Supplementary Information File

A library of chemically defined human *N*-glycans synthesized from microbial oligosaccharide precursors

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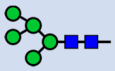
Supplementary Table 1. *N*-glycan structures produced in this study

Glycan Number	Symbolic Representation	Structure
2		Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc
3		Manα1-6(Manα1-2Manα1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc
4		Manα1-6(Manα1-3)Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc
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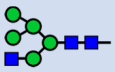
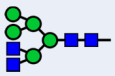
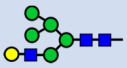
6		Manα1-6(Manα1-3)Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc
7		Manα1-6(Manα1-3)Manα1-6(GlcNAcβ1-2(GlcNAcβ1-4)Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc
8		Manα1-6(Manα1-3)Manα1-6(Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc
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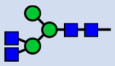
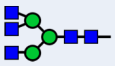
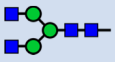
10		Manα1-6(Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc
11		Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc
12		Manα1-6(GlcNAcβ1-2(GlcNAcβ1-4)Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc
13		GlcNAcβ1-2(GlcNAcβ1-6)Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc
14		GlcNAcβ1-2Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc
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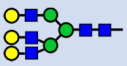
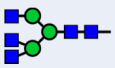
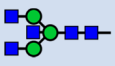
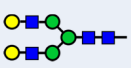
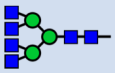
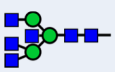
16		Galβ1-4GlcNAcβ1-2Manα1-6(Galβ1-4GlcNAcβ1-2(Galβ1-4GlcNAcβ1-4)Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc
17		GlcNAcβ1-2Manα1-6(GlcNAcβ1-2(GlcNAcβ1-4)Manα1-3)Manβ1-4 GlcNAcβ1-4GlcNAc
18		GlcNAcβ1-2Manα1-6(GlcNAcβ1-2Manα1-3)(GlcNAcβ1-4)Manβ1-4GlcNAcβ1-4GlcNAc
19		Galβ1-4GlcNAcβ1-2Manα1-6(Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc
20		GlcNAcβ1-2(GlcNAcβ1-6)Manα1-6(GlcNAcβ1-2(GlcNAcβ1-4)Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc
21		GlcNAcβ1-2Manα1-6(GlcNAcβ1-2(GlcNAcβ1-4)Manα1-3)(GlcNAcβ1-4)Manβ1-4GlcNAcβ1-4GlcNAc

Supplementary Table 2. Chemical shift assignments of the synthesized *N*-linked oligosaccharides

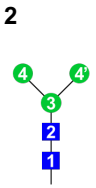
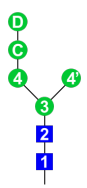
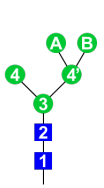
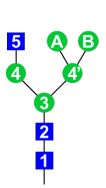
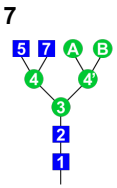
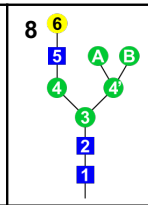
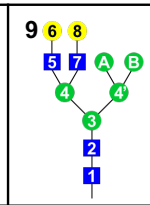
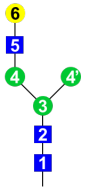
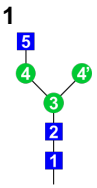
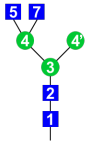
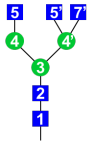
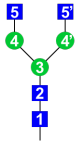
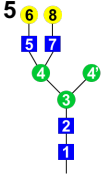
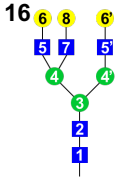
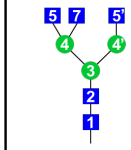
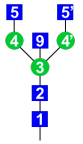
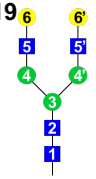
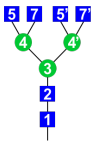
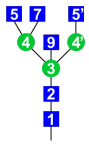
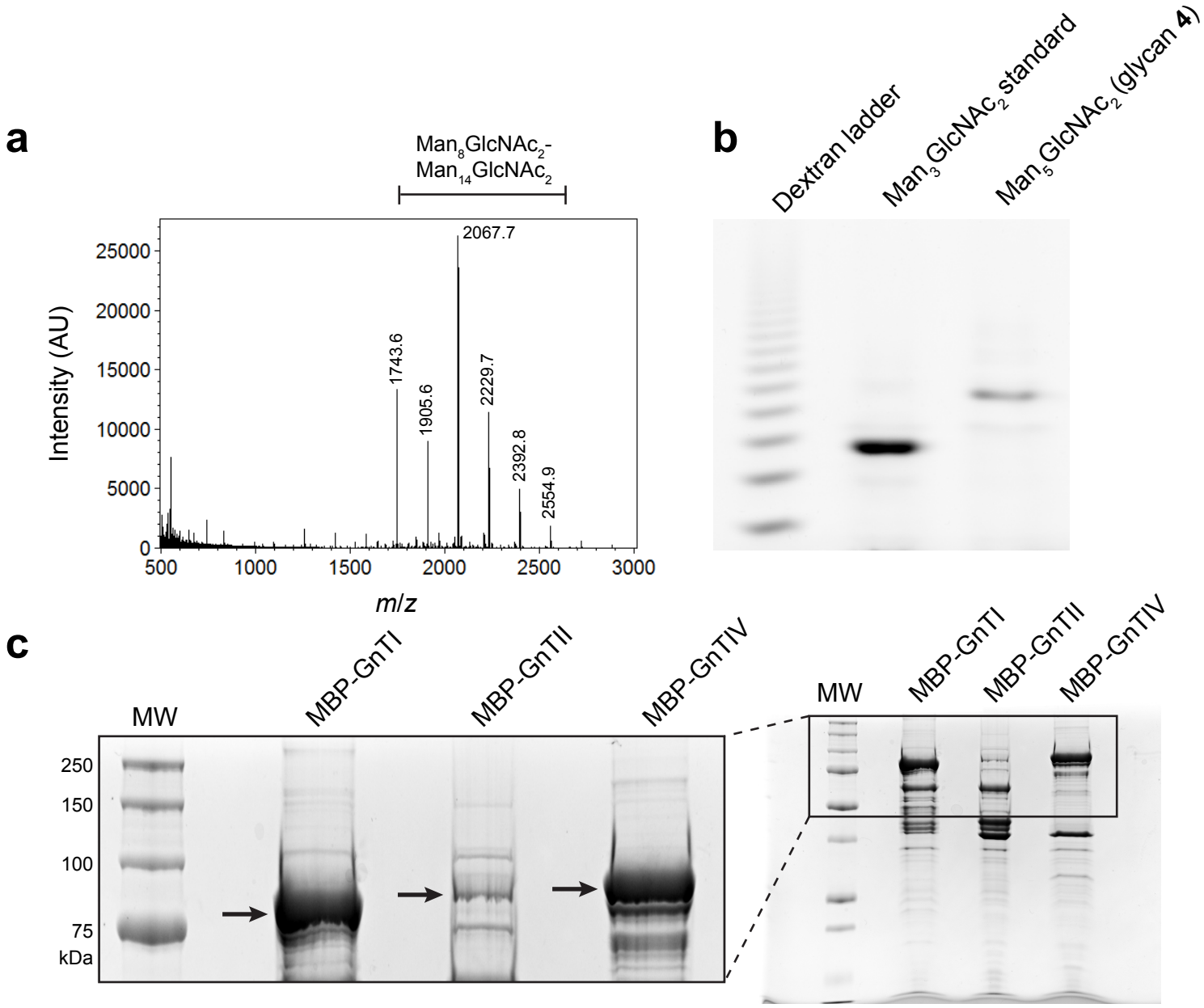
Residue	2	3	4	5	6	7	8	9	10	11
										
GlcNAc-1α	5.16	5.17	5.16	5.17	5.14	5.15	5.15	5.16	5.16	5.15
GlcNAc-1β	4.67	4.67	4.67	4.67	4.65	4.66	4.66	4.66	4.67	4.65
GlcNAc-2	4.58	4.58	4.57	4.57	4.55	4.56	4.55	4.56	4.58	4.57
Man-3	4.76	4.75	4.75	4.59	4.75	4.75	4.75	4.75	4.75	4.75
Man-4	5.08	5.32	5.06	-	5.08	5.07	5.08	5.08	5.07	5.08
Man-4’	4.89	4.89	4.84	-	4.84	4.84	4.84	4.84	4.87	4.88
Man-A	-	-	5.06	-	5.06	5.05	5.06	5.06	-	-
Man-B	-	-	4.88	-	4.88	4.87	4.88	4.88	-	-
Man-C	-	5.28	-	-	-	-	-	-	-	-
Man-D	-	5.02	-	-	-	-	-	-	-	-
GlcNAc-5	-	-	-	-	4.53	4.52	4.54	4.54	4.54	4.52
GlcNAc-5’	-	-	-	-	-	-	-	-	-	-
Gal-6	-	-	-	-	-	-	4.44	4.44	4.43	-
GlcNAc-7	-	-	-	-	-	4.50	-	4.52	-	-
Gal-8	-	-	-	-	-	-	-	4.43	-	-

Table 1. H1 chemical shifts. Glycans 2, 3, 5, 10-21 were analyzed at 25°C using a Varian Inova 600-MHz spectrometer (Complex Carbohydrate Research Center) with a cryoprobe. Glycans 4, and 6-9 were analyzed at 25°C using a Varian Inova 600-MHz spectrometer (Cornell) with a pulse field gradient probe. To calibrate spectra, HOD peak was set to δ_H = 4.77 ppm.

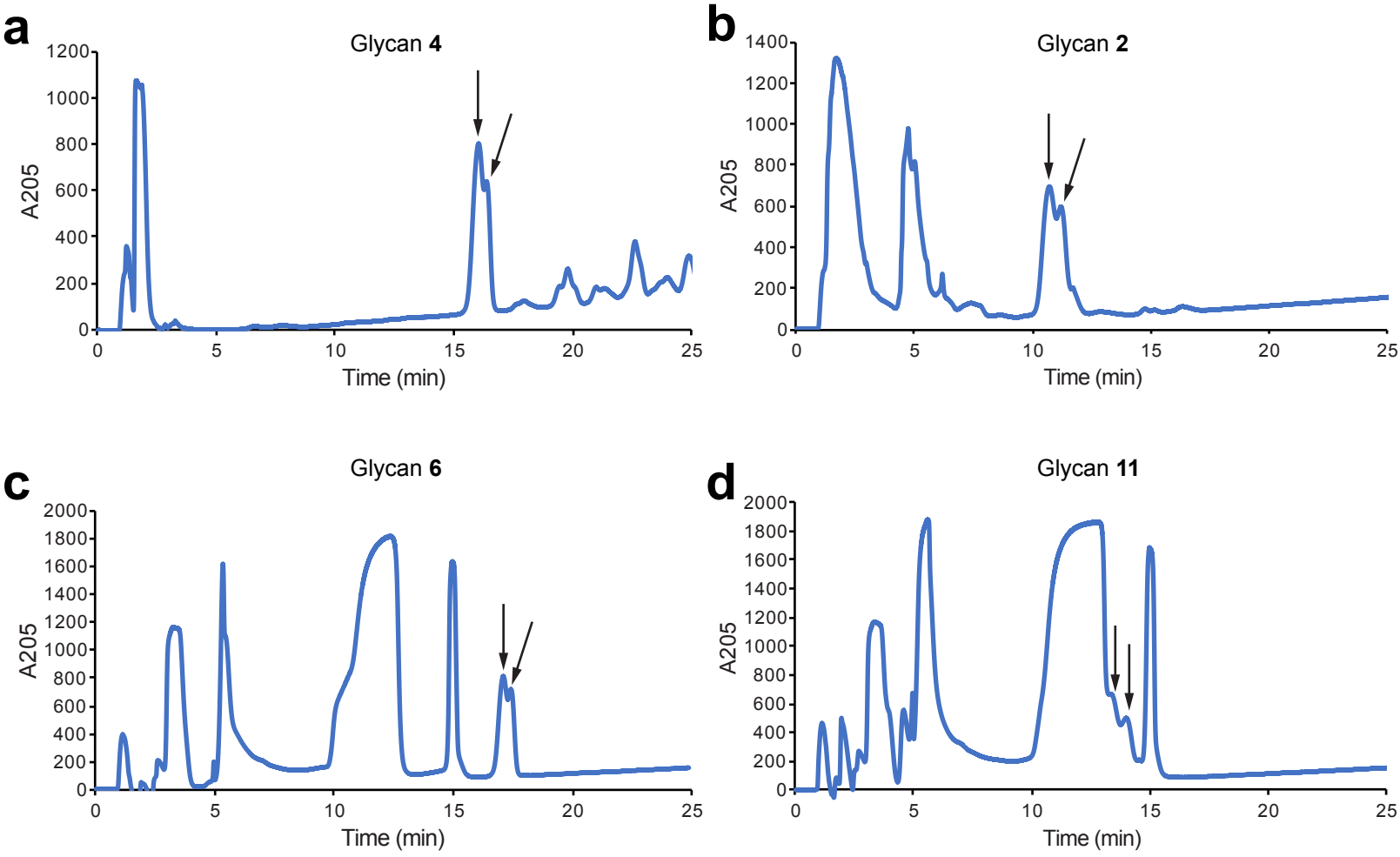
Supplementary Table 2. (continued)

Residue	12 	13 	14 	15 	16 	17 	18 	19 	20 	21 
GlcNAc-1α	5.15	5.17	5.17	5.17	5.17	5.17	5.16	5.17	5.17	5.17
GlcNAc-1β	4.67	4.67	4.67	4.68	4.67	4.68	4.67	4.67	4.67	4.66
GlcNAc-2	4.57	4.58	4.57	4.58	4.58	4.58	4.58	4.59	4.58	4.58
Man-3	4.75	4.75	[1]n.d.	[1]n.d.	[1]n.d.	[1]n.d.	4.67	4.75	4.74	4.66
Man4	5.08	5.1	5.09	5.09	5.09	5.09	5.04	5.1	5.1	5.03
Man-4’	4.88	4.84	4.9	4.89	4.89	4.89	4.98	4.91	4.84	4.98
GlcNAc-5	4.52	4.54	4.54	4.55	4.53	4.53	4.53	4.56	4.55	4.5
GlcNAc-5’	-	4.51	4.54	4.53	4.57	4.51	4.53	4.56	4.54	4.53
Gal-6	-	-	-	4.45	4.44	-	-	4.45	-	-
Gal-6’	-	-	-	-	4.44	-	-	4.45	-	-
GlcNAc-7	4.49	-	-	-	4.51	4.54	-	-	4.51	4.53
GlcNAc-7’	-	4.54	-	-	-	-	-	-	4.51	-
Gal-8	-	-	-	4.43	4.45	-	-	-	-	-
GlcNAc-9	-	-	-	-	-	-	4.44	-	-	4.44

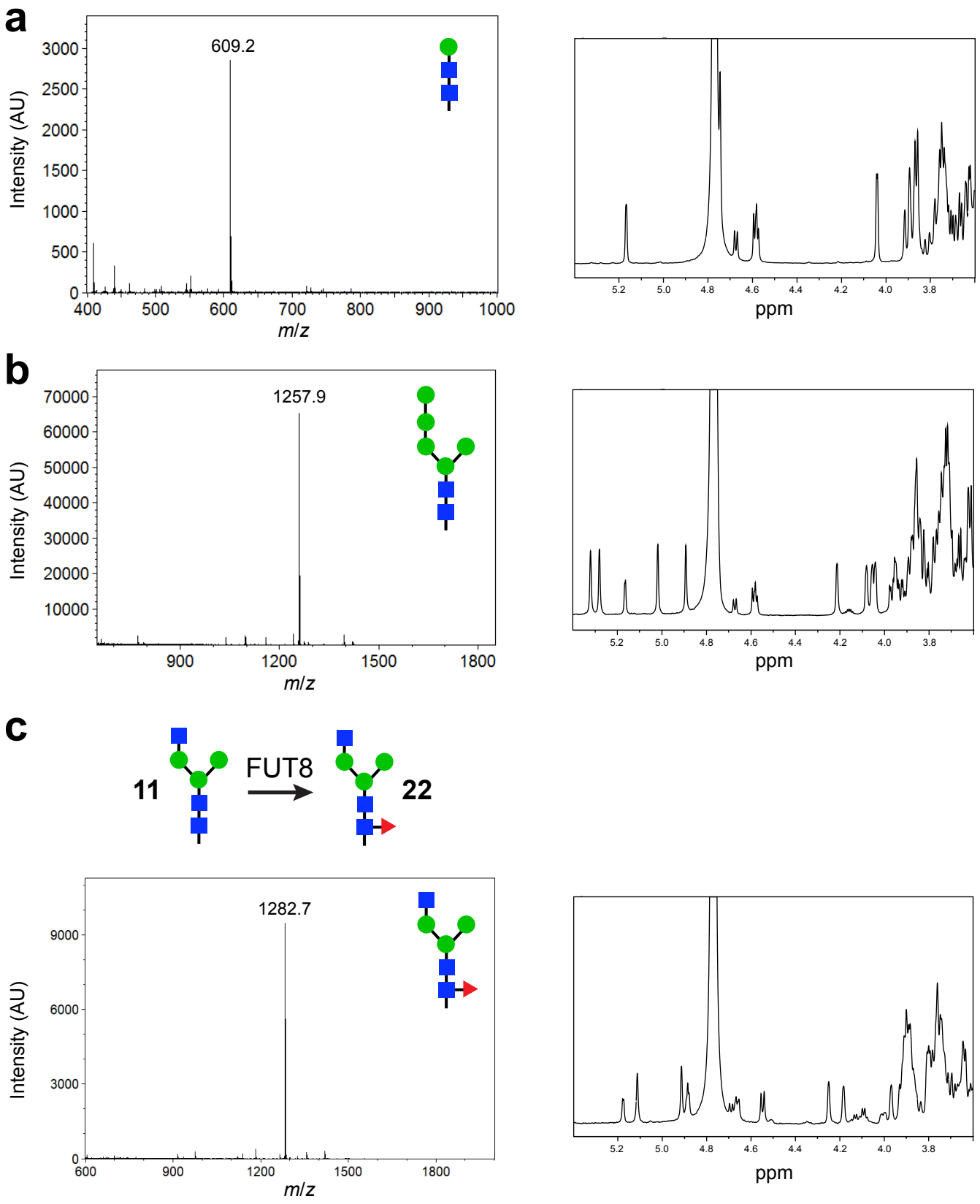
[1]n.d. - anomeric signal for Man3 overlaps with HOD peak at 25°C and is not visible in some proton spectra.



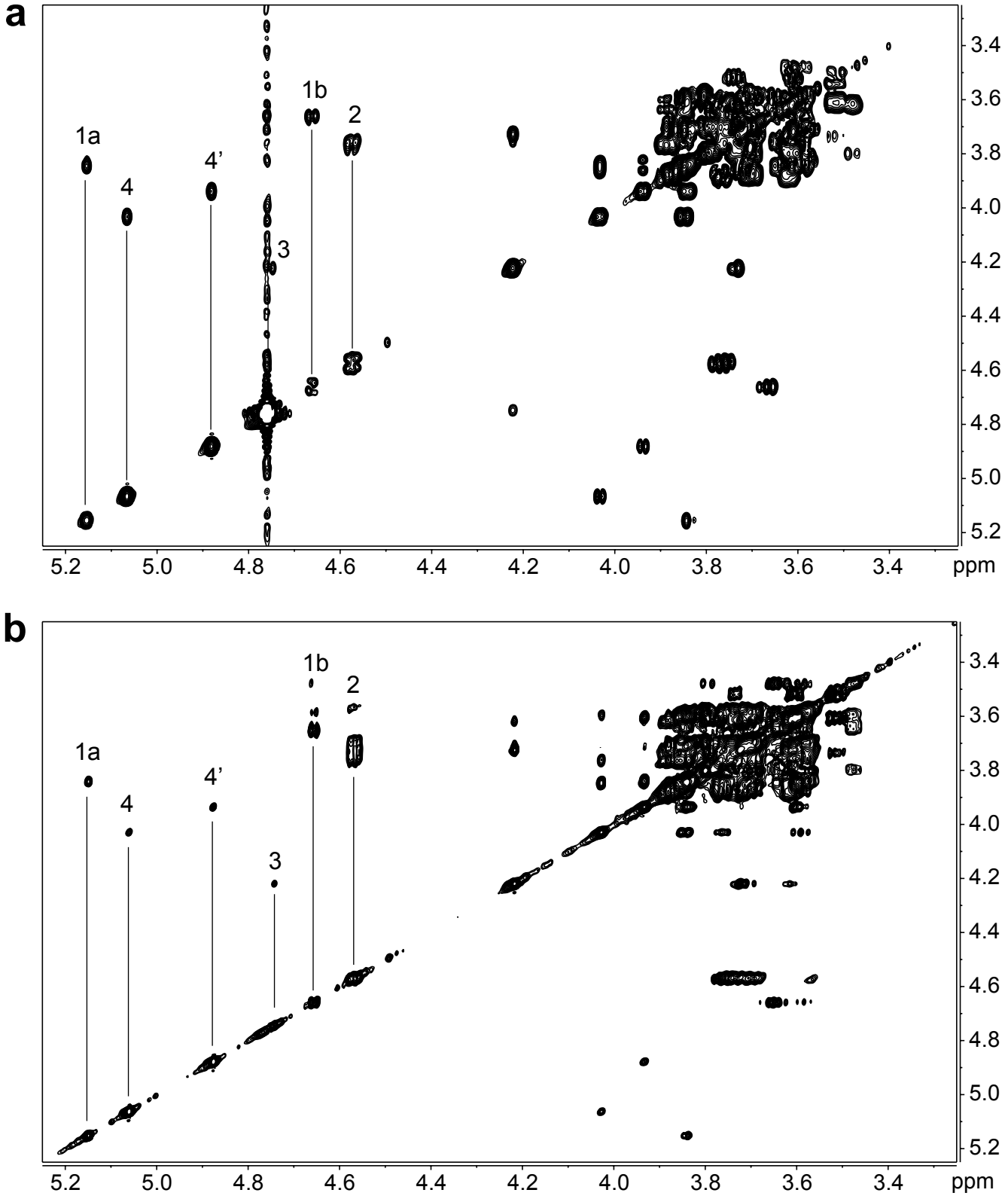
Supplementary Figure 1. MS and FACE analysis of yeast invertase-derived precursor oligosaccharides. (a) MALDI-TOF MS analysis of high-mannose oligosaccharides liberated from invertase by PNGase F. The predominant peak at 2067.7 *m*/*z* corresponds to $Man_{10}GlcNAc_2$. (b) FACE gel analysis of $Man_5GlcNAc_2$ produced by enzymatic deglycosylation of the oligosaccharide mixture shown in (a) demonstrates homogeneity and purity of glycan product. Commercially available $Man_3GlcNAc_2$ was used as a standard for quantification. (c) SDS-PAGE gel of fusion proteins comprised of the *E. coli* maltose-binding protein (MBP) and one of the following: *N. tabacum* GnTI, *H. sapiens* GnTII, or *B. taurus* GnTIV either GnTI, GnTII, or GnTIV, as indicated. All fusion proteins were expressed in *E. coli* Origami2(DE3) cells and purified by amylose affinity chromatography. Molecular weight (MW) ladder is shown at the left. Uncropped version of the SDS-PAGE gel is shown at the right, with the cropped section marked by the box.



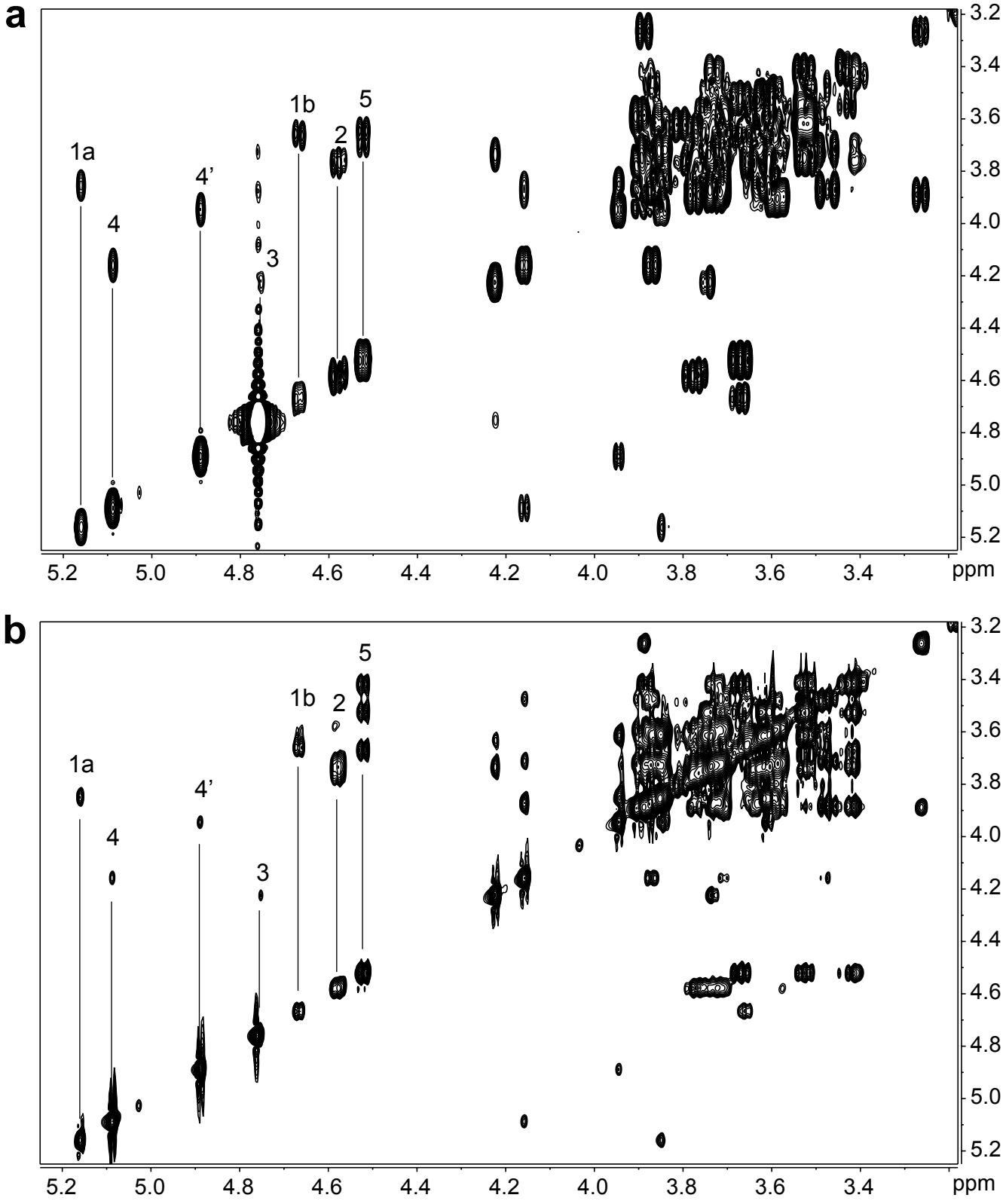
Supplementary Figure 2. Representative HPLC analysis of glycan precursors and products. (a) Partially purified glycan **4** (~150 mg isolated from invertase as described in the experimental procedures) was resuspended in 33% water, 67% acetonitrile, and resolved using a Waters XBridge BEH Amide column (130 Å, 5 µm, 4.6 mm X 100 mm). After an initial 5 min at 30% 20 mM ammonium formate in water, 70% acetonitrile, a linear gradient was run to 50% 20 mM ammonium formate in water, 50% acetonitrile over 20 min at room temp. Elution was monitored by absorbance at 205 nm, and fractions (~1.5 mL each) were analyzed by MALDI-TOF MS to verify the presence of glycan. This glycan eluted as a doublet with peaks at 16.0 and 16.4 min. Peaks eluting later in the gradient were high mannose contaminants as confirmed by MALDI-TOF MS. (b) Approximately 200 µg of dried glycan **2** (isolated from LLOs as described in the experimental procedures) was subjected to HPLC as described above. Glycan **2** eluted as a doublet, corresponding to alpha and beta anomers, with peaks at 10.7 and 11.2 min. (c) Glycan **6** was produced by subjecting HPLC-purified glycan **4** to an overnight reaction as described in the experimental procedures. The glycan **6** reaction was dried and subjected to HPLC as described above. This glycan eluted as a doublet with peaks at 17.1 and 17.4 min, making it well resolved from contaminant peaks and glycan **4**. (d) Glycan **11** was produced by subjecting HPLC-purified glycan **2** to an overnight reaction as described in the experimental procedures. The glycan **11** reaction was dried and subjected to HPLC as described for glycan **2**. Glycan **11** eluted as a doublet with peaks at 13.3 and 14.0 min. The shift in the elution time indicates addition of GlcNAc to glycan **2**; however, the assessment of precursor glycan **2** is prevented by overlap with the tail of a large peak corresponding to unreated UDP-GlcNAc. Doublet peaks corresponding to the glycan of interest are marked with arrows in each trace.



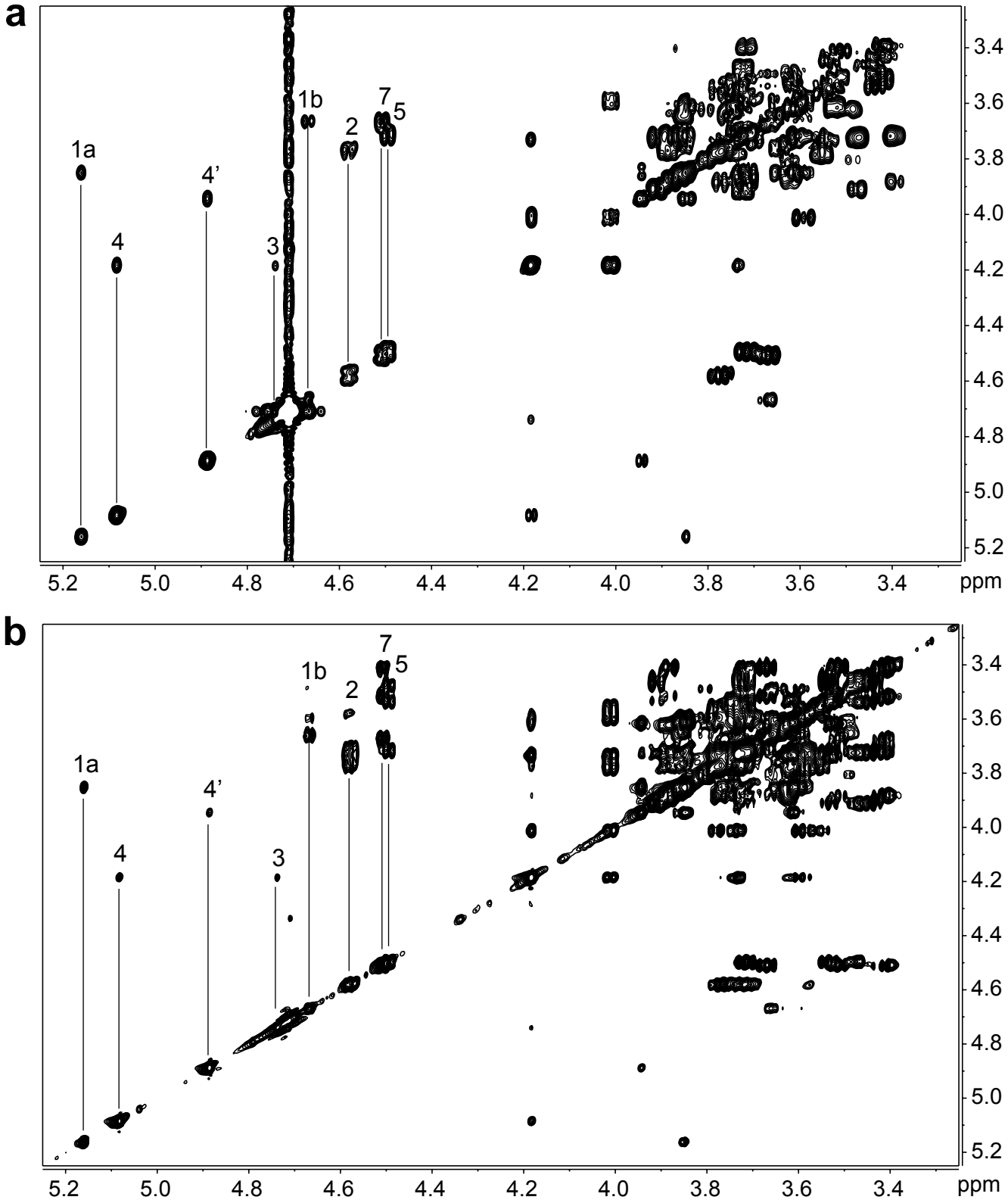
Supplementary Figure 3. Preparation of alternative precursor oligosaccharides for enzymatic remodeling. MALDI-TOF MS analysis (left panels) and 600-MHz ^{1}H NMR characterization (right panels) corresponding to: (a) $Man_1GlcNAc_2$ glycan synthesized by enzymatic deglycosylation of *S. cerevisiae* oligosaccharides; (b) the $Man_5GlcNAc_2$ glycan synthesized by glycoengineered *E. coli* cells; and (c) glycan 22, a fucosylated version of 11 generated by treatment with human FUT8 (R&D Systems), a commercially available α1,6-fucosyltransferase, in the presence of its natural donor substrate GDP-fucose.



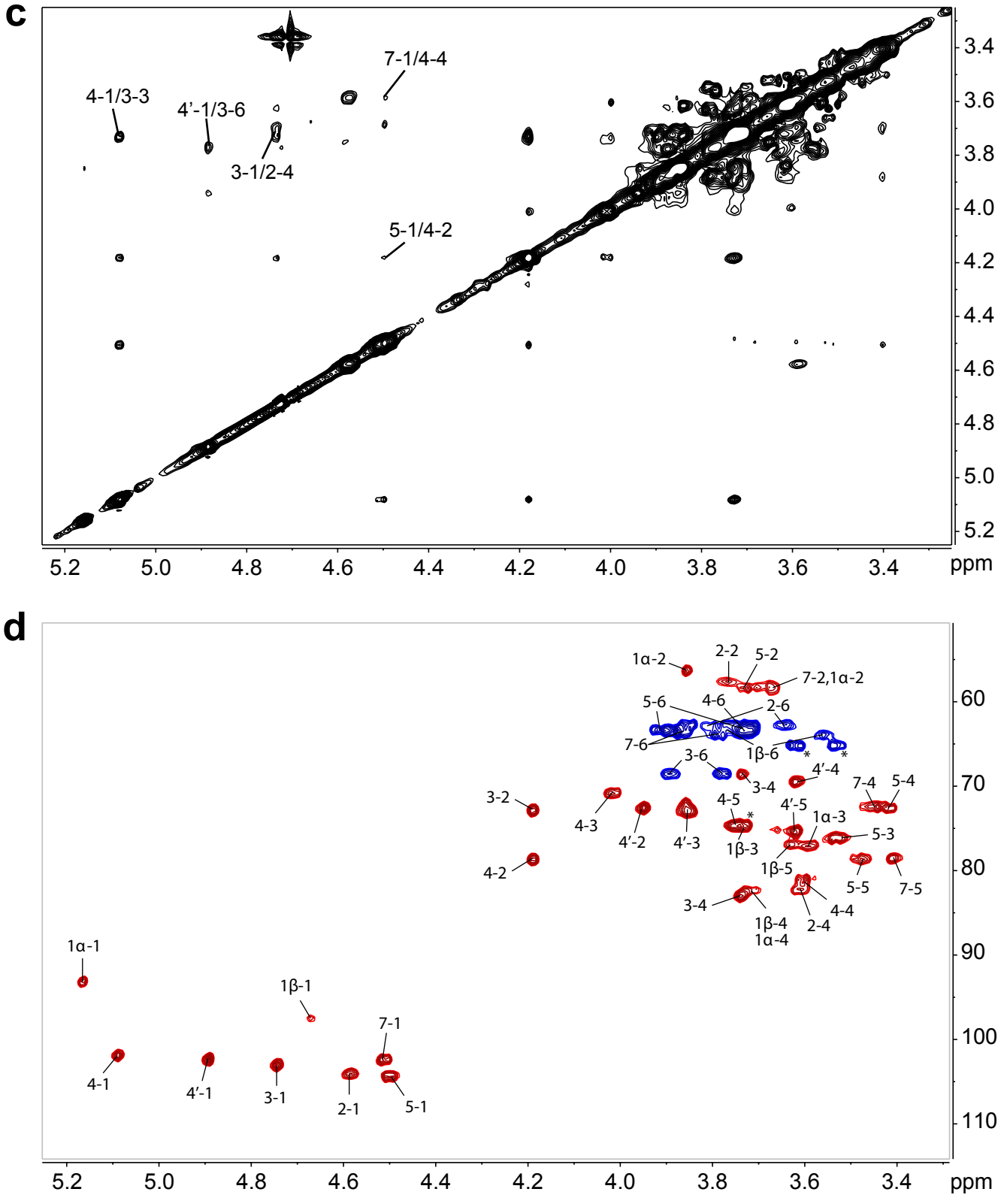
Supplementary Figure 4. (a) Partial ^{1}H-^{1}H-gradient COSY NMR spectrum of glycan **2**, acquired at 600 MHz and 25°C. Only the H1-H2-correlations are labeled for clarity. (b) Partial ^{1}H-^{1}H-zTOCSY NMR spectrum of glycan **2**, acquired at 600 MHz and 25°C. Only the correlations with H1 are labeled.



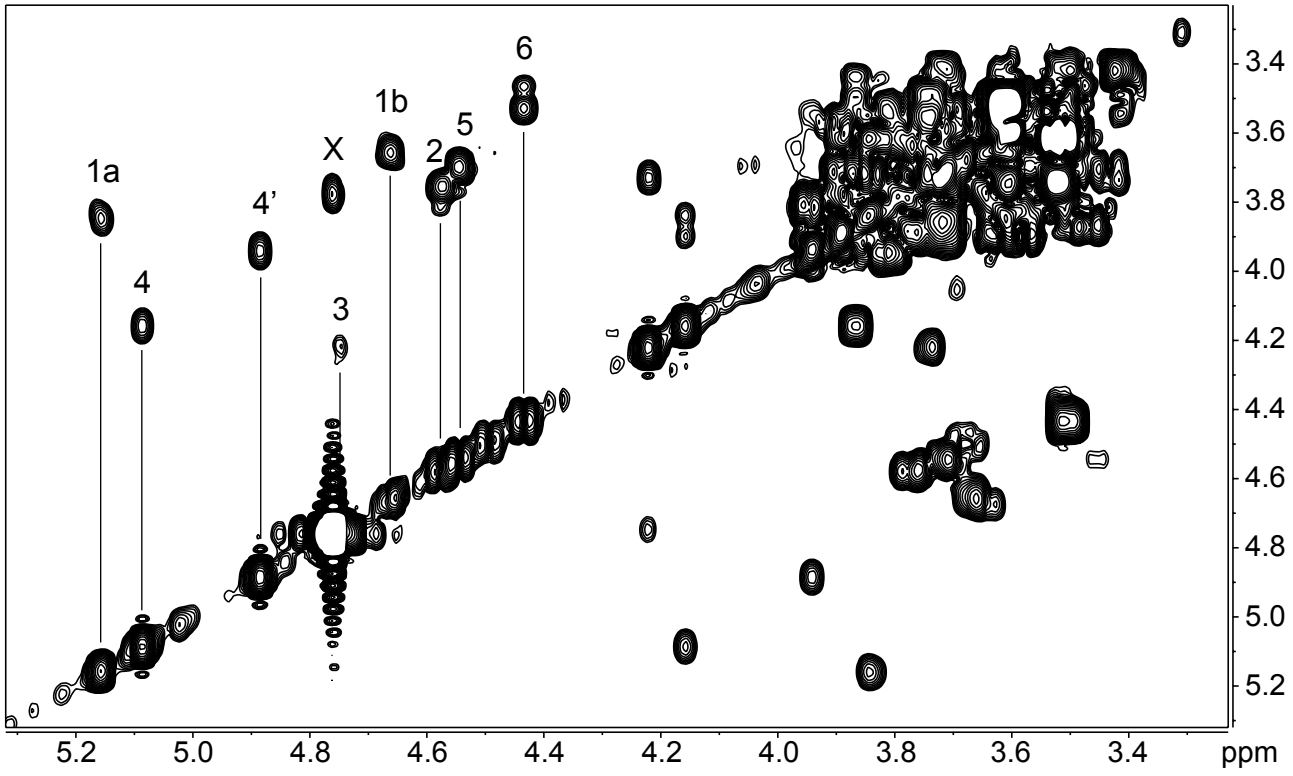
Supplementary Figure 5. (a) Partial ^{1}H-^{1}H-gradient COSY NMR spectrum of glycan **11**, acquired at 600 MHz and 25°C. Only the H1-H2-correlations are labeled. (b) Partial ^{1}H-^{1}H-zTOCSY NMR spectrum of glycan **11**, acquired at 600 MHz and 25°C. Only the correlations with H1 are labeled.



Supplementary Figure 6. (a) Partial ^{1}H-^{1}H-gradient COSY NMR spectrum of glycan **12**, acquired at 600 MHz and 30°C. Only the H1-H2-correlations are labeled. (b) Partial ^{1}H-^{1}H-zTOCSY NMR spectrum of glycan **12**, acquired at 600 MHz and 30°C. Only the correlations with H1 are labeled.



Supplementary Figure 6. (c) Partial ^{1}H-^{1}H-NOESY NMR spectrum of glycan **12**, acquired at 600 MHz and 30°C. Only the inter-residue correlations with H1 are labeled. (d) Partial ^{1}H-^{13}C-gradient HSQC NMR spectrum with multiplicity editing of glycan **12**, acquired at 600/150 MHz and 30°C. The signals labeled with asterisks are from contaminating glycerol.



Supplementary Figure 7. Partial ^{1}H-^{1}H-gradient COSY NMR spectrum of glycan **10**, acquired at 600 MHz and 25°C. Only the H1-H2-correlations are labeled. The "X" marks an artifact, which is only present in the top half of the spectrum.