Supporting Information

For

Isobaric labeling of intact gangliosides towards multiplexed LC-MS/MS based quantitative analysis

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Materials and Reagents.

The following glycosphingolipid standards and lipid extracts were purchased from Avanti Polar Lipids (Alabaster, AL): *D*-galactosyl-*β*-1,1'-*N*-stearoyl-*D*-erythro-sphingosine (GalCer d18:1/18:0, cat. no. 860844), *D*-galactosyl-*β*-1-1'-*N*-[2"(R)-hydroxystearoyl]-*D*-erythro-sphingosine (GalCer d18:1/18:0 (2ROH), cat. no. 860840), total gangliosides extract (porcine brain, cat. no. 860053), total lipid extract (porcine brain, cat. no. 131101). *N*-stearoyl-globotriaosylceramide (Gb3Cer d18:1/18:0, cat. no. 1529), *N*-stearoyl-sphingosyl-*β*-D-galactoside-3-sulfate (Sulfatide d18:1/18:0, cat. no. 1932), and ganglioside standard mixtures containing different acyl chain lengths and long chain bases (*e.g.* d18:1 and d20:1): monosialoganglioside GM1a (bovine, NH_4^+ salt, cat. no. 1061), monosialoganglioside GM3 (bovine buttermilk, NH_4^+ salt, cat. no. 1503), disialoganglioside GD3 (bovine buttermilk, NH_4^+ salt, cat. no. 1527), disialoganglioside GD1a (bovine, NH_4^+ salt, cat. no. 1062), disialoganglioside GD1b (bovine, NH_4^+ salt, cat. no. 1501), and trisialoganglioside GT1b (bovine, NH_4^+ salt, cat. no. 1063) were obtained from Matreya, LLC (State College, PA). Sodium metaperiodate ($NaIO_4$), Glycerin, sodium acetate (CH_3COONa), glacial acetic acid (CH_3COOH), aminoxyTMT^0 (cat. no. PI90400) and aminoxyTMT 6-plex reagent (cat. no. PI90401), and all solvents (at least HPLC or LC-MS grade) were obtained from Fisher Scientific (Pittsburgh, USA) unless stated otherwise.

Table S1. Structure of gangliosides discussed in the text. Symbolic representations are based on recommendations of the Consortium for Functional Glycomics (CFG)[1].

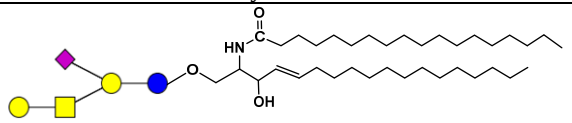
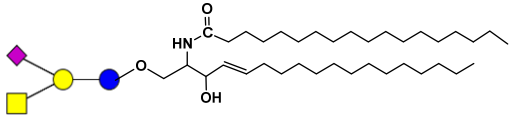
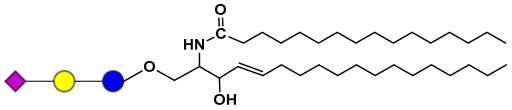
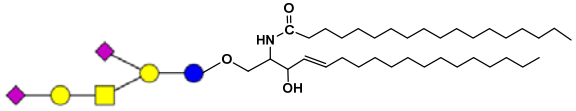
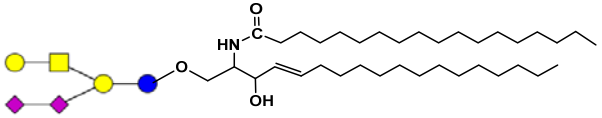
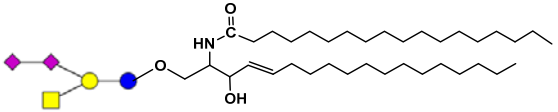
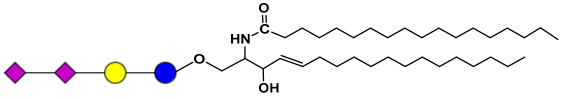
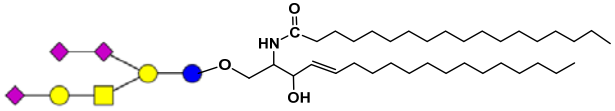
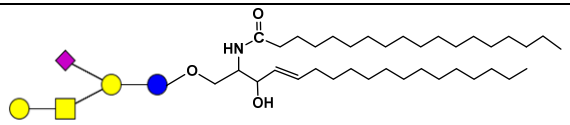
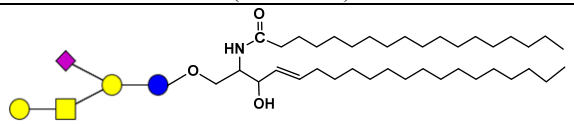
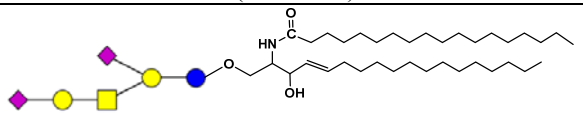
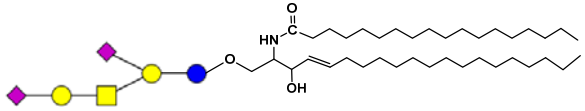
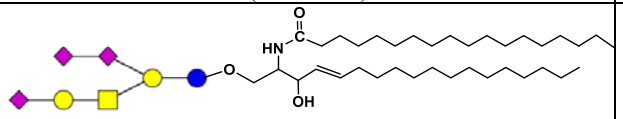
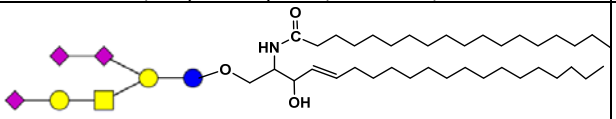
Ganglioside	LIPIDMAPS ID[2]	Structure/Systematic Name	Molecular Formula	Exact Mass (Da)
GM1a d18:1/18:0	LMSP0601AP02	 Galβ1-3GalNAcβ1-4(NeuAcα2-3)Galβ1-4Glcβ-Cer(d18:1/18:0)	$C_{73}H_{131}N_3O_{31}$	1545.8766
GM2 d18:1/18:0	LMSP0601AM02	 GalNAcβ1-4(NeuAcα2-3)Galβ1-4Glcβ-Cer(d18:1/18:0)	$C_{67}H_{121}N_3O_{26}$	1383.8238
GM3 d18:1/16:0	LMSP0601AJ01	 NeuAcα2-3Galβ1-4Glcβ-Cer(d18:1/16:0)	$C_{57}H_{104}N_2O_{21}$	1152.7131
GD1a d18:1/18:0	LMSP0601AS02	 NeuAcα2-3Galβ1-3GalNAcβ1-4(NeuAcα2-3)Galβ1-4Glcβ-Cer(d18:1/18:0)	$C_{84}H_{148}N_4O_{39}$	1836.9720
GD1b d18:1/18:0	LMSP0601AQ02	 Galβ1-3GalNAcβ1-4(NeuAcα2-8NeuAcα2-3)Galβ1-4Glcβ-Cer(d18:1/18:0)	$C_{84}H_{148}N_4O_{39}$	1836.9720
GD2 d18:1/18:0	LMSP0601AN02	 GalNAcβ1-4(NeuAcα2-8NeuAcα2-3)Galβ1-4Glcβ-Cer(d18:1/18:0)	$C_{78}H_{138}N_4O_{34}$	1674.9192
GD3 d18:1/16:0	LMSP0601AK01	 NeuAcα2-8NeuAcα2-3Galβ1-4Glcβ-Cer(d18:1/16:0)	$C_{68}H_{121}N_3O_{29}$	1443.8085
GT1b d18:1/18:0	LMSP0601AT02	 NeuAcα2-3Galβ1-3GalNAcβ1-4(NeuAcα2-8NeuAcα2-3)Galβ1-4Glcβ-Cer(d18:1/18:0)	$C_{95}H_{165}N_5O_{47}$	2128.0674

Table S2. Major ganglioside components in aminoxyTMT^0-labeled porcine brain gangliosides extract.

Ganglioside	Structure	Molecular Formula (aminoxyTMT^0 labeled)	Adduct	Theoretical m/z	Experimental m/z	Mass error (ppm)
GM1a d18:1/18:0	 Galβ1-3GalNAcβ1-4(NeuAcα2-3)Galβ1-4Glcβ-Cer(d18:1/18:0)	$C_{86}H_{153}N_7O_{31}$	$[M+2H]^{2+}$	891.0383	891.03625	2.35
GM1a d20:1/18:0	 Galβ1-3GalNAcβ1-4(NeuAcα2-3)Galβ1-4Glcβ-Cer(d20:1/18:0)	$C_{88}H_{157}N_7O_{31}$	$[M+2H]^{2+}$	905.0540	905.05261	1.53
GD1a d18:1/18:0	 NeuAcα2-3Galβ1-3GalNAcβ1-4(NeuAcα2-3)Galβ1-4Glcβ-Cer(d18:1/18:0)	$C_{110}H_{192}N_{12}O_{39}$	$[M+2H]^{2+}$	1153.6783	1153.67676	1.31
GD1a d20:1/18:0	 NeuAcα2-3Galβ1-3GalNAcβ1-4(NeuAcα2-3)Galβ1-4Glcβ-Cer(d20:1/18:0)	$C_{112}H_{196}N_{12}O_{39}$	$[M+2H]^{2+}$	1167.6939	1167.69238	1.32
GT1b d18:1/18:0	 NeuAcα2-3Galβ1-3GalNAcβ1-4(NeuAcα2-8NeuAcα2-3)Galβ1-4Glcβ-Cer(d18:1/18:0)	$C_{121}H_{209}N_{13}O_{47}$	$[M+2H]^{2+}$	1299.2260	1299.22449	1.15
GT1b d20:1/18:0	 NeuAcα2-3Galβ1-3GalNAcβ1-4(NeuAcα2-8NeuAcα2-3)Galβ1-4Glcβ-Cer(d20:1/18:0)	$C_{123}H_{213}N_{13}O_{47}$	$[M+2H]^{2+}$	1313.2416	1313.24048	0.87

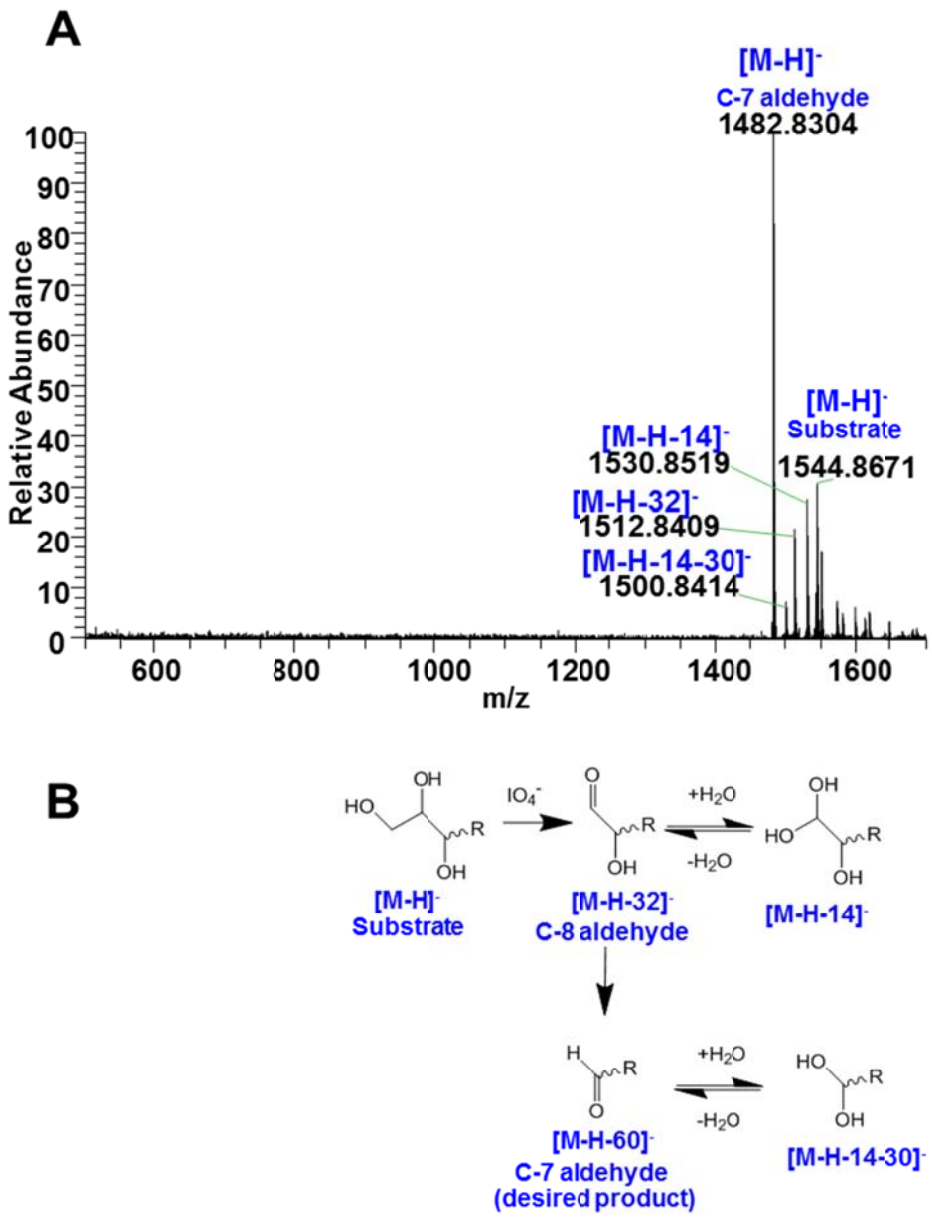


Figure S1. Demonstration of incomplete oxidation of ganglioside GM1a d18:1/18:0 after subjecting to 1mM $NaIO_4$ for only 30 minutes incubation in ice bath (~0^0C). (**A**) full scan mass range showing the presence of unwanted products of incomplete oxidation; (**B**) plausible origin of the observed side products, aldehydes are known to undergo geminal diol formation in aqueous medium[3] analogous to hemiacetals which could subsequently undergo dehydration to yield the observed side products. Notably, when incubation is done under optimum conditions (*i.e.* 60 minutes at ~0^0C as discussed in the text, almost exclusively C-7 aldehyde was obtained (see **Fig. 2a-b** of the main text).

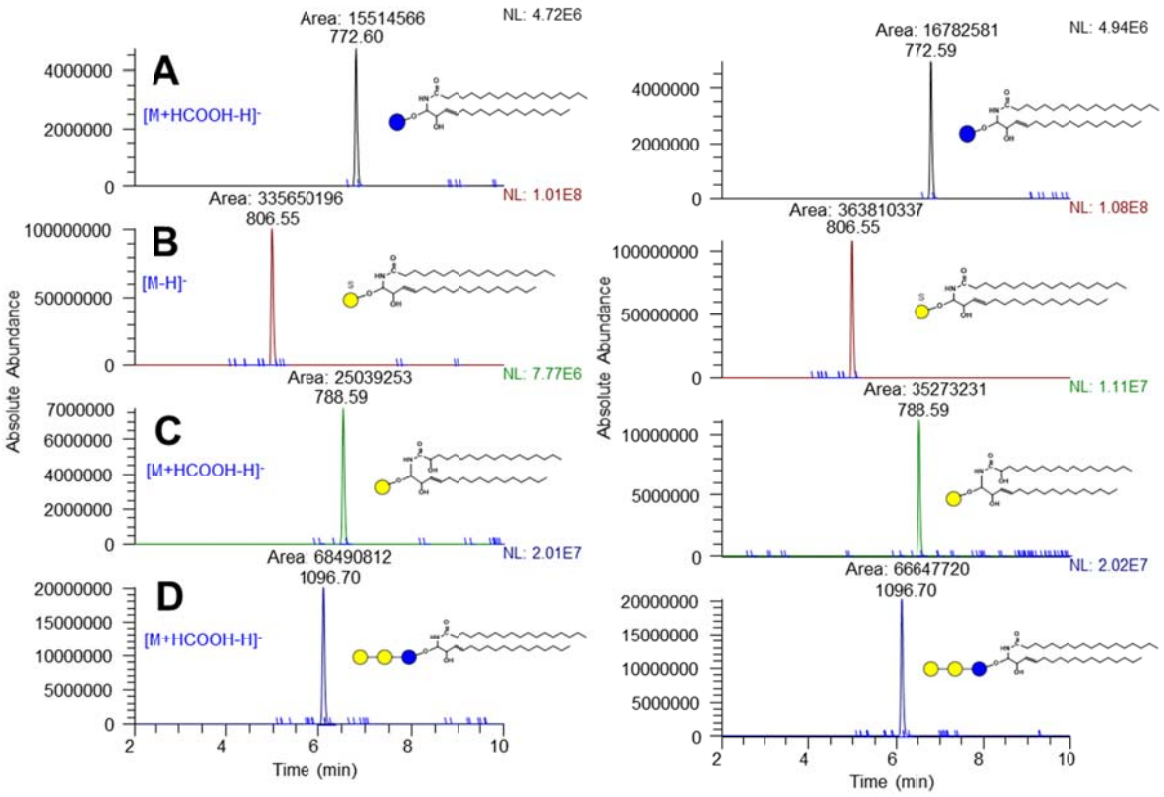


Figure S2. Chemoselectivity of $NaIO_4$ oxidation under mild conditions. Left column shows the extracted ion chromatograms of unoxidized glycosphingolipids in oxidation buffer alone. Right column shows the extracted ion chromatograms of the same compounds after 60 mins incubation with 1 mM $NaIO_4$. Comparable peak areas and absolute ion abundance were obtained between these two sets of data indicating the treatment does not significantly affect these structural motifs. Difference in the observed abundance and peak area could be attributed to sample handling including solid-phase extraction (SPE) and transfer of sample from one container to the other. (**A)**, GlcCer d18:1/18:0 (contains only vicinal diols); (**B)**, Sulfatide d18:1/18:0 (contains a sulfate group); (**C)**, GalCer d18:1/18:0(2ROH) (contains α-hydroxyl group in the fatty acyl chain); (**D)**, Gb3Cer d18:1/18:0 (contains vicinal diols and longer glycan chain). Symbols are defined in **Fig. 1** of the main text.

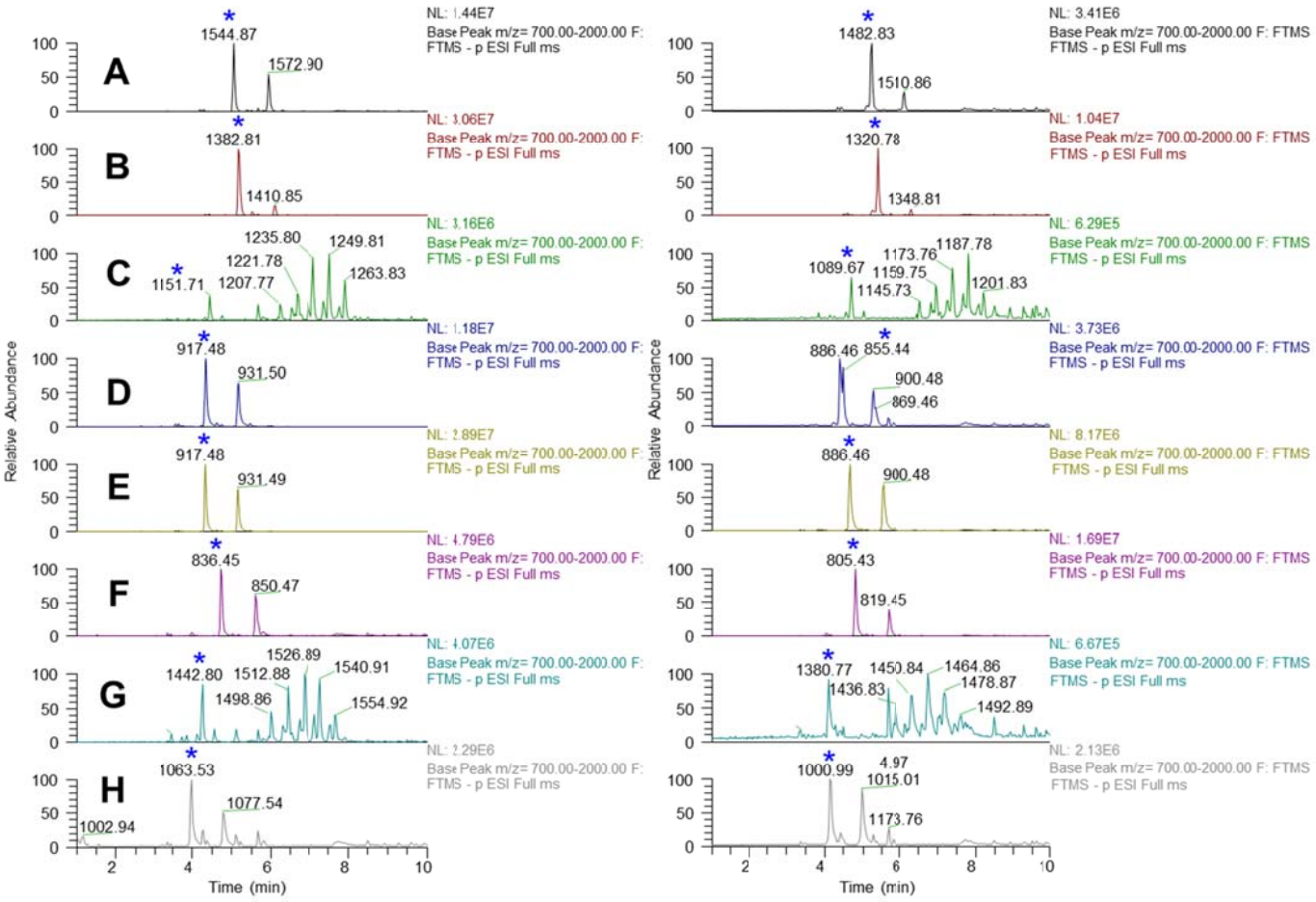


Figure S3. Left column represents the total ion chromatograms (TIC) of the unoxidized gangliosides with a mass range of *m/z* 700-2000. The right column represents the corresponding TIC of oxidized gangliosides. The major components of each mixture corresponding to the EIC shown in **Fig. 3** are marked with asterisk (*). In each case, a loss of 62.04 Da (from $[M-H]^-$) or 32.02 Da (from $[M-2H]^{2-}$) was observed. (**A)**, GM1a, $[M-H]^-$; (**B)**, GM2, $[M-H]^-$; (**C)**, GM3, $[M-H]^-$; (**D)**, GD1a, $[M-2H]^{2-}$; (**E)**, GD1b, $[M-2H]^{2-}$; (**F)**, GD2, $[M-2H]^{2-}$; (**G)**, GD3, $[M-H]^-$; (**H)**, GT1b, $[M-2H]^{2-}$.

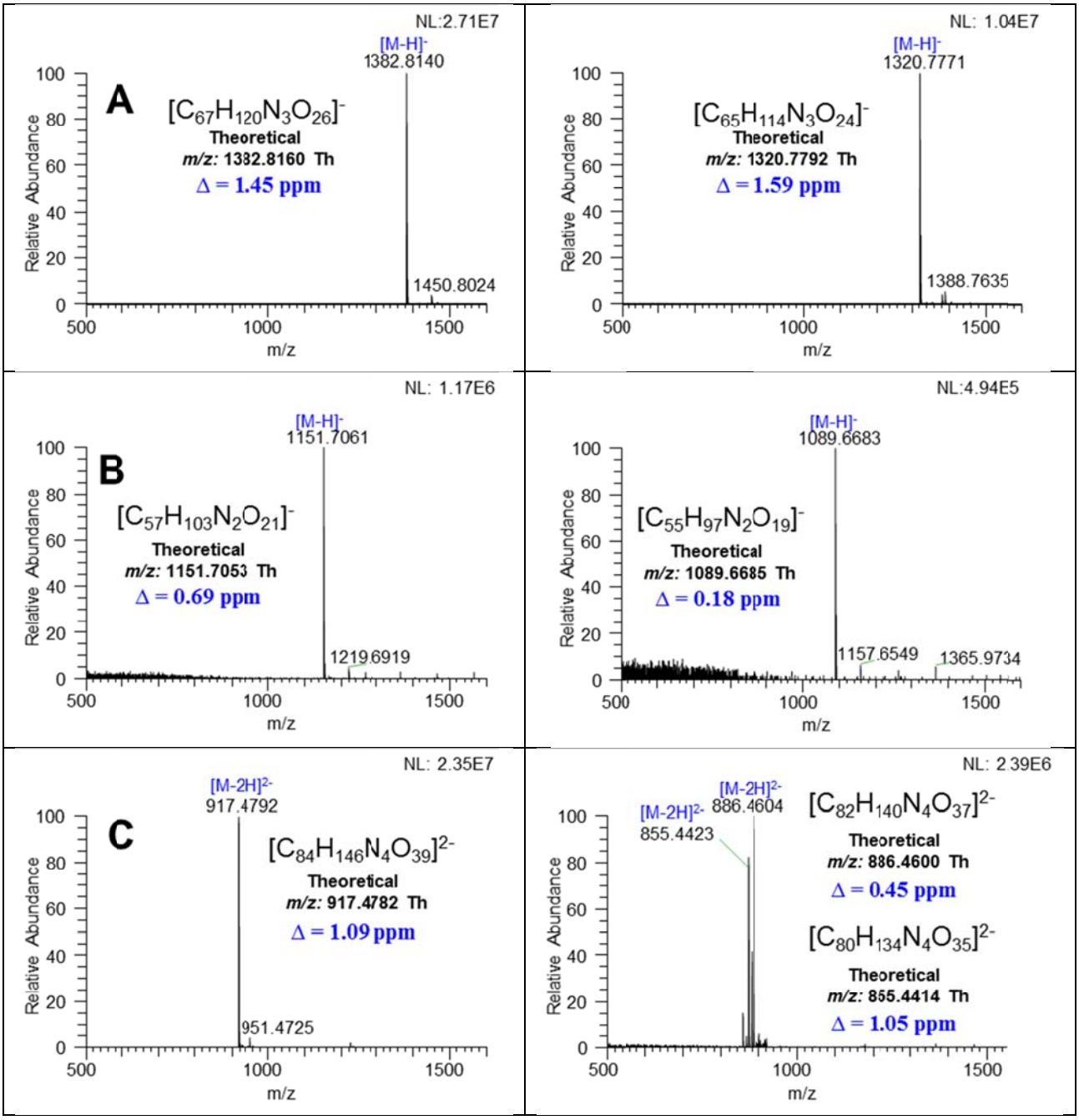


Figure S4. Full scan mass range of unoxidized (left column) and oxidized (right column) gangliosides based on the peak indicated by asterisk (*) in the right column of **Fig. S3**. (**A)**, GM2 d18:1/18:0; (**B)**, GM3 d18:1/16:0; (**C)**, GD1a d18:1/18:0; (**D)**, GD1b d18:1/18:0; (**E)**, GD2 d18:1/18:0; (**F)**, GD3 d18:1/16:0; (**G)**, GT1b d18:1/18:0. Δ indicates absolute mass error.

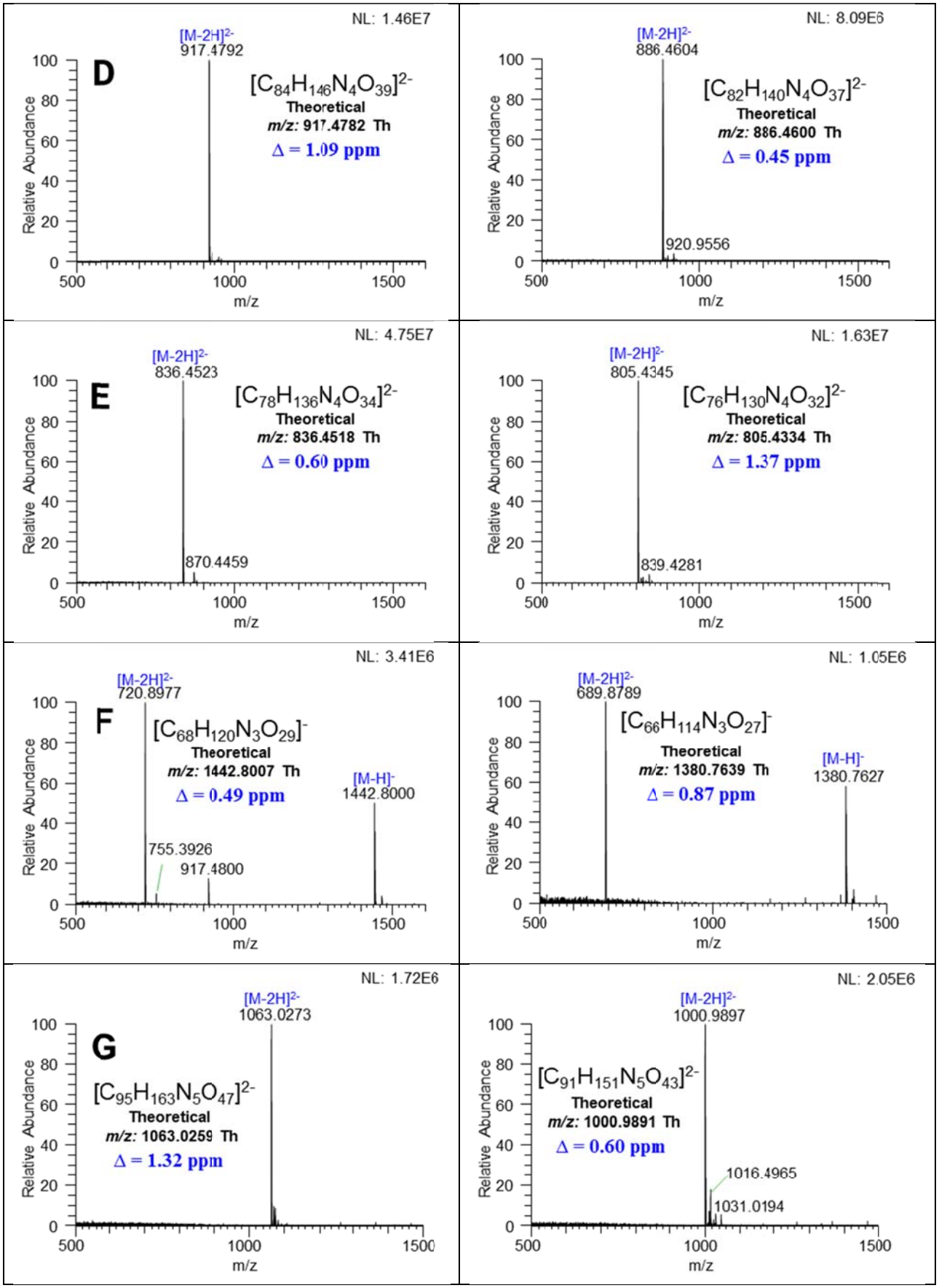


Figure S4 (Continued).

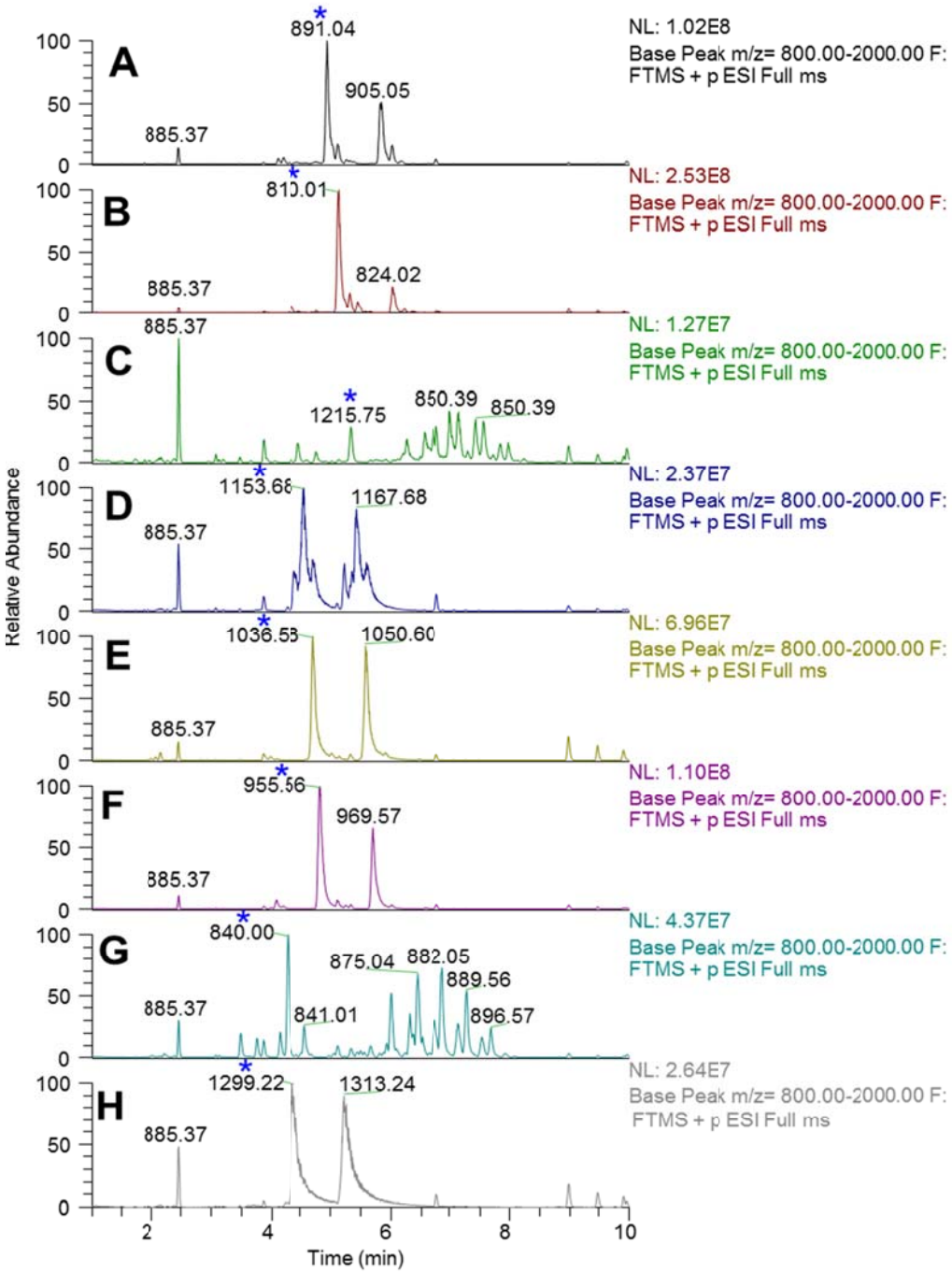


Figure S5. Total ion chromatograms of ganglioside standard mixtures labeled with aminoxyTMT^0. The major components of each mixture corresponding to the unoxidized and oxidized gangliosides whose EIC's are shown in **Fig. S3** are marked with asterisk (*). (**A)**, GM1a; (**B)**, GM2; (**C)**, GM3; (**D)**, GD1a; (**E)**, GD1b; (**F)**, GD2; (**G)**, GD3; (**H)**, GT1b. All masses shown are $[M+2H]^{2+}$, ion with *m/z* 885.37 and peaks above 8.5 min have been detected in the reagent blanks and not a component of ganglioside standards.

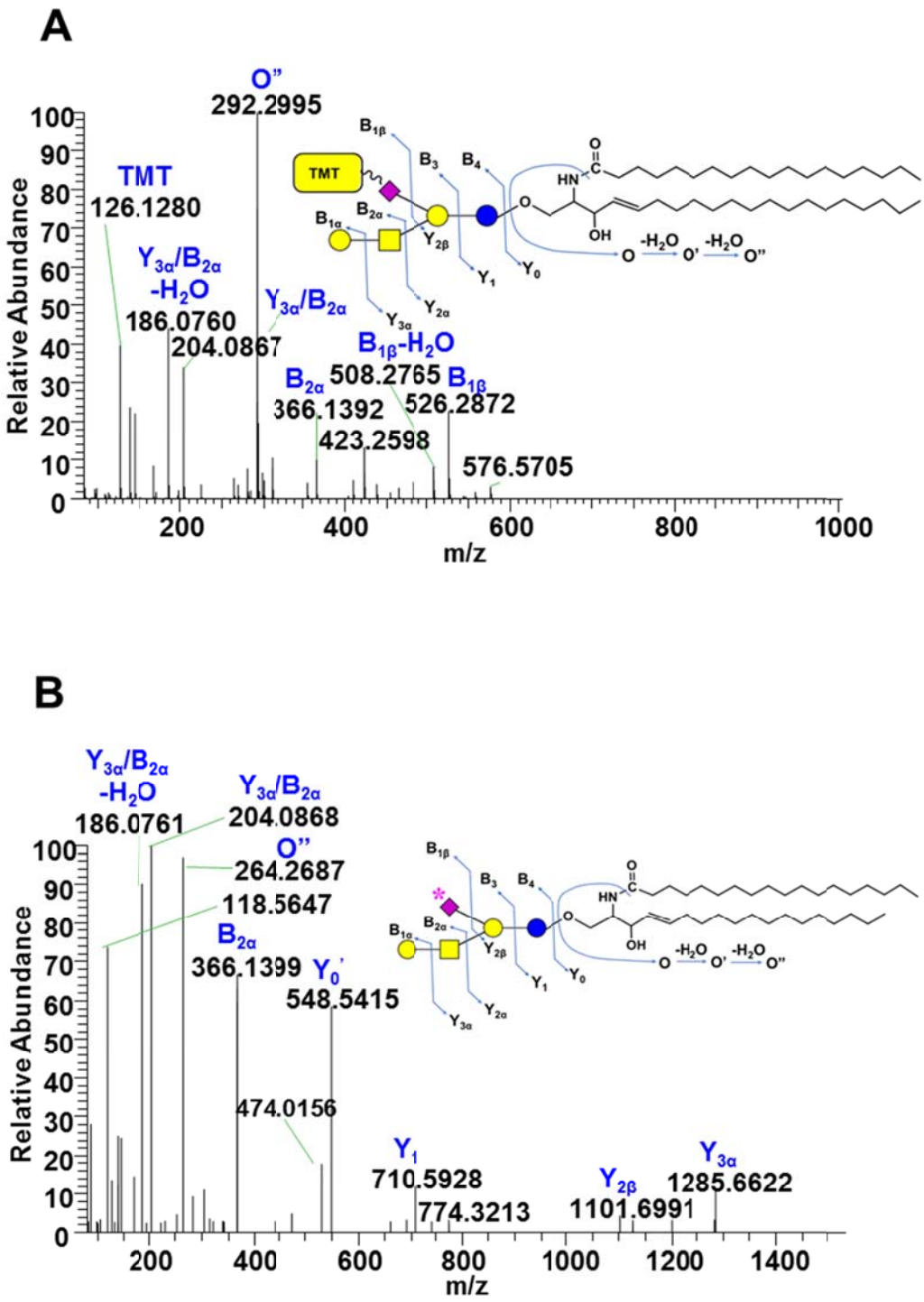


Figure S6. MS/MS spectrum of aminoxyTMT^0-labeled GM1a d20:1/18:0 using the $[M+2H]^{2+}$ (*m/z* 905.05) as precursor ion showing the *O"* ion at *m/z* 292.2995 diagnostic of the long chain base d20:1 (**A**). MS/MS spectrum of oxidized (unlabeled) GM1a d18:1/18:0 using the $[M+H]^+$ as precursor ion.

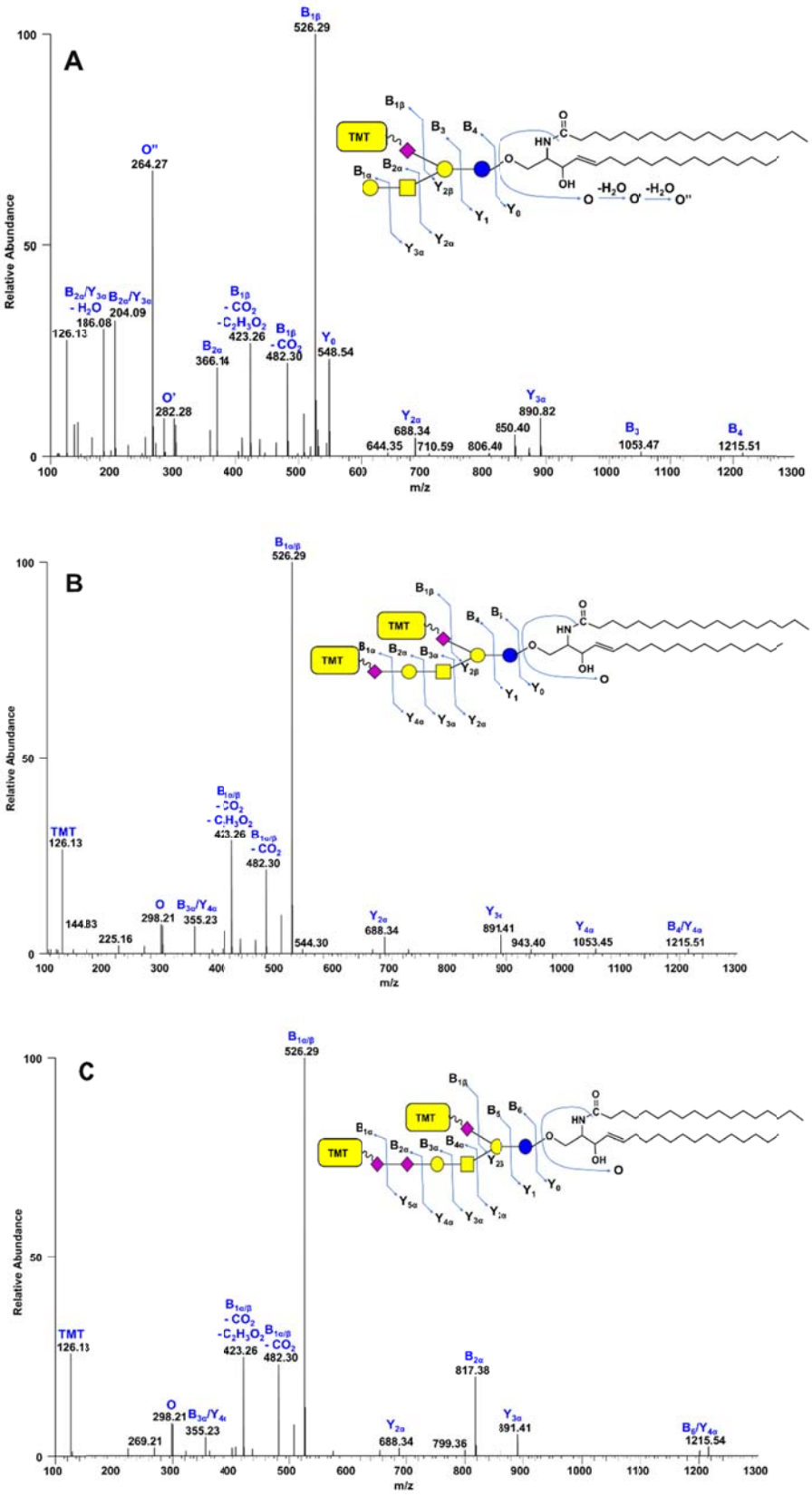


Figure S7. Representative HCD-MS/MS (Stepped NCE 22,26,30) of aminoxyTMT^0-labeled gangliosides from porcine brain extract GM1a d18:1/18:0 (**A**), GD1a d18:1/18:0 (**B**), and GT1b d18:1/18:0 (**C**). All precursor ions used are $[M+2H]^{2+}$.

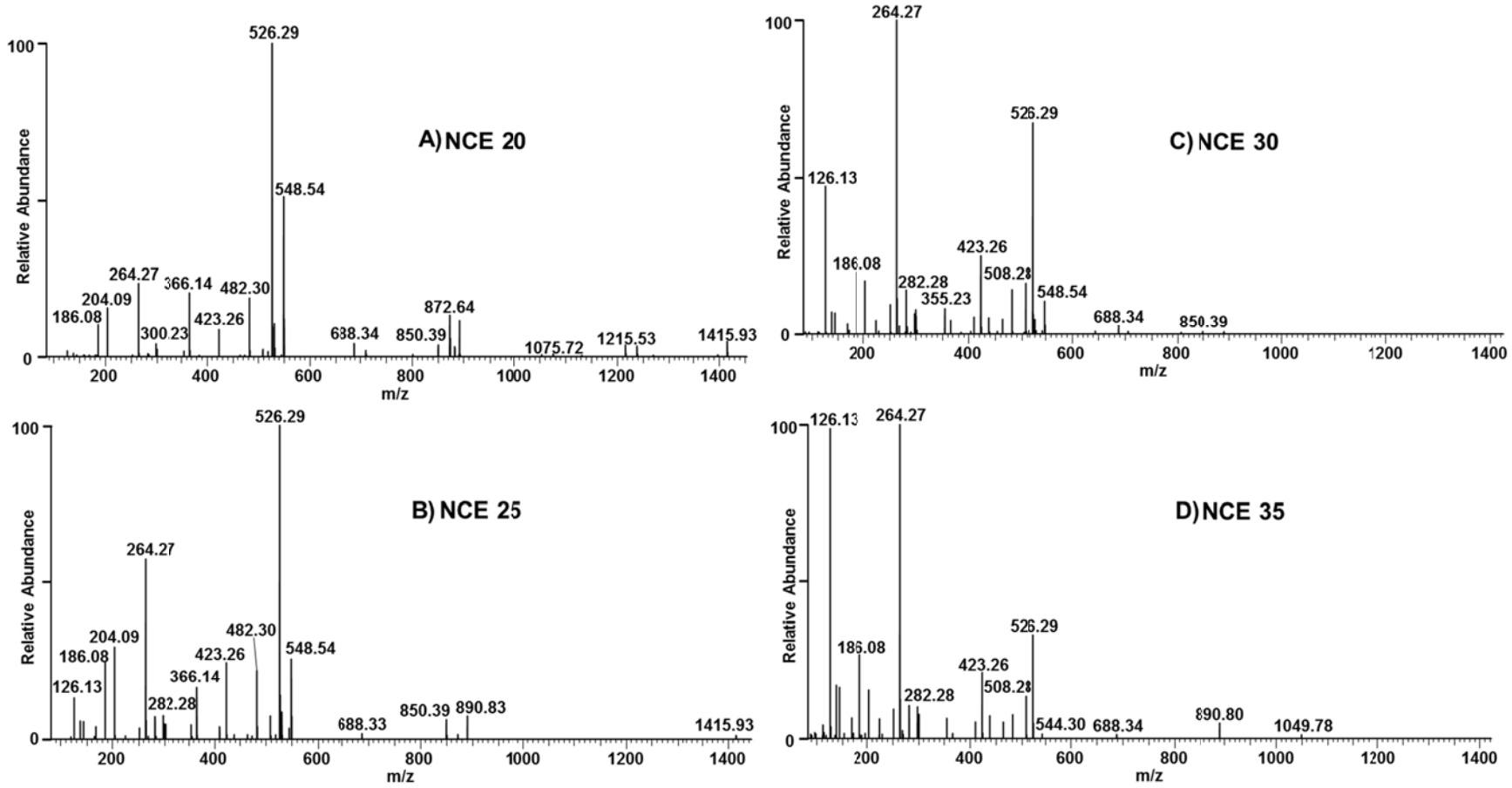


Figure S8. HCD-MS/MS spectra of aminoxyTMT^0-labeled ganglioside GM1a d18:1/18:0 at different normalized collision energy (NCE) values. (**A**) NCE 20; (**B**) NCE 25; (**C**) NCE 30; (**D**) NCE 35. Increasing the NCE values generate higher yield of reporter ion region along with a corresponding decrease in the intensity of glycan-informative fragments. Depending on the analytical purpose, appropriate collision energy values can be used.

References

(1) Varki, A.; Cummings, R. D.; Aebi, M.; Packer, N. H.; Seeberger, P. H.; Esko, J. D.; Stanley, P.; Hart, G.; Darvill, A.; Kinoshita, T.; Prestegard, J. J.; Schnaar, R. L.; Freeze, H. H.; Marth, J. D.; Bertozzi, C. R.; Etzler, M. E.; Frank, M.; Vliegenthart, J. F. G.; Lütteke, T.; Perez, S., et al. *Glycobiology* **2015**, *25*, 1323-1324.
(2) Fahy, E.; Subramaniam, S.; Murphy, R. C.; Nishijima, M.; Raetz, C. R.; Shimizu, T.; Spener, F.; van Meer, G.; Wakelam, M. J.; Dennis, E. A. *J. Lipid Res.* **2009**, *50 Suppl*, S9-14.
(3) Clark, G.; The Open, U.; Smart, L. E. *Mechanism and Synthesis*; Royal Society of Chemistry: Cambridge, 2003.