Supplementary Information

Experimental section

Enzymes. *N*-Glycosidase F (PNG*ase* F), was purchased from. ABS α2-3,6,8,9 sialidase (*Arthrobacter ureafaciens)*, BTG β1-3,4 galactosidase (bovine testis), BKF α1-2,3,4,6 fucosidase (bovine kidney), were purchased from Prozyme (Hayward, CA).

***N*-glycan release, reduction and preparation for PGC-LC-ESI-MS**

N-glycans were released and reduced from immobilised proteins as previously described (Jensen *et al*. 2012). Briefly, 10 µg aliquots of α1-acid glycoprotein/MM253 cell enriched membrane proteins were immobilised onto PVDF membrane (Millipore) and N-glycans released by overnight incubation with 2.5 U PNGase F in 10 µl water at 37 °C. Released glycans and glycan standards were reduced to alditols with 0.5 M $NaBH_4$ in 50 mM KOH for 3 hours at 50°C and desalted using strong cation exchange resin. In preparation for LC-MS samples were cleaned using PGC SPE.

Exoglycosidase digestion

Aliquots of released glycans were dried in a vacuum centrifuge before digestion with combinations of exoglycosidase enzymes. Each digestion was carried out in a total volume of 10 µl with 2 µl of 5x sodium acetate buffer, pH 5.5 (final concentration 50 mM). Enzymes were used at the following concentrations; ABS 2 µl (0.5 mU/µl), BTG 2 µl (0.5 mU/µl), BKF 1µl (4 µU/µl). at 37°C for 16 h as previously described (Royle *et al*. 2008).

PGC-LC-ESI-MS

N-glycans were analysed using a Hypercarb PGC column (3 µm, 100 mm × 180 µm, Thermo Scientific). Glycans were separated over a 85 min gradient of 0–45% (v/v) acetonitrile in 10 mM ammonium bicarbonate at a flow rate of 2 µL/min using a 1260 series HPLC (Agilent Technologies Inc. USA) interfaced with a LCD/ MSD Trap XCT Ultra (Agilent Technologies Inc., USA) mass spectrometer. The MS spectra were acquired in negative ion mode over a mass range of 440 to 2200 m/z. The following MS settings were used: drying gas temperature: 300°C, drying gas flow: 6 L/min, nebulizer gas: 12 psi, skimmer, trap drive and capillary exit were set at -40 V, -99.1 V and -166 V, respectively. Ions were detected in ion charge control (ICC) (target: 30,000 ions) with an accumulation time of 200 ms. Induced collision was performed at 35 % normalized collision energy and an isolation window of 4 m/z. Instrument control, data acquisition and processing were performed with Bruker DataAnalysis software version 4.0 (Bruker Daltonics, Germany).

Table S1. Summary of Prozyme *N*-glycan standards including detected and calculated masses.

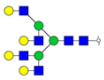
Structure		Mass		Enzyme Treatment
		Detected $[M-2H]^{2-}$	Calculated $[M-2H]^{2-}$	
M8		860.3	860.3	UNDIG/BTG
A2		658.3	658.2	BTG
A4		861.3	861.3	BTG
A2G(4)2		820.3	820.3	UNDIG
A4G(4)4		1185.4	1185.4	UNDIG

Table S2. Pool of *N*-glycan structures released from alpha-1-acid glycoprotein. For each glycan class the panel of exoglycosidases used to produce the target digest products is provided. For example, the A2 structure is the product of A2G2S1 and A2G2S2 after digestion with α-Sialidase ABS and β-Glactosidase BTG. The detected and calculate masses at different charge states is also shown.

Glycan Class	Structure	Mass				Enzyme Treatment
		Detected		Calculated		
		$[M-2H]^{2-}$	$[M-3H]^{3-}$	$[M-2H]^{2-}$	$[M-3H]^{3-}$	
Bi-antennary	A2	658.2		658.2		ABS,BTG
	A2G2	820.3		820.3		ABS
	A2G2S1	965.9		965.8		UNDIG
	A2G2S2	1111.4		1111.4		UNDIG
Tri-antennary	A3	759.8		759.8		ABS,BTG
	A3G3	1002.9		1002.9		ABS
	A3G3S2	1294		1293.9		UNDIG
	A3G3S3	1440	959.3	1439.5	959.3	UNDIG
Tetra-antennary	A4	861.4		861.3		ABS,BTG
	A4G4	1185.4		1185.4		ABS
	A4G4S2	1476.6		1476.5		UNDIG
	A4G4S3	1622.1	1081.1	1622.1	1081	UNDIG
	A4G4S4	1767.6	1178.2	1767.8	1178.1	UNDIG

Table S3. Selection of *N*-glycan structures released from the MM253 melanoma cell line. The detected and calculate masses at different charge states are shown alongside the array of exoglycosidases used to quantitated the relative abundances.

Glycan Class	Structure	Mass						Enzyme Treatment
		Detected			Calculated			
		$[M-H]^-$	$[M-2H]^{2-}$	$[M-3H]^{3-}$	$[M-H]^-$	$[M-2H]^{2-}$	$[M-3H]^{3-}$	
High Mannose	M5	1235.4			1235.4			UNDIG/ABS,BTG,BKF
	M6	1397.5	698.2		1397.5	698.3		UNDIG/ABS,BTG,BKF
	M7		779.3			779.3		UNDIG/ABS,BTG,BKF
	M8		860.3			860.3		UNDIG/ABS,BTG,BKF
	M9		941.3			941.3		UNDIG/ABS,BTG,BKF
Bi-antennary	A2		658.2			658.2		ABS,BTG,BKF
	A2G2S1		965.9			965.8		UNDIG
	A2G2S2		1111.4			1111.4		UNDIG
	FA2G2S1		1038.9			1038.9		UNDIG
	FA2G2S2		1184.4			1184.4		UNDIG
Tri-antennary	A3		759.8			759.8		ABS,BTG,BKF
	FA3G3S2		1367			1366.9		UNDIG
	FA3G3S3		1512.6			1512.5		UNDIG
Tetra-antennary	A4		861.3			861.3		ABS,BTG,BKF
	FA4G4S4			1226.8			1226.8	UNDIG

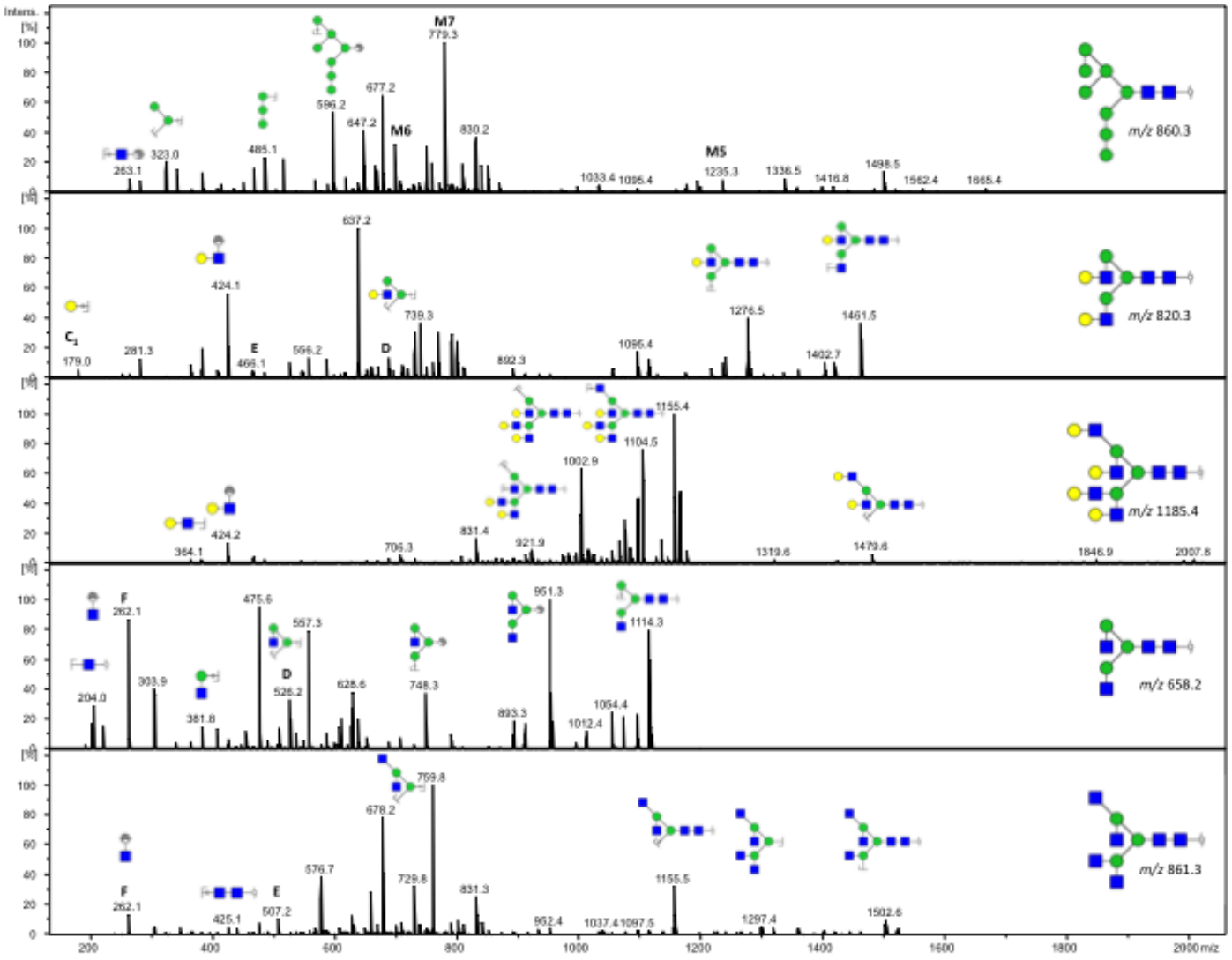


Figure S1. Comparison of annotated MS2 spectra for the reduced neutral *N*-glycan standards.

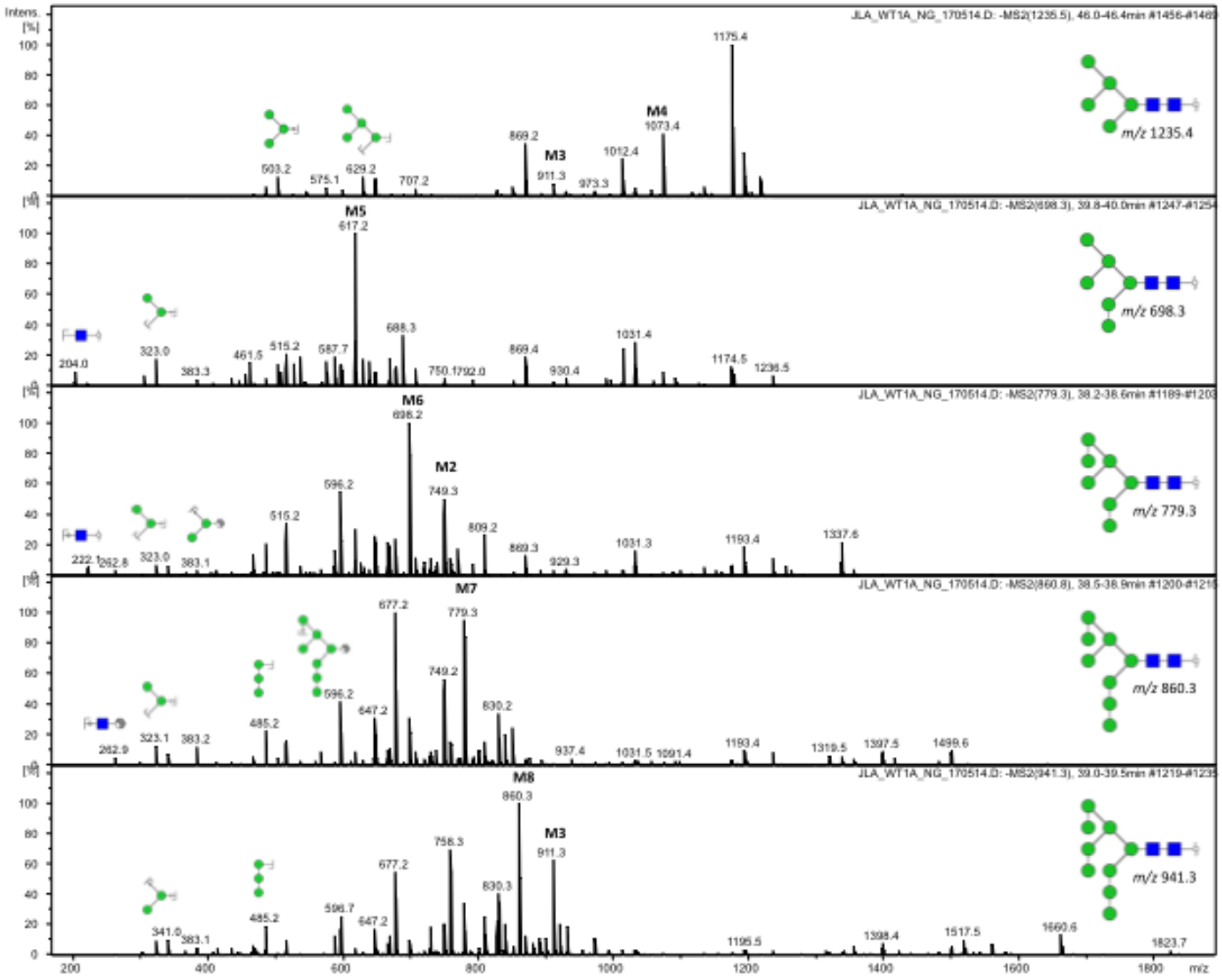


Figure S2: Annotated MS2 spectra of high-mannose glycans released from the MM253 cell line.

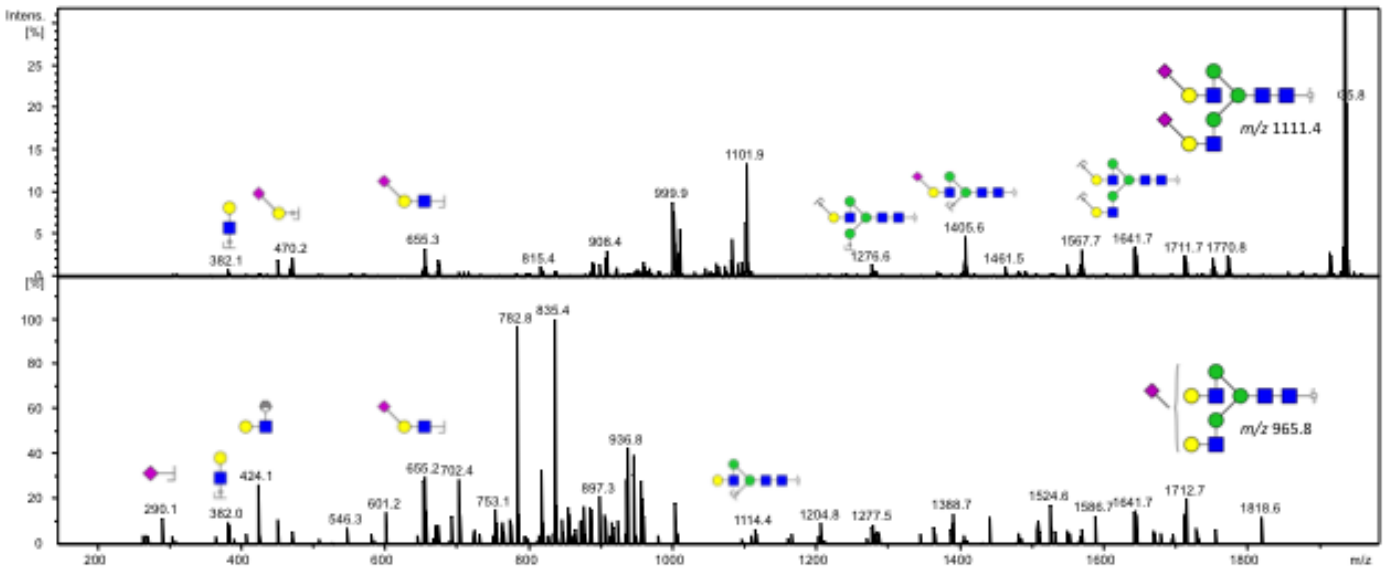


Figure S3: Annotated MS2 spectra of bi-antennary sialyated *N*-glycans from alpha-1-acid glycoprotein.

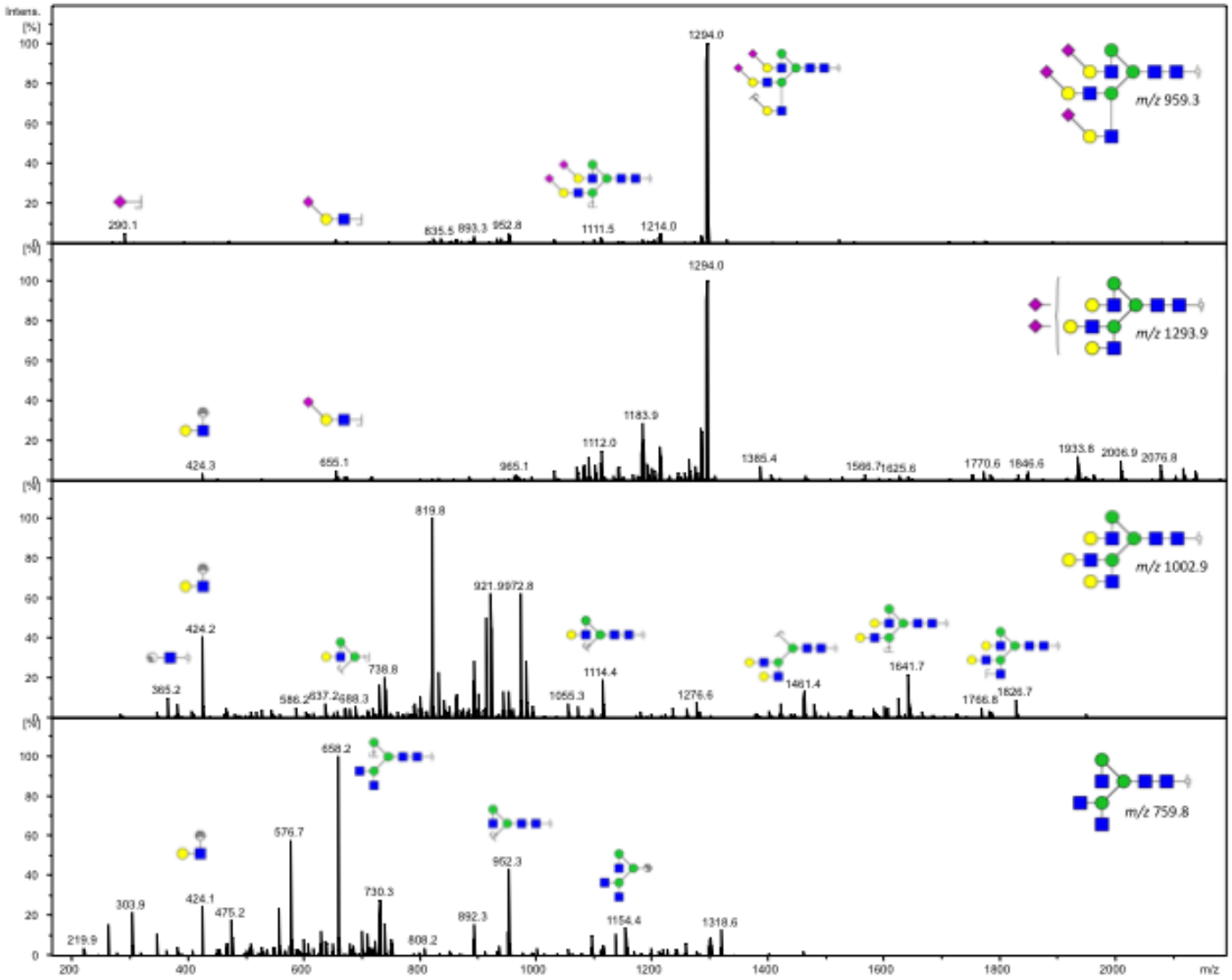


Figure S4: Annotated MS2 spectra of tri-antennary *N*-glycans released from alpha-1-acid glycoprotein.

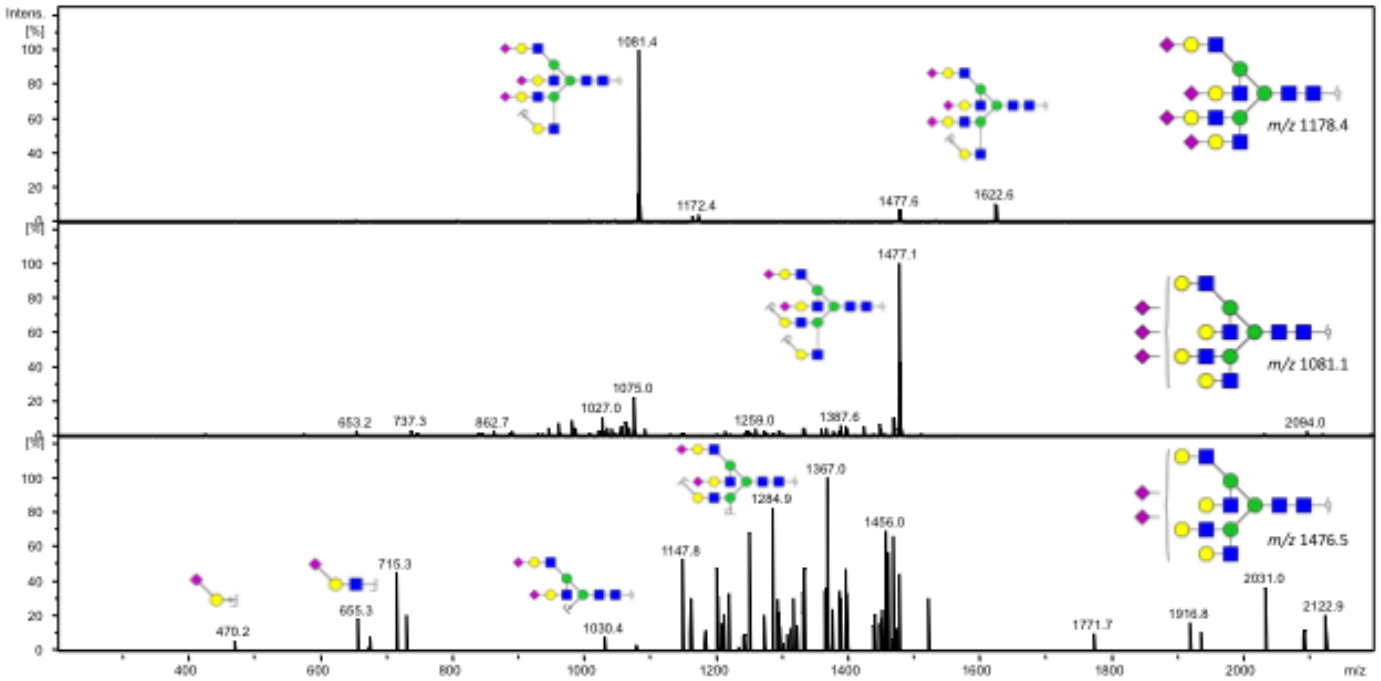


Figure S5: Annotated MS2 spectra of sialylated tetra-antennary *N*-glycans released from alpha-1-acid glycoprotein. (Sialic acid linkages have not been determined.)

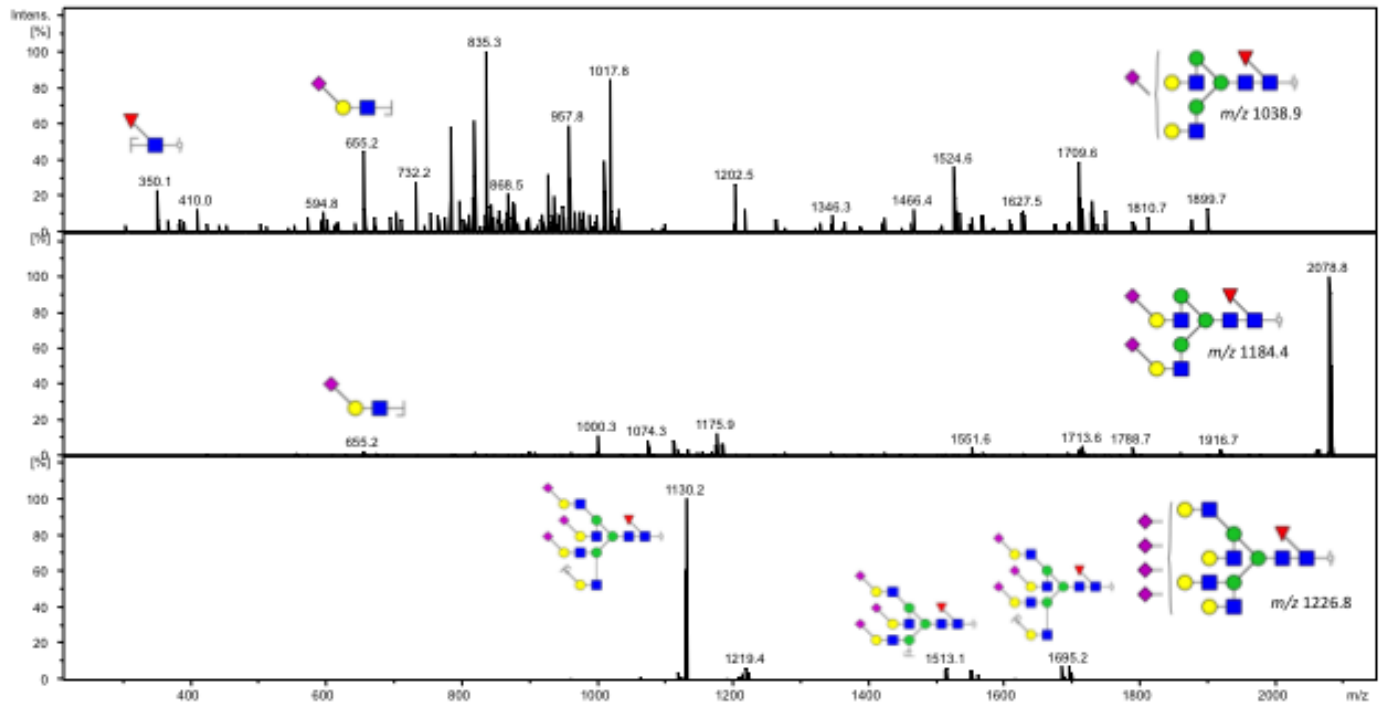


Figure S6: Annotated MS2 spectra of core fucosylated and sialylated *N*-glycan structures from MM253 melanoma cell line.