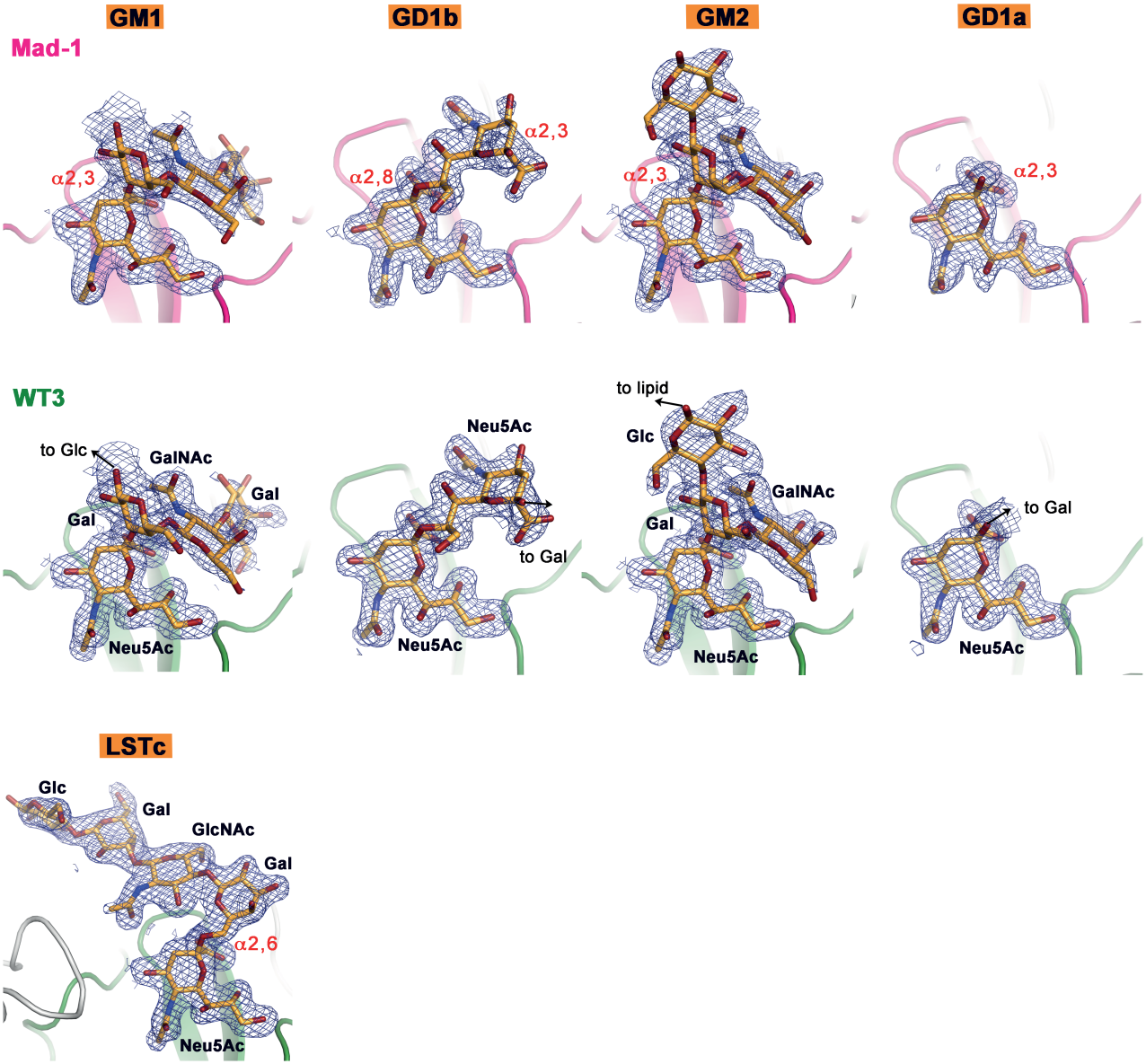

FIG. S1. Simulated annealing omit difference electron density maps for glycan complex structures of Mad-1 and WT3 VP1. Proteins are shown in cartoon representations and glycans as sticks colored according to the atom type (nitrogens in blue, oxygens in red and carbons in yellow). Fobs-Fcalc simulated annealing omit maps are shown at 3.0 sigma around 2.0 Å of the respective ligand.

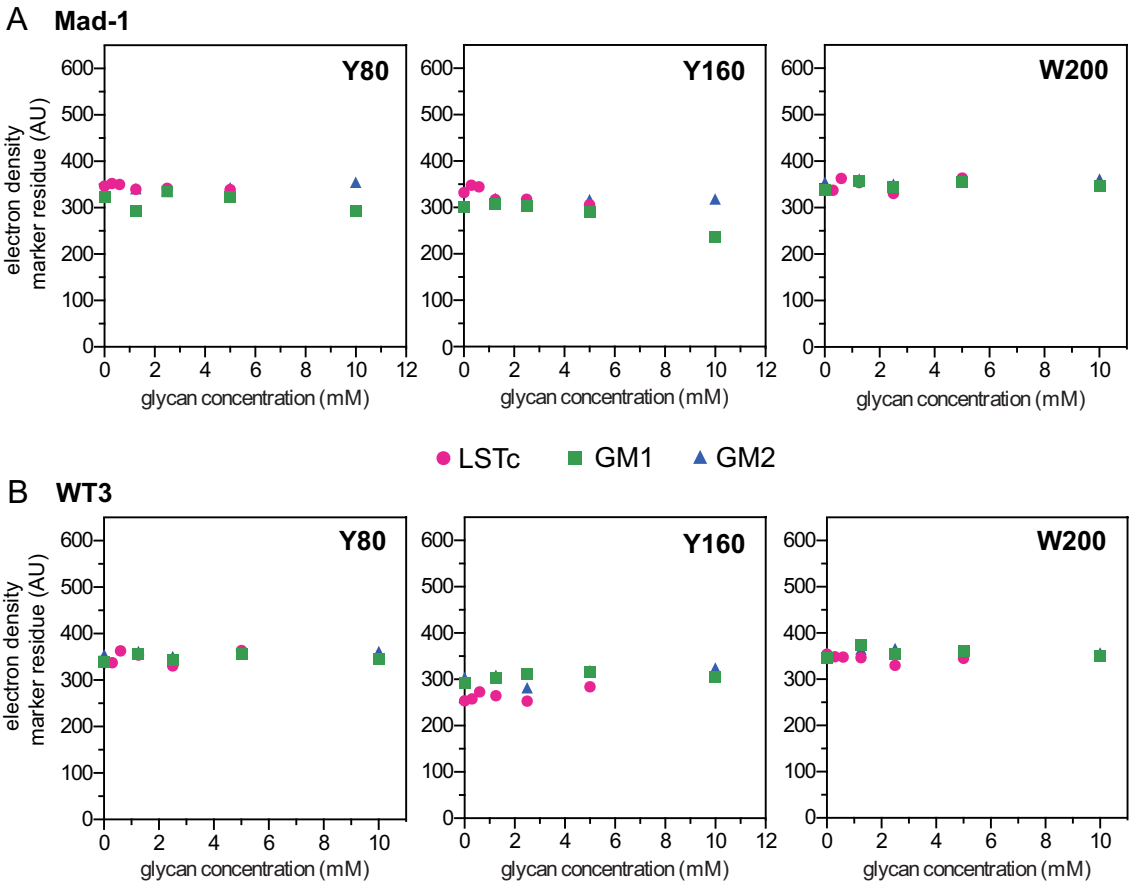

FIG. S2. Electron density integration within a 1.0 Å mask around marker residues of Mad-1 and WT3 VP1. In the JCPyV VP1 crystal environment only one binding site in the asymmetric unit is completely open towards the solvent, allowing engagement of all five ligands without steric hindrance or favorable interactions with a particular ligand. Therefore, statistic data analysis, calculation of the standard deviation, and cross-validation between binding sites was not possible for the electron density integration. Instead, Fobs-Fcalc simulated annealing omit electron densities maps for marker residues on the surface and in the hydrophobic core were integrated to test for the non-isomorphism of crystals and background differences of the electron density. Values for the electron density obtained by MAPMAN (53, 54) are plotted against the ligand concentrations, which were used in the concentration-dependent crystal soaking experiments. Representative examples are shown for experiments with LSTc, GM1, and GM2 glycans. The integrated electron density of marker atoms is independent of the ligand concentrations with standard deviations of about 1.5% to maximal 7% in rare cases.

Supplementary Tables

Table S1: Structures of native JCPyV WT3 VP1 and of JCPyV WT3 VP1 in complex with LSTc, GM1, GM2, GD1a and GD1a glycans.

Data collection and structural refinement statistics. The space group is C2 in all cases.

	WT3 VP1 native	GM1 (10 mM)	GM2 (20 mM)	GD1a (10 mM)	GD1b (10 mM)	LSTc (5 mM)
PDB accession code	4X0Y	4X0Z	4X10	4X11	4X12	4X13
Data Collection						
	X06SA	X06SA	X06SA	X06DA	X06DA	X06DA
a, *b*, *c* [Å]	150.5, 96.8, 128.2	150.3, 95.9, 128.9	150.4, 96.1, 128.8	150.1, 96.1, 128.0	150.2, 96.3, 128.33	150.1, 96.3, 128.1
β [°]	110.6	110.2	110.2	110.2	110.3	109.8
Resolution [Å]	30-1.70 (1.75-1.70)	30-1.85 (1.90-1.85)	30-1.90 (1.95-1.90)	30-2.25 (2.31-2.25)	30-1.90 (1.95-1.90)	30-2.00 (2.05-2.00)
Unique reflections	187,337 (13,674)	143,308 (10,293)	131,009 (9,366)	81,125 (5,921)	134,142 (9,504)	115,162 (8,278)
Total reflections	782,632 (57,482)	609,757 (41,002)	563,422 (38,910)	433,299 (27,562)	764,331 (46,179)	443,640 (32,140)
$CC_{1/2}$	99.2 (80.9)	99.6 (76.5)	99.4 (71.8)	99.5 (89.4)	99.6 (68.3)	99.6 (77.8)
R_{meas} [%]	12.5 (58.9)	11.2 (73.7)	13.8 (85.5)	12.3 (80.8)	13.0 (84.7)	11.5 (65.8)
I/σI	9.1 (2.6)	11.5 (2.1)	9.9 (2.0)	16.1 (2.5)	13.6 (2.1)	15.0 (2.5)
Completeness [%]	99.6 (98.4)	98.3 (96.0)	97.3 (94.3)	98.8 (89.1)	99.6 (95.9)	99.6 (97.3)
Wilson B-factor [$Å^2$]	22.1	23.3	24.8	24.9	21.8	22.6
Refinement						
Resolution [Å]	30-1.70 (1.75-1.70)	30-1.85 (1.90-1.85)	30-1.90 (1.95-1.90)	30-2.3 (2.36-2.30)	30.0-1.90 (1.95-1.90)	30-2.00 (2.05-2.00)
R_{work}/R_{free} [%]	15.7/17.8 (25.8/28.0)	16.3/19.1 (24.4/27.2)	16.4/19.6 (31.3/35.0)	16.1/20.2 (22.6/28.5)	15.8/18.7 (29.1/33.3)	15.6/19.2 (22.5/25.4)
No. of atoms						
protein / carbohydrate	10,108/-	10,026/182	10,079/160	9,866/63	10,029/123	10,022/193
water	1,274	1,284	1,181	809	1,034	1,063
Average B-Factor [$Å^2$]						
protein / carbohydrate	17.7/-	19.6/30.6	22.4/34.9	19.9/22.5	18.4/29.6	20.5/29.0
water	28.7	27.5	31.4	23.7	27.1	28.1
R.m.s. deviations						
bond length [Å]	0.009	0.010	0.008	0.009	0.008	0.009
bond angles [°]	1.408	1.437	1.350	1.320	1.336	1.356

Table S2: JCPyV Mad-1 VP1 pentamers in complex with LSTc, GM1, GM2, GD1a and GD1b glycans

Data collection and structural refinement statistics. The space group is C2 in all cases.

	GM1 (10 mM)	GM2 (20 mM)	GD1a (10 mM)	GD1b (10 mM)
PDB accession code	4X14	4X15	4X16	4X17
Data Collection				
	X06SA	X06SA	X06DA	X06DA
a, *b*, *c* [Å]	150.4, 95.6, 29.7	150.5, 95.7, 129.7	149.8, 95.6, 128.8	149.7, 95.4, 128.9
β [°]	110.2	110.3	110.3	110.4
Resolution [Å]	30-2.30 (2.36-2.30)	30-2.11 (2.16-2.11)	30-1.80 (1.85-1.80)	30-1.75 (1.80-1.75)
Unique reflections	75,740 (5,143)	99,141 (6,988)	157,148 (11,412)	170,305 (12,129)
Total reflections	318,028 (21,120)	413,312 (26,434)	892,253 (61,049)	970,603 (63,839)
$CC_{1/2}$	99.4 (79.9)	99.3 (77.7)	99.8 (73.5)	99.8 (79.9)
R_{meas} [%]	13.2 (76.9)	14.3 (75.7)	10.8 (86.9)	9.5 (71.0)
I/σI	11.2 (2.0)	9.1 (2.3)	15.4 (2.0)	15.5 (2.4)
Completeness [%]	98.8 (91.1)	99.3 (94.8)	99.7 (98.2)	99.6 (96.2)
Wilson B-factor [$Å^2$]	31.0	30.4	22.6	23.1
Refinement				
Resolution [Å]	30-2.30 (2.36-2.30)	30-2.11 (2.16-2.11)	30.0-1.80 (1.85-1.80)	30-1.75 (1.80-1.75)
R_{work}/R_{free} [%]	18.8/21.1 (35.1/36.3)	17.2/20.5 (28.7/31.0)	16.6/19.1 (29.4/32.1)	16.0/18.2 (26.9/29.4)
No. of atoms				
protein / carbohydrate	9,957/193	9,953/171	10,173/63	10,117/103
water	669	891	1,070	1,163
Average B-Factor [$Å^2$]				
protein / carbohydrate	27.2/41.8	25.4/39.1	19.4/27.9	18.7/30.8
water	29.1	32.2	28.9	29.3
R.m.s. deviations				
bond length [Å]	0.009	0.008	0.010	0.008
bond angles [°]	1.361	1.324	1.443	1.373

Table S3: Data collection statistics of data sets for the concentration-dependent density integration of LSTc. Crystals have the space group C2.

VP1	**WT3**	**WT3**	**WT3**	**WT3**	**Mad-1**	**Mad-1**	**Mad-1**	**Mad-1**	**Mad-1**	**Mad-1**
LSTc (mM)	0.31	0.63	1.25	2.5	-	0.31	0.63	1.25	2.5	5.0
beamline	X06DA	X06DA	X06DA	X06DA	X06SA	X06DA	X06DA	X06DA	X06DA	X06DA
Unit cell										
a, *b*, *c* [Å]	150.3, 96.8, 128.0	150.0, 96.5, 127.8	150.0, 96.9, 127.9	150.2, 96.7, 127.8	150.2, 96.7, 128.8	149.5, 97.3, 129.1	150.2, 96.5, 128.7	150.0, 96.4, 128.2	150.0, 96.5, 128.4	150.0, 96.4, 128.6
β [°]	110.5	110.3	110.2	110.1	110.5	110.2	110.4	110.4	110.4	110.3
Resolution [Å]	30-1.80 (1.85-1.80)	30-1.80 (1.85-1.80)	30-1.80 (1.85-1.80)	30-2.10 (2.15-2.10)	30-2.00 (2.06-2.00)	30-2.00 (2.05-2.00)	30-1.85 (1.90-185)	30-2.00 (2.05-2.00)	30-2.00 (2.05-2.00)	30-2.00 (2.05-2.00)
Unique reflections	158,177 (11,321)	157,331 (11,411)	157,639 (11,353)	99,844 (7,182)	114,410 (8,216)	115,891 (7,552)	145,564 (10,393)	115,158 (8,442)	113,914 (7,948)	115,576 (8,494)
Total reflections	662,748 (46,154)	662,524 (43,426)	667,378 (46,437)	424,630 (30,167)	482,593 (34,735)	481,090 (28,957)	616,941 (43,853)	486,667 (32,835)	488,740 (31,561)	488,619 (33,443)
$CC_{1/2}$	99.7 (70.4)	99.6 (77.0)	99.7 (79.7)	99.2 (75.2)	99.2 (75.4)	99.5 (80.3)	99.7 (72.6)	99.4 (72.2)	99.5 (74.2)	99.4 (64.3)
R_{meas} [%]	11.3 (76.4)	10.9 (59.8)	9.8 (62.1)	15.5 (68.9)	15.6 (74.2)	13.5 (77.9)	11.4 (77.5)	15.7 (78.5)	14.1 (71.5)	14.3 (80.7)
I/σI	12.6 (2.1)	11.7 (2.6)	14.0 (2.6)	11.7 (2.5)	8.5 (2.2)	9.5 (2.1)	11.2 (2.2)	9.7 (2.2)	10.7 (2.3)	10.5 (2.00)
Completeness [%]	99.6 (96.8)	99.7 (98.0)	99.6 (97.5)	99.6 (97.4)	98.7 (95.9)	98.9 (87.6)	99.5 (96.2)	99.6 (98.7)	98.1 (92.3)	99.7 (98.8)
Wilson B-factor [Å^2]	21.9	21.0	21.7	21.8	26.3	27.2	25.6	25.8	25.4	25.3

Table S4: Data collection statistics of data sets used for the concentration-dependent density integration of the GM1 glycan. Crystals have the space group C2.

VP1	**WT3**	**WT3**	**WT3**	**Mad-1**	**Mad-1**	**Mad-1**
GM1 (mM)	1.25	2.5	5	1.25	2.5	5
beamline	X06SA	X06SA	X06SA	X06SA	X06SA	X06SA
Unit cell						
a, *b*, *c* [Å]	150.4, 96.8, 128.5	150.5, 96.2, 129.0	150.6, 96.6, 129.3	150.4, 96.3, 128.9	150.4, 95.9, 129.5	150.0, 95.5,129.2
β [°]	110.3	110.3	110.3	110.5	110.4	110.2
Resolution [Å]	30-1.80 (1.85-1.80)	30-1.80 (1.85-1.80)	30-1.80 (1.85-1.81)	30-1.80 (1.85-1.80)	30-2.00 (2.06-2.00)	30-1.80 (1.85-1.80)
Unique reflections	158,084 (11,327)	158,032 (11,485)	158,601 (11,373)	155,805 (11,186)	114,858 (8,177)	154,151 (11,336)
Total reflections	659,532 (45,540)	660,134 (47,014)	662,871 (46,212)	661,296 (46,700)	481,966 (33,639)	639,240 (46,812)
$CC_{1/2}$	98.5 74.5	99.1 (71.0)	99.4 (80.8)	98.6 (81.6)	99.4 (78.8)	99.6 (82.1)
R_{meas} [%]	16.0 (77.5)	14.2 (76.3)	11.9 (64.1)	14.3 (55.0)	14.4 (77.2)	10.6 (65.3)
I/σI	7.0 (1.9)	7.9 (2.0)	9.4 (2.4)	7.7 (2.8)	8.9 (2.2)	11.0 (2.5)
Completeness [%]	99.5 (97.0)	99.7 (98.0)	99.5 (96.9)	98.1 (95.3)	99.3 (96.0)	97.9 (97.5)
Wilson B-factor [$Å^2$]	24.0	24.3	23.8	26.6	27.9	24.7

Table S5: Data collection statistics of the additional data sets used for the concentration dependent density integration for the GM2 glycan. Crystals have the space group C2.

VP1	**WT3**	**WT3**	**WT3**	**WT3**	**Mad-1**	**Mad-1**	**Mad-1**	**Mad-1**
GM2 (mM)	1.25	2.5	5	10	1.25	2.5	5	10
beamline	X06SA	X06SA	X06SA	X06SA	X06SA	X06SA	X06SA	X06SA
Unit cell								
a, *b*, *c* [Å]	150.3, 96.4, 128.2	151.3, 97.2, 128.8	150.8, 96.3, 128.6	150.9, 96.5, 128.7	150.1, 96.0, 128.9	150.2, 96.1, 129.0	150.3, 96.0, 129.3	150.1, 95.6, 129.3
β [°]	110.4	110.3	110.3	110.4	110.4	110.4	110.4	110.4
Resolution [Å]	30-1.80 (1.85-1.80)	30-2.11 (2.16-2.11)	30-2.00 (2.06-2.01)	30-1.70 (1.75-1.70	30-1.80 (1.85-1.80)	30-1.90 (1.95-1.90)	30-1.80 (1.85-1.80)	30-1.80 (1.85-1.80)
Unique reflections	152,248 (10,494)	99,824 (6,994)	115,134 (8,255)	188,271 (13,716)	154,257 (11,103)	134,315 (9,581)	157,972 (1,581)	157,529 (11,343)
Total reflections	637,365 (43,358)	414,098 (27,757)	481,083 (34,136)	788,337 (56,634)	663,743 (48,147)	563,375 (39,404)	664,477 (48,821)	662,083 (47,766)
$CC_{1/2}$	99.3 (82.9)	99.3 (78.5)	99.5 (79.7)	97.2 (69.1)	99.6 (75.7)	99.7 (78.8)	99.2 (79.7)	98.4 (86.2)
R_{meas} [%]	13.0 (61.4)	13.1 (63.6)	14.0 (73.1)	19.8 (76.7)	10.3 (76.3)	10.6 (73.3)	12.7 (60.8)	15.4 (51.9)
I/σI	8.8 (2.5)	9.7 (2.6)	11.48 (2.66)	5.4 (1.9)	10.7 (2.2)	11.4 (2.2)	8.4 (2.4)	7.1 (2.7)
Completeness [%]	96.0 (89.8)	99.4 (94.7)	99.5 (96.5)	99.7 (98.6)	97.6 (95.0)	99.5 (96.7)	99.7 (98.7)	99.6 (97.2)
Wilson B-factor [$Å^2$]	22.3	28.2	24.2	24.7	26.7	26.1	25.2	25.1

Table S6: Data collection statistics of the additional data sets used for the concentration dependent density integration for the GD1a glycan. Crystals have the space group C2.

VP1	**WT3**	**WT3**	**WT3**	**WT3**	**Mad-1**	**Mad-1**	**Mad-1**	**Mad-1**
GD1a (mM)	0.63	1.25	2.5	5	0.63	1.25	2.5	5
beamline	X06DA	X06DA	X06DA	X06DA	X06DA	X06DA	X06DA	X06DA
Unit cell								
a, *b*, *c* [Å]	150.2, 96.0, 128.1	149.9, 95.7, 128.1	150.5, 96.1, 128.5	150.3, 96.2, 128.2	150.1, 96.2, 128.5	149.8, 95.5, 128.5	149.8, 95.8, 128.7	149.9, 95.8, 128.8
β [°]	110.3	110.3	110.4	110.2	110.4	110.3	110.4	110.4
Resolution [Å]	30-2.05 (2.10-2.05)	30-1.85 (1.90-1.85)	30.0-1.90 (1.95-1.90)	30-2.40 (2.46-2.40)	30-1.60 (1.64-1.60)	30-1.65 (1.69-1.65)	30-1.90 (1.95-1.90)	30-1.75 (1.80-1.75)
Unique reflections	106,508 (7,661)	144,017 (10,301)	134,218 (9,235)	66,652 (4,604)	224,814 (16,388)	200,663 (14,146)	133,940 (9,734)	171,089 (12,331)
Total reflections	608,355 (40,545)	826,671 (52,526)	767,567 (49,522)	376,400 (23,692)	1,269,968 (86,946)	1,052,395 (68,886)	764,945 (48,815)	953,738 (62,373)
$CC_{1/2}$	99.3 (79.8)	99.7 (81.8)	99.7 (76.2)	99.2 (78.8)	99.9 (79.5)	99.9 (85.2)	99.7 (76.7)	99.8 (80.4)
R_{meas} [Å]	16.5 (79.7)	12.3 (12.3)	13.3 (90.7)	15.3 (74.0)	7.0 (75.7)	6.8 (64.6)	12.1 (81.5)	9.7 (77.7)
I/σI	13.0 (2.3)	14.04 (2.5)	13.5 (2.0)	14.4 (2.6)	19.1 (2.5)	18.9 (2.6)	14.7 (2.2)	15.1 (2.3)
Completeness [%]	99.6 (97.6)	99.6 (96.4)	99.3 (92.9)	99.2 (92.6)	99.7 (98.5)	98.5 (94.6)	99.7 (98.6)	99.7 (97.6)
Wilson B-factor [$Å^2$]	21.1	21.1	22.7	21.4	22.4	22.5	22.4	22.5

Table S7: Data collection statistics of the additional data sets used for the concentration dependent density integration for the GD1b glycan. Crystals have the space group C2.

VP1	**WT3**	**WT3**	**WT3**	**WT3**	**Mad-1**	**Mad-1**	**Mad-1**	**Mad-1**
GD1b (mM)	0.63	1.25	2.5	5	0.63	1.25	2.5	5
Data Collection	X06DA	X06DA	X06DA	X06DA	X06DA	X06DA	X06DA	X06DA
Unit cell								
a, *b*, *c* [Å]	145.0, 96.2, 128.0	150.4, 96.3, 128.4	150.2, 96.3, 128.2	150.1, 96.4, 128.2	149.8, 95.8, 128.4	149.8, 95.8, 128.4	149.8, 95.6, 128.8	149.9, 95.4, 128.9
β [°]	110.4	110.3	110.6	110.3	110.4	110.4	110.3	110.3
Resolution [Å]	30-1.85 (1.90-1.85)	30-1.95 (2.00- 1.95)	30-1.85 (1.90-1.85)	30-1.90 (1.95-1.90)	30-1.75 (1.80-1.75)	30-1.80 (1.85- 1.80)	30-1.90 (1.95-1.90)	30-2.10 (2.15-2.10)
Unique reflections	144,880 (10,544)	123,742 (8,470)	144,803 (10,370)	134,448 (9,647)	166,353 (11,910)	157,123 (11,513)	132,768 (9,444)	98,777 (7,210)
Total reflections	823,303 (54,911)	707,337 (45,494)	832,599 (53,184)	765,085 (47,900)	932,942 (61,102)	896,684 (58,683)	711,947 (43,508)	536,551 (35,871)
$CC_{1/2}$	99.5 (78.7)	99.6 (79.9)	99.7 (75.4)	99.6 (71.3)	99.8 (80.6)	99.8 (82.9)	99.5 (75.3)	99.5 (83.0)
R_{meas} (%)	15.3 (84.8)	15.2 (90.4)	12.2 (78.1)	13.1 (79.2)	9.6 (67.3)	10.3 (77.0)	13.5 (80.0)	13.3 (69.4)
I/σI	10.5 (2.3)	13.7 (2.8)	14.2 (2.3)	13.6 (2.2)	16.0 (2.6)	16.2 (2.4)	12.3 (2.1)	14.1 (2.6)
Completeness [%]	99.8 (98.5)	99.3 (92.3)	99.7 (96.9)	99.7 (97.4)	97.1 (94.4)	99.8 (98.8)	99.0 (95.5)	99.2 (97.8)
Wilson B-factor [$Å^2$]	21.2	23.9	22.5	21.7	22.6	22.1	22.0	23.6

Annotation for Tables S1-S7:

Values for the highest resolution bin are given in parentheses.

$CC_{1/2}$: correlation between intensities from random half-dataset

$R_{meas} = \frac{\sum_{hkl} \sqrt{\frac{n}{n-1}} \sum_{j=1}^{n} |I_{hkl,j} - \langle I_{hkl,j} \rangle|}{\sum_{hkl} \sum_{j}^{n} I_{hkl,j}}$, with n is the number of observation of the reflection and $\langle I_{h,k,l} \rangle$ the intensity of symmetry- (or Friedel-) related observations.

$R_{work} = \frac{\sum_{hkl} |F_{obs}(hkl) - F_{calc}(hkl)|}{\sum_{hkl} F_{obs}(hkl)}$, F_{obs} and F_{calc} are the observed and calculated structure factors, respectively. 5% of reflections were not used during structure refinement to calculate R_{free}.