Supplemental Material

Hidden in Plain Sight: Subtle Effects of the 8-oxoguanine Lesion on the Structure, Dynamics, and Thermodynamics of a 15-Base-Pair Oligodeoxynucleotide Duplex

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indicated assignments correspond directly to the parent strand. Note the disappearance of the G_8 aromatic proton resonance and significant movement of the A_7 and A_9 proton resonances in the **1-oxo** strand relative to the parent strand **1**.

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Supplementary Table 4: Chemical shift assignments for base and sugar protons on the parent and lesion duplexes. Note that for the purposes of brevity in comparison of lesion and parent duplexes, only those H2', H2", H3', and cytosine amino proton resonances that show significant movement (≥0.02 ppm) relative to the parent have been specifically included in this table; nonetheless, almost all of these could be identified in the spectrum except for those H3' that are swamped out by the resonance of residual water. H4' and H5' protons were not assigned.

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Supplementary Table 5: Representative results of a fit to Eqn. 4 of the amplitudes of each peak as a function of mixing time. The results for 100 mM total glycine are shown here. The results of these fits of individual peaks were then used to seed a simultaneous fit of all peaks and spectra, yielding the refined values in Supplementary Table 6.

Supplementary Table 6: Representative results of a simultaneous, multiparameter non-linear least-squares fit of a set of Lorentzian peaks to twenty-two spectra. This second fit was used to refine the results of Table 5 to deconvolute overlapping peaks and account for small peak volumes at some time points. The fit was implemented in Matlab using the *nlinfit* function. Each of the peaks was modeled using 5 free parameters (chemical shift, linewidth, $I_{z,eq}$, R_{1i} and k_{ex}). The results for 100 mM total glycine are shown here.

Supplementary Figure 6: Representative results of the mulitparameter Matlab fit to a set of spectra. The first of the 22 spectra in the set is shown here, corresponding to a mixing time of 520 μs as measured for the 100 mM total glycine sample. The residuals of the fit are shown in the lower panel. The data points are colored blue, and the fit spectrum is colored red. The peaks are numbered from the most downfield to upfield, as indicated above each with a green dot, corresponding to the numbering in Supplementary Tables 5 and 6.

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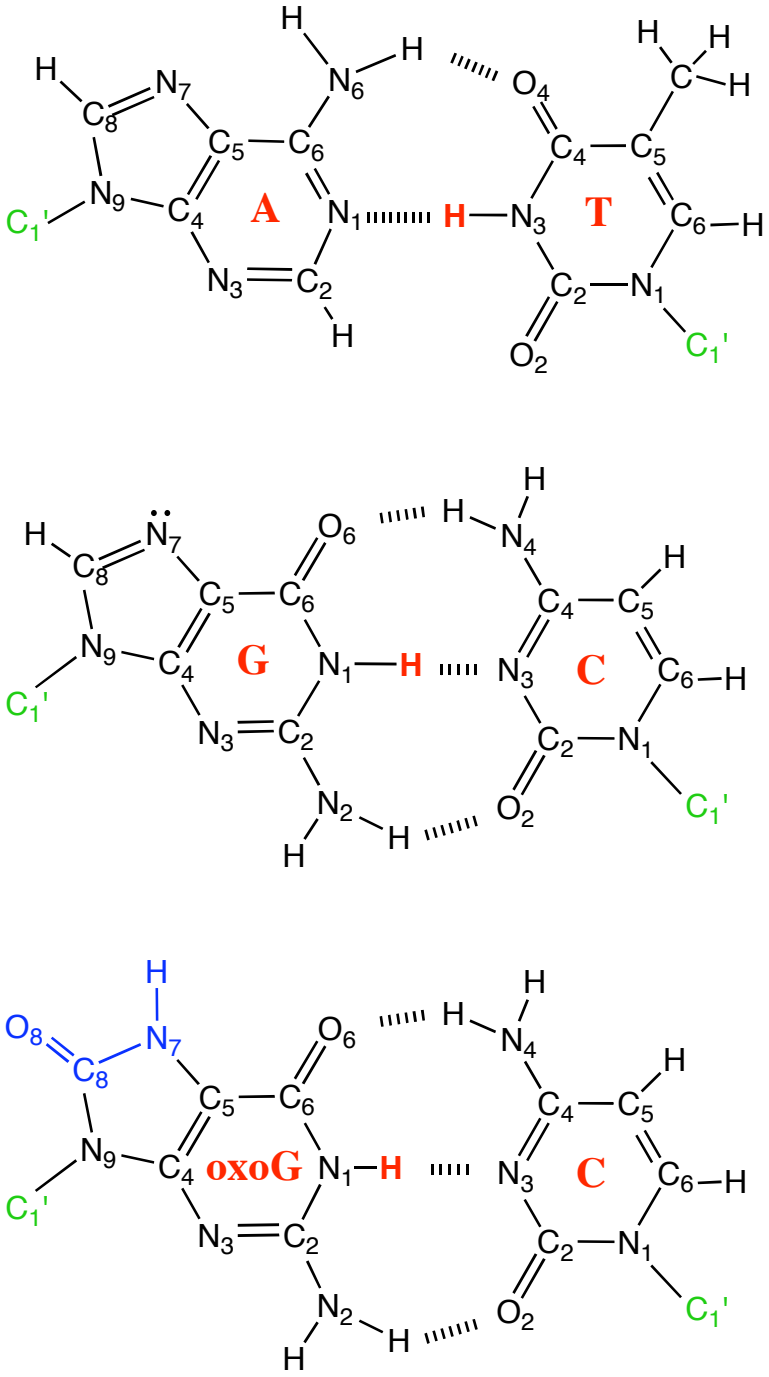
Supplementary Figure 7: Exchange rate of DNA imino protons in parent duplex **1-2** are correlated with their exchange rates in the lesion duplex **1oxo-2** over the whole range of glycine concentrations tested. The reference line demonstrates a perfect 1:1 correspondence. The exchange rate of the 8oxoG N1 imino proton is consistently ~1.4X higher than that of normal G8 in the parent duplex across this range of concentrations. The exchange rates of the other central guanines in the lesion duplex (G21, G6, G26, G12) are ~1.2X higher than that of the corresponding guanines in the parent duplex, but the thymine exchange rates are slightly lower.


A
T
G
C
oxoG
C

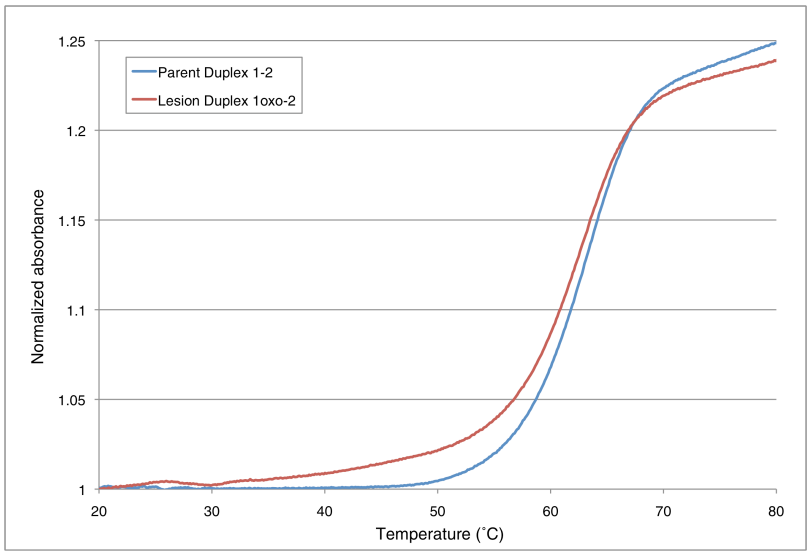
Supplementary Table 1: Mixing times for measurement of water relaxation (R_{1w}).

3	1.2 ms
4	0.4 s
5	10 s
6	1.6 s
7	2.8 s
8	3.6 s
9	15 s
10	0.8 s
11	3.2 s
12	2 s
13	4 s
14	1.2 s
15	2.4 s
16	6 s

Supplementary Table 2: Mixing times for measurement of imino exchange (k_{ex}).

17	520 µs
18	600 ms
19	10 ms
20	150 ms
21	1.5 ms
22	350 ms
23	500 ms
24	1ms
25	250 ms
26	1.5 s
27	3 ms
28	75 ms
29	300 ms
30	20 ms
31	1 s
32	400 ms
33	100 ms
34	750 ms
35	200 ms
36	450 ms
37	50 ms
38	2 s

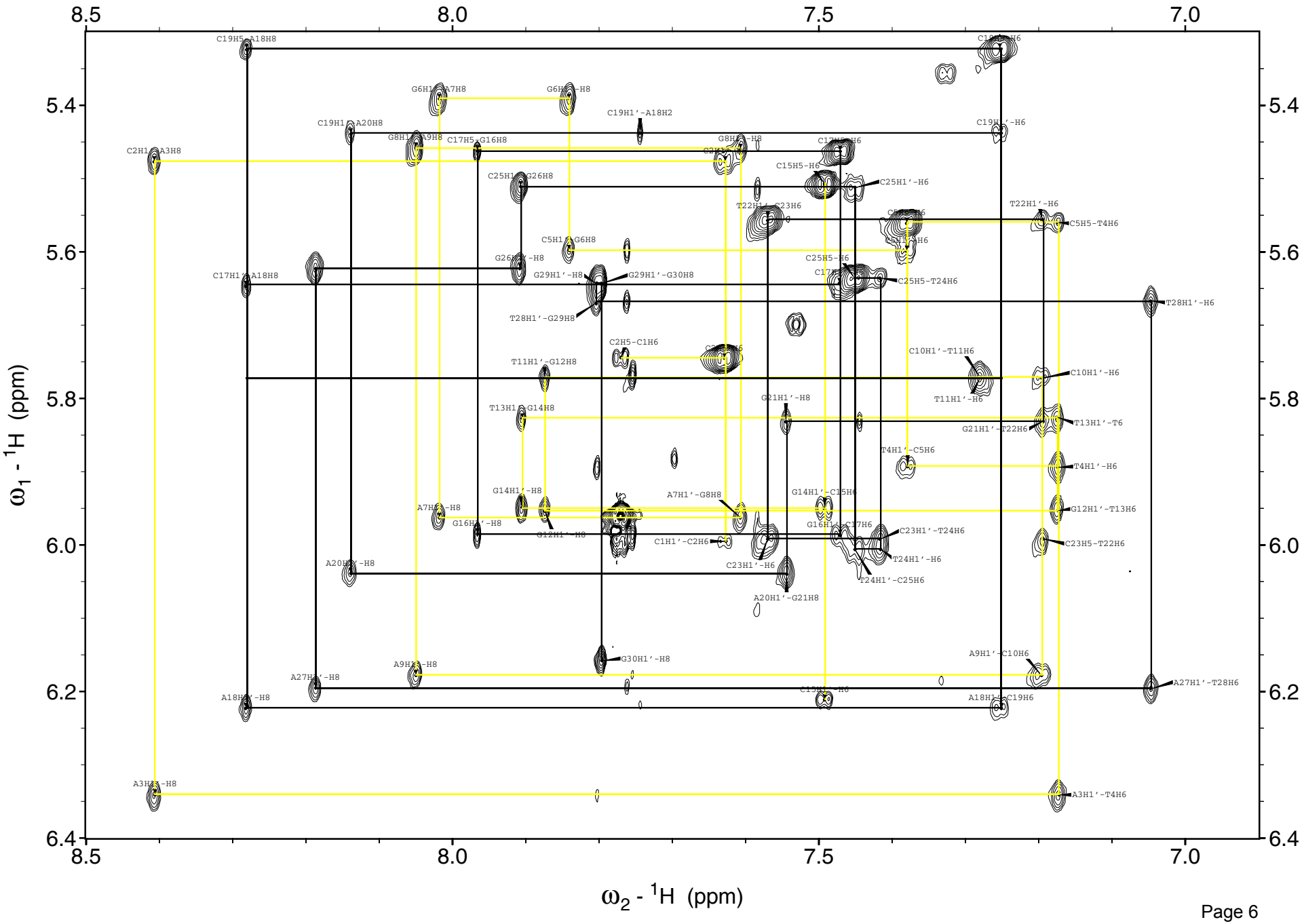
Supplemental Figure 2

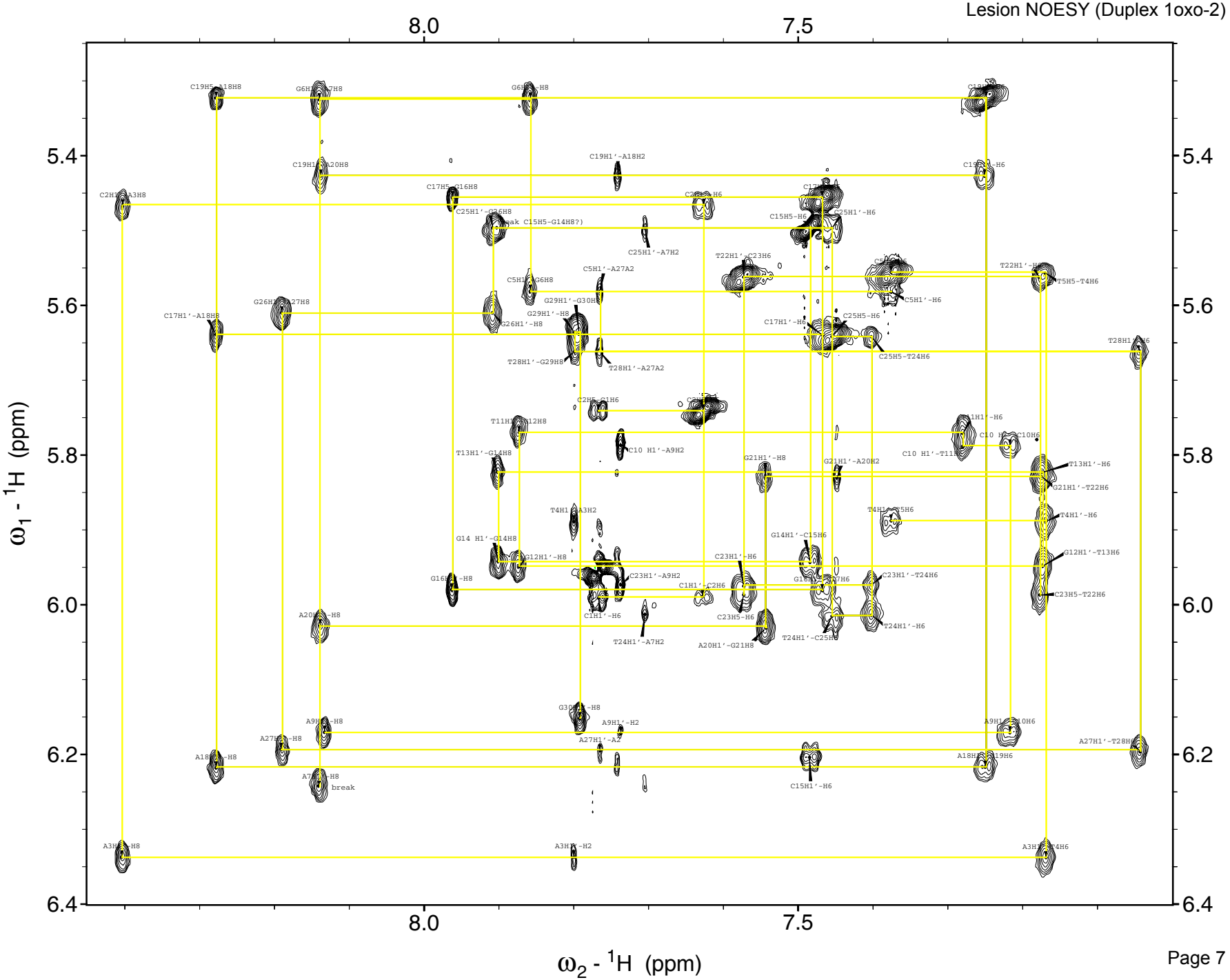


Supplemental Table 3: Thermodynamic Data for the ssDNA ⇔ dsDNA Equilibrium

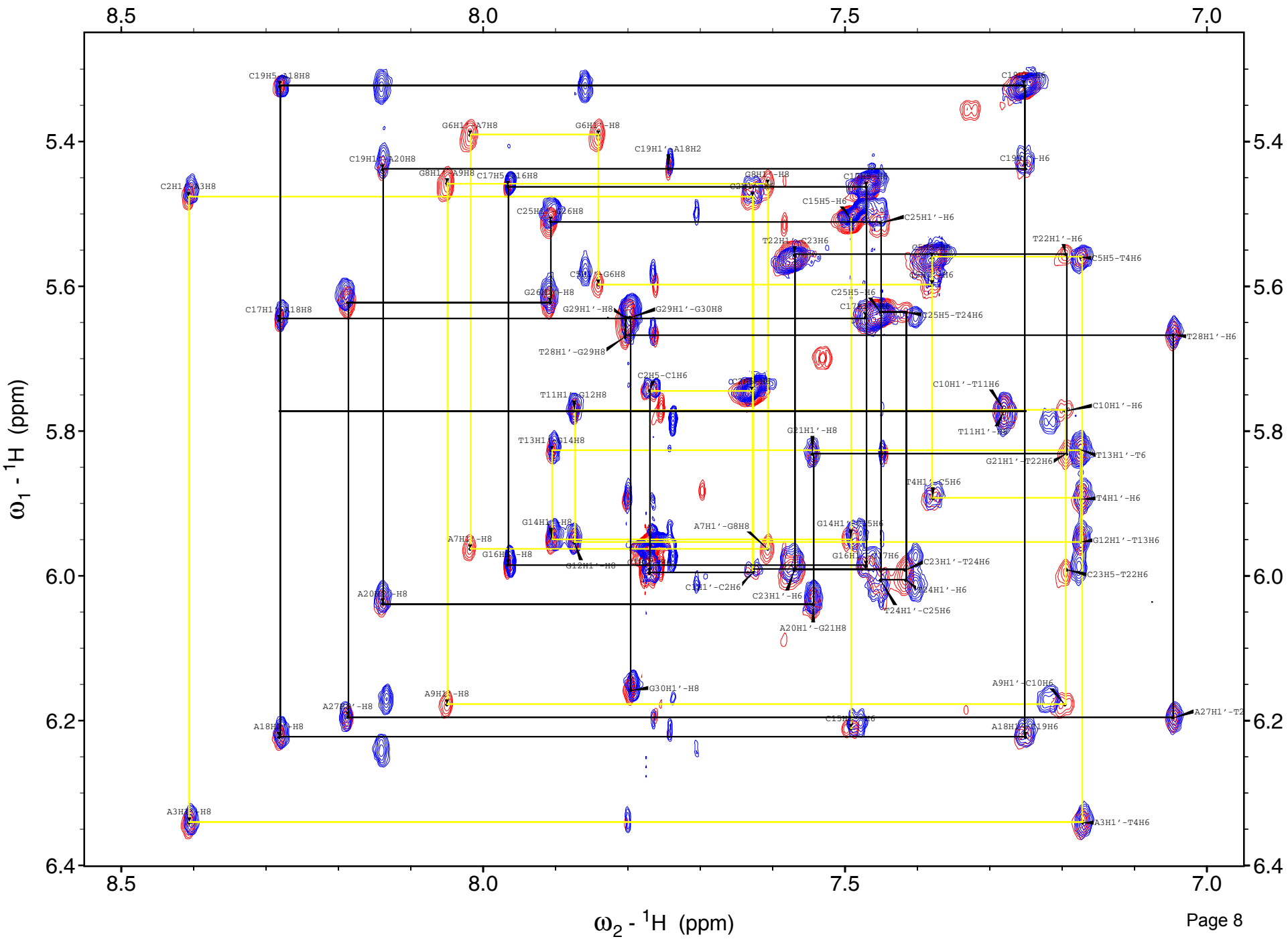
	T_m at 21 uM duplex in ˚C	ΔH˚ kcal/mol	ΔS˚ cal/mol K	ΔG˚ at 25˚C kcal/mol
1-2	64.4 ± 0.2	-126 ± 4	-349 ± 10	-21.4 ± 0.7
1oxo-2	63.4 ± 0.2	-124 ± 5	-345 ± 13	-21.0 ± 0.9
Difference due to 8oxoG	ΔT_m = -1.0 ± 0.3	ΔΔH˚ = +2 ± 6	ΔΔS˚ = +4 ± 16	ΔΔG˚ = +0.4 ± 1.1

Supplementary Figure 3
Parent NOESY (Duplex 1-2)




8.0
7.5
5.4
5.6
5.8
6.0
6.2
6.4
ω1 - 1H (ppm)
ω2 - 1H (ppm)
C19H5-A18H8
G6H1'-A7H8
G6H1'-H8
C19H1'-A18H2
C19H1'-A20H8
C17H5-G16H8
C25H1'-G26H8
(weak C15H5-G14H8?)
C25H1'-A7H2
C15H5-H6
C25H1'-H6
C2H5-A3H8
C5H1'-A27A2
C5H1'-G26H8
G29H1'-G30H8
G29H1'-H8
G26H1'-H8
T22H1'-C23H6
C5H1'-H6
T22H1'-H6
T5H5-T4H6
G26H1'-A27H8
C17H1'-A18H8
C17H1'-H6
C25H5-H6
C25H5-T24H6
T28H1'-G29H8
T28H1'-A27A2
T28H1'-H6
C2H5-C1H6
T11H1'-G12H8
C10 H1'-A9H2
C10 H1'-H6
C10 H1'-T11H6
C10H5
T13H1'-G14H8
G21H1'-H8
G21H1'-A20H2
T13H1'-H6
G21H1'-T22H6
T4H1'-A3H2
T4H1'-C5H6
T4H1'-H6
G14 H1'-C14H8
G14H1'-C15H6
G12H1'-H8
C23H1'-H6
G12H1'-T13H6
G16H1'-H8
C23H1'-A9H2
C1H1'-C2H6
G16H1'-A17H6
C23H1'-T24H6
C23H5-T22H6
A20H1'-H8
C1H1'-H6
C23H5-H6
T24H1'-H6
T24H1'-A7H2
A20H1'-G21H8
T24H1'-C25H6
G30H1'-H8
A9H1'-H8
A9H1'-H2
A9H1'-C10H6
A27H1'-H8
A27H1'-A2
A27H1'-T28H6
A18H1'-H8
A18H1'-C19H6
A7H1'-H8
break
C15H1'-H6
A3H1'-H8
A3H1'-H2
A3H1'-T4H6

Supplementary Figure 5
NOESY Overlay Parent (red) and Lesion (blue) Duplexes



Lesion Strand 1 or 1oxo

BASE	Number	location	distance	ppm 1	ppm 1oxo	delta ppm
BASE PROTONS						
G8	H8	base	0	7.607	N/A	
A9	H8	base	1	8.05	8.133	-0.083
A7	H8	base	1	8.018	8.14	-0.122
G6	H8	base	2	7.841	7.859	-0.018
C10	H6	base	2	7.197	7.218	-0.021
T11	H6	base	3	7.28	7.28	0.000
C5	H6	base	3	7.379	7.376	0.003
G12	H8	base	4	7.874	7.874	0.000
T4	H6	base	4	7.174	7.17	0.004
A3	H8	base	5	8.407	8.403	0.004
T13	H6	base	5	7.175	7.173	0.002
G14	H8	base	6	7.905	7.901	0.004
C2	H6	base	6	7.629	7.628	0.001
C15	H6	base	7	7.492	7.484	0.008
C1	H6	base	7	7.77	7.767	0.003
C10	H5	base	2	5.101	5.122	-0.021
C5	H5	base	3	5.566	5.556	0.010
C2	H5	base	6	5.745	5.741	0.004
C15	H5	base	7	5.508	5.497	0.011
T11	5CH3	base	3	1.531	1.531	0.000
T4	5CH3	base	4	1.444	1.439	0.005
T13	5CH3	base	5	1.45	1.447	0.003
G8/oxoG8	imino	base	0	12.550	12.830	-0.280
G6	imino	base	2	12.660	12.720	-0.060
T11	imino	base	3	13.650	13.660	-0.010
G12	imino	base	4	12.440	12.450	-0.010
T4	imino	base	4	13.530	13.530	0.000
T13	imino	base	5	13.680	13.680	0.000
G14	imino	base	6	12.860	12.860	0.000

Lesion Strand 1 or 1oxo

SUGAR PROTONS

G8	*H1'*	*sugar*	*0*	*5.459*	*missing*	
A7	H1'	sugar	1	5.963	6.243	-0.280
A9	H1'	sugar	1	6.178	6.172	0.006
G6	H1'	sugar	2	5.391	5.323	0.068
C10	H1'	sugar	2	5.772	5.788	-0.016
T11	H1'	sugar	3	5.771	5.768	0.003
C5	H1'	sugar	3	5.598	5.582	0.016
T4	H1'	sugar	4	5.893	5.888	0.005
G12	H1'	sugar	4	5.952	5.949	0.003
T13	H1'	sugar	5	5.827	5.823	0.004
A3	H1'	sugar	5	6.341	6.336	0.005
G14	H1'	sugar	6	5.95	5.942	0.008
C2	H1'	sugar	6	5.477	5.467	0.010
C15	H1'	sugar	7	6.211	6.204	0.007
C1	H1'	sugar	7	5.996	5.991	0.005
G8	H2"	sugar	0	2.649	*3.156*	-0.507
G8	H2'	sugar	0	2.504	*2.276*	0.228
A7	H2'	sugar	1	2.596	2.498	0.098
A7	H2"	sugar	1	2.836	3.128	-0.292
A9	H2'	sugar	1	2.622	2.617	0.005
A9	H2"	sugar	1	2.868	2.863	0.005
G6	H2'	sugar	2	2.701	2.699	0.002
G6	H2"	sugar	2	2.622	2.634	-0.012
oxo/G8	H3'	sugar	0	4.975	5.088	-0.113
A9	H3'	sugar	1	4.98	4.983	-0.003
A7	H3'	sugar	1	5.028	5.014	0.014
G6	H3'	sugar	2	4.964	4.956	0.008

Complementary Strand 2 (or 2 opposite 1oxo)

BASE	Number	location	distance	ppm 2	ppm 2 (oxo)	delta ppm
BASE PROTONS						
C23	H6	base	0	7.569	7.573	-0.004
T24	H6	base	1	7.416	7.402	0.014
T22	H6	base	1	7.195	7.177	0.018
G21	H8	base	2	7.544	7.544	0.000
C25	H6	base	2	7.451	7.454	-0.003
G26	H8	base	3	7.908	7.908	0.000
A20	H8	base	3	8.14	8.138	0.002
A20	H2	base	3	7.445	7.448	-0.003
A27	H8	base	4	8.187	8.189	-0.002
C19	H6	base	4	7.253	7.25	0.003
A18	H8	base	5	8.281	8.278	0.003
T28	H6	base	5	7.047	7.044	0.003
G29	H8	base	6	7.804	7.799	0.005
C17	H6	base	6	7.471	7.468	0.003
G30	H8	base	7	7.796	7.792	0.004
G16	H8	base	7	7.966	7.963	0.003
C23	H5	base	0	5.525	5.544	-0.019
C25	H5	base	2	5.636	5.641	-0.005
C19	H5	base	4	5.324	5.324	0.000
C17	H5	base	6	5.463	5.456	0.007
T24	5CH3	base	1	1.606	1.555	0.051
T22	5CH3	base	1	1.218	1.211	0.007
T28	5CH3	base	5	1.396	1.394	0.002
T22	imino	base	1	13.580	13.590	-0.010
T24	imino	base	1	13.720	13.630	0.090
G21	imino	base	2	12.720	12.750	-0.030
G26	imino	base	3	12.580	12.600	-0.020
T28	imino	base	5	13.640	13.630	0.010
G29	imino	base	6	12.860	12.860	0.000
C23	N4b	base	0	6.921	6.864	0.057
C23	N4a	base	0	8.242	8.357	-0.115

Complementary Strand 2 (or 2 opposite 1oxo)

SUGAR PROTONS

C23	H1'	sugar	0	5.992	5.974	0.018
T24	H1'	sugar	1	6.006	6.015	-0.009
T22	H1'	sugar	1	5.556	5.563	-0.007
G21	H1'	sugar	2	5.832	5.829	0.003
C25	H1'	sugar	2	5.512	5.495	0.017
G26	H1'	sugar	3	5.623	5.611	0.012
A20	H1'	sugar	3	6.037	6.028	0.009
C19	H1'	sugar	4	5.436	5.427	0.009
A27	H1'	sugar	4	6.195	6.195	0.000
T28	H1'	sugar	5	5.667	5.662	0.005
A18	H1'	sugar	5	6.222	6.216	0.006
G29	H1'	sugar	6	5.643	5.636	0.007
C17	H1'	sugar	6	5.646	5.639	0.007
G30	H1'	sugar	7	6.158	6.152	0.006
G16	H1'	sugar	7	5.985	5.988	-0.003

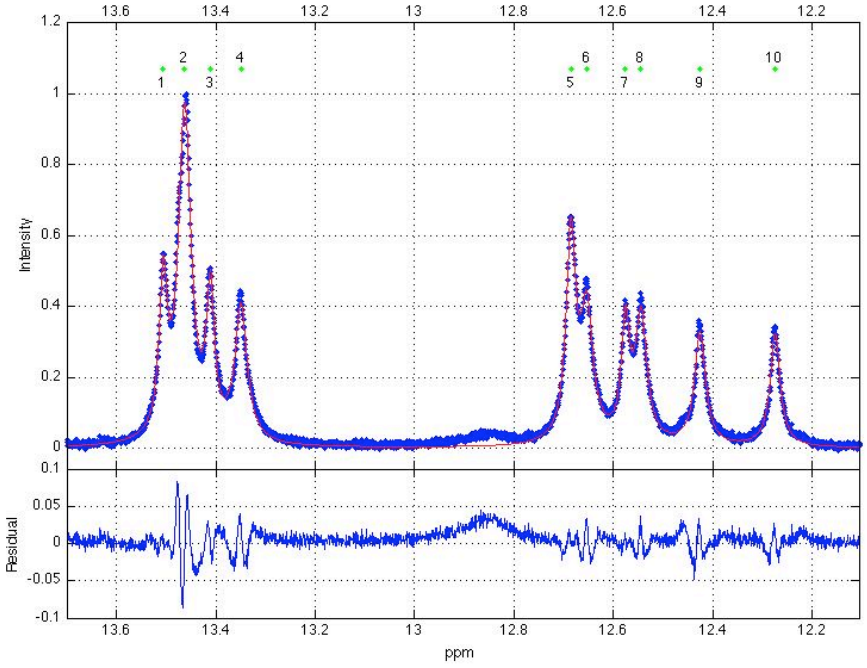
Supplementary Table 5

#Pk	ppm	width	Amp	err	R1i	err	**kex**	**err**
1	13.506	0.01	3.8	0.03	6.38	0.27	**2.41**	**0.09**
2	13.463	0.016	7	0.03	5.05	0.19	**1.31**	**0.04**
3	13.411	0.01	3.5	0.02	7.46	0.22	**3.07**	**0.08**
4	13.349	0.015	3	0.02	6.57	0.26	**2.28**	**0.08**
5	12.685	0.012	4.5	0.03	11.98	0.26	**6.27**	**0.13**
6	12.654	0.015	3.4	0.03	10.94	0.51	**3.41**	**0.15**
7	12.576	0.01	2.8	0.03	7.18	0.53	**1.77**	**0.12**
8	12.544	0.014	3.1	0.02	12.09	0.79	**2.51**	**0.16**
9	12.426	0.013	2.4	0.03	10.41	0.91	**2.33**	**0.20**
10	12.275	0.012	2.3	0.02	9.24	0.71	**2.27**	**0.17**

Supplementary Table 6

#Pk	ppm	err	width	err	Intens.	err	R1i	err	**kex**	**err**
1	13.507	0.0001	0.0086	0.0001	0.08	0.00082	6.56	0.19	**2.82**	**0.07**
2	13.463	0.0001	0.0170	0.0001	0.355	0.0013	5.16	0.08	**1.30**	**0.02**
3	13.412	0.0001	0.0113	0.0001	0.098	0.001	8.44	0.23	**3.61**	**0.09**
4	13.349	0.0001	0.0163	0.0001	0.137	0.00095	7.03	0.2	**2.36**	**0.06**
5	12.686	0.0001	0.0113	0.0001	0.147	0.0014	13.33	0.2	**7.38**	**0.10**
6	12.654	0.0001	0.0153	0.0001	0.137	0.0015	12.1	0.46	**3.30**	**0.12**
7	12.577	0.0001	0.0089	0.0001	0.057	0.001	6.61	0.48	**1.65**	**0.11**
8	12.546	0.0001	0.0172	0.0002	0.142	0.0014	13.98	0.82	**2.49**	**0.15**
9	12.426	0.0001	0.0146	0.0001	0.099	0.00082	11.65	0.75	**2.28**	**0.15**
10	12.274	0.0001	0.0142	0.0001	0.096	0.00078	9.59	0.56	**2.09**	**0.12**

Supplementary Figure 6



Supplemental Figure 7