Solution Structure of an Unique G-quadruplex Scaffold Adopted by a Guanosine-rich Human Intronic Sequence

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Supplementary Materials

Stacked T19•G8•A10 triple stabilizes edge-wise G8-A9-A10 loop. We observe a T19•G8•A10 triple in the *chl1* intronic G-quadruplex, in which residue G8 forms a sheared base pair with A10 and, simultaneously, base pairs with the terminal residue T19, coordinated by single hydrogen bond (Figure 5A). Formation of the sheared G8•A10 base pair is reflected in observed NOE's for its constitutive bases G8 and A10. The position of G8 base is defined by observed cross-peaks between G8(imino)-G18(imino) (peak s, Figure 3B) and G8(H8)-G7(H1', H2', H2'', H3') proton pairs. The position of the A10 base is defined by the observed cross-peaks between A10(H2)-G7(imino) and A10(H1´)-G7 (imino) (peaks q and r, Figure 3B) proton pairs, as well as cross-peaks between A10(H8)-G11(H8), A10(H8)-G11(H1´) and A10(H2)-G11(H1') proton pairs.

The pairing alignment between G8(imino) and T19(carbonyl) is reflected in the slow exchange of the imino proton of G8, which is observed as a narrow resonance at 9.95 ppm (Figure 1A). The position of G8 with respect to T19 is further supported by observation of cross-peaks between G8(imino)-T19(H1', H2', H2'') proton pairs, whereas its position on top of the G-tetrad layer is evidenced by the observed cross-peak between G8(imino)-G18(imino) proton pair (peak s, Figure 3b).

Table S1. Proton chemical shifts of *chl1* intronic G-quadruplex[1].

	H1'	H2'	H2''	H3'	H4'	H5'/H5''	H6/H8	CH_3	H2	H1
G1	5.88	2.47	2.55	4.93			7.30			11.74
G2	6.20	2.55	3.01	4.96			7.73			11.66
G3	4.82	1.66	2.26	4.42			7.56			10.54
T4	5.82	1.79	1.90	4.38	4.36		6.81	0.74		
G5	6.12	3.72	2.91	4.88			7.33			11.81
G6	6.00	2.54	2.89	5.02			8.04			11.88
G7	6.01	1.42	2.39	4.84			7.10			11.41
G8	5.27	2.78	2.54	4.93			8.05			9.95
A9	5.63	2.30	2.12	4.59			8.00		6.21	
A10	5.89	2.66	2.86	4.77			7.90		7.85	
G11	5.99	2.81	2.70	5.62			6.81			11.02
G12	6.07	3.03	2.71	5.02			7.49			11.10
G13	6.22	3.00	2.71	4.84			7.67			11.56
G14	6.61	2.72	2.72	5.19			8.03			11.16
T15	6.54	2.78	2.44	5.15		4.40/4.63	7.93	2.01		
G16	6.04	2.93	3.09	4.52			7.45			11.86
G17	6.01	2.93	3.09	5.14			8.35			11.70
G18	6.34	2.67	3.00	4.93	4.59		7.59			11.48
T19	6.22	2.26	2.44	4.53			7.26	1.64		

[1]Buffer: 50 mM K^+, 5 mM phosphate, pH 6.8, 25 ^oC.

Supplementary Figure Captions

Figure S1. Imino Proton NMR Spectra of *chl1* Intronic 19-mer Sequence in 50 mM K^+, 5 mM Phosphate-H_2O buffer, pH 6.8, at 25 ^oC, following single residue substitutions at the A9 position in the G8-A9-A10 edge-wise loop.

Sequences contain (A) A9 control, (B) G9 replacing A9, (C) T9 replacing A9 and (D) C9 replacing A9.

Figure S2. Imino Proton NMR Spectra of *chl1* Intronic 19-mer Sequence in 50 mM K^+, 5 mM Phosphate-H_2O buffer, pH 6.8, at 25 ^oC, following single residue substitutions at the T4 and T15 positions.

Sequences contain (A) T4 control, (B) A4 replacing T4, (C) C4 replacing T4, (D) G4 replacing T4, and (E) C15 replacing T15.

Figure S3. Imino Proton NMR Spectra of *chl1* intronic 19-mer sequence in 50 mM K^+, 5 mM Phosphate-H_2O buffer, pH 6.8, at 25 ^oC, following single residue substitutions at the G3 position in the G3-T4 edge-wise loop.

Sequences contain (A) G3 control, (B) I3 replacing G3, (C) T3 replacing G3, and (D) A3 replacing G3.

Figure S4. Imino Proton NMR Spectra of *chl1* Intronic 19-mer Sequence in 50 mM K^+, 5 mM phosphate-H_2O buffer, pH 6.8, at 25 ^oC, following single residue substitutions at the 3'-end T19 position.

Sequences contain (A) T19 control, (B) C19 replacing T19, (C) A19 replacing T19, and (D) G19 replacing T19.

Figure S5. A Proposed Model of a Higher Order RNA Structure, where a Constrained Guanosine as part of a G-quadruplex, could Facilitate the Splicing Reaction at a Quadruplex-Duplex Junction.

(A) A proposed model of a higher order RNA structure where a constrained guanosine as part of a G-quadruplex could participate in the splicing reaction at a quadruplex-duplex junction. The G-quadruplex is shown on the right, while the duplex is on the left, with

connecting loop segments linking the ends of the duplex shown as dashed lines. The dinucleotide spanning the scissible site is in biscuit color, with the guanosine (in biscuit color) intercalated within a G-G step (in cyan) of the intronic G-quadruplex. (B) An expanded view of panel (A), highlighting the postulated in-line attack by a 5’-OH on the scissible phosphate, as shown by a red arrow. Phosphorus and oxygen atoms are colored in yellow and red, respectively.

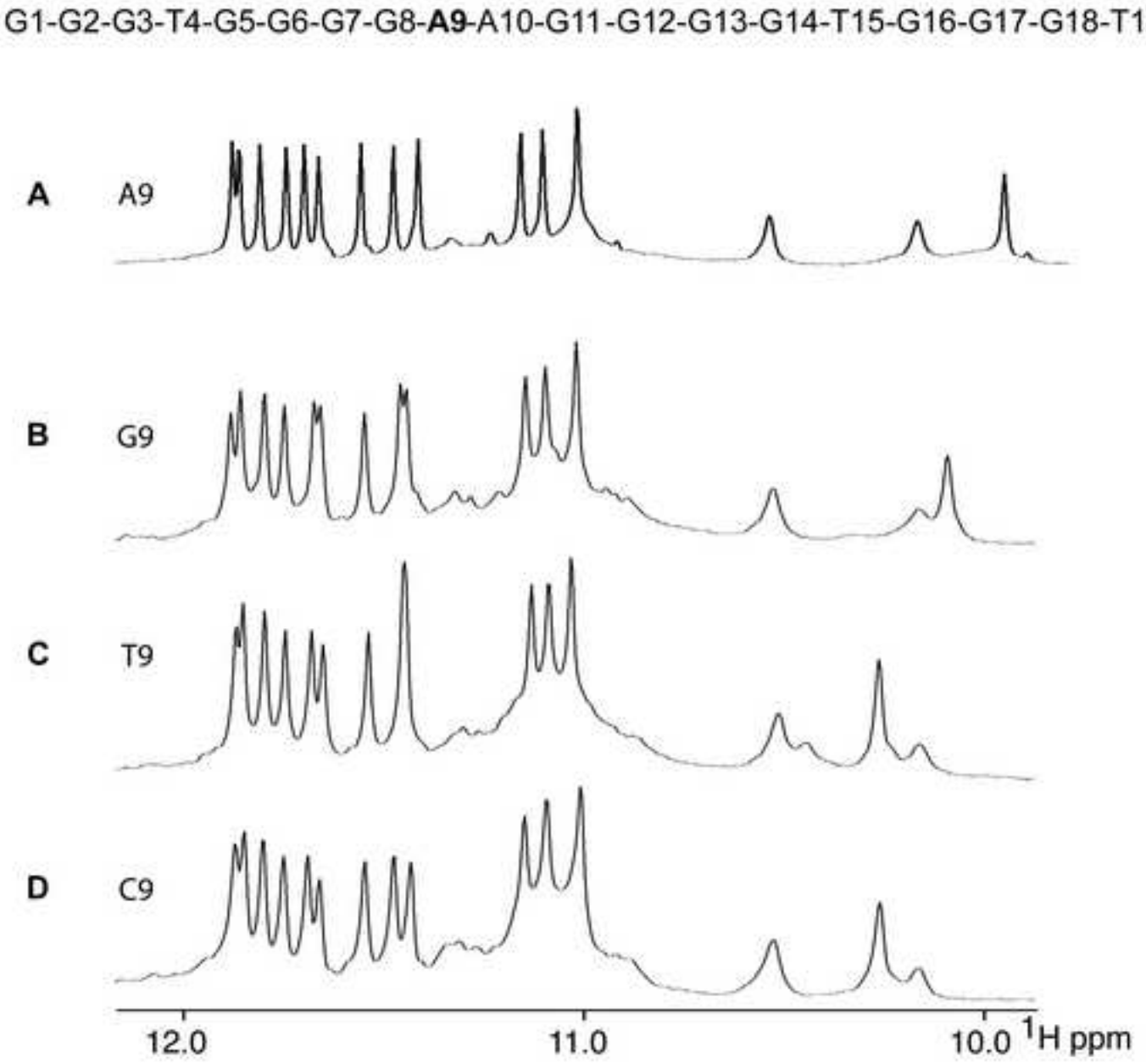
G1-G2-G3-T4-G5-G6-G7-G8-A9-A10-G11-G12-G13-G14-T15-G16-G17-G18-T19
A
A9
B
G9
C
T9
D
C9
12.0
11.0
10.0
^{1}H ppm

Figure S1

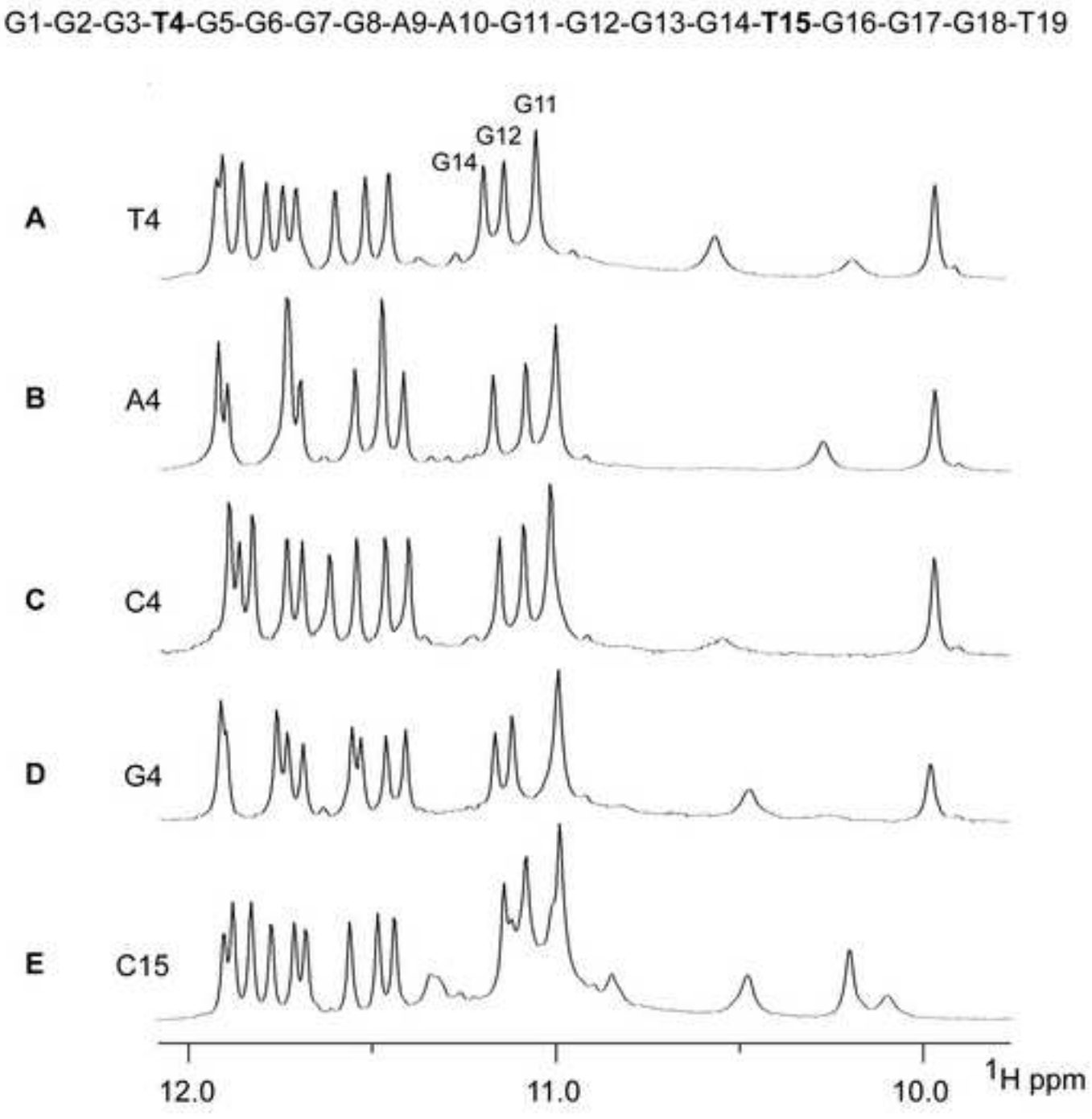
G1-G2-G3-T4-G5-G6-G7-G8-A9-A10-G11-G12-G13-G14-T15-G16-G17-G18-T19
G11
G12
G14
A
T4
B
A4
C
C4
D
G4
E
C15
12.0
11.0
10.0
1H ppm

Figure S2

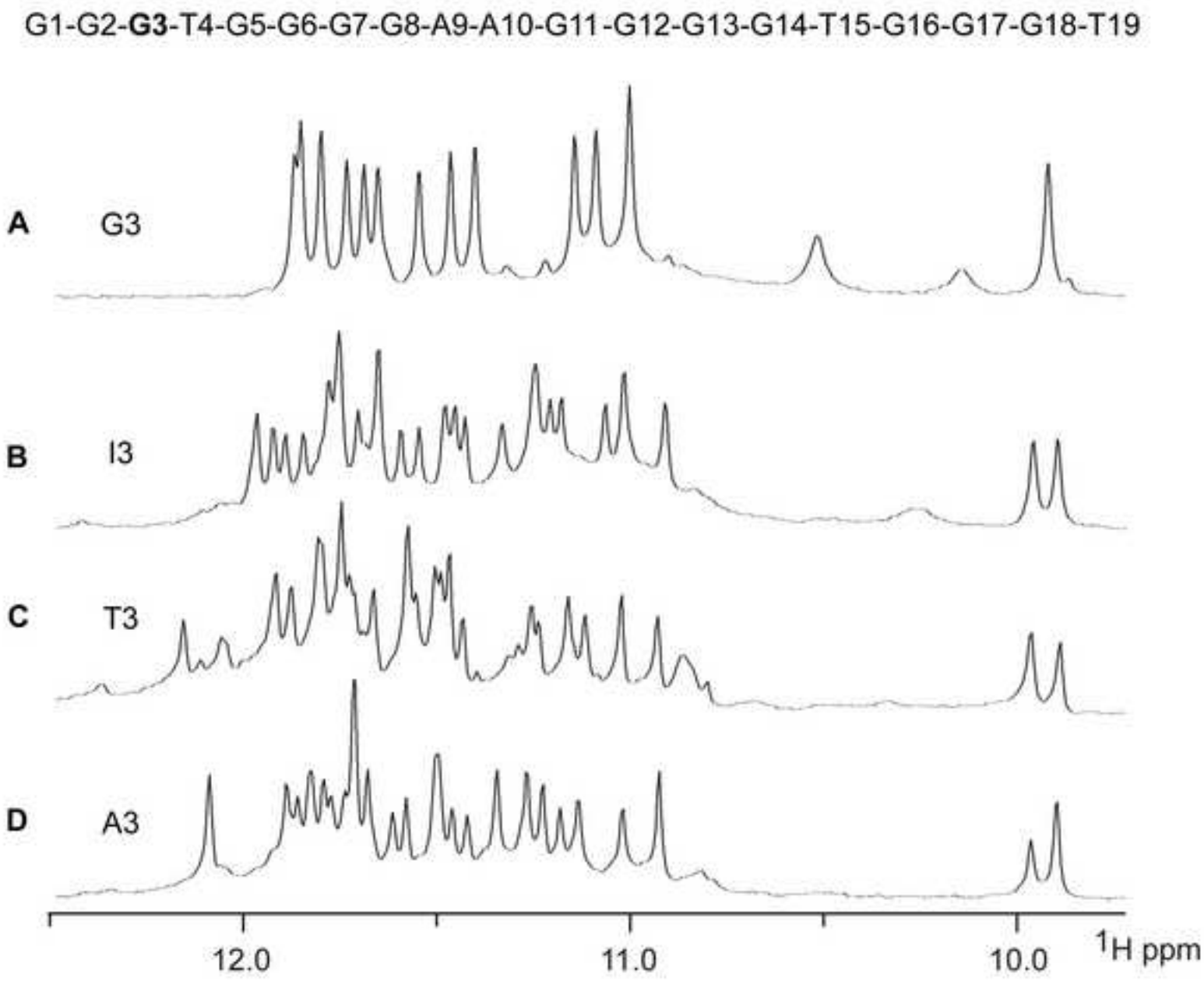
G1-G2-G3-T4-G5-G6-G7-G8-A9-A10-G11-G12-G13-G14-T15-G16-G17-G18-T19
A
G3
B
I3
C
T3
D
A3
12.0
11.0
10.0
1H ppm

Figure S3

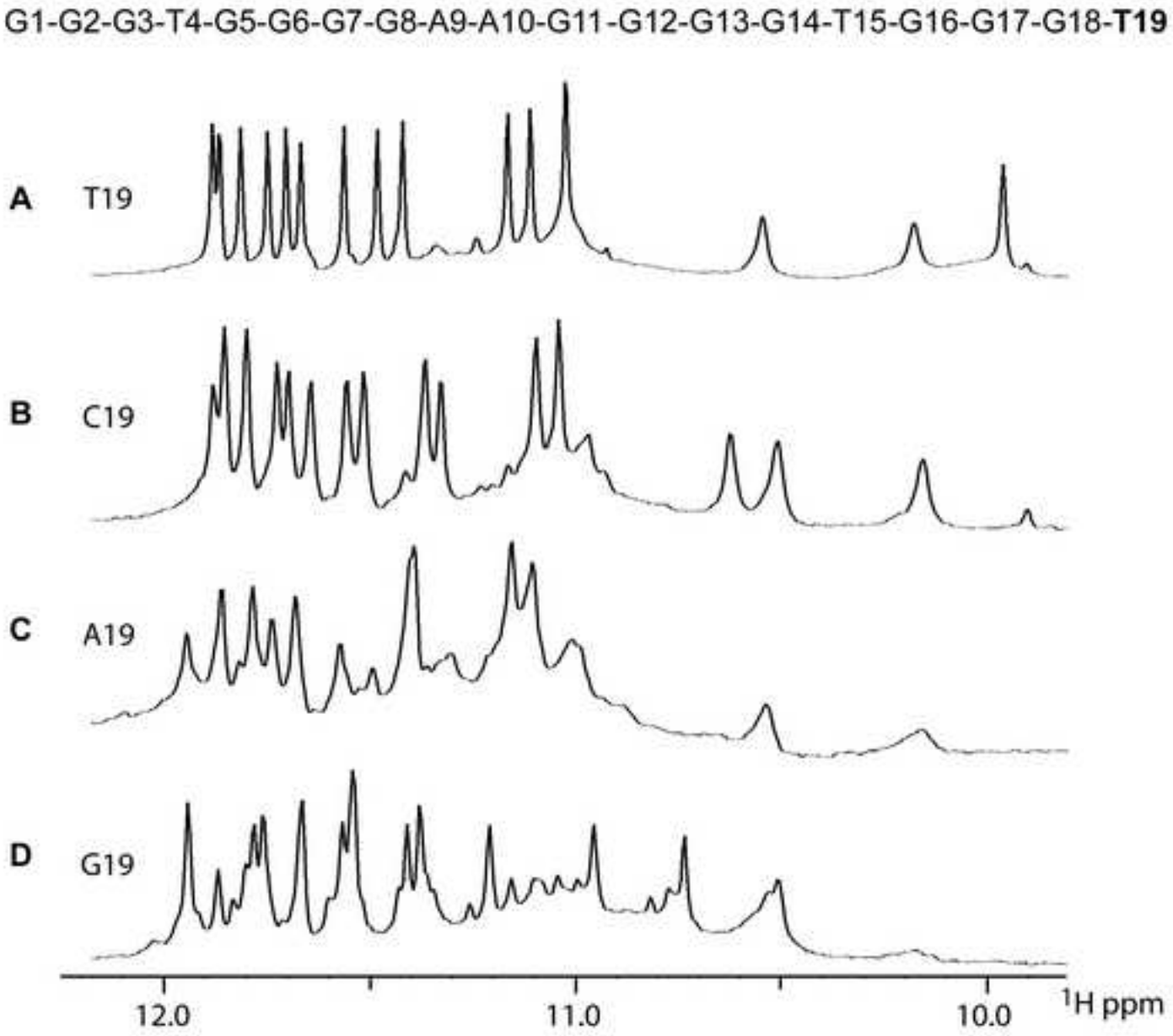
G1-G2-G3-T4-G5-G6-G7-G8-A9-A10-G11-G12-G13-G14-T15-G16-G17-G18-T19
A
T19
B
C19
C
A19
D
G19
12.0
11.0
10.0
1H ppm

Figure S4

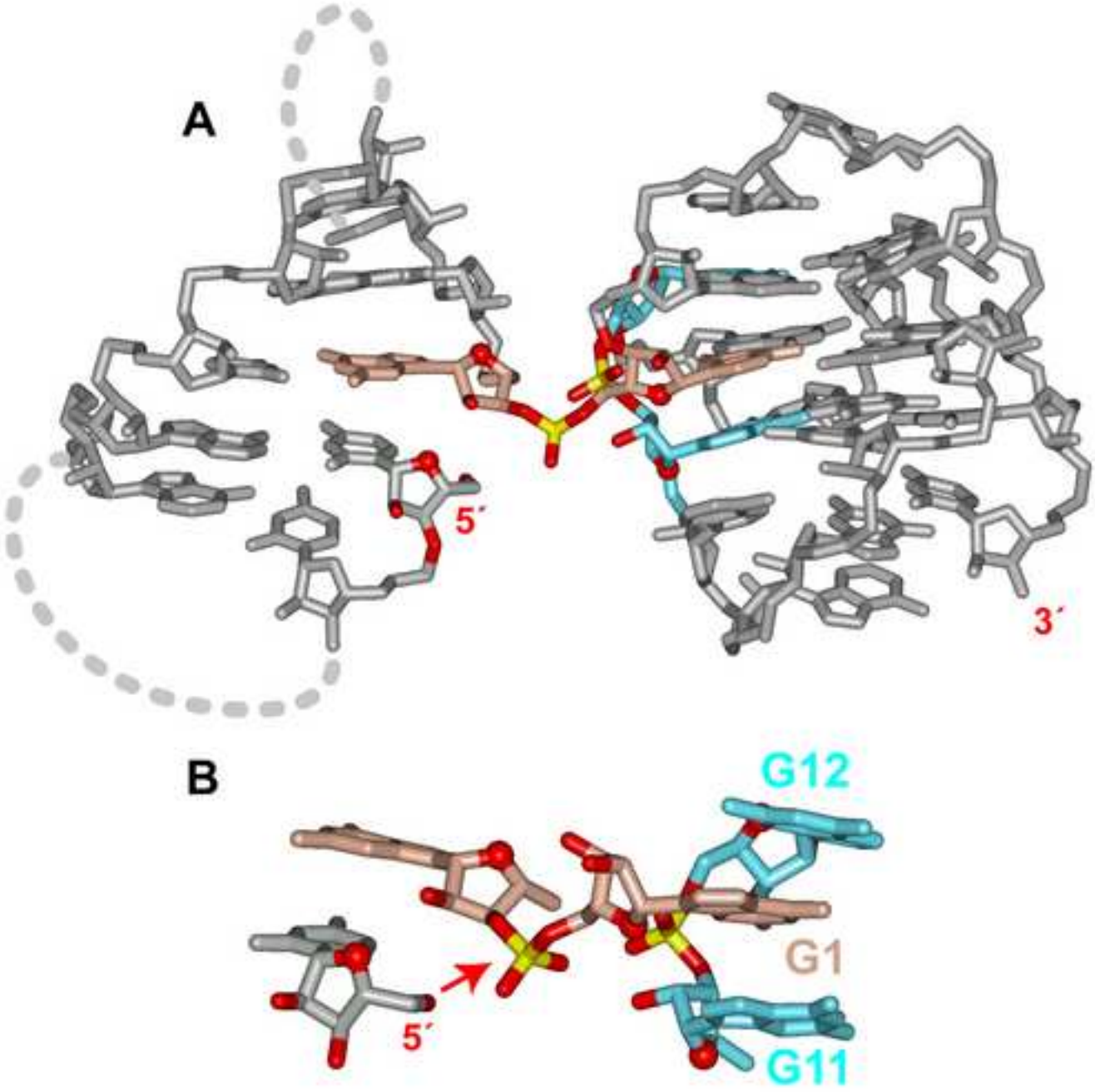

Figure S5