SUPPLEMENTARY INFORMATION

Simple Gd^{3+}-Neu5*N*Ac complexation results in NMR chemical shift asymmetries of structurally equivalent complex-type *N*-glycan branches

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Experimental details

NMR spectroscopy: All measurements were acquired on a Bruker AVANCE-III HD 700 spectrometer (^{1}H frequency: 700.13 MHz) equipped with a 5 mm QCI (quadruple resonance ^{1}H/^{13}C/^{15}N/^{31}P) CryoProbe^{TM}. NMR samples contained 200 micrograms of *N*-glycan in 300 microliters D_2O in a 5 mm Shigemi NMR tube. The temperature was set to 293 K. Two-dimensional (2D) proton (COSY, TOCSY, NOESY) spectra were acquired in the phase-sensitive mode using the States-TPPI method for TOCSY and the QF mode for COSY experiments, using a time domain data point density of 512 t_1 x 4096 t_2 complex points and 64 transients per complex t_1 increment. 2D ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HSQC-TOCSY, and ^{1}H-^{13}C HMBC spectra were recorded over 512 (^{1}H) x 4096 (^{13}C) complex points to obtain spectral widths of 13 (^{1}H) and 165 ppm (^{13}C). The NOESY mixing time was 150 ms.

Spectra were processed by NMRPipe[13] and analyzed using SPARKY. Proton and carbon resonance assignment for all residues were obtained using a combination of 2D COSY and TOCSY experiments for the ^{1}H signals, and by 2D ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HSQC-TOCSY, and ^{1}H-^{13}C HMBC for the ^{13}C signals, as well as with reference to the chemical shift data obtained from undecasacharides reported previously by Sato and Kajihara.[11]

The assignment of the Gd^{3+} interacting residues was performed based on the observed magnitude of the chemical shift changes. The carbon-bonded protons closest to the Gd^{3+} site generally provided the largest chemical shift changes, in ppm.

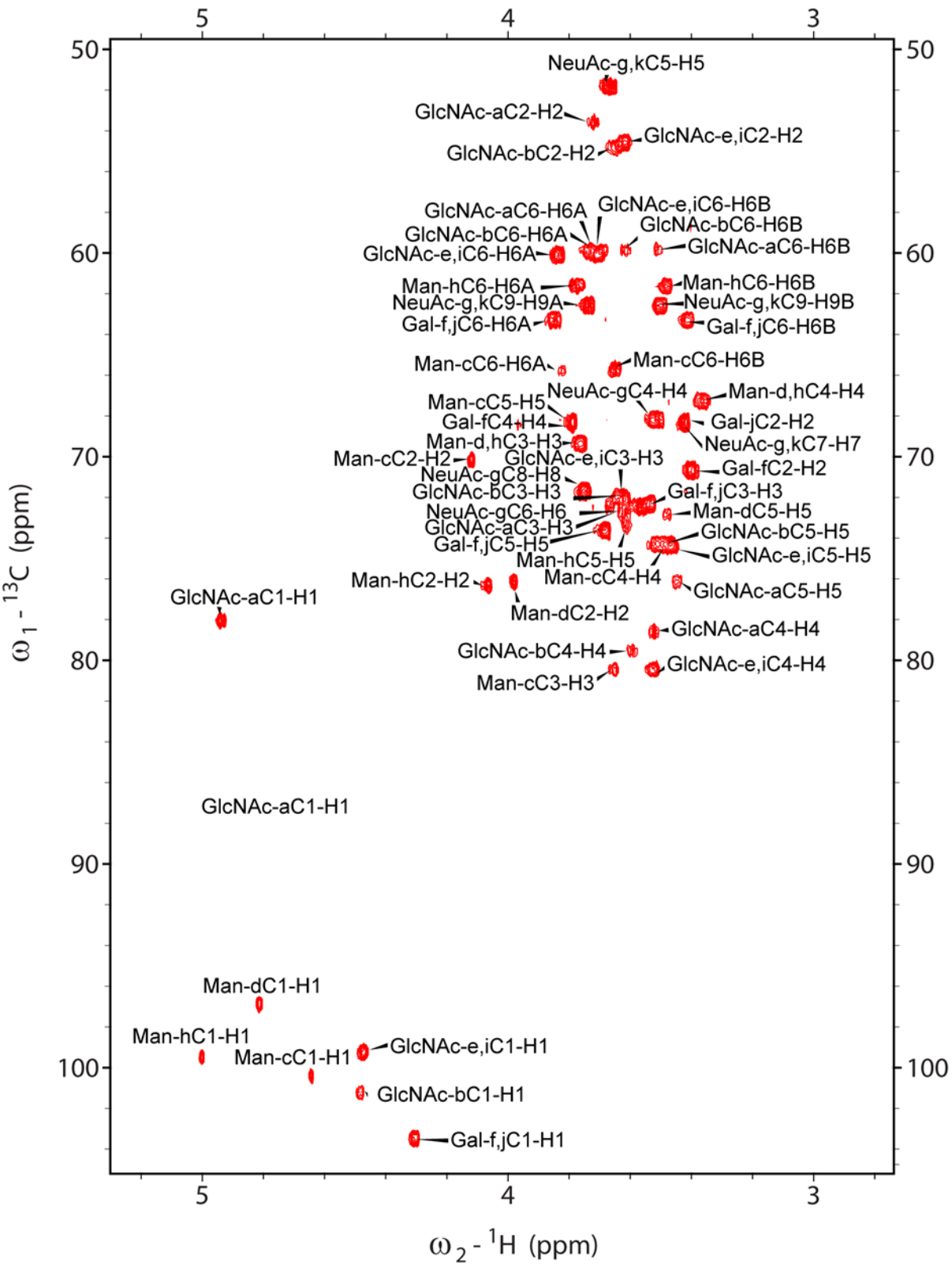

Figure S1. ^{1}H-^{13}C HSQC NMR (^{1}H: 700 MHz) spectra of disialo-*N*-glycan labeled with DOTA, **1-DOTA** at 293K in D_2O solution.

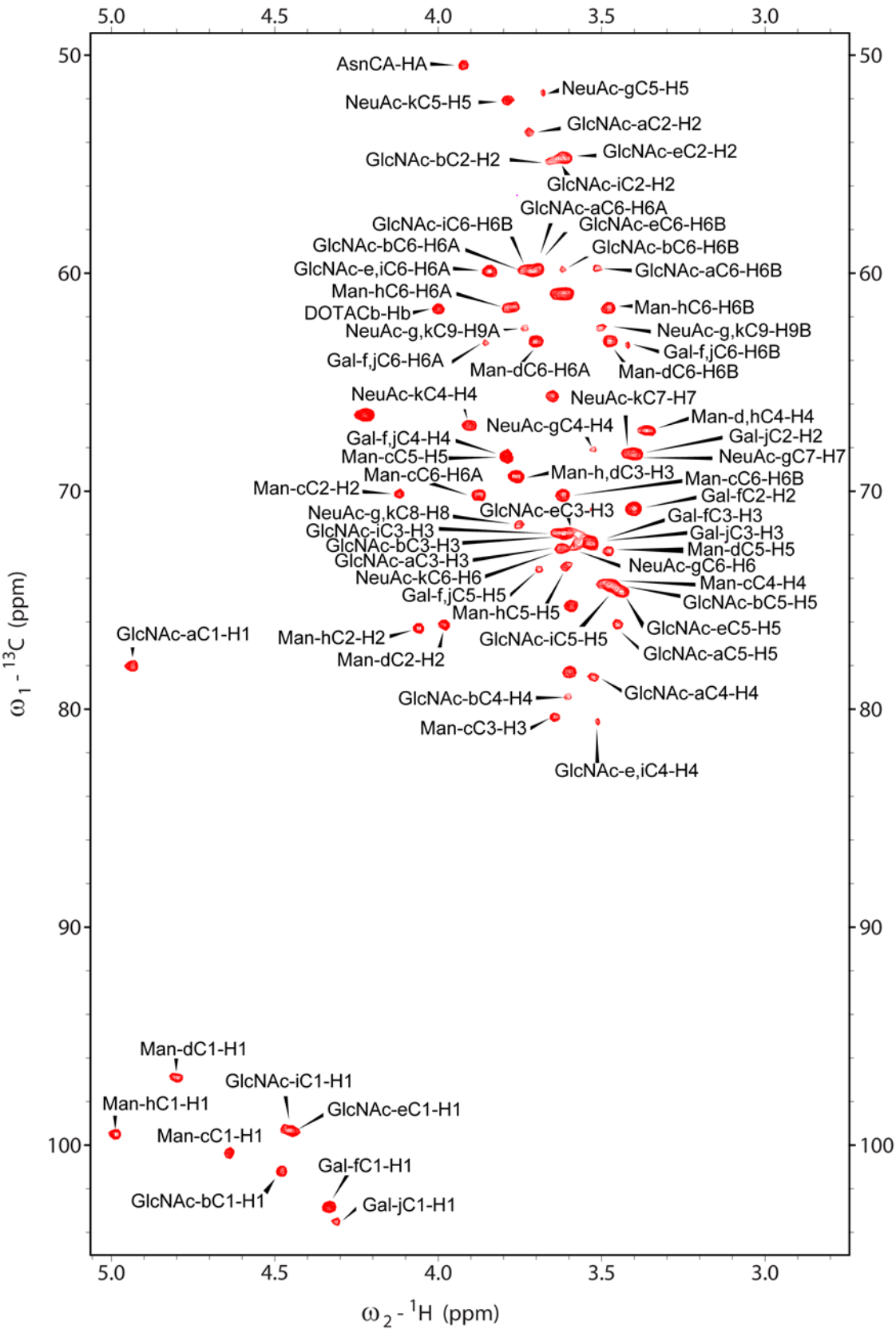

Figure S2. ^{1}H-^{13}C HSQC NMR (^{1}H: 700 MHz) spectra of disialo-*N*-glycan labeled with DOTA, **1-DOTA** at 293K in D_2O solution after the addition of Gd^{3+}.

Table S1. ^{1}H and ^{13}C chemical shifts in ppm measured in D_2O for **1-DOTA** with Gd^{3+} at 293 K.

Functional group and its positions	^{1}H (ppm)	^{13}C (ppm)	Functional group and its positions	^{1}H (ppm)	^{13}C (ppm)
Asn H	8.34				
α	3.85	50.77			
β	2.79	34.88			
GlcNAc-a 1	4.95	77.97	Man-h 1	4.99	99.50
2	3.73	53.55	2	4.07	76.30
3	3.63	72.71	3	3.77	69.31
4	3.53	78.49	4	3.36	67.16
5	3.46	76.12	5	3.63	73.46
6A	3.72	59.84	6A	3.78	61.60
6B	3.52	59.84	6B	3.49	61.60
GlcNAc-b 1	4.48	101.20	Gal-f 1	4.34	102.90
2	3.66	54.87	2	3.41	70.87
3	3.61	71.91	3	3.55	72.35
4	3.61	79.51	4	3.80	68.47
5	3.49	74.24	5	3.68	73.54
6A	3.75	59.86	6A	3.70	63.22
6B	3.63	59.86	6B	3.46	63.22
GlcNAc-e 1	4.42	99.51	Gal-j 1	4.31	103.50
2	3.62	54.74	2	3.40	68.35
3	3.62	71.91	3	3.53	72.35
4	3.53	80.61	4	3.79	68.47
5	3.46	74.56	5	3.69	73.54
6A	3.85	59.87	6A	3.69	63.22
6B	3.71	59.87	6B	3.49	63.22
GlcNAc-i 1	4.47	99.31	NeuNAc-g 3a	2.54	39.99
2	3.62	54.74	3b	1.60	39.99
3	3.62	71.91	4	3.53	68.14
4	3.53	80.61	5	3.68	51.83
5	3.46	74.56	6	3.62	72.68
6A	3.85	59.87	7	3.42	68.37
6B	3.71	59.87	8	3.76	71.63
			9a	3.74	62.52
			9b	3.52	62.52
Man-c 1	4.64	100.40	NeuNAc-k 3a	2.12	39.29
2	4.13	70.12	3b	1.71	39.29
3	3.65	80.44	4	3.90	67.22
4	3.48	74.34	5	3.79	52.14
5	3.80	68.47	6	3.60	72.28
6A	3.83	65.73	7	3.43	68.37
6B	3.66	65.73	8	3.86	70.13
			9a	3.78	60.48
			9b	3.70	60.48
Man-d 1	4.80	96.93			
2	3.99	76.17			
3	3.77	69.31			
4	3.37	67.19			
5	3.49	72.64			
6A	3.71	63.15			
6B	3.48	63.15			

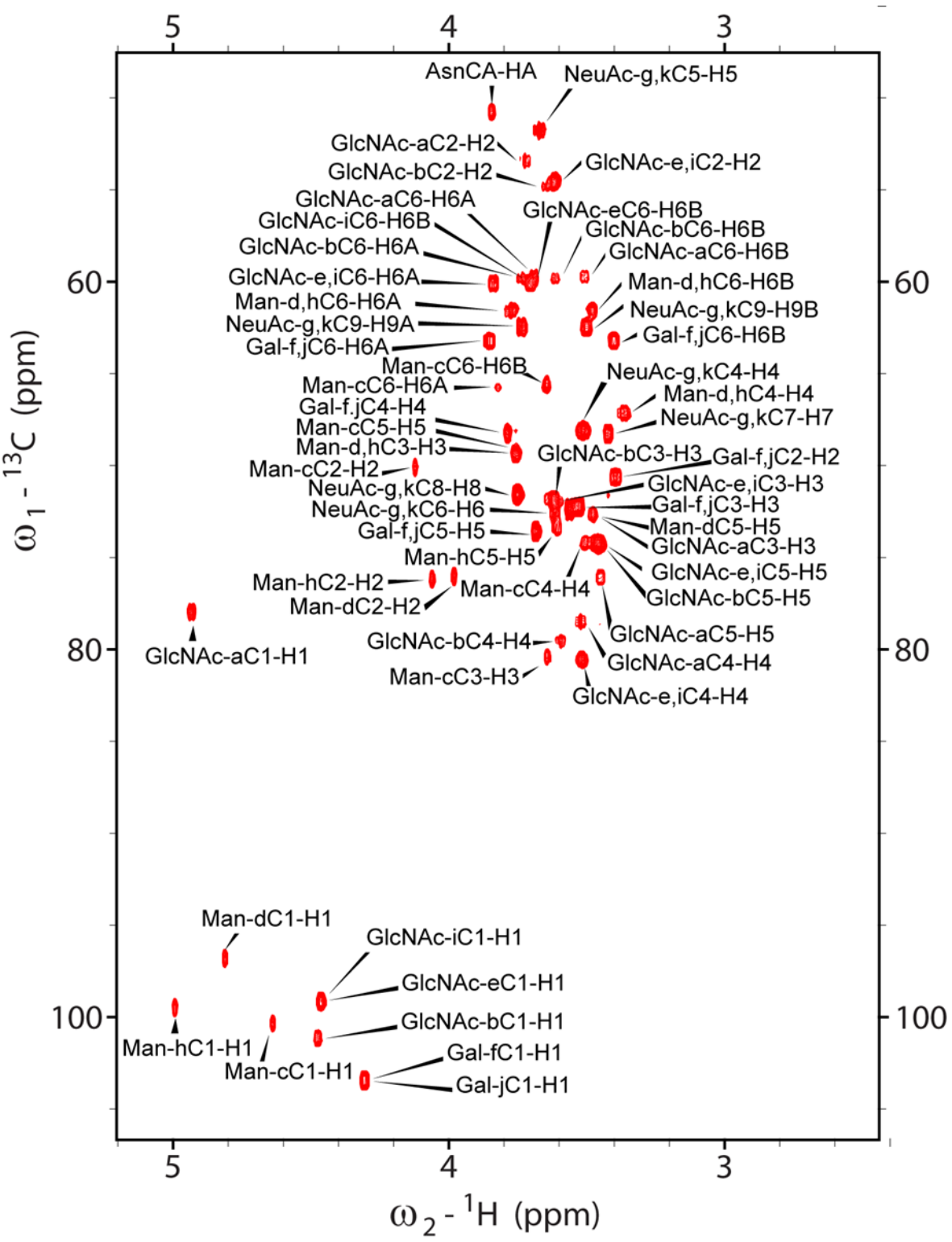

Figure S3. ^{1}H-^{13}C HSQC NMR (^{1}H: 700 MHz) spectra of disialo-*N*-glycan **1** at 293K in D_2O solution.

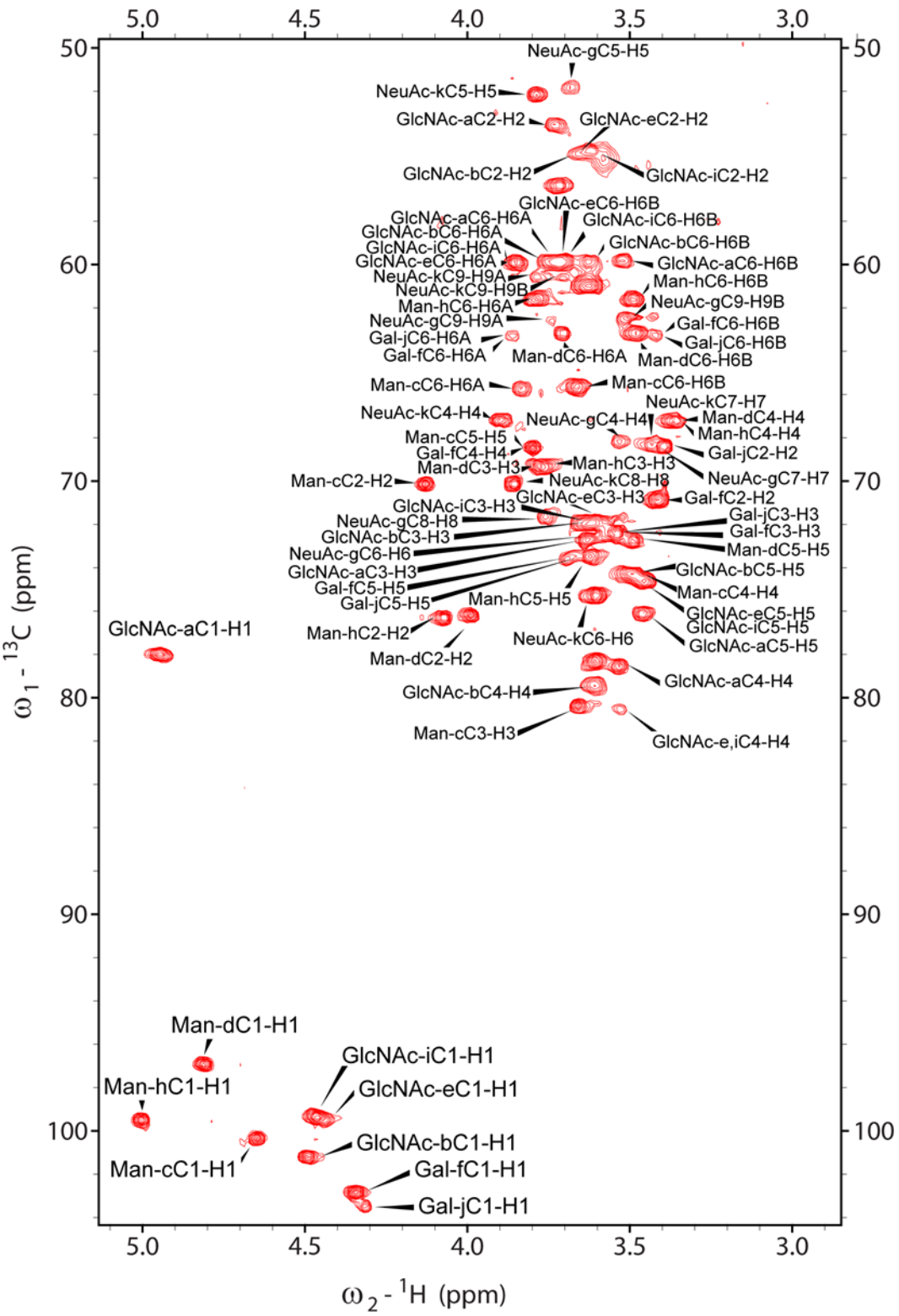

Figure S4. ^{1}H-^{13}C HSQC NMR (^{1}H: 700 MHz) spectra of disialo-*N*-glycan **1** at 293K in D_2O solution after the addition of Gd^{3+}.

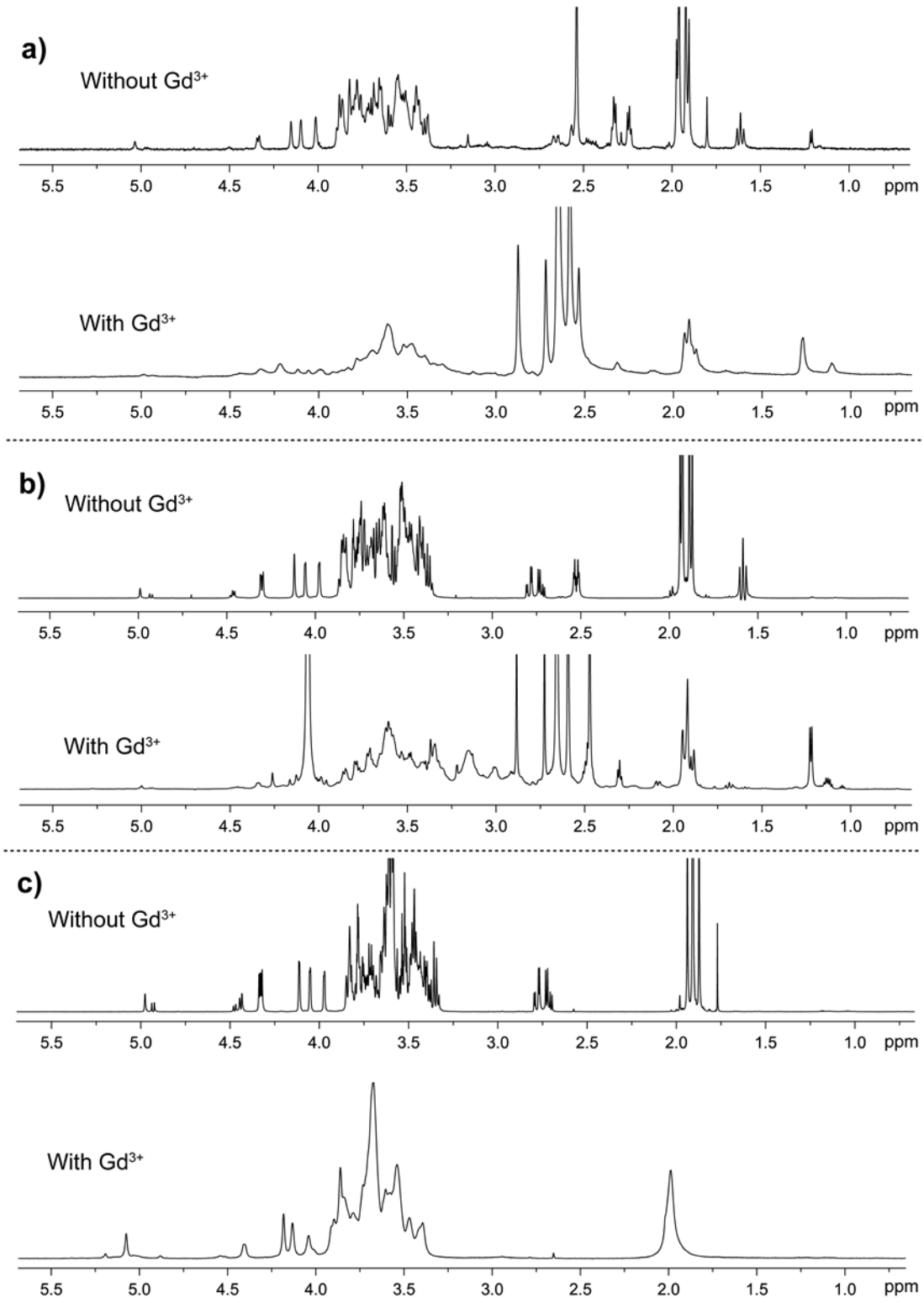

Figure S5. ^{1}H NMR (700 MHz) spectra of the *N*-glycans at 293K in D_2O solution and after the addition of Gd^{3+}. (a) Disialo-*N*-glycan labeled with DOTA, **1-DOTA**; (b) disialo-*N*-glycan **1**; (c) asialo-*N*-glycan **2**.

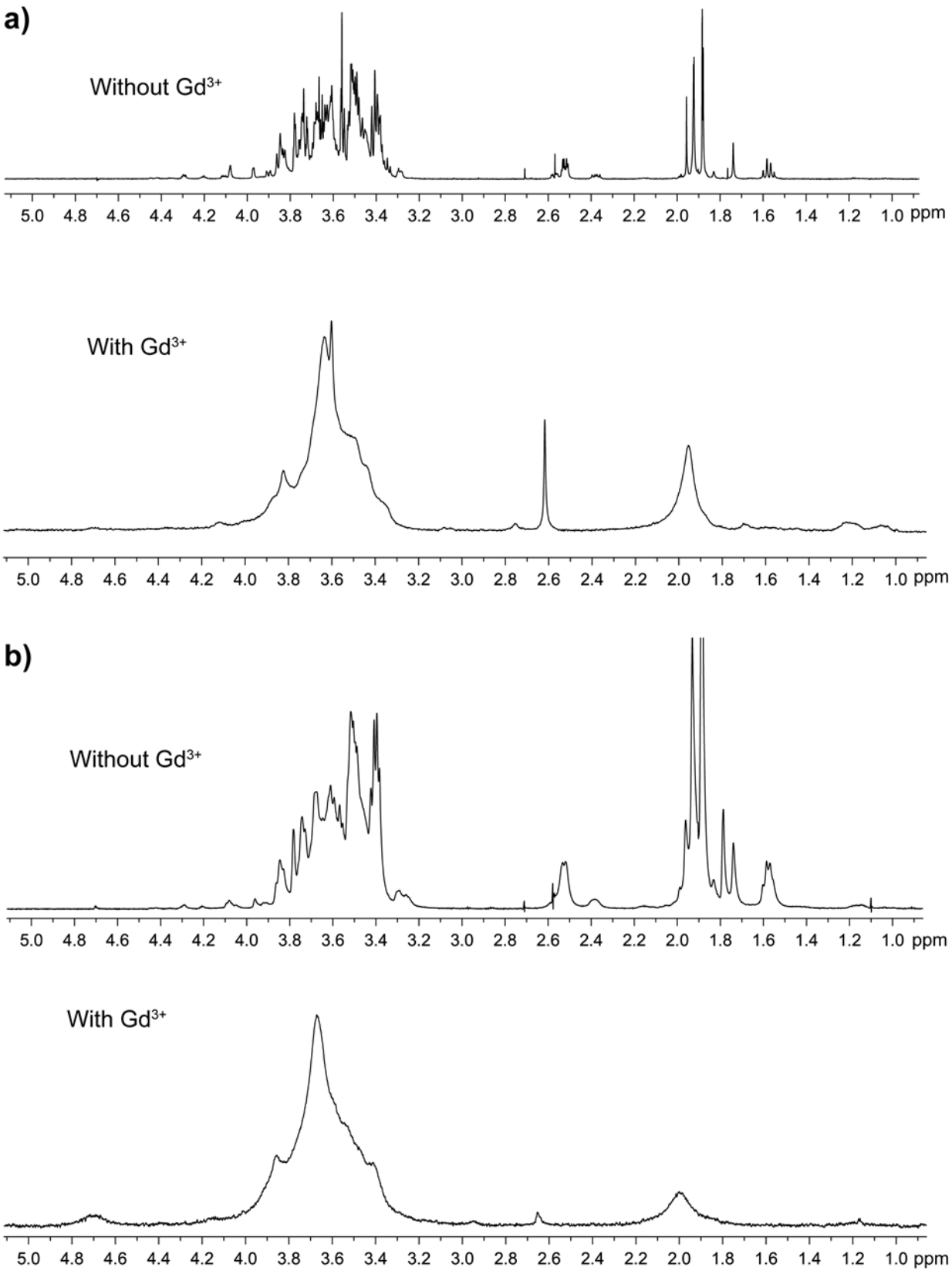

Figure S6. ^{1}H NMR (700 MHz) spectra of the *N*-glycans at 293K in D_2O solution and after the addition of Gd^{3+}. (a) Tri-antenary sialo-*N*-glycan **3**; and (b) tetra-antenary sialo-*N*-glycan **4**.