SUPPORTING INFORMATION FOR:

Nanodiscs and Electrospray Ionization Mass Spectrometry. A Novel Tool for Screening Glycolipids Against Proteins

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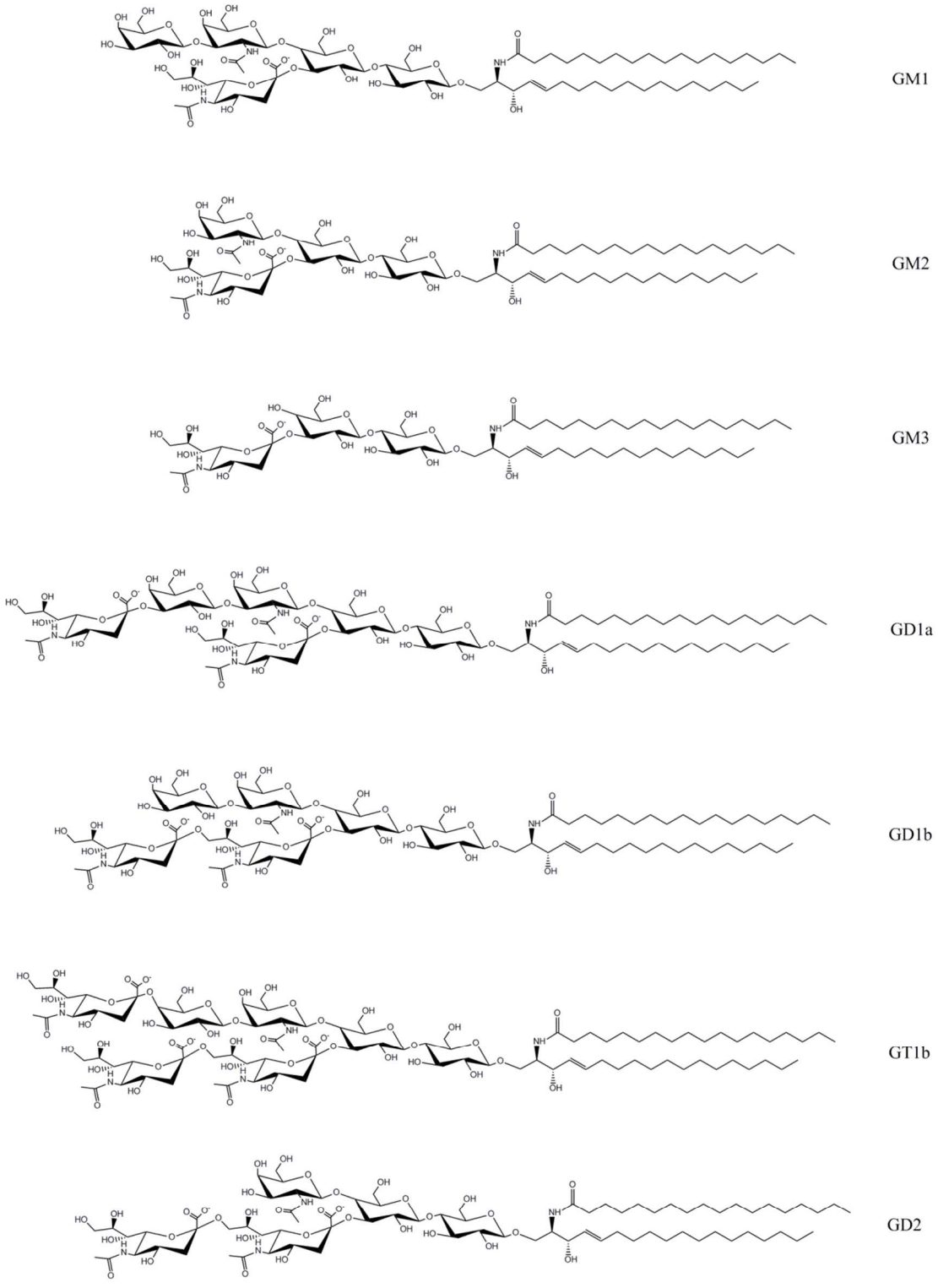


Figure S1. Structures of the gangliosides (GM1, GM2, GM3, GD1a, GD1b, GT1b and GD2) that were incorporated into nanodiscs (NDs) for the present study.

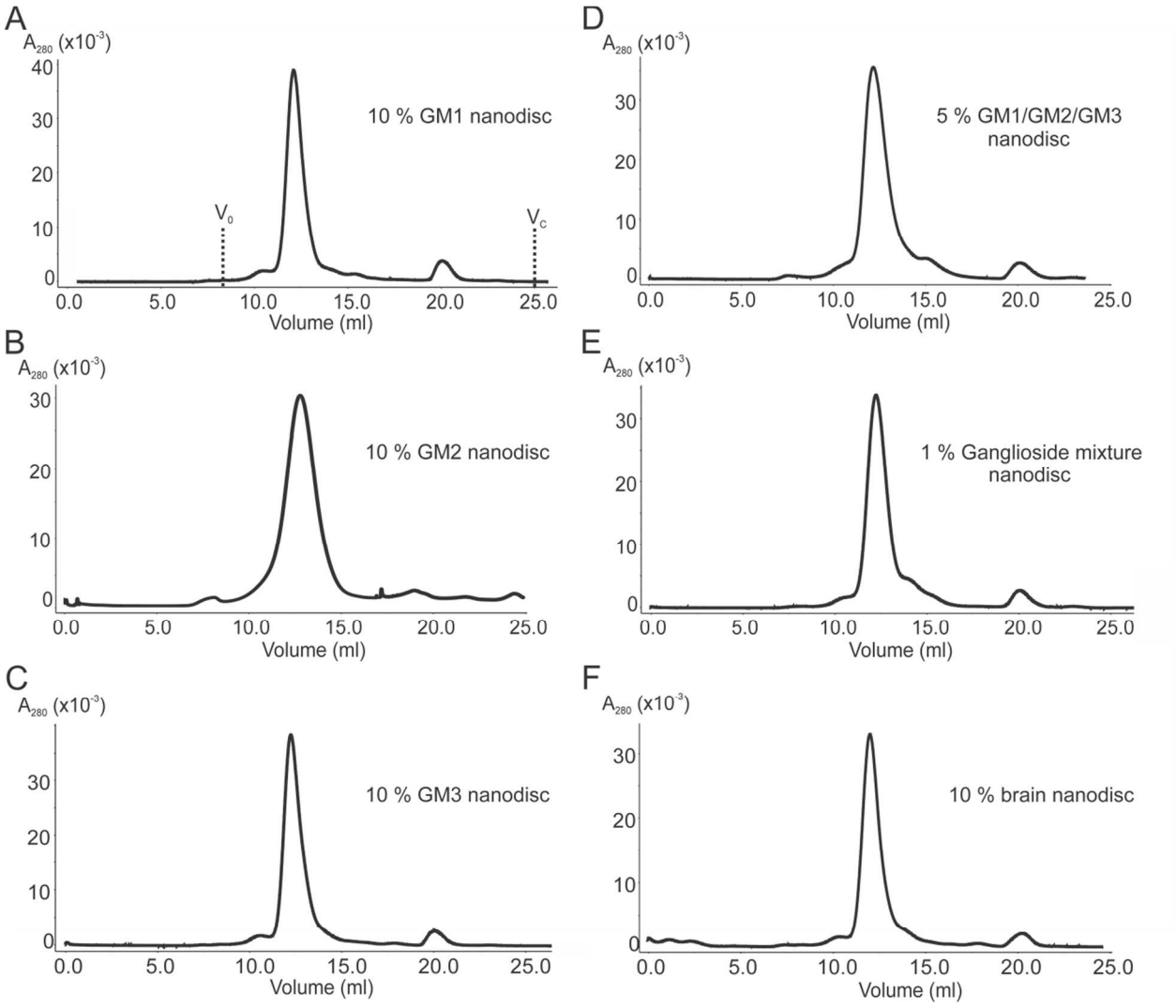


Figure S2. Size exclusion chromatography for 10% GM1 ND (A), 10% GM2 ND (B), 10% GM3 ND (C), 5% GM1/2/3 ND (D), 1% ganglioside mixture ND (7G ND) (E), and the ND containing gangliosides extracted from pig brain (pig ND) (F). The void volume (V_0) and total column volume (V_C) are shown as an example for the 10% GM1 ND (A).

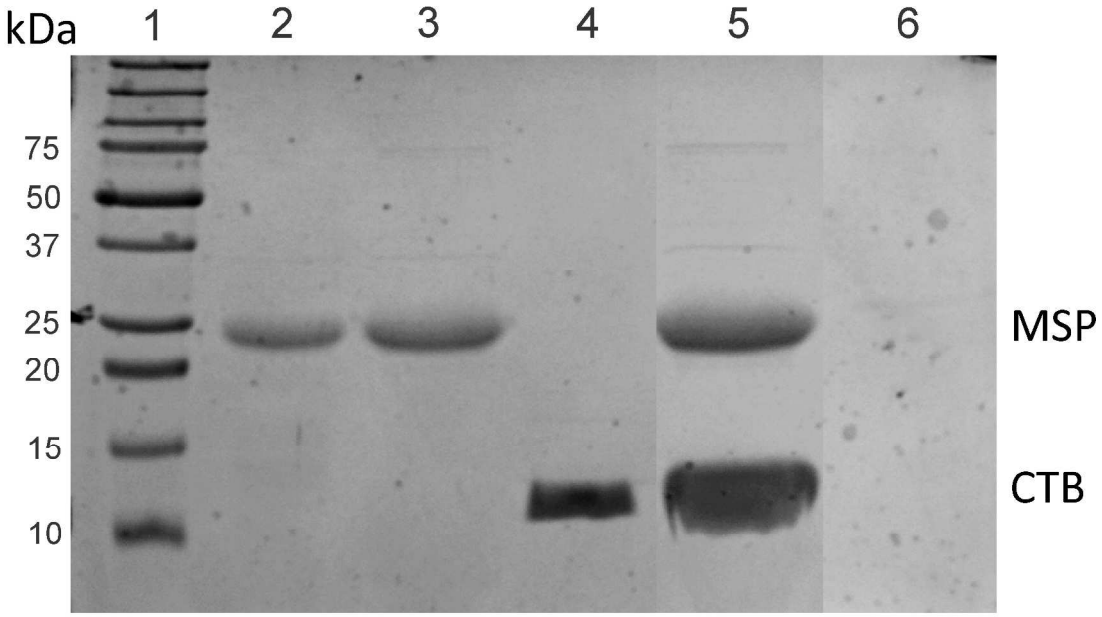


Figure S3. Evidence for binding of CTB_5 binding to GM1-containing NDs in solution. SDS-PAGE showing molecular weight markers (lane 1) and controls for MSP alone (lane 2), 10% GM1 ND (lane 3), CTB_5 (lane 4), and species that are ≥100 kDa (lane 5) and ≤100 kDa (lane 6) in solutions of CTB_5 and 10% GM1 ND. These data suggest that CTB_5 is fully bound to the GM1-containing NDs and the absence of free CTB_5-GM1 complexes in solution.

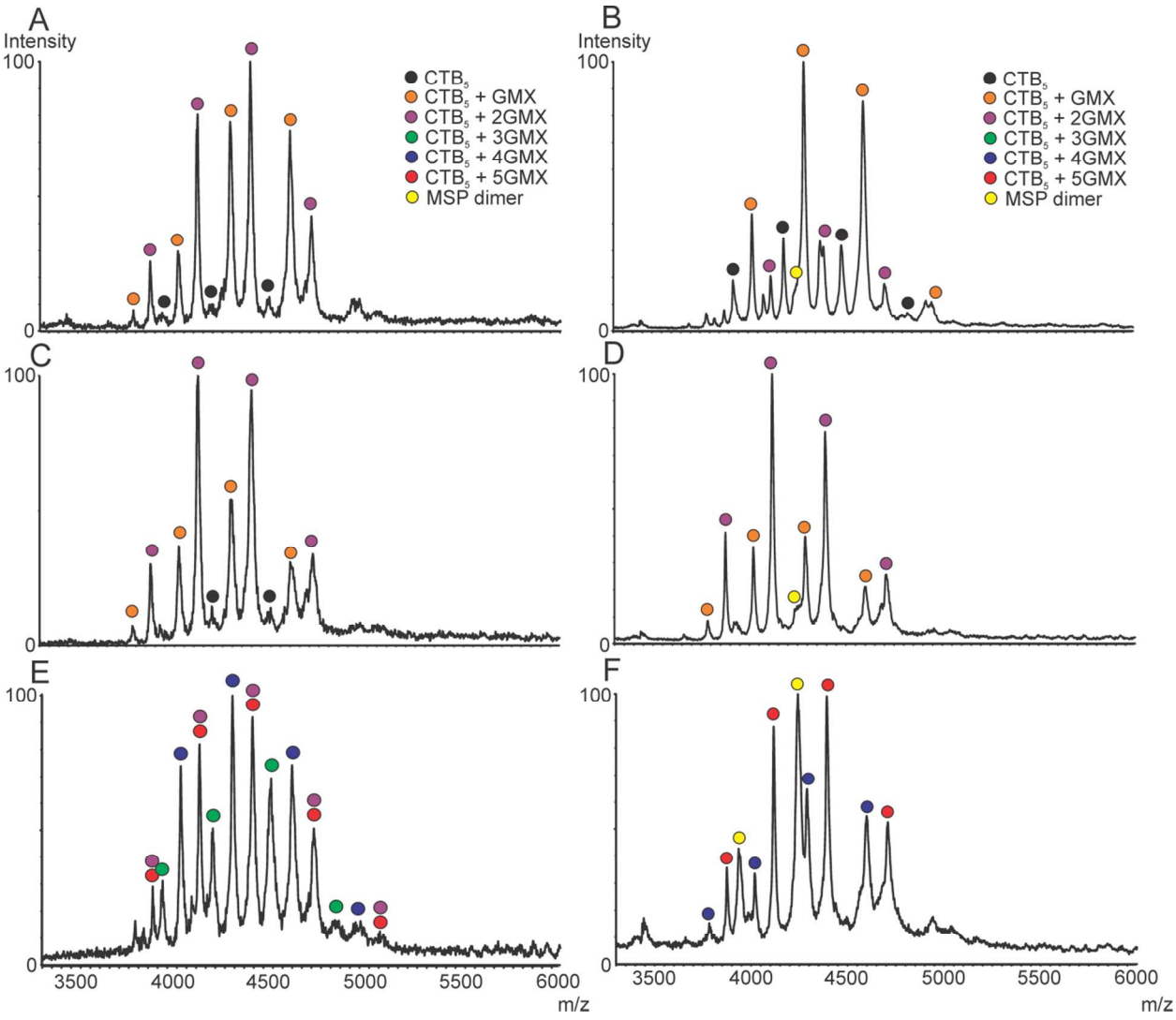


Figure S4. ESI-MS analysis of CTB_5 (5 µM) upon addition of 5 µM 1% GM1/GM2/GM3 ND (A), 1% GM1 + 1% GM2 ND + 1% GM3 ND (B), 2.5% GM1/2/3 ND (C), 2.5% GM1 ND + 2.5% GM2 ND + 2.5% GM3 ND (D), 5% GM1/2/3 ND (E) and 5% GM1 ND + 5% GM2 ND + 5% GM3 ND (F). The (CTB_5 + 5GM1), (CTB_5 + 4GM1), (CTB_5 + 3GM1), (CTB_5 + 2GM1), (CTB_5 + GM1), CTB_5 and MSP1E1 dimer ions are identified by the red, blue, green, purple, orange, black and yellow circles, respectively.

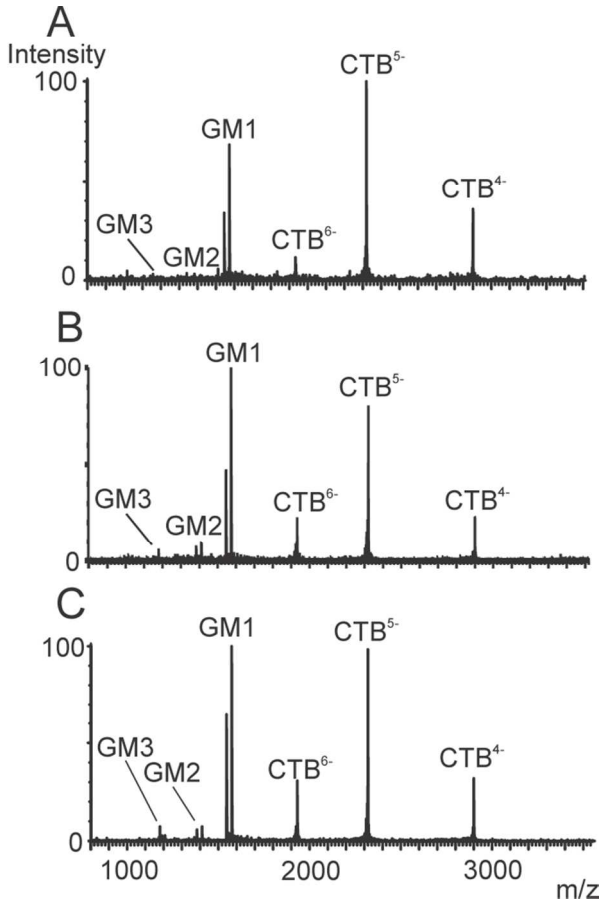


Figure S5. CID mass spectra measured for $(CTB_5 + 2GMX)^{13-}$ (A), $(CTB_5 + 2GMX)^{14-}$ (B) and $(CTB_5 + 5GMX)^{15-}$ (C) ions produced by ESI performed on solutions of CTB_5 (5 μM) and 5 μM 1% GM1 + GM2 + GM3 ND (A), 2.5% GM1 + GM2 + GM3 ND (B), and 5% GM1 + GM2 + GM3 ND (C). The collision energy used was 125 V in each case.

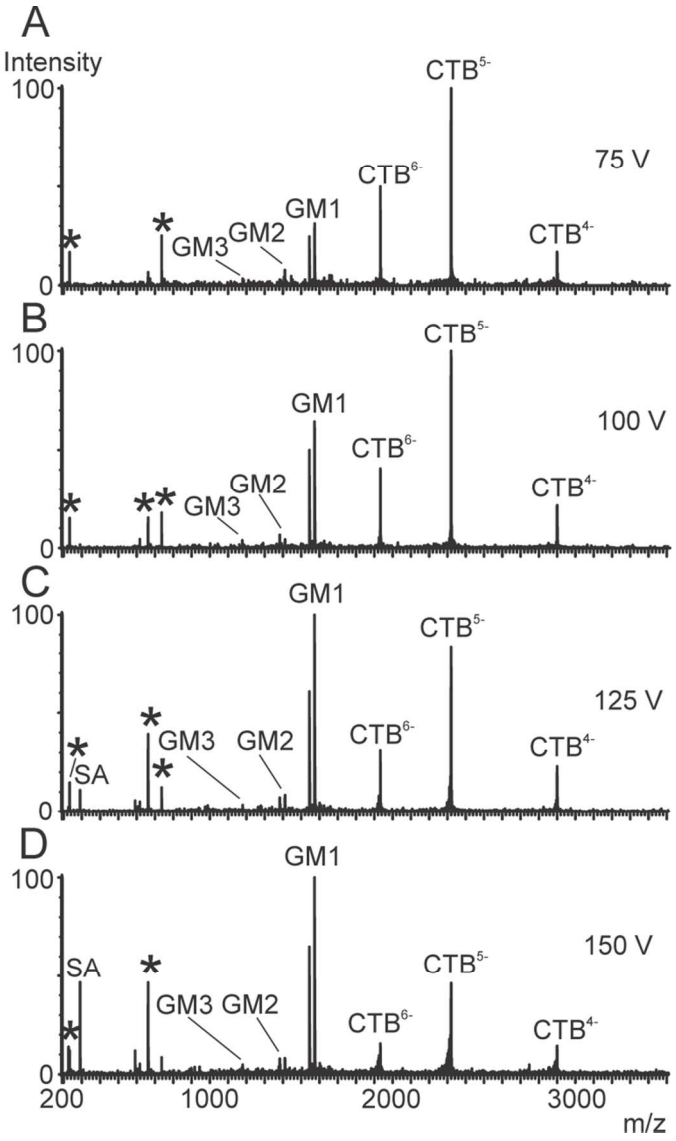


Figure S6. CID mass spectra measured for $(CTB_5 + 5GMX)^{15-}$ ions produced by ESI performed on solutions CTB_5 (5 μM) and 5 μM 5% GM1 + 5% GM2 + 5% GM3 ND. The isolation window was centred at 4393 m/z. The collision energies used were 75V (A), 100V (B), 125V (C) and 150V (D). * denotes peaks corresponding to DMPC ions or DMPC fragmentation products.

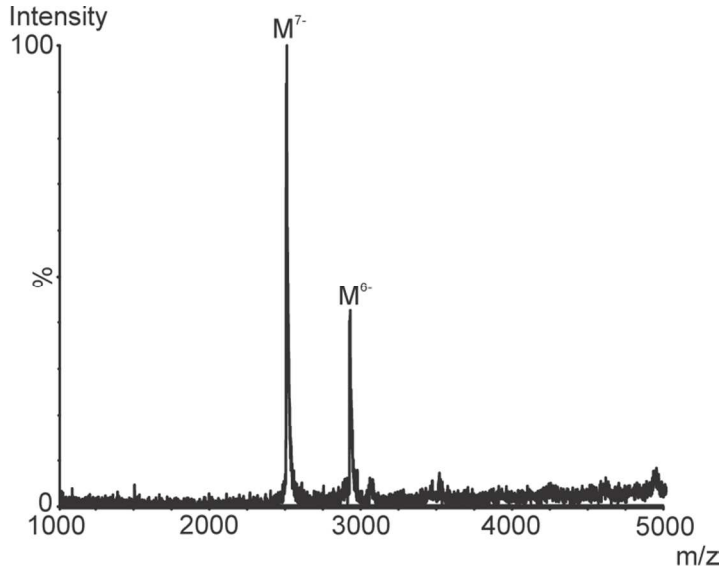


Figure S7. ESI-MS analysis of aqueous ammonium acetate solution of myoglobin (5 μM) and 5% GM1 + GM2 + GM3 ND (5 μM).

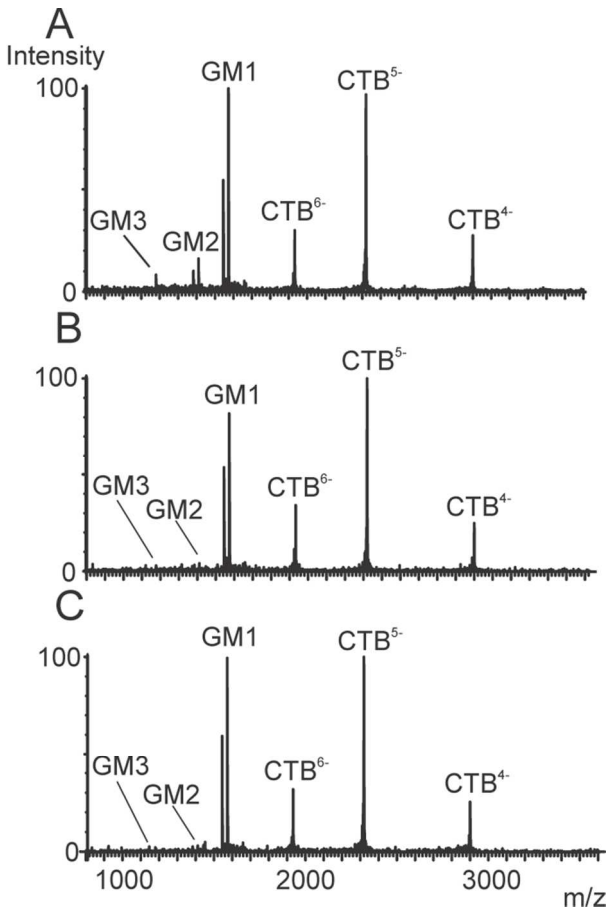


Figure S8. CID mass spectra measured for $(CTB_5 + 2GMX)^{14-}$ (A), $(CTB_5 + 2GMX)^{14-}$ (B) and $(CTB_5 + 5GMX)^{15-}$ (C) ions produced by ESI performed on solutions of CTB_5 (5 μM) and 5 μM of 1% GM1/GM2/GM3 ND (A), 2.5% GM1/GM2/GM3 ND (B), and 5% GM1/GM2/GM3 ND (C). The collision energy used was 125V in each case.

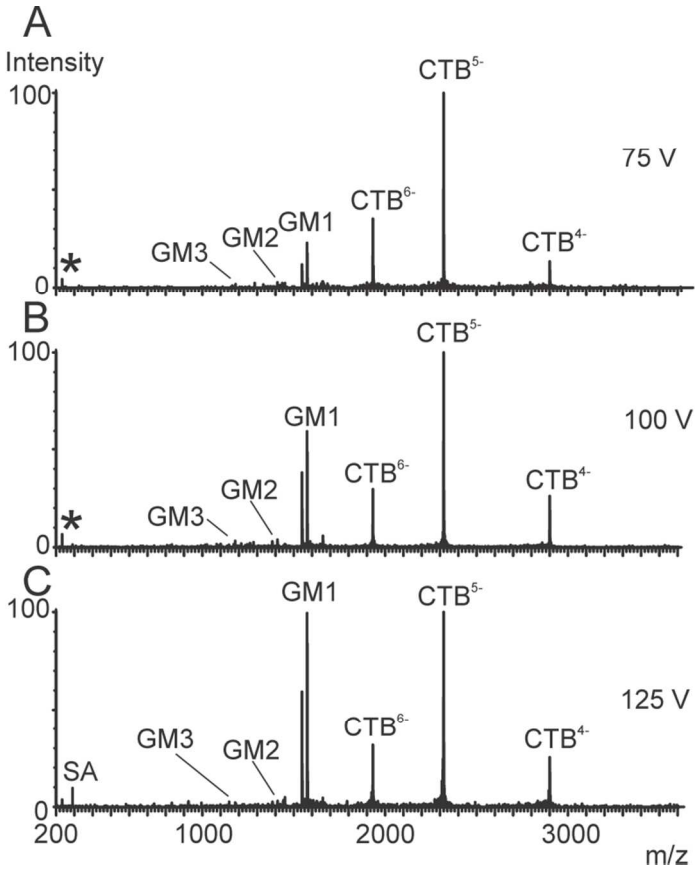


Figure S9. CID mass spectra measured for $(CTB_5 + 5GMX)^{15-}$ and $(CTB_5 + 2GMX)^{14-}$ produced by ESI performed on solutions CTB_5 (5 μM) and 5 μM of 5% GM1/GM2/GM3 ND. The isolation window was centred at 4380 m/z. The collision energies used were 75V (A), 100V (B) and 125V (C). * denotes peaks corresponding to DMPC ions or DMPC fragmentation products.

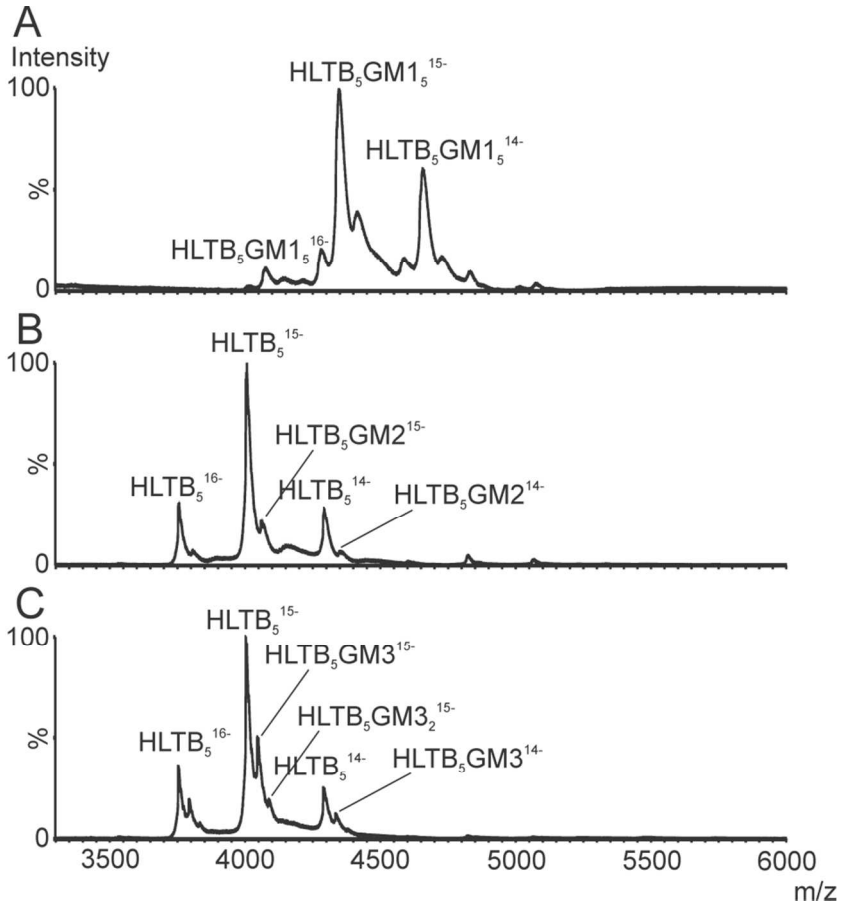
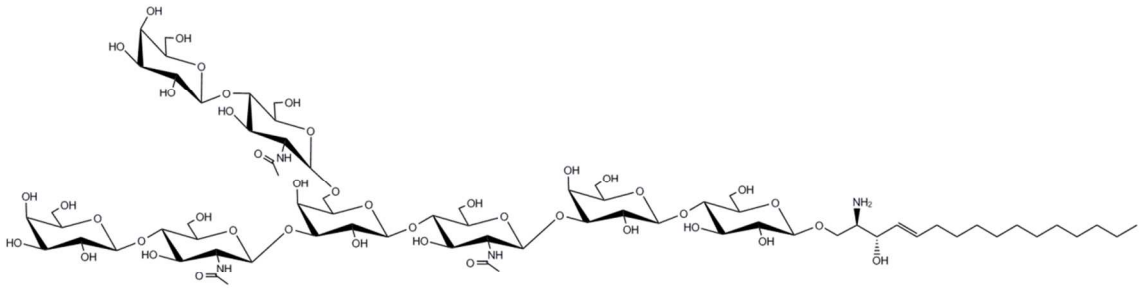
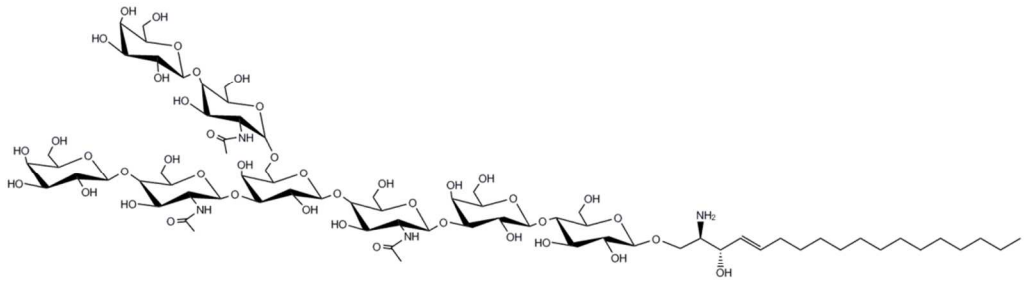


Figure S10. ESI-MS analysis of aqueous ammonium acetate solutions of $HLTB_5$ (5 μM) and 50 μM of the oligosaccharide of GM1 (A), GM2 (B) and GM3 (C).



Molecular Weight: 1691.72



Molecular Weight: 1719.77

Figure S11. Structures of the two isoforms of the putative neolacto GSL ligand of CTB_5.