Anthranilic Acid (AA) as a Versatile Fluorescent Tag and Linker for Functional Glycomics

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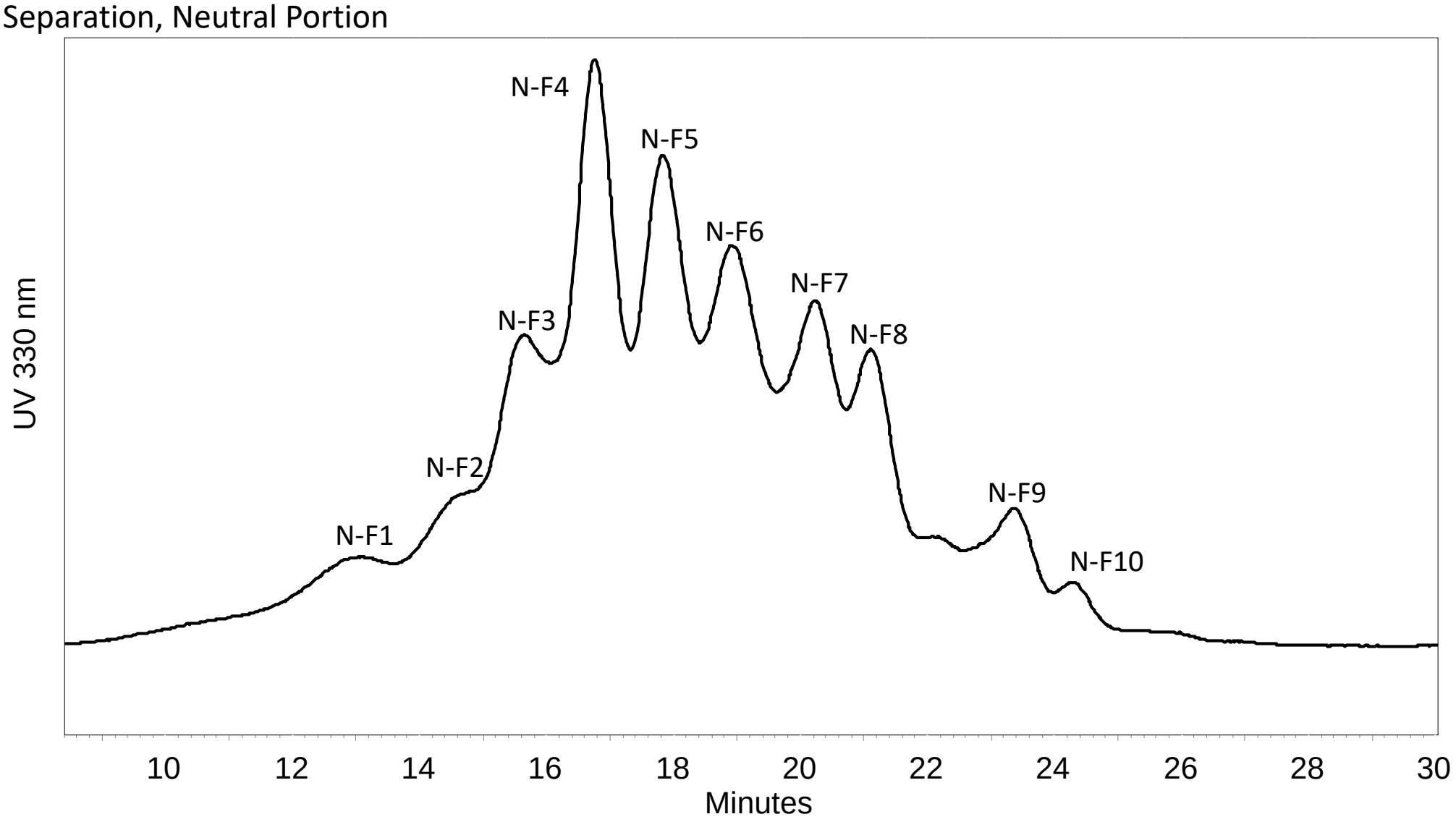
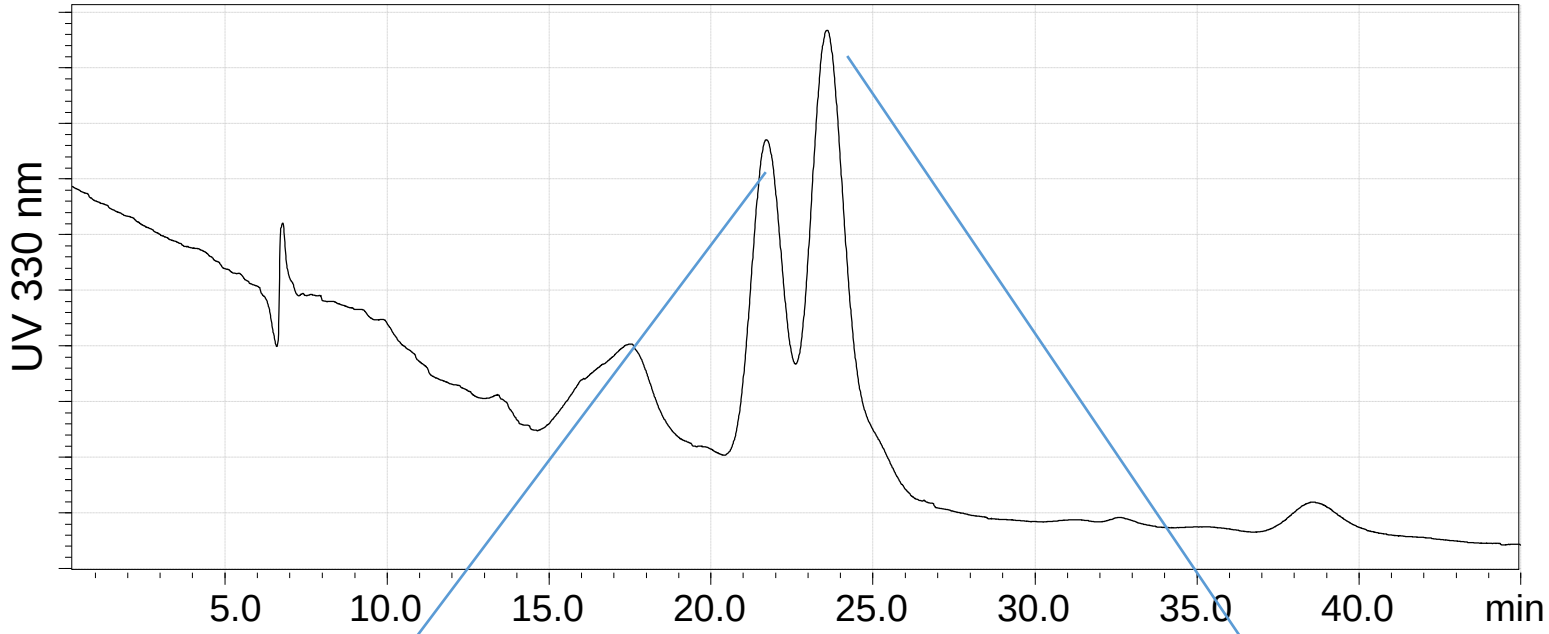
Supporting Information

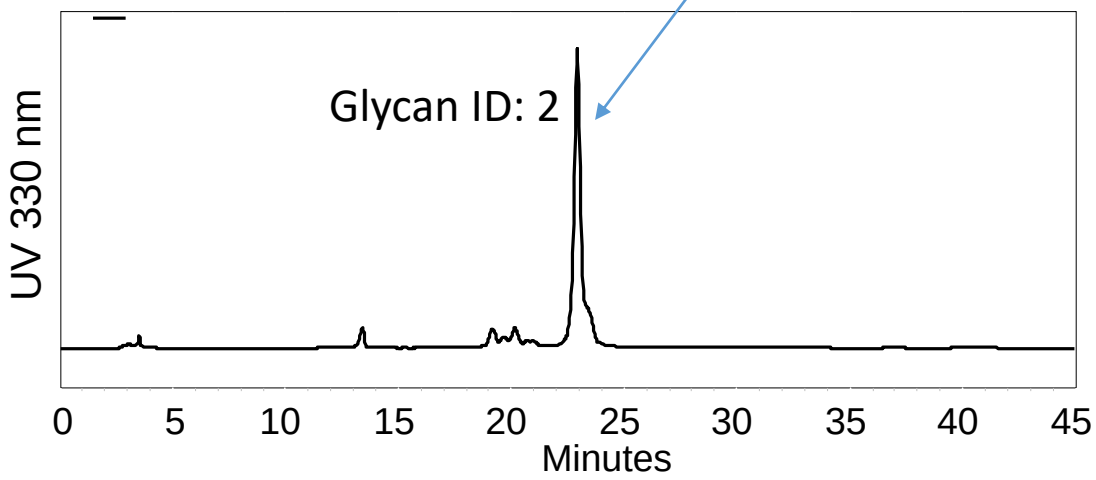
Figure S1: Summary of HPLC profiles of chicken egg N-glycan AA conjugates

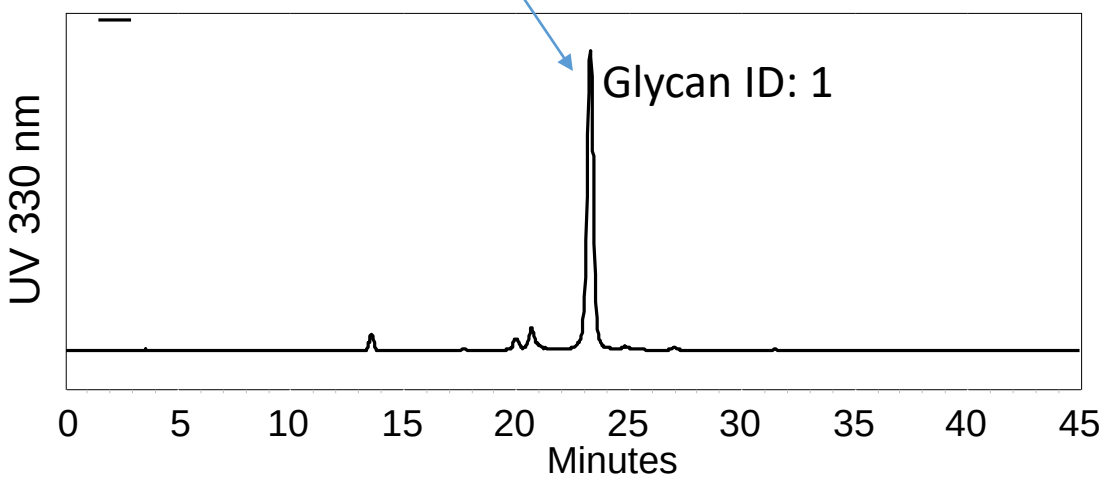


N-F1 from WAX HPLC, then C18 HPLC



After C18 , then amino HPLC separation





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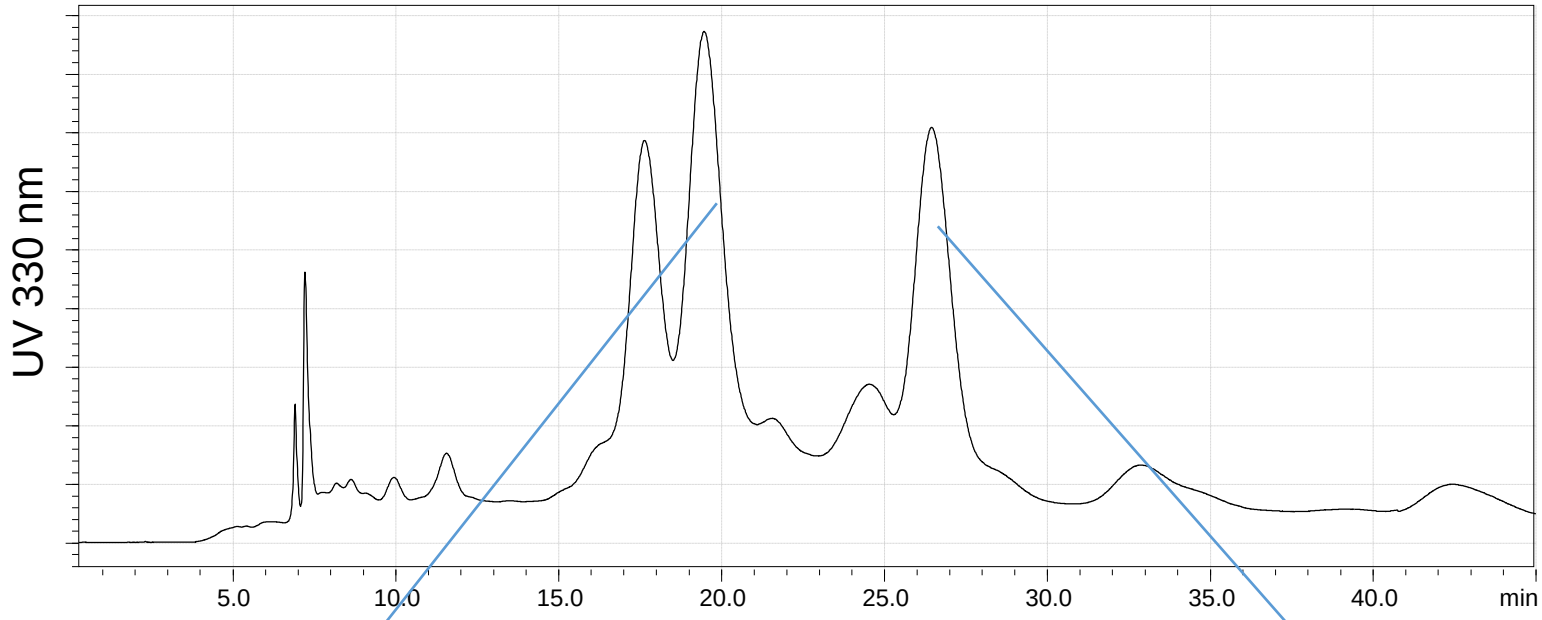
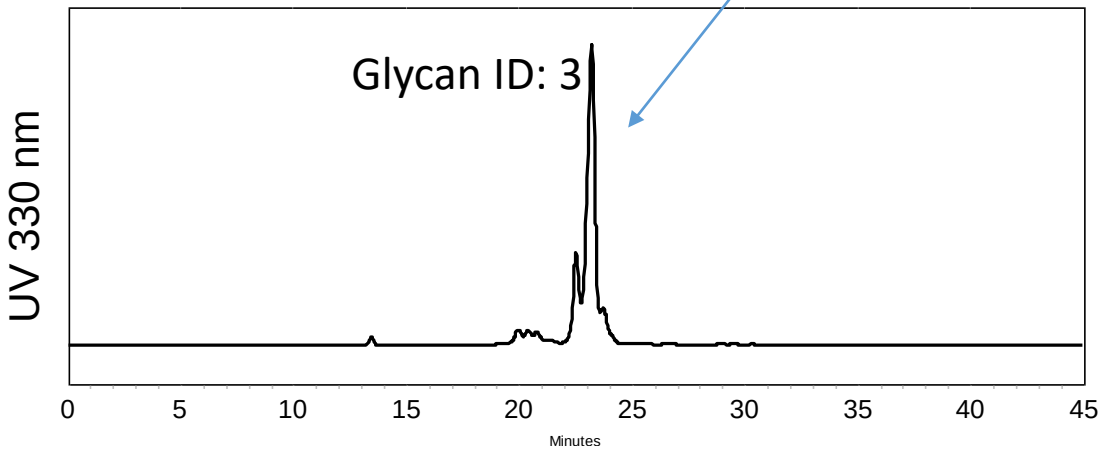
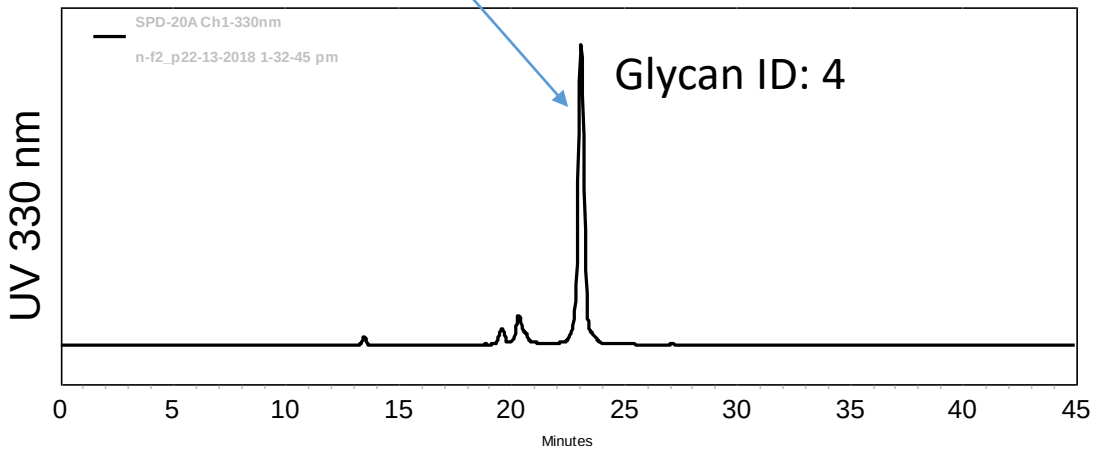
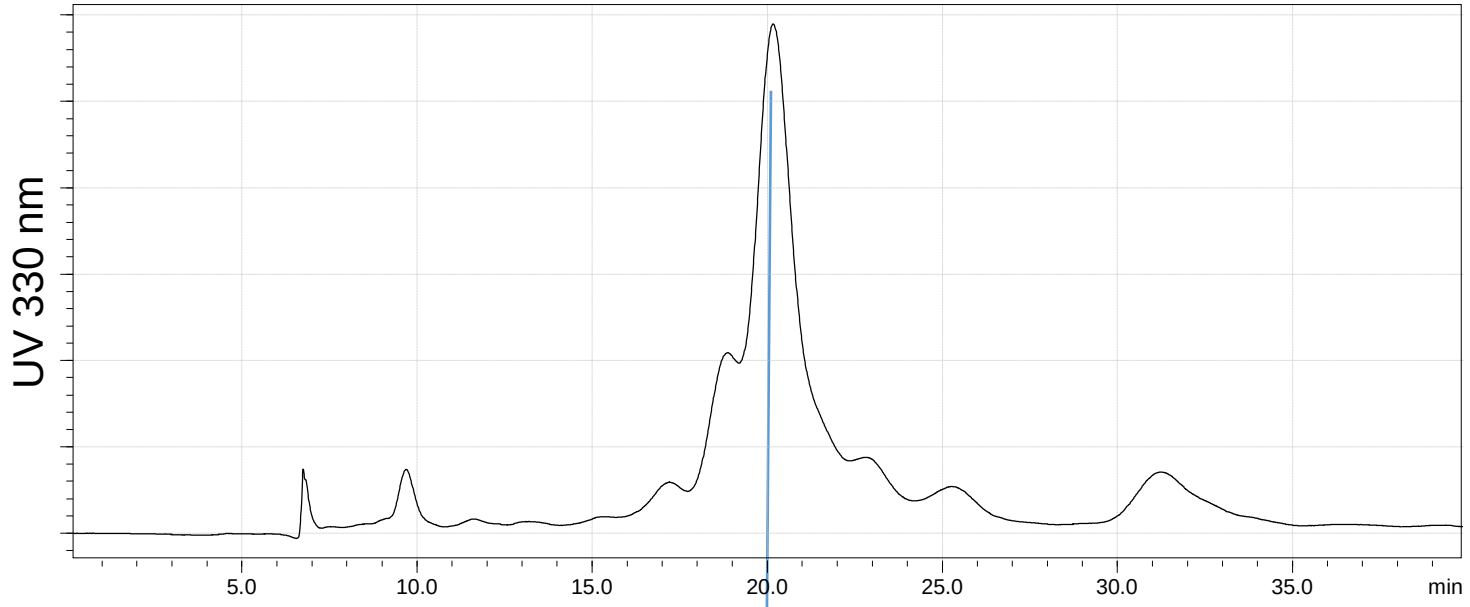


After C18 , then amino HPLC separation

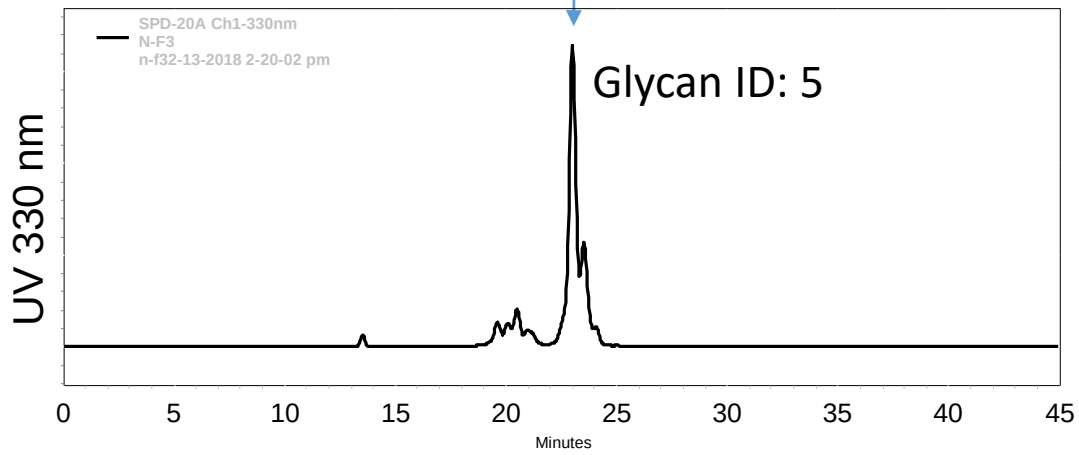




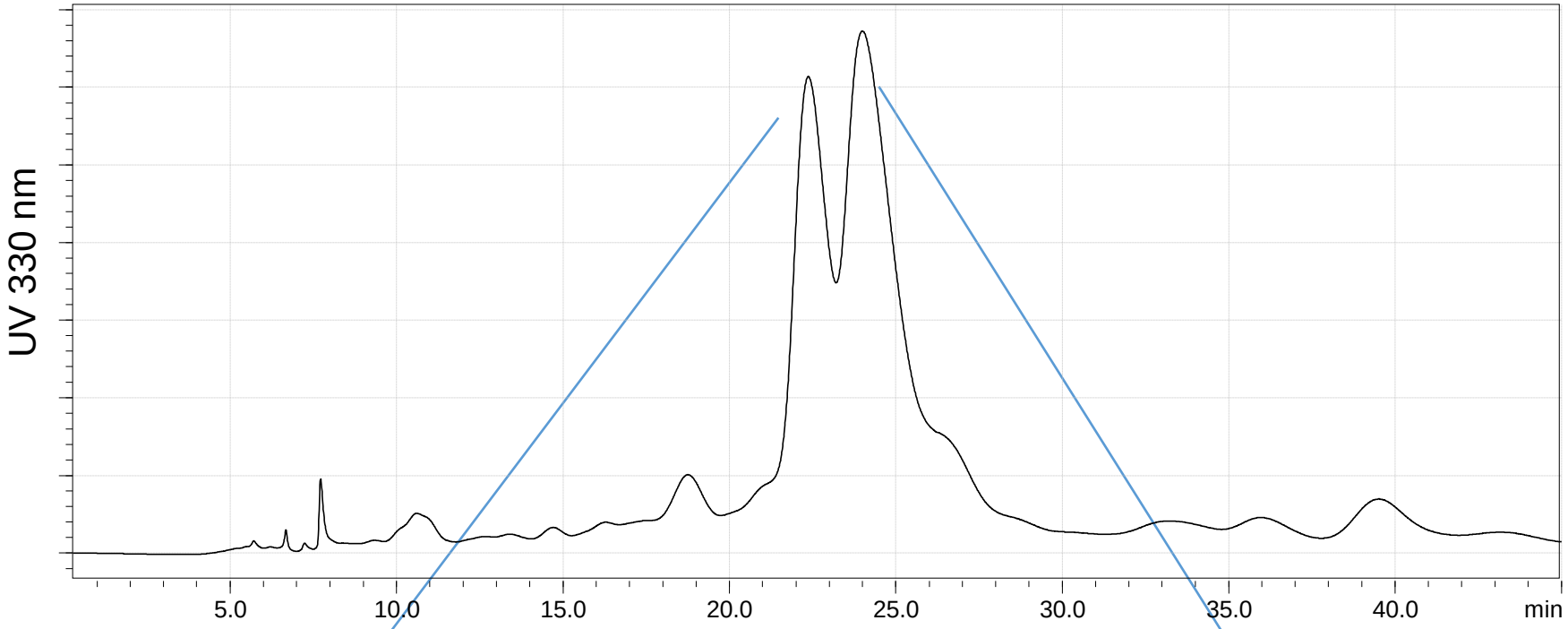
N-F3 from WAX HPLC, then C18 HPLC



After C18 , then amino HPLC separation



F4 from WAX HPLC, then C18 HPLC



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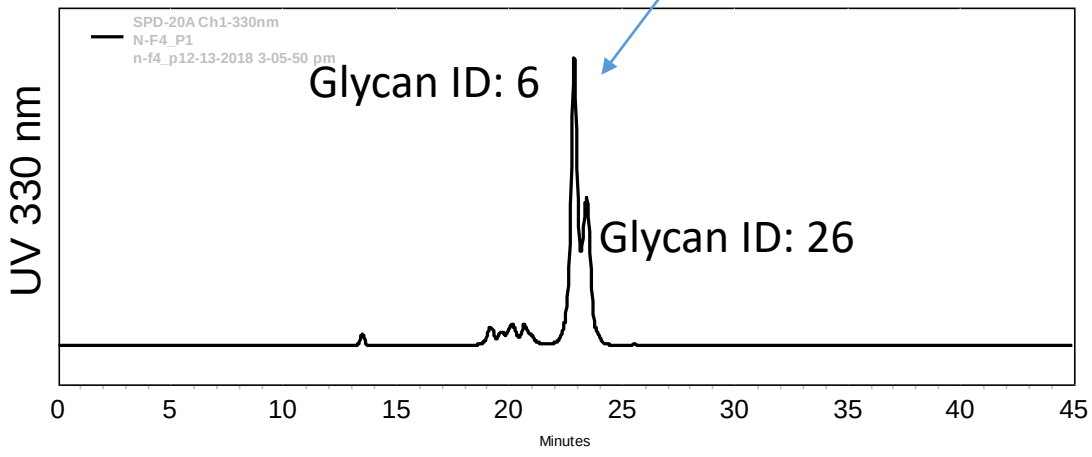

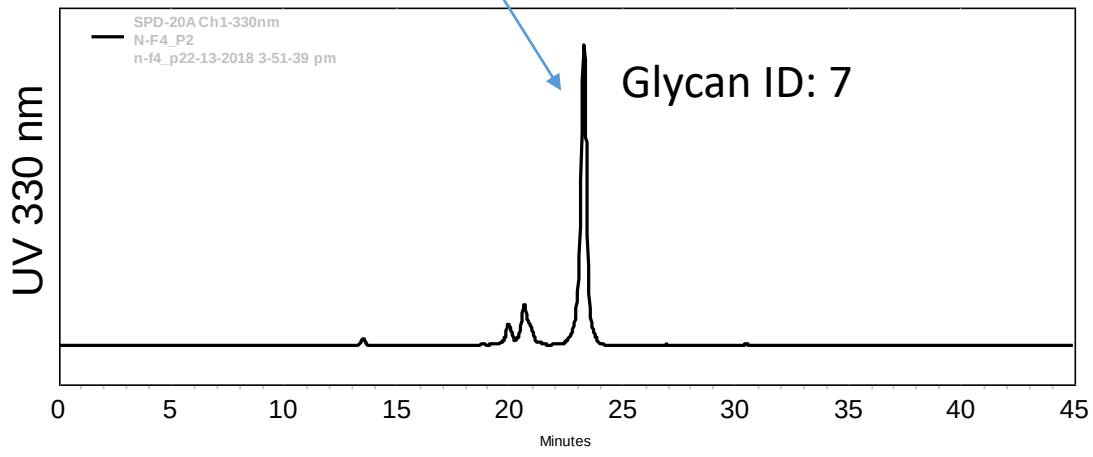

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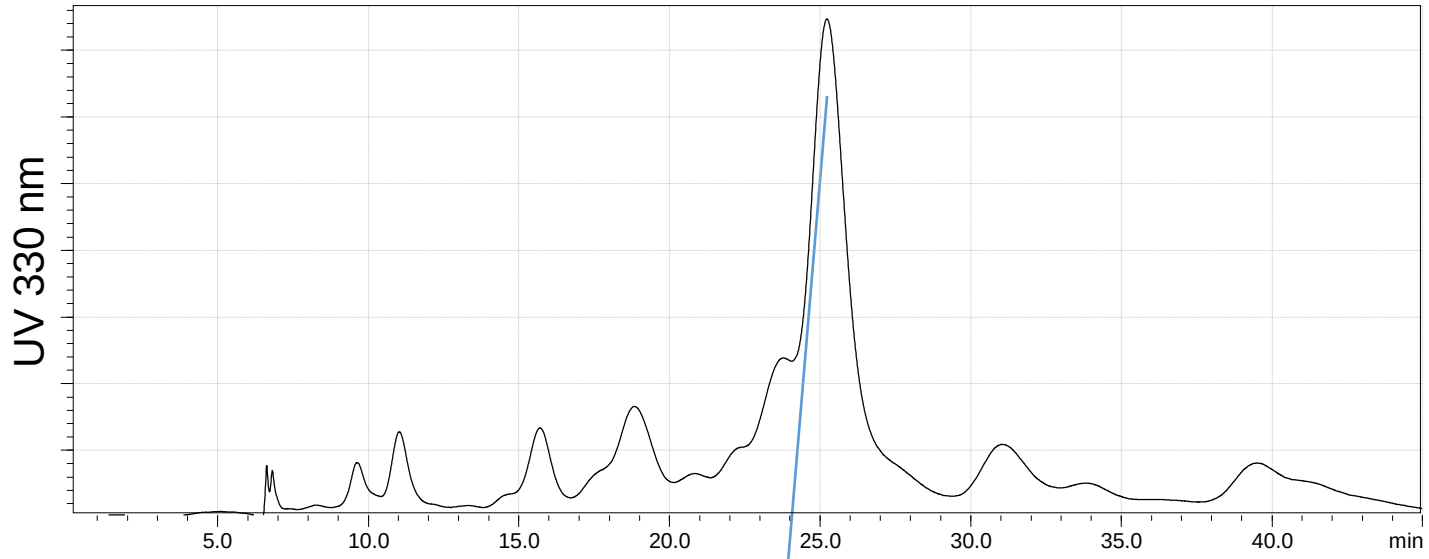
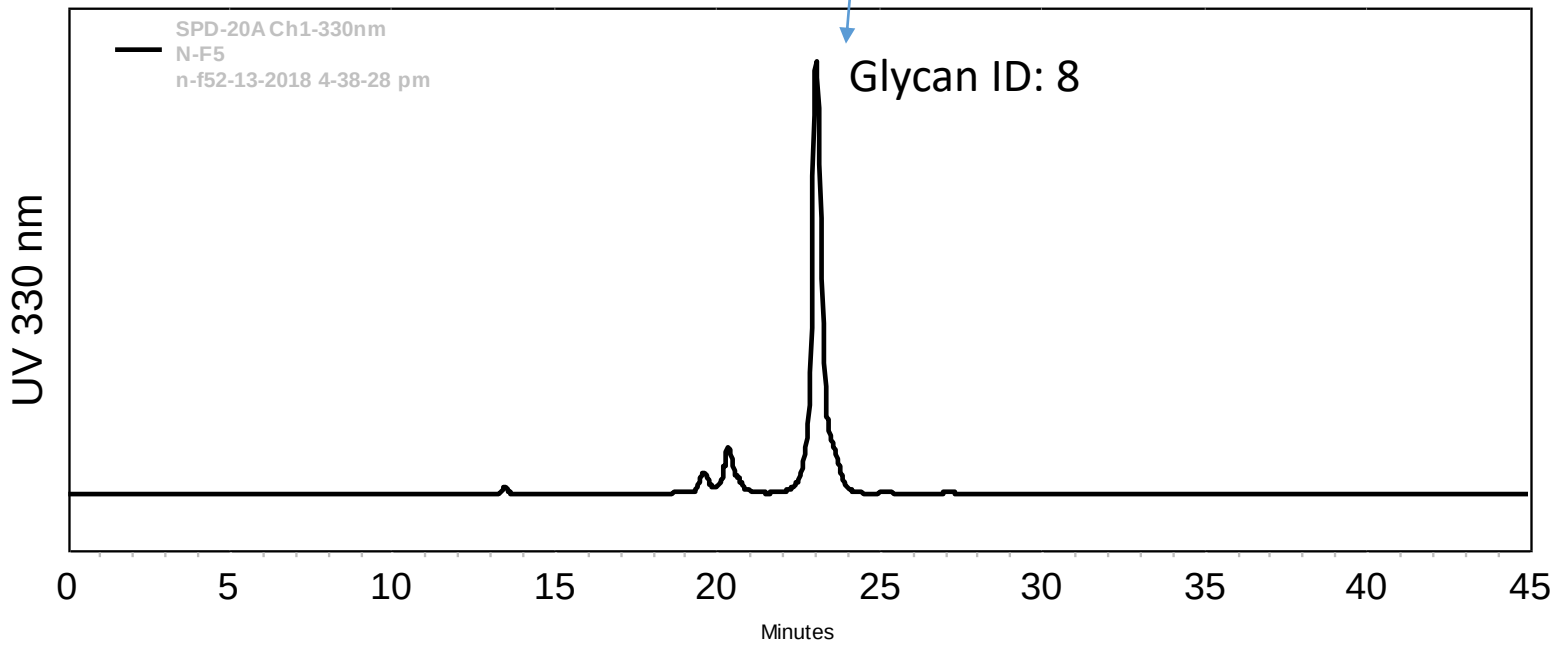
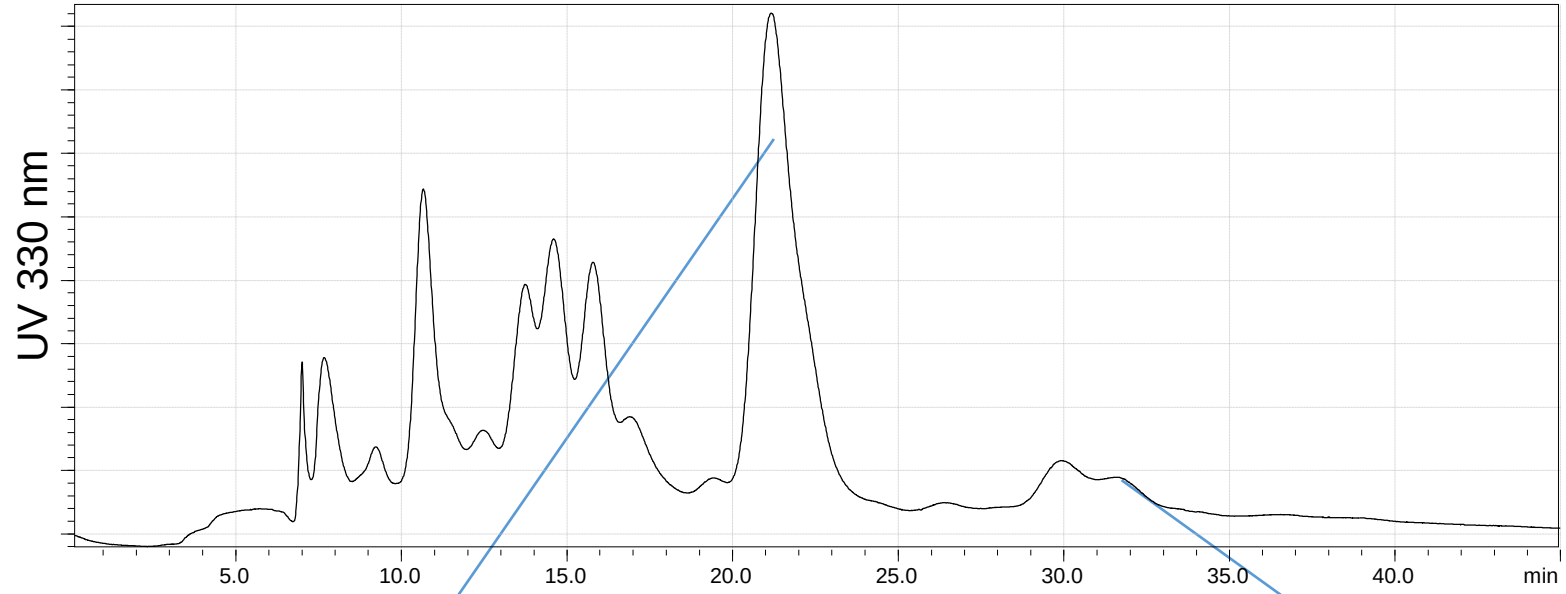


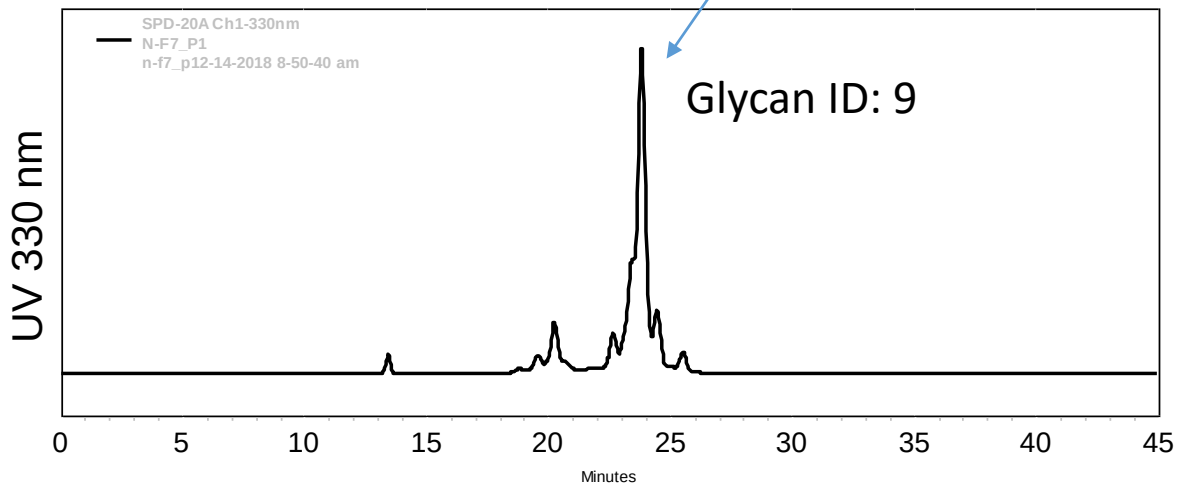
After C18 , then amino HPLC separation

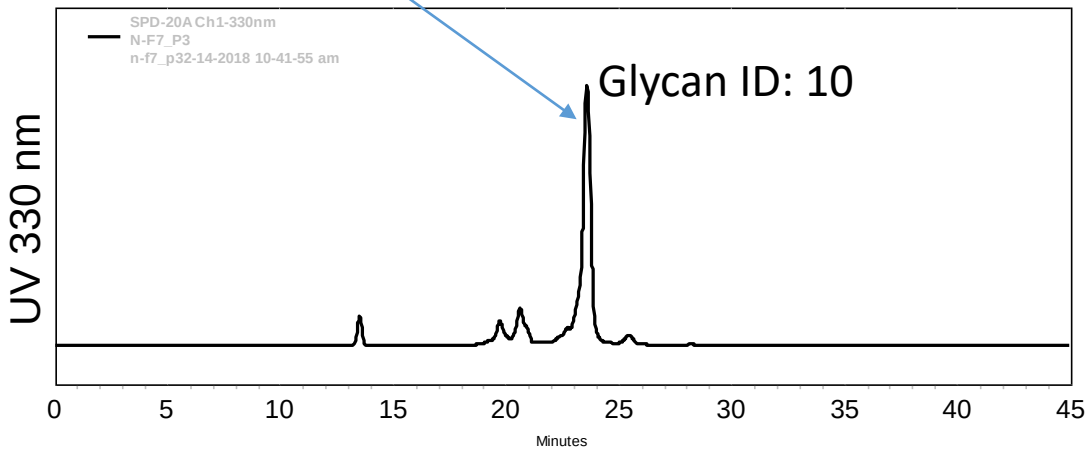


N-F7 from WAX HPLC , then C18 HPLC

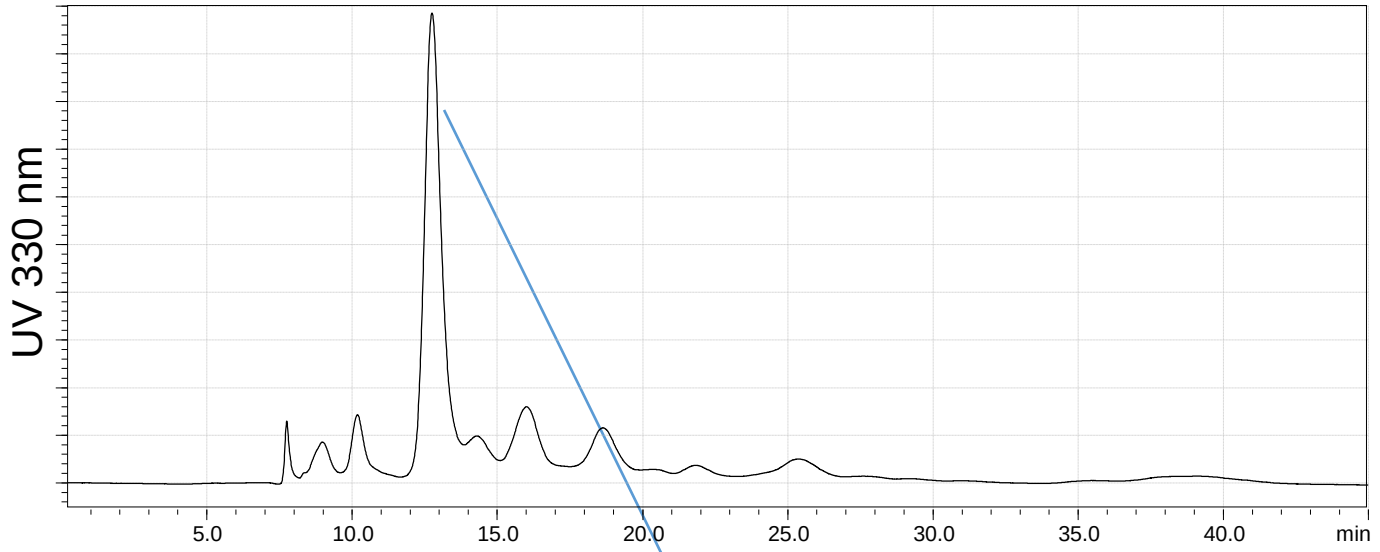


After C18 , then amino HPLC separation

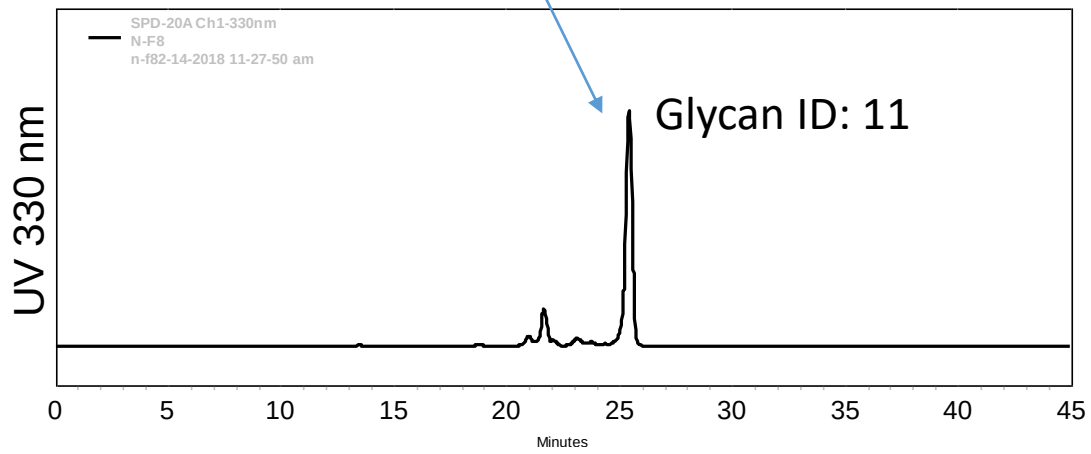




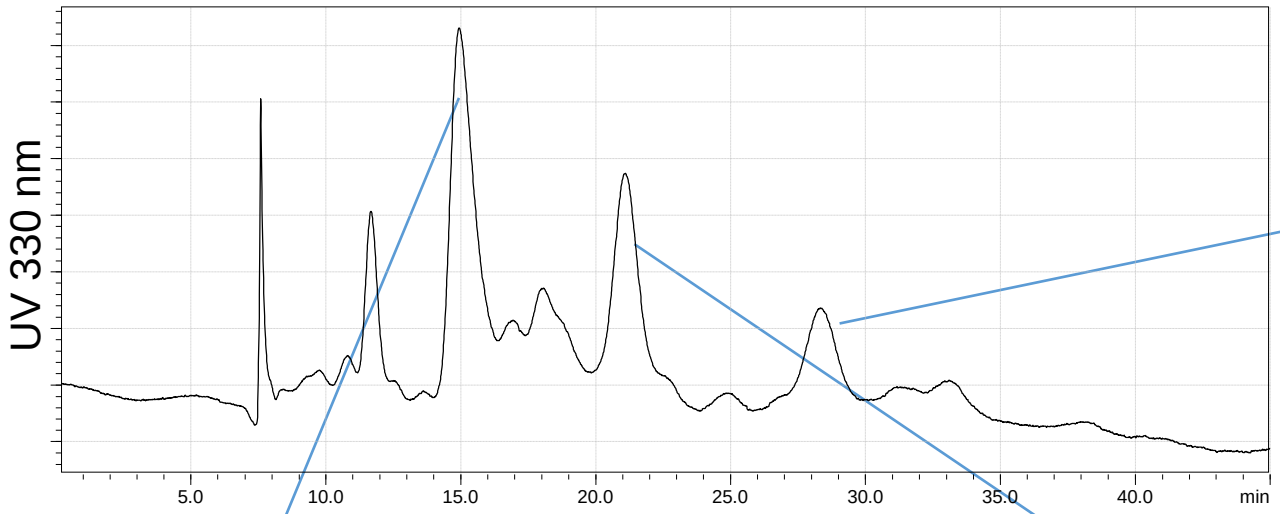
N-F8 from WAX HPLC, then C18 HPLC

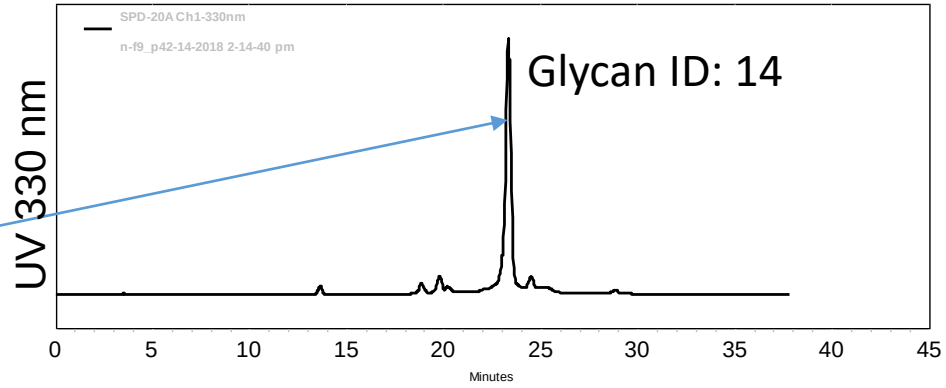


After C18 , then amino HPLC separation



N-F9 from WAX HPLC , then C18 HPLC





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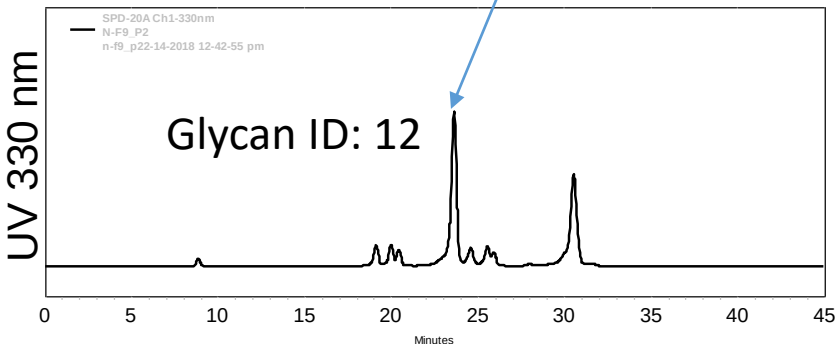
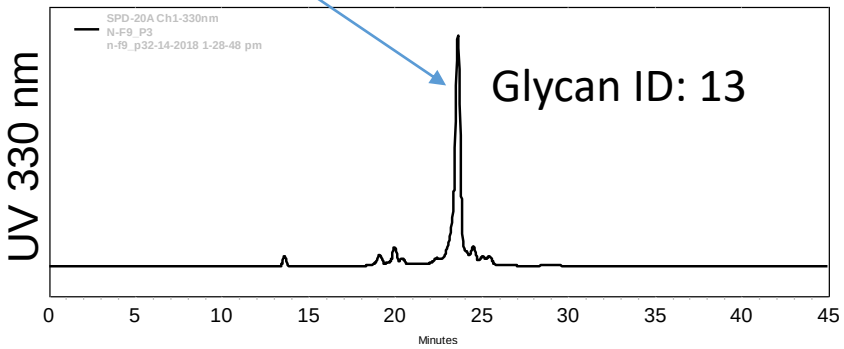
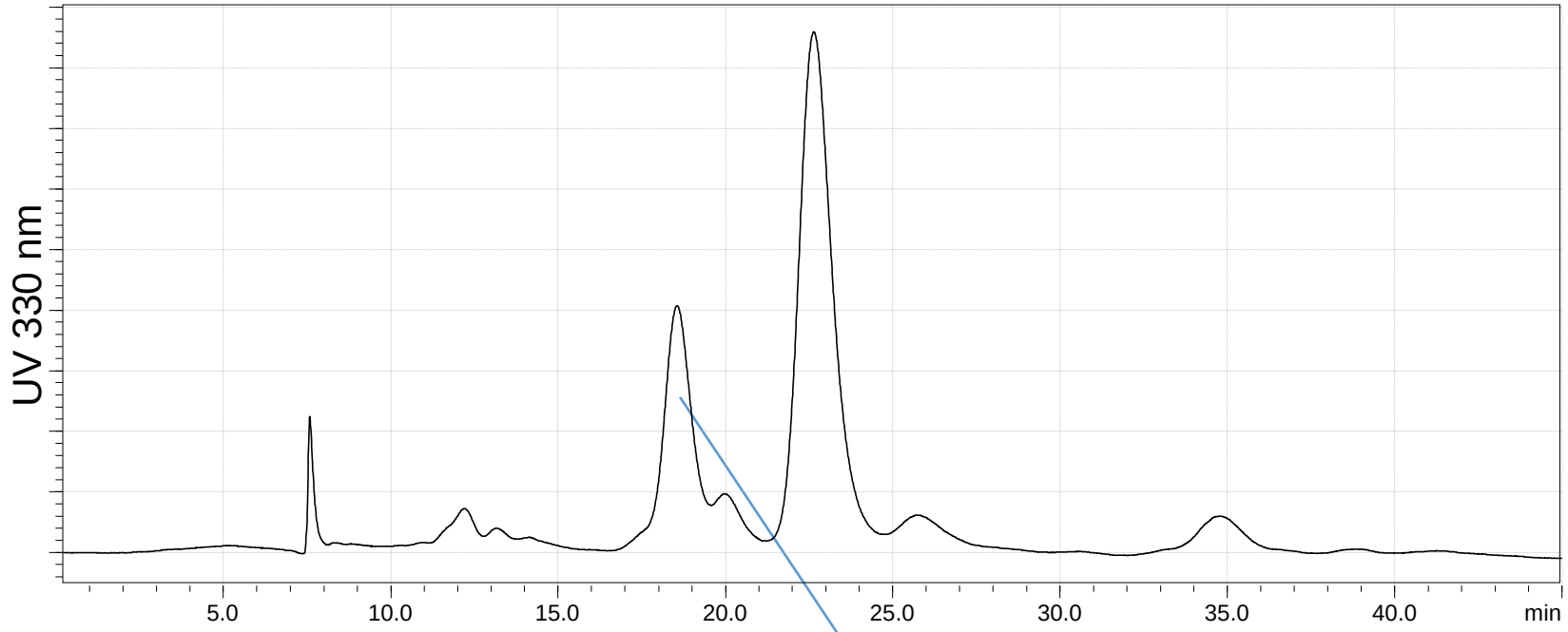
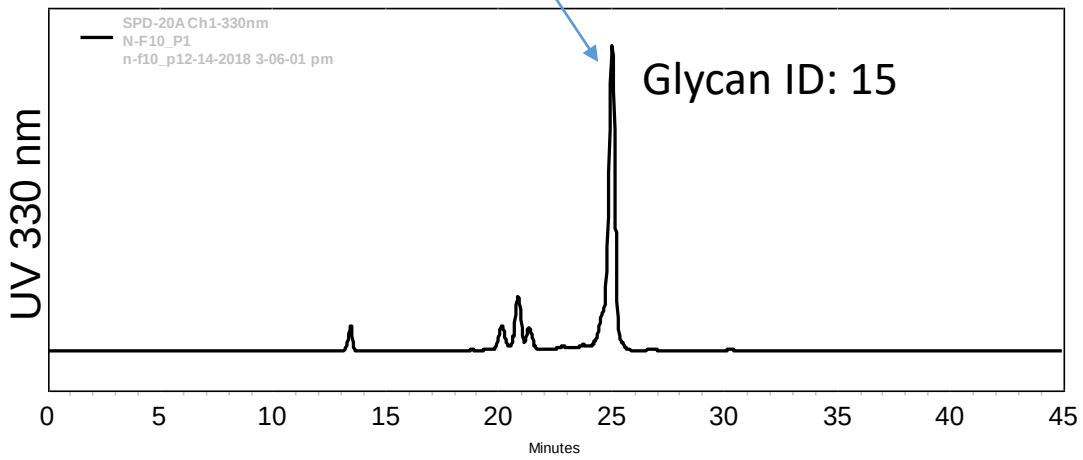




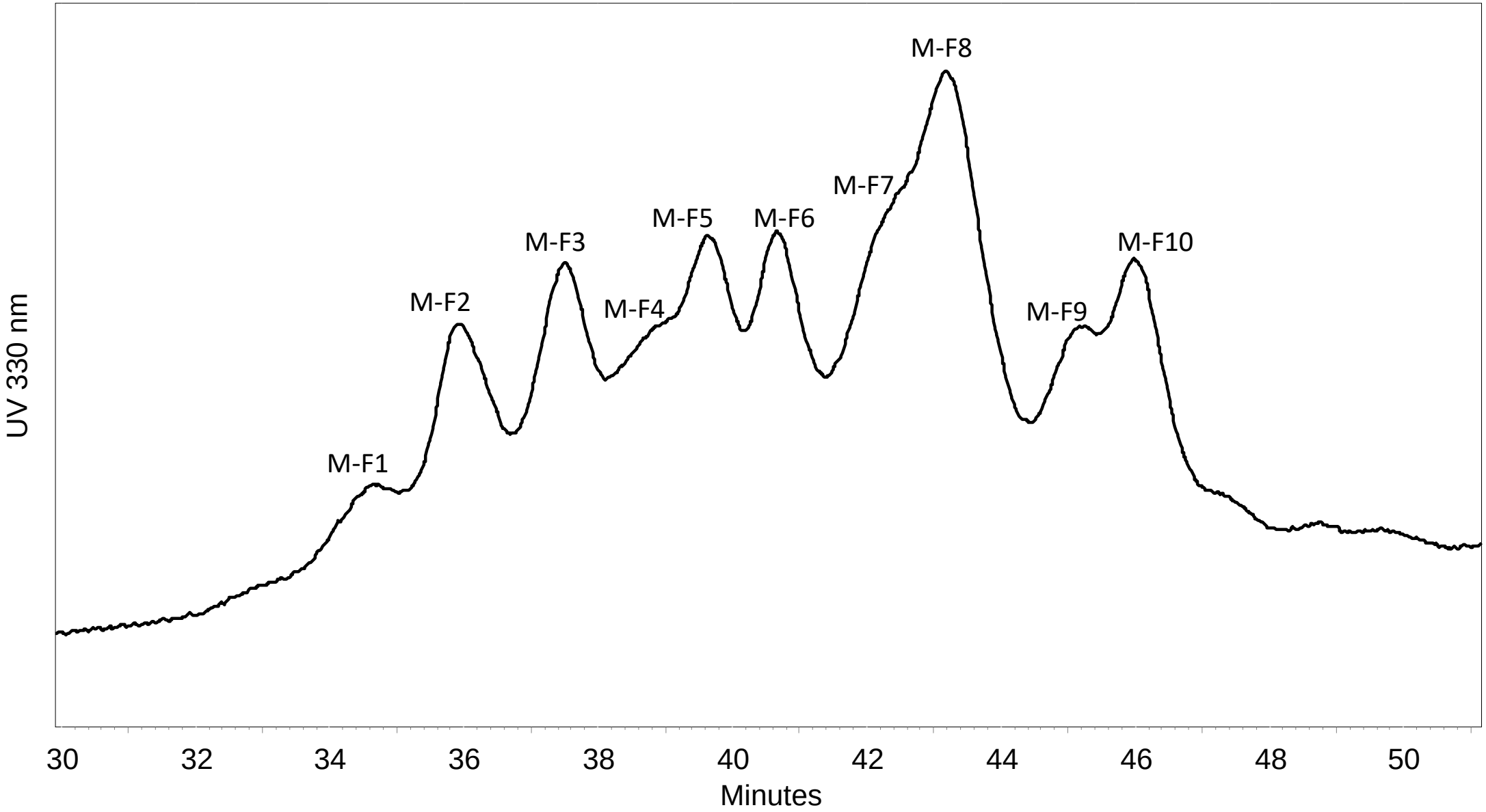
N-F10 from WAX HPLC, then C18 HPLC



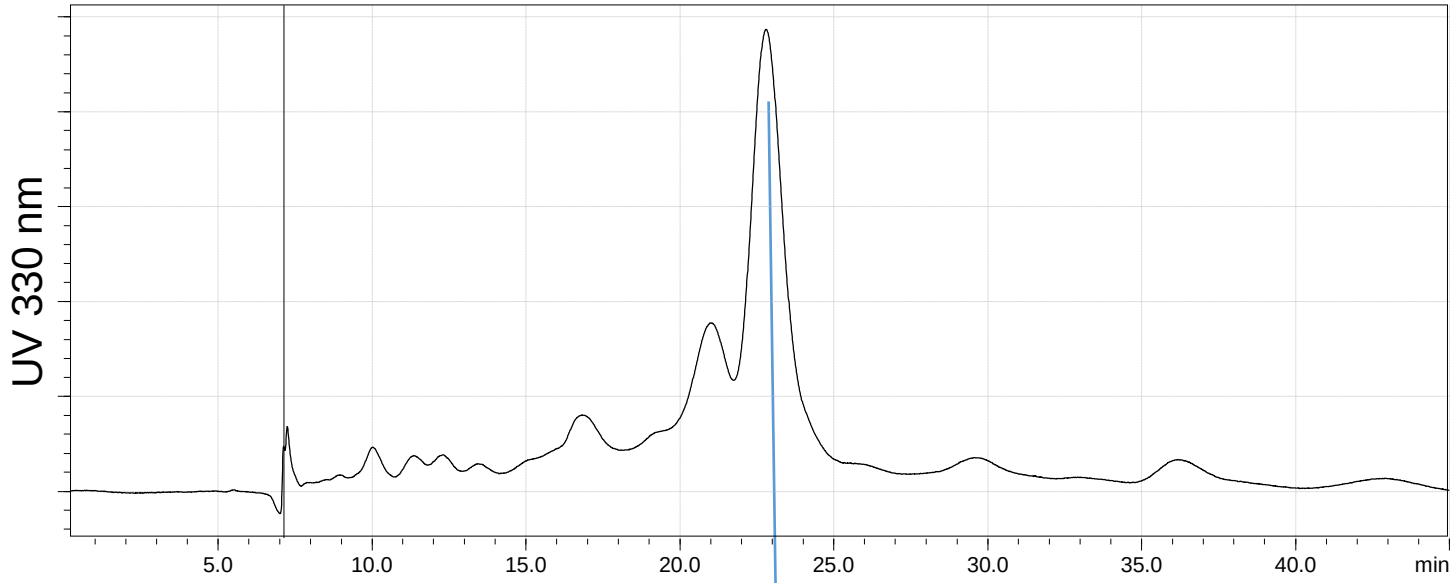
After C18 , then amino HPLC separation



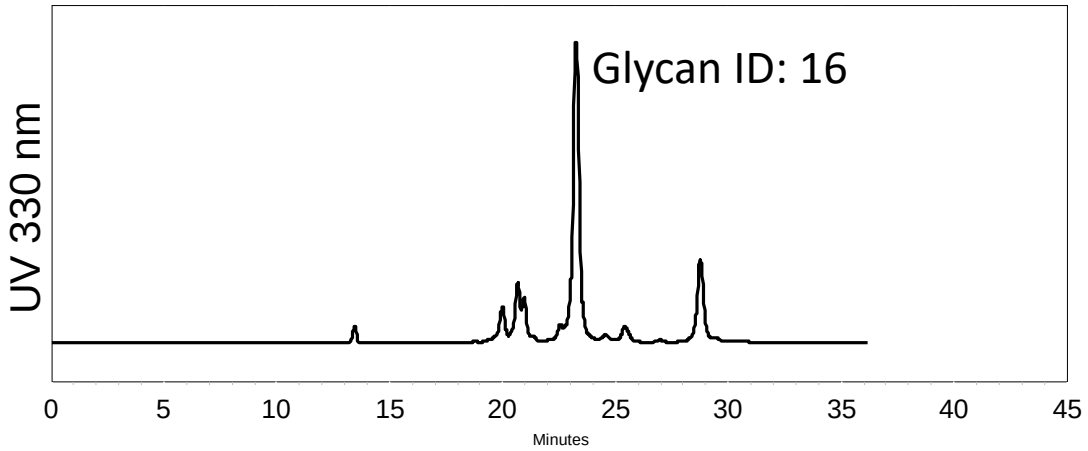
WAX Separation, mono-sialic acids Portion



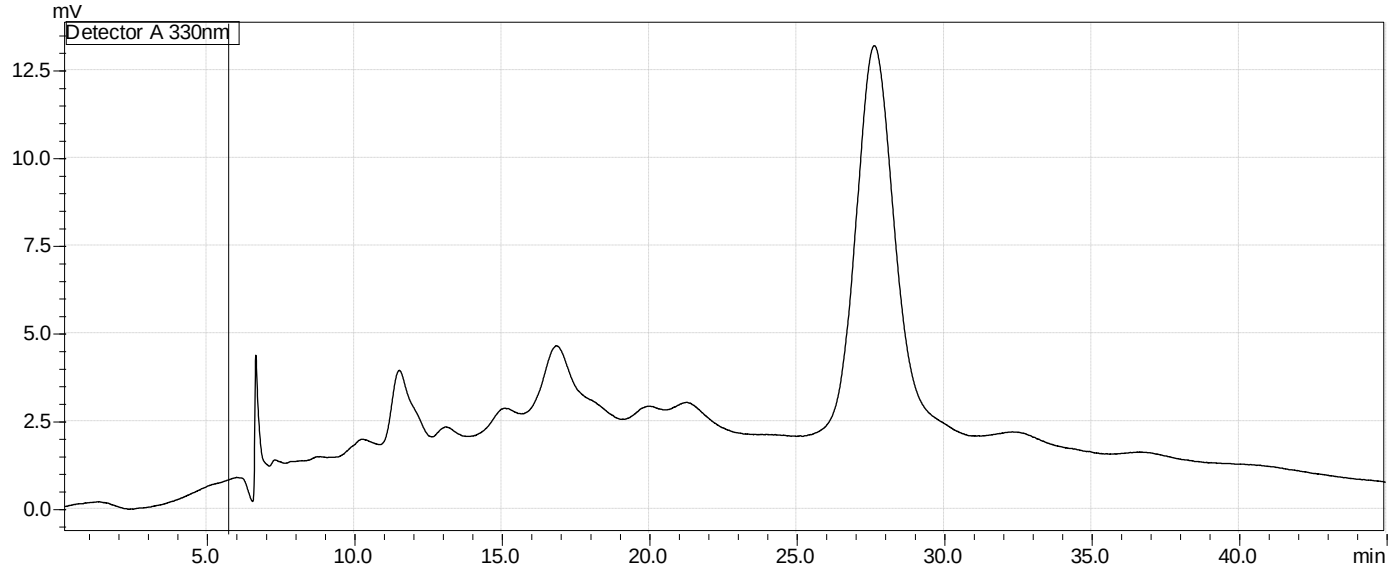
M-F3 from WAX HPLC, then C18 HPLC



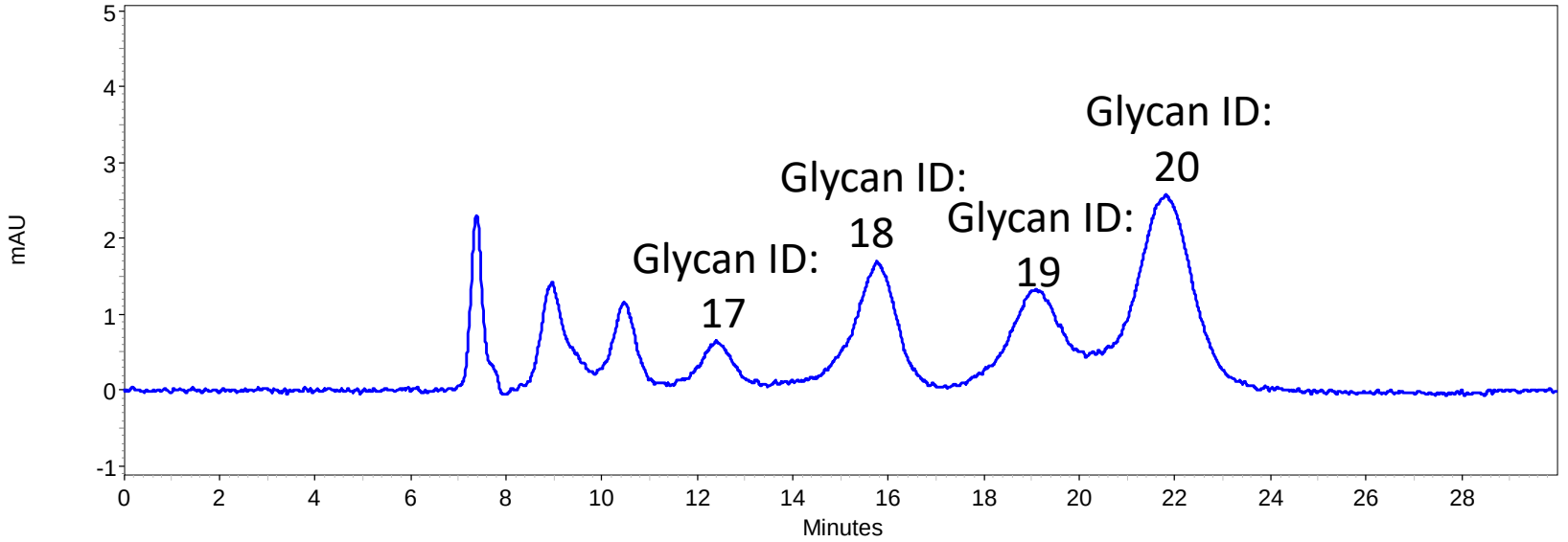
After C18 , then amino HPLC separation



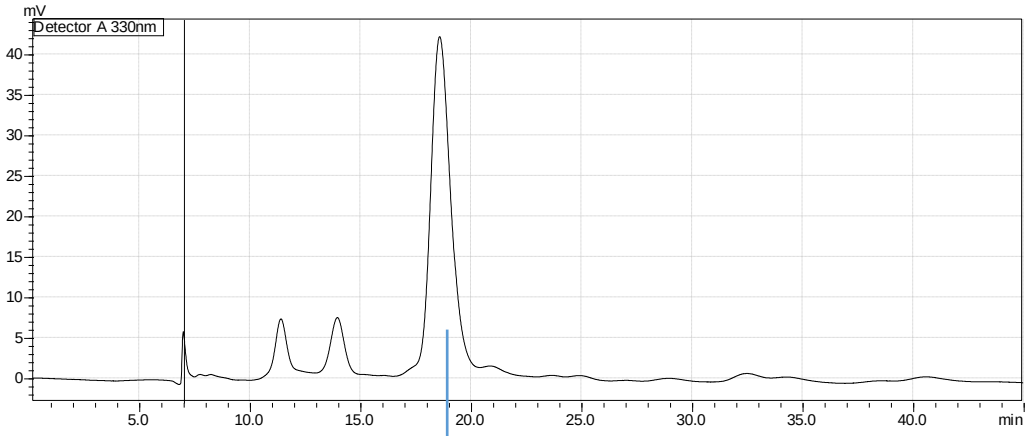
M-F6 from WAX HPLC, then C18 HPLC



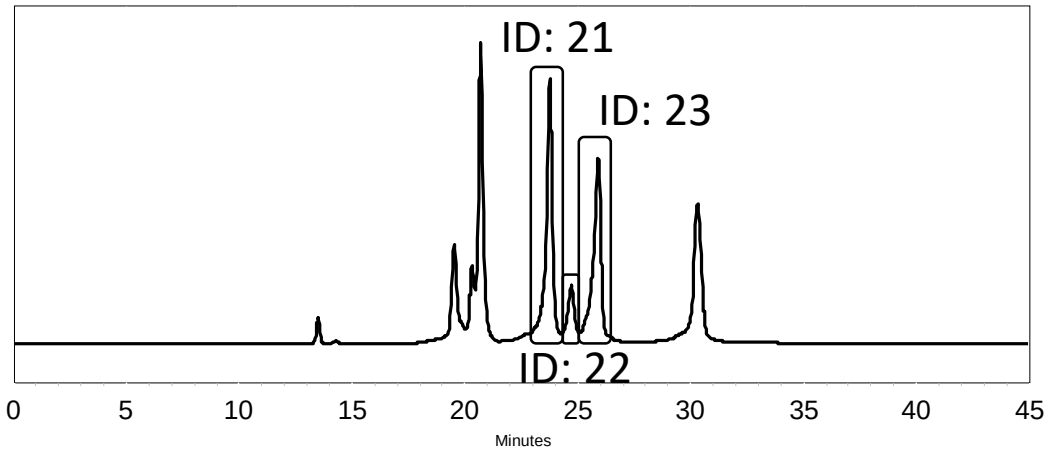
After C18 , then amino HPLC separation



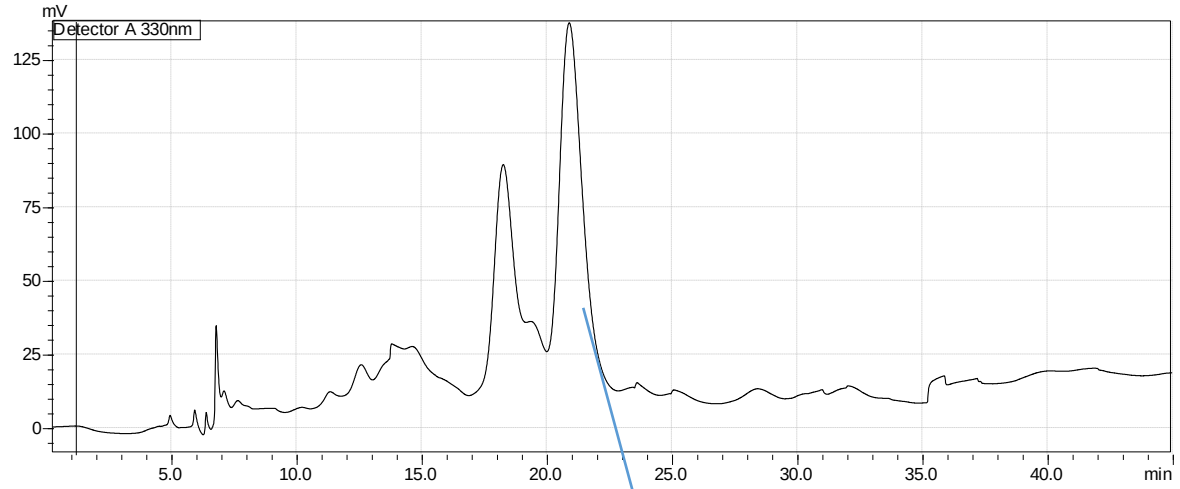
M-F8 from WAX HPLC, then C18 HPLC



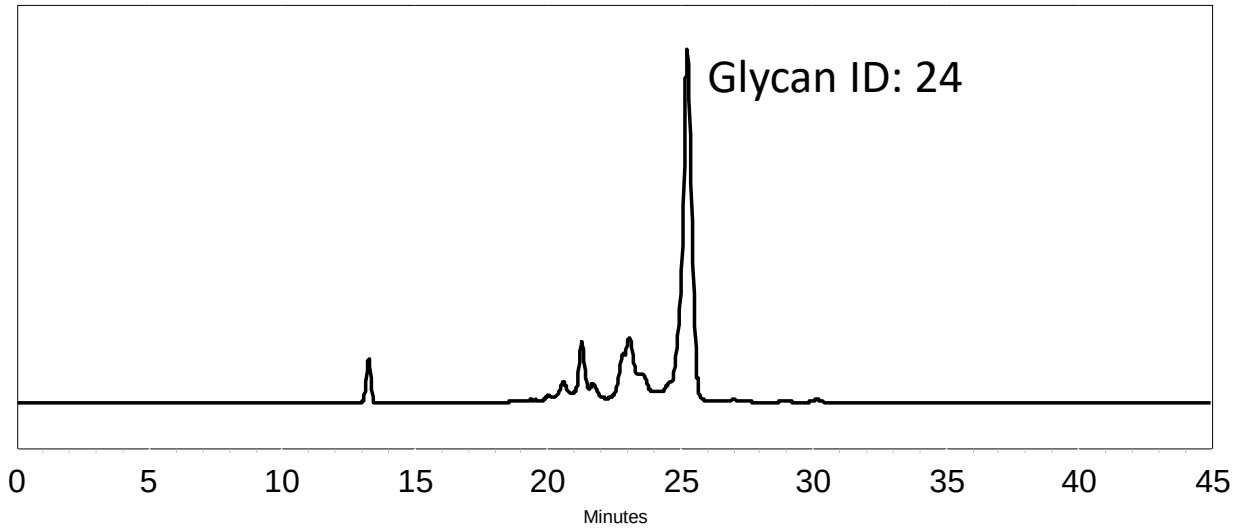
After C18 , then amino HPLC separation



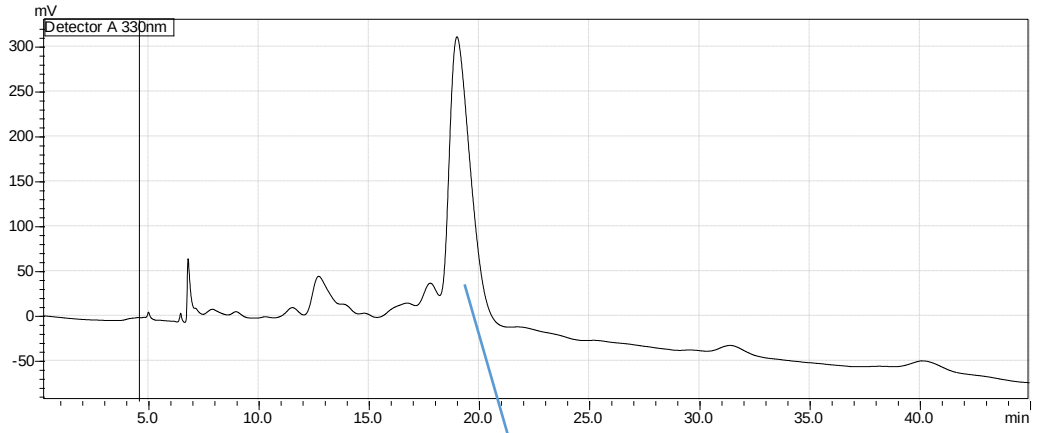
M-F9 from WAX HPLC, then C18 HPLC



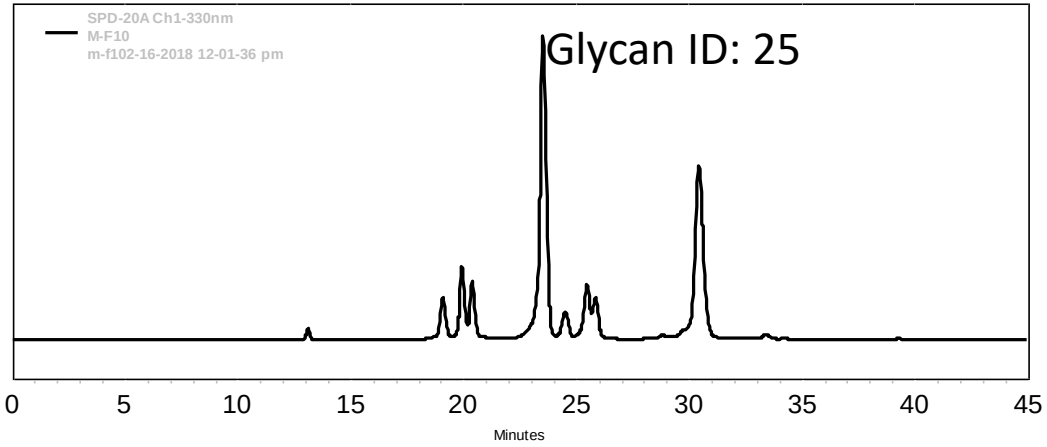
After C18 , then amino HPLC separation



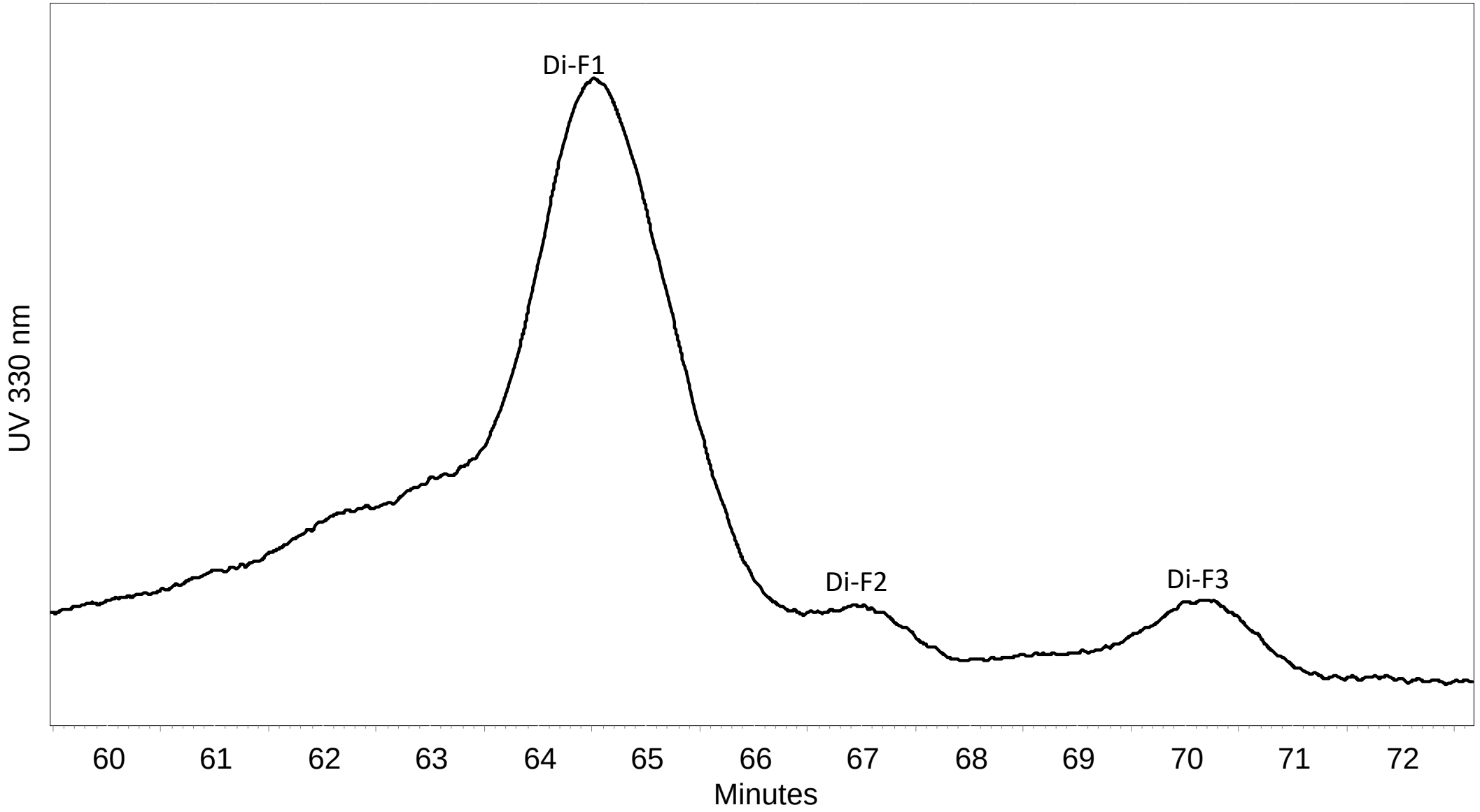
M-F10 from WAX HPLC, then C18 HPLC



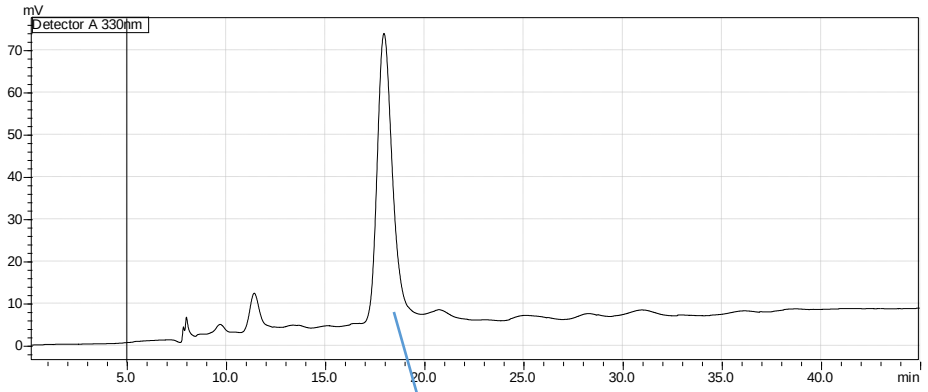
After C18 , then amino HPLC separation



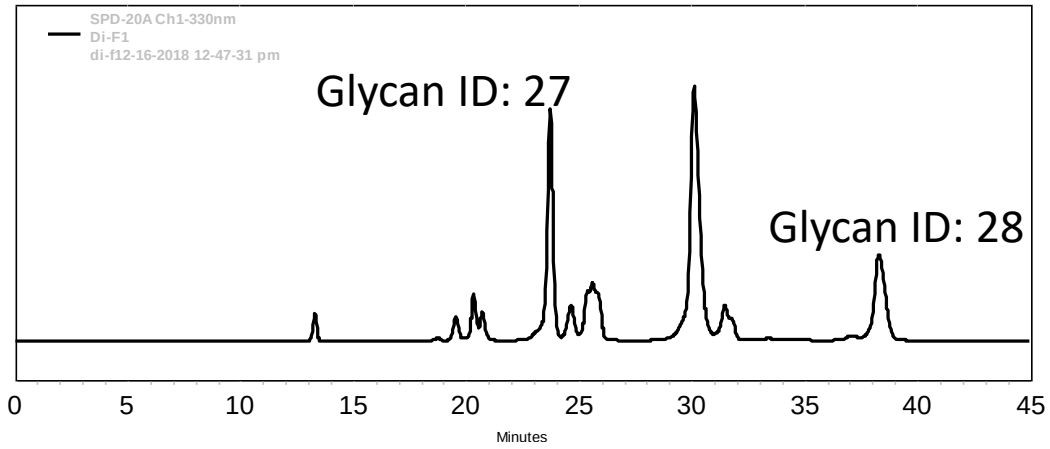
WAX Separation, di-sialic acids Portion



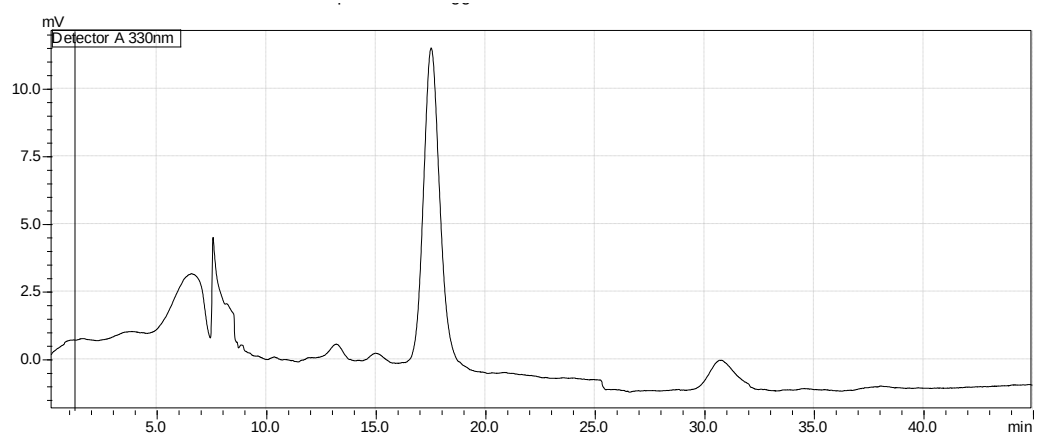
Di-F1 from WAX HPLC, then C18 HPLC



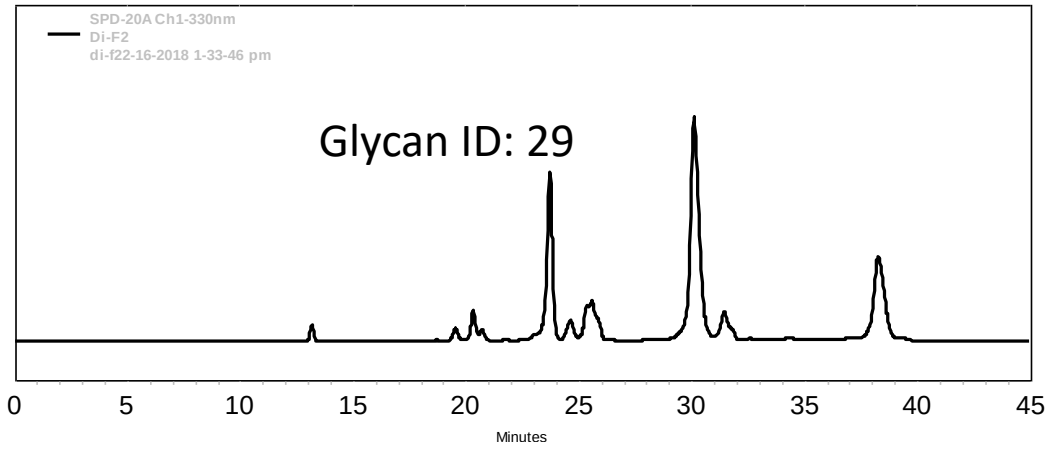
After C18 , then amino HPLC separation



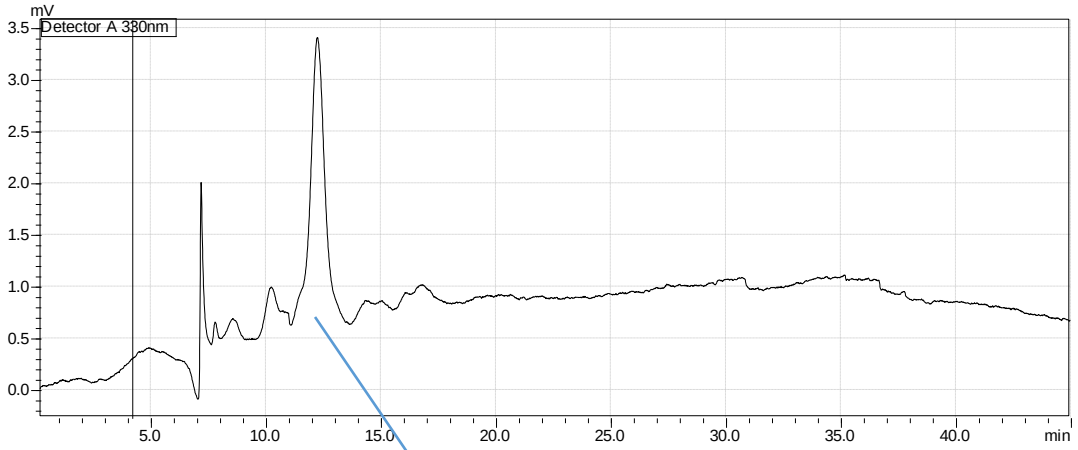
Di-F2 from WAX HPLC, then C18 HPLC



After C18 , then amino HPLC separation



Di-F3 from WAX HPLC, then C18 HPLC



After C18 , then amino HPLC separation

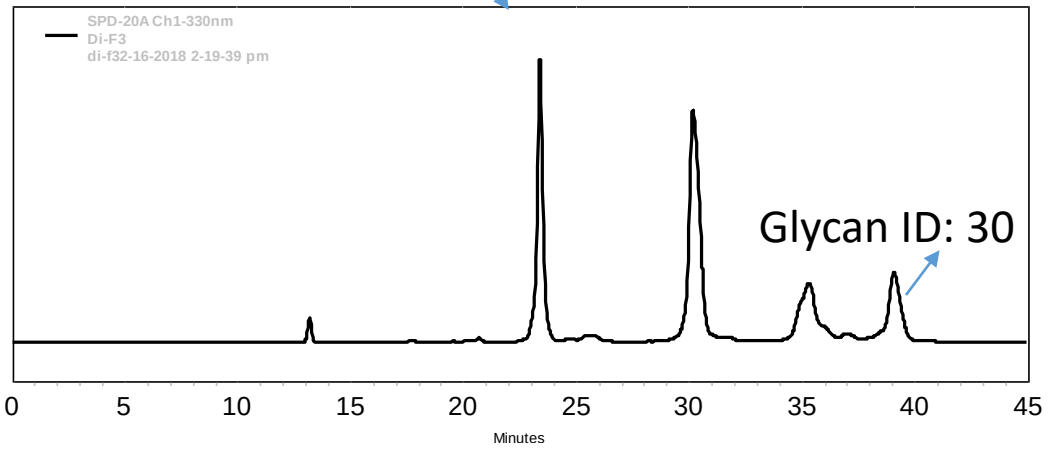


Figure S2 Plant lectin binding on the glycan microarray of purified LNnT-AA(Chart ID 1-6, concentration: 200 μM, 100 μM, 50 μM, 25 μM, 10 μM, 5 μM, respectively and same below) and LNnT-AEAB (Chart ID 7-12), 2'-FL-AA (Chart ID 13-18), 2'-FL-AEAB (Chart ID 19-24). Error bars represent standard deviation of 6 replicates. **a**) binding of *Aleuria aurantia lectin*; **b**) binding of *Erythrina cristagalli lectin*.

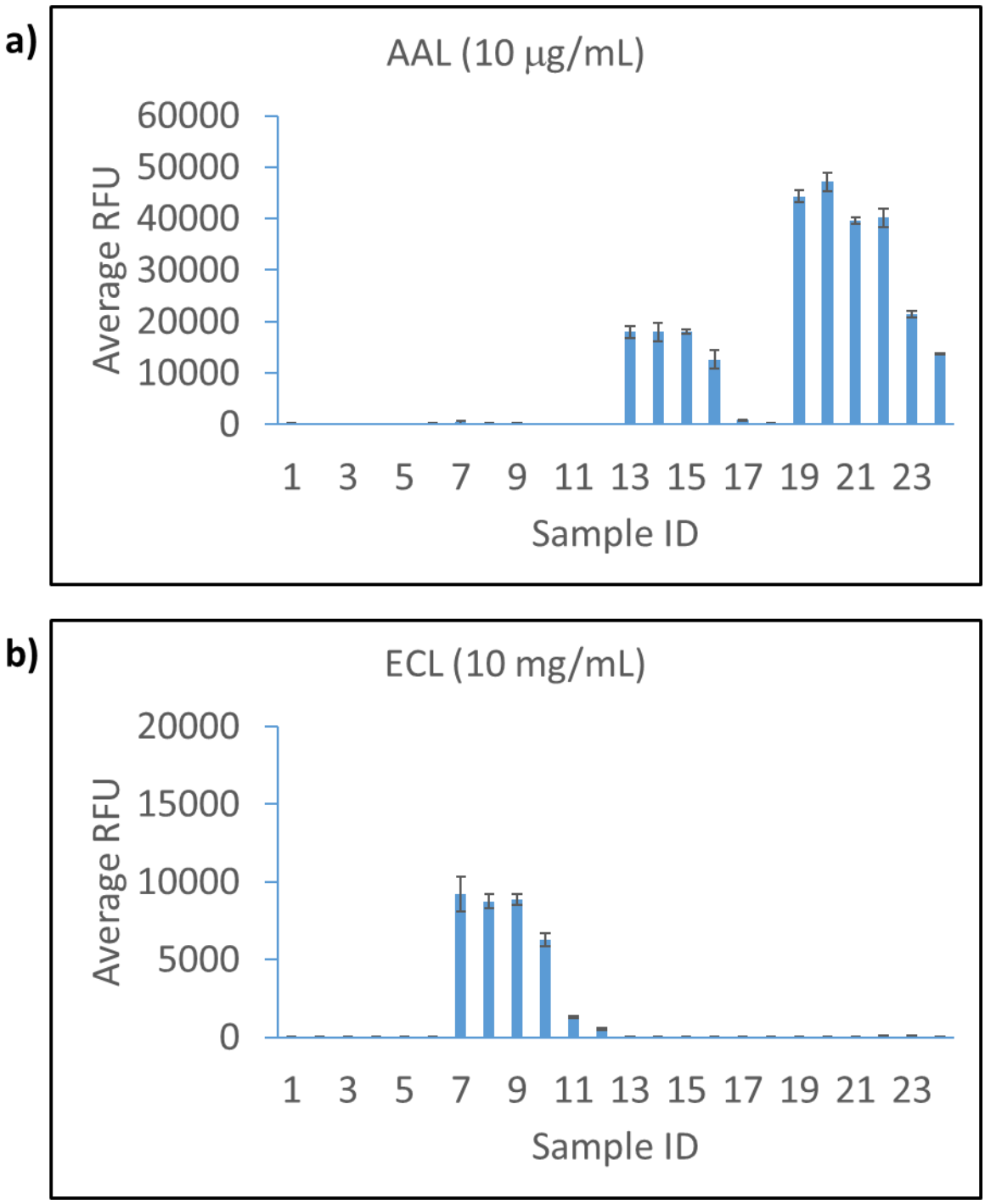


Figure S3: Click chemistry of LNnT-AZAB with propargylamine

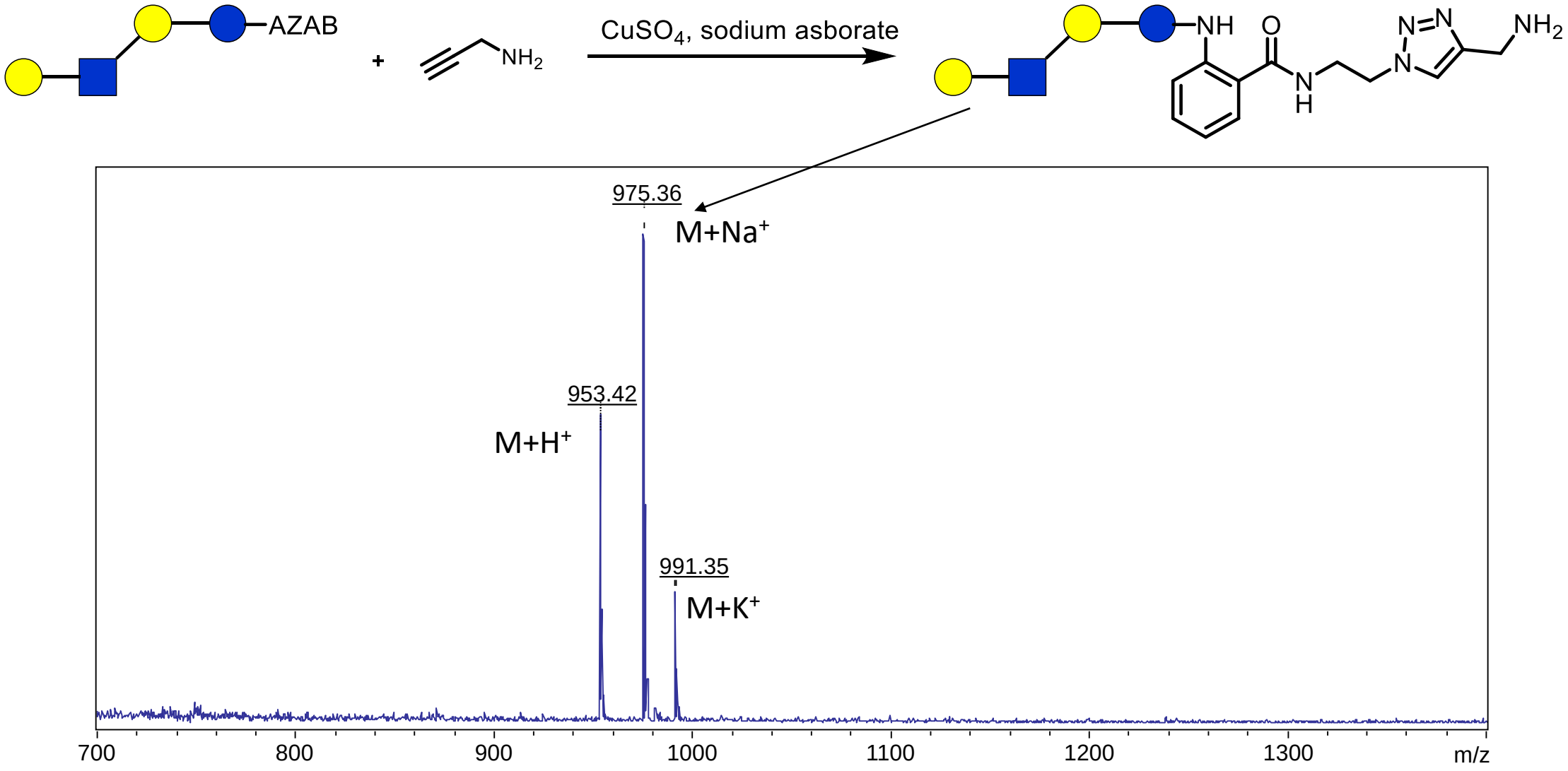


Table S1 Summary of purified chicken egg N-glycan-AA conjugates compositions for conversion to glycan-AEAB conjugates.

Glycan ID	Structure Composition
1	H4N8
2	H3N7
3	H5N7
4	H3N8
5	H3N7
6	H3N7
7	H4N8
8	H5N7
9	H5N5
10	H3N7
11	H9N2
12	H4N4
13	H4N4
14	H3N5
15	H7N2
16	H4N8
17	H5N5
18	H4N5
19	H4N6
20	H5N5
21	H5N4
22	H5N4Sia1
23	H6N3
24	H8N2
25	H4N4
26	H5N7
27	H5N4
28	H5N4Sia2
29	H5N4
30	H6N3

Table S2 Summary tables for microarray and lectin binding data

Biotin-AAL(10ug/ml), Cy5-SA (1ug/ml), LNnT & 2'-FL				
Sample ID	**Detail**	**Average**	**STDEV**	**%CV**
1	LNnT-AA-200	227.5	134.2498	59.01089
2	LNnT-AA-100	67	15.18771	22.66823
3	LNnT-AA-50	77	18.38478	23.87633
4	LNnT-AA-25	73.5	5.446712	7.410492
5	LNnT-AA-10	56.25	5.737305	10.19965
6	LNnT-AA-5	146.25	130.0471	88.9211
7	LNnT-AEAB-200	383	349.5025	91.25392
8	LNnT-AEAB-100	195.25	97.16438	49.76409
9	LNnT-AEAB-50	97	90.10364	92.89035
10	LNnT-AEAB-25	68.25	12.44655	18.23671
11	LNnT-AEAB-10	48.5	6.244998	12.87628
12	LNnT-AEAB-5	93	27.98809	30.09472
13	2FL-AA-200	17964.5	1107.589	6.165429
14	2FL-AA-100	17956.25	1842.499	10.26105
15	2FL-AA-50	18004	363.129	2.016935
16	2FL-AA-25	12584.75	1766.956	14.04046
17	2FL-AA-10	875.75	84.23133	9.618193
18	2FL-AA-5	204.75	30.01527	14.65947
19	2FL-AEAB-200	44347	1210.533	2.729685
20	2FL-AEAB-100	47115.5	1800.935	3.822383
21	2FL-AEAB-50	39536.5	677.8109	1.714393
22	2FL-AEAB-25	40172	1745.578	4.34526
23	2FL-AEAB-10	21427.25	659.005	3.075546
24	2FL-AEAB-5	13703.5	123.2383	0.89932

Biotin-ECL(10ug/ml), Cy5-SA (1ug/ml), LNnT & 2'-FL				
Sample ID	**Detail**	**Average**	**STDEV**	**%CV**
1	LNnT-AA-200	77	3	3
2	LNnT-AA-100	70	8	12
3	LNnT-AA-50	51	4	7
4	LNnT-AA-25	43	5	11
5	LNnT-AA-10	28	2	8
6	LNnT-AA-5	26	2	7
7	LNnT-AEAB-20(	9227	1107	12
8	LNnT-AEAB-10(	8746	435	5
9	LNnT-AEAB-50	8863	364	4
10	LNnT-AEAB-25	6265	427	7
11	LNnT-AEAB-10	1304	60	5
12	LNnT-AEAB-5	553	53	10
13	2FL-AA-200	42	6	14
14	2FL-AA-100	39	4	10
15	2FL-AA-50	54	15	27
16	2FL-AA-25	31	2	5
17	2FL-AA-10	37	5	14
18	2FL-AA-5	36	3	8
19	2FL-AEAB-200	71	4	6
20	2FL-AEAB-100	61	3	5
21	2FL-AEAB-50	44	8	18
22	2FL-AEAB-25	65	21	33
23	2FL-AEAB-10	83	31	37
24	2FL-AEAB-5	47	15	31

Biotin ConA (1 ug/ml)_Cy5 SA (1ug/ml), Egg N-Glycans				
Structure Composition	**Glycan ID**	**Average**	**STDEV**	**%CV**
H4N8	1	388	54	14
H3N7	2	63	10	16
H5N7	3	52	12	24
H3N8	4	38	5	12
H3N7	5	25	1	4
H3N7	6	39	3	6
H4N8	7	31	4	13
H5N7	8	31	9	30
H5N5	9	15783	1952	12
H3N7	10	2479	378	15
H9N2	11	12666	1755	14
H4N4	12	5353	2074	39
H4N4	13	11429	364	3
H3N5	14	2306	844	37
H7N2	15	15586	249	2
H4N8	16	32	4	11
H5N5	17	23	3	13
H4N5	18	34	6	19
H4N6	19	167	4	2
H5N5	20	56	22	39
H5N4	21	8228	473	6
H5N4Sia1	22	8300	438	5
H6N3	23	19413	1292	7
H8N2	24	15246	1511	10
H4N4	25	15093	1762	12
H5N7	26	3193	203	6
H5N4	27	3743	1019	27
H5N4Sia2	28	3401	680	20
H5N4	29	6057	575	10
H6N3	30	1529	379	25

Biotin ECL (10 ug/ml)_Cy5 SA (1ug/ml), Egg N-Glycans				
Structure Composition	**Glycan ID**	**Average**	**STDEV**	**%CV**
H4N8	1	8822	0	4
H3N7	2	81	15	19
H5N7	3	11896	1366	11
H3N8	4	66	9	14
H3N7	5	77	10	13
H3N7	6	75	3	4
H4N8	7	9647	1149	12
H5N7	8	56	8	13
H5N5	9	72	13	18
H3N7	10	68	9	13
H9N2	11	48	2	5
H4N4	12	10203	434	4
H4N4	13	15441	1295	8
H3N5	14	31	6	21
H7N2	15	53	3	5
H4N8	16	14915	479	3
H5N5	17	597	116	19
H4N5	18	9073	575	6
H4N6	19	20723	2294	11
H5N5	20	11871	2284	19
H5N4	21	16711	1045	6
H5N4Sia1	22	11355	158	1
H6N3	23	13297	1468	11
H8N2	24	22	5	21
H4N4	25	16185	1779	11
H5N7	26	10325	903	9
H5N4	27	9144	1107	12
H5N4Sia2	28	33	4	12
H5N4	29	10959	1846	17
H6N3	30	5638	649	12

Biotin RCA1 (10 ug/ml)_Cy5 SA (1ug/ml), Egg N-Glycans				
Structure Composition	**Glycan ID**	**Average**	**STDEV**	**%CV**
H4N8	1	16928	543	3
H3N7	2	70	12	18
H5N7	3	18674	517	3
H3N8	4	54	4	8
H3N7	5	64	10	15
H3N7	6	46	7	15
H4N8	7	22397	937	4
H5N7	8	59	8	13
H5N5	9	52	6	12
H3N7	10	70	9	13
H9N2	11	64	5	8
H4N4	12	20624	2133	10
H4N4	13	24405	957	4
H3N5	14	30	7	24
H7N2	15	78	3	4
H4N8	16	24201	204	1
H5N5	17	2397	849	35
H4N5	18	17031	405	2
H4N6	19	20513	619	3
H5N5	20	20802	2336	11
H5N4	21	23873	2348	10
H5N4Sia1	22	28289	673	2
H6N3	23	23348	450	2
H8N2	24	30	3	11
H4N4	25	20690	2936	14
H5N7	26	19433	2362	12
H5N4	27	17453	2238	13
H5N4Sia2	28	22464	2390	11
H5N4	29	20664	4836	23
H6N3	30	13742	1796	13

Biotin SNA (10 ug/ml)_Cy5 SA (1ug/ml), Egg N-Glycans				
Structure Composition	**Glycan ID**	**Average**	**STDEV**	**%CV**
H4N8	1	178.5	26.63957	14.92413
H3N7	2	39.75	9.945686	25.02059
H5N7	3	28.75	0.5	1.73913
H3N8	4	33.25	3.685557	11.08438
H3N7	5	20.25	2.986079	14.74607
H3N7	6	25.75	4.924429	19.124
H4N8	7	25.75	2.217356	8.61109
H5N7	8	24	3.162278	13.17616
H5N5	9	24.75	2.061553	8.329506
H3N7	10	19.75	2.061553	10.43824
H9N2	11	43.25	3.5	8.092486
H4N4	12	17.25	4.193249	24.30869
H4N4	13	43.5	9.036961	20.77462
H3N5	14	23	0.816497	3.549985
H7N2	15	249.5	54.75095	21.94427
H4N8	16	27.75	4.112988	14.82158
H5N5	17	21	4.546061	21.64791
H4N5	18	22.75	2.217356	9.746619
H4N6	19	31.5	9.983319	31.69308
H5N5	20	25.25	3.685557	14.59627
H5N4	21	20.75	1.892969	9.122744
H5N4Sia1	22	10711	853.4112	7.967615
H6N3	23	21.75	3.593976	16.52403
H8N2	24	52	16.83251	32.37021
H4N4	25	24.5	2.516611	10.27188
H5N7	26	27.5	2.380476	8.656277
H5N4	27	23	3.91578	17.02513
H5N4Sia2	28	8749.5	832.8587	9.518929
H5N4	29	22.75	0.5	2.197802
H6N3	30	22.25	4.5	20.22472