

Supplementary information

to

Discovery and structural characterization of fucosylated oligomannosidic N-glycans in mushrooms

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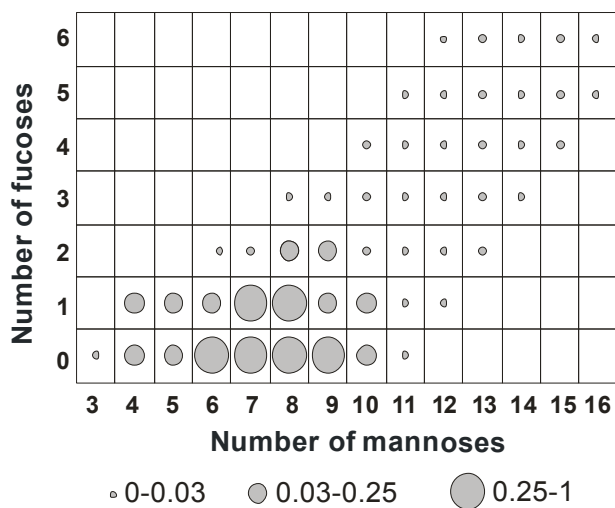
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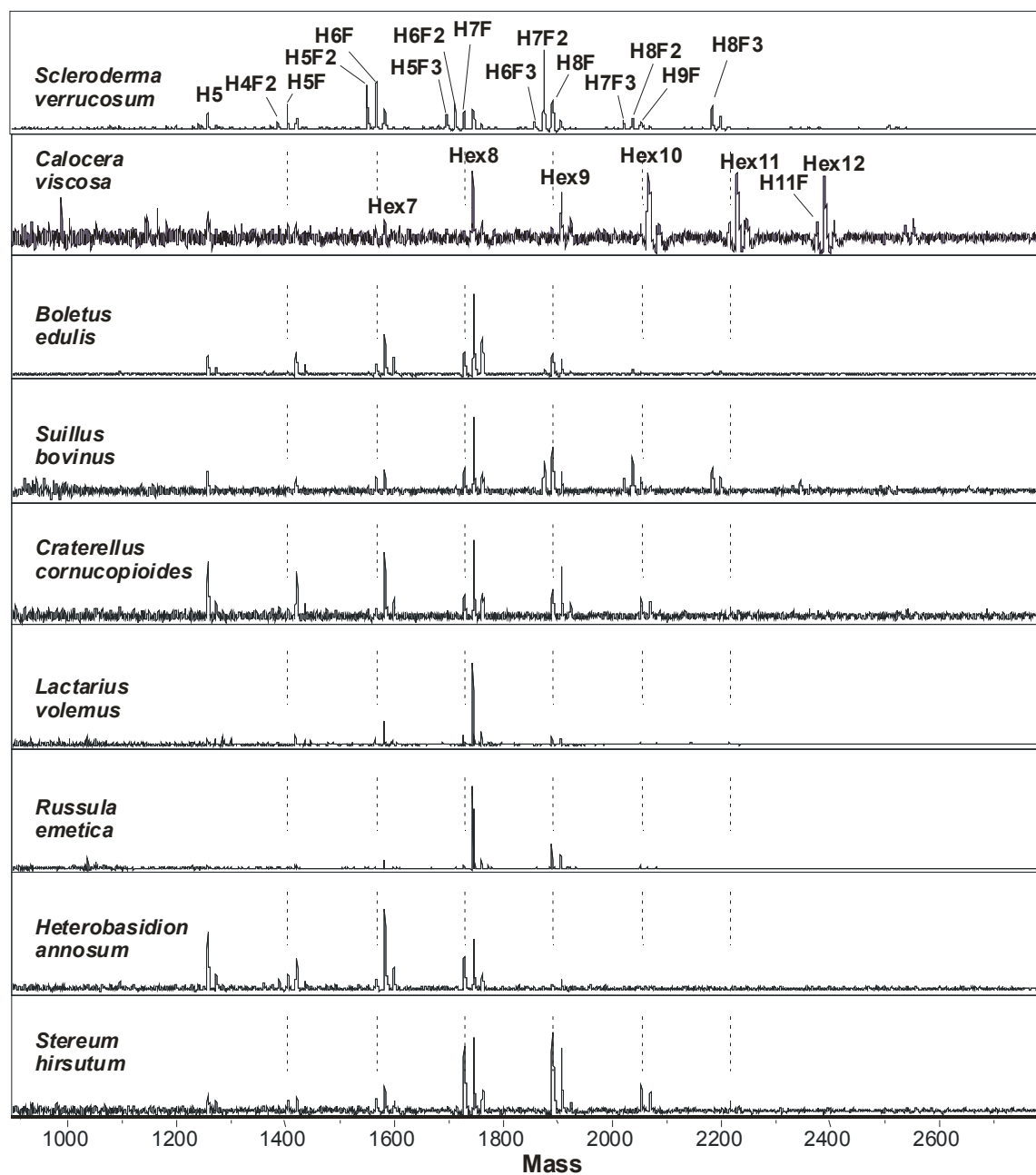
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Supplementary Figures:

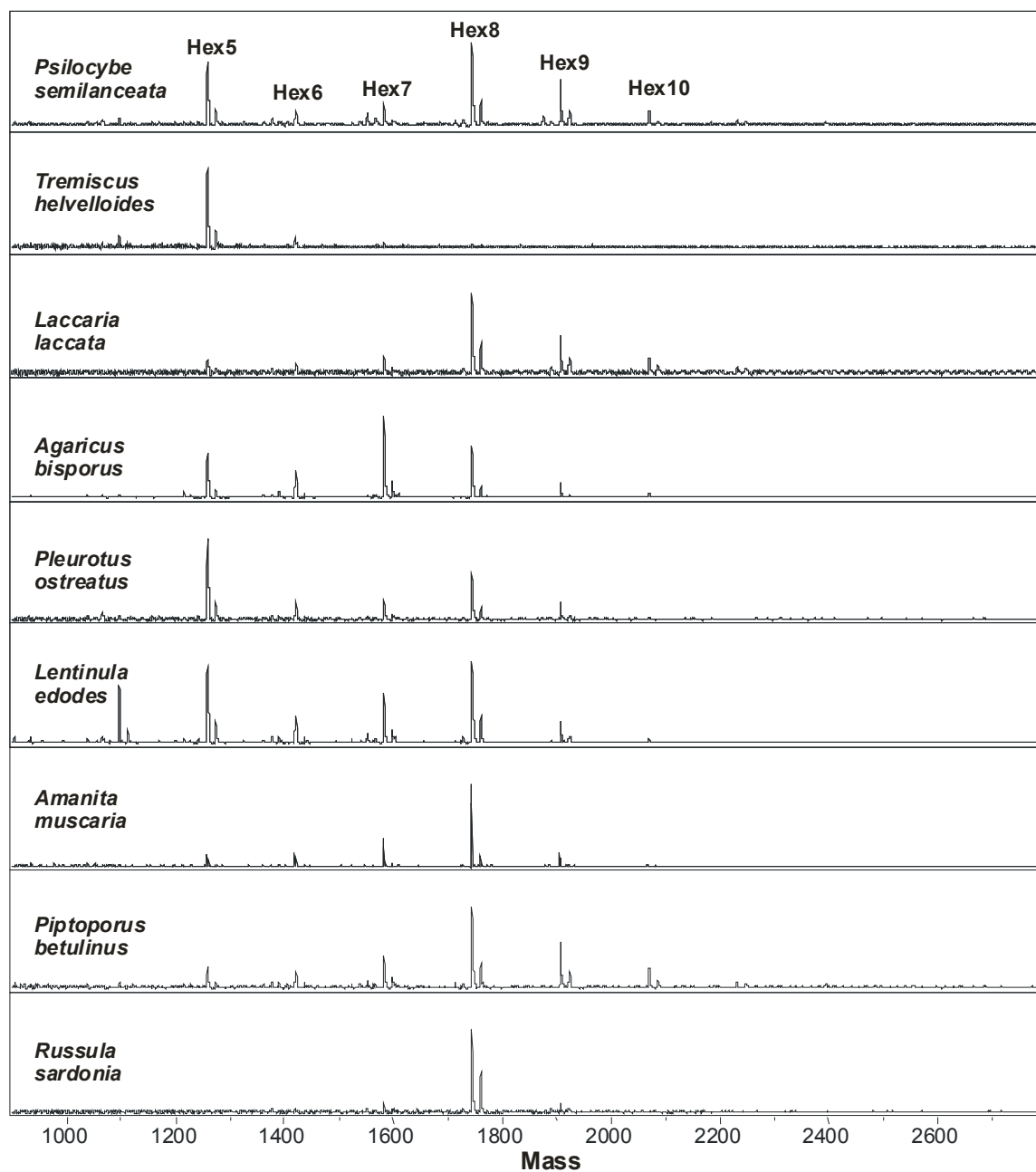
Supplemental Fig. 1: The plot depicts the correlation of the number of mannose and fucose residues. The spot size indicates the relative peak height in the MALDI spectrum



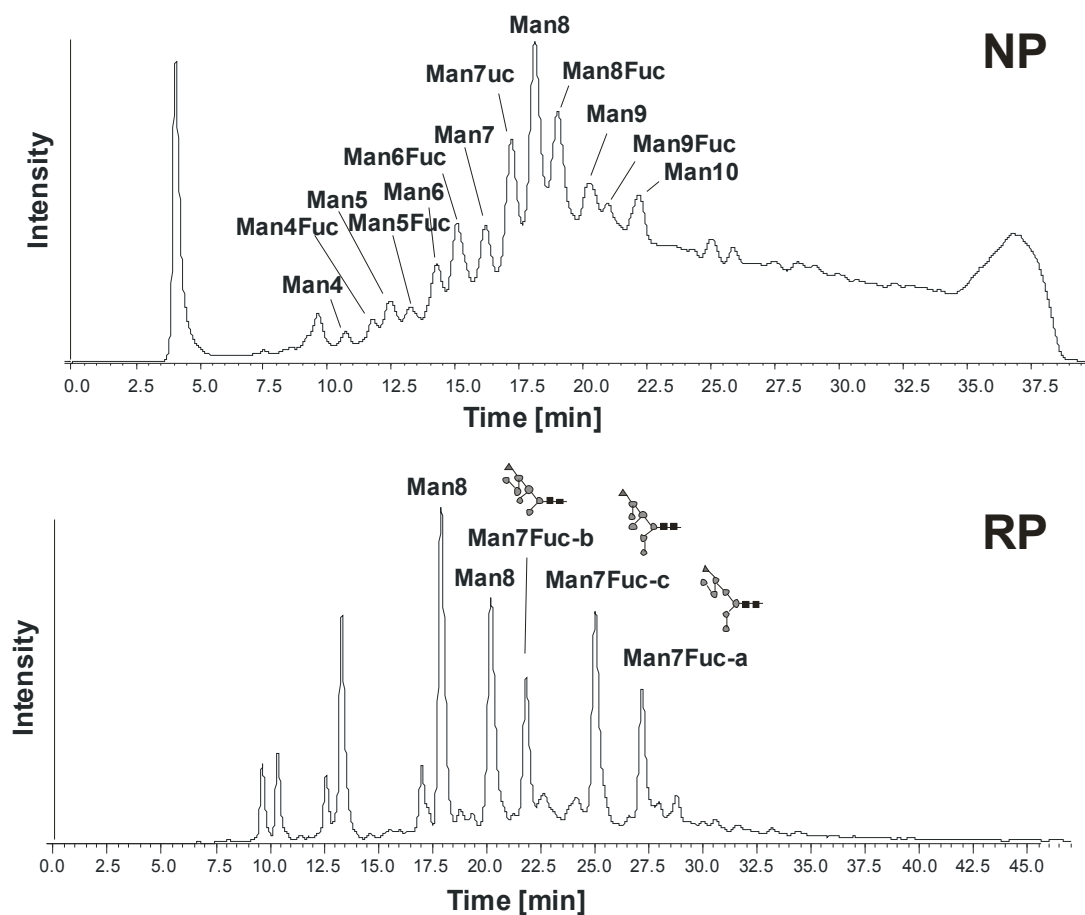
Supplemental Fig. 2: MALDI-TOF-MS spectra for mushrooms species containing fucosylated N-glycans. Hex (or H for brevity) stands for hexose or *bona fide* mannose, F for fucose residues. The dashed lines delineate the positions of glycans with one fucose residue.



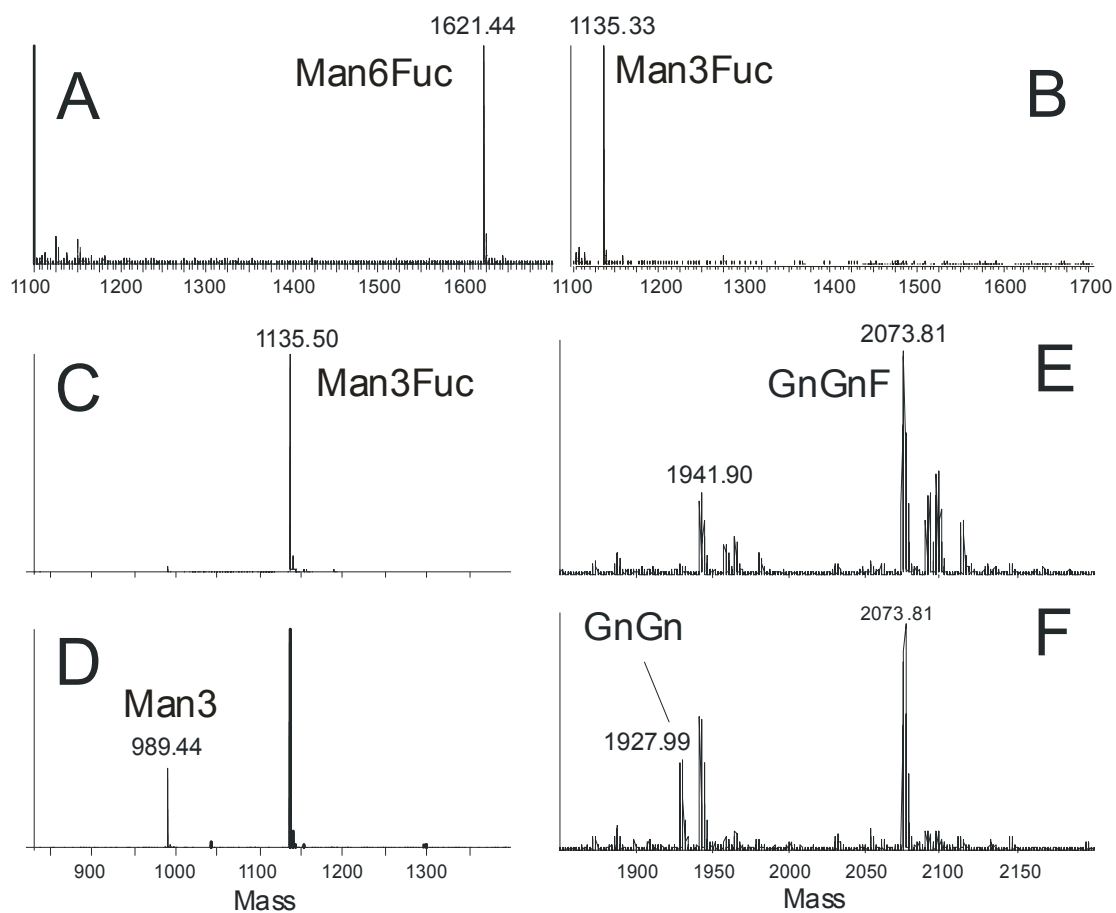
Supplemental Fig. 3: MALDI-TOF-MS spectra for mushrooms species containing non-fucosylated N-glycans only.



Supplemental Fig. 4: Preparation of individual glycan structures. PA-labelled glycans from 5 kg of chanterelles were first fractionated by normal-phase HPLC on an amide-silica column. Fractions were analyzed by mass spectrometry and those containing fucosylated N-glycans were further separated by RP-HPLC. Panel **A** shows a typical normal-phase chromatogram. Panel **B** depicts the preparation of the Man7Fuc isomers by RP-HPLC.



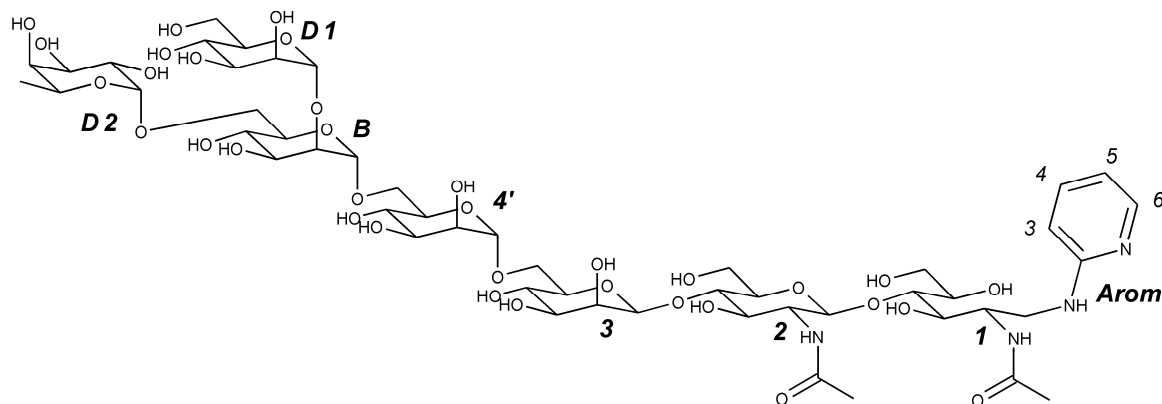
Supplemental Fig. 5: Glycosidase digestions of Man6Fuc. Panels **A** and **B** show the spectra of Man6Fuc before and after digestion with α -mannosidase, which generated Man3Fuc as the smallest product. The thereby obtained Man3Fuc was treated with bovine kidney α -fucosidase. Panels **C** and **D** depict the results of digestion of Man3Fuc with bovine kidney α -fucosidase. Panels **E** (control) and **F** (digested sample) show the effect of a five-fold lower dose of fucosidase on the favourable substrate IgG glycopeptide Ser-(GnGnF⁶-)Asn



Supplementary NMR data

^1H NMR chemical shifts (ppm) of isolated oligosaccharides

1.) Man4Fuc

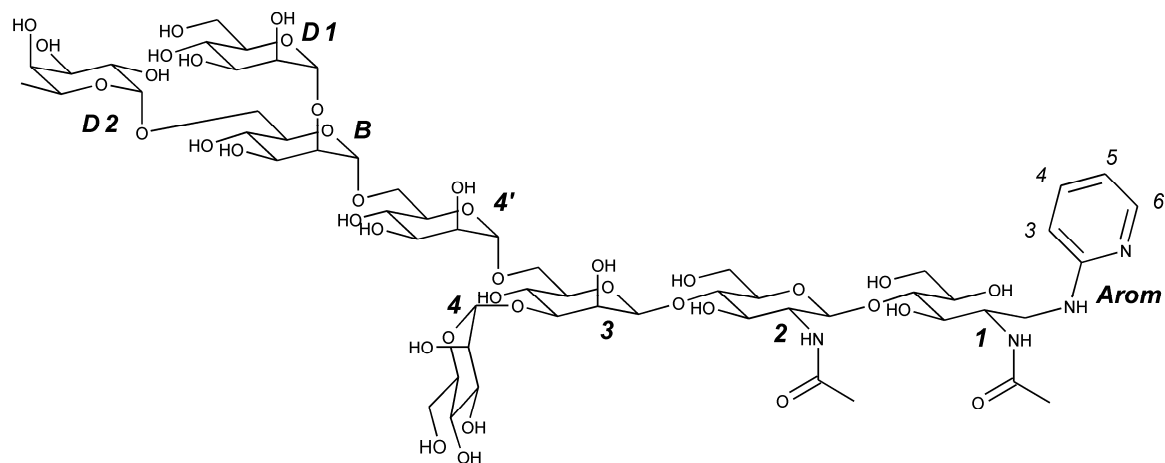


unit	pos. 1	pos. 2	pos. 3	pos. 4	pos. 5	pos. 6a	pos. 6b	Ac
arom.	-	-	6.61	7.52	6.64	7.81	-	-
1	3.74 & 3.64	4.35	3.91	3.85	3.91	3.82	3.75	1.95
2	4.64	3.75	3.80	3.74	3.52	3.83	3.76	2.07
3*	nd	nd	nd	nd	nd	nd	nd	-
4'	4.89	3.94	3.89	nd	nd	4.00	3.69	-
B	5.14	4.02	3.88	nd	nd	3.80	3.75	-
D1	5.04	4.06	3.80	nd	nd	3.87	3.73	-
D2	4.92	3.78	3.84	3.77	4.14	1.21	-	-

nd not determined

* only determined for Man5Fuc and Man6Fuc at 308 K

2.) Man5Fuc

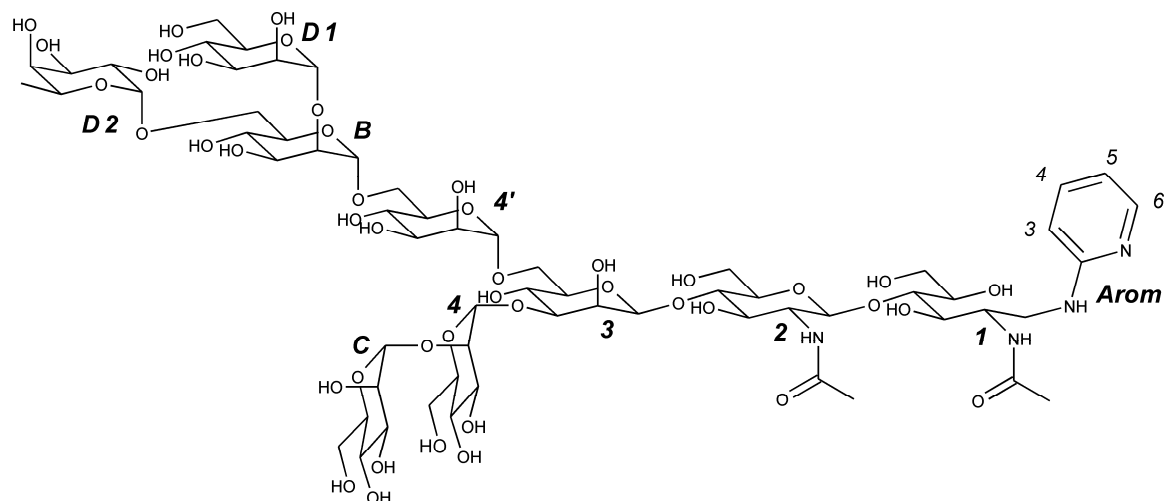


unit	pos. 1	pos. 2	pos. 3	pos. 4	pos. 5	pos. 6a	pos. 6b	Ac
arom.	-	-	6.67	7.57	6.74	7.98	-	-
1	3.74 & 3.64	4.35	3.91	3.85	3.91	3.82	3.75	1.94
2	4.64	3.76	3.80	3.74	3.52	3.83	3.76	2.06
3*	4.77	4.10	3.78	3.72	3.64	3.92	3.86	-
4	5.10	4.06	3.79	nd	nd	3.81	3.70	-
4'	4.92	3.95	3.85	nd	nd	3.98	3.64	-
B	5.15	4.05	3.88	nd	nd	3.85	3.75	-
D1	5.04	4.06	3.79	nd	nd	3.85	3.71	-
D2	4.92	3.80	3.84	3.78	4.15	1.21	-	-

nd not determined

* at 308 K

3.) Man6Fuc

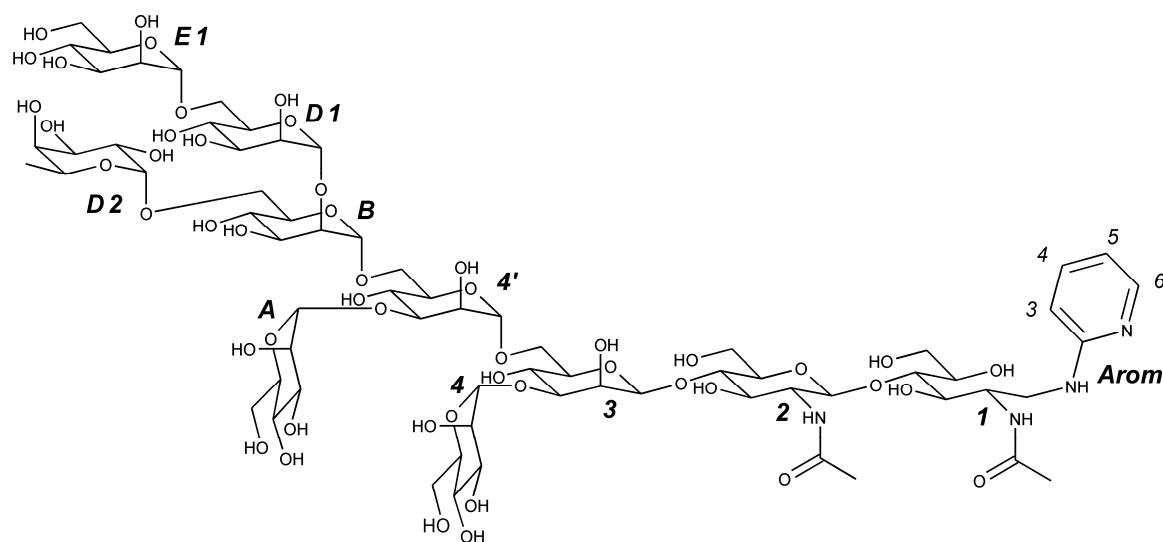


unit	pos. 1	pos. 2	pos. 3	pos. 4	pos. 5	pos. 6a	pos. 6b	Ac
arom.	-	-	6.67	7.56	6.73	7.98	-	-
1	3.74 & 3.64	4.35	3.91	3.85	3.91	3.82	3.75	1.94
2	4.64	3.77	3.81	3.74	3.52	3.83	3.76	2.05
3*	4.79	4.14	3.73	3.70	3.65	3.93	3.83	-
4	5.35	4.10	4.01	3.67	3.74	3.85	3.75	-
4'	4.92	3.98	3.88	3.68	nd	3.95	3.66	-
B	5.13	4.03	3.90	nd	nd	3.95	3.66	-
C	5.06	4.02	3.80	nd	nd	3.82	3.68	-
D1	5.05	4.07	3.78			3.88	3.70	-
D2	4.92	3.79	3.84	3.78	4.15	1.22	-	-

nd not determined

* at 308 K

4.) Man7Fuc-a

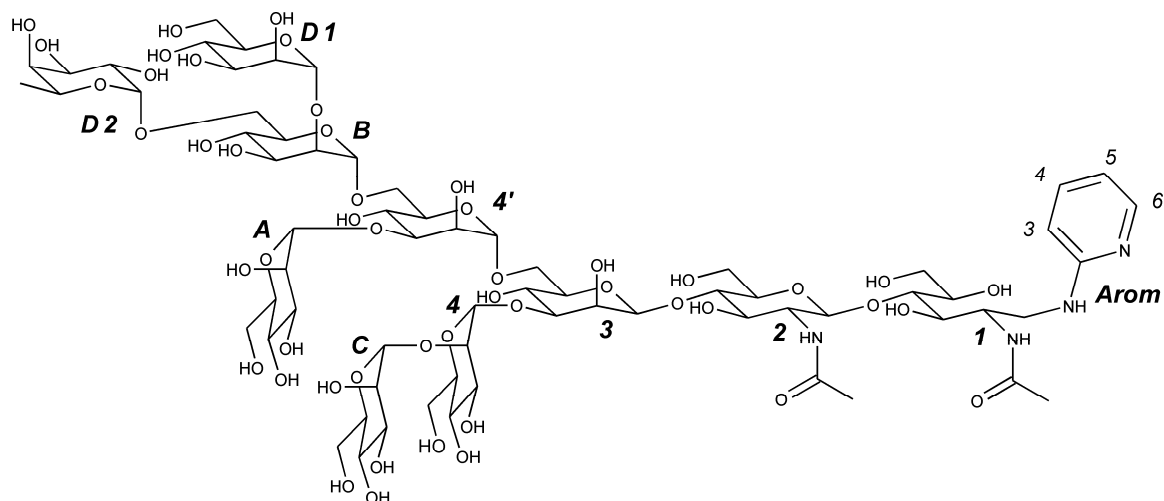


unit	pos. 1	pos. 2	pos. 3	pos. 4	pos. 5	pos. 6a	pos. 6b	Ac
arom.	-	-	6.73	7.64	6.76	7.95	-	-
1	3.74 & 3.64	4.34	3.91	3.85	3.91	3.82	3.75	1.93
2	4.63	3.76	3.80	3.72	3.51	3.83	3.76	2.06
3*	nd	nd	nd	nd	nd	nd	nd	-
4	5.10	4.04	3.80	nd	nd	3.80	3.70	-
4'	4.88	4.00	3.82	nd	nd	4.02	3.70	-
A	5.09	3.93	3.84	nd	nd	nd	nd	-
B	5.14	3.95	3.90	nd	nd	3.90	3.70	-
D1	5.05	4.05	3.80	nd	nd	3.95	3.75	-
D2	4.92	3.79	3.84	3.77	4.13	1.24	-	-
E1	4.90	3.95	3.93	3.62	3.70	3.85	3.70	-

nd not determined

* only determined for Man5Fuc and Man6Fuc at 308 K

5.) Man7Fuc-b

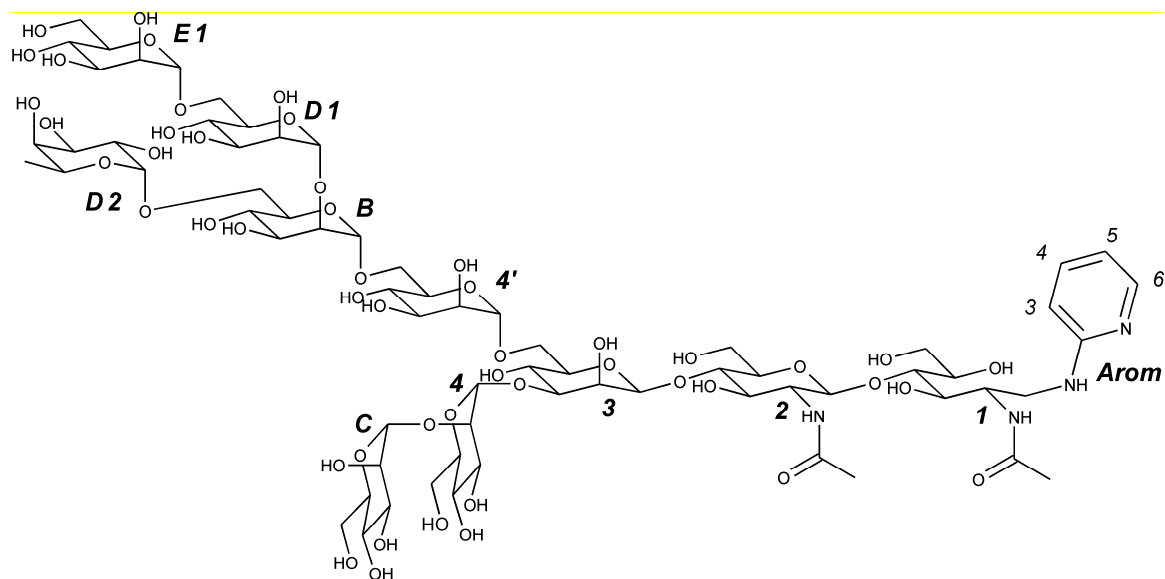


unit	pos. 1	pos. 2	pos. 3	pos. 4	pos. 5	pos. 6a	pos. 6b	Ac
arom.	-	-	6.73	7.66	6.77	7.95	-	-
1	3.74 & 3.64	4.34	3.91	3.85	3.91	3.82	3.75	1.94
2	4.63	3.74	3.80	3.73	3.51	3.83	3.76	2.05
3*	nd	nd	nd	nd	nd	nd	nd	-
4	5.35	4.09	4.02	3.67	3.69	3.85	3.72	-
4'	4.86	3.95	3.83	nd	nd	4.00	3.60	-
A	5.08	3.91	3.87	nd	nd	nd	nd	-
B	5.14	3.95	3.90	nd	nd	3.90	3.70	-
C	5.06	3.98	3.82	nd	nd	3.82	3.69	-
D1	5.04	4.07	3.79	nd	nd	3.89	3.70	-
D2	4.92	3.81	3.86	3.90	4.14	1.24	-	-

nd not determined

* only determined for Man5Fuc and Man6Fuc at 308 K

6.) Man7Fuc-c



unit	pos. 1	pos. 2	pos. 3	pos. 4	pos. 5	pos. 6a	pos. 6b	Ac
arom.	-	-	6.73	7.64	6.76	7.95	-	-
1	3.74 & 3.64	4.35	3.91	3.85	3.91	3.82	3.75	1.94
2	4.64	3.75	3.80	3.74	3.52	3.83	3.76	2.07
3*	nd	nd	nd	nd	nd	nd	nd	-
4	5.36	4.11	4.00	3.68	3.72	3.88	3.73	-
4'	4.89	3.96	3.90	nd	nd	3.98	3.66	-
B	5.13	3.98	3.88	nd	nd	3.95	3.69	-
C	5.06	3.99	3.82	nd	nd	3.82	3.68	-
D1	5.05	4.08	3.78	nd	nd	3.93	3.74	-
D2	4.92	3.78	3.84	3.78	4.12	1.24	-	-
E1	4.91	3.95	3.93	3.60	3.70	3.85	3.70	-

nd not determined

* only determined for Man5Fuc and Man6Fuc at 308 K