Supporting Information to

The virulence factor LecB varies in clinical isolates: consequences for ligand binding and drug discovery

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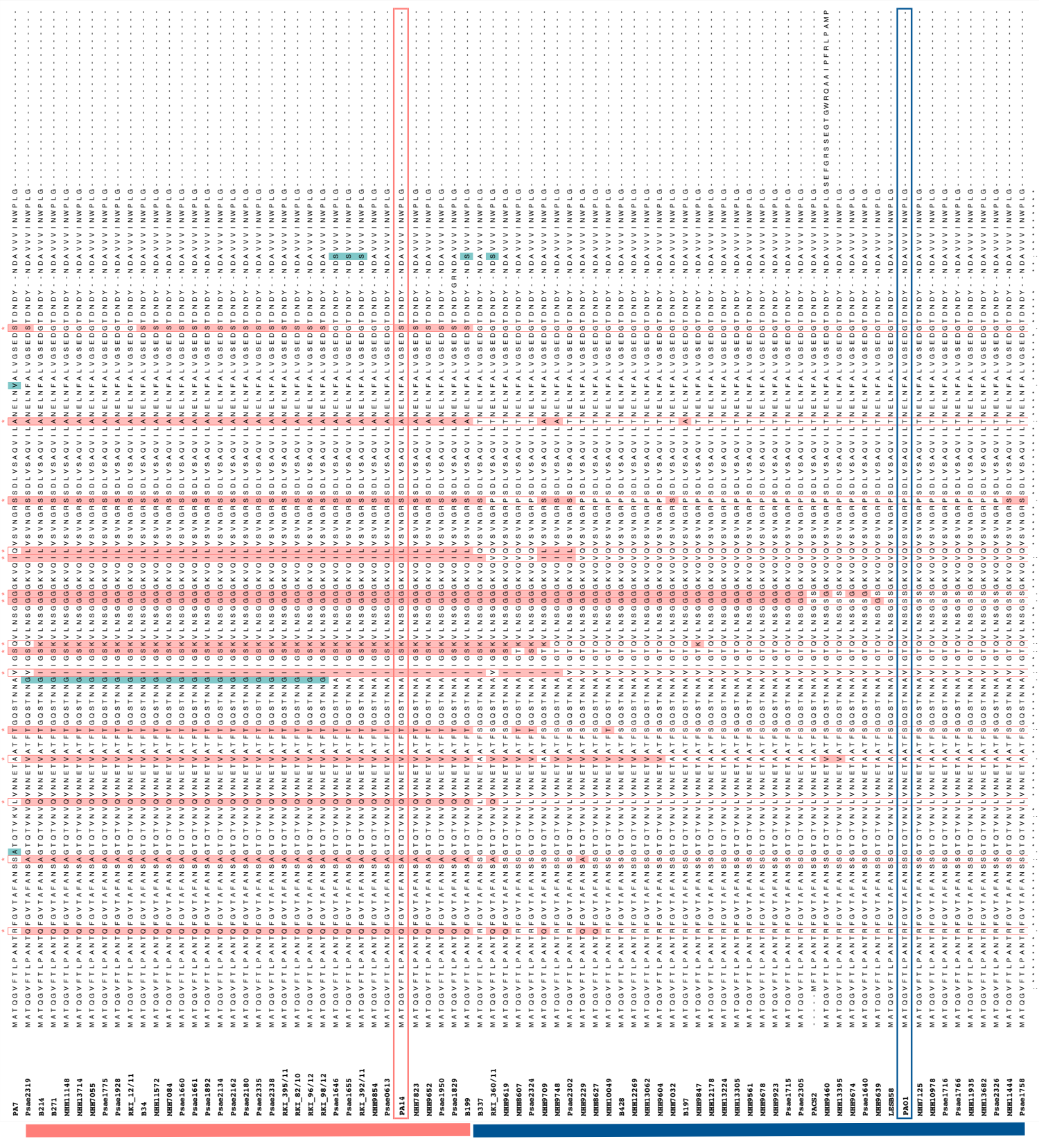


Figure S1: Multiple sequence alignment (MUSCLE) of LecB sequences and color coded PA14-like strains (red) and PAO1-like strains (blue). The point mutations present in the sequence of $LecB_{PA14}$ compared to $LecB_{PAO1}$ are highlighted with asterisks (red). Additional point mutations that are not present in PA14 are highlighted in green. Amino acid numbering corresponds to the crystal structure of $LecB_{PAO1}$, which lacks methionine.

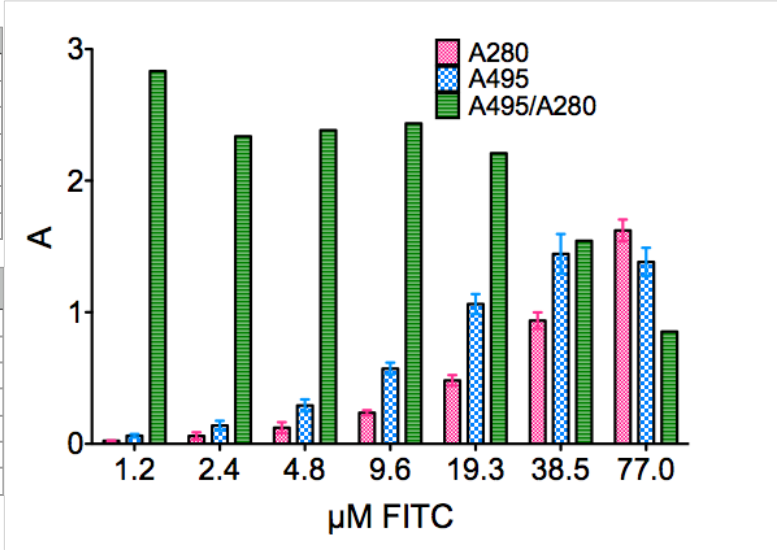
UV straight calibration line

A280

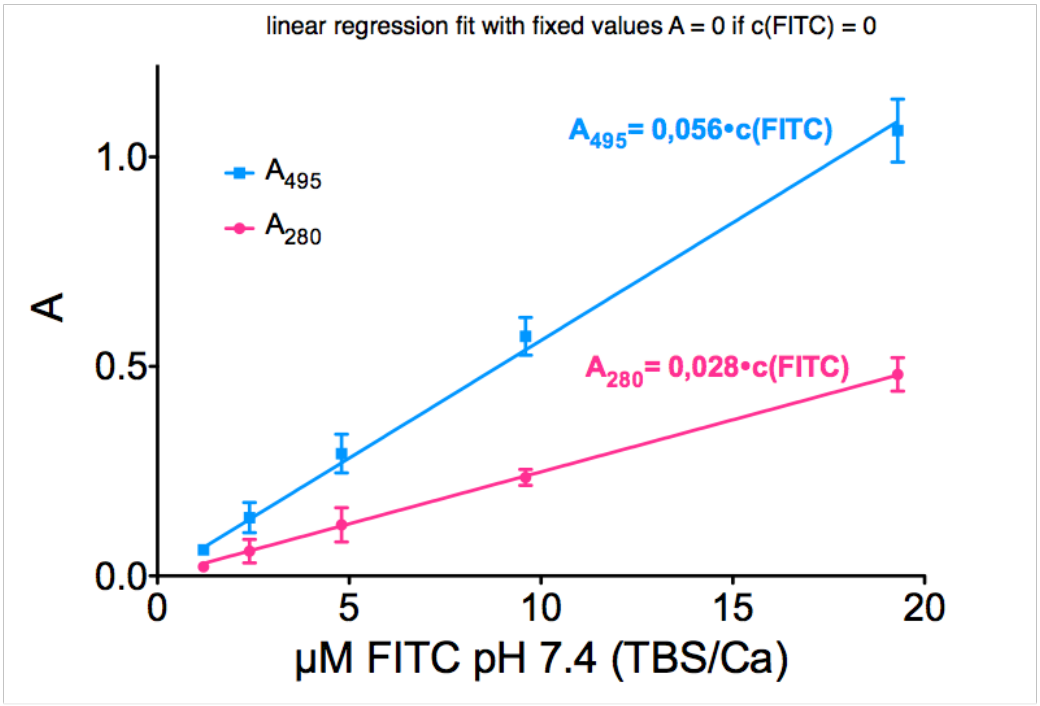
µg/mL	A280	A280	A280	M A280	STD A280	µM
30	1,690	1,646	1,528	1,621	0,084	77,046
15	0,919	1,004	0,884	0,936	0,062	38,523
7,5	0,473	0,525	0,446	0,481	0,040	19,261
3,75	0,245	0,247	0,213	0,235	0,019	9,631
1,87	0,166	0,117	0,084	0,122	0,041	4,803
0,93	0,049	0,091	0,038	0,059	0,028	2,388
0,47	0,020	0,021	0,025	0,022	0,003	1,207

A495

µg/mL	A495	A495	A495	M A495	STD A495	rA495/ A280
30	1,375	1,495	1,280	1,383	0,108	0,853
15	1,523	1,270	1,538	1,444	0,151	1,543
7,5	1,108	1,105	0,977	1,063	0,075	2,209
3,75	0,599	0,598	0,520	0,572	0,045	2,435
1,87	0,317	0,320	0,238	0,292	0,047	2,384
0,93	0,149	0,172	0,095	0,139	0,040	2,337
0,47	0,068	0,071	0,048	0,062	0,013	2,833



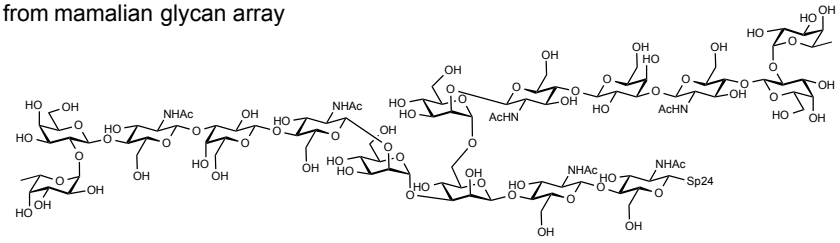
LINEAR RANGE: 0-20 µM FITC in TBS/Ca



$$C_{labeled\ LecB} = \frac{A_{280} - 0.028 \cdot \frac{A_{495}}{0.056}}{6990}$$

Figure S2: concentration determination of FITC-labeled lectin $LecB_{PAO1}$ and $LecB_{PA14}$ by UV absorbance measurements

bi-antennary H type II blood group antigen **543**
from mamalian glycan array



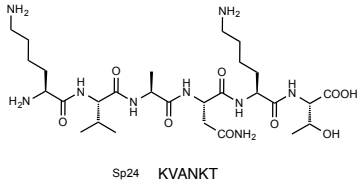


Figure S3: Structure of the best ligand identified by glycan array screening (compound 543).

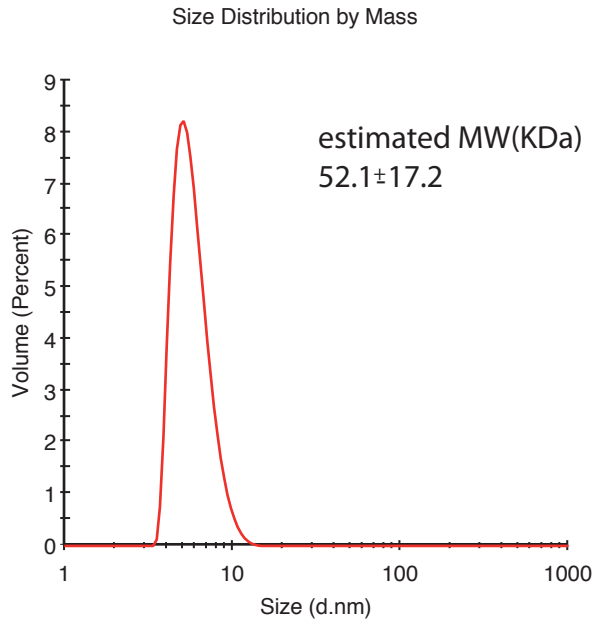


Figure S4: Dynamic light scattering of $LecB_{PA14}$ suggested a tetramer in solution.

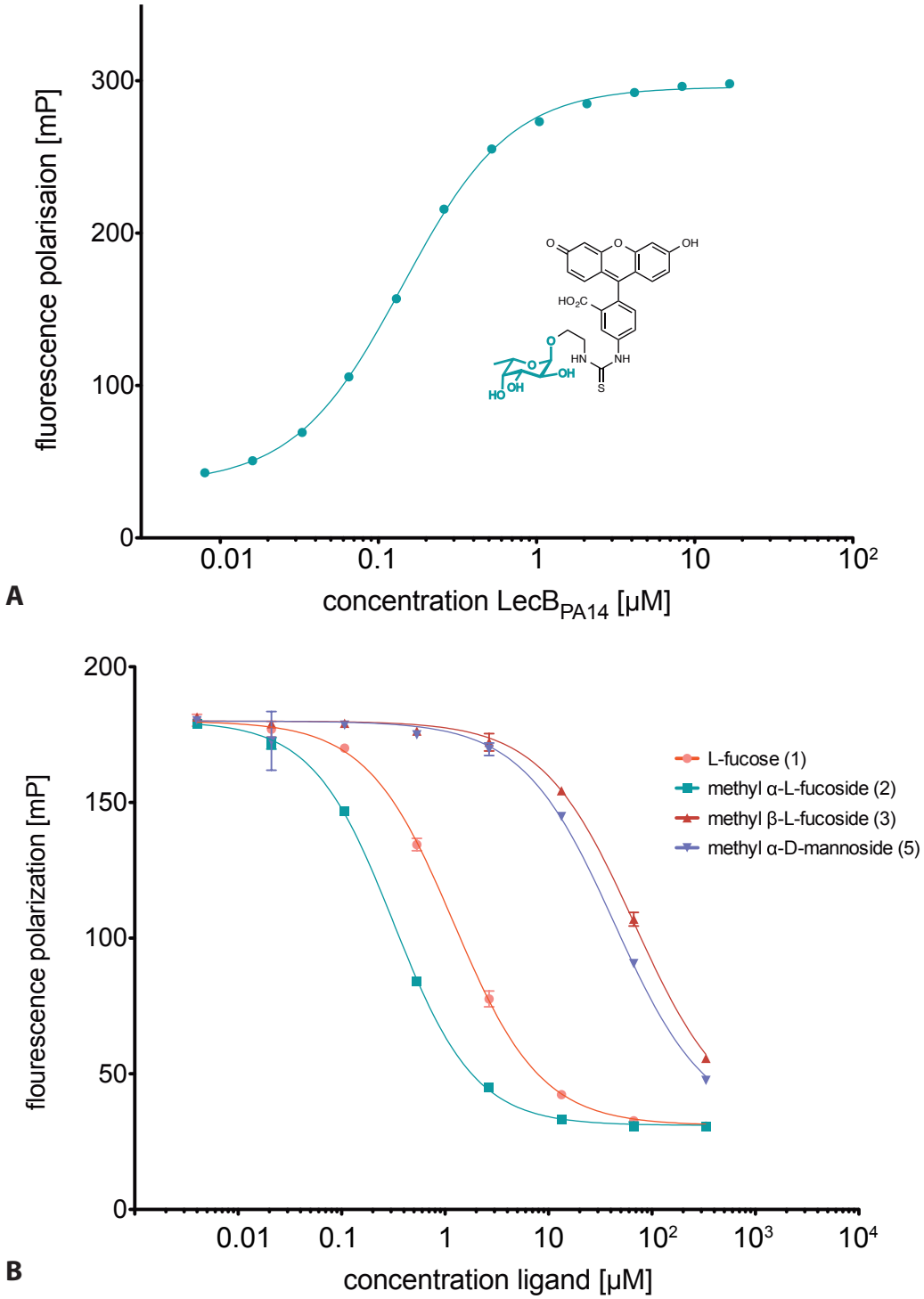
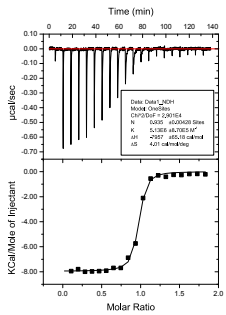
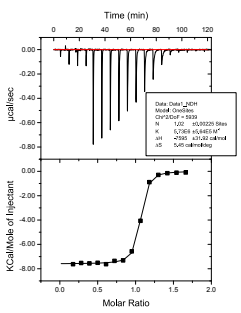
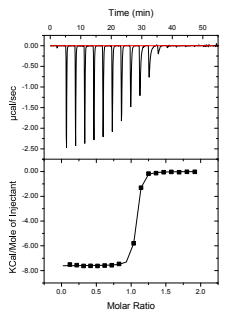
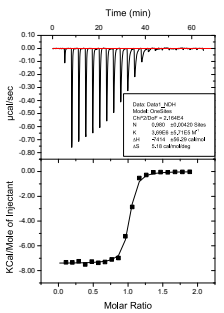
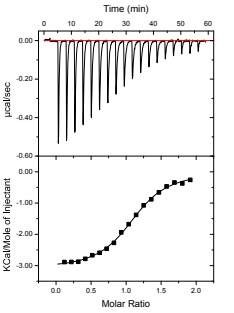
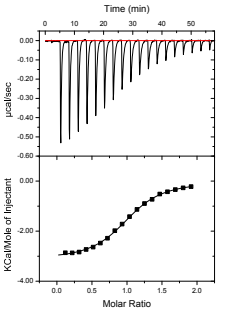
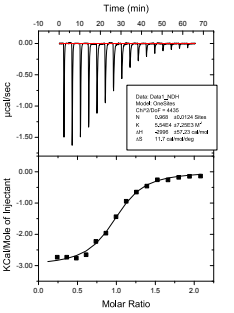


Figure S5: (A) Titration of fluorescein-labeled fucose-based reporter ligand N-(fluorescein-5-yl)-N'-(α-L-fucopyranosyl ethylen)-thiocarbamide (Hauck *et al*, ACS Chem. Biol., 2013) at 10 nM with LecB$_{PA14}$ and determination of the fluorescence polarization. (B) Competitive inhibition of the binding of N-(fluorescein-5-yl)-N'-(α-L-fucopyranosyl ethylen)-thiocarbamide (10 nM) to LecB$_{PA14}$ (100 nM) with various ligands. One representative titration of triplicates is shown and error bars shown here represent standard deviation of triplicates on one plate.

	LecB$_{PA14}$ 91 µM	LecB$_{PA14}$ 91 µM	LecB$_{PA14}$ 236 µM	LecB$_{PA14}$ 110 µM
	methyl α-L-fucoside (**2**) 800 µM	methyl α-L-fucoside (**2**) 800 µM	methyl α-L-fucoside (**2**) 2.3 mM	methyl α-L-fucoside (**2**) 1 mM
	TBS supplemented with 1 mM $CaCl_2$	TBS supplemented with 1 mM $CaCl_2$	TBS supplemented with 1 mM $CaCl_2$	TBS supplemented with 1 mM $CaCl_2$
methyl α-L-fucoside (2)				
Ka [1/M]	5,13E+06	5,73E+06	5,94E+06	3,69E+06
△H [cal/mol]	-7957	-7593	-7612	-7414
△s [cal/molK]	4,01	5,45	5,47	5,18
Kd [M]	1,95E-07	1,75E-07	1,68E-07	2,71E-07
N	0,935	1,02	1,03	0,980
△G [cal/mol]	-9,15E+03	-9,22E+03	-9,24E+03	-8,96E+03

	LecB$_{PA14}$ 236 µM	LecB$_{PA14}$ 236 µM	LecB$_{PA14}$ 499 µM
	methyl α-D-mannoside (**5**) 2.3 mM	methyl α-D-mannoside (**5**) 2.3 mM	methyl α-D-mannoside (**5**) 5.0 mM
	TBS supplemented with 1 mM $CaCl_2$	TBS supplemented with 1 mM $CaCl_2$	TBS supplemented with 1 mM $CaCl_2$
methyl α-D-mannoside (5)			
Ka [1/M]	7,18E+04	6,20E+04	5,54E+04
△H [cal/mol]	-3112	-3153	-2996
△s [cal/molK]	11,8	11,3	11,7
Kd [M]	1,39E-05	1,61E-05	1,81E-05
N	1,11	1,02	0,968
△G [cal/mol]	-6,63E+03	-6,52E+03	-6,48E+03

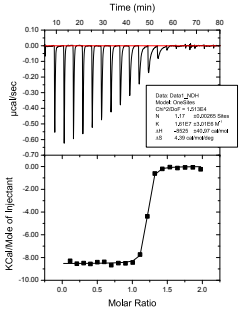
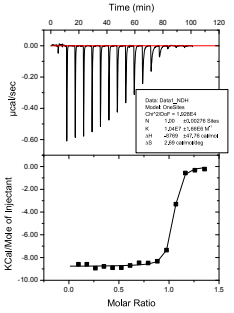
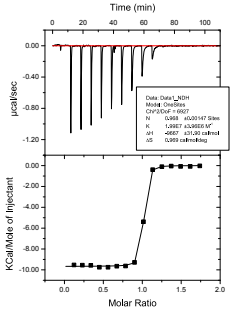
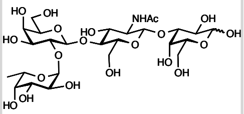
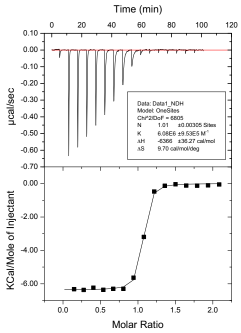
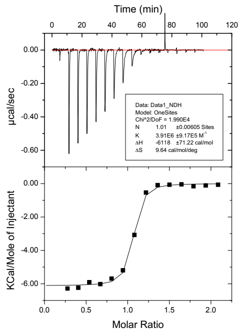
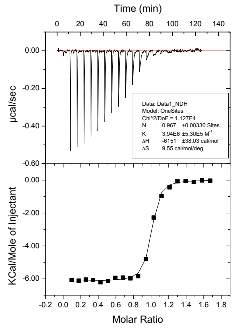
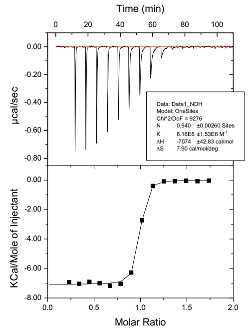
	LecB$_{PA14}$ 84.1 µM	LecB$_{PA14}$ 94 µM	LecB$_{PA14}$ 124 µM
	Lewis^a (**12**) 800 µM	Lewis^a (**12**) 650 µM	Lewis^a (**12**) 1.1 mM
	TBS supplemented with 1 mM $CaCl_2$	TBS supplemented with 1 mM $CaCl_2$	TBS supplemented with 1 mM $CaCl_2$
Lewis^a			
Ka [1/M]	1,61E+07	1,04E+07	1,99E+07
△H [cal/mol]	-8521	-8769	-9667
△s [cal/molK]	4,39	2,69	0,969
Kd [M]	6,21E-08	9,62E-08	5,03E-08
N	1,17	1,00	0,968
△G [cal/mol]	-9,83E+03	-9,57E+03	-9,96E+03

Figure S6: Isothermal microcalorimetry of LecB$_{PA14}$ with methyl α-L-fucoside (**2**), methyl α-D-mannoside (**5**) and with Lewis^a (**12**)

	LecB$_{PA14}$ 94 µM	LecB$_{PA14}$ 94 µM	LecB$_{PA14}$ 124 µM	LecB$_{PA14}$ 124 µM
	H-type II (**15**) 1 µM	H-type II (**15**) 1 µM	H-type II (**15**) 1 µM	H-type II (**15**) 1.1 µM
	TBS supplemented with 1 mM $CaCl_2$	TBS supplemented with 1 mM $CaCl_2$	TBS supplemented with 1 mM $CaCl_2$	TBS supplemented with 1 mM $CaCl_2$
 **H type II**				
Ka [1/M]	6.08E+06	3.91E+06	3.94E+06	8.16E+06
△H [cal/mol]	-6366	-6118	-6151	-7074
△s [cal/molK]	9.7	9.6	9.6	7.9
Kd [M]	1.64E-07	2.56E-07	2.54E-07	1.23E-07
N	1.01	1.01	0.97	0.94
△G [cal/mol]	-9258	-8992	-8998	-9429

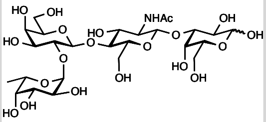
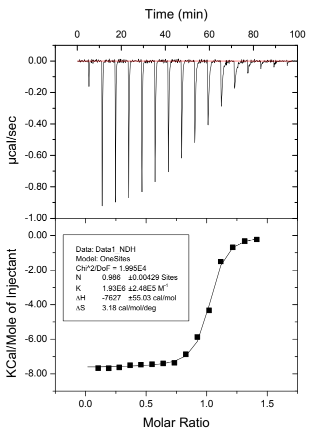
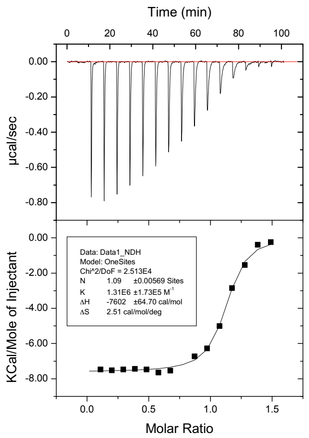
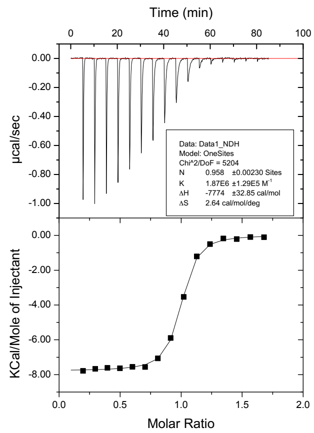
	LecB$_{PAO1}$ 139 µM	LecB$_{PAO1}$ 132 µM	LecB$_{PAO1}$ 124 µM
	H-type II (**15**) 1 µM	H-type II (**15**) 1 µM	H-type II (**15**) 1.3 µM
	TBS supplemented with 1 mM $CaCl_2$	TBS supplemented with 1 mM $CaCl_2$	TBS supplemented with 1 mM $CaCl_2$
 **H type II**			
Ka [1/M]	1.93E+06	1.31E+06	1.87E+06
△H [cal/mol]	-7627	-7602	-7774
△s [cal/molK]	3.2	2.5	2.6
Kd [M]	5.18E-07	7.63E-07	5.35E-07
N	0.99	1.09	0.96
△G [cal/mol]	-8575	-8350	-8561

Figure S7: Isothermal microcalorimetry of LecB$_{PA14}$ (top row) and LecB$_{PAO1}$ (bottom row) with H-type II antigen (**15**).

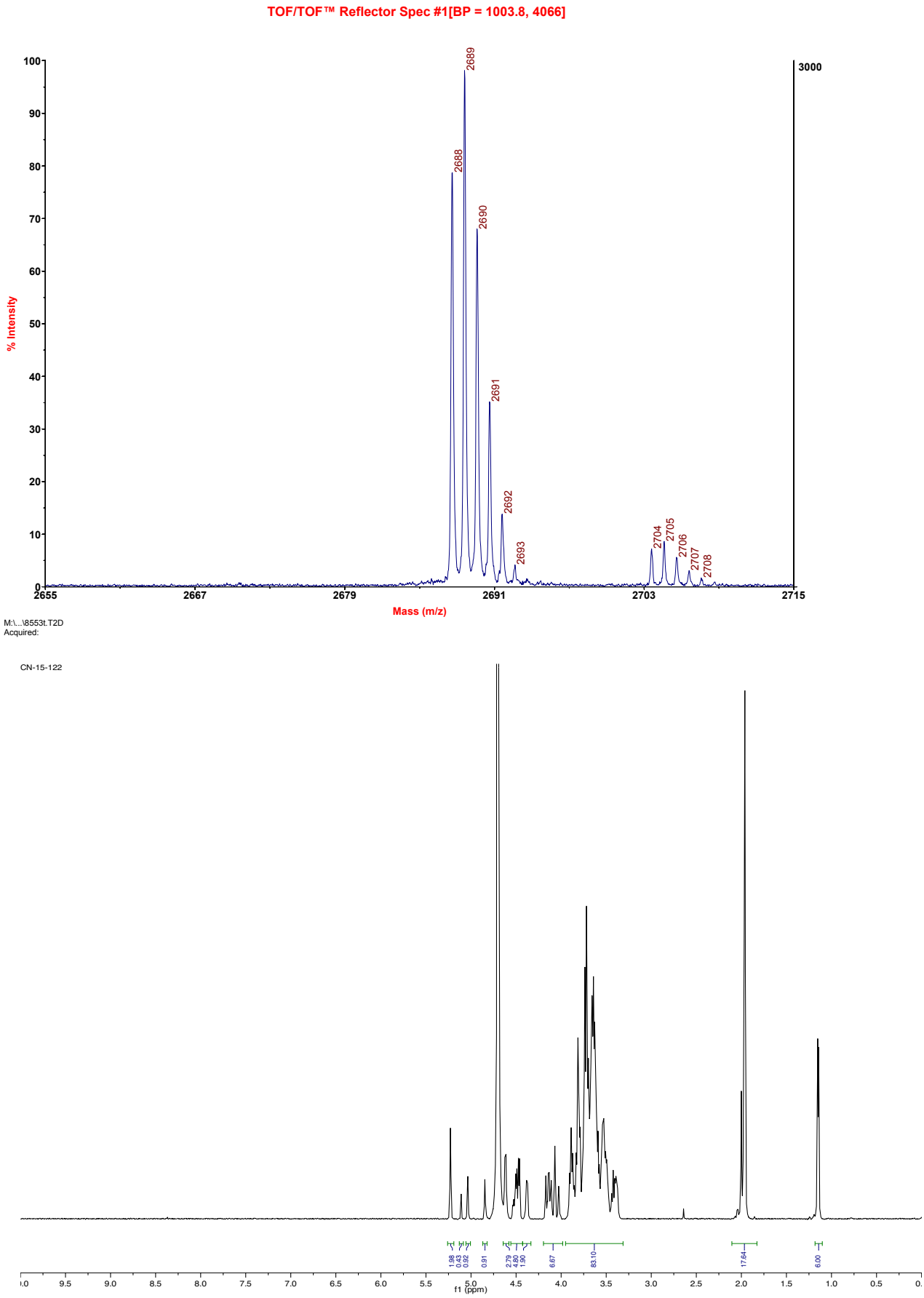


Figure S8: MALDI-MS (top) and 1H-NMR (bottom) of divalent N-glycan **23**.

Generic Display Report

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Comment			

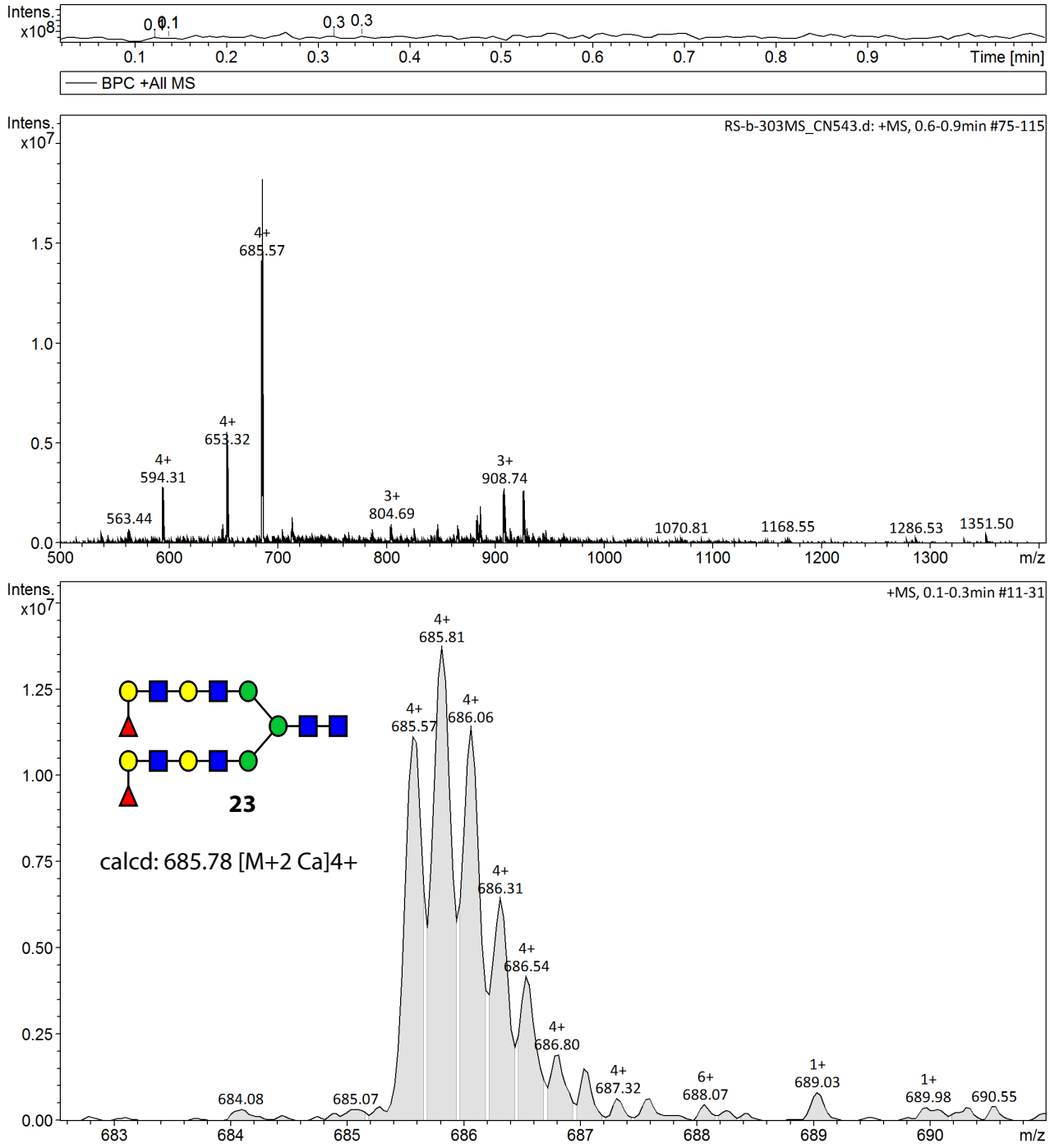

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Figure S9: ESI-MS of of divalent N-glycan **23** in presence of 300 μM $CaCl_2$.

Generic Display Report

Analysis Info

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Comment			

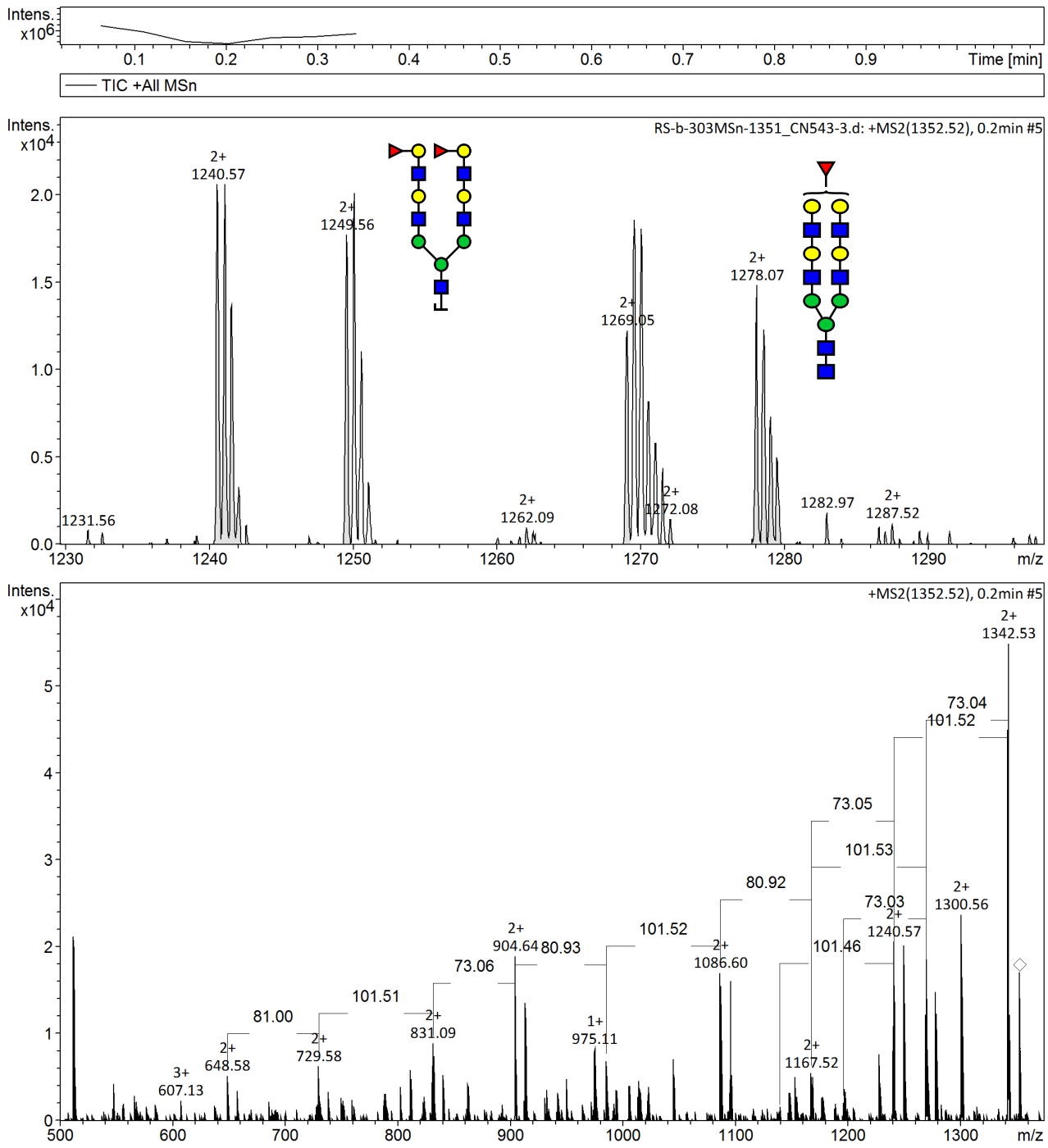

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Figure S10: ESI-MS^2 of divalent N-glycan **23** in presence of 300 μM $CaCl_2$. m/z 1342.5 corresponds to $[M\text{-}H_2O\text{+}Ca]^{2+}$, loss of Fuc (**Δ**m/z = 73), Hex (**Δ**m/z = 81) and GlcNAc (**Δ**m/z = 101.5).

Generic Display Report

Analysis Info

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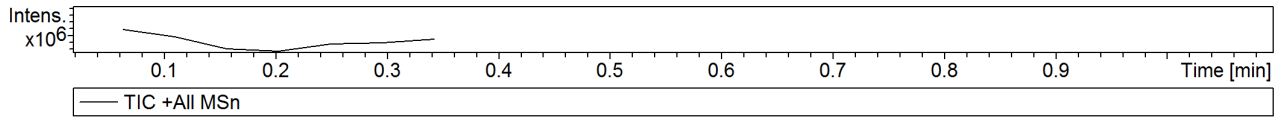
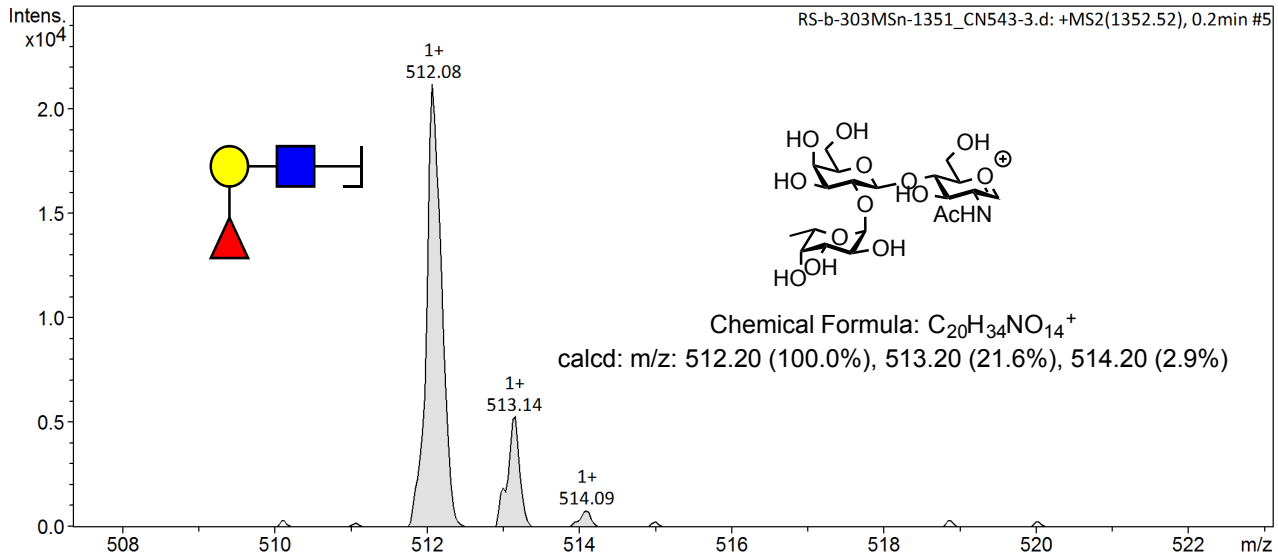
Sample Name	RS-b-303AutoMSN-CN543	Instrument	amaZon SL
Comment			





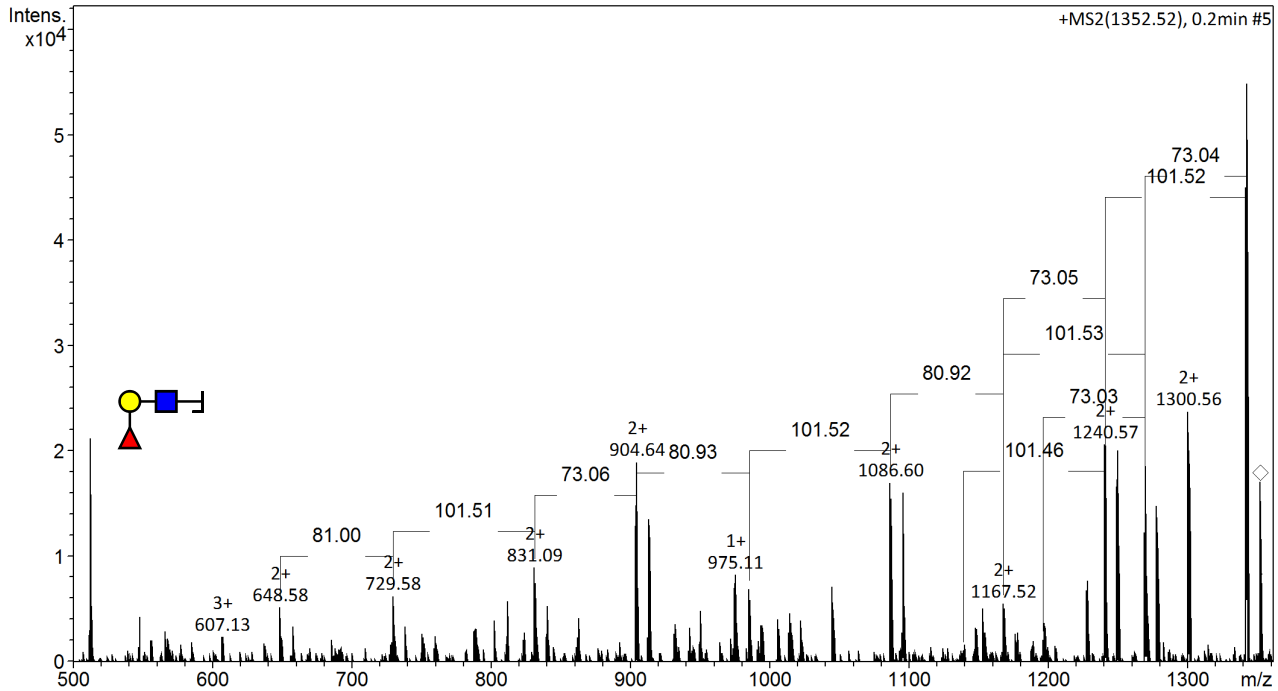


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Figure S11: ESI-MS^2 of divalent N-glycan **23** in presence of 300 μM $CaCl_2$. Mass peak at m/z = 512 is indicative for H-type antigen.

Table S1: Data Collection and Refinement Statistics for $LecB_{PA14}$ structures

Data-collection statistics																
Complex	**Native**				**Me-α-L-Fuc (2)**				**Man-α1,3-Man (11)**				**Lewis[a] (12)**			
Crystallization conditions	22% Peg8K, 0.2 M $AMSO_4$, 0.1 M Tris pH 8.5				26% Peg8K, 1 M $AMSO_4$, 0.1M Citric acid pH 3.8				24% Peg8K, 0.2 M $AMSO_4$, 0.1M Tris pH 8.5				22% Peg8K, 0.2 M $AMSO_4$, 0.1M Tris pH 8.5			
cryoprotectant	10% glycerol added				none				10% glycerol added				Increase Peg8K to 30%			
Beamline ESRF	BM30A				BM30A				ID23-1				BM30A			
Wavelength (Å)	0.92053				0.92053				0.8726				0.98021			
Spacegroup	$P2_1$				$P2_12_12_1$				$P2_1$				$P2_1$			
Unit cell: a,b,c (Å) α,β,γ (°)	52.9 49.6 75.5 90.0 93.4 90.0				52.5 65.6 109.0 90.0 90.0 90.0				53.0 49.8 75.3 90.0 93.3 90.0				53.0 63.8 64.5 90.0 91.2 90.0			
Resolution (outer shell), (Å)	41.42-1.7 (1.73-1.70)				37.84-1.55 (1.58-1.55)				44.52-1.4 (1.42-1.40)				27.82-1.50 (1.53-1.50)			
Measured/ Unique reflections	138777/43091				352300/55470				242799/76843				237721/66333			
Average multiplicity	3.2 (3.2)				6.4 (4.6)				3.2 (2.4)				3.6 (2.7)			
R_{merge}	0.079 (0.295)				0.067 (0.392)				0.037 (0.178)				0.028 (0.147)			
Completeness (%)	99.7 (99.0)				99.9 (98.5)				99.4 (94.9)				96.5 (72.4)			
Mean I / σI	10.1 (3.4)				20.9 (3.4)				21 (5.4)				26.9 (5.6)			
CC1/2	0.992 (0.831)				0.999 (0.867)				0.999 (0.931)				0.999 (0.959)			
R_{cryst}/ R_{free}	15.7/19.7				12.8/15.3				12.2/16.3				13.0/15.5			
nb reflections/free reflections	40973/2104				52637/2763				72808/4016				62930/3384			
R_{msd} bonds, (Å)	0.017				0.015				0.017				0.015			
R_{msd} angles (°)	1.60				1.64				1.80				1.71			
Rmsd chiral ($Å^3$)	0.099				0.10				0.113				0.095			
Chain	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Protein atoms Bfac ($Å^2$)	835 7.6	831 7.3	844 5.2	838 5.6	838 7.6	838 7.6	817 8.0	817 8.0	843 9.3	843 8.8	846 7.3	828 7.7	852 10.3	843 10.2	844 8.9	839 8.7
Calcium Bfac ($Å^2$)	2 13.1	2 13.4	2 4.4	2 5.2	2 5.5	2 5.9	2 6.6	2 6.8	2 8.9	2 9.0	2 5.3	2 5.6	2 9.9	2. 8.4	2 6.5	2 7.1
Ligand atoms Bfac ($Å^2$)	6 17.0	6 14.6	6 6.7	6 5.4	12 6.4	12 7.0	12 7.0	12 7.2	11 15.4	23 16.0	23 15.0	23 14.6	45 20.3	36 17.3	47 15.7	47 18.0
Water molecules Bfac ($Å^2$)	174 20.0	154 18.3	169 18.6	151 19.5	218 20.7	205 20.1	188 21.6	147 22.0	176 22.0	177 22.4	185 22.3	143 22.2	221 24.9	219 24.5	232 23.4	191 23.4
Ramachandran plot : Allowed /Favored /Outliers (%)	100 / 97.1 / 0				100 / 97.2 / 0				99.6 / 97.1 / 2				100 / 97.1 / 0			
PDBcode	5A6Q				5A6X				5A6Y				5A6Z			