

Supplementary Information

Direct Comparison of Derivatization Strategies For LC-MS/MS Analysis of N-Glycans

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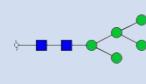
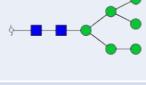
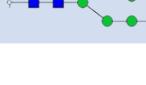
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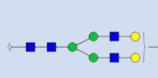
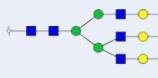
References. (p. 7)

Table S-1. Distribution of differently derivatized glycans released from ribonuclease B analyzed by LC-MS.

	NMR *	Procainamide	TMT	Native	2AB	RFMS	Permetylation
	57	68 ± 4	78 ± 6	85 ± 6	86 ± 4	50 ± 2	73 ± 22
	31	26 ± 1	18 ± 1	15 ± 2	13.1 ± 0.1	28 ± 2	22 ± 2
	4	4 ± 2	2.1 ± 0.1	-----	0.4 ± 0.1	8.4 ± 0.6	3.4 ± 1.2
	7	2.1 ± 0.2	2.2 ± 0.1	-----	-----	10.4 ± 0.9	1.8 ± 0.6
	1	0.1 ± 0.0	0.1 ± 0.0	-----	-----	3.1 ± 0.0	0.1 ± 0.0

* NMR data from Fu *et al.*¹

Table S-2. Distribution of differently derivatized glycans released from fetuin analyzed by LC-MS.

	NMR *	Procainamide	TMT	Native	2AB	RFMS	Permetylation
	0.9	17.6 ± 0.6	4.3 ± 0.4	2.4 ± 0.4	12.1 ± 0.7	8 ± 1	1.1 ± 0.1
	15.8	29 ± 1	36 ± 3	38.9 ± 0.5	29.4 ± 0.8	32 ± 3	19 ± 1
	19.4	15.8 ± 0.1	14 ± 2	1.2 ± 0.3	21 ± 1	18 ± 2	2.1 ± 0.3
	55	33.4 ± 0.3	38 ± 3	58 ± 2	37.1 ± 0.3	40 ± 8	66 ± 4
	7.7	4.6 ± 0.1	7.8 ± 0.7	-----	-----	2.5 ± 0.5	12 ± 2

* NMR data from Green *et al.*²

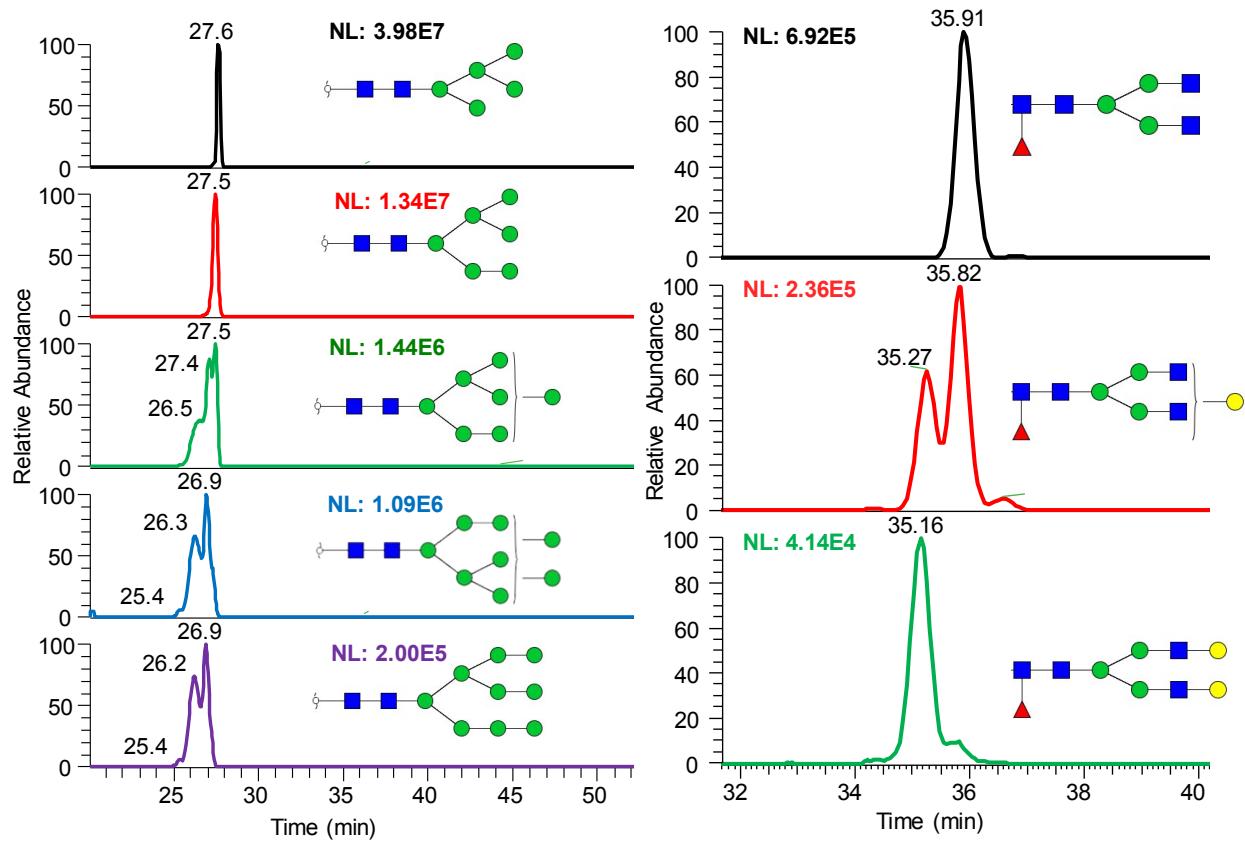
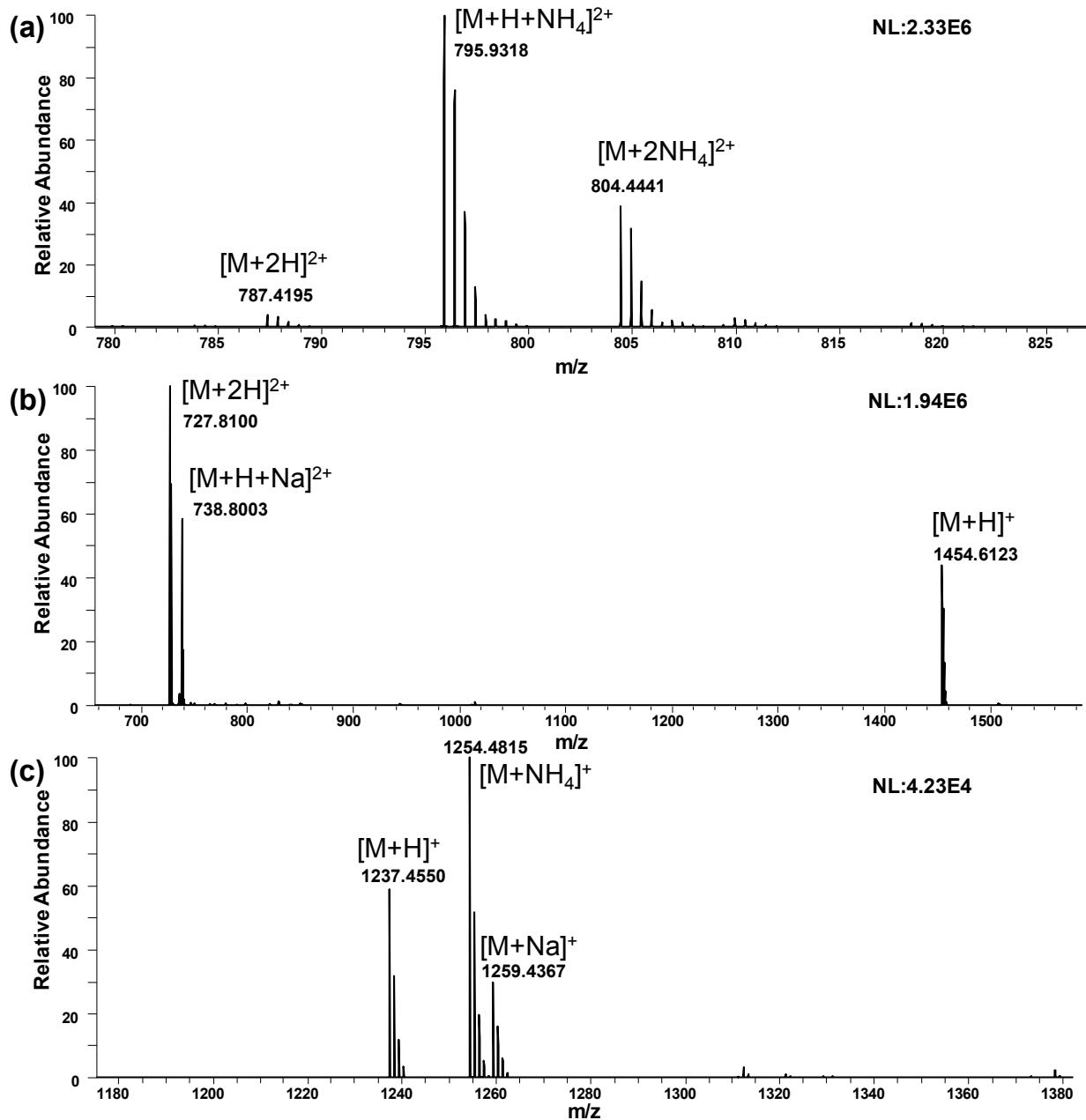


Figure S-1. EICs of glycans released from RNase B and IgG, labeled with RFMS, separated on a C18 nano column and detected using a LTQ Orbitrap Velos MS.



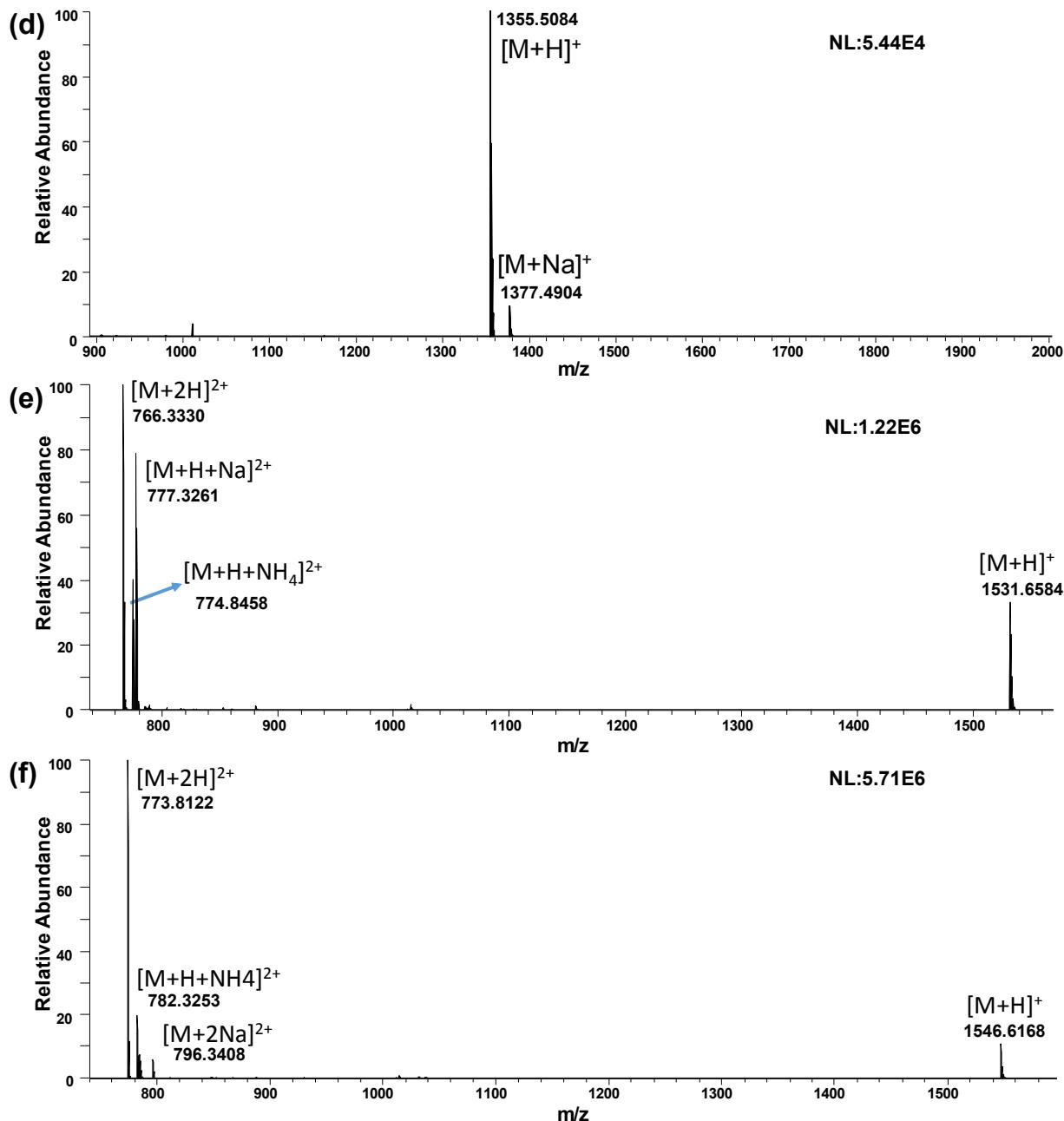


Figure S-2. Full MS scan of **a**-permethylated, **b**-procainamide labeled, **c**-native reduced, **d**-2-AB labeled, **e**-AminoxyTMT labeled and **f**-RFMS labeled Man5. The permethylated glycans, shown in panel **a**, were separated using a C18 column, while all other glycans, shown in panels **b-f**, were separated using a HILIC column. In every case an Exactive Orbitrap MS was used for detection.

References

- (1). Fu, D.; Chen, L.; O'Neill, R. A., A detailed structural characterization of ribonuclease B oligosaccharides by ^1H NMR spectroscopy and mass spectrometry. *Carbohydr. Res.* **1994**, 261, (2), 173-86.
- (2). Green, E. D.; Adelt, G.; Baenziger, J. U.; Wilson, S.; Van Halbeek, H., The asparagine-linked oligosaccharides on bovine fetuin. Structural analysis of N-glycanase-released oligosaccharides by 500-megahertz ^1H NMR spectroscopy. *J. Biol. Chem.* **1988**, 263, (34), 18253-68.