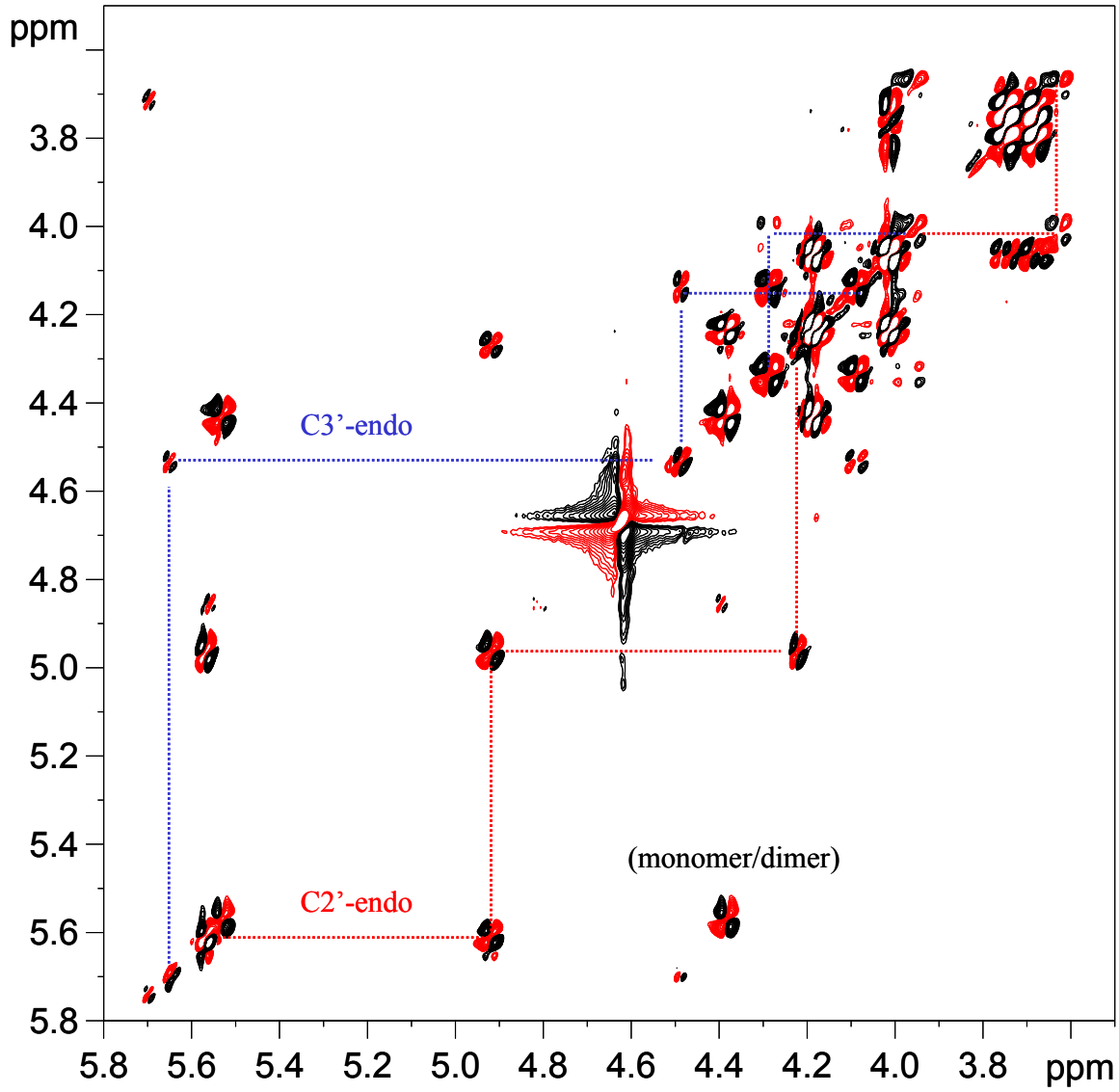

Figure S6. ^{1}H DQF-COSY. 2D gradient-enhanced DQF-COSY (homonuclear shift correlation spectrum with double quantum filter) experiment was performed for 1.0 M Na_2(5′-GMP) in D_2O at 298.0 K using the Bruker pulse program COSYDFETGP. A total of 16 scans and a relaxation delay of 6.0 s were collected for a spectrum with 2403.8 Hz on either dimension. The final data matrix was 2048 (F2) × 512 (F1).

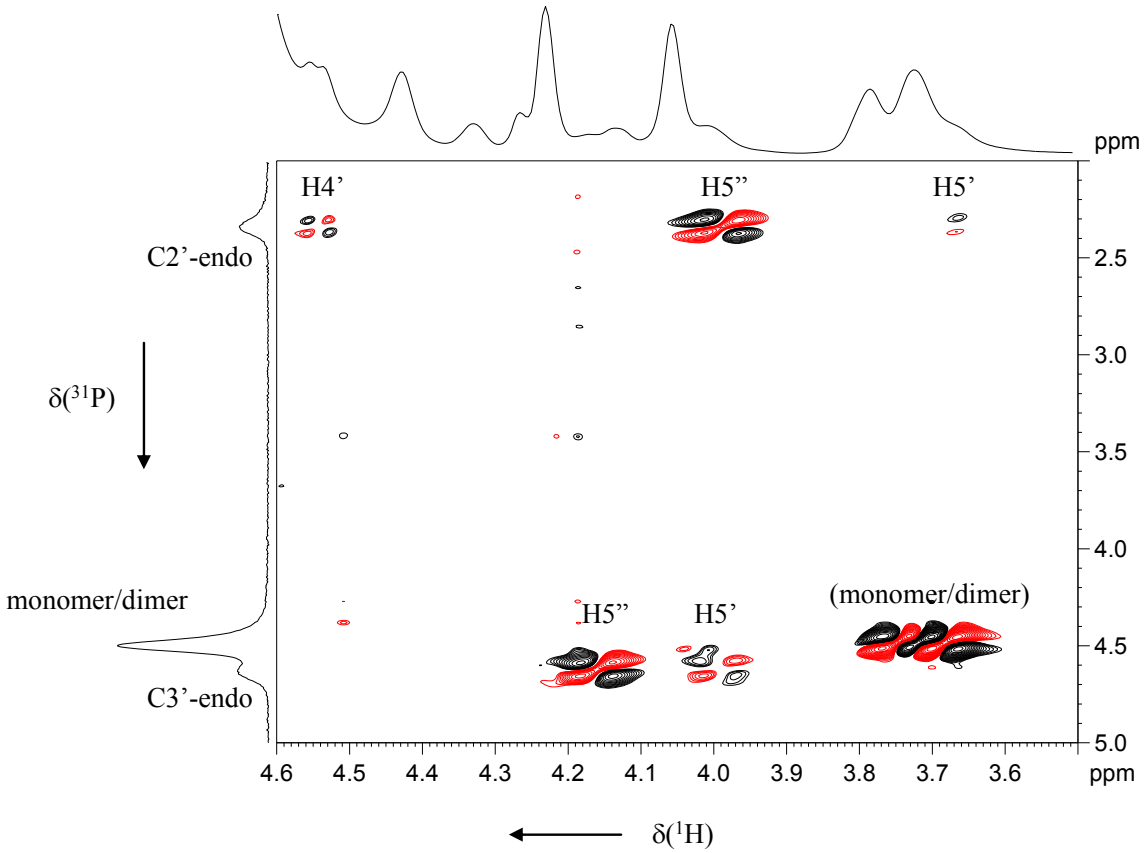

Figure S7. ^{1}H-^{31}P COSY. Phase-sensitive ^{1}H-^{31}P COSY (proton-detected 2D heteronuclear shift correlation) was performed on 1.0 M Na_2(5′-GMP) in D_2O at 298.0 K. The ^{1}H 90° and 180° pulse widths of ^{31}P at a pulse power of 0.0 dB were 22.0 μs and 44.0 μs, respectively. ^{31}P chemical shift was externally referenced to 85% H_3PO_4(aq) at 298.0 K. The spectral widths were 2403.8 Hz (F2) and 971.8 Hz (F1). A total of 20 scans were collected with a relaxation delay of 10.0 s, and the final data matrix was 1024 (F2) × 256 (F1).

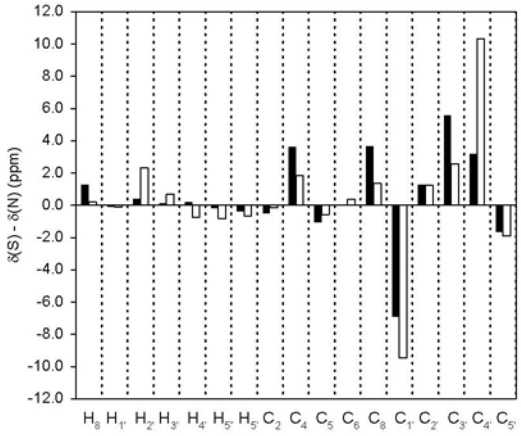

Figure S8: Comparison between experimental and computed chemical shift differences between the two 5′-GMP sugar pucker conformations. The closed and open bars represent experimental and computed data, respectively. Computations were performed at the B3LYP/6-311++G(d,p) level.