

HOBs methods for enhancing resolution and sensitivity in small DNA oligonucleotide acid NMR studies

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Supporting Information

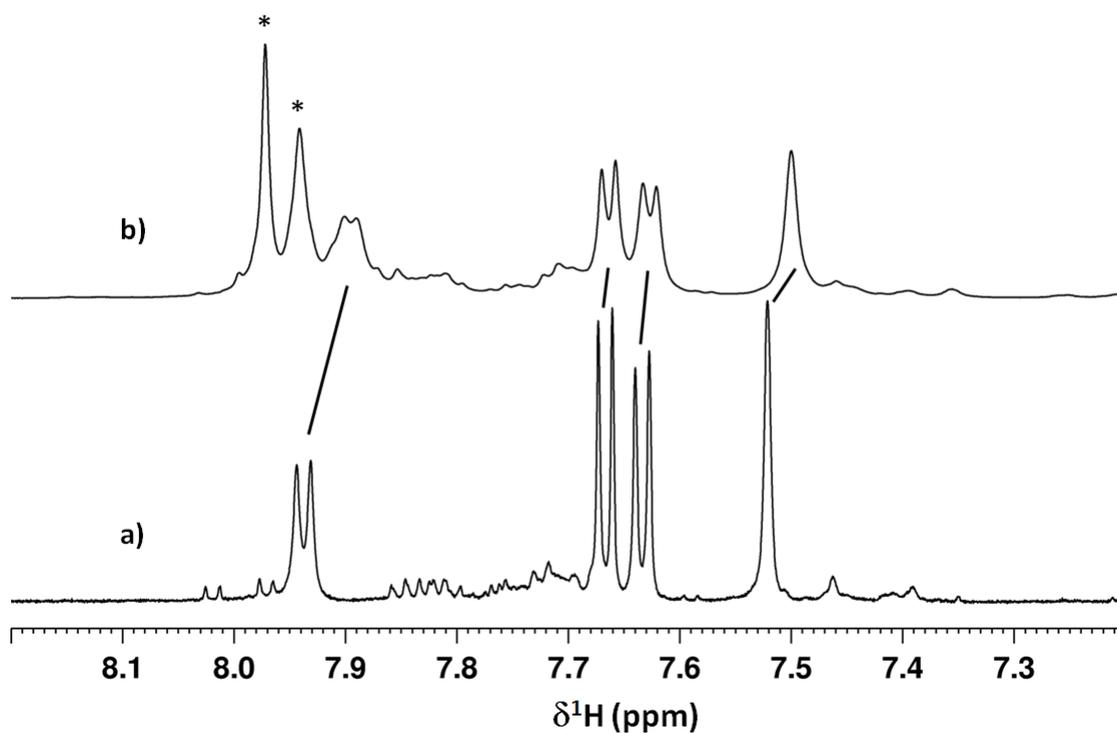


Figure S1: Aromatic region of the 600 MHz 1D ^1H NMR spectrum of d(GCCTGC) in D_2O . **a)** Aged sample; **b)** freshly dissolved sample. Asterisks indicate G H8 resonances which exchange with deuterium over extended periods of time. Note that sample concentrations differ in the two cases resulting in linewidth and small chemical shift differences for those proton resonances that are common to both spectra.

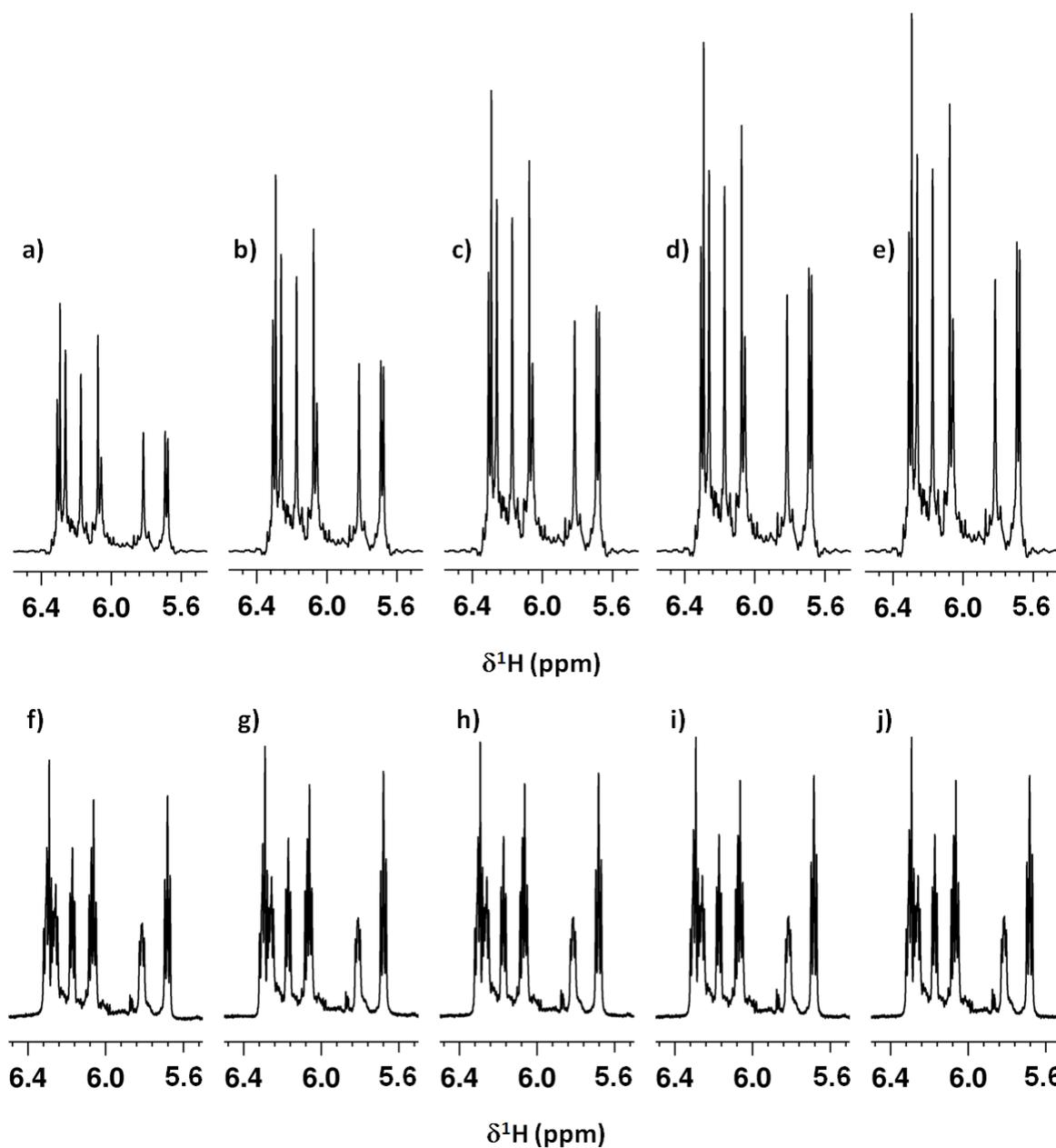


Figure S2: Effects on the appearance of the H1'/H5 resonance region of the 1D ^1H -HOBS NMR spectrum (**a-e**) and standard 1D ^1H NMR spectrum (**f-j**) of d(GCCTGC) using different relaxation delays, d_1 . **a)** and **f)**: $d_1 = 1$ s; **b)** and **g)**: $d_1 = 2$ s; **c)** and **h)**: $d_1 = 3$ s; **d)** and **i)**: $d_1 = 4$ s; **e)** and **j)**: $d_1 = 5$ s. For the HOBS data, the H1'/H5 resonance region of the NMR spectrum was selected using a 600 Hz band-selective reburp 180° pulse offset from the transmitter frequency by 710.5 Hz. Presaturation of the residual HOD resonance was achieved at the transmitter frequency using low power irradiation during part of d_1 in each case. The receiver gain was set to 2050 for the series **a) – e)** and to 228 for the series **f) – j)**. All spectra in each series are presented with the same vertical scaling factor.

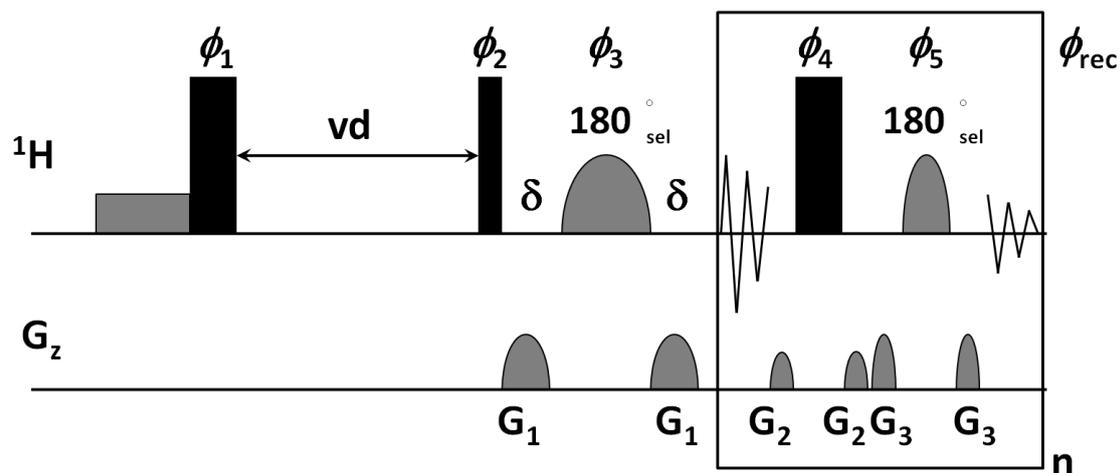


Figure S3: Pulse sequence used for acquiring T_1 inversion recovery data using a HOBS data acquisition scheme with presaturation to suppress the residual H_2O signal from the aqueous sample. \mathbf{vd} is a variable delay set according to a list of inter-pulse delay values (see main text for details). $\phi_1 = x, -x$; $\phi_2 = \phi_3 = x, -x, -x, x, y, -y, -y, y$; $\phi_4 = x, -x$; $\phi_5 = -x, x$; $\phi_{\text{rec}} = x, -x, -x, x, y, -y, -y, y$; $G_1 = 60\%$, $G_2 = 23\%$ and $G_3 = 41\%$ with a 1 ms duration (δ). Sine-shaped gradient pulses were used throughout. Narrow black bars correspond to hard 90° pulses, wide black bars correspond to hard 180° pulses and soft 180° pulses are as indicated. A band-selective reburp pulse was used for selective inversion of magnetization. A low-powered pulse was applied at the solvent resonance offset during the recycle delay for suppression purposes.

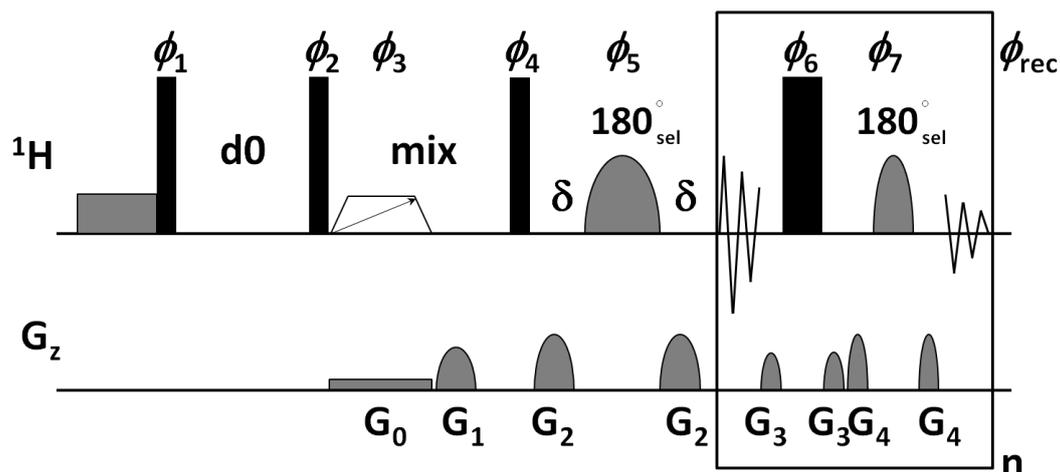


Figure S4: Pulse sequence used for acquiring zero-quantum suppressed 2D band-selective NOESY NMR spectra with homonuclear broadband proton decoupling. $\phi_1 = x, -x$; $\phi_2 = x [8], -x [8]$; $\phi_3 = x$; $\phi_4 = x, x, -x, -x, y, y, -y, -y$; $\phi_5 = x, -x, -x, x, y, -y, -y, y$; $\phi_6 = -x, x$; $\phi_7 = x, -x$; $\phi_{\text{rec}} = x, -x, -x, x, y, -y, -y, y$; $G_0 = 10\%$, $G_1 = 40\%$, $G_2 = 60\%$, $G_3 = 23\%$ and $G_4 = 41\%$ with a 1 ms duration (δ). Sine-shaped gradient pulses were used throughout. Narrow black bars correspond to hard 90° pulses, wide black bars correspond to hard 180° pulses and soft 180° pulses are as indicated. A band-selective reburp pulse was used for selective inversion of magnetization. A low-powered pulse was applied at the solvent resonance offset during the recycle delay for suppression purposes. A smoothed-CHIRP pulse of duration 20 ms was applied during the mixing time for ZQC suppression.

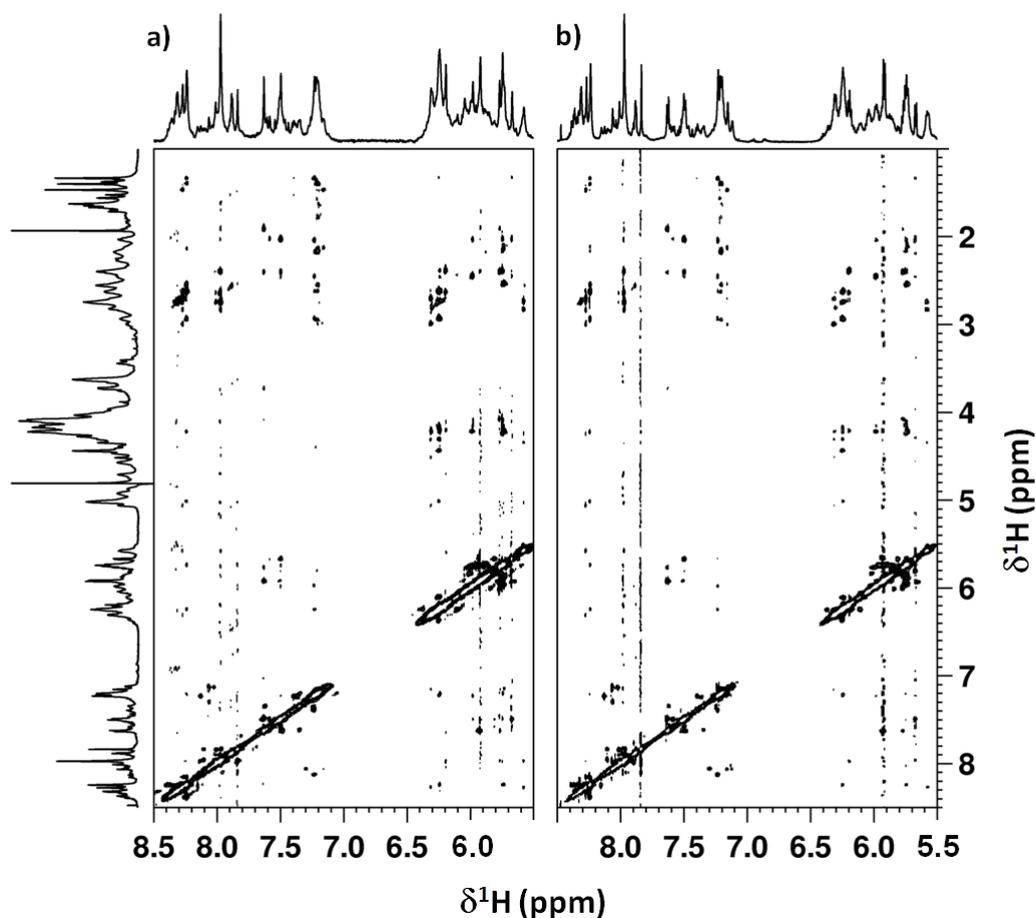


Figure S5: Combined data sets from band-selected- **(a)** and HOBS- **(b)** 2D [^1H , ^1H] NOESY for the 10-mer DNA duplex d(CGATATATCG)₂. Separate excitation regions were used for each half of each data set which were then co-added following full data processing. Band-selective reburp pulses were used to select H1'/H5 (bandwidth = 600 Hz) and aromatic/H6 (bandwidth = 990 Hz) regions separately. 1D ^1H NMR spectra appropriate to each type of data acquisition scheme are shown as ω_2 projections in each case. The full 1D ^1H NMR spectrum is shown as the ω_1 projection.

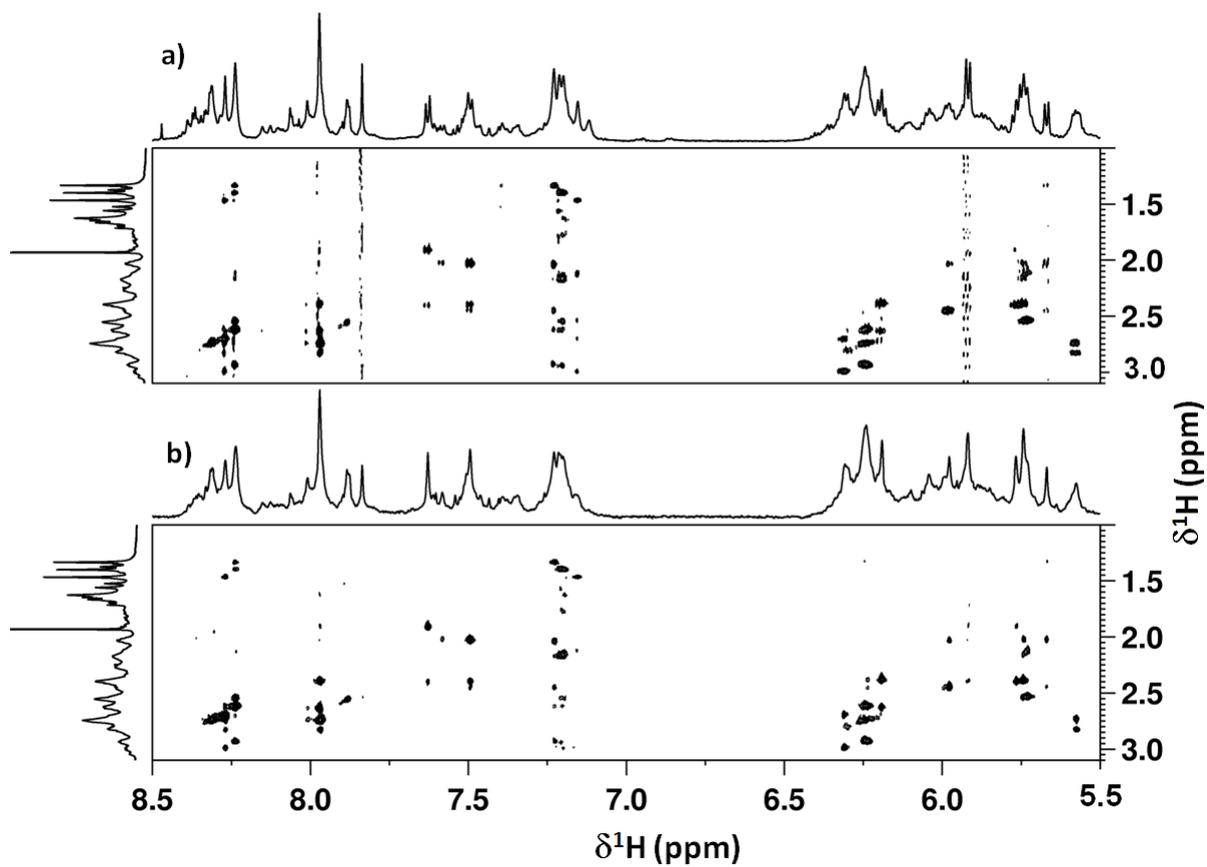


Figure S6: Expansion of the aromatic and H1'/H5 to H2'/H2''/CH₃ cross-peak regions of combined data sets from band selective- **(a)** and HOBS- **(b)** 2D [¹H, ¹H] NOESY NMR spectra for the 10-mer DNA duplex d(CGATATATCG)₂. Other features are as detailed in the caption to **Figure S5**.

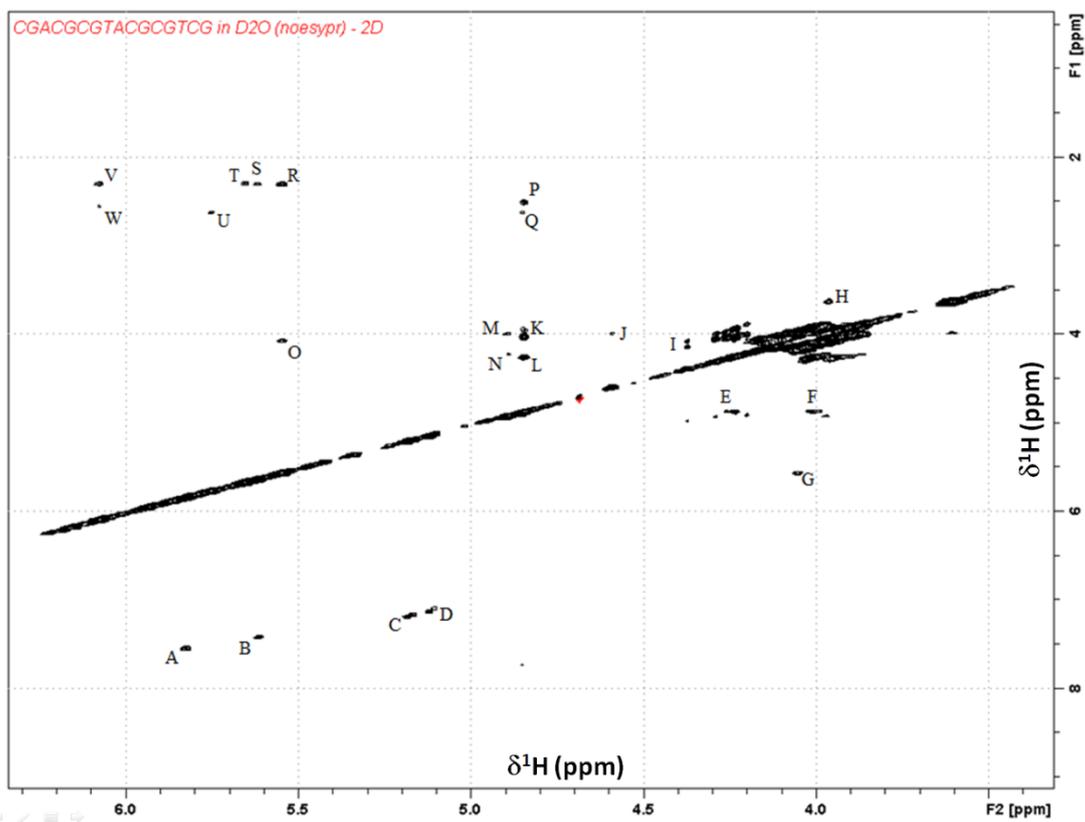


Figure S7: Strip plot in the H1'/H5/H3'/H4'/H5'/H5'' resonance region of the 600 MHz 2D [^1H , ^1H] NOESY NMR spectrum of the 16-mer DNA duplex $d(\text{CGACGCGTACGCGTTCG})_2$ in D_2O displayed with a high threshold level. Peaks used in the volume integration study described in the main text are shown labelled for ease of visualizing the reported data.

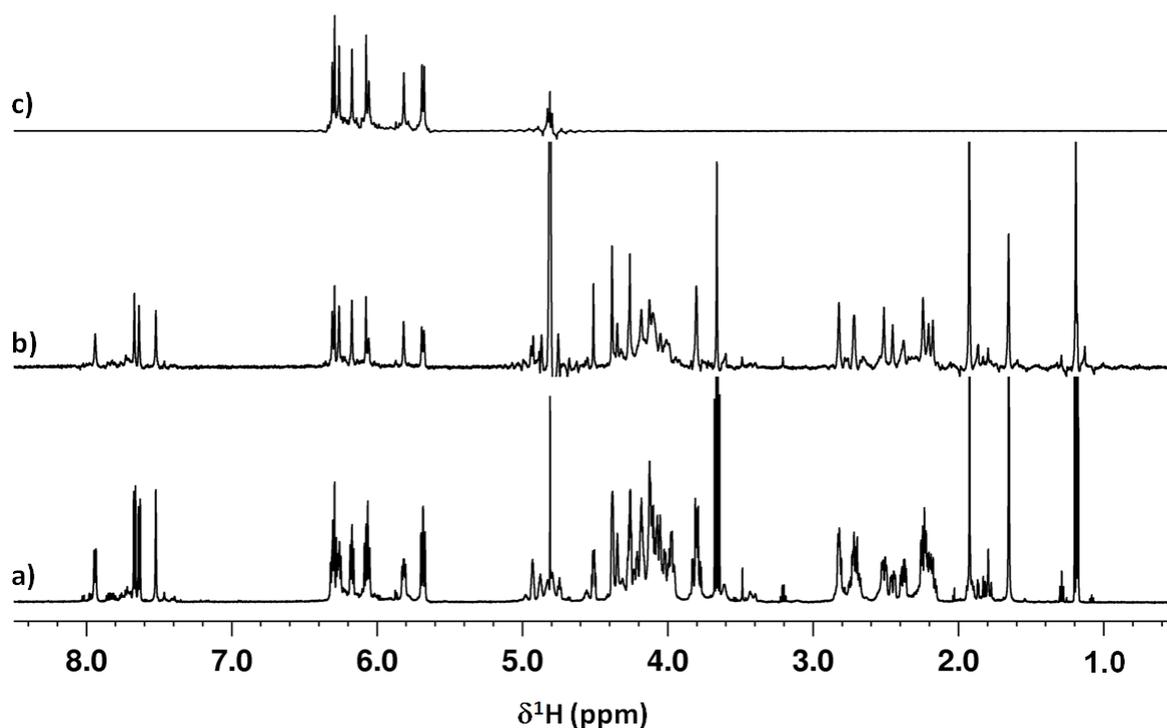


Figure S8: 600 MHz 1D ^1H NMR spectra for d(GCCTGC) in D_2O using various data acquisition schemes. **a)** Standard 1D ^1H NMR spectrum using a single pulse/acquire acquisition scheme incorporating solvent presaturation – number of transients (ns) = 16; **b)** Pure-shift (HOBB) 1D ^1H NMR spectrum with solvent presaturation – ns = 4096; **c)** HOBS -1D ^1H NMR spectrum using a 600 Hz band-selective reburp pulse offset by 710.5 Hz from the transmitter frequency – ns = 64. No solvent presaturation was used during the acquisition of the HOBS data.

Table S1: HOBS-¹H T₁ inversion-recovery relaxation data for H5 and H1' resonances from the oligonucleotide d(GCCTGC).

H5/H1' Resonance Region										
Peak ^a	T ₁ by Integration (s)					T ₁ by Peak Picking (s)				
	#1	#2	#3	Avg.	St. Dev.	#1	#2	#3	Avg.	St. Dev.
1	1.845	1.852	1.846	1.848	0.004	1.673	1.670	1.665	1.669	0.004
2	2.059	2.051	2.054	2.055	0.004	2.166	2.165	2.177	2.169	0.007
3	1.742	1.746	1.747	1.745	0.003	1.707	1.699	1.712	1.706	0.007
4	1.852	1.853	1.861	1.855	0.005	1.918	1.905	1.924	1.916	0.010
5	1.908	1.910	1.931	1.916	0.013	1.852	1.860	1.857	1.856	0.004
6	2.405	2.386	2.347	2.379	0.030	2.520	2.512	2.614	2.549	0.057
7	2.114	2.119	2.125	2.119	0.006	1.878	1.885	1.893	1.885	0.008
8	2.882	2.853	2.854	2.863	0.016	2.804	2.822	2.842	2.823	0.019
9	2.699	2.751	2.807	2.752	0.054	2.986	2.980	2.976	2.981	0.005

^aPeak Assignments: 1 – C⁶H1', δ¹H = 6.308 ppm; 2 – C³H1', δ¹H = 6.292 ppm; 3 – C²H1', δ¹H = 6.291 ppm; 4 – G⁵H1', δ¹H = 6.173 ppm; 5 – G¹H1', δ¹H = 6.075 ppm; 6 – C³H5, δ¹H = 6.057 ppm; 7 – T⁴H1', δ¹H = 5.816 ppm; 8 – C⁶H5, δ¹H = 5.691 ppm; 9 – C²H5, δ¹H = 5.675 ppm. Measurements are reported from experiments carried out in triplicate.

Table S2: Standard-¹H T₁ inversion-recovery relaxation data for H5 and H1' resonances from the oligonucleotide d(GCCTGC).

H5/H1' Resonance Region										
Peak ^a	T ₁ by Integration (s)					T ₁ by Peak Picking (s)				
	#1	#2	#3	Avg.	St. Dev.	#1	#2	#3	Avg.	St. Dev.
1	---	---	---	---	---	1.849	1.826	1.819	1.831	0.016
2	---	---	---	---	---	2.072	2.060	2.051	2.061	0.011
3	---	---	---	---	---	1.725	1.702	1.686	1.704	0.020
4	1.864	1.842	1.845	1.850	0.012	1.872	1.848	1.841	1.854	0.016
5	---	---	---	---	---	1.912	1.879	1.863	1.885	0.025
6	---	---	---	---	---	2.543	2.505	2.464	2.504	0.040
7	2.048	2.043	2.058	2.050	0.008	2.007	2.005	2.001	2.004	0.003
8	---	---	---	---	---	2.870	2.817	2.780	2.822	0.045
9	---	---	---	---	---	2.779	2.774	2.711	2.755	0.038

^aPeak Assignments: 1 – C⁶H1', δ¹H = 6.308 ppm; 2 – C³H1', δ¹H = 6.292 ppm; 3 – C²H1', δ¹H = 6.291 ppm; 4 – G⁵H1', δ¹H = 6.173 ppm; 5 – G¹H1', δ¹H = 6.075 ppm; 6 – C³H5, δ¹H = 6.057 ppm; 7 – T⁴H1', δ¹H = 5.816 ppm; 8 – C⁶H5, δ¹H = 5.691 ppm; 9 – C²H5, δ¹H = 5.675 ppm. Measurements are reported from experiments carried out in triplicate. Integration was carried out only for those peaks that were clearly resolved.

Table S3: HOBS-¹H T₁ inversion-recovery relaxation data for aromatic resonances from the oligonucleotide d(GCCTGC).

Aromatic Resonance Region										
Peaks ^a	T ₁ by Integration (s)					T ₁ by Peak Picking (s)				
	#1	#2	#3	Avg.	St. Dev.	#1	#2	#3	Avg.	St. Dev.
1	1.658	1.663	1.642	1.654	0.011	1.384	1.390	1.395	1.390	0.006
2	1.382	1.381	1.384	1.382	0.002	1.384	1.406	1.395	1.395	0.011
3	1.589	1.583	1.577	1.583	0.006	1.624	1.639	1.629	1.631	0.008
4	1.587	1.582	1.581	1.583	0.003	1.631	1.637	1.629	1.632	0.004

^aPeak Assignments: 1 – C³H6, δ¹H = 7.940 ppm; 2 – C²H6, δ¹H = 7.670 ppm; 3 – C⁶H6, δ¹H = 7.630 ppm; 4 – T⁴H6, δ¹H = 7.520 ppm. Measurements are reported from experiments carried out in triplicate.

Table S4: Standard-¹H T₁ inversion-recovery relaxation data for aromatic resonances from the oligonucleotide d(GCCTGC).

Aromatic Resonance Region										
Peaks ^a	T ₁ by Integration (s)					T ₁ by Peak Picking (s)				
	#1	#2	#3	Avg.	St. Dev.	#1	#2	#3	Avg.	St. Dev.
1	1.609	1.599	1.601	1.603	0.005	1.605	1.585	1.566	1.585	0.020
2	1.396	1.382	1.382	1.387	0.008	1.404	1.384	1.377	1.388	0.014
3	1.627	1.607	1.611	1.615	0.011	1.642	1.605	1.589	1.612	0.027
4	1.566	1.561	1.565	1.564	0.003	1.597	1.583	1.584	1.588	0.008

^aPeak Assignments: 1 – C³H6, δ¹H = 7.940 ppm; 2 – C²H6, δ¹H = 7.670 ppm; 3 – C⁶H6, δ¹H = 7.630 ppm; 4 – T⁴H6, δ¹H = 7.520 ppm. Measurements are reported from experiments carried out in triplicate.