SUPPORTING INFORMATION FOR:

Gangliosides are Ligands for Human Noroviruses

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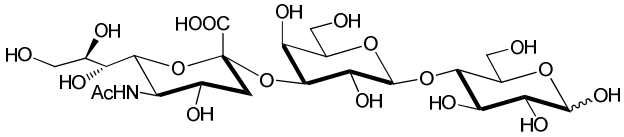
Table S1. Intrinsic association constants ($K_{a,int}$) for the NoV VA387 P dimer and the histo-blood group type 3 oligosaccharides (H3, A3 and B3) in an aqueous ammonium acetate solution (200 mM) at pH 7 and 25 °C measured using the direct ESI-MS assay performed in negative ion mode.[a]

Ligand	$K_{a,int}$ (M^{-1})	$K_{a,int}$ (M^{-1}) [b]
H3	720 ± 80	650 ± 65
A3	760 ± 60	800 ± 50
B3	1500 ± 230	1500 ± 150

a. The reported errors are one standard deviation. b. Values taken from reference S1.

Table S2. Summary of results from screening of ganglioside oligosaccharide against the P particle of human NoV VA387 in an aqueous ammonium acetate solution (200 mM) at pH 7 and 25 °C using the CaR-ESI-MS assay.

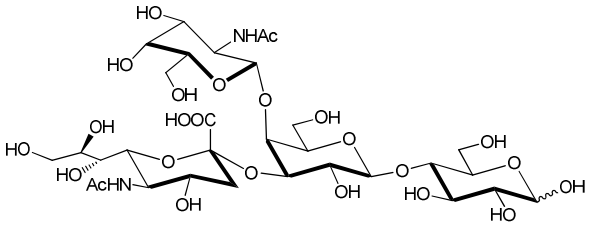
Oligosaccharide	Binder (+) / Non-binder (-)
GM3	+
GM2	+
GM1a	+
GM1b	+
GD3	+
GD2	+
GD1a	–
GD1b	–
GT3	–
GT2	–
GT1a	–
GT1c	–
fucosyl-GM1	+
asialo-GM1	–
asialo-GM2	–



GM3

MW 633.21 Da

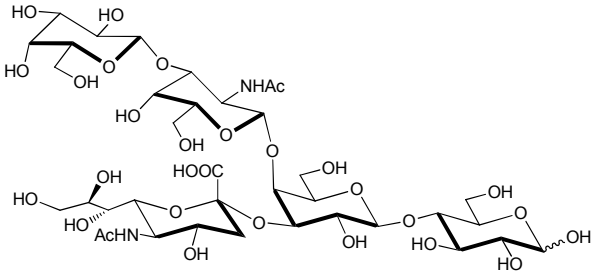
α-D-Neu5Ac-(2,3)-β-D-Gal-(1,4)-D-Glc



GM2

MW 836.29 Da

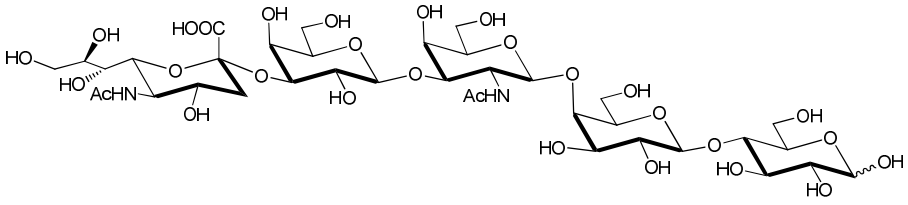
β-D-GalNAc-(1,4)-[α-D-Neu5Ac-(2,3)]-β-D-Gal-(1,4)-D-Glc



GM1a

MW 998.34 Da

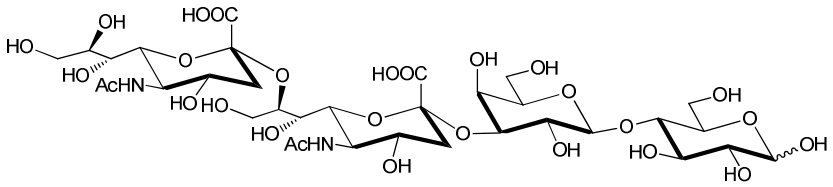
β-D-Gal-(1,3)-β-D-GalNAc-(1,4)-[α-D-Neu5Ac-(2,3)]-β-D-Gal-(1,4)-D-Glc



GM1b

MW 998.34 Da

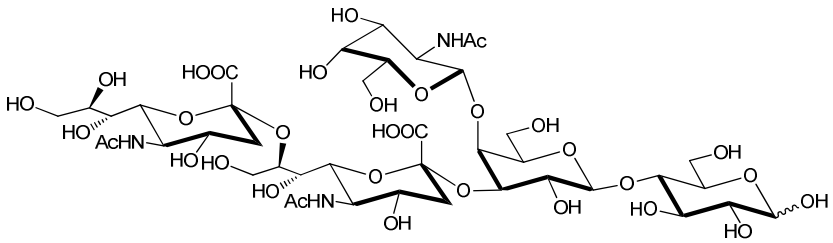
α-D-Neu5Ac-(2,3)-β-D-Gal-(1,3)-β-D-GalNAc-(1,4)-β-D-Gal-(1,4)-D-Glc



GD3

MW 924.31 Da

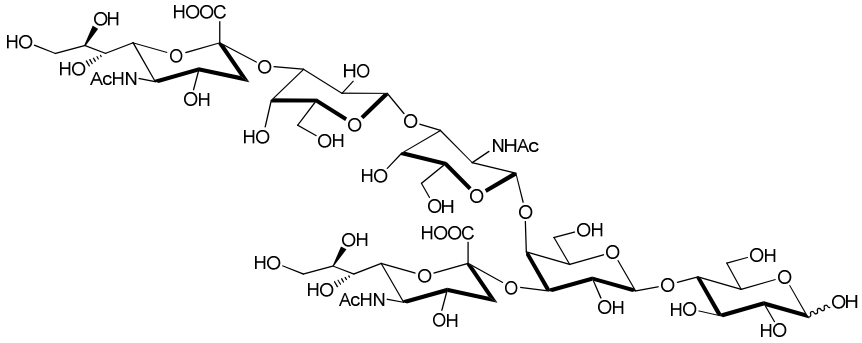
α-D-Neu5Ac-(2,8)-α-D-Neu5Ac-(2,3)-β-D-Gal-(1,4)-D-Glc



GD2

MW 1127.39 Da

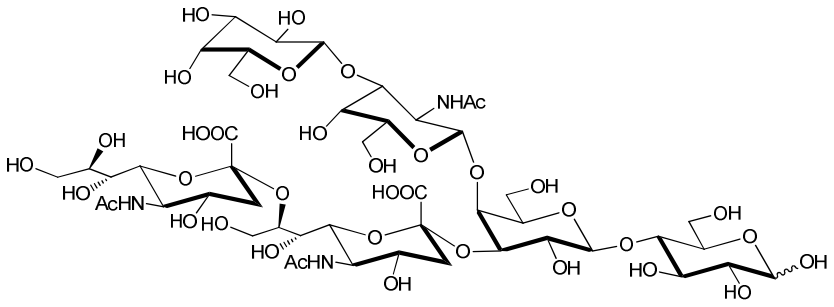
β-D-GalNAc-(1,4)-[α-D-Neu5Ac-(2,8)-α-D-Neu5Ac-(2,3)]-β-D-Gal-(1,4)-D-Glc



GD1a

MW 1289.44 Da

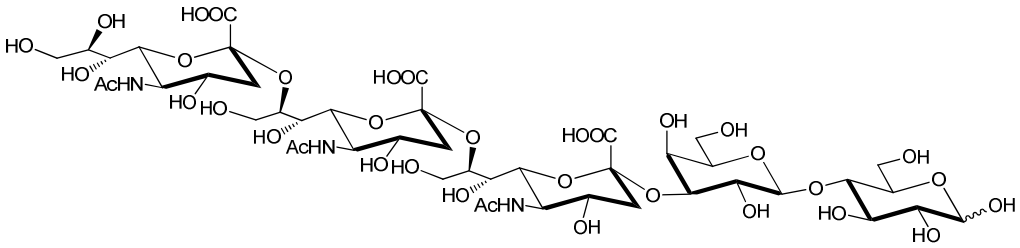
α-D-Neu5Ac-(2,3)-β-D-Gal-(1,3)-β-D-GalNAc-(1,4)-[α-D-Neu5Ac-(2,3)]-
β-D-Gal-(1,4)-D-Glc



GD1b

MW 1289.44 Da

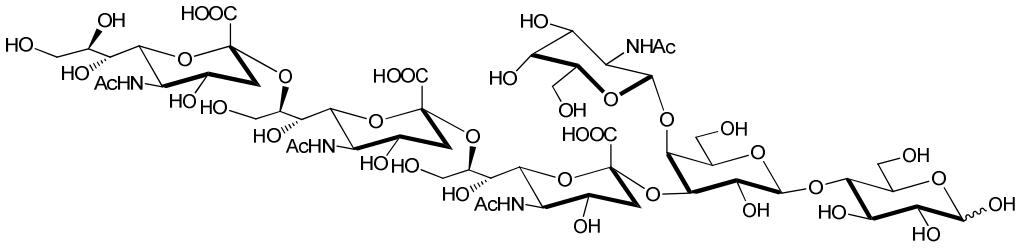
β-D-Gal-(1,3)-β-D-GalNAc-(1,4)-[α-D-Neu5Ac-(2,8)-α-D-Neu5Ac-(2,3)]-β-D-Gal-(1,4)-D-Glc



GT3

MW 1215.40 Da

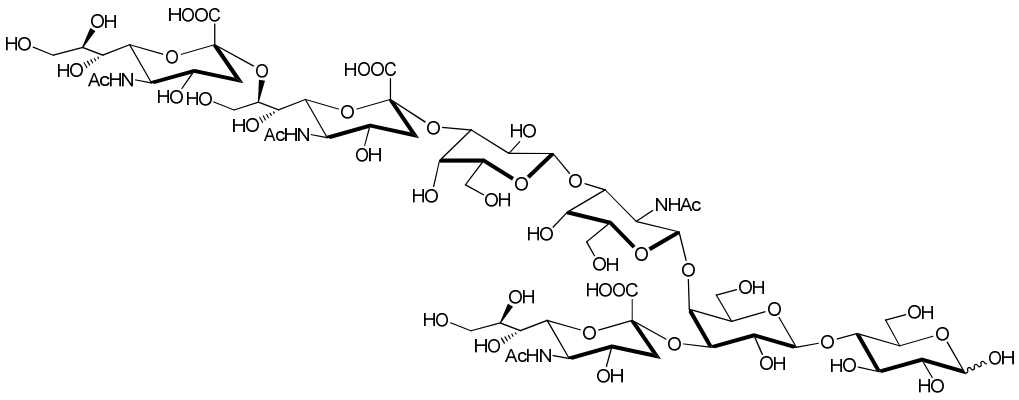
α-D-Neu5Ac-(2,8)-α-D-Neu5Ac-(2,8)-α-D-Neu5Ac-(2,3)-β-D-Gal-(1,4)-D-Glc



GT2

MW 1418.48 Da

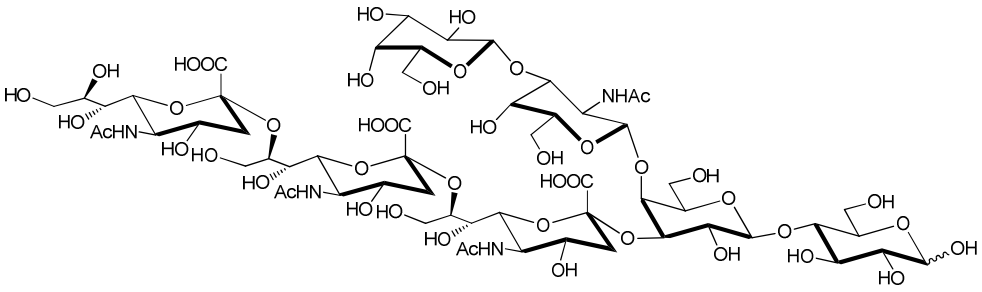
β-D-GalNAc-(1,4)-[α-D-Neu5Ac-(2,8)-α-D-Neu5Ac-(2,8)-α-D-Neu5Ac-(2,3)]-β-D-Gal-(1,4)-D-Glc



GT1a

MW 1580.53 Da

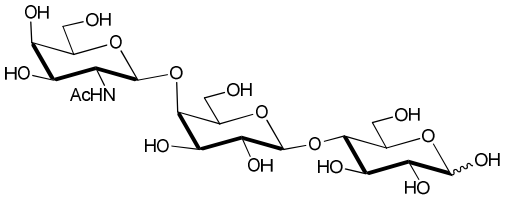
α-D-Neu5Ac-(2,8)-α-D-Neu5Ac-(2,3)-β-D-Gal-(1,3)-β-D-GalNAc-(1,4)-[α-D-Neu5Ac-(2,3)]-β-D-Gal-(1,4)-D-Glc



GT1c

MW 1580.53 Da

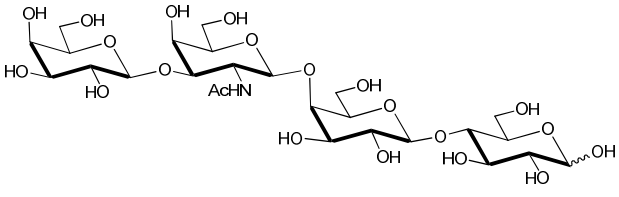
β-D-Gal-(1,3)-β-D-GalNAc-(1,4)-[α-D-Neu5Ac-(2,8)-α-D-Neu5Ac-(2,8)-α-D-Neu5Ac-(2,3)]-β-D-Gal-(1,4)-D-Glc



asialo-GM2

MW 545.20 Da

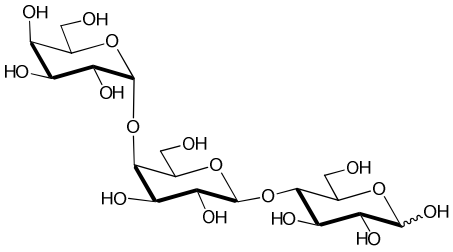
β-D-GalNAc-(1,4)-β-D-Gal-(1,4)-D-Glc



asialo-GM1

MW 707.25 Da

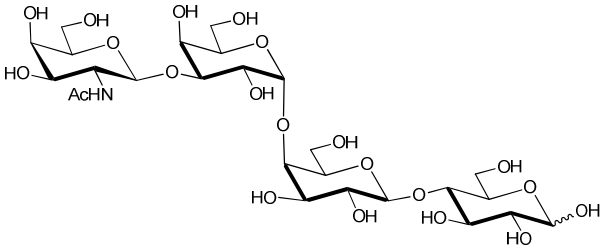
β-D-Gal-(1,3)-β-D-GalNAc-(1,4)-β-D-Gal-(1,4)-D-Glc



Gb3

MW 545.20 Da

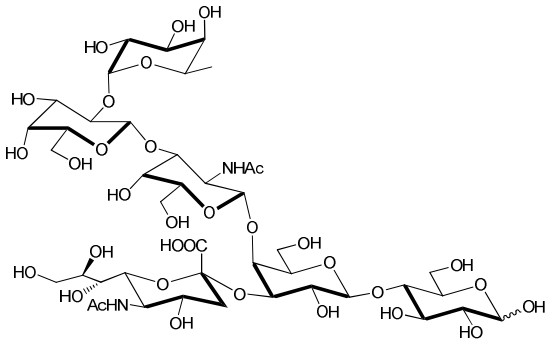
α-D-Gal-(1,4)-β-D-Gal-(1,4)-D-Glc



Gb4

MW 707.25 Da

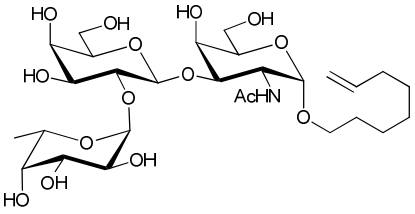
β-D-GalNAc-(1,3)-α-D-Gal-(1,4)-β-D-Gal-(1,4)-D-Glc



fucosyl-GM1 (Fuc-GM1)

MW 1144.40 Da

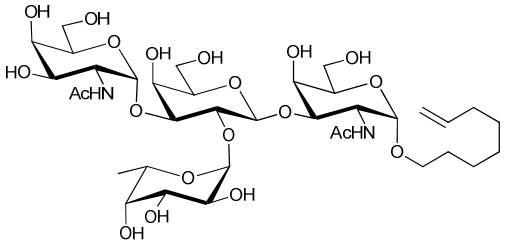
α-L-Fuc-(1,2)-β-D-Gal-(1,3)-β-D-GalNAc-(1,4)-[α-D-Neu5Ac-(2,3)]-β-D-Gal-(1,4)-D-Glc



H type 3 trisaccharide (H3)

MW 639.31 Da

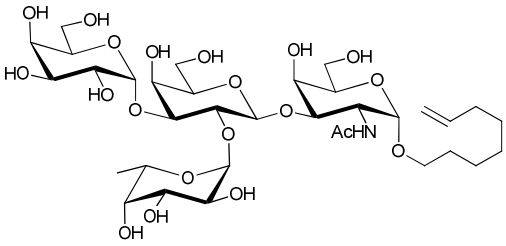
α-L-Fuc-(1,2)-β-D-Gal-(1,3)-α-D-GalNAc-$O(CH_2)_6CH=CH_2$



A type 3 tetrasaccharide (A3)

MW 842.39 Da

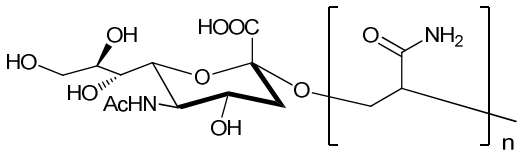
α-D-GalNAc-(1,3)-[α-L-Fuc-(1,2)]-β-D-Gal-(1,3)-α-D-GalNAc-$O(CH_2)_6CH=CH_2$



B type 3 tetrasaccharide (B3)

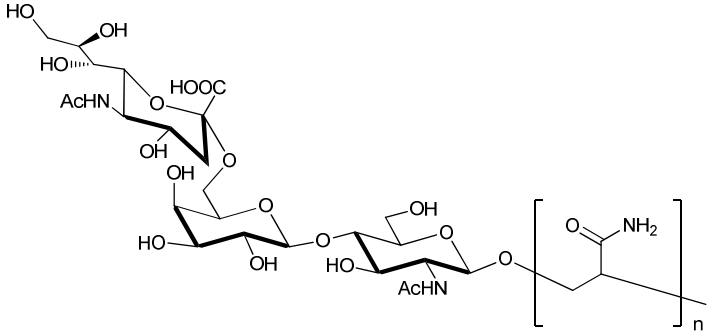
MW 801.36 Da

α-D-Gal-(1,3)-[α-L-Fuc-(1,2)]-β-D-Gal-(1,3)-α-D-GalNAc-$O(CH_2)_6CH=CH_2$



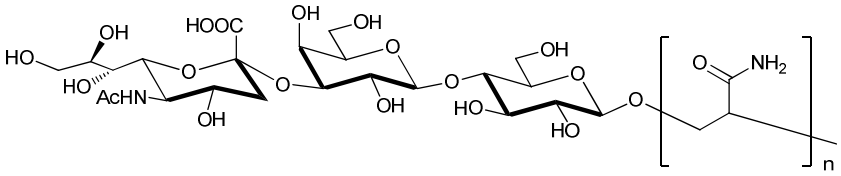
Neu5Ac-PAA

α-D-Neu5Ac-2-PAA



6'-sialylacNAc-PAA

α-D-Neu5Ac-(2,6)-β-D-Gal-(1,4)-β-D-GlcNAc-PAA



GM3-PAA

α-D-Neu5Ac-(2,3)-β-D-Gal-(1,4)-β-D-Glc-PAA

Figure S1. Structures of the twenty-component carbohydrate library consisting of the oligosaccharides of gangliosides, globosides and HBGAs and the structures of polyacrylamide (PAA)-conjugated sialic acid-containing oligosaccharides used for the ELISA binding measurements.

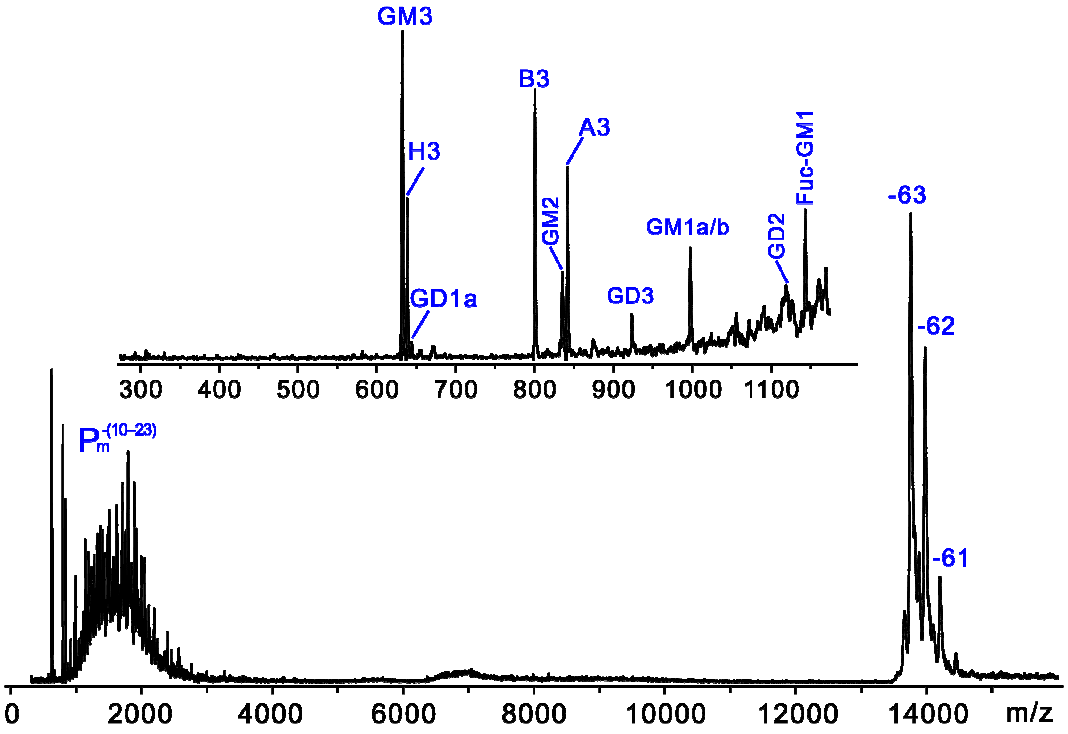


Figure S2. CID mass spectrum acquired in negative ion mode for an aqueous ammonium acetate solution (200 mM, pH 7 and 25 °C) of P particle (3 μM) and a twenty-component (10 μM each) carbohydrate library (GM1a, GM1b, GM2, GM3, GD1a, GD1b, GD2, GD3, GT1a, GT1c, GT2, GT3, Fuc-GM1, asialo-GM1, asialo-GM2, Gb3 and Gb4, as well as the H3, B3 and A3 oligosaccharides) using a broad (200 *m/z*) quadrupole isolation window centered at *m/z* 13,900. A Trap voltage of 200 V was used.

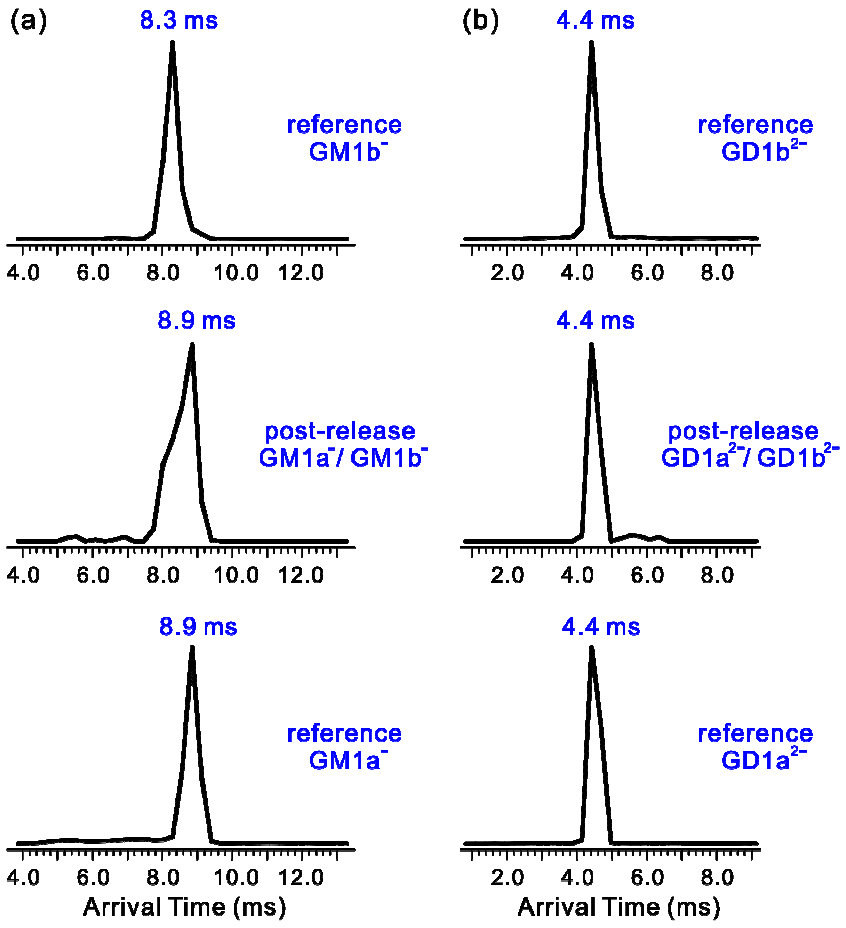


Figure S3. (a) Arrival time distributions measured for the (a) deprotonated ions of GM1a/GM1b (m/z 997.3) following release from the P particle (post-release) and the deprotonated GM1a and GM1b ions obtained directly from solution (reference) and (b) double deprotonated GD1a/GD1b ions (m/z 644.1) following release from the P particle (post-release) and the GD1a and GD1b ions obtained directly from solution (reference).

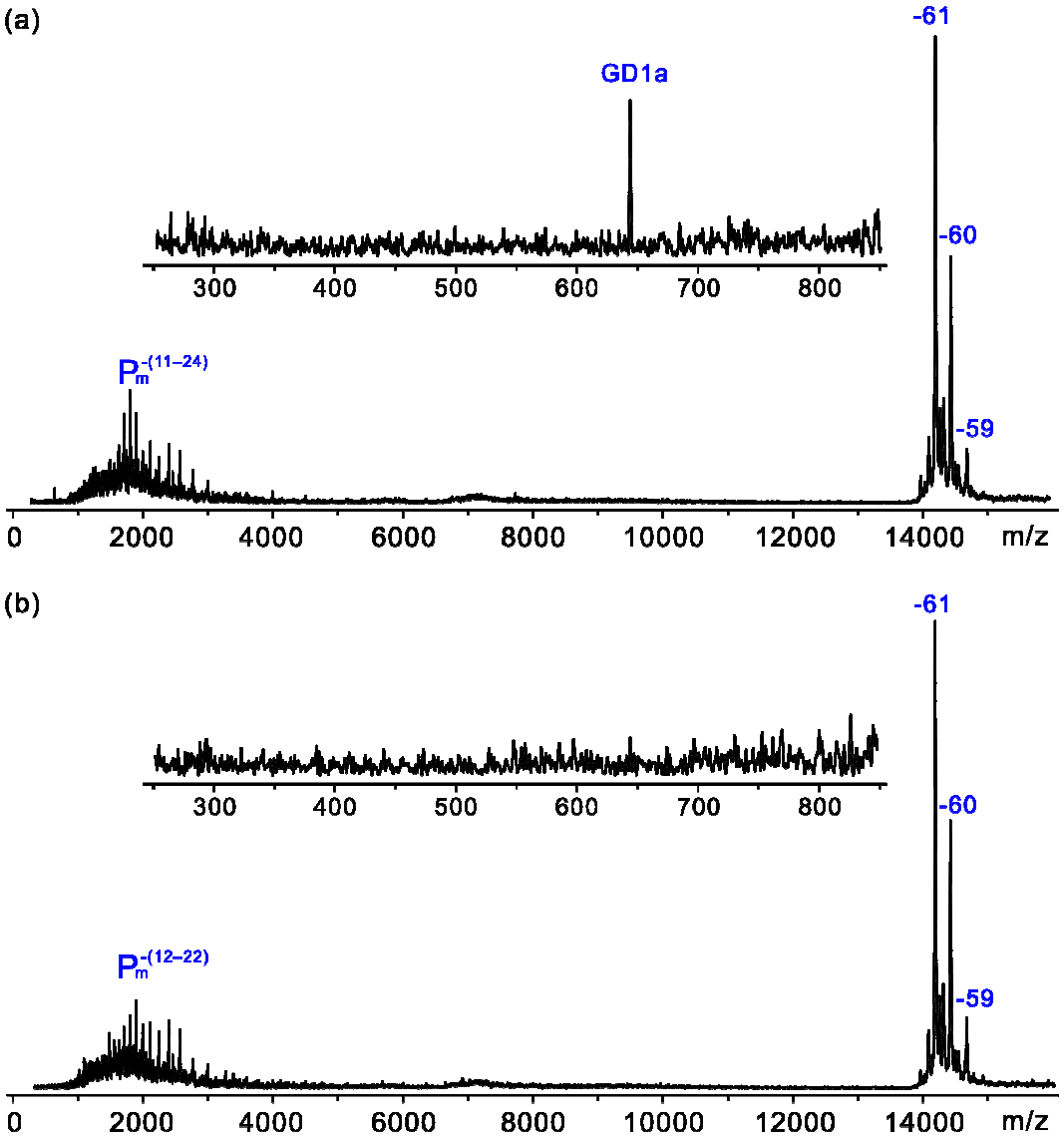


Figure S4. CID mass spectra acquired in negative ion mode for an aqueous ammonium acetate solutions (200 mM, pH 7 and 25 °C) of P particle (3 μM) and 10 μM of (a) GD1a and (b) GD1b using a broad (200 *m/z*) quadrupole isolation window centered at *m/z* 14,350. A Trap voltage of 200 V was used.

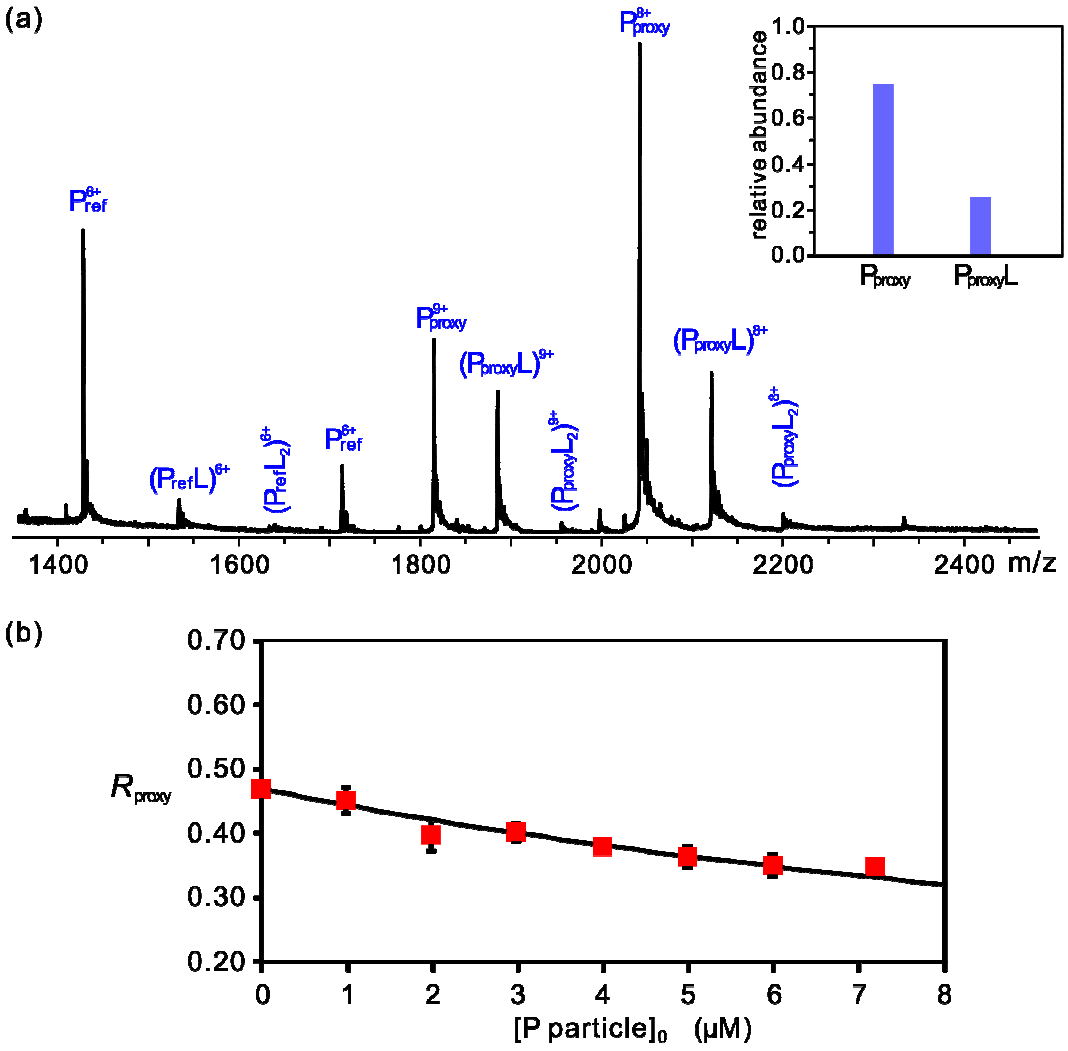


Figure S5. (a) Representative ESI mass spectrum measured in positive ion mode for aqueous ammonium acetate solution (160 mM, pH 7 and 25 °C) of P_{proxy} (Galectin-3, 3.0 μM), P_{ref} (ubiquitin, 1.0 μM), GM3 trisaccharide (40 μM) and NoV P particle (6.0 μM, 24-mer). Inset corresponds to normalized distribution of GM3 bound to P_{proxy} after correction for nonspecific ligand binding. (b) Plot of abundance ratio of GM3 trisaccharide-bound P_{proxy} to free P_{proxy} (R_{proxy}) versus P particle concentration. The solution conditions for each measurement were the same as in (a), but with the addition of P particle. The curve represents the best fit of eq 5 to the experimental data.

References

S1. Han, L.; Kitov, P. I.; Kitova, E. N.; Tan, M.; Wang, L.; Xia, M.; Jiang, X.; Klassen, J. S. *Glycobiology* **2013**, *23*, 276.