SUPPLEMENTAL MATERIAL

SUPPLEMENTARY TABLE 1

Code number[1]	Oligosaccharide[2]	Purity[3]	Production method[4]	Starting material	Reference[5]
1	6',6''',6'''''-*tri*methyl-(1→4)-α-D-*hexa*galacturonic acid	>99%	Synthesis	D-galactose	Clausen *et al*.
2	6',6'',6''',6''''-*tetra*methyl-(1→4)-α-D-*hexa*galacturonic acid	>99%	Synthesis	D-galactose	Clausen *et al*.
3	6',6''''-*di*methyl-(1→4)-α-D-*hexa*galacturonic acid	>99%	Synthesis	D-galactose	Clausen *et al*.
4	6',6'',6'''-*tri*methyl-(1→4)-α-D-*hexa*galacturonic acid	>99%	Synthesis	D-galactose	Clausen *et al*.
5	6,6'''',6'''''-*tri*methyl-(1→4)-α-D-*hexa*galacturonic acid	>99%	Synthesis	D-galactose	Clausen *et al*.
6	(1→4)-α-D-*hexa*galacturonic acid	>99%	Synthesis	D-galactose	Clausen, M.H.
7	4''''''- (4,5-anhydro-α-D-galacturonyl)- (1→4)-α-D-*octa*galacturonic acid	>99%	Enz. F.&P.	Polygalacturonic acid, lime	Guillaumie *et al.*
8	(1→5)-α-L-arabinobiose	~95%	Enz. F.&P.	de-branched arabinan, sugar beet	Megazyme
9	(1→5)-α-L-arabinotriose	~95%	Enz. F.&P.	de-branched arabinan, sugar beet	Megazyme
10	(1→5)-α-L-arabinotetraose	~95%	Enz. F.&P.	de-branched arabinan, sugar beet	Megazyme
11	(1→5)-α-L-arabinopentaose	~95%	Enz. F.&P.	de-branched arabinan, sugar beet	Megazyme
12	(1→5)-α-L-arabinohexaose	~95%	Enz. F.&P.	de-branched arabinan, sugar beet	Megazyme
13	(1→5)-α-L-arabinoheptaose	~95%	Enz. F.&P.	de-branched arabinan, sugar beet	Megazyme
14	(1→5)-α-L--arabinooctaose	~95%	Enz. F.&P.	de-branched arabinan, sugar beet	Megazyme
15	2'-*E*-feruloylyl-α-(1→5)-L-arabinofuranobiose	>98%	Enz. F.&P.	Sugar beet pulp	Ralet *et al* 1994
16	2'-*E*-feruloylyl-α-(1→5)-L-arabinofuranotriose	>98%	Enz. F.&P.	Sugar beet pulp	Ralet *et al* 1994
17	D-galactose	>99%	na	na	Sigma-Aldrich
18	(1→4)-β-D-galactobiose	~90%	Enz. F.&P.	Galactan	Megazyme
19	(1→4)-β-D-galactotetraose	>99%	Synthesis	D-galactose	Clausen, M. H.
20	6'-α-D-galactosyl- (1→4)-β-D-galactotriose	>99%	Synthesis	D-galactose	Clausen, M. H.
21	6'-β-D-galactosyl- (1→4)-β-D-galactotriose	>99%	Synthesis	D-galactose	Clausen, M. H.
22	4',6'-α-D-*di*galactosyl- (1→4)-β-D-galactobiose	>99%	Synthesis	D-galactose	Clausen, M. H.
23	β-D-galactosyl-(1→4)-D-glucose (lactose, milk sugar)	>99%	na	na	Sigma-Aldrich
24	6'-*E*-feruloylyl- (1→4)-β-D-galactobiose	>98%	Enz. F.&P.	Sugar beet galactan	Ralet *et al* 1994
25	D-Mannose	>99%	na	na	Sigma-Aldrich

26	(1→4)-β-D-mannobiose	~95%	Enz. F.&P.	Mannan	Megazyme
27	(1→4)-β-D-mannotriose	~95%	Enz. F.&P.	Mannan	Megazyme
28	(1→4)-β-D-mannotetraose	~95%	Enz. F.&P.	Mannan	Megazyme
29	(1→4)-β-D-mannopentaose	>95%	Enz. F.&P.	Mannan	Megazyme
30	(1→4)-β-D-mannohexaose	>95%	Enz. F.&P.	Mannan	Megazyme
31	6-α-D-galactosyl- (1→4)-β-D-mannobiose	~95%	Enz. F.&P.	galactomannan, carob	Megazyme
32	6-α-D-galactosyl- (1→4)-β-D-mannotriose	>95%	Enz. F.&P.	galactomannan, carob	Megazyme
33	Galman VF	~95%	Enz. F.&P.	galactomannan, carob	Farkas, V.
34	6'',6'''-α-D-*di*galactosyl- (1→4)-β-D-mannopentaose	~95%	Enz. F.&P.	galactomannan, carob	Megazyme
35	Glcman 1	~95%	Enz. F.&P.	Konjac	Farkas, V.
36	Glcman 2	~95%	Enz. F.&P.	Barley	Farkas, V.
37	(1→4)-β-D-xylobiose	>95%	A.hyd. F.&P.	arabinoxylan	Megazyme
38	(1→4)-β-D-xylotriose	>95%	A.hyd. F.&P.	arabinoxylan	Megazyme
39	(1→4)-β-D-xylotetraose	>95%	A.hyd. F.&P.	arabinoxylan	Megazyme
40	(1→4)-β-D-xylopentaose	>95%	A.hyd. F.&P.	arabinoxylan	Megazyme
41	(1→4)-β-D-xylohexaose	>95%	A.hyd. F.&P.	arabinoxylan	Megazyme
42	D-glucoronyl-α-(1→2)-[(1→4)-β-D-xylotriose] (aldouronic acid)	~95%	Enz. F.&P.	arabinoxylan	Megazyme
43	L-arabinosyl-α-(1→2)-[(1→4)-β-D-xylobiose]	~95%	Enz. F.&P.	Xyloglucan	Megazyme
44	β-D-xylosyl-(1→6)-D-glucose (isoprimeverose)	~95%	Enz. F.&P.	Xyloglucan	Megazyme
45	β-X-(1→4)-β-X-(1→4)-β-X-(1→4)-G (xyloglucan heptamer)	~90%	Enz. F.&P.	Xyloglucan	Megaxyme
46	β-X-(1→4)-β-L-(1→4)-β-X-(1→4)-G (xyloglucan octamer)	~90%	Enz. F.&P.	Xyloglucan	Farkas & Maclachlan **1988**
47	β-L-(1→4)-β-L-(1→4)-β-X-(1→4)-G (xyloglucan nonamer)	~90%	Enz. F.&P.	Xyloglucan	Farkas & Maclachlan **1988**
48	XXXGXXXG	~80%	Enz. F.&P.	Xyloglucan	Megazyme
49	D-glucose	>99.5%	na	Biomass	Sigma-Aldrich
50	(1→4)-β-D-glucobiose (cellobiose)	≥98%	na	na	Sigma-Aldrich
51	(1→4)-β-D-glucotriose (cellotriose)	~95%	A.hyd. F.&P.	Cellulose acetate	Megazyme
52	(1→4)-β-D-glucotetraose (cellotetraose)	~95%	A.hyd. F.&P.	Cellulose acetate	Megazyme
53	(1→4)-β-D-glucopentaose (cellopentaose)	~94%	A.hyd. F.&P.	Cellulose acetate	Megazyme
54	(1→4)-β-D-glucohexaose (cellohexaose)	~94%	A.hyd. F.&P.	Cellulose acetate	Megazyme
55	(1→3)-β-D-glucobiose (laminaribiose)	~95%	A.hyd. F.&P.	Curdlan	Megazyme
56	(1→3)-β-D-glucotriose (laminaritriose)	~95%	A.hyd. F.&P.	Curdlan	Megazyme
57	(1→3)-β-D-glucotetraose (laminaritetraose)	~95%	A.hyd. F.&P.	Curdlan	Megazyme
58	(1→3)-β-D-glucopentaose (laminaripentaose)	~95%	A.hyd. F.&P.	Curdlan	Megazyme
59	(1→3)-β-D-glucohexaose (laminarihexaose)	~95%	A.hyd. F.&P.	Curdlan	Megazyme
60	3'-β-D-glucosyl- (1→4)-β-D-glucobiose	>95%	Enz. F.&P.	β-glucan (barley)	Megazyme
61	4'-β-D-glucosyl- (1→3)-β-D-glucobiose	95%	Enz. F.&P.	β-glucan (barley)	Megazyme
62	3'-β-D-glucosyl- (1→4)-β-D-glucotriose	~90%	Enz. F.&P.	β-glucan (barley)	Megazyme
63	3-β-[(1→4)-β-D-glucotriosyl]-D-glucose	95%	Enz. F.&P.	β-glucan (barley)	Megazyme
64	3'-β-[(1→4)-β-D-glucobiosyl]- (1→4)-β-D-glucobiose	~90%	Enz. F.&P.	β-glucan (barley)	Megazyme
65	Mlg5a	~90%	Enz. F.&P.	β-glucan (barley)	Megazyme

66	Mlg6a	~90%	Enz. F.&P.	β-glucan (barley)	Megazyme
67	(1→4)-α-D-glucobiose (maltose)	≥98%	na	Potato	Sigma-Aldrich
68	(1→4)-α-D-glucotriose (maltotriose)	95%	na	na	Sigma-Aldrich
69	(1→4)-α-D-glucohexaose (maltohexaose)	≥98%	na	na	Sigma-Aldrich
70	6’’-α-D-glucosyl- (1→4)-α-D-glucotriose	~95%	Enz. F.&P.	Pullulan	Megazyme
71	6’’-α-{6**-α-D-glucosyl-[(1→4)-α-D-glucotriosyl]}-(1→4)-α-D-glucotriose	~95%	Enz. F.&P.	Pullulan	Megazyme
72	6’,6’’’’-α-*di*-[(1→4)-α-D-glucobiosyl]- (1→4)-α-D-glucohexaose	>99%	Synthesis	D-glucose	Damager *et al*.
73	N-Acethyl-2-deoxy-2-amino-D-glucose	>95%	na	na	Sigma-Aldrich
74	N,N’-diacethyl-2,2’-dideoxy-2,2’-diamino- (1→4)-β-D-glucobiose	~95%	Acid hydrol/frac	Chitin	Megazyme
75	N,N’,N’’-triacethyl-2,2’,2’’-trideoxy-2,2’,2’’-triamino- (1→4)-β-D-glucotriose	~95%	Acid hydrol/frac	Chitin	Megazyme
76	N,N’,N’’,N’’’-tetraacethyl-2,2’,2’’,2’’’-tetradeoxy-2,2’,2’’,2’’’-tetraamino- (1→4)-β-D-glucotetraose	~95%	Acid hydrol/frac	Chitin	Megazyme
77	N,N’,N’’,N’’’,N’’’’-pentaacethyl-2,2’,2’’,2’’’,2’’’’-pentadeoxy-2,2’,2’’,2’’’,2’’’’-pentaamino- (1→4)-β-D-glucopentaose	~95%	Acid hydrol/frac	Chitin	Megazyme
78	N,N’,N’’,N’’’,N’’’’,N’’’’’-hexaacethyl-2,2’,2’’,2’’’,2’’’’,2’’’’’-hexadeoxy-2,2’,2’’,2’’’,2’’’’,2’’’’’-hexaamino- (1→4)-β-D-glucohexaose	~95%	Acid hydrol/frac	Chitin	Megazyme

[1] Oligosaccharides are numbered according to figure 2. [2] Oligosaccharide name refers to the structure of the unconjugated glycan i.e. the reducing end sugar residue is intact. [3] Purities are determined by the suppliers and are approximate percentages. Production methods: Synthesis, chemical synthesis from monosaccharides; Enz. F.&P, enzymatic digestion followed by fractionation and purification; A.hyd. F.&P., acidic hydrolysis followed by fractionation and purification; na, data not available.

SUPPLEMENTARY TABLE 2

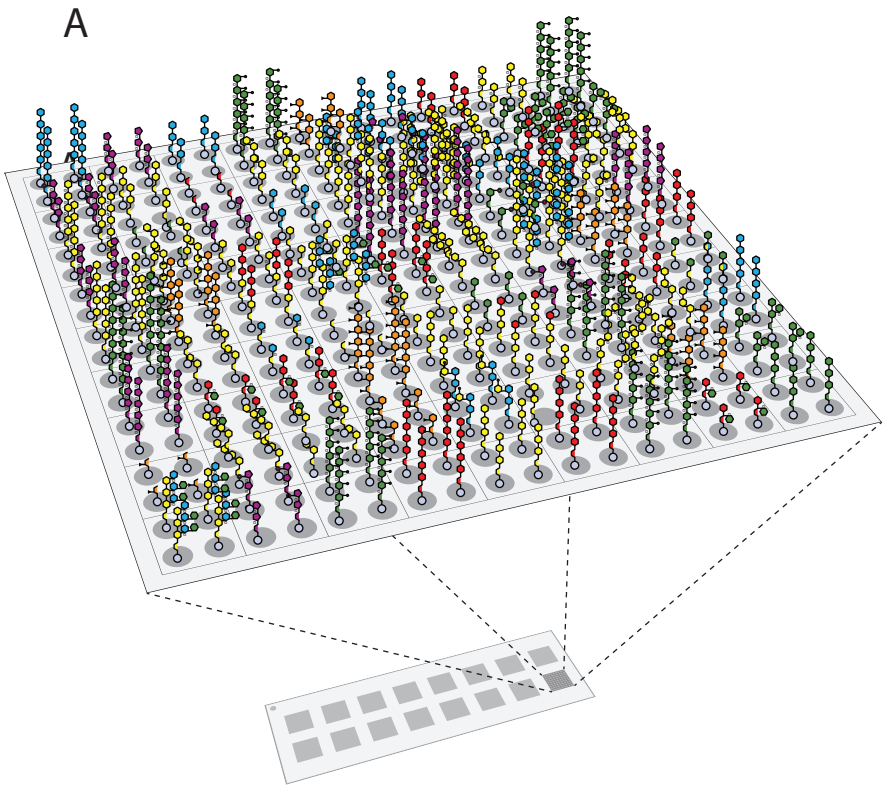
Conjugate no (see **Table S1**)	Mass of the oligosaccharide (g/mol)	Mass of the conjugate (m/*z*)	Average no. of residues on BSA
42	511.23	69,078.9	4.67
10	546.56	71070.6	8.01
14	1075,20	73099.1	5.96
52	666.58	74843.4	12.23
54	828.72	79909.4	15.95
76	830.80	69035.6	2.82
18	342.30	69567.7	8.40
32	666.58	71927.0	7.85
57	666.58	67798.9	1.66
67	342.30	71672.3	14.54
-	513.23	70577.7	7,57
70	666.58	73033.4	9.51
69	828.72	78212.2	13.90
72	1639,44	69938.9	1.98
63	666.58	69037.1	3.52
28	666.58	72060.6	8.05
30	828.72	73395.0	8.09
39	546.56	72614.9	10.83
45	1061.92	82198.2	14.60
48	2105.82	68557,7	0.89*

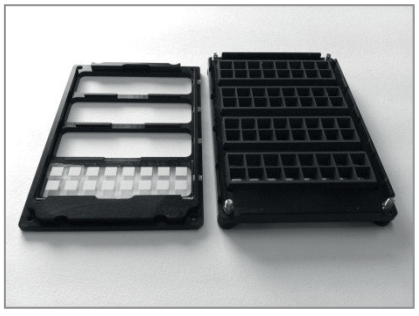
SUPPLEMENTARY FIGURE LEGENDS

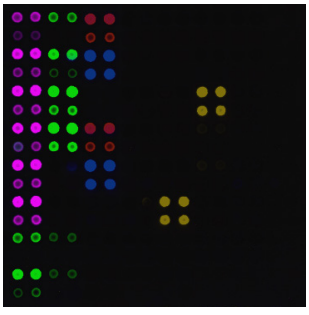
SUPPLEMENTARY FIGURE 1. Microarray layout and handling. (**A**) A typical oligosaccharide microarray printed using a piezoelectric robot onto a 16-pad nitrocellulose-coated glass slide. Each of the 16 pads is 6 x 6 mm and can accommodate approximately at least 324 spots. Typically, each oligosaccharide is represented by 4 spots (two replicates of two concentrations). (**B**) Multi-chamber incubation equipment is crucial for high throughput analysis. In this example, 64 arrays can be probed separately and simultaneously. (**C**) A composite image showing five identical arrays with the layout shown in (**A**) and probed with five monoclonal antibodies (mAbs): mAb LM6 (anti-(1→5)-α-L-arabinan); mAb LM10 (anti-(1→4)-β-D-xylan); mAb LM15 (anti-xyloglucan); mAb LM24 (anti-xyloglucan); mAb BS-400-2 (anti-(1→3)-β-D-glucan).

SUPPLEMENTARY FIGURE 2. Competitive inhibition studies. The binding of selected monoclonal antibodies (mAbs) and carbohydrate binding modules (CBMs) to selected oligosaccharides was tested by competitive inhibition binding studies in which micoarrays were probed with in the presence of un-conjugated oligosaccharides. (**A**) Examples of competitive microarray assays. Binding of the xylan-binding CBM4-2 and the anti-AGP mAb LM14 to immobilized glycan were inhibited by 2 mg-mL (1→4)-β-D-glucotetraose (structure 52) and glucoronyl- (1→2)-α-[(1→4)-β-D-xylotriose] (Structure 42) respectively, whilst LM14 binding was not inhibited by (1→4)-β-D-xylotetraose. (**B**) Heatmap showing the inhibitory effects of several haptens used from 0 to 10 mg/mL on the binding of mAbs LM14, LM21 (anti-mannan) and LM22 (anti-mannan/galactomannan). The structures of haptens and immobilised glycans are as listed in **Supplementary Table 1.**

Supplemental Figure S1

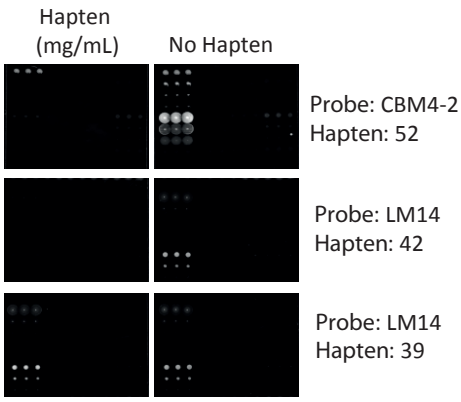






Supplemental Figure S2

A



B

Hapten concentraction (mg/ml)					Hapten	Immob. glycan	Probe
10	2	0,4	0,08	0			
76	88	100	93	90	39	42	LM14
5	6	34	65	90	42	42	LM14
19	18	31	39	59	30	30	LM21
24	30	43	55	76	32	30	LM21
21	30	57	73	85	34	32	LM22
9	13	16	22	48	32	32	LM22
25	37	56	68	97	28	32	LM22
22	33	45	62	94	30	32	LM22
1	1	1	2	70	41	39	CBM4-2
0	0	3	13	70	41	62	CBM4-2