

Employment of tandem MS for the accurate and specific identification of oligosaccharide structures

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Captions for the Supplementary Figures

Figure S1. A two-dimensional plot with m/z on the vertical and nanoLC MS retention time on the horizontal axis. Second panel correspond to a partially zoomed in version.

Figure S2. The MassHunter interface for the automated compound search in the structural library.

Figure S3. NanoLC chromatograms of pooled HMOs (upper panel), from a single human sample (middle panel) and from a single orangutan sample (lower panel) with some structures annotated. a) Base peak chromatograms from MS scan and b) MS/MS scan.

Figure S1

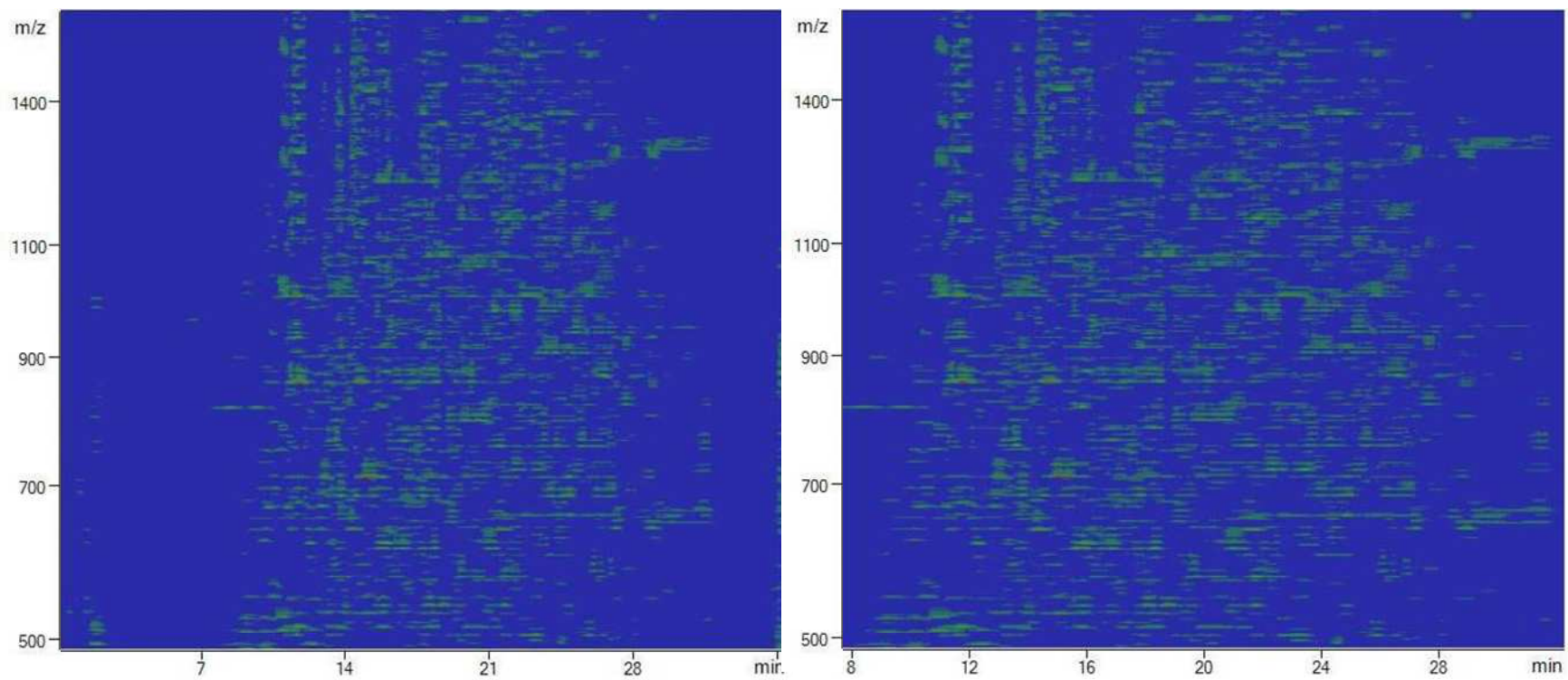


Figure S2.

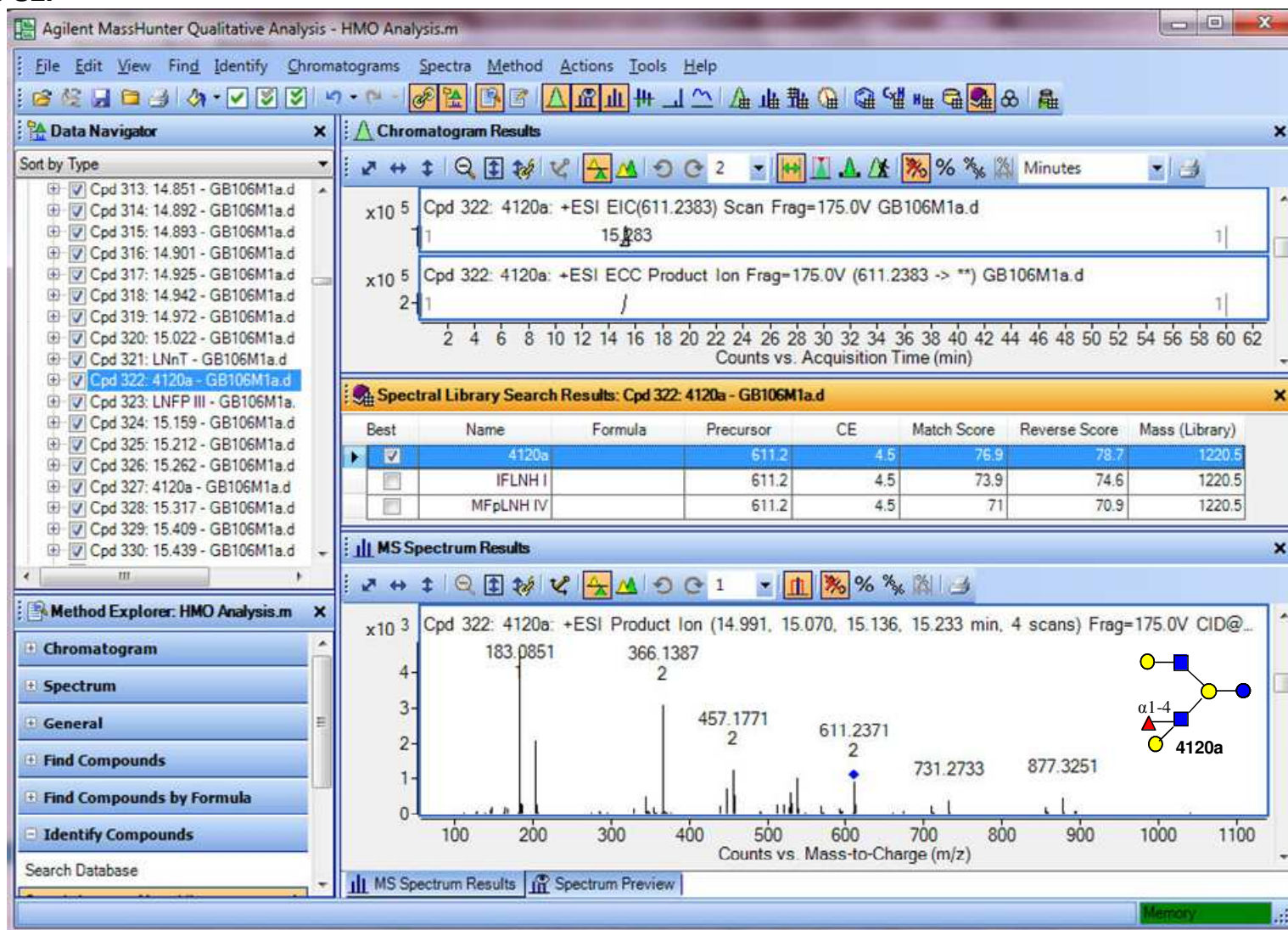
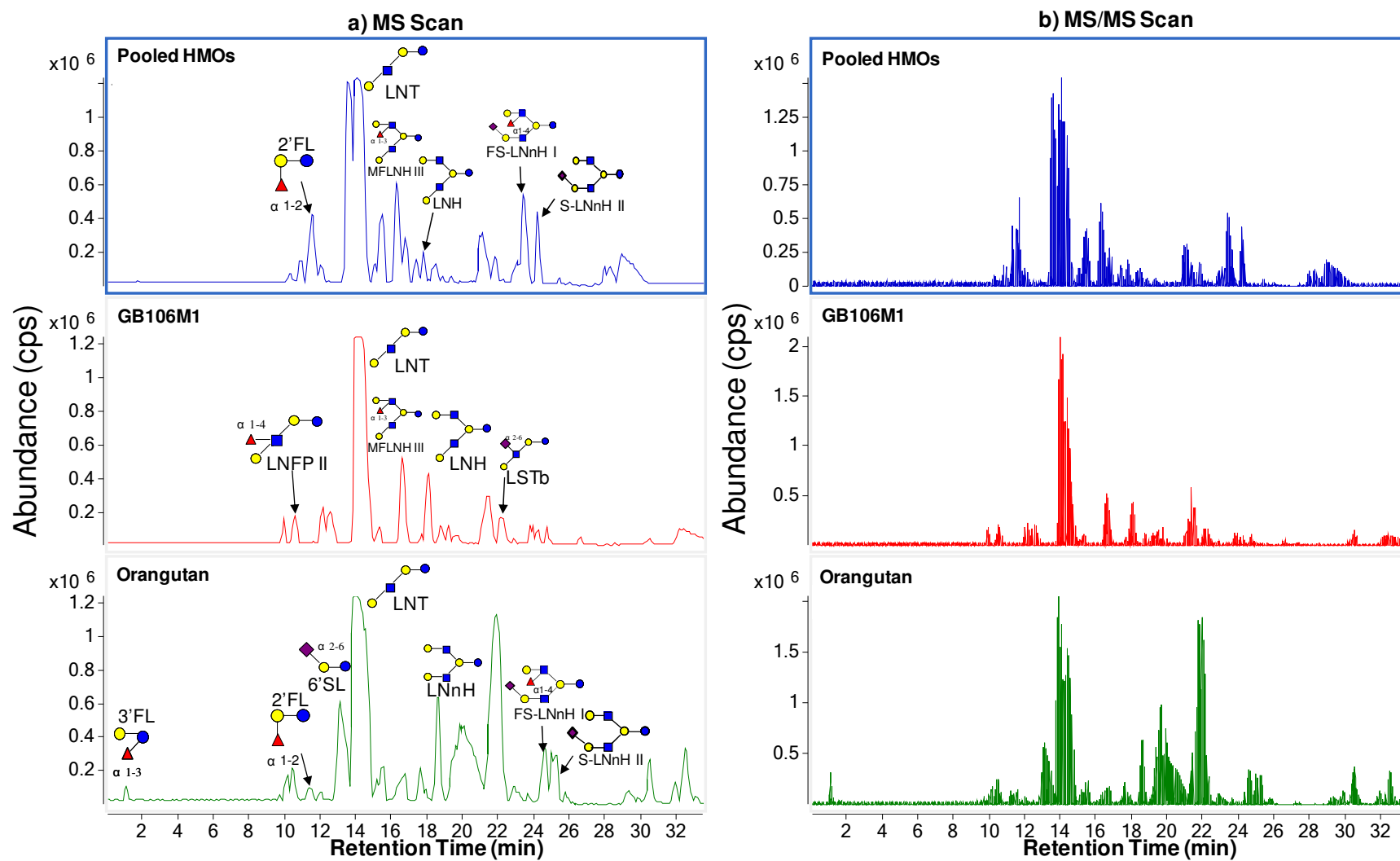


Figure S3.



Captions for the Supplementary Tables

Table S1. The automated search result for isomer a) MFLNH III and b) IFLNH III.

Table S2. The automated search result for milk oligosaccharides from a single mother with five consecutive runs.

Table S3. a) The OS found in Orangutan milk sample using retention time and accurate mass from Chip/Q-TOF; b) The automated library search result for Orangutan OS employing the HMO database.

Table S1**a)**

Name	Precursor	CE	Match Score	Reverse Score	Mass (Library)
MFLNH III	611.2	4.5	80.5	82.4	1220.5
MFLNH I	611.2	4.5	78.4	81.7	1220.5
IFLNH III	611.2	4.5	64.9	69.7	1220.5

b)

Name	Precursor	CE	Match Score	Reverse Score	Mass (Library)
IFLNH III	611.2	4.5	65.6	75	1220.5

Table S2

a)

Cpd	Name	RT	m/z	Mass (DB)	Score (DB)	Hits (DB)	Height
1	2'FL	11.44	491.196	490.19	71.75	2	8693
2	3'SL	19.75	636.235	635.227	88.17	1	81133
3	4021b	26.04	683.756	1365.492	64.94	1	28947
4	4120a	14.96	611.238	1220.454	75.61	4	359673
	4120a	15.29	1221.463	1220.454	81.72	3	103253
5	4121b	20.95	756.781	1511.55	65.46	2	26685
6	5130a	19.18	793.8	1585.586	81.17	4	370684
7	5130b	20.19	793.8	1585.586	71.27	4	26872
8	6041a	26.42	1048.885	2095.756	61.35	1	9594
9	6140a	23.41	976.365	1950.719	68.82	1	57286
10	6'SL	12.48	636.235	635.227	87.65	1	216619
	6'SL	12.95	636.235	635.227	81.02	1	41129
11	DFLNHc	20.25	684.263	1366.512	67.13	1	14065
12	DFLNO I	17.28	866.831	1731.644	73.14	5	35032
13	DSLNT	32.07	646.738	1291.455	79.87	1	208210
	DSLNT	32.69	646.738	1291.455	76.8	1	171906
	DSLNT	33.31	646.738	1291.455	73.79	1	131312
14	F-LNH II, MFLNH III	16.54	611.238	1220.454	81.68	3	849074
15	F-LSTc	21.21	1147.423	1146.417	69.22	1	9003
16	FS-LNH I	23.28	756.781	1511.55	66.36	1	27511
17	FS-LNnH I	23.96	756.781	1511.55	76.55	2	202029
18	FS-LNO	24.87	939.345	1876.682	69.37	1	22644
19	IFLNH III	16.64	1221.463	1220.454	85.64	5	238055
20	LNDFH II	10.25	1002.38	1001.38	71.23	1	19200
21	LNFP II	10.19	856.326	855.322	78.08	1	430899
	LNFP II	10.82	856.326	855.322	78.9	1	457871
22	LNFP III	13.85	856.326	855.322	81.14	2	161192
	LNFP III	14.32	856.326	855.322	77.43	2	39863
23	LNH	18.55	538.209	1074.396	79.19	2	227167
24	LNnH	18.82	1075.401	1074.396	73.04	2	22827
	LNnH	18.99	538.209	1074.396	68.75	2	88255
25	LNT	13.94	710.272	709.264	92.95	1	5820999
26	LNnT	14.68	710.272	709.264	92.47	1	3592858
27	LSTa	25.30	501.188	1000.359	65.14	2	30637
	LSTa	25.27	1001.367	1000.359	84.59	1	45151
28	LSTb	22.07	1001.367	1000.359	82	3	260778
	LSTb	22.63	1001.367	1000.359	78.4	3	125809
29	p-LNH	22.12	538.209	1074.396	65.86	2	67537

30	S-LNH	23.77	683.756	1365.492	78.74	1	166481
31	SLNnH II	24.51	683.756	1365.492	73.21	2	151093

b)

Cpd	Name	RT	m/z	Mass (DB)	Score (DB)	Hits (DB)	Height
1	2'FL	11.47	491.196	490.19	74.65	1	7853
2	3'SL	19.59	636.236	635.227	89.2	1	79817
	3'SL	20.06	636.236	635.227	84.31	1	25406
3	4021b	26.14	683.755	1365.492	62.64	1	17644
4	4120a	15.16	1221.464	1220.454	81.81	3	94735
5	4121b	20.98	756.783	1511.55	70.64	2	24543
6	5130a	19.10	793.8	1585.586	81.75	4	305810
7	5130b	20.22	793.8	1585.586	70.02	5	31978
8	6041a	26.50	1048.88	2095.756	60.93	1	8487
9	6140a	23.36	976.365	1950.719	69.41	1	78922
10	6'SL	12.47	636.236	635.227	86.56	1	245059
	6'SL	12.85	636.236	635.227	83.5	1	140403
11	DFLNHa	13.80	684.264	1366.512	62.04	2	25199
12	DFLNHc	20.10	684.264	1366.512	60.08	1	12565
13	DFLNO I	17.15	866.829	1731.644	70.5	5	27721
14	DSLNT	32.56	646.738	1291.455	77.31	1	140734
	DSLNT	33.17	1292.461	1291.455	71.71	1	16726
	DSLNT	33.34	646.738	1291.455	79.72	1	212402
	DSLNT	34.01	646.738	1291.455	73.51	1	136539
15	F-LNH II, MFLNH III	16.66	611.238	1220.454	80.75	4	909918
16	F-LSTc	21.14	574.216	1146.417	60.85	1	9815
	F-LSTc	21.22	1147.421	1146.417	65.48	1	8668
17	FS-LNH I	23.55	756.783	1511.55	66.38	2	27755
18	FS-LNH II	26.36	756.783	1511.55	62.15	1	14874
19	FS-LNnH I	24.27	756.783	1511.55	78.48	2	118321
20	FS-LNO	24.88	939.344	1876.682	64.46	1	20921
21	IFLNH III	16.58	1221.464	1220.454	84.58	5	197372
22	LNDFH II	10.39	1002.382	1001.38	71.43	1	17219
23	LNFP II	10.12	856.327	855.322	77.69	1	330563
	LNFP II	10.86	856.327	855.322	78.08	1	90536
24	LNFP III	13.76	856.327	855.322	83.02	2	144211
	LNFP III	14.17	856.327	855.322	79.72	2	31973
25	LNH	18.69	1075.403	1074.396	71	2	23067
26	LNnH	19.78	538.209	1074.396	72.65	2	30956
27	LNT	13.83	710.271	709.264	93.26	1	4866233
	LNT	14.63	710.271	709.264	91.7	1	2699338

28	LSTa	25.43	1001.365	1000.359	85.13	1	43879
	LSTa	25.45	501.189	1000.359	61.6	2	25751
29	LSTb	22.11	1001.365	1000.359	84.38	3	336822
	LSTb	22.72	1001.365	1000.359	77.74	3	94283
	LSTb	22.85	501.189	1000.359	62.84	1	32778
30	LSTc	22.38	501.189	1000.359	69.41	3	82662
31	S-LNH	23.77	683.755	1365.492	73.89	1	128768
32	SLNnH II	24.43	683.755	1365.492	75.77	3	154222

c)

Cpd	Name	RT	m/z	Mass (DB)	Score (DB)	Hits (DB)	Height
1	2'FL	11.44	491.195	490.19	77.13	2	7267
2	3'FL	1.64	491.195	490.19	61.71	1	4989
3	3'SL	20.03	636.238	635.227	89.7	1	91224
	3'SL	20.40	636.238	635.227	73.3	1	11003
4	4021b	26.19	683.757	1365.492	62.94	1	24185
5	4120a	14.86	611.238	1220.454	74.07	3	284226
	4120a	15.28	1221.464	1220.454	82.77	3	94027
6	4121b	20.96	756.783	1511.55	67.73	2	28110
7	5130a	19.13	793.8	1585.586	82.69	4	374543
8	6041a	26.56	1048.883	2095.756	64.05	1	11338
9	6140a	23.43	976.367	1950.719	68.56	1	72239
10	6'SL	12.58	636.238	635.227	87.2	1	221496
	6'SL	13.03	636.238	635.227	77.41	1	58726
11	DFLNHa	13.81	684.264	1366.512	60.59	2	25658
12	DFLNHc	20.16	684.264	1366.512	62.85	2	12423
13	DFLNO I	17.20	866.829	1731.644	73.43	5	33535
14	DSLNT	33.17	646.738	1291.455	78.3	1	40249
	DSLNT	33.83	646.738	1291.455	78.39	1	176237
	DSLNT	34.07	1292.46	1291.455	71.04	1	13909
15	F-LNH I, MFLNH I	17.06	611.238	1220.454	71.41	4	277540
16	F-LNH II, MFLNH III	16.43	611.238	1220.454	78.95	3	746213
17	F-LSTc	21.32	1147.423	1146.417	61.32	1	8547
18	FS-LNH I	23.59	756.783	1511.55	68.75	2	27915
19	FS-LNH II	26.40	756.783	1511.55	62.27	1	11099
20	FS-LNnH I	24.27	756.783	1511.55	79.8	2	229865
21	FS-LNO	24.99	939.347	1876.682	66.64	1	21619
22	IFLNH III	16.56	1221.464	1220.454	84.14	4	230174
23	LNDFH II	10.17	1002.383	1001.38	72.13	1	17420
24	LNFP II	10.57	856.327	855.322	78.31	1	434466

	LNFP II	11.07	856.327	855.322	77.82	1	10358
25	LNFP III	13.78	856.327	855.322	83.34	2	176782
	LNFP III	14.40	856.327	855.322	72.95	2	20982
26	LNH	17.85	538.208	1074.396	71.48	1	588731
27	LNnH	18.04	1075.403	1074.396	76.2	2	156645
	LNnH	18.51	538.208	1074.396	78.34	2	213243
28	LNT	14.16	710.273	709.264	93.49	1	5263069
29	LNnT	14.80	710.273	709.264	86.63	1	1154642
30	LSTa	25.45	1001.366	1000.359	86.72	1	49653
31	LSTb	22.32	1001.366	1000.359	84.06	3	373052
	LSTb	22.40	501.188	1000.359	75.42	3	89080
	LSTb	22.83	1001.366	1000.359	78.17	3	89600
32	S-LNH	23.77	683.757	1365.492	78.39	1	175437
33	SLNnH II	24.49	683.757	1365.492	78.54	2	176593

d)

Cpd	Name	RT	m/z	Mass (DB)	Score (DB)	Hits (DB)	Height
1	3'FL	1.63	491.198	490.19	74.83	2	6182
2	2'FL	11.41	491.198	490.19	73.36	2	9500
3	3'SL	20.07	636.236	635.227	89.87	1	82469
	3'SL	20.45	636.236	635.227	84.54	1	23549
4	4021b	25.76	683.754	1365.492	64.58	1	27102
	4021b	26.47	683.754	1365.492	62.4	1	22505
5	4120a	15.43	611.239	1220.454	72.19	4	332646
6	4121b	21.01	756.783	1511.55	72.27	2	27779
7	5130a	19.14	793.799	1585.586	82.3	4	439503
8	6140a	23.33	976.364	1950.719	65.62	1	53212
9	6'SL	12.65	636.236	635.227	87.16	1	292326
	6'SL	13.18	636.236	635.227	70.64	1	86984
10	DFLNHb	13.78	684.262	1366.512	61.63	2	30395
11	DFLNHc	20.21	684.262	1366.512	60.39	1	11707
12	DFLNO I	17.14	866.827	1731.644	75.1	5	35555
13	DSLNT	34.09	646.737	1291.455	75.39	1	46776
	DSLNT	34.73	646.737	1291.455	82.03	1	85841
	DSLNT	34.88	1292.46	1291.455	63.36	1	7528
14	F-LNH II, MFLNH III	16.24	611.239	1220.454	79.83	3	1016071
	F-LNH II, MFLNH III	16.53	1221.463	1220.454	84.22	4	247009
	F-LNH II, MFLNH III	16.83	611.239	1220.454	78.76	5	1181284
15	F-LSTc	21.43	1147.42	1146.417	68.18	1	7490
16	FS-LNH I	23.73	756.783	1511.55	71.54	2	25921

17	FS-LNH II	26.39	756.783	1511.55	63.34	1	16642
18	FS-LNnH I	24.36	756.783	1511.55	75.83	2	201603
19	FS-LNO	24.88	939.344	1876.682	71.22	1	23716
20	IFLNH III	17.44	611.239	1220.454	77.43	4	134557
	IFLNH III	17.66	1221.463	1220.454	74.47	2	30456
21	LNDFH II	10.08	1002.38	1001.38	71.63	1	15519
22	LNFP II	10.62	856.327	855.322	78.37	1	494396
	LNFP II	11.05	856.327	855.322	67.18	1	5206
23	LNFP III	13.72	856.327	855.322	82.77	2	175578
	LNFP III	14.33	856.327	855.322	77.76	1	16974
24	LNH	17.72	538.208	1074.396	73.31	2	1089701
	LNH	17.99	1075.401	1074.396	80.25	2	169560
25	LNnH	18.59	1075.401	1074.396	75.52	2	28543
26	LNT	13.71	710.272	709.264	92.39	1	4648647
27	LNnT	14.30	710.272	709.264	92.81	1	4434954
28	LSTa	25.58	1001.366	1000.359	84.78	1	50133
29	LSTb	22.29	1001.366	1000.359	81.25	3	265552
	LSTb	22.74	1001.366	1000.359	82.62	3	152742
30	LSTc	22.46	501.189	1000.359	72.16	3	127703
31	MFpLNH IV	15.21	1221.463	1220.454	83.53	3	97227
32	S-LNH	23.81	683.754	1365.492	79.48	1	100683
33	SLNnH II	24.42	683.754	1365.492	74.38	3	67150
	SLNnH II	25.06	683.754	1365.492	73.21	1	202694

e)

Cpd	Name	RT	m/z	Mass (DB)	Score (DB)	Hits (DB)	Height
1	2'FL	11.38	491.194	490.19	78.87	2	9100
2	3'FL	1.62	491.194	490.19	72.43	2	6507
3	3'SL	20.27	636.236	635.227	89.27	1	86879
4	4021b	26.20	683.754	1365.492	69.07	1	25236
5	4120a	14.90	611.238	1220.454	75.83	5	396081
	4120a	15.37	611.238	1220.454	80.27	6	339535
6	4121b	21.03	756.782	1511.55	68.41	2	27103
7	5130a	19.13	793.798	1585.586	83.73	4	234966
	5130a	19.90	793.798	1585.586	72.47	4	23352
8	6140a	23.25	976.365	1950.719	69.23	1	58492
9	6'SL	12.68	636.231	635.227	87.09	1	290074
	6'SL	13.25	636.236	635.227	82.98	1	42080
10	DFLNHb	13.76	684.262	1366.512	61	1	25403
11	DFLNO I	17.19	866.827	1731.644	69.05	5	38082

12	DSLNT	29.79	646.736	1291.455	81.41	1	37489
	DSLNT	30.48	646.736	1291.455	77.36	1	22213
	DSLNT	31.12	646.736	1291.455	71.98	1	13608
13	F-LNH II, MFLNH III	16.26	611.238	1220.454	78.97	3	1105525
	F-LNH II, MFLNH III	16.58	1221.464	1220.454	85.22	5	202896
	F-LNH II, MFLNH III	16.90	611.238	1220.454	73.49	4	919127
14	F-LSTc	21.38	1147.42	1146.417	68.39	1	8149
15	FS-LNH I	23.86	756.782	1511.55	74.49	4	47172
16	FS-LNH II	26.47	756.782	1511.55	62.73	1	11939
17	FS-LNnH I	24.42	756.782	1511.55	74.32	2	289317
18	FS-LNO	24.83	939.344	1876.682	70.22	1	24847
19	IFLNH III	17.52	611.238	1220.454	72.26	2	140974
	IFLNH III	17.71	1221.464	1220.454	63.27	1	22074
20	LNDFH II	10.12	1002.383	1001.38	71.45	1	19572
21	LNFP II	10.45	856.326	855.322	78.26	2	460447
	LNFP II	11.00	856.326	855.322	77.17	1	10258
22	LNFP III	13.74	856.326	855.322	82.28	2	158204
	LNFP III	14.40	856.326	855.322	74.04	2	17177
23	LNH	18.04	538.207	1074.396	83.19	2	661642
24	LNnH	18.66	1075.401	1074.396	69.76	2	26314
	LNnH	18.69	538.207	1074.396	75.71	2	201956
25	LNT	13.74	710.272	709.264	92.78	1	5191929
26	LNnT	14.38	710.272	709.264	93.44	1	4720548
27	LSTa	25.58	1001.366	1000.359	87.48	1	44727
	LSTa	25.78	501.188	1000.359	77.08	2	38408
28	LSTb	22.37	1001.366	1000.359	83.54	3	294053
	LSTb	22.95	1001.366	1000.359	80.93	3	65627
29	LSTc	22.46	501.188	1000.359	72.24	3	109861
30	MFpLNH IV	15.17	1221.464	1220.454	84.5	3	91364
31	S-LNH	23.75	683.754	1365.492	79.32	1	244024
	S-LNH	24.04	1366.496	1365.492	71.27	2	9207
32	SLNnH II	24.52	683.754	1365.492	73.68	3	210021
	SLNnH II	25.17	683.754	1365.492	70.1	1	146542

Table S3a

Cpd	Mass (exp)	Mass (cal)	Error	Hex	Fuc	HexNAc	NeuAc	NeuGc	RT	Abund.
1	490.1887	490.1897	-0.001	2	1				1.65	582284
2	490.1906	490.1897	0.0009	2	1				11.41	5849170
3	490.1897	490.1897	0	2	1				13.80	525393
4	506.1858	506.1846	0.0012	3					10.13	4928841
5	506.1841	506.1846	-5E-04	3					14.12	206650
6	635.231	635.2272	0.0038	2			1		13.17	20769672
7	635.2312	635.2272	0.004	2			1		20.10	16991408
8	651.2249	651.2221	0.0028	2				1	12.91	1381356
9	651.227	651.2221	0.0049	2				1	19.58	1711533
10	652.2548	652.2425	0.0123	3	1				20.03	1698727
11	676.2517	676.2538	-0.002	1		1	1		12.98	117598
12	709.2629	709.264	-0.001	3		1			10.49	222000
13	709.2658	709.264	0.0018	3		1			12.02	1903066
14	709.2679	709.264	0.0039	3		1			14.08	113585448
15	781.2829	781.2851	-0.002	2	1		1		17.44	567460
16	797.2789	797.28	-0.001	2	1			1	23.89	88937
17	838.3065	838.3066	-1E-04	2		1	1		23.18	408372
18	855.3261	855.3219	0.0042	3	1	1			10.48	6385175
19	855.3238	855.3219	0.0019	3	1	1			13.63	1413876
20	871.321	871.3168	0.0042	4		1			15.49	5851423
21	912.3479	912.3434	0.0045	3		2			17.91	991866
22	1000.3657	1000.3594	0.0063	3		1	1		22.92	5013286
23	1000.3655	1000.3594	0.0061	3		1	1		23.14	2208376
24	1016.3532	1016.3543	-0.001	3		1		1	22.72	381166
25	1017.3778	1017.3747	0.0031	4	1	1			13.21	1512615
26	1074.4055	1074.3962	0.0093	4		2			15.14	546984
27	1074.4056	1074.3962	0.0094	4		2			18.61	27681444
28	1074.4013	1074.3962	0.0051	4		2			21.71	1361048
29	1162.4115	1162.4122	-7E-04	4		1	1		22.40	157723
30	1220.4546	1220.4541	0.0005	4	1	2			12.40	168922
31	1220.4696	1220.4541	0.0155	4	1	2			15.17	5590550
32	1220.4501	1220.4541	-0.004	4	1	2			16.60	123765
33	1220.4674	1220.4541	0.0133	4	1	2			17.62	7873134
34	1291.4528	1291.4548	-0.002	3		1	2		25.58	473713
35	1365.504	1365.4916	0.0124	4		2	1		25.02	9484554
36	1366.5242	1366.512	0.0122	4	2	2			13.74	2566509
37	1381.498	1381.4865	0.0115	4		2		1	24.92	708389
38	1382.5057	1382.5069	-0.001	5	1	2			16.61	175307

39	1439.5317	1439.5284	0.0033	5		3		23.04	244919
40	1439.5401	1439.5284	0.0117	5		3		23.66	2146217
41	1511.548	1511.5495	-0.002	4	1	2	1	21.26	172680
42	1511.5599	1511.5495	0.0104	4	1	2	1	24.55	7155953
43	1527.549	1527.5444	0.0046	5		2	1	24.45	607964
44	1585.583	1585.5863	-0.003	5	1	3		19.03	146124
45	1585.5885	1585.5863	0.0022	5	1	3		20.42	166548
46	1656.5943	1656.587	0.0073	4		2	2	34.23	338378
47	1731.6421	1731.6442	-0.002	5	2	3		16.94	175233
48	1804.6575	1804.6606	-0.003	6		4		24.35	307189
49	1876.6776	1876.6817	-0.004	5	1	3	1	24.95	127137

Table S3b

Cpd	Name	RT	<i>m/z</i>	Mass (DB)	Score (DB)	Hits (DB)	Height
1	3'FL	1.67	491.1962	490.1897	87.87	2	65222
2	2'FL	11.28	491.1962	490.1897	86.72	2	235638
	2'FL	11.77	491.1962	490.1897	85.76	2	97925
3	3'SL	19.98	636.2397	635.2272	91.12	1	1331767
	3'SL	20.85	636.2397	635.2272	90.58	1	940155
4	4120a	15.13	611.2378	1220.4541	81.5	5	236350
	4120a	15.16	1221.4623	1220.4541	80.17	5	57913
5	5130a	19.08	793.7991	1585.5863	70.77	4	19404
6	6'SL	13.08	636.2397	635.2272	90.13	1	738048
	6'SL	13.55	636.2397	635.2272	82.08	1	459420
7	6'SLN	13.01	677.2592	676.2538	80.03	2	9092
8	DFLNHc	13.77	684.2649	1366.512	64.31	2	282607
9	DFLNO I	17.00	866.8282	1731.6442	71	4	26563
10	DSLNT	29.68	646.7379	1291.4548	62.14	1	16409
11	F-LNH II, MFLNH III	16.48	611.2378	1220.4541	75.92	3	202881
12	FS-LNnH I	24.46	756.785	1511.5495	82.48	2	487771
13	IFLNH III	17.65	1221.4623	1220.4541	81.66	4	35088
	IFLNH III	17.69	611.2378	1220.4541	78.51	4	389281
14	LDFT	13.95	637.257	636.2476	81.89	1	349257
15	LNDFH II	10.34	1002.3856	1001.3798	68	1	9246
16	LNFP II	10.51	856.3296	855.3219	80.9	1	588506
17	LNH	17.75	538.2086	1074.3962	71.93	2	251438
18	LNnH	18.56	538.2086	1074.3962	85.3	2	778278
	LNnH	18.67	1075.4053	1074.3962	82.36	2	102000
19	LNT	13.97	710.2738	709.264	92.99	1	5870987
20	LNnT	14.70	710.2738	709.264	89.16	1	3198692
21	LSTb	22.80	1001.369	1000.3594	83.98	3	157786
	LSTb	23.39	1001.369	1000.3594	79.6	3	78250
	LSTb	23.41	501.1885	1000.3594	71.7	3	120728

22	LSTc	22.71	501.1885	1000.3594	73.06	2	92299
23	S-LNH	24.42	683.7568	1365.4916	71.36	3	29759
24	SLNnH II	25.07	683.7568	1365.4916	77.79	1	235450
