

Supplementary Information 2

O-Glycomics – Structural Elucidation

Integrated N- and O-Glycomics of Acute Myeloid Leukemia (AML) Cell Lines

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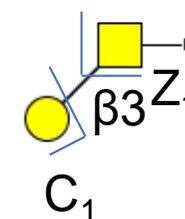
* Correspondence: c.huber@sbg.ac.at, m.wuhrer@lumc.nl, t.zhang@lumc.nl

Glycan 1

H1N1

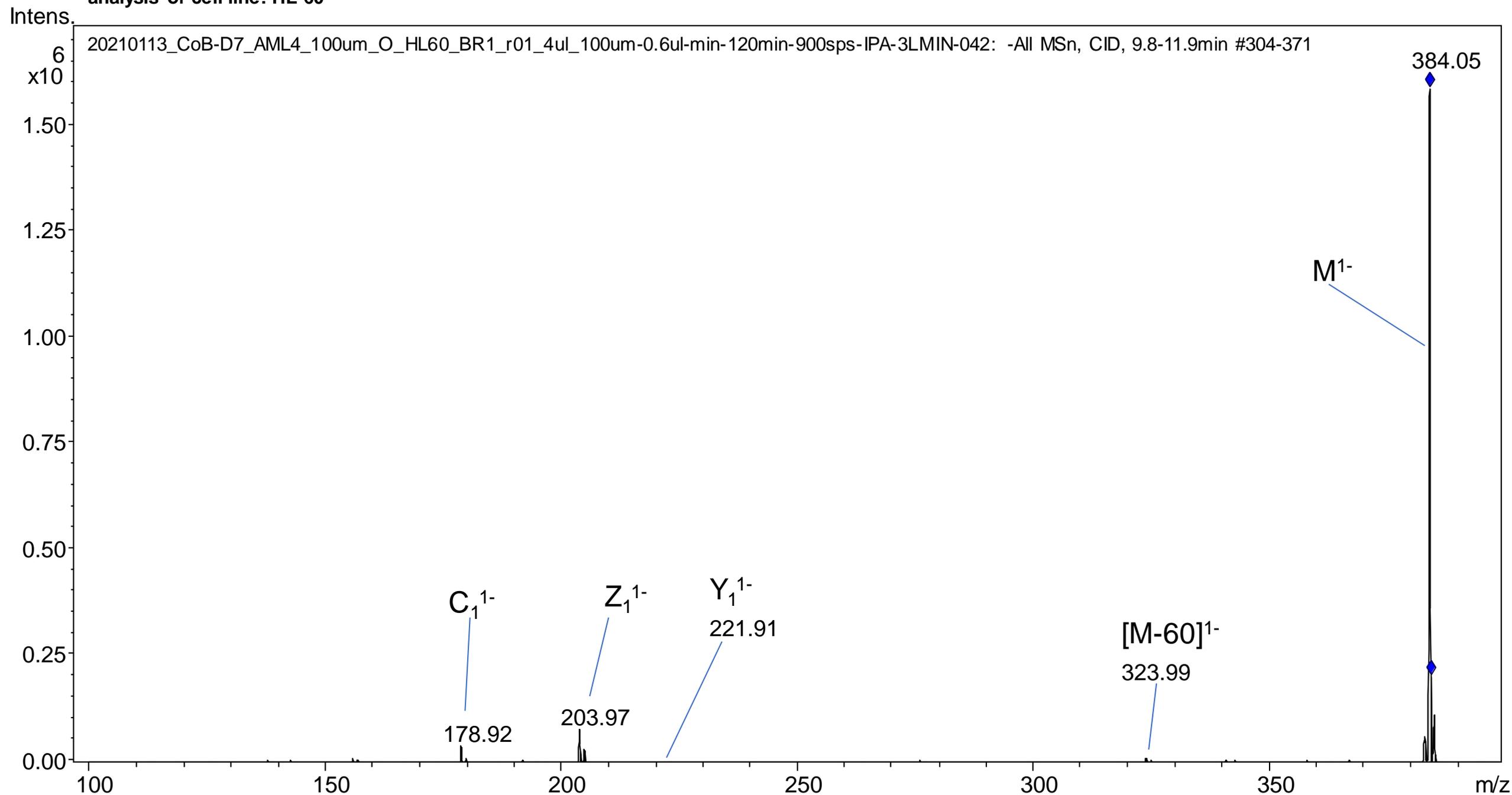
Monoisotopic mass: 385.16 Da
Charge observed: 1-
Theoretical ion: m/z 384.15
Observed ion: m/z 384.05
Mass deviation: m/z 0.10
Retention time: 10.6 min

UniCarb-DB: #171



Depicted MS² was obtained from analysis of cell line: HL-60

20210113_CoB-D7_AML4_100um_O_HL60_BR1_r01_4ul_100um-0.6ul-min-120min-900sps-IPA-3LMIN-042: -All MSn, CID, 9.8-11.9min #304-371



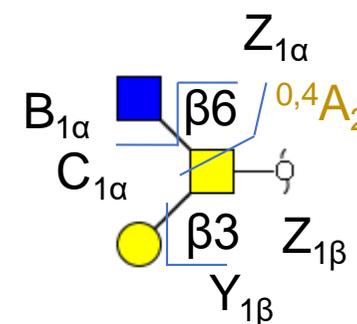
Glycan 2

H1N2

Depicted MS² was obtained from analysis of cell line: HL-60

Monoisotopic mass: 588.24 Da
Charge observed: 1-
Theoretical ion: *m/z* 587.23
Observed ion: *m/z* 587.19
Mass deviation: *m/z* 0.04
Retention time: 33.3 min

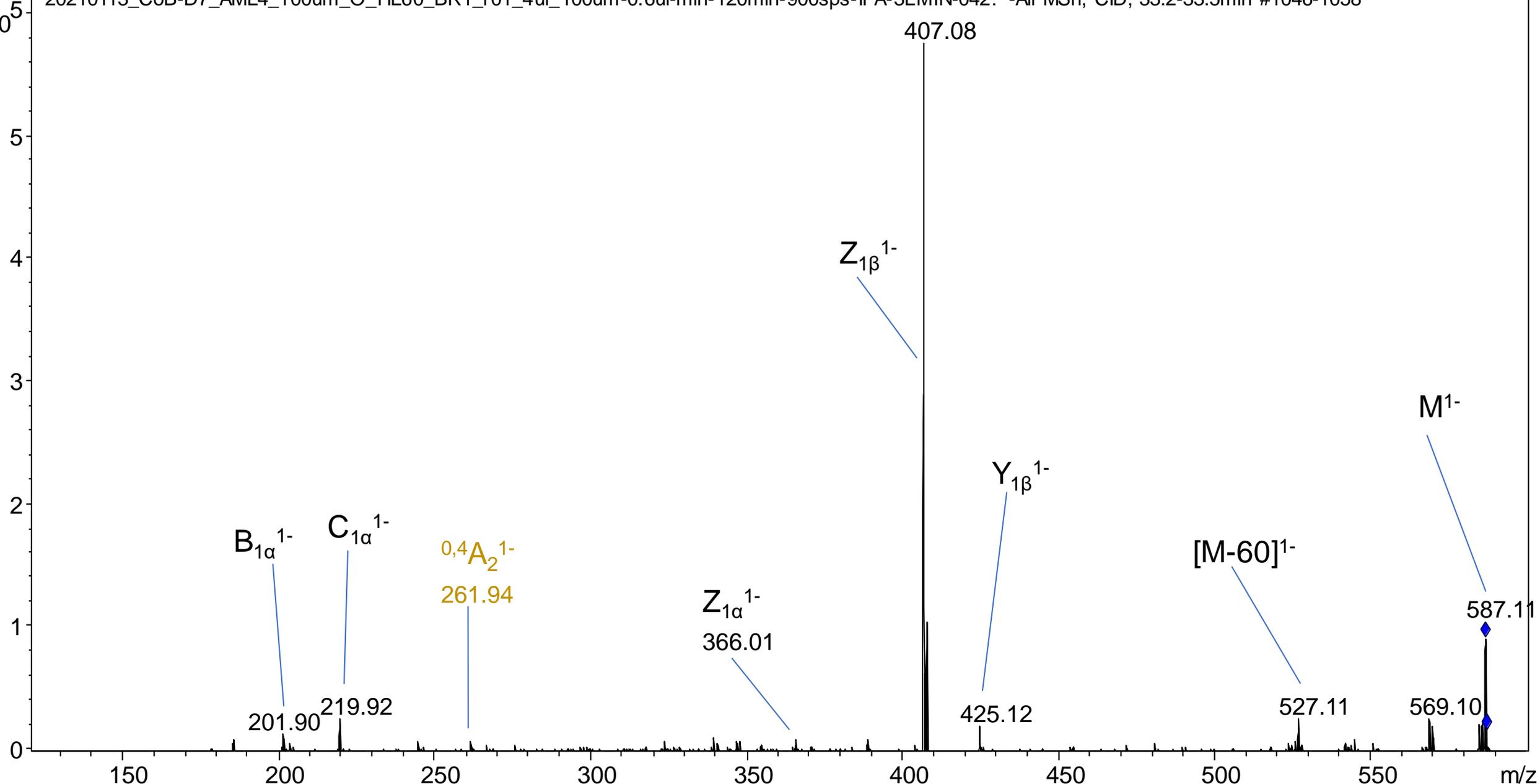
UniCarb-DB: #177



Intens.

x10⁵

20210113_CoB-D7_AML4_100um_O_HL60_BR1_r01_4ul_100um-0.6ul-min-120min-900sps-IPA-3LMIN-042: -All MSn, CID, 33.2-33.5min #1046-1058



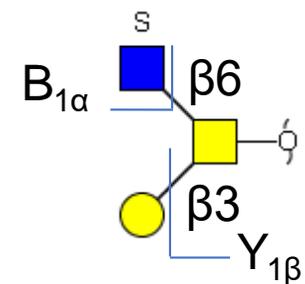
Glycan 3

H1N2Su1

Depicted MS² was obtained from analysis of cell line: MV4-11

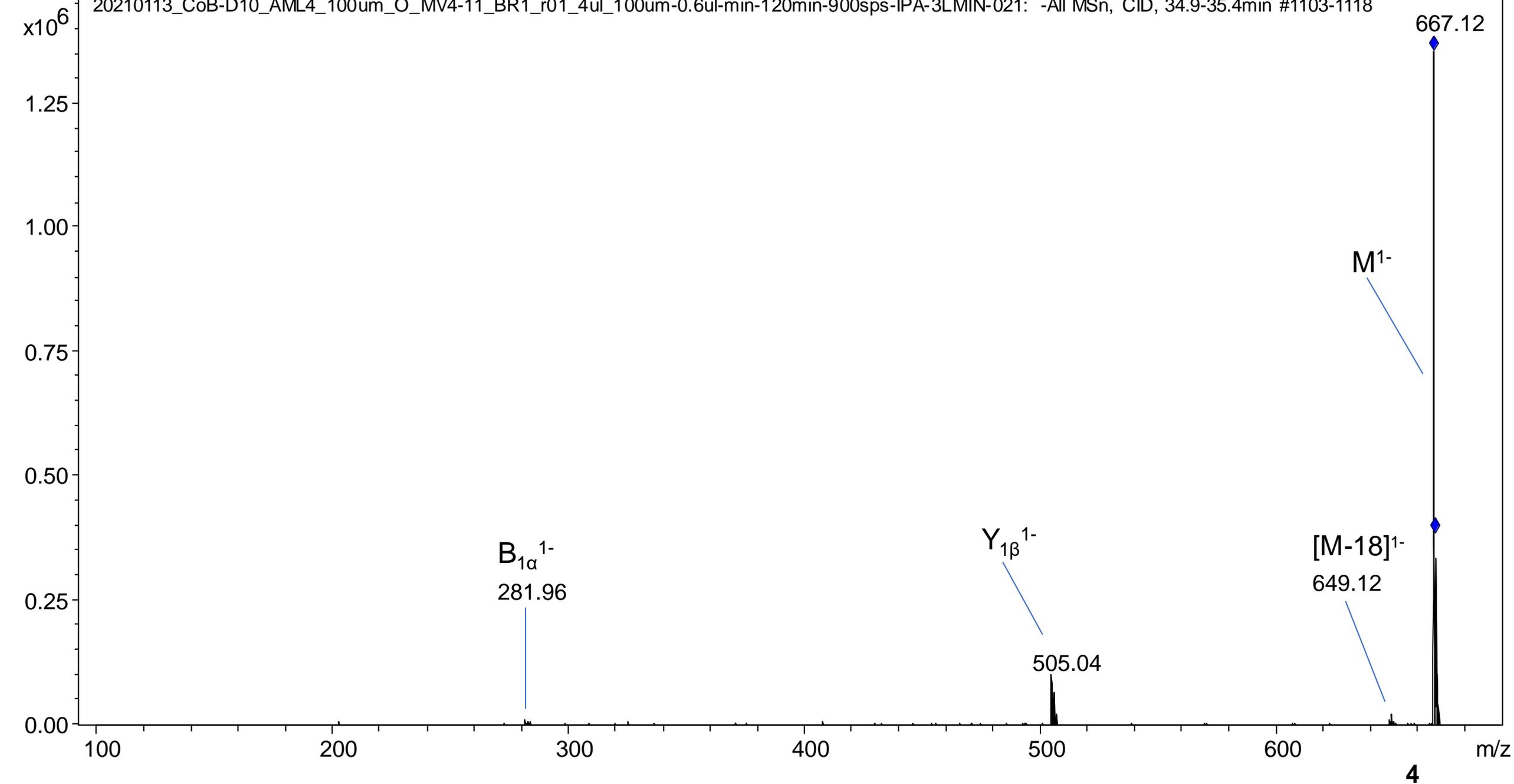
Monoisotopic mass: 668.20 Da
Charge observed: 1-
Theoretical ion: *m/z* 667.19
Observed ion: *m/z* 667.11
Mass deviation: *m/z* 0.07
Retention time: 35.0 min

UniCarb-DB: #3039



Intens.

20210113_CoB-D10_AML4_100um_O_MV4-11_BR1_r01_4ul_100um-0.6ul-min-120min-900sps-IPA-3LMIN-021: -All MSn, CID, 34.9-35.4min #1103-1118



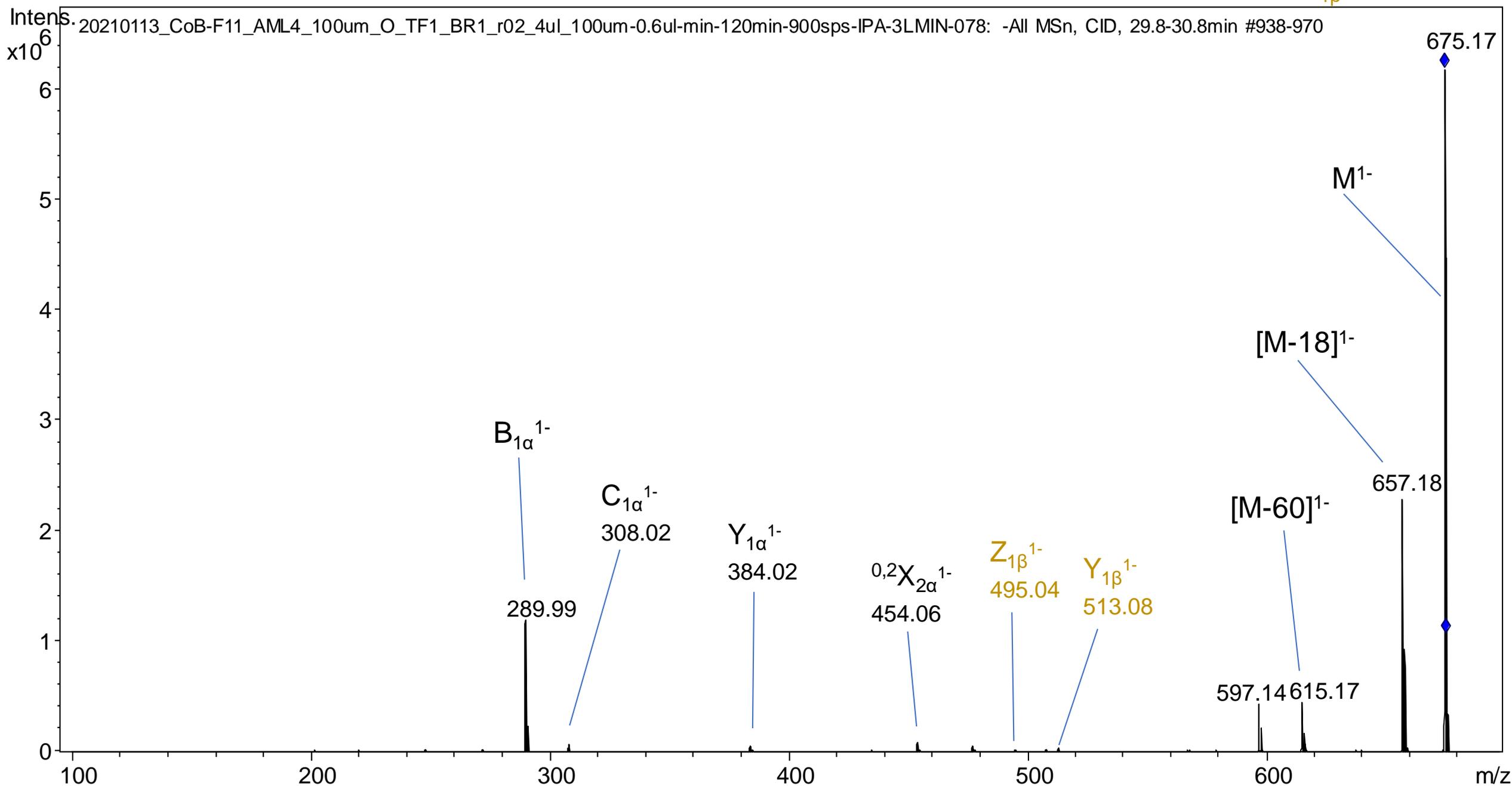
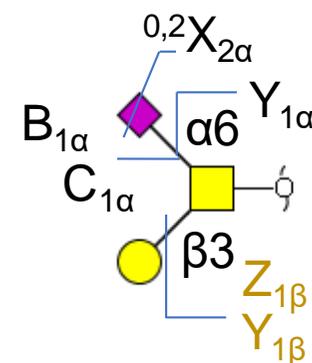
Glycan 4a

H1N1S1

Depicted MS² was obtained from analysis of cell line: TF-1

Monoisotopic mass: 676.26 Da
Charge observed: 1-
Theoretical ion: *m/z* 675.25
Observed ion: *m/z* 675.17
Mass deviation: *m/z* 0.08
Retention time: 30.1 min
Note: α -2,6 linkage of sialic acid confirmed by neuraminidase S and A treatment

UniCarb-DB: #41



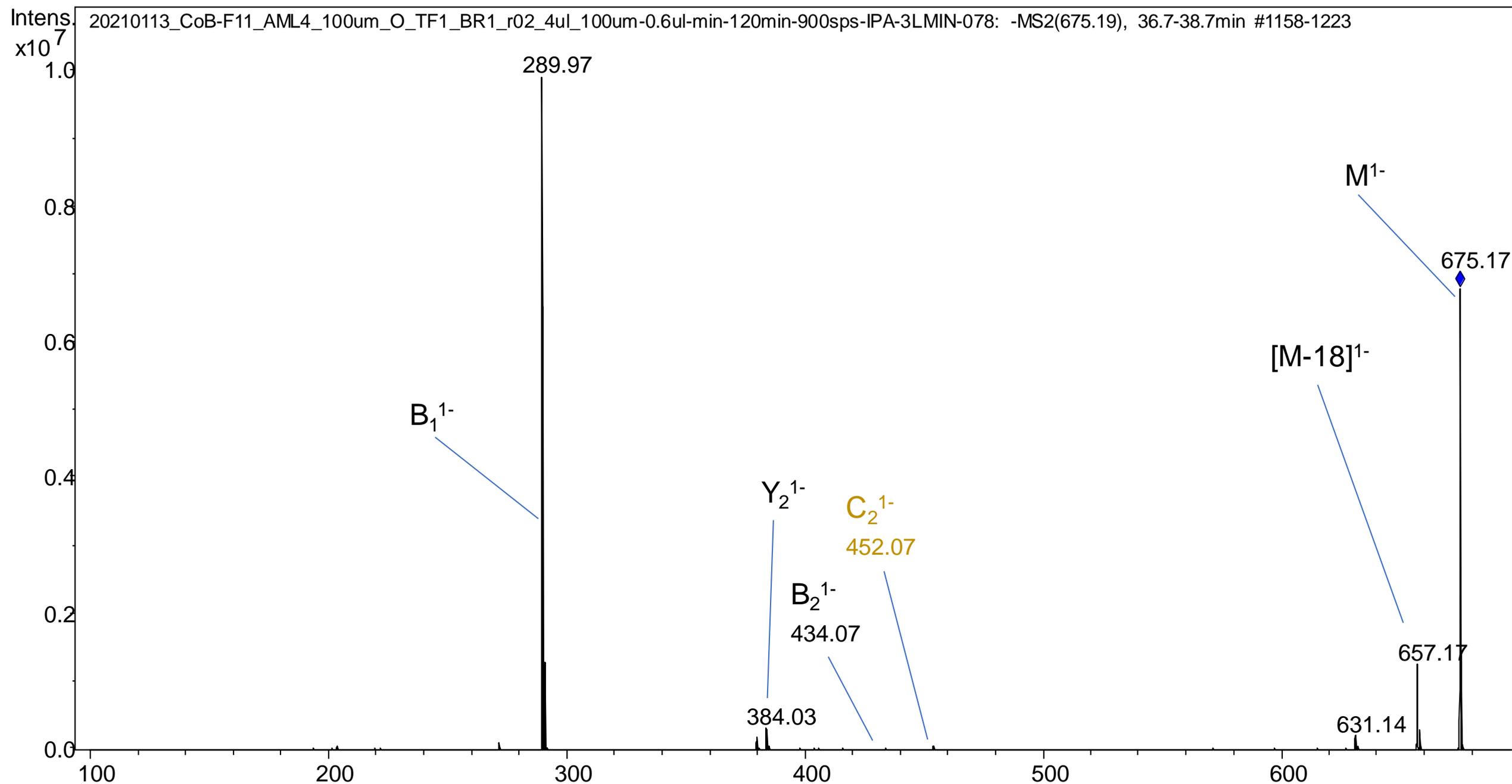
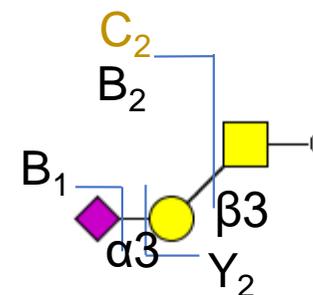
Glycan 4b

H1N1S1

Depicted MS² was obtained from analysis of cell line: TF-1

Monoisotopic mass: 676.26 Da
Charge observed: 1-
Theoretical ion: *m/z* 675.25
Observed ion: *m/z* 675.18
Mass deviation: *m/z* 0.07
Retention time: 37.1 min
Note: α -2,3 linkage of sialic acid confirmed by neuraminidase S and A treatment

UniCarb-DB: #146



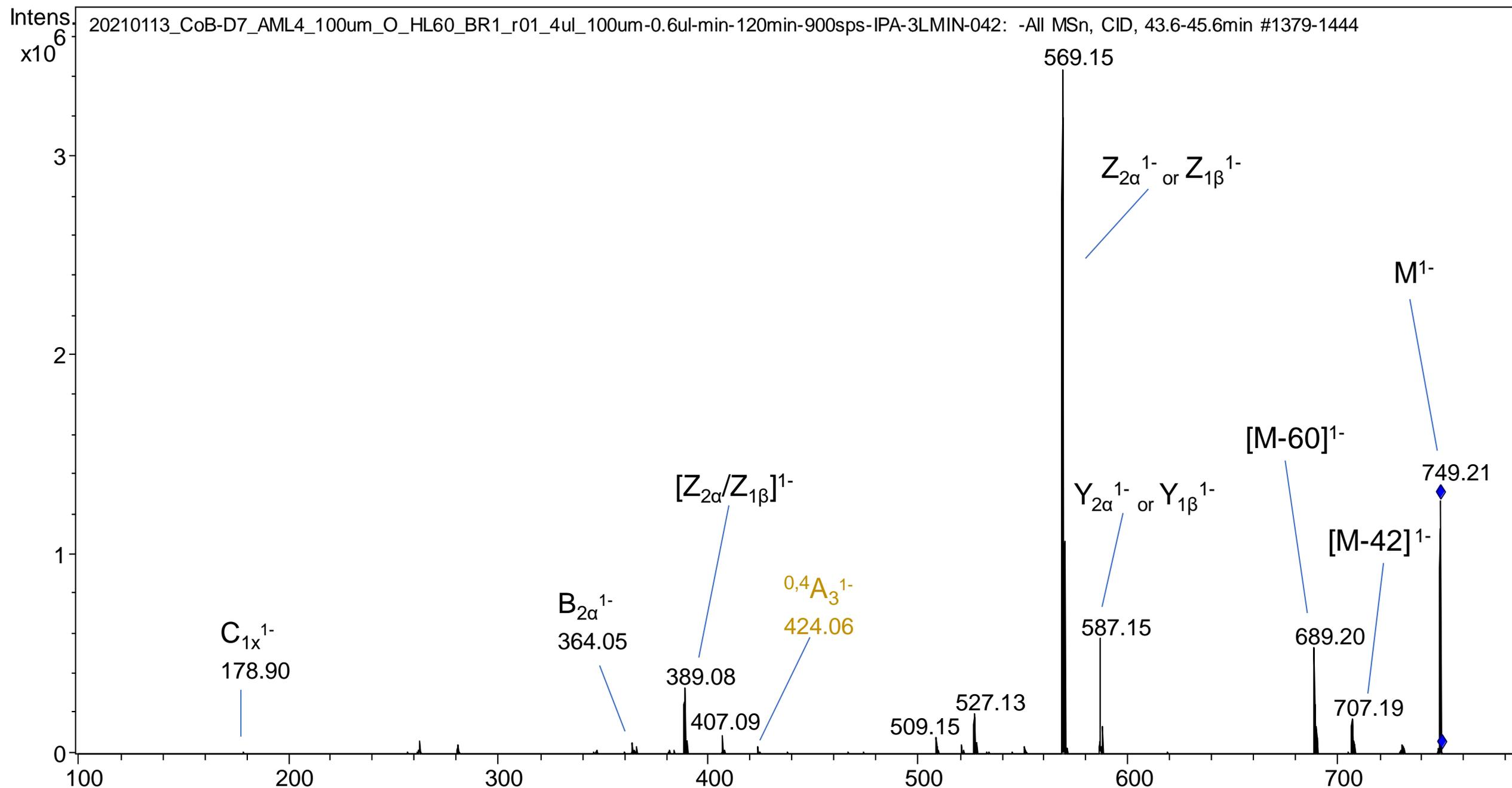
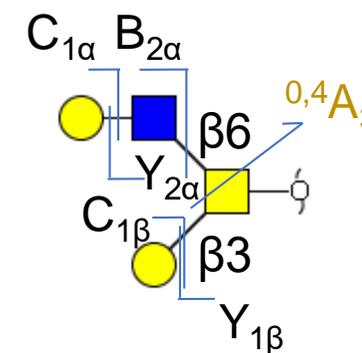
Glycan 5

H2N2

Depicted MS² was obtained from analysis of cell line: HL-60

Monoisotopic mass: 750.29 Da
Charge observed: 1-
Theoretical ion: *m/z* 749.28
Observed ion: *m/z* 749.23
Mass deviation: *m/z* 0.05
Retention time: 43.9 min

UniCarb-DB: #24



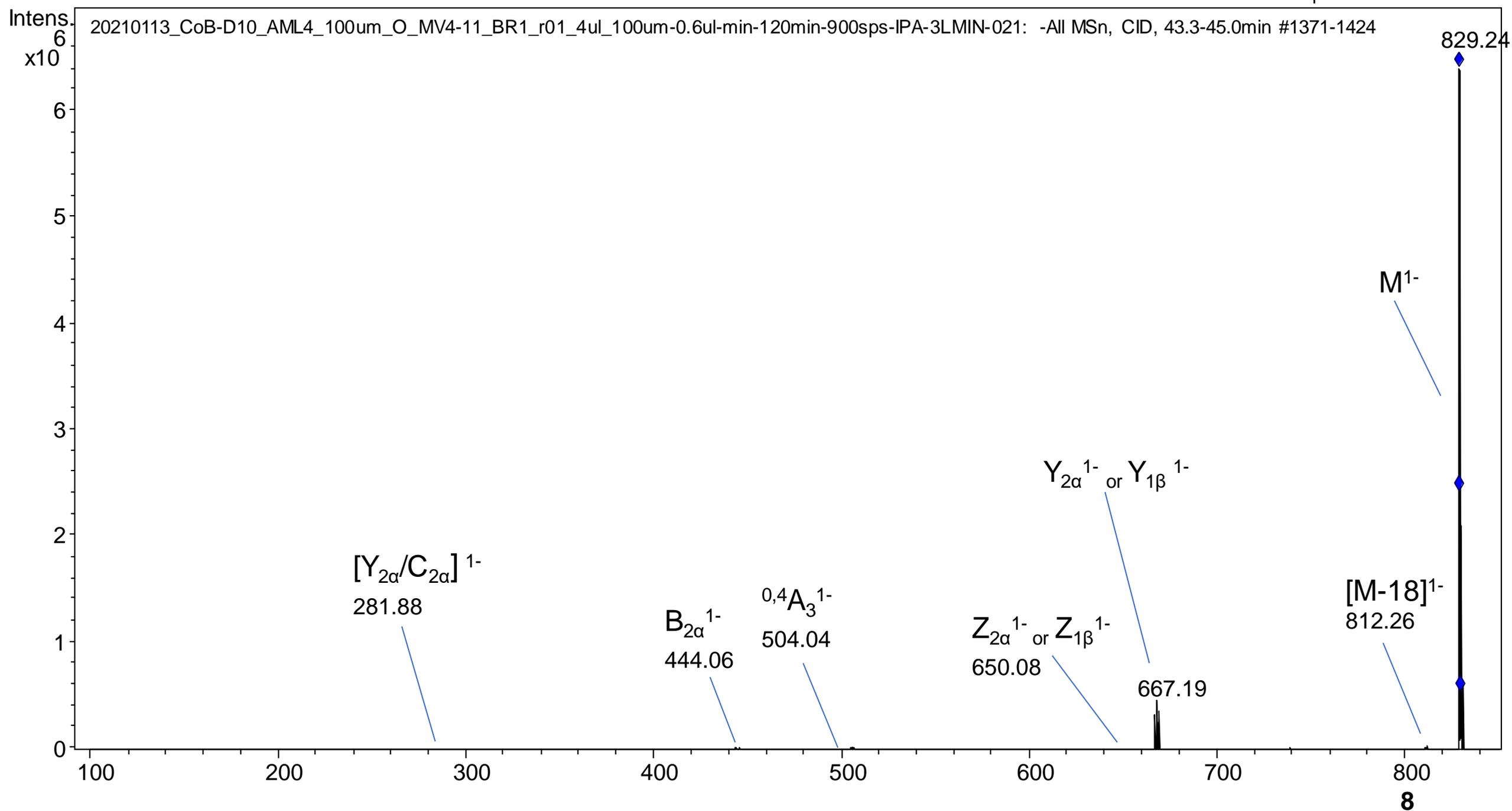
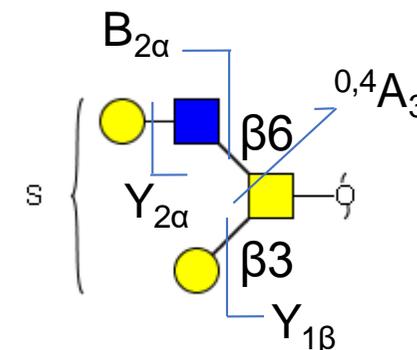
Glycan 6

H2N2Su1

Depicted MS² was obtained from analysis of cell line: MV4-11

Monoisotopic mass: 830.25 Da
Charge observed: 1-
Theoretical ion: *m/z* 829.24
Observed ion: *m/z* 829.22
Mass deviation: *m/z* 0.02
Retention time: 43.7 min

UniCarb-DB: #104



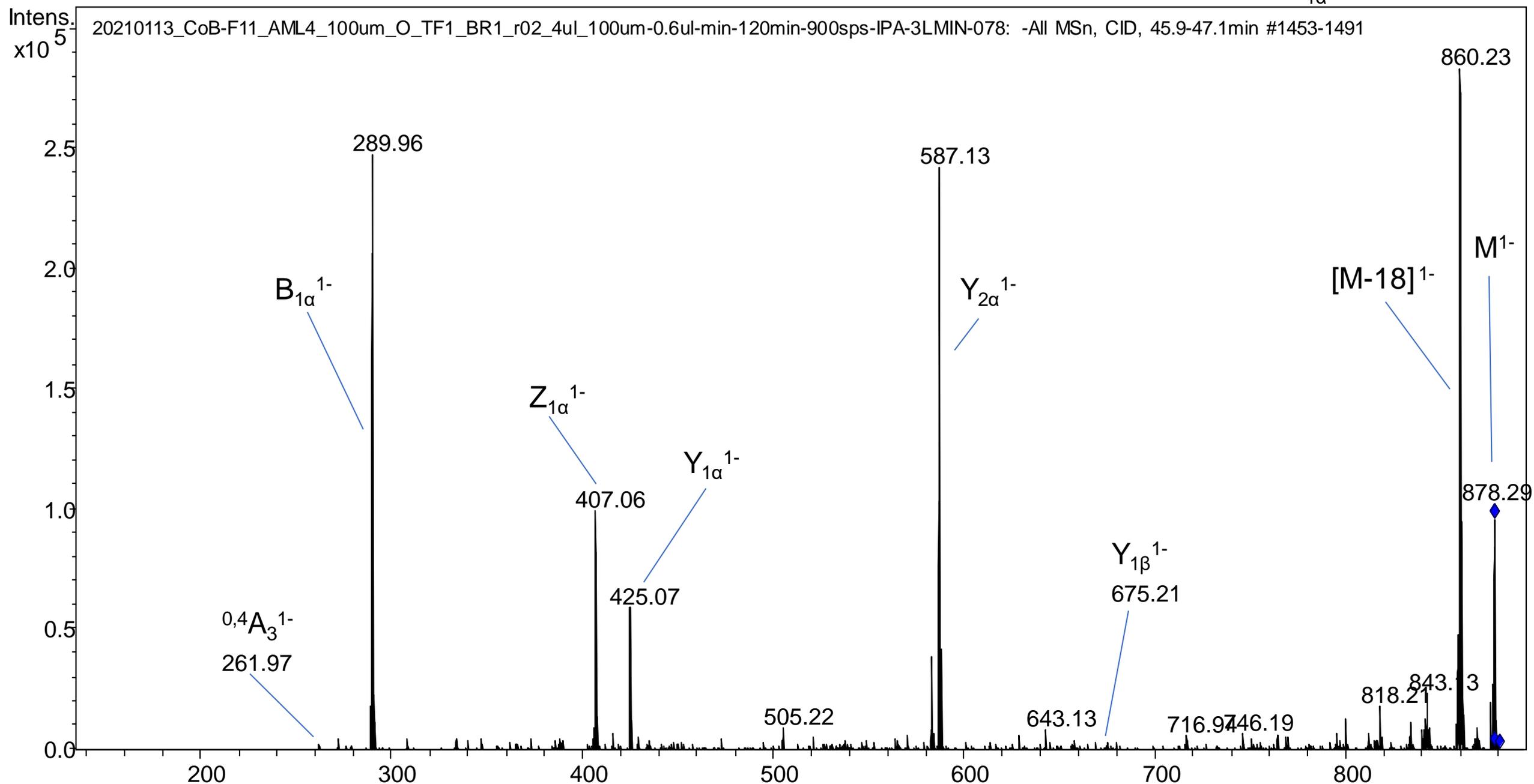
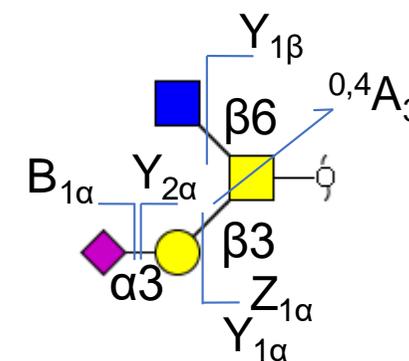
Glycan 7

H1N2S1

Depicted MS² was obtained from analysis of cell line: TF-1

Monoisotopic mass: 879.34 Da
Charge observed: 1-
Theoretical ion: *m/z* 878.33
Observed ion: *m/z* 878.27
Mass deviation: *m/z* 0.05
Retention time: 46.0 min
Note: α -2,3 linkage of sialic acid confirmed by neuraminidase S and A treatment

UniCarb-DB: #191



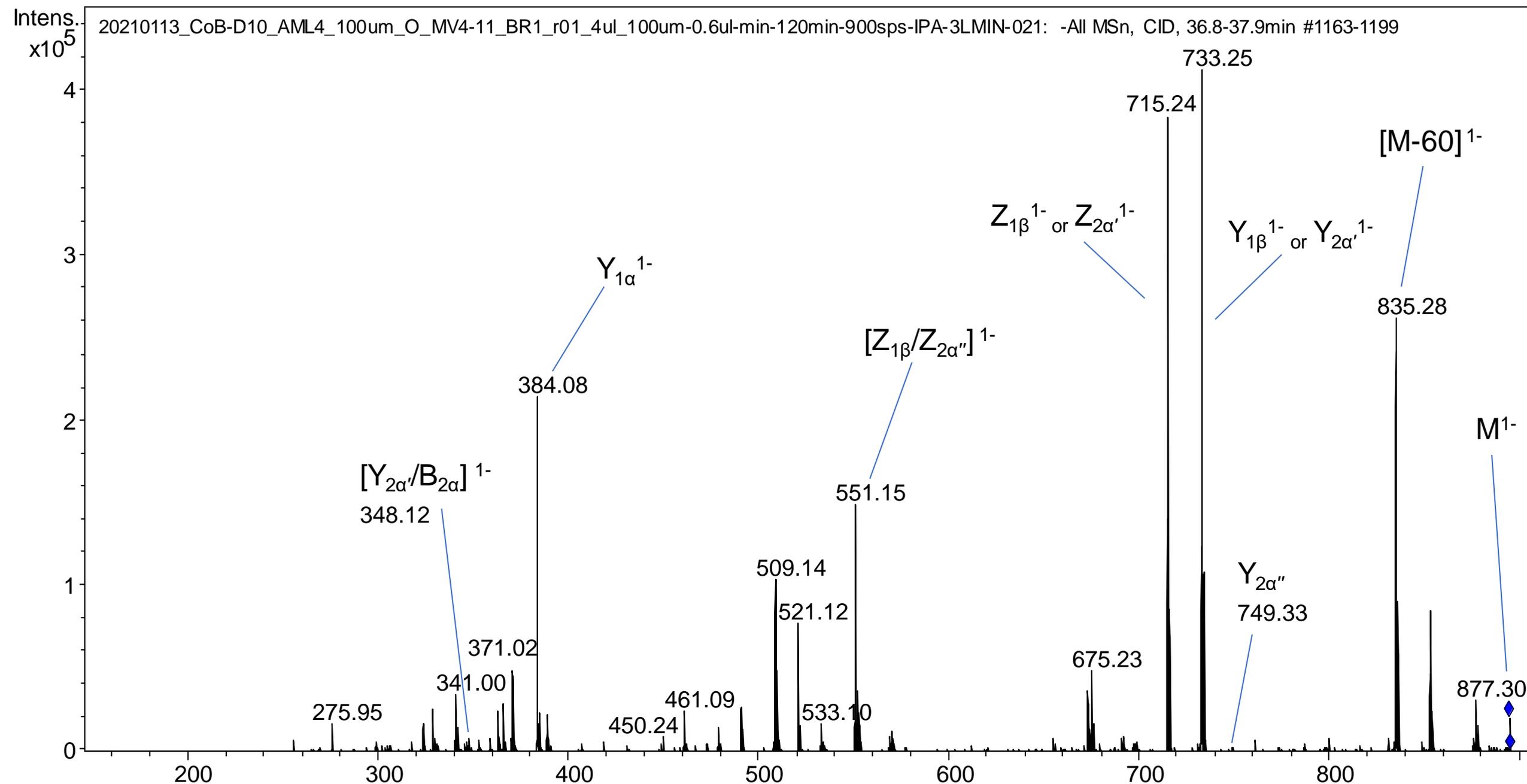
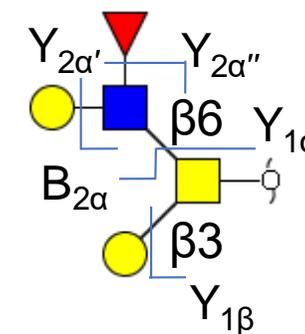
Glycan 8

H2N2F1

Depicted MS² was obtained from analysis of cell line: MV4-11

Monoisotopic mass: 896.35 Da
Charge observed: 1-
Theoretical ion: *m/z* 895.34
Observed ion: *m/z* 895.28
Mass deviation: *m/z* 0.06
Retention time: 37.0 min
Note: Characteristic *m/z* 348 ion for Lewis a

UniCarb-DB: #33



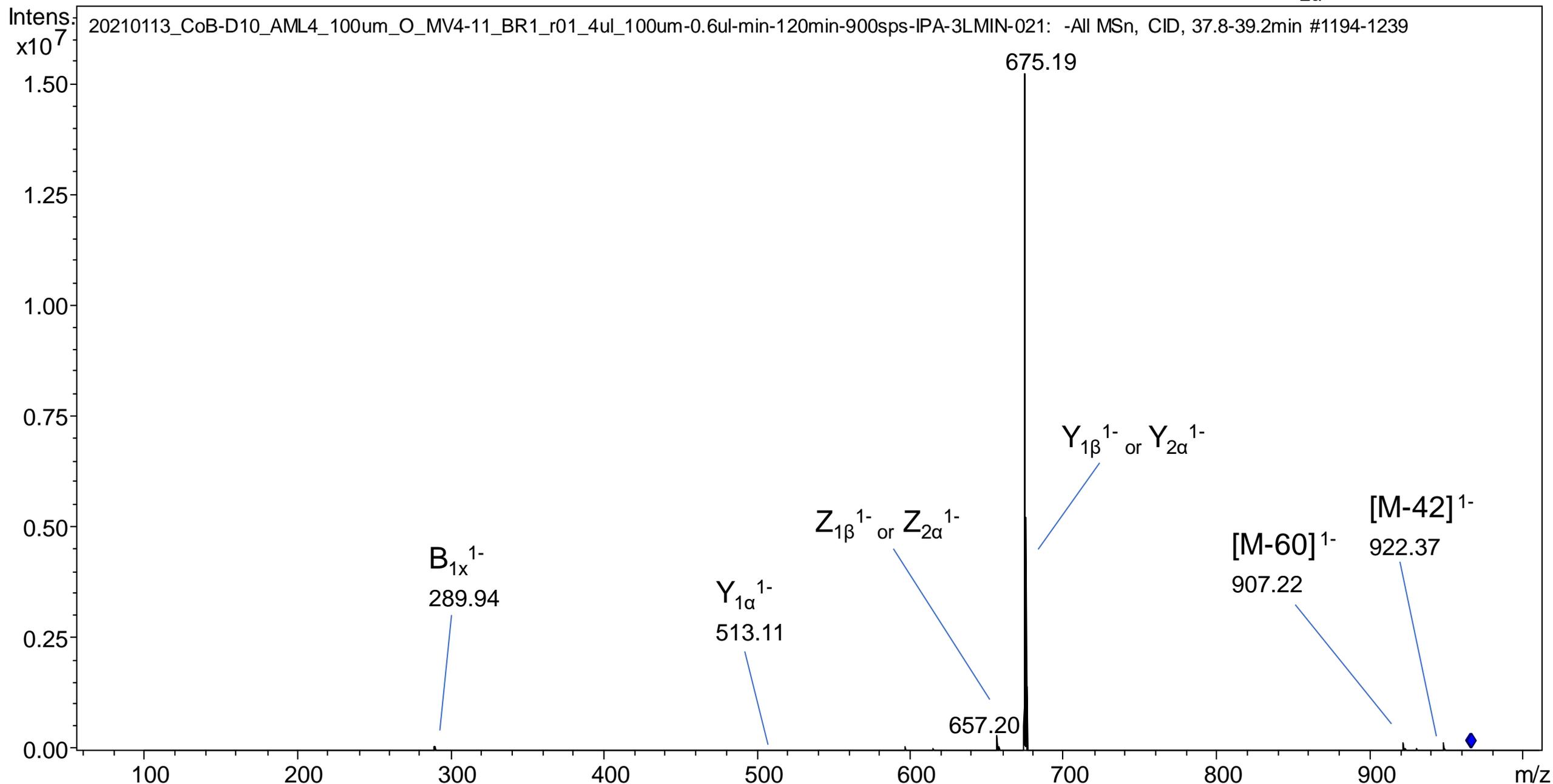
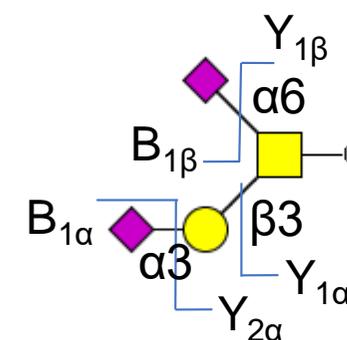
Glycan 9

H1N1S2

Depicted MS² was obtained from analysis of cell line: MV4-11

Monoisotopic mass: 967.35 Da
Charge observed: 1-
Theoretical ion: *m/z* 966.34 *m/z*
Observed ion: *m/z* 966.32 *m/z*
Mass deviation: *m/z* 0.02 *m/z*
Retention time: 38.1 min
Note: Linkage of sialic acids confirmed by neuraminidase S and A treatment

UniCarb-DB: #47



Glycan 10b

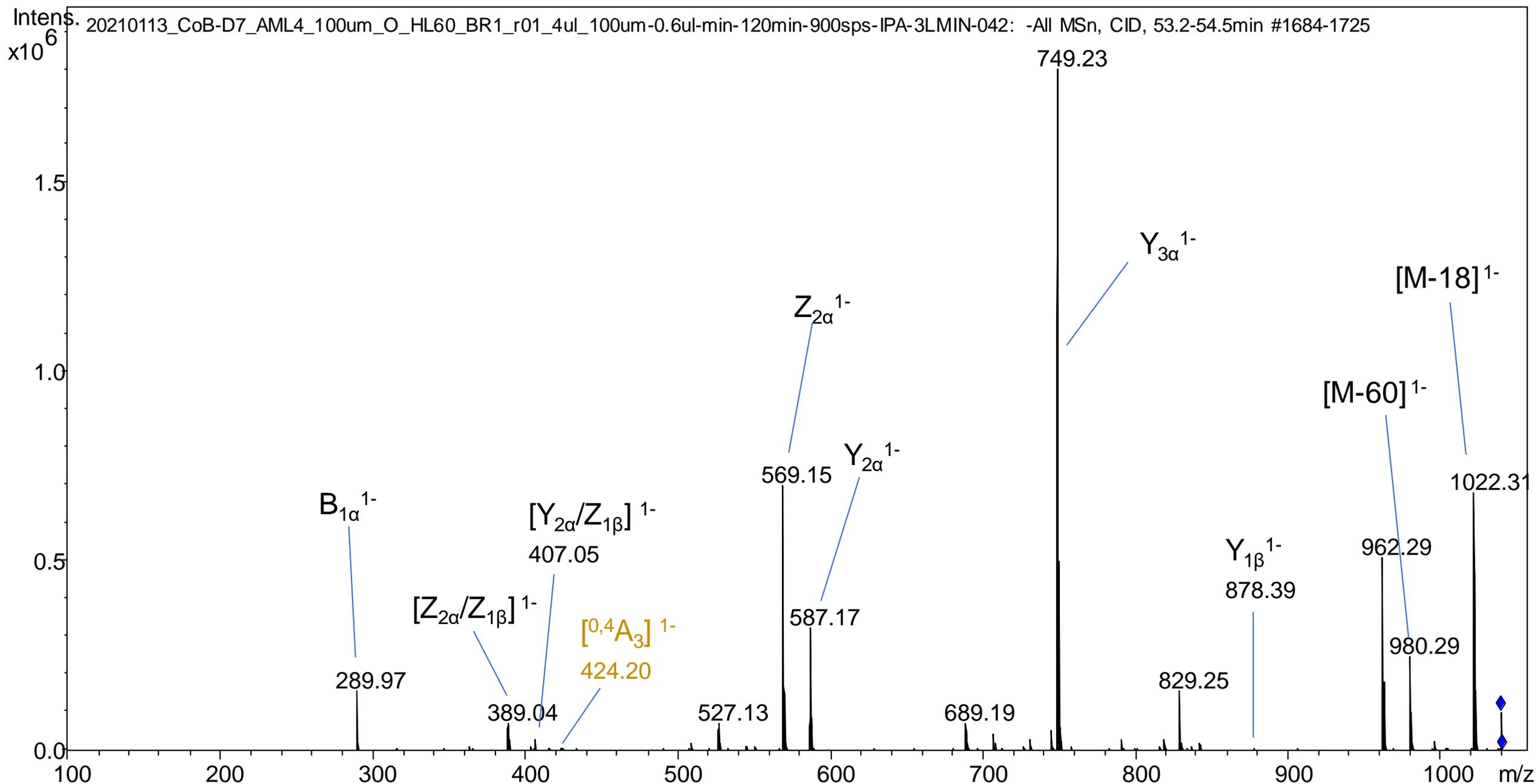
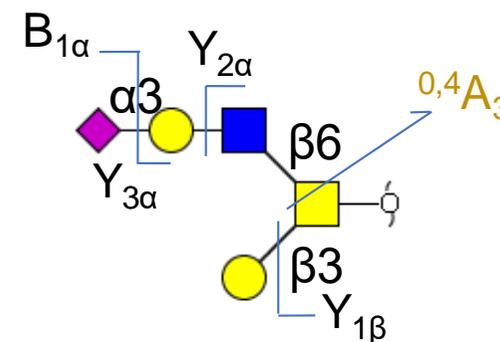
H2N2S1

Depicted MS² was obtained from analysis of cell line: HL-60

Monoisotopic mass: 1041.40 Da
Charge observed: 1-
Theoretical ion: *m/z* 1040.39
Observed ion: *m/z* 1040.33
Mass deviation: *m/z* 0.06
Retention time: 61.3 min
Note: α -2,3 linkage of sialic acid confirmed by neuraminidase S and A treatment

UniCarb-DB: #2643

Elution order of isomers in line with Jin, Chunsheng, et al. *Mol. & Cell. Proteomics* 16.5 (2017): 743-758.

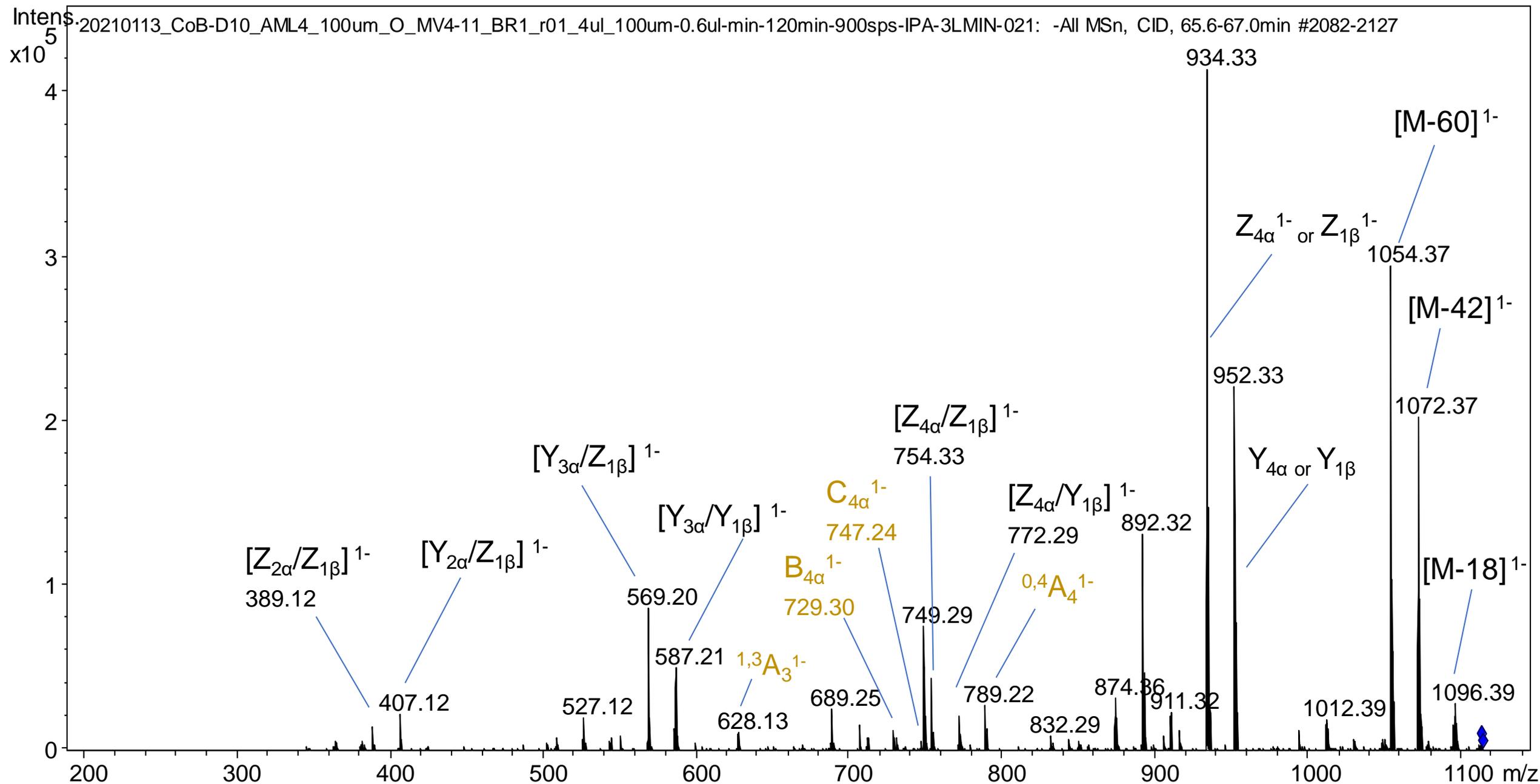
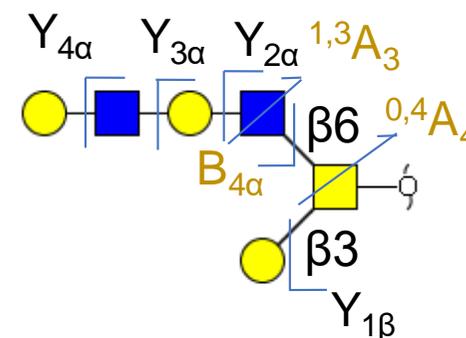


Glycan 11

H3N3

Depicted MS² was obtained from analysis of cell line: MV4-11

Monoisotopic mass: 1115.43 Da
Charge observed: 1-
Theoretical ion: *m/z* 1114.42
Observed ion: *m/z* 1114.38
Mass deviation: *m/z* 0.04
Retention time: 65.8 min



Glycan 12b

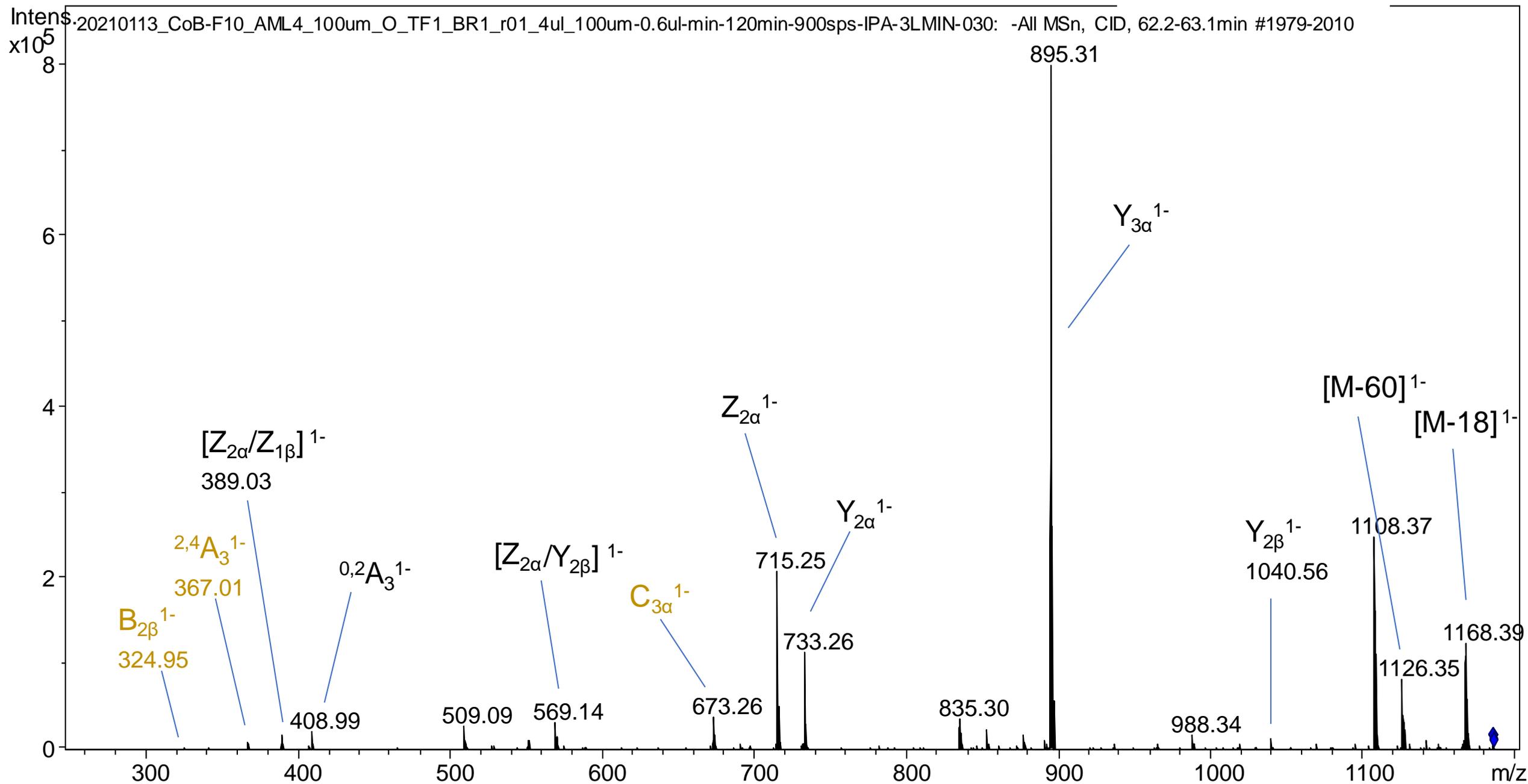
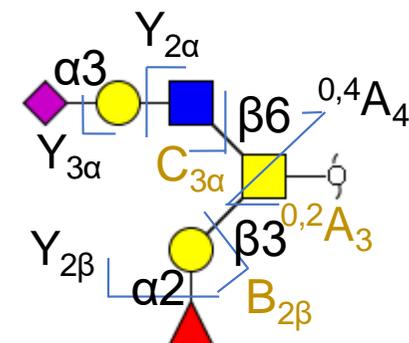
H2N2F1S1

Depicted MS² was obtained from analysis of cell line: TF-1

Monoisotopic mass: 1187.45 Da
Charge observed: 1-
Theoretical ion: *m/z* 1186.44
Observed ion: *m/z* 1186.40
Mass deviation: *m/z* 0.04
Retention time: 62.5 min

Note: α -2,3 linkage of sialic acids confirmed by neuraminidase S and A treatment; *m/z* 325 and *m/z* 409 fragment indicate a H-epitope

UniCarb-DB: #165

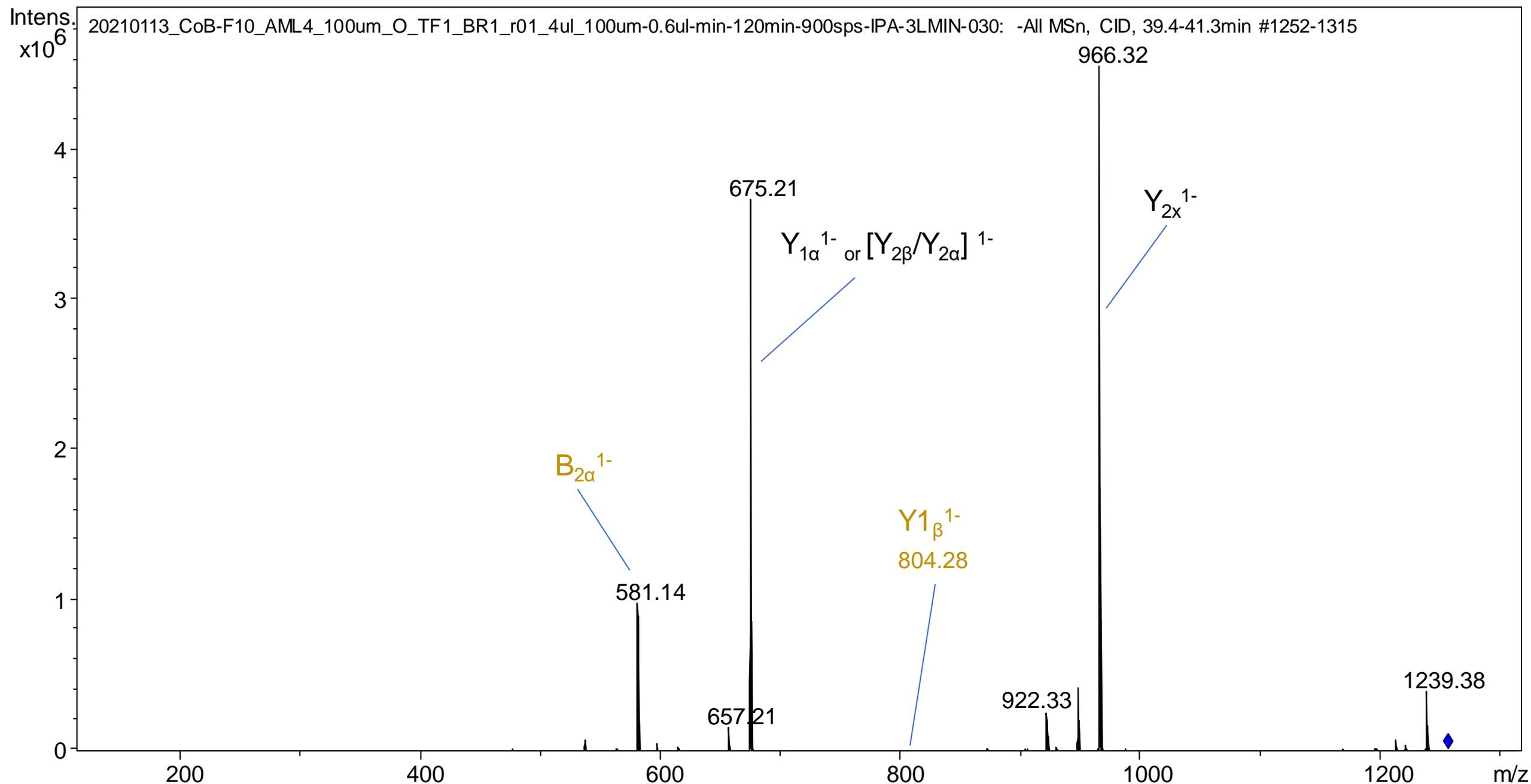
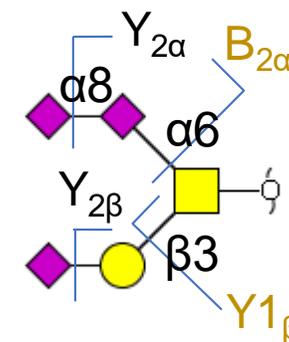


Glycan 13

H1N1S3

Depicted MS² was obtained from analysis of cell line: TF-1

Monoisotopic mass: 1258.45 Da
Charge observed: 1-
Theoretical ion: *m/z* 1257.44
Observed ion: *m/z* 1257.40
Mass deviation: *m/z* 0.04
Retention time: 39.9 min
Note: *m/z* 581 indicates two sialic acids linked to each other



Glycan 14

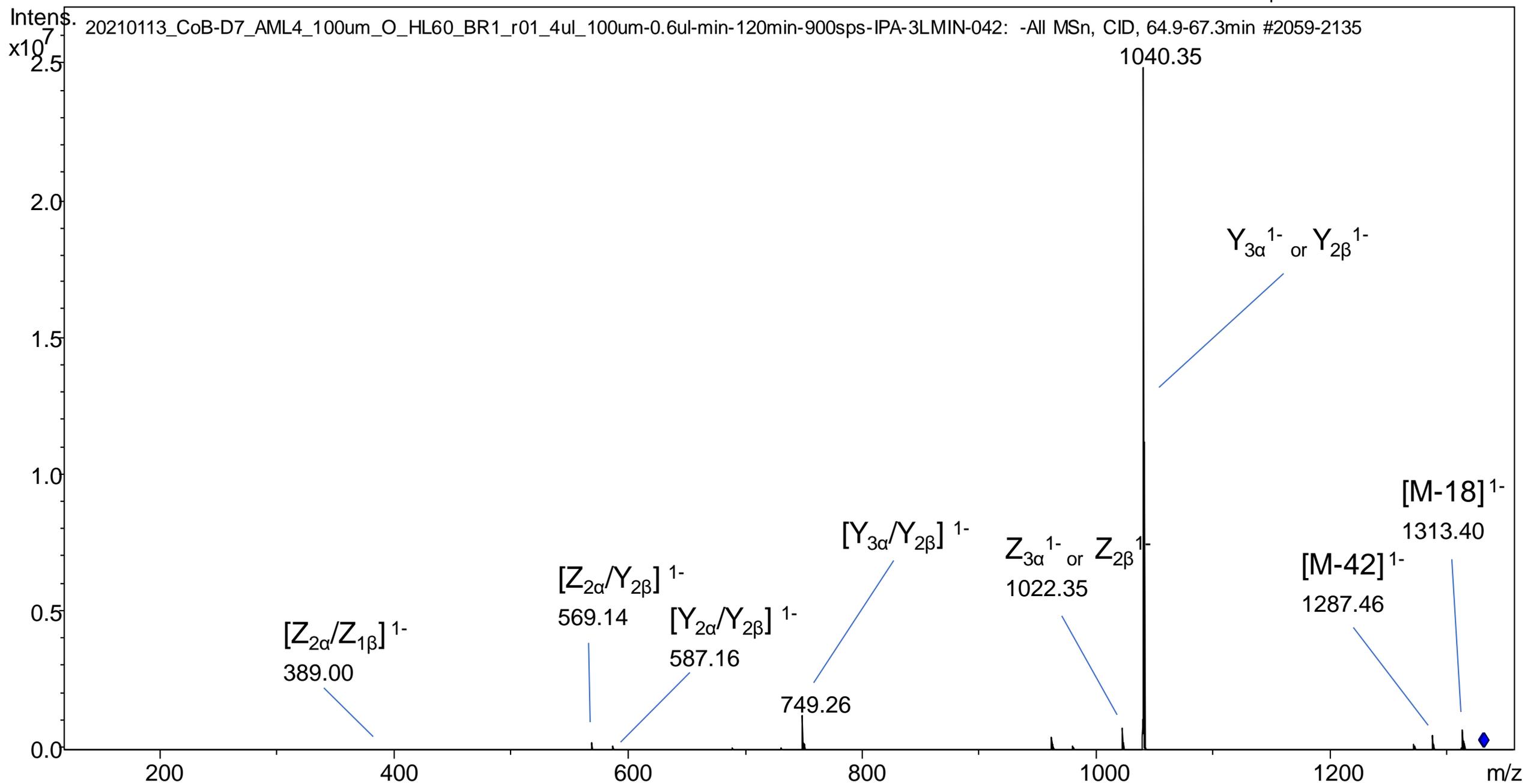
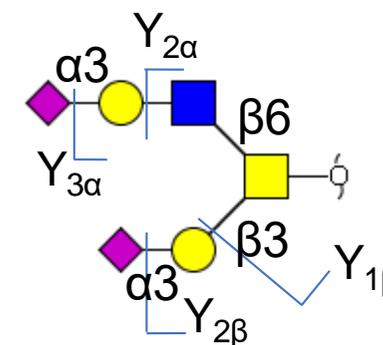
H2N2S2

Depicted MS² was obtained from analysis of cell line: HL-60

Monoisotopic mass: 1332.48 Da
Charge observed: 1-
Theoretical ion: *m/z* 1331.47
Observed ion: *m/z* 1331.44
Mass deviation: *m/z* 0.03
Retention time: 65.4 min

UniCarb-DB: #502

Note:: α -2,3 linkage of both sialic acids confirmed by neuraminidase S and A treatment as no species were detected with α -2,6 as a digested H2N2S1



Glycan 16

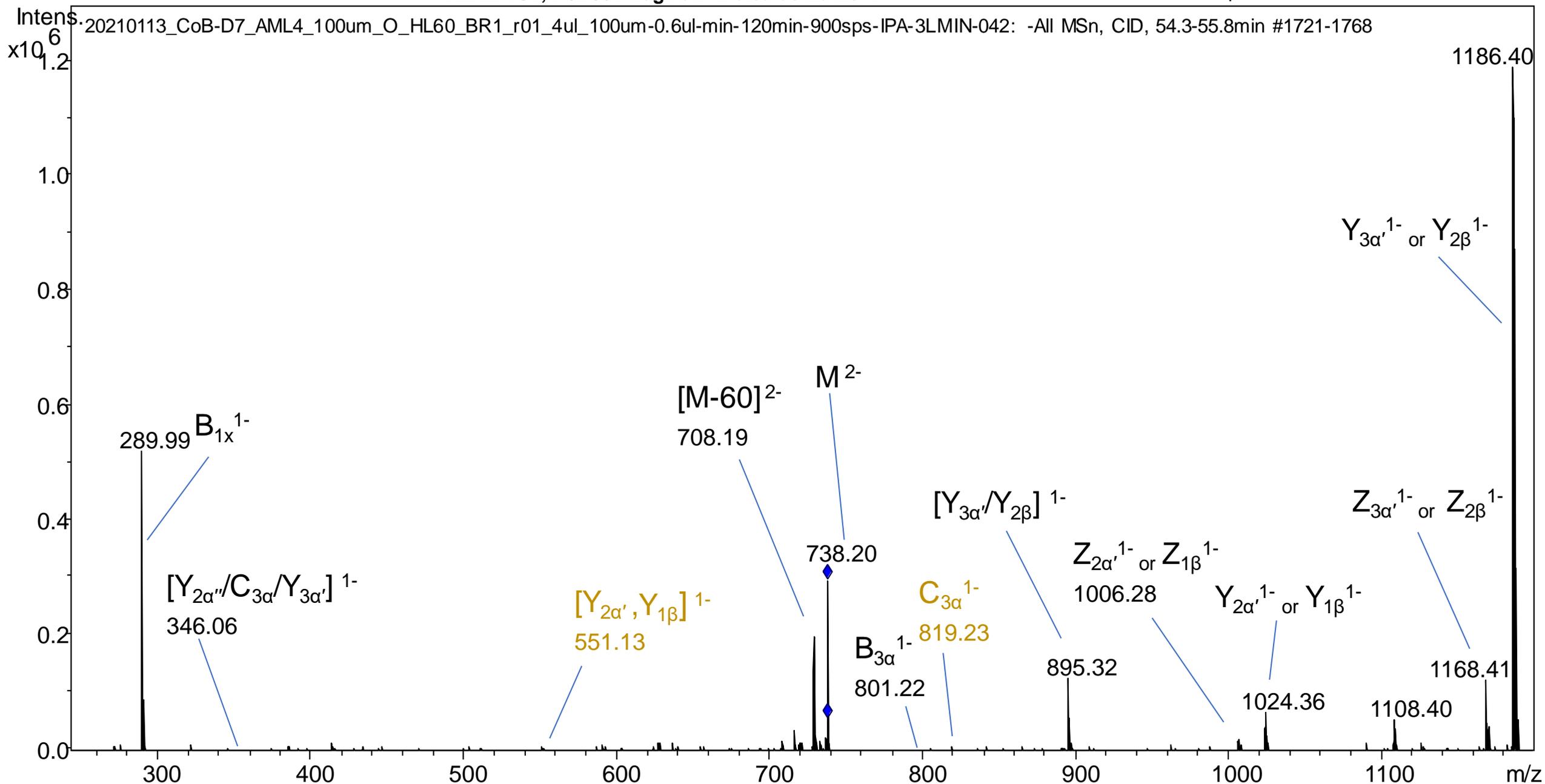
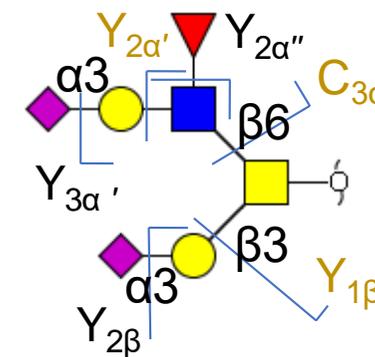
H2N2F1S2

Depicted MS² was obtained from analysis of cell line: HL-60

Monoisotopic mass: 1478.54 Da
Charge observed: 2-
Theoretical ion: *m/z* 738.26
Observed ion: *m/z* 738.21
Mass deviation: *m/z* 0.05
Retention time: 54.6 min

UniCarb-DB: #61

Note: α -2,3 linkage of both sialic acids confirmed by neuraminidase S and A treatment as no species were detected with α -2,6 as a digested H2N2F1S1; *m/z* 364 fragment indicates Lewis x



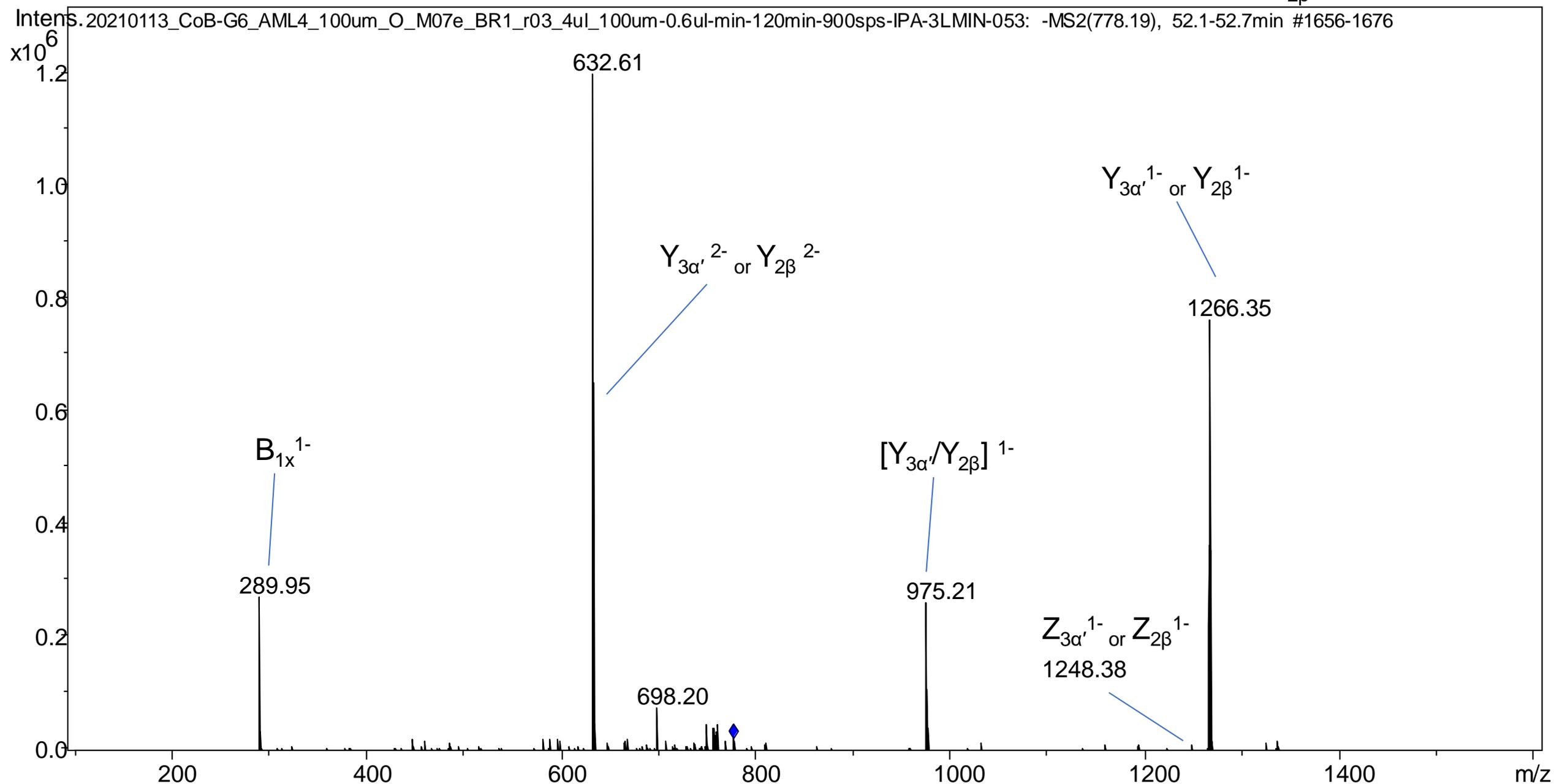
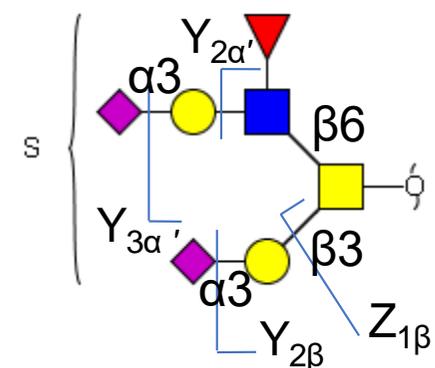
Glycan 17

H2N2F1S2Su1

Depicted MS² was obtained from analysis of cell line: M-07e

Monoisotopic mass: 1558.50 Da
Charge observed: 2-
Theoretical ion: *m/z* 778.25
Observed ion: *m/z* 778.20
Mass deviation: *m/z* 0.05
Retention time: 51.6 min

UniCarb-DB: #2947

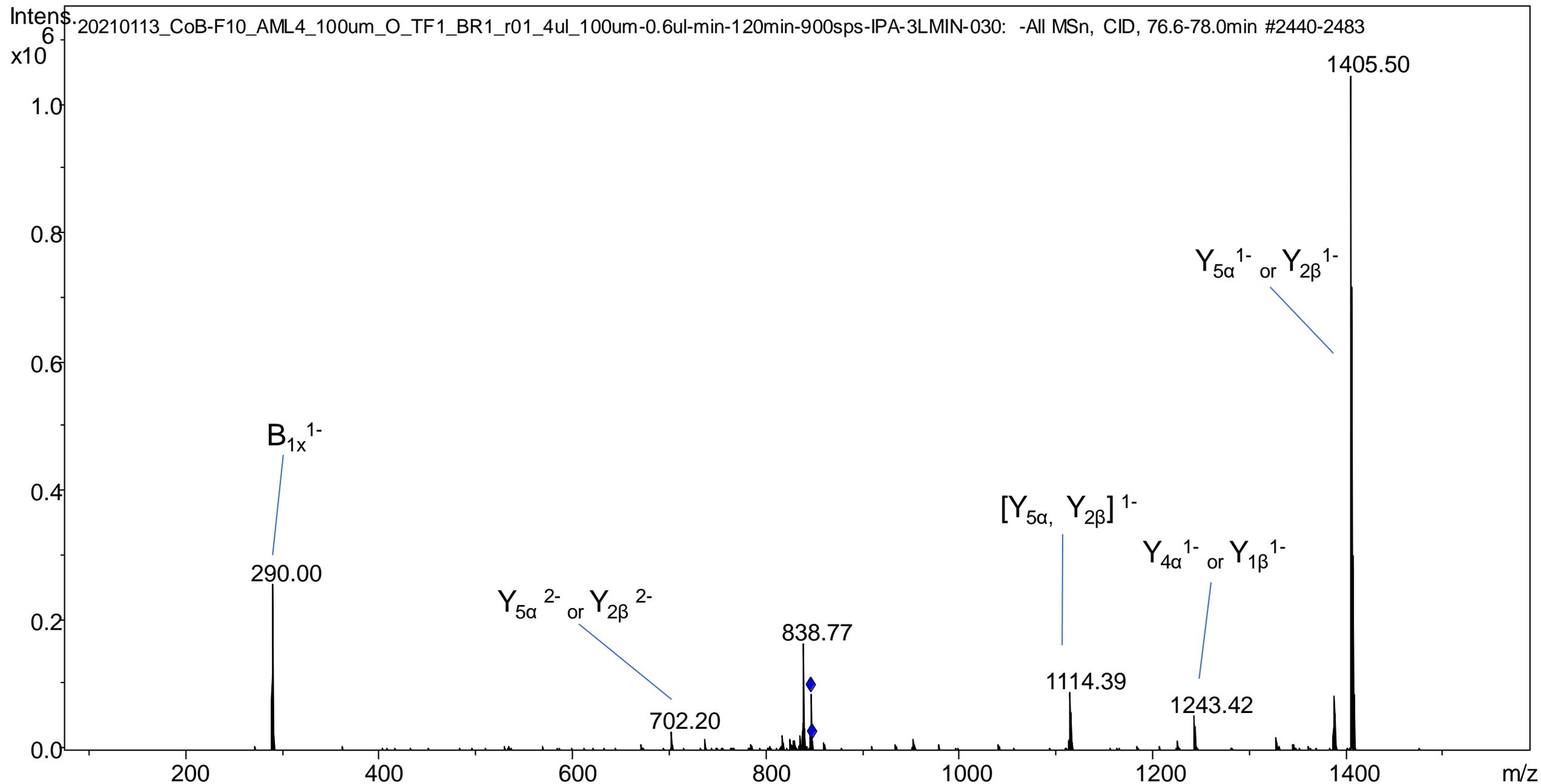
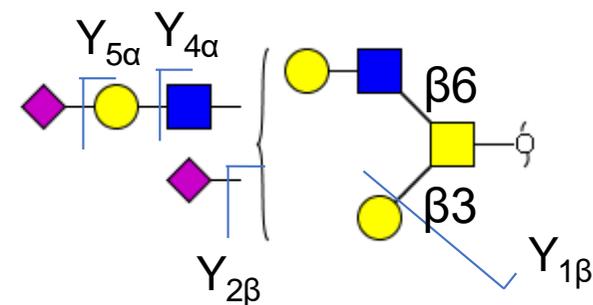


Glycan 18a

H3N3S2

Depicted MS² was obtained from analysis of cell line: TF-1

Monoisotopic mass: 1697.61 Da
Charge observed: 2-
Theoretical ion: *m/z* 847.80
Observed ion: *m/z* 847.76
Mass deviation: *m/z* 0.04
Retention time: 77.0 min



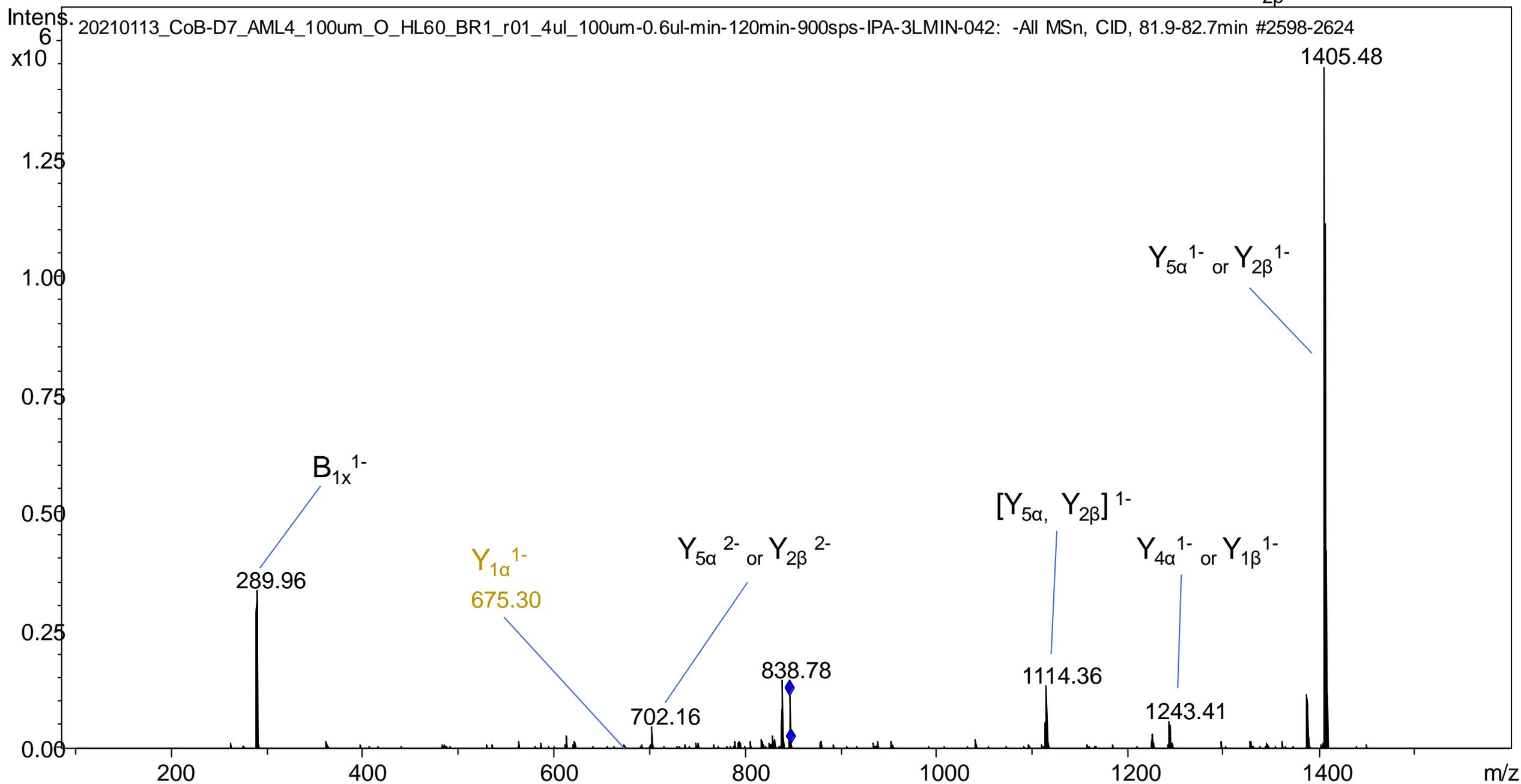
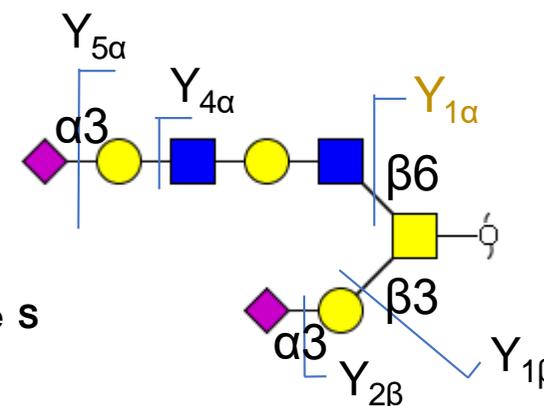
Glycan 18b

H3N3S2

Depicted MS² was obtained from analysis of cell line: HL-60

Monoisotopic mass: 1697.61 Da
Charge observed: 2-
Theoretical ion: *m/z* 847.80
Observed ion: *m/z* 847.75
Mass deviation: *m/z* 0.05
Retention time: 82.1 min

Note: α -2,3 linkage of both sialic acids confirmed by neuraminidase S and A treatment as no species were detected with α -2,6 as H3N3S1



Glycan 19

H3N3F1S2

Depicted MS² was obtained from analysis of cell line: HL-60

Monoisotopic mass: 1843.67 Da
Charge observed: 2-
Theoretical ion: *m/z* 920.79
Observed ion: *m/z* 920.77
Mass deviation: *m/z* 0.02
Retention time: 69.2 min

