Supporting Information For

Novel Conformation of an RNA Structural Switch

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CONTENTS

Methods

RNA Preparation and Purification. Oligonucleotides, rGACGAGUGUCA, rGACGAGUGAGA, and rCUCGAGUGUCA, were purchased from Dharmacon RNA Technologies or IDT. Oligonucleotides with a modified G, rGACGA^{Br}GUGUCA, rGACGA^{Br}GUGAGA, rCUCGA^{Br}GUGUCA, rGACGA^{Me}GUGUCA, rGACGA^{Me}GUGAGA, and rCUCGA^{Me}GUGUCA, were synthesized as previously described.[1,2] NMR buffer is 20 mM sodium phosphate with 0.05 mM Na_2EDTA and 80 mM NaCl at pH ~6.1, which has been filter-sterilized with Corning 0.22 μm PES filters. Samples for NMR spectra were dissolved in RNase-free water, dialyzed against NMR buffer for 48 h at 4 ºC, dried down and resuspended with RNase-free 90% water/10% D_2O, in a volume equal to that removed from dialysis. For spectra in D_2O, oligonucleotides were lyophilized and resuspended in 99.96% D_2O three times, then lyophilized and resuspended in 99.996% D_2O from Cambridge Isotopes.

NMR Spectroscopy. NMR spectra were taken on a Varian Inova spectrometer at 600 MHz. Spectra of modified and unmodified self-complementary duplexes (rGACGAGUGUCA)$_2$, (rGACGA^{Br}GUGUCA)$_2$, and (rGACGA^{Me}GUGUCA)$_2$ were acquired as previously described.[3] Spectra of non-self-complementary duplexes 5'GACGAGUGAGA / 3'ACUGUGAGCUC and 5'GACGA^{Br}GUGAGA / 3'ACUGU^{Br}GAGCUC were acquired in 95% H_2O/5% D_2O. 2D NOESY spectra at 1 °C with mixing times of 50, 100, 150, and 400 msec were acquired along with TOCSY (mix time = 28 msec) and ^{13}C-^{1}H HMQC spectra. Additional NOESYs at -4 °C were acquired with mixing times of 50 and 150 msec. Spectra of non-self-complementary 5'GACGA^{Me}GUGAGA / 3'ACUGU^{Me}GAGCUC were acquired in 100% D_2O. NOESY spectra at 1 °C with mixing times of 50, 150, and 400 msec were acquired along with a TOCSY (mix time = 12 msec) spectrum. NMRPipe[4] was used for data processing and Sparky[5] was used for peak assignments and integration. Previously published assignments[3] for (rGACGAGUGUCA)$_2$, (rGACGA^{Br}GUGUCA)$_2$, and (rGACGA^{Me}GUGUCA)$_2$ are listed in Table S1 along with assignments made for the non-self-complementary duplexes.

Restraint Generation. Distance restraints for modeling of (rGACGAGUGUCA)$_2$ were generated from cross-peaks in 75 ms mixing time NOESY spectra using $(1/r)^6$ scaling with distance bounds that allow a three-fold error in the NOE volume determined for non-exchangeable protons, or ±40% of distances derived for exchangeable protons. A majority of restraints were obtained from spectra of (rGACGA^{Br}GUGUCA)$_2$, with ambiguous restraints assigned as described in the text from spectra of non-self-complementary 5'GACGA^{Br}GUGAGA / 3'ACUGU^{Br}GAGCUC. Other NOEs, especially those involving G6H8, were obtained from spectra of (rGACGAGUGUCA)$_2$, (rGACGA^{Me}GUGUCA)$_2$, 5'GACGAGUGAGA / 3'ACUGUGAGCUC, and 5'GACGA^{Me}GUGAGA / 3'ACUGU^{Me}GAGCUC. The cross-peaks for H5-H6 (2.45 Å), H1´-H2´ (2.75 Å), GH1-C amino, and UH3-AH2 in the Watson-Crick stems were used for reference volumes. Watson-Crick hydrogen-bond restraints were applied

between stem bases G1-C10*, A2-U9*, C3-G8*, G8-C3*, U9-A2*, and C10-G1* as indicated by imino proton cross-peaks in NOESY spectra. Dihedral angle restraints were determined based on sugar proton and phosphorus scalar couplings taken from TOCSY, NOESY, and ^{1}H-^{31}P HETCOR spectra. Strong H3´-H4´ peaks and the absence of H1´-H2´ peaks in the TOCSY spectra indicated C3´-endo sugar puckers ($\delta \sim 81°$). H4´-H5´/H5´´ J-couplings less than 2 Hz indicated γ was not in the trans or g^- conformation and so the γ dihedral angle was restrained to g^+ ($\gamma \sim 60°$). ^{31}P(n+1)-H3´(n) J-couplings greater than 10 Hz indicated $\varepsilon \sim -115°$ (excluding g^+). Weak ^{31}P-H5´/H5´´ cross-peaks in ^{1}H-^{31}P HETCOR spectra (J-coupling < 6 Hz) indicated β in the trans conformation (~165°). Couplings within the stem residues were within these typical A-form ranges. Consequently, backbone dihedrals in the stems, including α and ζ, were restrained to A-form values: α (-65 ± 90°), β (165 ± 75°), γ (60 ± 60°), ε (-115 ± 125°), ζ (-70 ± 90°) as defined previously.[6] Backbone angles from C3 ζ through G8 γ were not restrained with the exceptionof δ and γ. δ was restrained to 140 ± 20° (C2'-endo sugar pucker) for residues 4-7, as indicated by J(H1´-H2´) ≥ 6 Hz and J(H3´-H4´) < 2 Hz for these four residues. γ was restrained to the trans conformation (180 ± 60°) for G6 as indicated by J(H4´-H5´/H5´´) greater than 6 Hz; γ was restrained to the g^+ conformation (60 ± 60°) for residues 4, 5, and 8 as indicated by J(H4´-H5´/H5´´) less than 2 Hz (Figure S1). A strong U7 ^{31}P-H5´/H5´´ cross-peak in a ^{1}H-^{31}P HETCOR spectrum indicates β is in a conformation other than trans at least some of the time.[3] This is consistent with $\beta = -125 \pm 20°$ in 16 of the 19 accepted structures and with this extra-helical residue being flexible as indicated by narrow aromatic resonances. NOEs observed for A5H8-G4*H8 and G6H2'-A5H4' are consistent with the model, however these restraints were not applied in the simulated annealing. G6H2'-A5H4' is likely due to spin diffusion through G6H5'/5''. Assignments for H5´ and H5´´ were not stereospecific. α and ζ were not restrained although the phosphorous shifts of A5 and U7 are notably shifted from A-form. Glycosidic bonds were set to be *anti* ($\chi = 255 \pm 85°$) for all residues not exhibiting large H8/H6-H1´ NOE cross-peaks. G4 and G6 had large H8-H1´ cross-peaks so were set to be *syn* ($\chi = 20 \pm 80°$).

Structure Calculation. Simulated annealing and molecular dynamics calculations of (rGACGAGUGUCA)$_2$ were carried out over 100 ps using distance and dihedral angle restraints generated from NMR data. The structures were calculated with the program AMBER (version 10, ff99 force field)[7] with the following protocol using Generalized-Born implicit solvent with ion screening equivalent to salt concentration of 0.1 M, dielectric radii offset by 0.13 Å, nonbonded interaction cutoff of 15 Å, and step size 1 fs: (1) the system was heated to 2000 K for 5 ps with tight temperature coupling and NMR restraints ramped from 10% to 100% during the first 3 ps, (2) the temperature was gradually reduced to 100 K over the next 93 ps with weak temperature coupling, (3) final cooling to 0 K for 2 ps with tight temperature coupling. Full NOE and dihedral scale factors were 30 kcal/mol Å^2 and 30 kcal/mol rad^2. The procedure was repeated 600 times with different velocity seeds in an A-form starting structure derived from minimization of coordinates generated in the program *nucgen*. Of these 600 simulations, 27 did not violate the NMR restraints. These 27 structures were subjected to another round of simulated annealing in which the system was heated to only 600 K and hydrogen bonds restraining the G4/G6* and G6/G4* pairs were removed. The 19 resulting structures with no distance violations greater than 0.1 Å and restraint energies less than 2.5 kcal/mol are deposited with the RCSB Protein Data Bank with ID code 2LX1. NOEs corresponding to the major conformation were obtained from 100 ms (D_2O) and 150 ms (H_2O) NOESY spectra of the unmodified duplex and were used to generate 118 distance restraints to compare to the final structure. The lack of violations of these restraints (Table S4) confirms the accuracy of the model generated using data from brominated and methylated duplexes.

Table S1. Proton chemical shifts of six duplexes studied. Imino, H6/H8, H2/H5, H1', and A5H5'/H5" protons only. GAGU means $(5'GACGAGUGUCA)_2$; GAGUnc means 5'GACGAGUGAGA/3'ACUGUGAGCUC. "major" means major conformation. "8Br" and "8Me" mean 8-bromo and 8-methyl substitution, respectively, in the G6 and G6* position.

	Original Duplex (black in Figure 1)											Modified End of Modified Duplex (green in Figure 1)										
	G1	A2	C3	G4	A5*	G6*	U7*	G8*	U9*	C10*	A11*											
GAGU-major																						
imino	11.94			10.17		13.38		12.99	14.55													
H6/H8	8.11	7.95	7.16	7.62	7.60	7.82	8.09	7.80	7.86	7.70	8.01											
H2/H5		7.58	4.98		7.68		6.01		5.12	5.56	7.28											
H1'	5.85	5.95	5.06	5.66	5.73	5.89	6.21	5.06	5.46	5.49	5.92											
H5'					2.80																	
H5"					3.63																	
GAGU-8Br																						
imino	11.92			10.28		13.32		12.98	14.49													
H6/H8	8.10	7.94	7.12	7.57	7.62		8.12	7.80	7.85	7.68	7.98											
H2/H5		7.57	4.97		7.70		6.00		5.14	5.55	7.26											
H1'	5.83	5.92		5.64	5.75	5.93	6.23	5.20	5.45	5.47	5.89											
H5'					2.86																	
H5"					3.67																	
GAGU-8Me																						
imino	11.92			10.25		13.18		12.98	14.52													
H6/H8	8.09	7.93	7.13	7.57	7.60		8.11	7.77	7.82	7.68	7.98											
H2/H5		7.55	4.94		7.68		6.00		5.14	5.54	7.25											
H1'	5.83	5.91		5.64	5.74	5.74	6.24	5.21	5.44	5.46	5.89											
H5'					2.76																	
H5"					3.61							C1*	U2*	C3*	G4*	A5	G6	U7	G8	A9	G10	A11
GAGUnc-major																						
imino	11.93			10.19		13.36		12.98	14.53				14.43		10.29		13.15		12.11		12.85	
H6/H8	8.10	7.93	7.15	7.61	7.60	7.80	8.07	7.79	7.83	7.68	7.99	8.17	8.00	7.65	7.54	7.56	7.78	8.03	7.72	7.74	6.93	7.74
H2/H5		7.57	4.98	0.00	7.70		6.00		5.10	5.55	7.27	6.02	5.35	5.48		7.70		5.99		7.23		7.90
H1'	5.84	5.93	5.03	5.64	5.72	5.86	6.18	5.04	5.47	5.47	5.90	5.60	5.44	5.24	5.62	5.68	5.87	6.17	5.07	5.84	5.41	5.94
H5'					2.78											2.74						
H5"					3.62											3.60						
GAGUnc-8Br																						
imino	11.93			10.20		13.38		12.98	14.50				14.42		10.31		13.24		12.11		12.84	
H6/H8	8.10	7.95	7.12	7.58	7.59		8.14	7.80	7.85	7.69	7.98	8.16	8.00	7.62	7.51	7.54		8.09	7.75	7.72	6.93	7.73
H2/H5		7.57	4.98		7.67		6.01		5.15	5.55	7.26	6.02	5.34	5.46		7.67		6.00		7.22		7.89
H1'	5.83	5.92		5.63	5.75	5.93	6.24	5.20	5.45	5.47	5.90	5.59	5.44	5.23	5.62	5.70	5.92	6.22	5.20	5.85	5.41	5.94
H5'					2.89											2.83						
H5"					3.67											3.63						
GAGUnc-8Me																						
imino																						
H6/H8	8.08	7.93	7.13		7.55		8.11	7.77	7.83	7.68	7.98	8.15	7.99	7.61		7.60		8.07	7.72	7.68	6.91	7.72
H2/H5		7.54	4.93				5.99		5.14	5.53	7.25	6.00	5.33	5.44				5.98		7.20		7.88
H1'	5.82	5.90	5.02		5.70	5.73	6.24	5.21	5.44	5.45	5.88	5.58	5.42	5.21		5.74	5.72	6.23	5.21	5.83	5.39	5.92
H5'					2.71											2.77						
H5"					3.57											3.60						

Table S2. Dihedral and distance restraints derived from NMR data and used in simulated annealing calculation for 5'GACGAGUGUCA/3'ACUGUGAGCAG. For every restraint listed there is an equivalent restraint originating on the opposite strand of the self-complementary duplex.

Dihedral Restraints

2 ADE ALPHA: (1 RG5 O3')-(2 RA P)-(2 RA O5')-(2 RA C5') -155.0 25.0
3 CYT ALPHA: (2 RA O3')-(3 RC P)-(3 RC O5')-(3 RC C5') -155.0 25.0
9 URA ALPHA: (8 RG O3')-(9 RU P)-(9 RU O5')-(9 RU C5') -155.0 25.0
10 CYT ALPHA: (9 RU O3')-(10 RC P)-(10 RC O5')-(10 RC C5') -155.0 25.0
11 ADE ALPHA: (10 RC O3')-(11 RA3 P)-(11 RA3 O5')-(11 RA3 C5') -155.0 25.0
2 ADE BETA: (2 RA P)-(2 RA O5')-(2 RA C5')-(2 RA C4') 125.0 205.0
3 CYT BETA: (3 RC P)-(3 RC O5')-(3 RC C5')-(3 RC C4') 125.0 205.0
9 URA BETA: (9 RU P)-(9 RU O5')-(9 RU C5')-(9 RU C4') 125.0 205.0
10 CYT BETA: (10 RC P)-(10 RC O5')-(10 RC C5')-(10 RC C4') 125.0 205.0
11 ADE BETA: (11 RA3 P)-(11 RA3 O5')-(11 RA3 C5')-(11 RA3 C4') 125.0 205.0
1 GUA GAMMA: (1 RG5 O5')-(1 RG5 C5')-(1 RG5 C4')-(1 RG5 C3') -20.0 140.0
2 ADE GAMMA: (2 RA O5')-(2 RA C5')-(2 RA C4')-(2 RA C3') 0.0 120.0
3 CYT GAMMA: (3 RC O5')-(3 RC C5')-(3 RC C4')-(3 RC C3') 0.0 120.0
4 ADE GAMMA: (4 RA O5')-(4 RA C5')-(4 RA C4')-(4 RA C3') 0.0 120.0
5 ADE GAMMA: (5 RA O5')-(5 RA C5')-(5 RA C4')-(5 RA C3') 0.0 120.0
6 ADE GAMMA: (6 RA O5')-(6 RA C5')-(6 RA C4')-(6 RA C3') 120.0 240.0
8 ADE GAMMA: (8 RA O5')-(8 RA C5')-(8 RA C4')-(8 RA C3') 0.0 120.0
9 URA GAMMA: (9 RU O5')-(9 RU C5')-(9 RU C4')-(9 RU C3') 0.0 120.0
10 CYT GAMMA: (10 RC O5')-(10 RC C5')-(10 RC C4')-(10 RC C3') 0.0 120.0
11 ADE GAMMA: (11 RA3 O5')-(11 RA3 C5')-(11 RA3 C4')-(11 RA3 C3') 0.0 120.0
1 GUA DELTA: (1 RG5 C5')-(1 RG5 C4')-(1 RG5 C3')-(1 RG5 O3') 40.0 120.0
2 ADE DELTA: (2 RA C5')-(2 RA C4')-(2 RA C3')-(2 RA O3') 40.0 120.0
3 CYT DELTA: (3 RC C5')-(3 RC C4')-(3 RC C3')-(3 RC O3') 40.0 120.0
4 GUA DELTA: (4 RG C5')-(4 RG C4')-(4 RG C3')-(4 RG O3') 120.0 160.0
5 ADE DELTA: (5 RA C5')-(5 RA C4')-(5 RA C3')-(5 RA O3') 120.0 160.0
6 GUA DELTA: (6 RG C5')-(6 RG C4')-(6 RG C3')-(6 RG O3') 120.0 160.0
7 URA DELTA: (7 RU C5')-(7 RU C4')-(7 RU C3')-(7 RU O3') 120.0 160.0
8 GUA DELTA: (8 RG C5')-(8 RG C4')-(8 RG C3')-(8 RG O3') 40.0 120.0
9 URA DELTA: (9 RU C5')-(9 RU C4')-(9 RU C3')-(9 RU O3') 40.0 120.0
10 CYT DELTA: (10 RC C5')-(10 RC C4')-(10 RC C3')-(10 RC O3') 40.0 120.0
11 ADE DELTA: (11 RA3 C5')-(11 RA3 C4')-(11 RA3 C3')-(11 RA3 O3') 40.0 120.0
1 GUA EPSILN: (1 RG5 C4')-(1 RG5 C3')-(1 RG5 O3')-(2 RA P) -240.0 10.0
2 ADE EPSILN: (2 RA C4')-(2 RA C3')-(2 RA O3')-(3 RC P) -240.0 10.0
3 CYT EPSILN: (3 RC C4')-(3 RC C3')-(3 RC O3')-(4 RG P) -240.0 10.0
8 GUA EPSILN: (8 RG C4')-(8 RG C3')-(8 RG O3')-(9 RU P) -240.0 10.0
9 URA EPSILN: (9 RU C4')-(9 RU C3')-(9 RU O3')-(10 RC P) -240.0 10.0
10 CYT EPSILN: (10 RC C4')-(10 RC C3')-(10 RC O3')-(11 RA3 P) -240.0 10.0
1 GUA ZETA: (1 RG5 C3')-(1 RG5 O3')-(2 RA P)-(2 RA O5') -160.0 20.0
2 ADE ZETA: (2 RA C3')-(2 RA O3')-(3 RC P)-(3 RC O5') -160.0 20.0
8 GUA ZETA: (8 RG C3')-(8 RG O3')-(9 RU P)-(9 RU O5') -160.0 20.0
9 URA ZETA: (9 RU C3')-(9 RU O3')-(10 RC P)-(10 RC O5') -160.0 20.0
10 CYT ZETA: (10 RC C3')-(10 RC O3')-(11 RA3 P)-(11 RA3 O5') -160.0 20.0
1 GUA CHI: (1 RG5 O4')-(1 RG5 C1')-(1 RG5 N9)-(1 RG5 C4) 170.0 340.0
2 ADE CHI: (2 RA O4')-(2 RA C1')-(2 RA N9)-(2 RA C4) 170.0 340.0
3 CYT CHI: (3 RC O4')-(3 RC C1')-(3 RC N1)-(3 RC C2) 170.0 340.0
4 GUA CHI: (4 RG O4')-(4 RG C1')-(4 RG N9)-(4 RG C4) -60.0 100.0
5 ADE CHI: (5 RA O4')-(5 RA C1')-(5 RA N9)-(5 RA C4) 170.0 280.0
6 GUA CHI: (6 RG O4')-(6 RG C1')-(6 RG N9)-(6 RG C4) -60.0 110.0
7 URA CHI: (7 RU O4')-(7 RU C1')-(7 RU N1)-(7 RU C2) 170.0 340.0
8 GUA CHI: (8 RG O4')-(8 RG C1')-(8 RG N9)-(8 RG C4) 170.0 340.0
9 URA CHI: (9 RU O4')-(9 RU C1')-(9 RU N1)-(9 RU C2) 170.0 340.0
10 CYT CHI: (10 RC O4')-(10 RC C1')-(10 RC N1)-(10 RC C2) 170.0 340.0
11 ADE CHI: (11 RA3 O4')-(11 RA3 C1')-(11 RA3 N9)-(11 RA3 C4) 170.0 340.0

Distance Restraints (Å)	**From 8-Br G6 or 8-Me G6** used in structure calculation	**From unmodified duplex** not used in structure calculation
From H2O spectrum		
# 1 RG5 H1 20 RU H3	3.21 7.51	2.21 5.17
# 1 RG5 H1 21 RC H42	1.54 3.60	
# 1 RG5 H1 22 RA3 H2	2.52 5.90	2.24 5.22
# 1 RG5 H22 22 RA3 H1'	2.00 5.00	
# 2 RA H1' 1 RG5 H22	2.00 5.00	
# 2 RA H2 1 RG5 H1	2.13 4.97	2.53 5.91
# 3 RC H3' 4 RG H1	1.66 4.20	2.02 4.72
# 3 RC H41 4 RG H1	2.22 5.20	
# 3 RC H42 20 RU H3	2.10 4.92	
# 3 RC H5 4 RG H1	1.76 4.10	
# 3 RC H6 4 RG H1	2.27 5.29	2.56 5.98
# 4 RG H1' 17 RG H1	3.00 6.00	3.00 7.00
# 4 RG H8 19 RG H1	2.43 5.67	2.29 5.35
# 5 RA H1' 6 RG H1	3.00 6.00	2.06 4.80
# 5 RA H2 6 RG H1	3.50 18.50	
# 5 RA H8 6 RG H1	1.68 3.92	1.90 4.42
# 8 RG H1 6 RG H1	2.00 4.68	2.05 4.79
# 8 RG H1 9 RU H3	2.14 5.00	1.92 4.48
# 8 RG H1 14 RC H42	1.41 3.31	
# 8 RG H8 6 RG H1	3.50 18.50	
# 9 RU H1' 8 RG H22	2.00 5.00	2.23 5.21
# 9 RU H1' 9 RU H3	2.83 6.61	
# 9 RU H3 13 RA H2	1.72 4.02	
# 9 RU H3 14 RC H42	2.10 4.92	
# 10 RC H1' 9 RU H3	2.82 6.58	
# 10 RC H41 9 RU H3	2.93 6.83	
# 10 RC H42 9 RU H3	2.31 5.41	
# 10 RC H5 9 RU H3	3.09 7.19	2.43 5.67
# 11 RA3 H1' 12 RG5 H22	2.00 5.00	
# 15 RG H1 5 RA H2	2.02 4.72	2.58 6.02
# 15 RG H8 6 RG H1	1.63 3.79	1.72 4.02
# 16 RA H2 6 RG H1	3.50 18.50	
# 13 RA H2 8 RG H1		2.26 5.28
From D2O Spectrum		
# 1 RG5 H1' 1 RG5 H3'	2.70 3.89	
# 1 RG5 H1' 1 RG5 H4'	2.72 3.91	
# 1 RG5 H1' 1 RG5 H8	2.86 4.12	2.65 3.82
# 1 RG5 H1' 22 RA3 H2	3.19 4.60	3.24 4.67
# 1 RG5 H2' 1 RG5 H8	2.90 4.18	2.61 3.76
# 2 RA H1' 1 RG5 H2'	3.00 4.32	
# 1 RG5 H2' 2 RA H8	2.00 2.88	2.07 2.98
# 1 RG5 H3' 1 RG5 H8	2.40 3.46	2.24 3.23
# 1 RG5 H4' 1 RG5 H8	3.11 4.48	
# 2 RA H1' 2 RA H2	4.70 6.77	
# 2 RA H1' 2 RA H8	3.17 4.56	3.22 4.63
# 2 RA H2' 3 RC H5	3.26 4.69	
# 2 RA H2' 3 RC H6	2.27 3.26	2.17 3.13
# 2 RA H1' 2 RA H3'	2.70 3.89	
# 2 RA H3' 2 RA H8	2.35 3.38	
# 2 RA H3' 3 RC H6	2.71 3.90	2.58 3.72
# 2 RA H1' 2 RA H4'	2.70 3.89	
# 2 RA H4' 2 RA H8	3.16 4.55	
# 2 RA H8 1 RG5 H8	3.20 4.61	
# 2 RA H2 21 RC H1'	2.67 3.84	2.71 3.90
# 2 RA H2 3 RC H1'		3.24 4.67

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#   3  RC  H2'  3  RC   H6          3.61  5.20                  3.37  4.85
#   3  RC  H3'  3  RC   H6          2.51  3.61                  2.53  3.65
#   3  RC  H3'  4  RG   H8          3.50 11.50
#   3  RC  H4'  3  RC   H6          3.58  5.16                  3.38  4.87
#   3  RC   H6  4  RG   H8          5.00 25.00

#   4  RG  H1'  4  RG  H2'          2.66  3.84
#   4  RG  H1'  4  RG  H3'          2.90  4.18
#   4  RG  H1'  4  RG  H4'          2.90  4.18
#   4  RG  H1'  4  RG   H8          1.98  2.86                  2.02  2.92
#   4  RG  H2'  4  RG   H8          3.00  6.00                  3.00  7.00
#   4  RG  H3'  4  RG   H8          3.50 11.50                  3.00  7.00
#   4  RG  H3'  5  RA  H2'          2.00  3.80
#   4  RG  H3'  5  RA   H8          3.50 11.50                  3.00  7.00
#   4  RG  H4'  4  RG   H8          3.00  8.00
#   4  RG  H2'  5  RA   H8                                      3.50 23.50

#   5  RA  H1'  5  RA  H2'          2.49  3.59
#   5  RA  H1'  5  RA  H3'          2.96  4.26
#   5  RA  H1'  5  RA  H4'          2.81  4.04                  2.98  4.30
#   5  RA  H1'  5  RA   H8          3.09  4.45                  3.16  4.55
#   5  RA  H2'  5  RA   H8          2.52  3.64                  2.66  3.83
#   5  RA  H4'  5  RA   H8          3.79  5.46                  3.69  5.32
#   5  RA   H8  5  RA  H3'                                      3.00  7.00
#   5  RA   H8  5  RA  H5'                                      3.37  4.86

#   6  RG  H2'  5  RA  H4'          2.88  4.15                  3.07  4.43
#   6  RG  H1'  6  RG  H2'          2.44  3.52
#   6  RG  H2'  6  RG  H3'          2.05  2.95
#   6  RG  H2'  6  RG  H4'          2.77  3.98
#   6  RG  H2'  8  RG   H8          2.78  4.01                  2.56  3.68
#   6  RG  H1'  6  RG  H3'          3.58  5.16
#   6  RG  H1'  6  RG  H4'          2.71  3.91
#   6  RG  H1'  5  RA   H2                                      3.50 23.50
#   8  RG   H8  6  RG  H1'                                      2.86  4.12
#   6  RG  H3'  8  RG   H8                                      3.82  5.51
#   6  RG  H2'  6  RG H5"                                       2.04  2.94

#   7  RU  H1'  7  RU  H2'          2.58  3.72
#   7  RU  H1'  7  RU   H6          2.66  3.83                  2.95  4.25
#   7  RU  H3'  7  RU  H2'          2.05  2.95
#   7  RU  H2'  7  RU   H6          1.93  2.78                  2.14  3.08
#   6  RG  H1'  7  RU  H3'          2.37  3.42
#   7  RU  H3'  7  RU   H6          2.83  4.08
#   7  RU  H3'  8  RG   H8          3.01  4.33
#   7  RU  H3'  7  RU  H4'          2.05  2.95
#   7  RU   H5  7  RU  H2'          2.60  7.00
#   7  RU  H1'  7  RU  H4'                                      3.15  4.54

#   8  RG  H1'  8  RG  H4'          2.68  3.86
#   8  RG  H1'  8  RG   H8          2.94  4.24                  3.05  4.39
#   8  RG  H2'  8  RG   H8          3.17  4.57
#   9  RU   H5  8  RG  H2'          2.88  4.15
#   8  RG  H2'  9  RU   H6          1.97  2.84                  1.97  2.84
#   8  RG  H1'  8  RG  H3'          3.00  4.32
#   8  RG  H3'  9  RU   H6          2.30  3.31

#   9  RU  H1'  9  RU  H3'          2.64  3.80
#   9  RU  H1'  9  RU   H6          3.01  4.33
#   9  RU  H2'  9  RU   H6          2.59  3.73
#   9  RU  H2' 10  RC   H6          1.97  2.83                  2.06  2.96
#   9  RU  H3'  9  RU   H6          2.16  3.11                  1.94  2.80
#   9  RU  H3' 10  RC   H6          2.42  3.49
#   9  RU   H5  8  RG   H8          3.02  4.36                  3.30  4.75
```

```
#   10  RC    H5   9   RU    H5          2.97   4.28
#   10  RC    H1'  9   RU    H2'         2.74   3.95
#   10  RC    H1'  10  RC    H3'         3.00   4.32
#   10  RC    H1'  10  RC    H6          3.00   4.32          3.04   4.38
#   10  RC    H2'  10  RC    H6          2.46   3.54
#   10  RC    H2'  11  RA3   H8          2.07   2.99
#   10  RC    H3'  10  RC    H6          2.21   3.18
#   10  RC    H3'  11  RA3   H8          2.28   3.29
#   10  RC    H5   9   RU    H2'         2.63   3.79
#   10  RC    H5   9   RU    H3'         3.07   4.42
#   10  RC    H5   9   RU    H6          3.61   5.20
#   10  RC    H5   10  RC    H2'         3.44   4.96
#   10  RC    H5   10  RC    H3'         2.92   4.20
#   10  RC    H6   11  RA3   H8          3.32   4.79

#   11  RA3   H1'  10  RC    H2'         3.46   4.98
#   11  RA3   H1'  11  RA3   H2          3.52   5.06
#   11  RA3   H1'  11  RA3   H3'         2.80   4.03
#   11  RA3   H1'  11  RA3   H4'         2.72   3.92
#   11  RA3   H1'  11  RA3   H8          3.00   4.32          2.97   4.27
#   11  RA3   H1'  11  RA3   H2'         2.13   3.07
#   11  RA3   H2'  11  RA3   H8          2.57   3.71
#   11  RA3   H3'  11  RA3   H8          2.17   3.12
#   11  RA3   H4'  11  RA3   H8          3.13   4.51
G6H8 restraints from -8Me
sample
#    6  RG    H8   5   RA    H2          5.00  20.00
#    6  RG    H8   5   RA    H2'         4.00  19.00
#    6  RG    H8   5   RA    H4'         3.00   7.00          4.17   6.01
#    6  RG    H8   5   RA    H8          3.00   7.00
#    6  RG    H8   6   RG    H1'         1.80   2.90          2.11   3.04
#    6  RG    H8   6   RG    H2'         3.20  23.20          3.17   4.57
#    6  RG    H8   7   RU    H1'         3.20  23.20
#    6  RG    H8   7   RU    H6          3.50  11.50
#    6  RG    H8   8   RG    H8          3.00   5.00
Watson-Crick Hydrogen bonds
#    1  RG5   H1   21  RC    N3          1.80   2.40
#    1  RG5   O6   21  RC    H42         1.80   2.40
#    1  RG5   H22  21  RC    O2          1.80   2.40
#    2  RA    N1   20  RU    H3          1.80   2.40
#    2  RA    H62  20  RU    O4          1.80   2.40
#    3  RC    N3   19  RG    H1          1.80   2.40
#    3  RC    H42  19  RG    O6          1.80   2.40
#    3  RC    O2   19  RG    H22         1.80   2.40
G4-G6* hydrogen bonds used in
initial 600 simulated annealing
calculations
#    6  RG    H1   15  RG    N7          1.80   2.80
#    6  RG    H22  15  RG    O6          1.80   2.80
#    6  RG    O6   15  RG    H8          1.80   3.30
```

Table S3. AMBER input file for simulated annealing calculation of structures.

```
simulated annealing protocol, 100 ps
 &cntrl
   nstlim=100000, pencut=0.001, nmropt=1,  ntpr=1000, ntt=1, ntwx=1000, igb=1, saltcon=0.1, offset=0.13, cut=15.0, ntb=0,
vlimit=10,
   TEMPI=2000.0, ig=71272,
 /
 &ewald
   eedmeth=5,
 /
#
```

```
#Simple simulated annealing algorithm:
#
#from steps 0 to 5000: heat the system to 2000K
#from steps 5001-98000: re-cool to low temperatures with long tautp
#from steps 98001-100000: final cooling with short tautp
#
 &wt type='TEMP0', istep1=0,istep2=5000,value1=2000.,  value2=2000.,    /
 &wt type='TEMP0', istep1=5001, istep2=98000, value1=2000.0, value2=100.0,     /
 &wt type='TEMP0', istep1=98001, istep2=100000, value1=0.0,  value2=0.0,     /

 &wt type='TAUTP', istep1=0,istep2=5000,value1=0.4,  value2=0.4,     /
 &wt type='TAUTP', istep1=5001,istep2=98000,value1=4.0, value2=4.0,     /
 &wt type='TAUTP', istep1=98001,istep2=99000,value1=1.0, value2=1.0,     /
 &wt type='TAUTP', istep1=99001,istep2=100000,value1=0.1, value2=0.05,    /

 &wt type='REST', istep1=0,istep2=3000,value1=0.1, value2=1.0,  /
 &wt type='REST', istep1=3001,istep2=100000,value1=1.0, value2=1.0,  /

 &wt type='END'  /
LISTOUT=POUT
DISANG=RSTr
```

Table S4. Structure statistics for 5'GACGAGUGUCA/3'ACUGUGAGCAG.

	8-Br G6 duplex	**Unmodified duplex (major conformation)**
Distance restraints		
Total	261	118
Intraresidue	130	52
Interresidue	131	40
Long range (i-j >= 2)	30	26
Violations (>0.1Å)	0	0 (relative to structure from 8-Br G6 restraints)
Hydrogen bond restraints	16	
Dihedral angle restraints	106	
Violations (>2°)	0 (excluding 5' terminal gamma)	
RMSD to restraints		
NOEs	0.003 Å	
Dihedral angles	0.1°	
Energies (kcal/mole)		
Total (system)	-5004±2	
NOE	1.4±0.2	
Dihedral	0.18±0.01	
Structures calculated/accepted	600/19	
RMSD relative to mean structure (Å)		
Heavy atoms	0.86	
All atoms	0.89	

Figure S1. H-H TOCSY at 1 °C of (rGACGA^{Br}GUGUCA)$_2$. Mixing time is 36 ms. Red dots indicate H4'-H5' and H4'-H5'' cross-peaks, or lack of cross-peak, for residues 4-7. Blue Xs indicate all H5'-H5" cross-peaks. Only G6 shows a strong scalar H4'-H5' and H4'-H5'' coupling, indicating that for this residue $\gamma \sim 180°$.

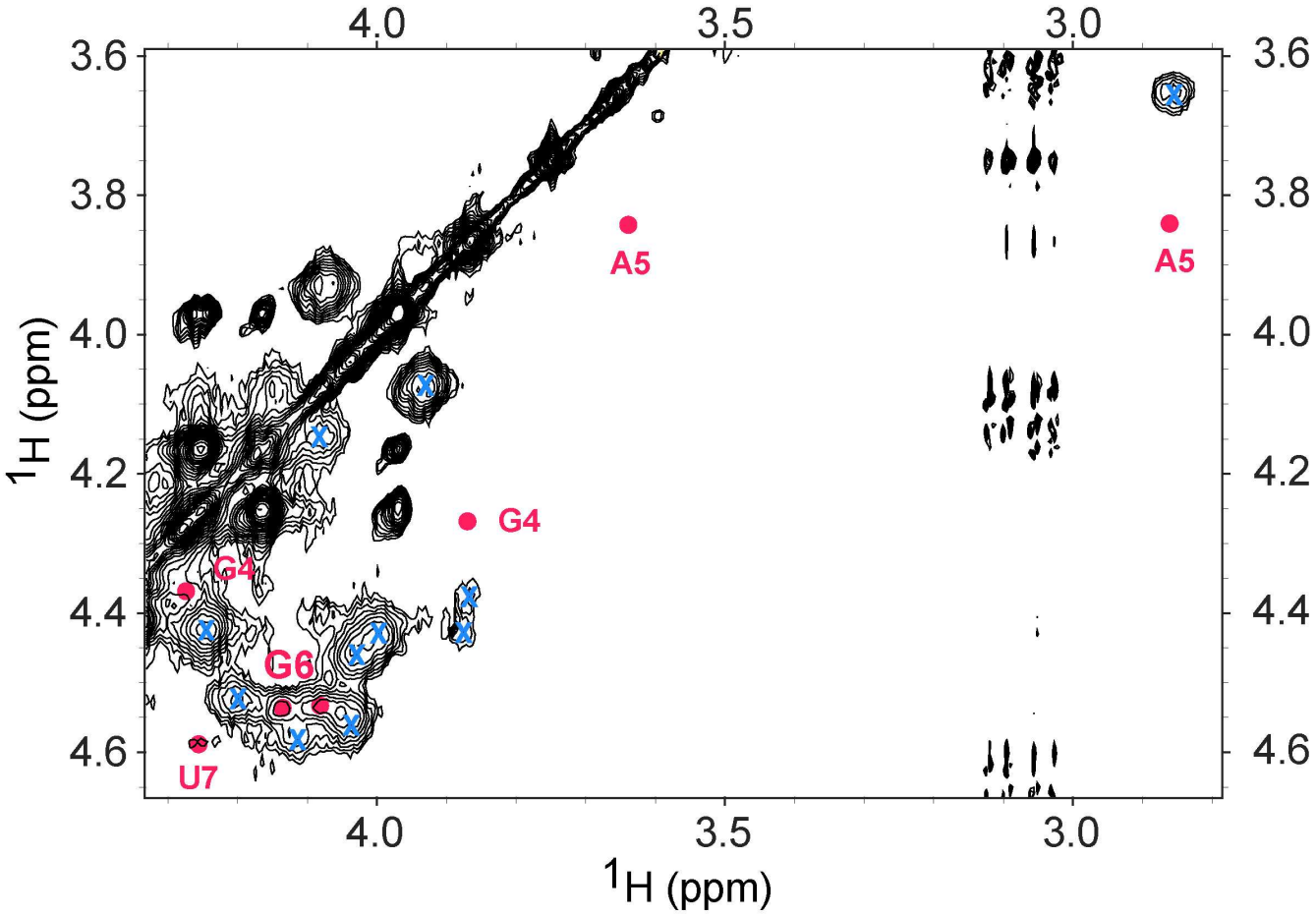


Figure S2. Same 1D imino NMR spectra as in Figure 1 with the addition of 8-Me G6 modified duplex (rGACGA^{Me}GUGUCA)$_2$ (bottom).

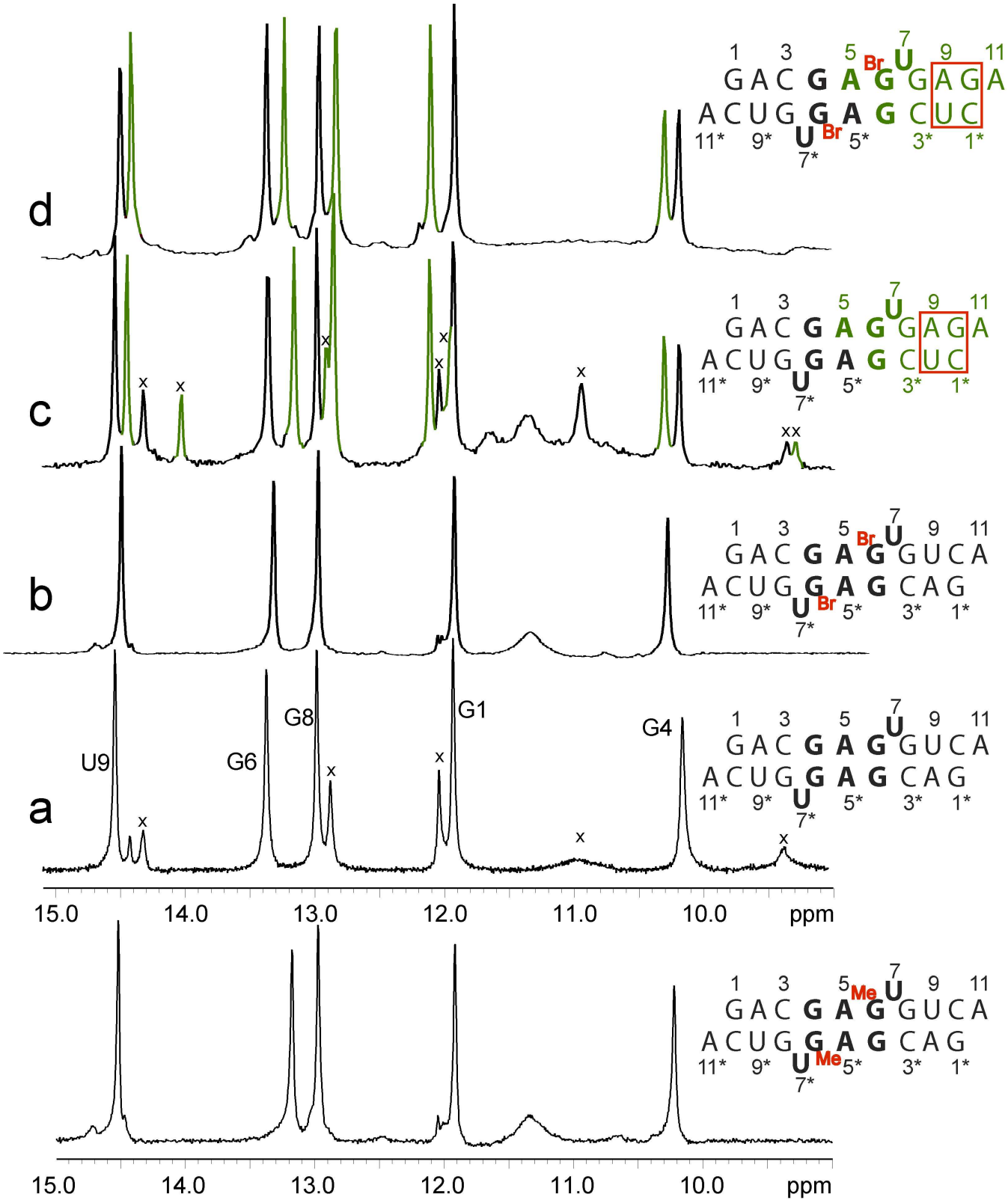


Figure S3. Correlation plot of NOE distances obtained from brominated and unmodified duplexes, (r GACGA^{Br}GUGUCA)$_2$ and (r GACGAGUGUCA)$_2$, respectively. Loop nucleotides include G4-U7.

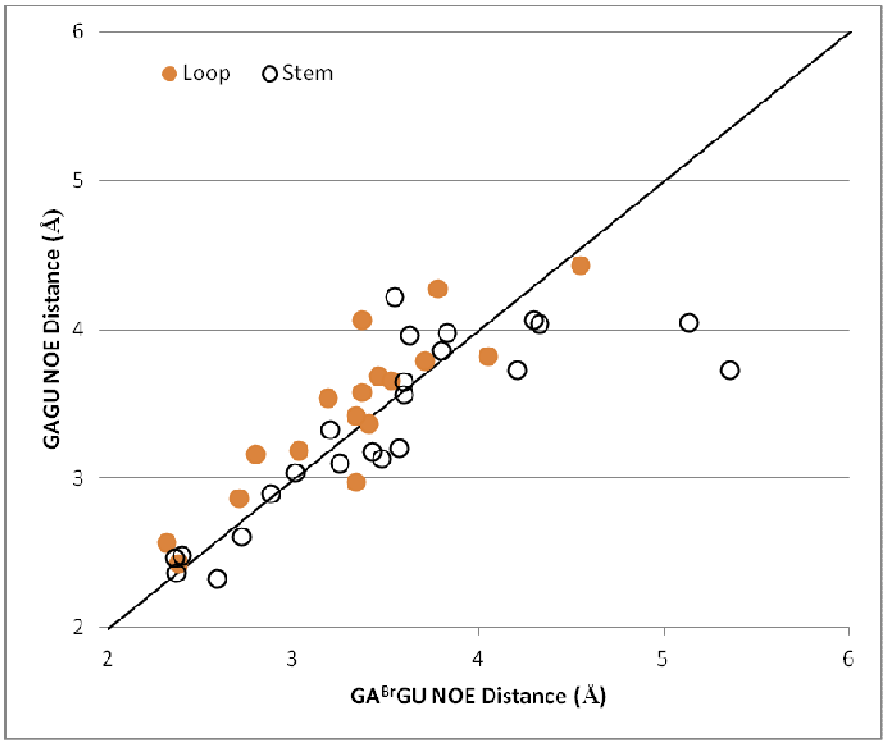


Figure S4. 2D NOE spectra showing sugar-sugar cross-peaks due to inverted A5 sugar residues. All spectra acquired at 1 °C. GAGU 8-Br G6 is (rGAC<u>GA^{Br}GU</u>GUCA)$_2$, and GAGU-NSC 8-Br G6 is 5'GAC<u>GA^{Br}GU</u>GAGA / 3'ACUG<u>U^{Br}GAG</u>CUC (non-self-complementary).

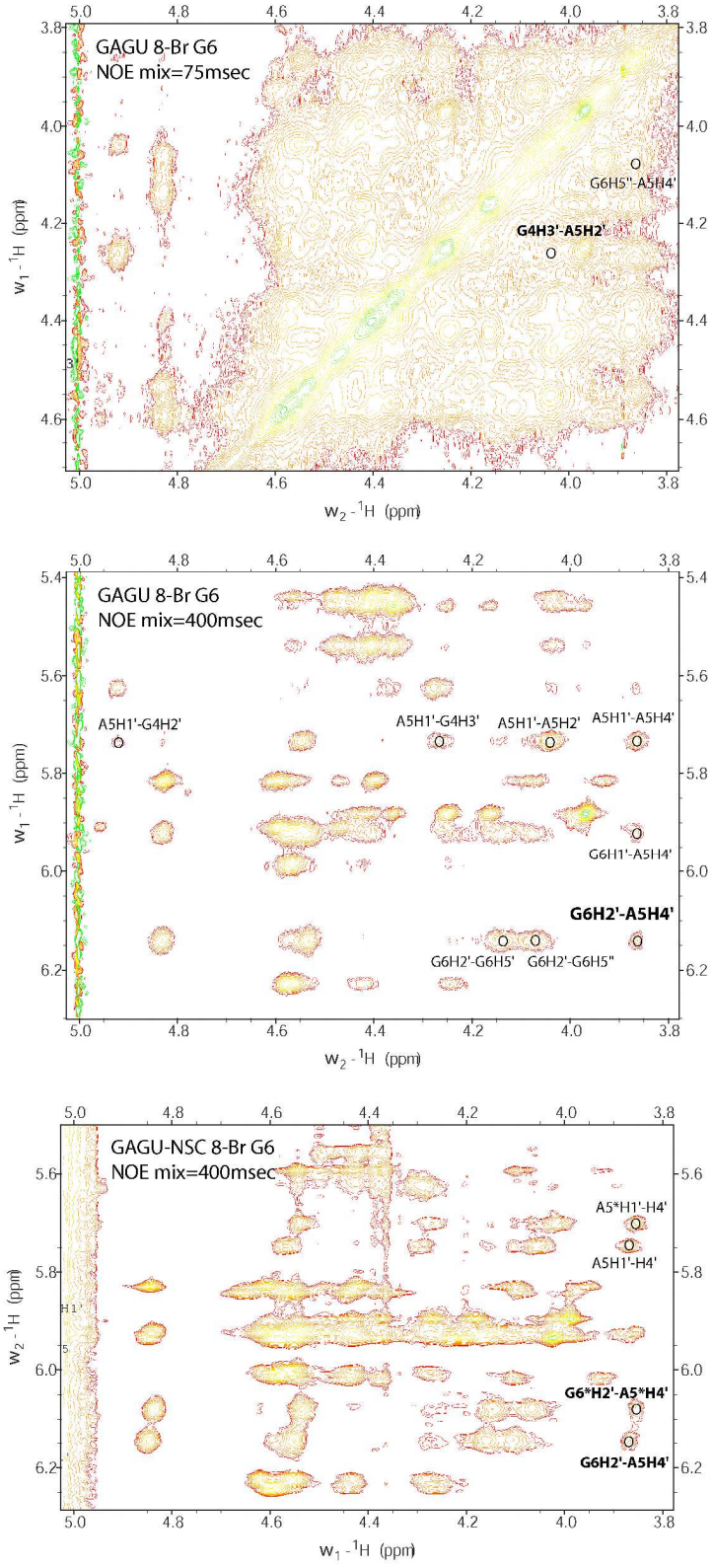
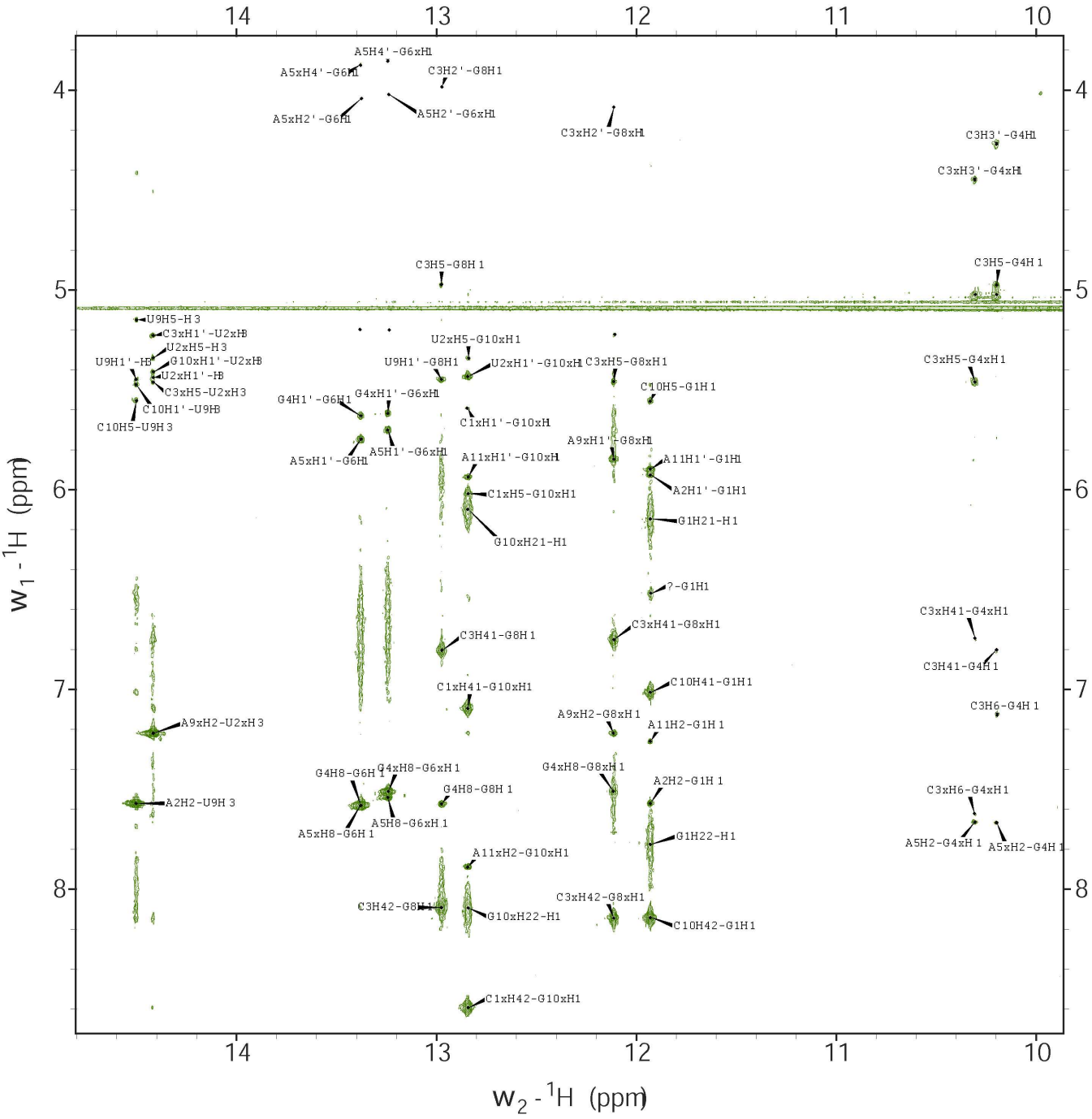


Figure S5. 2D Watergate-NOESY spectrum of 5'GAC<u>GA^{Br}GU</u>GAGA / 3'ACUG<u>U^{Br}GAG</u>CUC at 1 °C and 150 msec mixing time showing imino cross-peaks to aromatic and sugar protons.



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