

Supplemental Tables

Supplemental Table I. Chemical shifts arising from Interferon-Hyp-polysaccharide-1 glycosyl and Hyp residues at 55°C. Two sets of chemical shifts for Hyp residues resulted from base hydrolysis during preparation of Hyp-AGs. Residue designations correspond to those in Figures 4 and 5

Residues		C-1/H-1	C-2/H2	C-3/H-3	C-4/H-4	C-5/H-5	C-6/H-6
		ppm					
A ₁	t-Ara	107.542/5.087	81.047/4.135	76.665/3.960	84.171/4.099	61.440/3.833,3.723	
A ₂	5-Ara	109.179/5.259	81.353/4.228	76.951/4.019	82.473/4.260	67.076/3.891,3.816	
A ₃	3-Ara	109.179/5.261	81.200/4.220	83.877/3.955	84.098/4.147	61.440/3.833,3.723	
A ₄	t-Ara	107.542/5.087	81.047/4.135	76.665/3.960	84.171/4.099	61.440/3.833,3.723	
A ₅	5-Ara	109.179/5.259	81.353/4.228	76.951/4.019	82.473/4.260	67.076/3.891,3.816	
A ₆	3-Ara	109.179/5.261	81.200/4.220	83.877/3.955	84.098/4.147	61.440/3.833,3.723	
A ₇	t-Ara	109.179/5.264	81.352/4.228	76.665/3.960	84.171/4.099	61.440/3.833,3.723	
A ₈	t-Ara	109.179/5.264	81.352/4.228	76.665/3.960	84.171/4.099	61.440/3.833,3.723	
R ₁	t-Rha	100.746/4.797	70.451/3.948	70.582/3.750	72.223/3.538	69.178/4.021	16.628/1.267
R ₂	t-Rha	100.746/4.797	70.451/3.948	70.582/3.750	72.223/3.538	69.178/4.021	16.628/1.267
UA ₁	4-GlcUA	103.135/4.511	73.431/3.391	74.791/3.728	79.477/3.625	74.484/3.585	
UA ₂	4-GlcUA	103.135/4.511	73.090/3.361	75.858/3.716	79.477/3.625	74.484/3.585	
G _a	3,6-Gal(s)	103.310/4.536	70.009/3.664	80.378/3.728	68.524/4.146	73.567/3.923	69.169/4.041,3.921
G _b	3,6-Gal(s)	103.546/4.487	70.009/3.664	80.378/3.728	68.524/4.146	73.562/3.923	69.169/4.041,3.921
G _c	3-Gal(s)	103.556/4.447	70.838/3.607	80.378/3.728	68.590/4.112	72.497/3.691	61.068/3.784
G _d	3-Gal(s)	103.201/4.406	70.947/3.557	80.378/3.728	68.590/4.112	72.497/3.691	61.068/3.784
G ₁	3,6-Gal(b)	101.439/4.571	70.030/3.689	82.129/3.854	68.546/4.232	72.869/3.662	69.465/4.041,3.921
G ₂	3,6-Gal(b)	103.920/4.691	70.030/3.689	81.939/3.812	68.546/4.232	72.869/3.662	70.098/4.041,3.921
G ₃	6-Gal(b)	104.363/4.644	71.920/3.539	72.869/3.662	69.092/3.903	74.943/3.730	70.435/4.041,3.921
G ₄	3,6-Gal(b)	103.800/4.703	70.030/3.689	82.135/3.868	68.542/4.232	72.869/3.662	69.185/4.041,3.921
G ₅	3,6-Gal(b)	103.800/4.703	70.030/3.689	81.821/3.893	68.542/4.232	72.869/3.662	69.986/4.041,3.921
G ₆	t-Gal(b)	103.695/4.725	71.920/3.539	72.869/3.662	69.092/3.903	75.357/3.712	61.068/3.784
	set 1		59.978/4.356	35.130/2.650,2.214	78.020/4.786	51.889/3.683,3.569	
Hyp	set 2		59.978/4.244	34.895/2.564,2.452	77.303/4.708	51.999/3.774,3.483	

Supplemental Table II. Chemical shifts arising from Interferon-Hyp-polysaccharide-1 glycosyl and Hyp residues at 25°C. Two sets of chemical shifts for Hyp residues and G₁ resulted from base hydrolysis during preparation of Hyp-AGs. Residue designations correspond to those shown in Figures 4 and 5

Residues		C-1/H-1	C-2/H2	C-3/H-3	C-4/H-4	C-5/H-5	C-6/H-6
		ppm					
A ₁	t-Ara	107.411/5.075	80.899/4.128	76.559/3.946	83.941/4.089	61.232/3.824,3.718	
A ₂	5-Ara	109.250/5.252	81.316/4.224	76.721/4.009	82.330/4.243	66.747/3.875,3.795	
A ₃	3-Ara	109.250/5.264	81.018/4.223	83.781/3.938	83.880/4.053	61.213/3.825,3.695	
A ₄	t-Ara	107.411/5.075	80.899/4.128	76.559/3.946	83.941/4.089	61.232/3.824,3.718	
A ₅	5-Ara	109.250/5.252	81.316/4.224	76.721/4.009	82.330/4.243	66.747/3.875,3.795	
A ₆	3-Ara	109.250/5.264	81.018/4.223	83.781/3.938	83.880/4.053	61.213/3.825,3.695	
A ₇	t-Ara	109.250/~5.241	81.298/4.207	76.547/3.939	83.788/4.119	61.189/3.827,3.696	
A ₈	t-Ara	109.250/~5.241	81.298/4.207	76.547/3.939	83.788/4.119	61.189/3.827,3.696	
R ₁	t-Rha	100.801/4.732	70.203/3.934	70.115/3.756	71.950/3.430	69.137/4.020	16.442/1.255
R ₂	t-Rha	100.801/4.732	70.203/3.934	70.115/3.756	71.950/3.430	69.137/4.020	16.442/1.255
UA ₁	4-GlcUA	102.685/4.505	73.283/3.367	74.286/3.570	79.245/3.577	76.191/3.741	
UA ₂	4-GlcUA	102.685/4.505	72.855/3.337	75.483/3.516	79.175/3.589	75.476/3.712	
G _a	3,6-Gal(s)	103.149/4.532	69.879/3.658	80.185/3.758	68.468/4.141	73.139/3.948	69.394/4.026,3.913
G _b	3,6-Gal(s)	103.149/4.492	69.879/3.663	80.185/3.724	68.505/4.106	73.139/3.948	69.394/4.026,3.913
G _c	3-Gal(s)	103.615/4.432	70.652/3.527	80.196/3.646	69.278/3.925	75.030/3.692	60.970/3.768
G _d	3-Gal(s)	103.251/4.388	70.738/3.581	80.196/3.662	69.278/3.944	75.030/3.692	60.970/3.768
	3,6-Gal(b)						
G ₁	Set 1	101.257/4.565	69.870/3.681	81.853/3.859	68.488/4.239	75.097/3.680	69.201/4.026,3.913
	Set 2	101.89/4.485					
G ₂	3,6-Gal(b)	103.868/4.686	70.115/3.763	81.801/3.869	68.462/4.248	75.097/3.680	69.171/4.026,3.913
G ₃	6-Gal(b)	104.305/4.622	69.857/3.615	72.456/3.663	69.171/3.924	74.885/3.689	69.433/4.031,3.913
G ₄	3,6-Gal(b)	103.851/4.708	70.135/3.774	81.689/3.882	68.478/4.238	76.758/3.994	69.171/4.026,3.913
G ₅	3,6-Gal(b)	103.772/4.723	70.235/3.777	81.668/3.879	68.478/4.252	76.758/3.994	69.171/4.026,3.913
G ₆	t-Gal(b)	103.887/4.660	70.333/3.774	71.734/3.714	68.940/3.955	75.032/3.772	60.968/3.778
Hyp	set 1	173.98	59.800/4.336	35.186/2.635,2.173	77.970/4.780	51.980/3.675,3.540	
	set 2		59.800/4.236	34.952/2.524,2.438	77.364/4.693	52.010/3.765,3.450	

Supplemental Table III. NOE assignments of Interferon-Hyp-polysaccharide-1 at 25°C. Italicized sections highlight the NOEs between Hyp and Gal residue G1, bolded sections highlight non-sequential NOEs and corresponding residues, sequential NOEs are neither bolded nor italicized. When two distinct proton pair sets produced the same NOEs both pairs are presented.

NOEs	Assignments
<i>4.790/4.579</i>	<i>Hyp-H4/G₁-H1</i>
<i>4.791/4.240</i>	<i>Hyp-H4/G₁-H4</i>
<i>4.576/3.532</i>	<i>G₁-H1/Hyp-H5</i>
<i>4.789/3.863</i>	<i>Hyp-H4/G₁-H3</i>
5.241/4.526	A₇-H1 or A₈-H1/G_a-H1
5.233/4.500	A₇-H1 or A₈-H1/G_b-H1 or UA₁-H1 or UA₂-H1
5.262/4.511	A₆-H1, or A₃-H1/UA₁-H1, or UA₂-H1
4.709/4.146	G₄-H1/G_a-H4, or A₁-H2, or A₄-H2
4.518/4.245	UA₁-H1 or UA₂-H1 or G_a-H1/A₆-H4 or A₂-H4 or G₂-H4
5.237/4.694	A₇-H1 or A₈-H1/G₂-H1
5.265/4.700	A₃-H1 or A₆-H1 /G₄-H1
4.690/4.245	G ₂ -H1/G ₁ -H4
4.677/4.217	G ₆ -H1/G ₅ -H4
4.710/4.038	G ₄ -H1/G ₃ -H61
4.706/3.913	G ₄ -H1/G ₃ -H62
4.392/4.251	G ₄ -H1/G ₅ -H4
4.436/4.237	G _c -H1/G ₄ -H4
4.620/3.870	G ₃ -H1/G ₂ -H3
4.616/3.767	G ₃ -H1/G ₂ -H2
4.013/5.085	A ₂ -H3/A ₁ -H1, or A ₅ -H3/A ₄ -H1
5.265/3.645	A ₃ -H1/G _a -H2, or A ₆ -H1/G _b -H2
4.220/3.647	A ₃ -H2/G _a -H2, or A ₆ -H2/G _b -H2
5.241/3.653	A ₇ -H1/G _c -H3 or A ₈ -H1/G _d -H3
3.726/4.683	G _b -H3/G ₂ -H1
3.520/4.045	UA ₂ -H3/G _b -H62
4.436/4.702	G _c -H1/G ₄ -H1
4.574/4.687	G ₁ -H1/G ₂ -H1
4.724/3.568	R ₁ -H1/UA ₁ -H4 or R ₂ -H1/UA ₂ -H4
4.724/3.373	R ₁ -H1/UA ₁ -H2
4.029/3.566	R ₁ -H5/UA ₁ -H3
3.373/4.020	UA ₁ -H2/G _a -H61
3.372/3.879	UA ₁ -H2/G _a -H62
3.371/3.921	UA ₁ -H2/G _a -H5
3.374/4.137	UA ₁ -H2/G _a -H4
4.505/4.032	UA ₁ -H1/G _a -H61
4.505/3.888	UA ₁ -H1/G _a -H62
3.351/3.891	UA ₂ -H2/G _b -H61
3.348/4.045	UA ₂ -H2/G _b -H62
4.506/3.912	UA ₂ -H1/G _b -H61
4.247/5.086	A ₂ -H4/A ₁ -H1 or A ₅ -H4/A ₄ -H1
3.886/5.086	A ₂ -H51/A ₁ -H1 or A ₅ -H51/A ₄ -H1
3.798/5.086	A ₂ -H52/A ₁ -H1 or A ₅ -H52/A ₄ -H1
4.540/4.039	G _a -H1/G ₁ -H61

4.490/4.245	G ₆ -H1/G ₂ -H4
4.394/3.902	G ₄ -H1/G ₅ -H61
4.395/4.028	G ₄ -H1/G ₅ -H62
4.440/4.037	G _c -H1/G ₄ -H61
4.440/3.891	G _c -H1/G ₄ -H62
4.618/4.241	G ₃ -H1/G ₂ -H4
5.084/4.245	A ₁ -H1/A ₂ -H4, or A ₄ -H1/A ₅ -H4
5.084/3.801	A ₁ -H1/A ₂ -H51, or A ₄ -H1/A ₅ -H51
5.085/3.887	A ₁ -H1/A ₂ -H52, or A ₄ -H1/A ₅ -H52

Supplemental Table IV. Chemical shifts (collected at 55 °C) arising from Interferon-Hyp-polysaccharide-2 glycosyl and Hyp residues. Residue designations correspond to those shown in Figure 5

Residues		C-1/H-1	C-2/H2	C-3/H-3	C-4/H-4	C-5/H-5	C-6/H-6
		Ppm					
A ₁	t-Ara	108.0/5.08	81.4/4.21	77.1/3.94	84.4/4.13	61.6/3.71,3.82	
A ₂	5-Ara	109.4/5.24	81.4/4.21	77.5/4.00	84.4/4.13	67.1/3.79,3.88	
A ₃	3-Ara	109.4/5.24	81.1/4.12	82.9/4.24	84.0/4.09	61.6/3.71,3.82	
A ₄	t-Ara	109.4/5.24	81.4/4.21	77.1/3.94	84.4/4.13	61.6/3.71,3.82	
A ₅	3-Ara	109.4/5.24	81.1/4.12	82.9/4.24	84.0/4.09	61.6/3.71,3.82	
R ₁	t-Rha	101.1/4.79	71.0/3.93	70.7/3.69	72.5/3.53	68.2/4.02	17.0/1.25
UA ₁	t-GlcUA	102.9/4.52	73.8/3.39	76.2/3.78	71.3/3.53	75.8/3.52	
UA ₂	4-GlcUA	102.9/4.52	73.1/3.34	77.0/3.75	79.5/3.62	74.8/3.58	
UA ₃	t-GlcUA	102.9/4.52	73.8/3.39	76.2/3.78	71.3/3.53	75.8/3.52	
UA ₄	t-GlcUA	102.9/4.52	73.8/3.39	76.2/3.78	71.3/3.53	75.8/3.52	
G _a	3,6-Gal(s)	103.5/4.49	70.4/3.66	80.5/3.72	68.5/4.13	74.0/3.92	69.5/4.03,3.92
G _b	3,6-Gal(s)	103.5/4.49	70.4/3.66	80.5/3.72	68.5/4.13	74.0/3.92	69.5/4.03,3.92
G _c	6-Gal(s)	104.0/4.43	71.9/3.53	73.1/3.60	68.2/3.93	75.1/3.73	69.5/4.03,3.92
G _d	6-Gal(s)	103.7/4.39	71.9/3.53	73.1/3.60	68.2/3.93	75.1/3.73	69.5/4.03,3.92
G ₁	3,6-Gal(b)	101.8/4.56	70.4/3.66	82.2/3.83	68.5/4.22	73.0/3.68	70.3/4.03,3.92
G ₂	3,6-Gal(b)	104.2/4.68	70.4/3.66	82.2/3.85	68.5/4.22	73.0/3.68	70.3/4.03,3.92
G ₃	6-Gal(b)	104.2/4.68	71.9/3.53	73.1/3.68	68.2/3.93	75.1/3.73	71.0/4.03,3.92
G ₄	3,6-Gal(b)	104.2/4.68	70.4/3.66	82.2/3.86	68.5/4.22	73.0/3.68	69.5/4.03,3.92
G ₅	3,6-Gal(b)	104.2/4.71	70.4/3.66	82.2/3.88	68.5/4.22	73.0/3.68	69.5/4.03,3.92
G ₆	t-Gal(b)	104.2/4.71	71.9/3.53	73.1/3.64	68.2/3.93	76.0/3.71	61.2/3.77
	set 1		60.5/4.34	35.2/2.62,2.20	78.4/4.78	52.0/3.56,3.67	
Hyp	set 2		60.2/4.23	35.0/2.44,2.55	77.6/4.69	52.3/3.47,3.77	

Supplemental Table V. Chemical shifts (collected at 60 °C) arising from Ala-Hyp-polysaccharide-2 glycosyl and Hyp residues. The residue labeling corresponds to the labeling in Figure 5.

Residues		C-1/H-1	C-2/H2	C-3/H-3	C-4/H-4	C-5/H-5	C-6/H-6
		ppm					
A ₁	t-Ara	110.3/5.25	82.3/4.21	77.7/3.95	85.1/4.13	62.4/3.71,3.82	
A ₂	t-Ara	110.3/5.25	82.3/4.21	77.7/3.95	85.1/4.13	62.4/3.71,3.82	
R ₁	t-Rha	101.7/4.81	71.4/3.95	71.4/3.78	73.2/3.42	70.1/4.02	17.6/1.25
R ₂	t-Rha	101.7/4.81	71.4/3.95	71.4/3.78	73.2/3.42	70.1/4.02	17.6/1.25
UA ₁	4-GlcUA	103.7/4.51	74.4/3.45	77.4/3.73	80.2/3.63	75.5/3.56	
UA ₂	4-GlcUA	103.7/4.51	74.4/3.45	77.4/3.73	80.2/3.63	75.5/3.56	
G _a	3,6-Gal _{sc}	104.5/4.47	71.7/3.65	81.7/3.71	69.6/4.11	74.7/3.91	70.5/4.04,3.94
G _b	3,6-Gal _{sc}	104.6/4.48	71.7/3.65	81.7/3.71	69.6/4.11	74.7/3.91	70.5/4.04,3.94
G ₁	3,6-Gal _{bb}	102.5/4.57	71.6/3.65	83.0/3.87	69.7/4.23	74.8/3.77	70.5/4.04,3.94
G ₂	3,6-Gal _{bb}	105.0/4.64	71.6/3.65	83.0/3.85	69.7/4.23	74.8/3.77	70.5/4.04,3.94
G ₃	6-Gal _{bb}	105.0/4.67	72.7/3.54	74.0/3.65	70.0/3.92	74.8/3.77	70.5/4.04,3.94
G ₄	3-Gal _{bb}	105.0/4.68	71.6/3.65	83.0/3.87	69.7/4.12	76.0/3.70	62.3/3.78
G ₅	3-Gal _{bb}	105.0/4.69	71.6/3.65	83.0/3.87	69.7/4.12	76.0/3.70	62.3/3.78
G ₆	t-Gal _{bb}	105.0/4.72	72.7/3.54	74.0/3.65	70.5/3.93	76.2/3.70	62.3/3.78
	set 1		61.0/4.32	36.5/2.62,2.19	79.1/4.77	52.9/3.54,3.64	
Hyp	set 2		61.3/4.21	36.1/2.40,2.55	78.4/4.68	52.9/3.47,3.77	

Supplemental Figure Legends

Supplemental Figure 1. A TOSCY spectrum of Interferon-Hyp-polysaccharide-1 collected at 55°C aided in the assignment of ^1H chemical shifts and Cross-peaks A indicated side-chain Gal residues G_c and G_d were substituted at position 3.

Supplemental Figure 2. DOSY spectrum of Interferon-Hyp-polysaccharide-1 collected at 25°C used to calculate the diffusion coefficient.

Supplemental Figure 3. NOESY spectrum of Interferon-Hyp-polysaccharide-1 (25 °C).

Supplemental Figure 4. ^1H NMR Spectrum of Hyp-AG Interferon-Hyp-polysaccharide-2 collected at 55 °C. Signals A and B were assigned to H-1 of α -L-Ara, signal C to H-1 α -L-Rha, signal D (shoulder of C) to H-4 of Hyp, and signal E to the β -D-Gal residues of the galactan backbone. Signal F was assigned to H-1 of β -D-Gal linked to Hyp, and signal G to H-1 of side-chain β -D-Gal and β -D-GlcUA residues.

Supplemental Figure 5. 2D TOCSY spectrum of Interferon-Hyp-polysaccharide-2 collected at 55 °C. The spectrum was used for ^1H chemical shift assignments. Based on our earlier work (15) Signal A indicated the side-chain Gal residues G_c and G_d were unsubstituted at position 3.

Supplemental Figure 6. HSQC Spectrum of Hyp-AG Interferon-Hyp-polysaccharide-2 collected at 55 °C. All labelling corresponds to those featured in Figure 2.

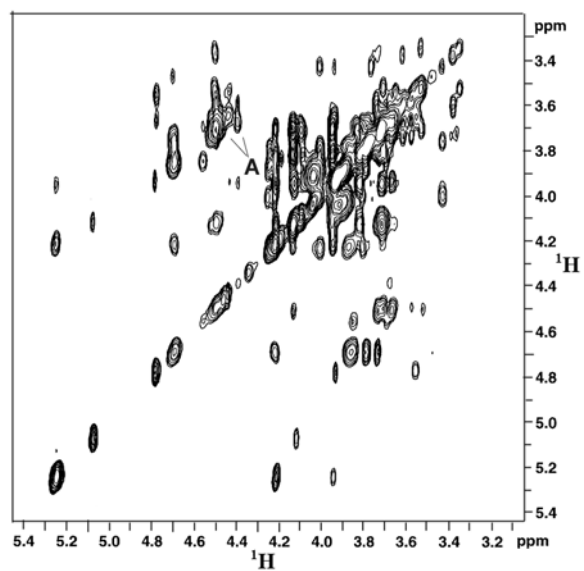
Supplemental Figure 7. HMBC Spectrum of Hyp-AG Interferon-Hyp-polysaccharide-2 collected at 55°C. All labelling corresponds to those featured in Figure 3.

Supplemental Figure 8. HSQC spectrum of Ala-Hyp-polysaccharide-2 collected at 60 °C. All labelling corresponds to those featured in Figure 2.

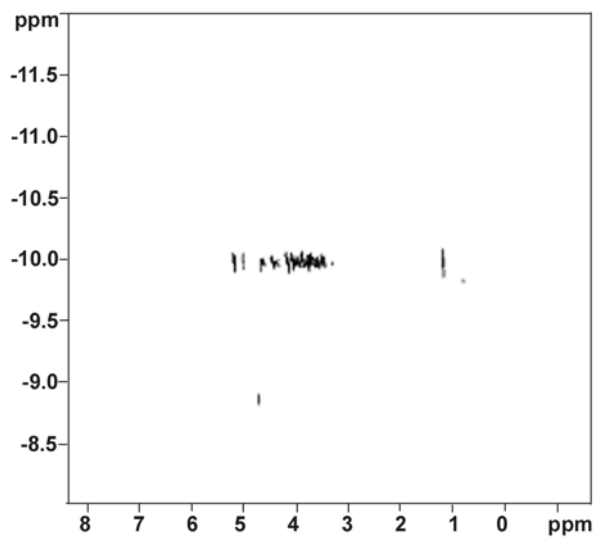
Supplemental Figure 9. HMBC spectrum of Ala-Hyp-polysaccharide-2 collected at 60 °C. All labelling corresponds to those featured in Figure 3.

Supplemental Figure 10. A stable folded conformer of Interferon Hyp-polysaccharide-1 depicts a 'reverse-turn' galactan backbone (dark blue) with carboxylate chelation of a calcium ion (yellow) by sidechain glucuronic acid residues (purple). Peripheral α -L-arabinosyl trisaccharides are depicted by light blue 5-membered rings and sidechain Gal residues by light blue 6-membered rings. Not all residues are visible in this presentation.

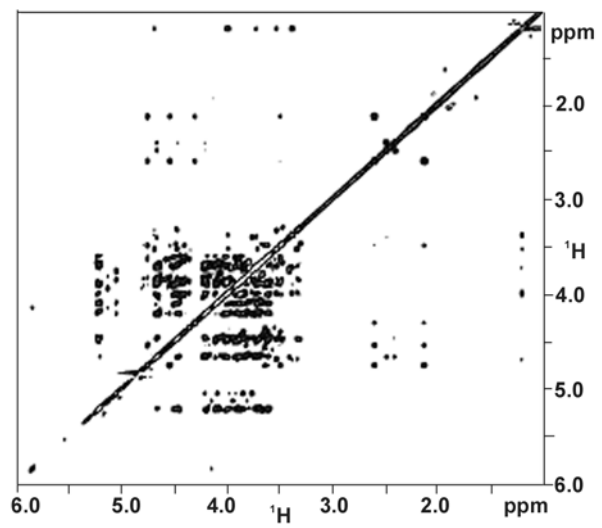
Supplemental Figure 1



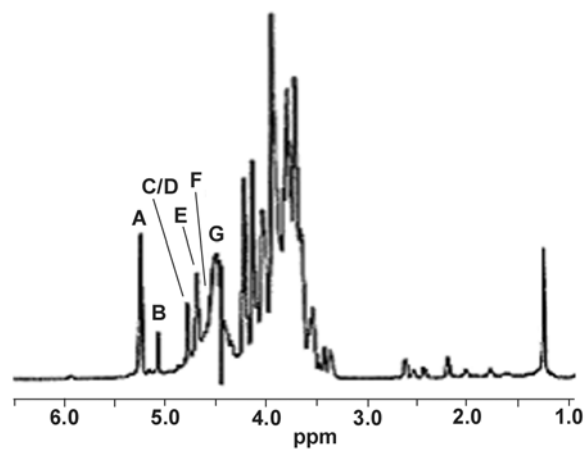
Supplemental Figure 2



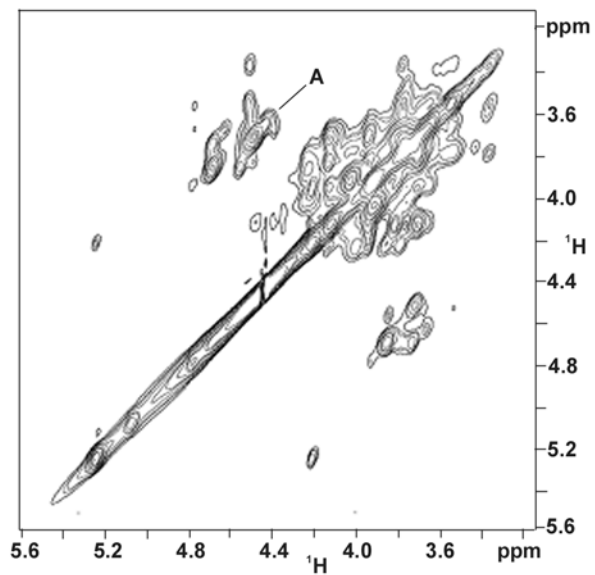
Supplemental Figure 3



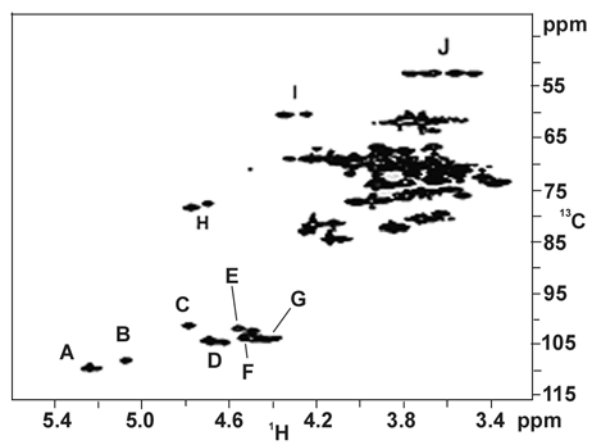
Supplemental Figure 4



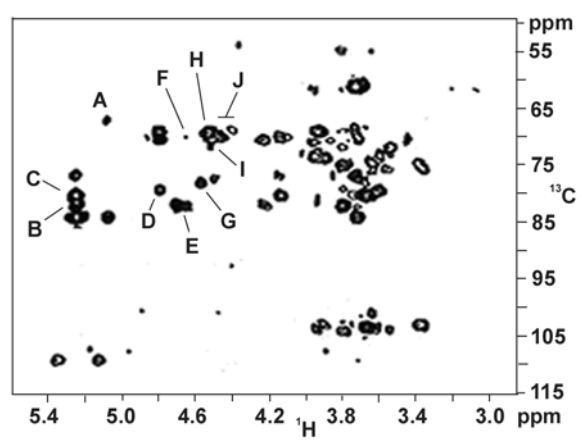
Supplemental Figure 5



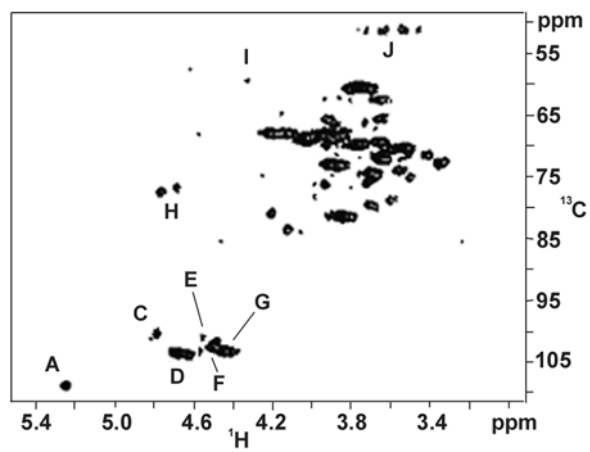
Supplemental Figure 6



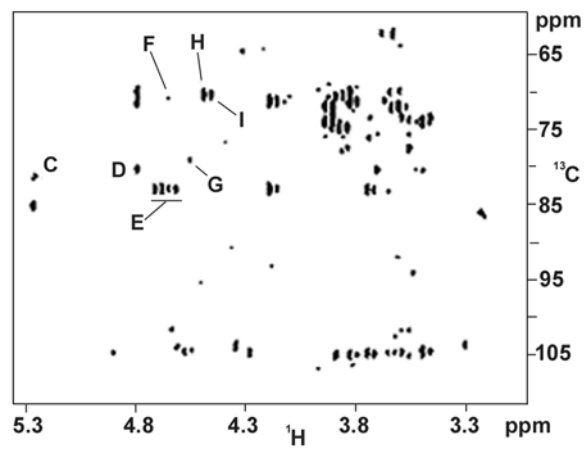
Supplemental Figure 7



Supplemental Figure 8



Supplemental Figure 9



Supplemental Figure 10

