

Supplementary Information 2

O-Glycomics – Structural Elucidation

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

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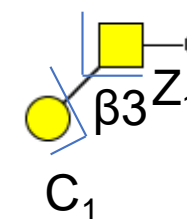
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Glycan 1

H1N1

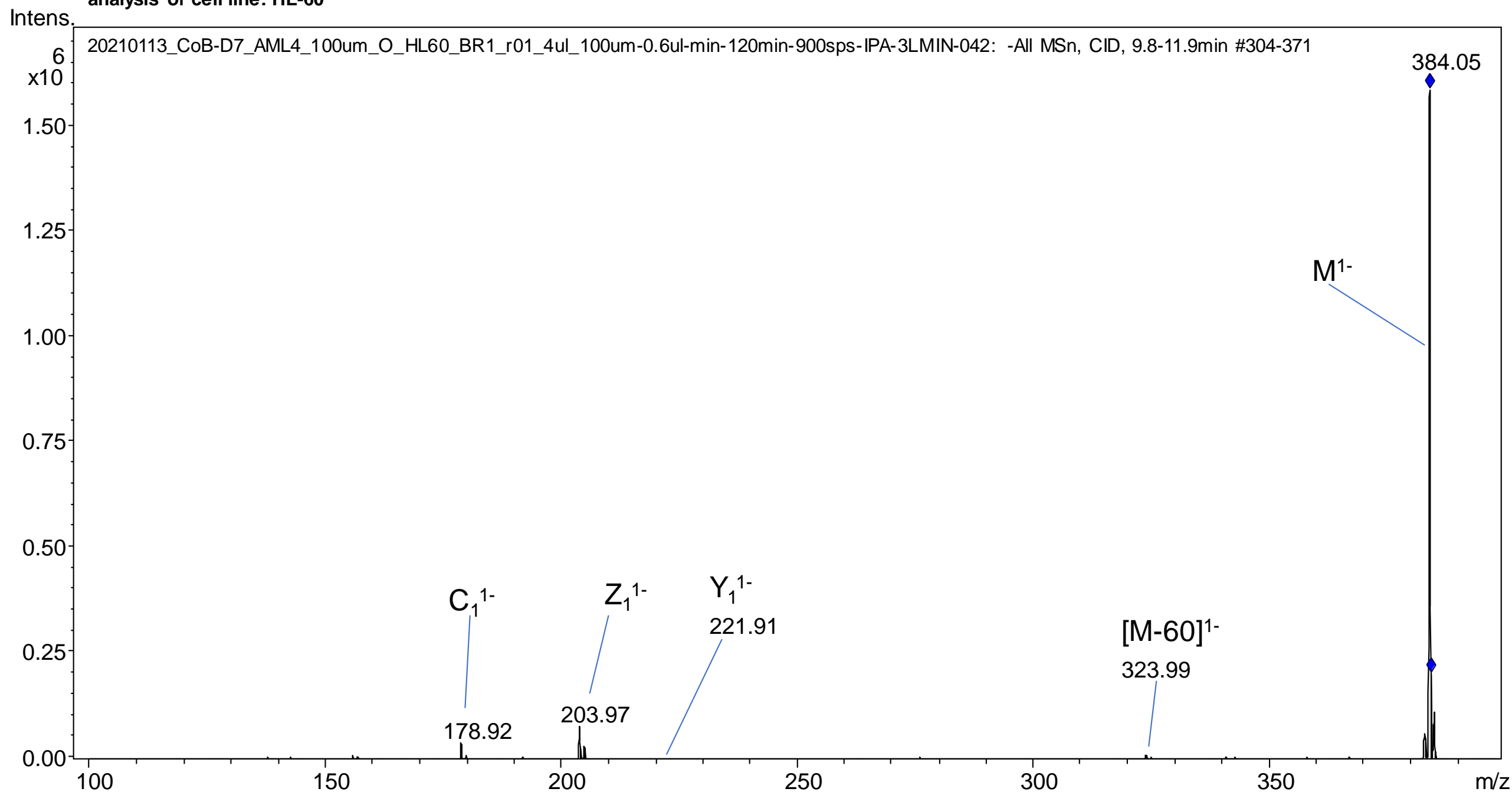
Monoisotopic mass: 385.16 Da
Charge observed: 1-
Theoretical ion: m/z 384.15
Observed ion: m/z 384.05
Mass deviation: m/z 0.10
Retention time: 10.6 min

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



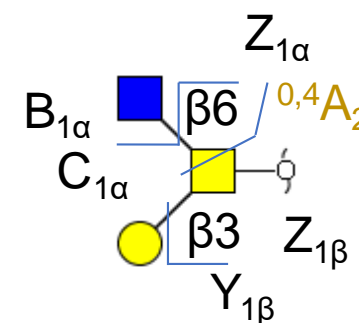
Glycan 2

H1N2

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

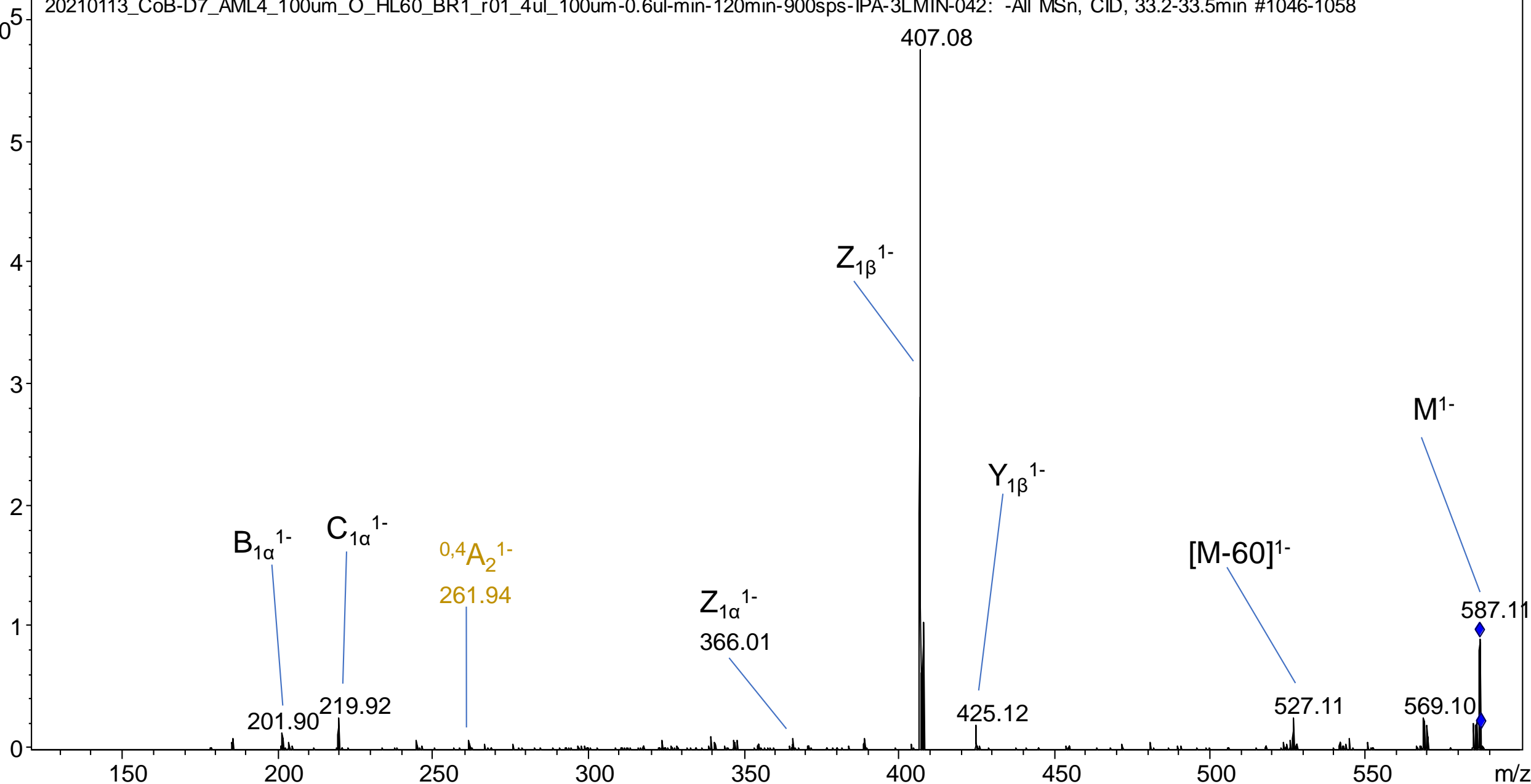
Monoisotopic mass: 588.24 Da
Charge observed: 1-
Theoretical ion: *m/z* 587.23
Observed ion: *m/z* 587.19
Mass deviation: *m/z* 0.04
Retention time: 33.3 min

UniCarb-DB: #177



Intens.

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



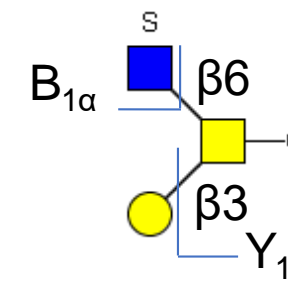
Glycan 3

H1N2Su1

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

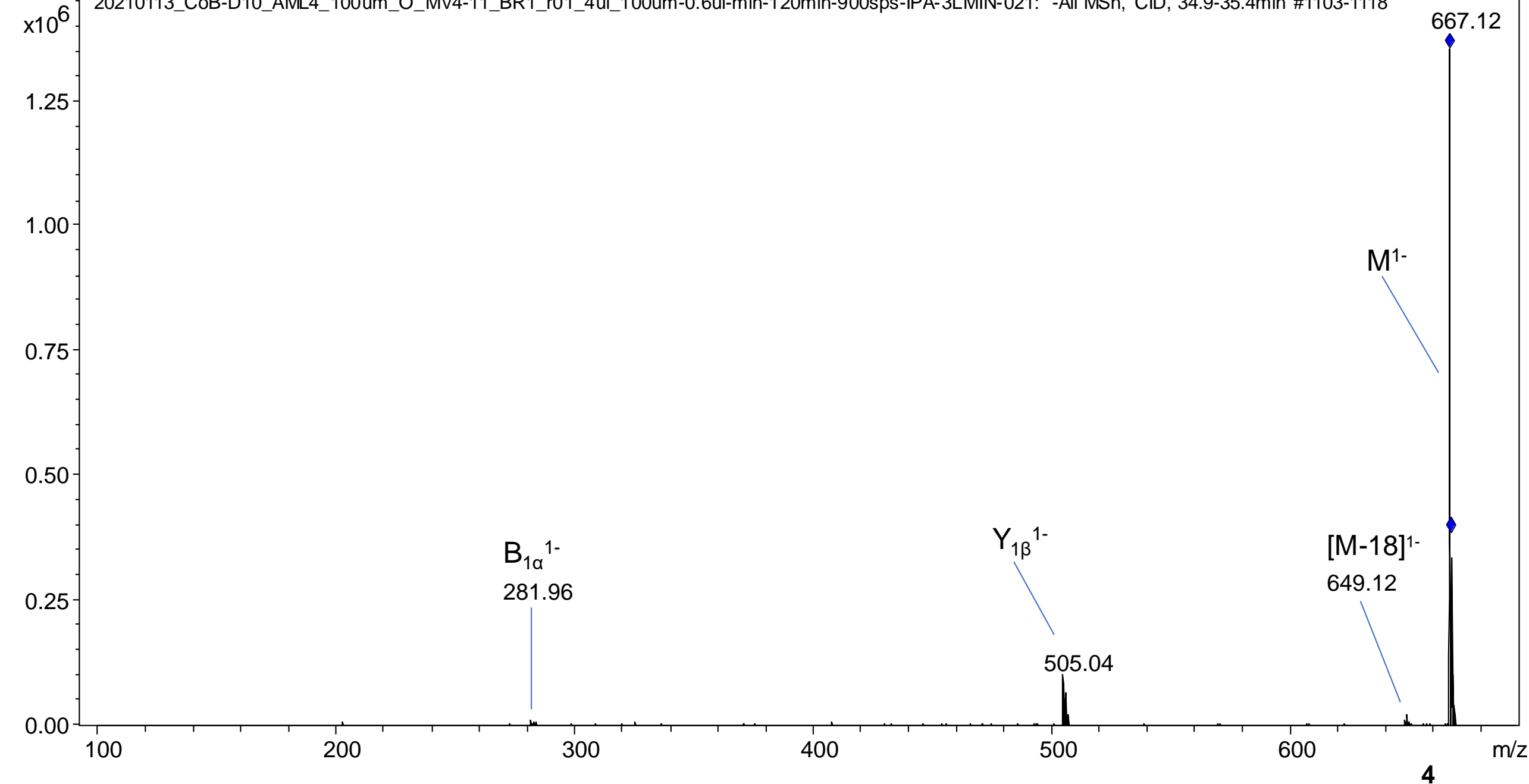
Monoisotopic mass: 668.20 Da
Charge observed: 1-
Theoretical ion: *m/z* 667.19
Observed ion: *m/z* 667.11
Mass deviation: *m/z* 0.07
Retention time: 35.0 min

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



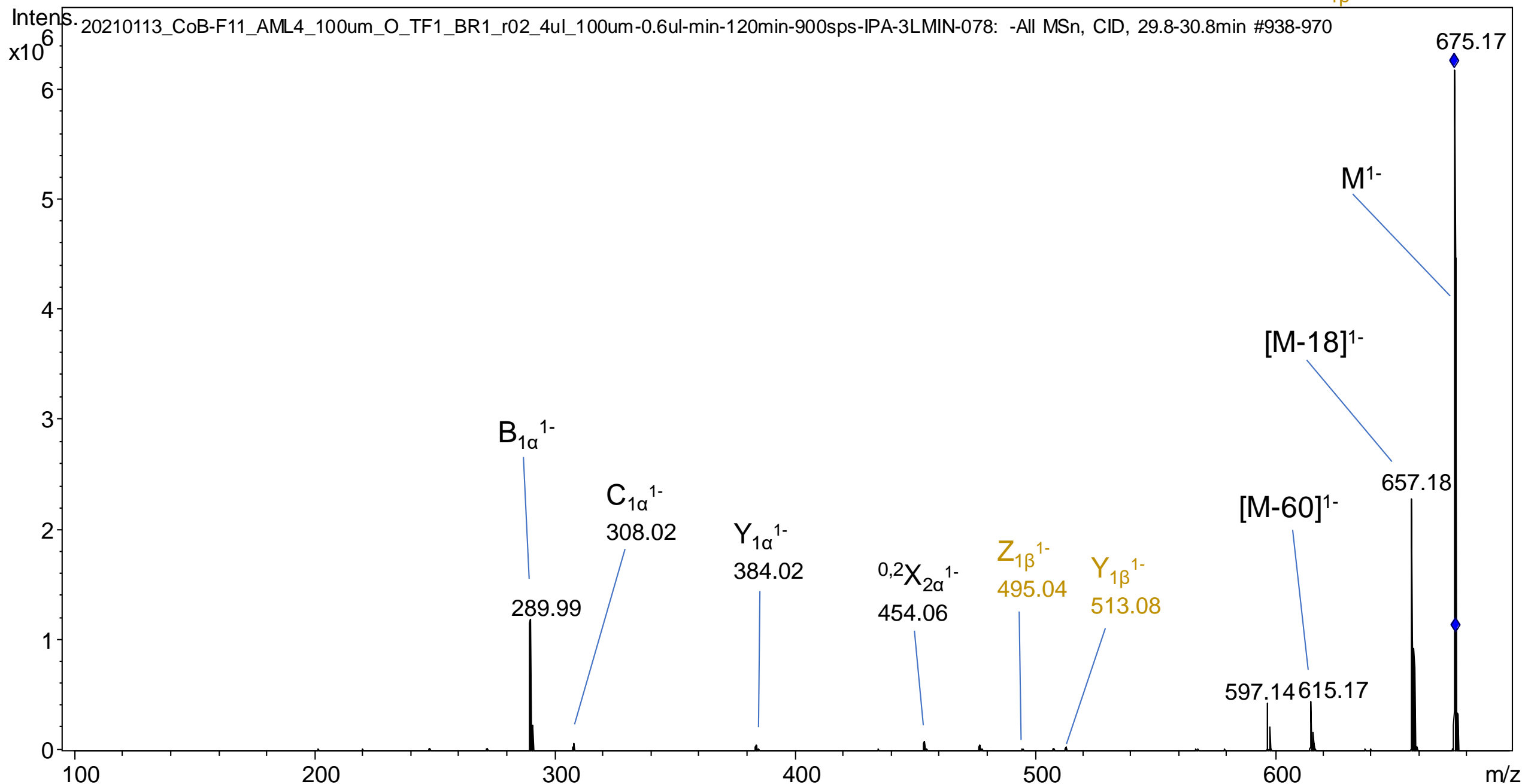
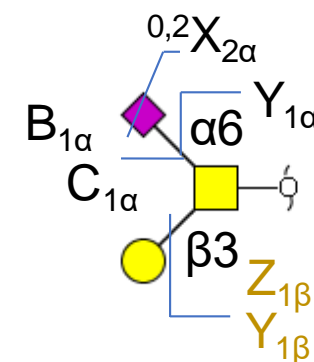
Glycan 4a

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



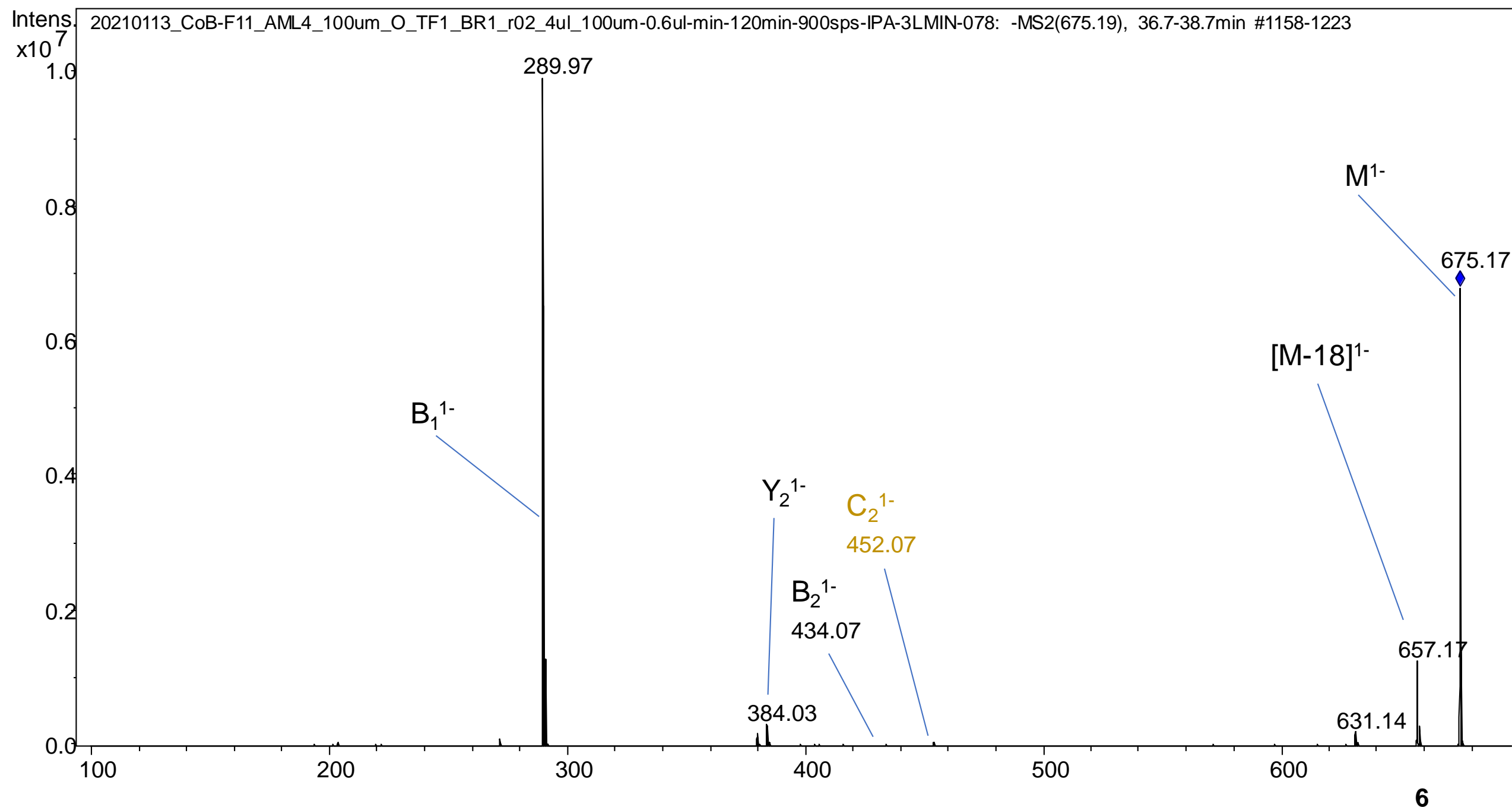
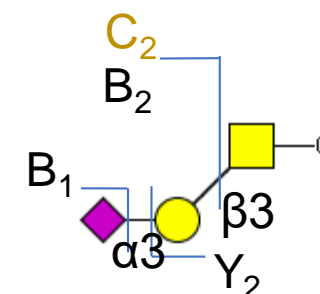
Glycan 4b

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

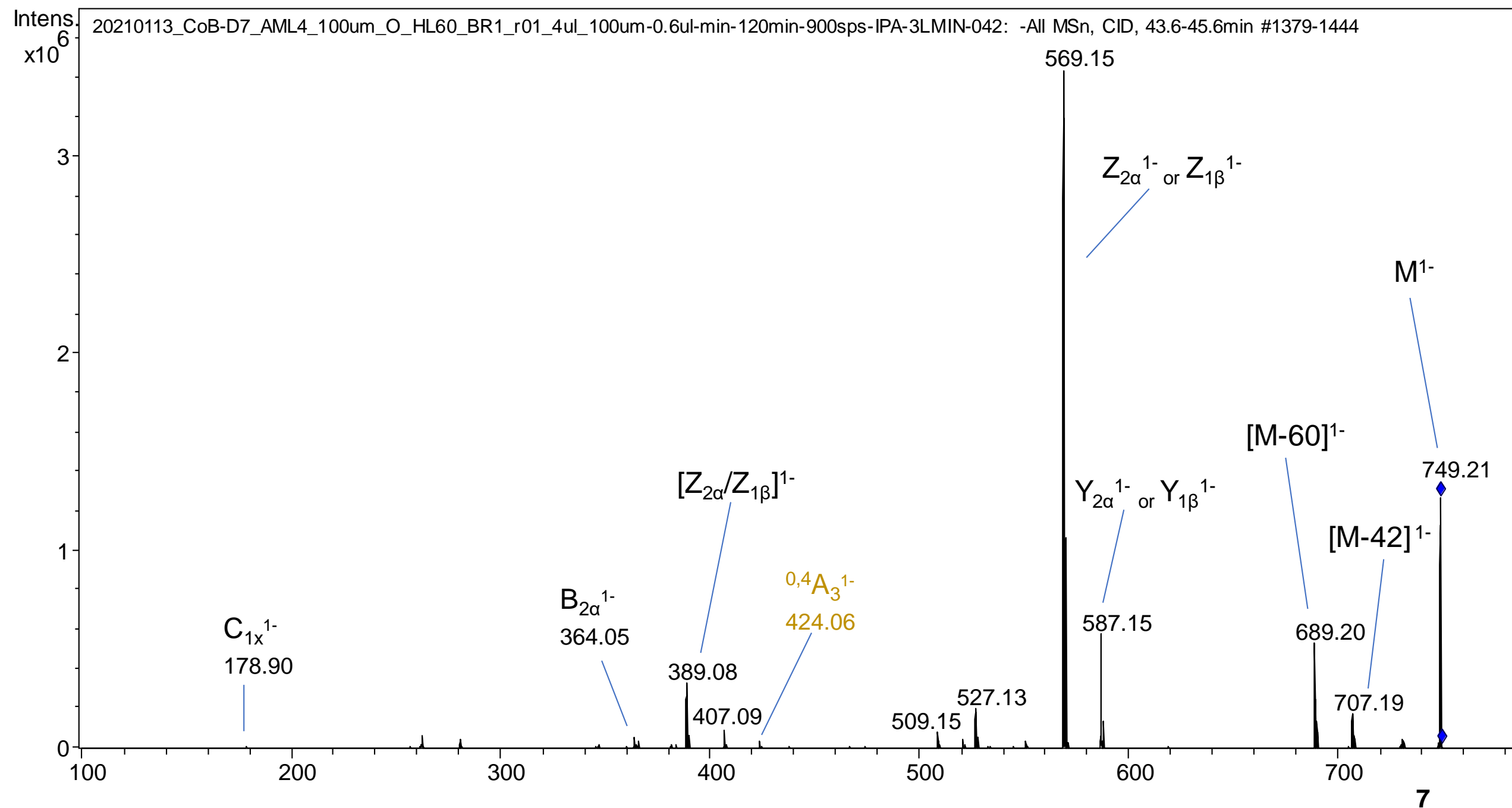
UniCarb-DB: #146



$$\text{H}_2\text{N}_2$$

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

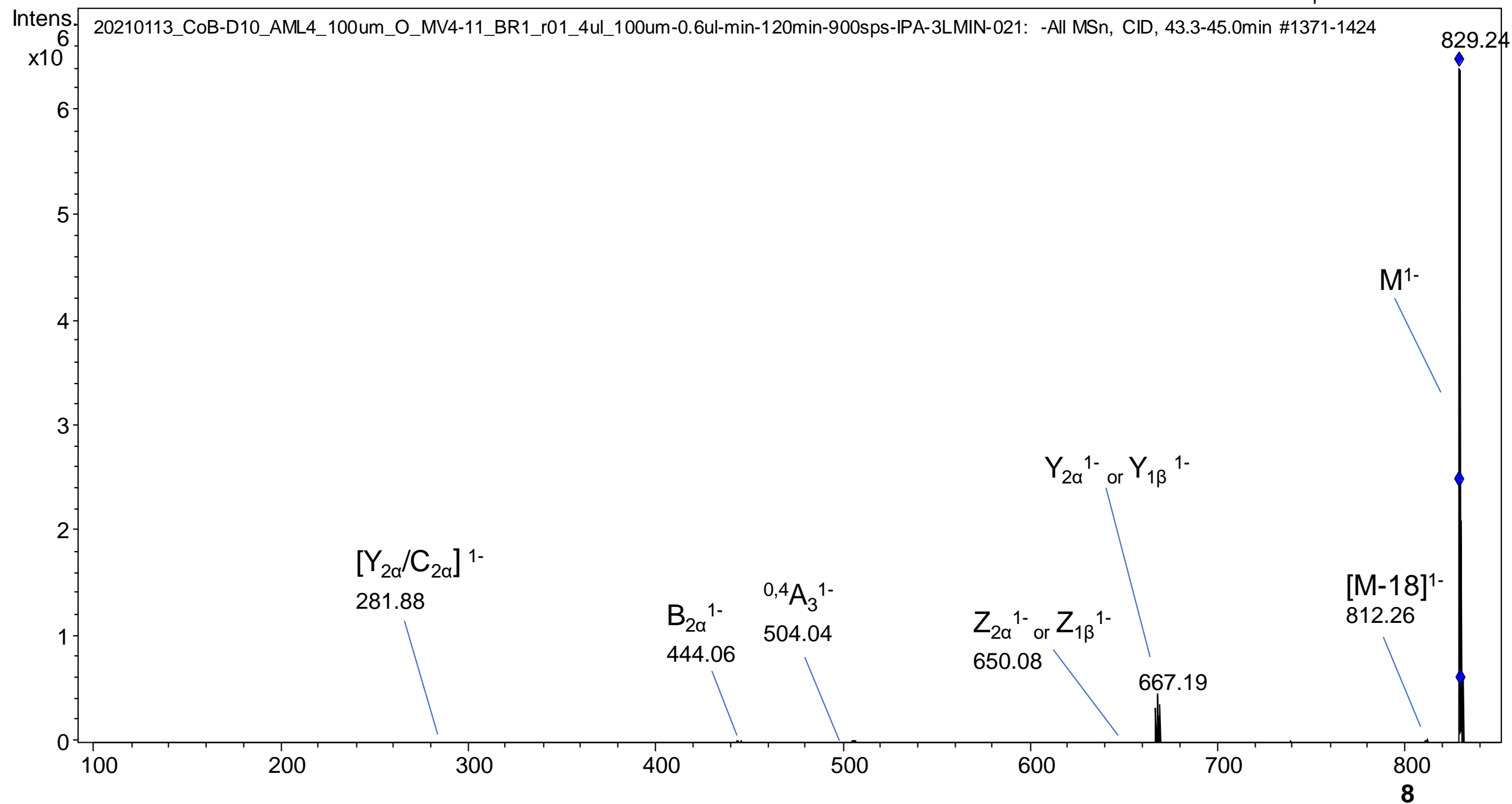
Diagram illustrating a 2D hexagonal lattice structure. The nodes are labeled $C_{1\alpha}$, $B_{2\alpha}$, $C_{1\beta}$, and $Y_{2\alpha}$. The edges are labeled β_6 , β_3 , and $Y_{1\beta}$. A yellow circle is labeled $0,4A_3$.



H2N2Su1

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

The diagram shows the electronic configuration for the S state. It features two yellow circles representing electrons, one labeled $B_{2\alpha}$ and the other $Y_{2\alpha}$. A blue square represents an electron labeled β_6 , which is connected by a diagonal line to another yellow circle labeled β_3 . This β_3 electron is further connected to a yellow square labeled $Y_{1\beta}$. A bracket on the left groups the $B_{2\alpha}$ and $Y_{2\alpha}$ electrons under the label S . A diagonal line connects the β_6 electron to the label $0,4A_3$.

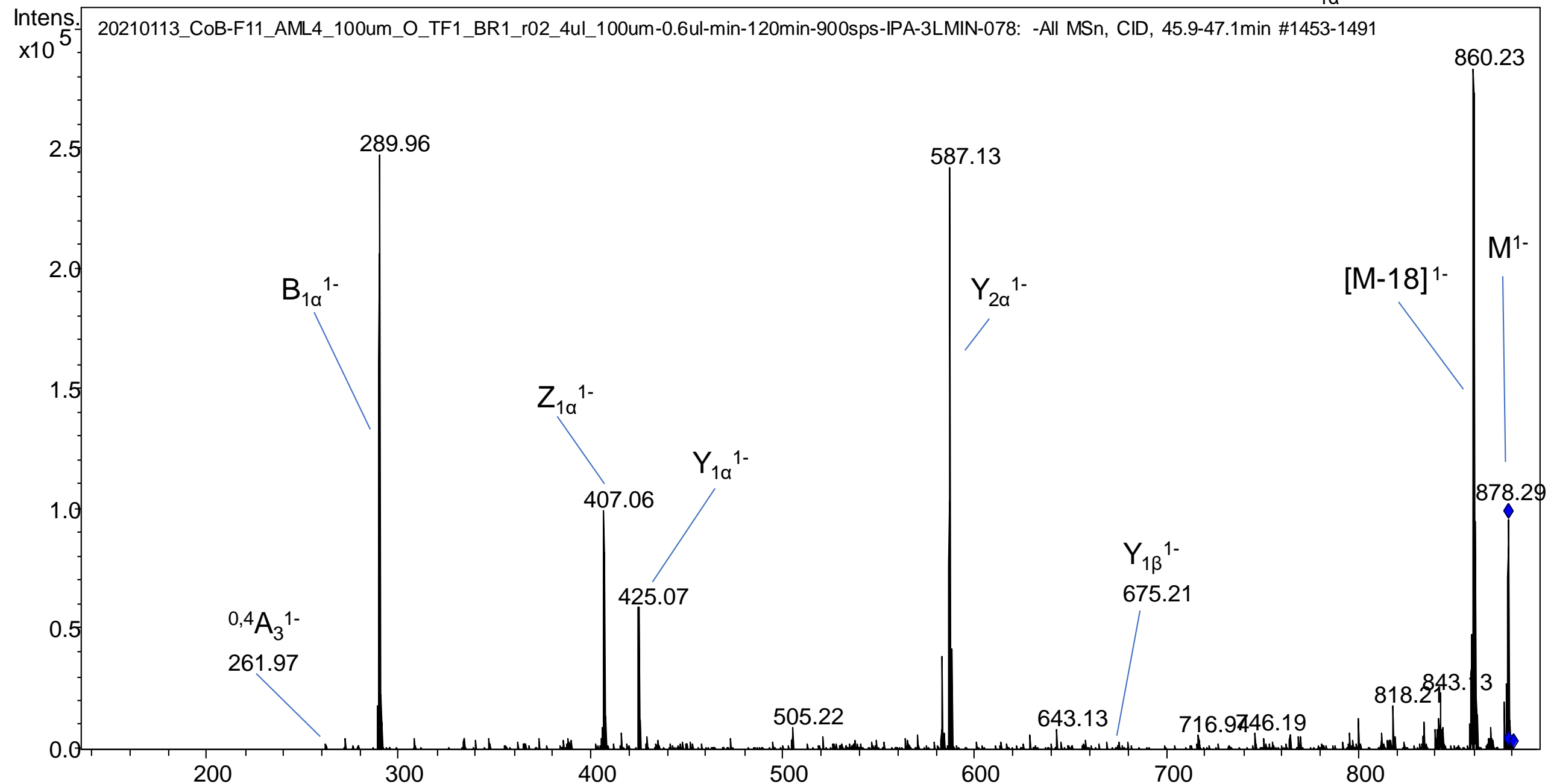
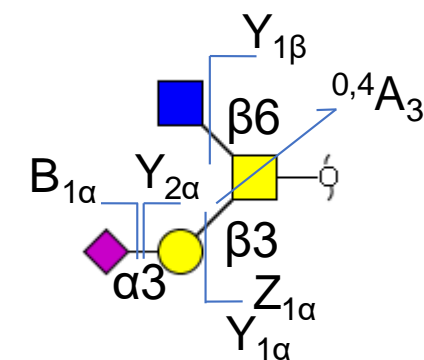


H1N2S1

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



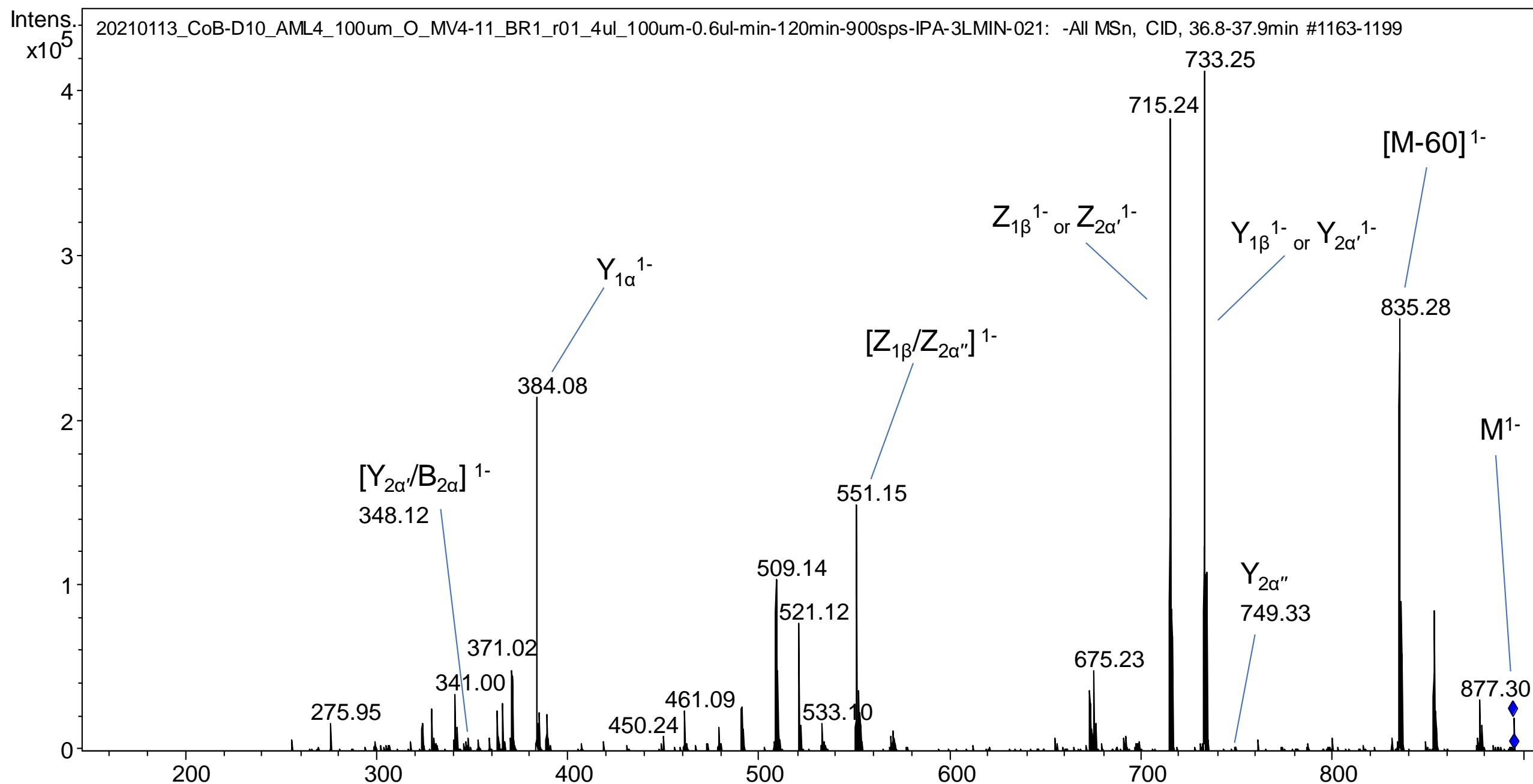
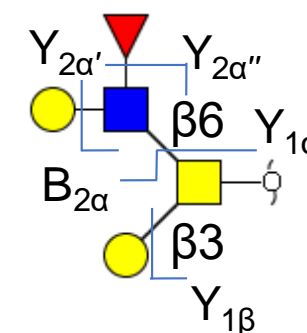
Glycan 8

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

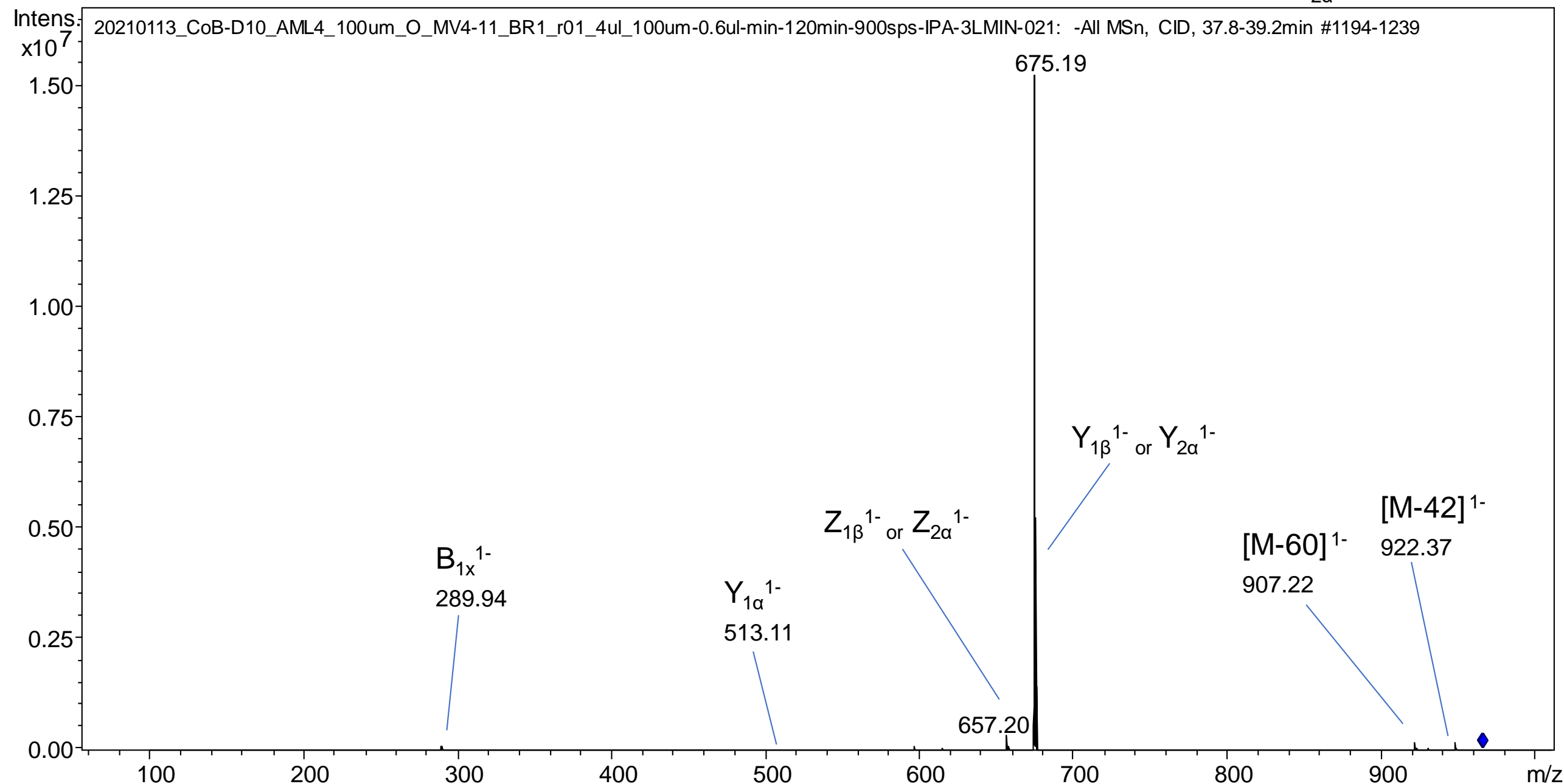
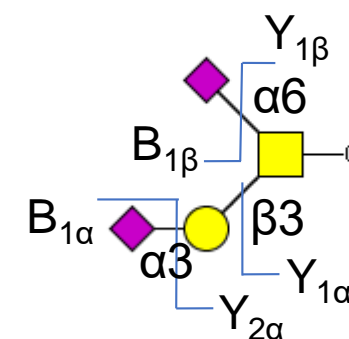


H1N1S2

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 10a

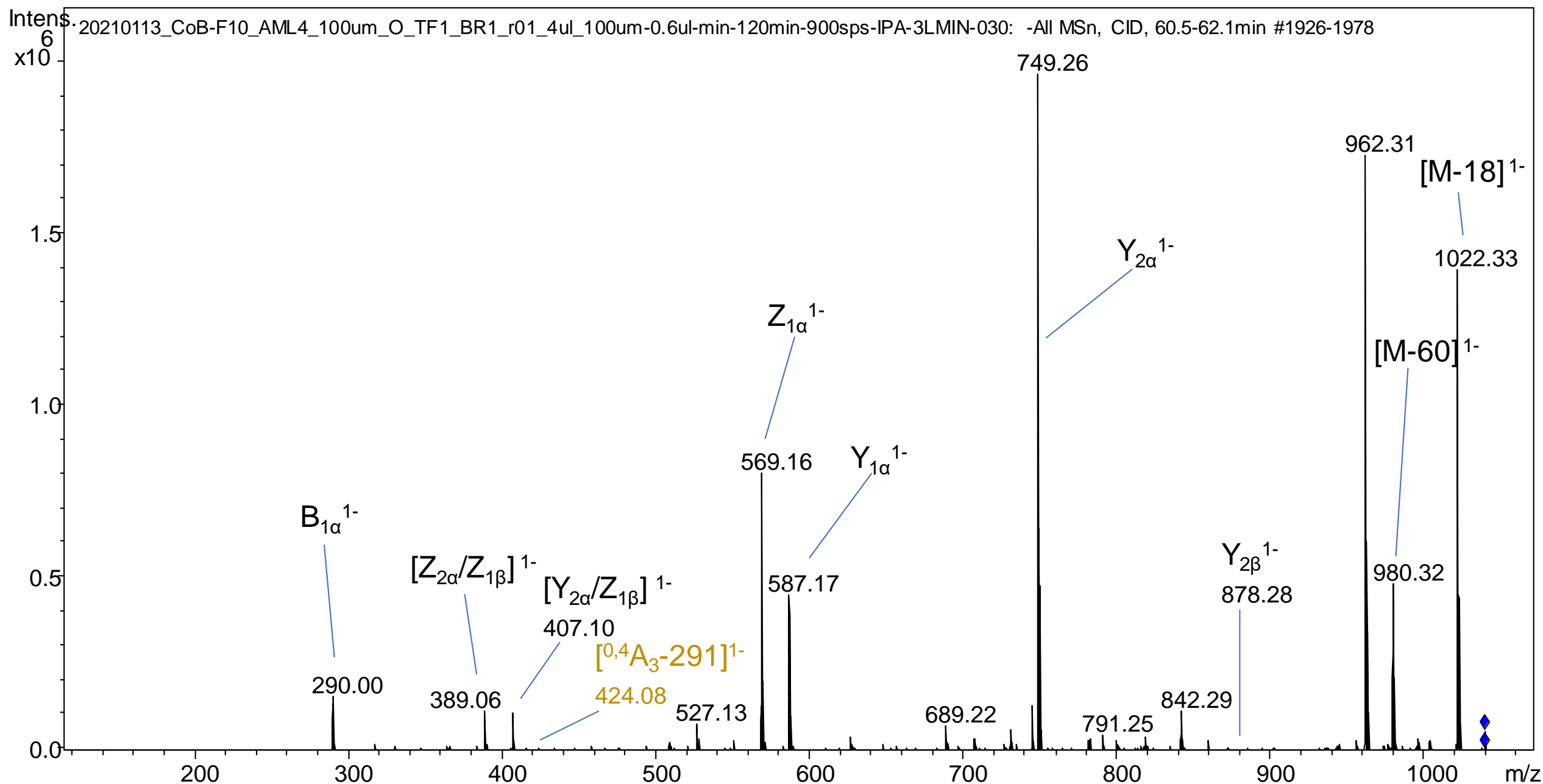
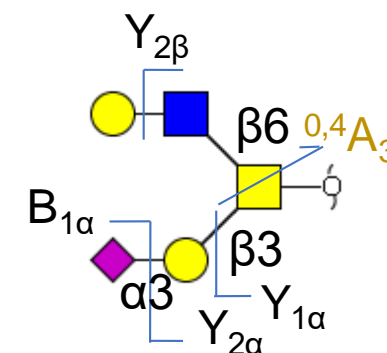
H2N2S1

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

Monoisotopic mass: 1041.40 Da
Charge observed: 1-
Theoretical ion: *m/z* 1040.39
Observed ion: *m/z* 1040.35
Mass deviation: *m/z* 0.04
Retention time: 53.5 min
Note: α -2,3 linkage of sialic acid confirmed
by neuraminidase S and A treatment

UniCarb-DB: #48

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



Glycan 10b

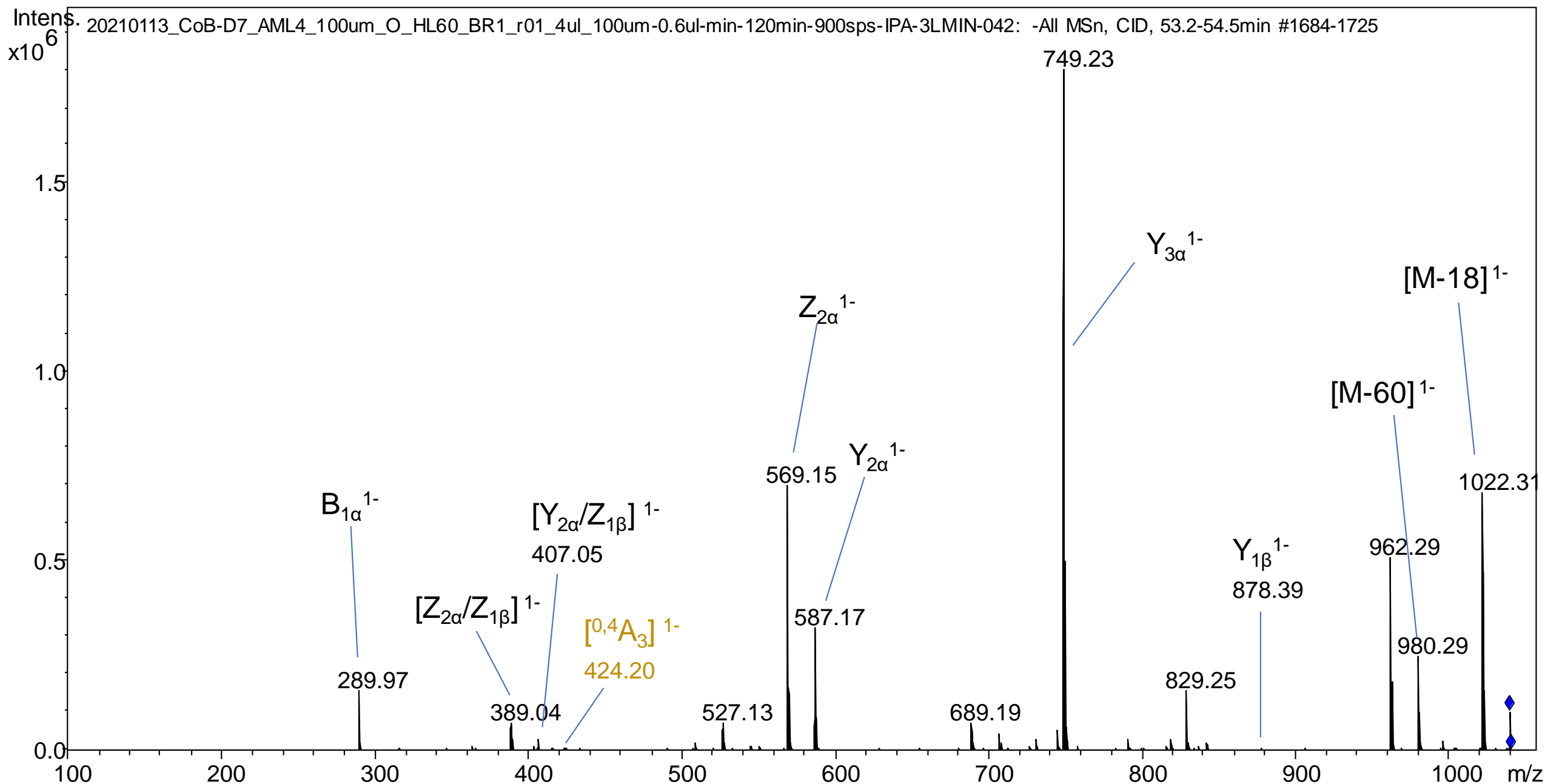
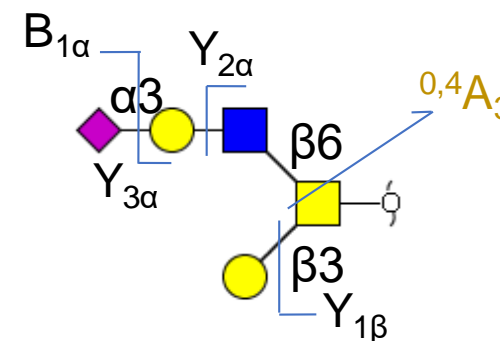
H2N2S1

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.

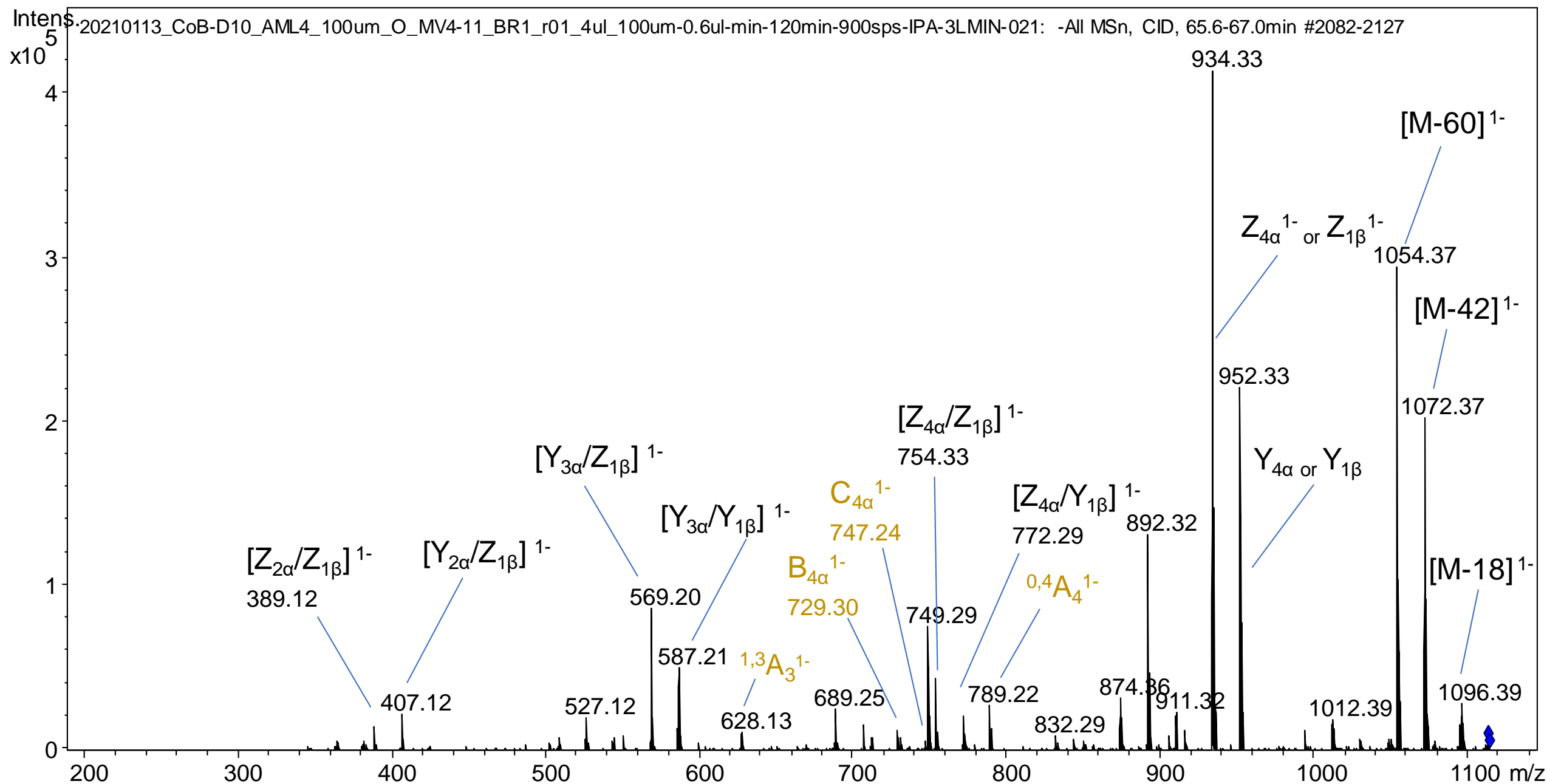
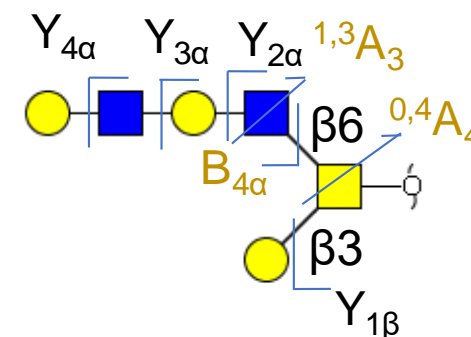


Glycan 11

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

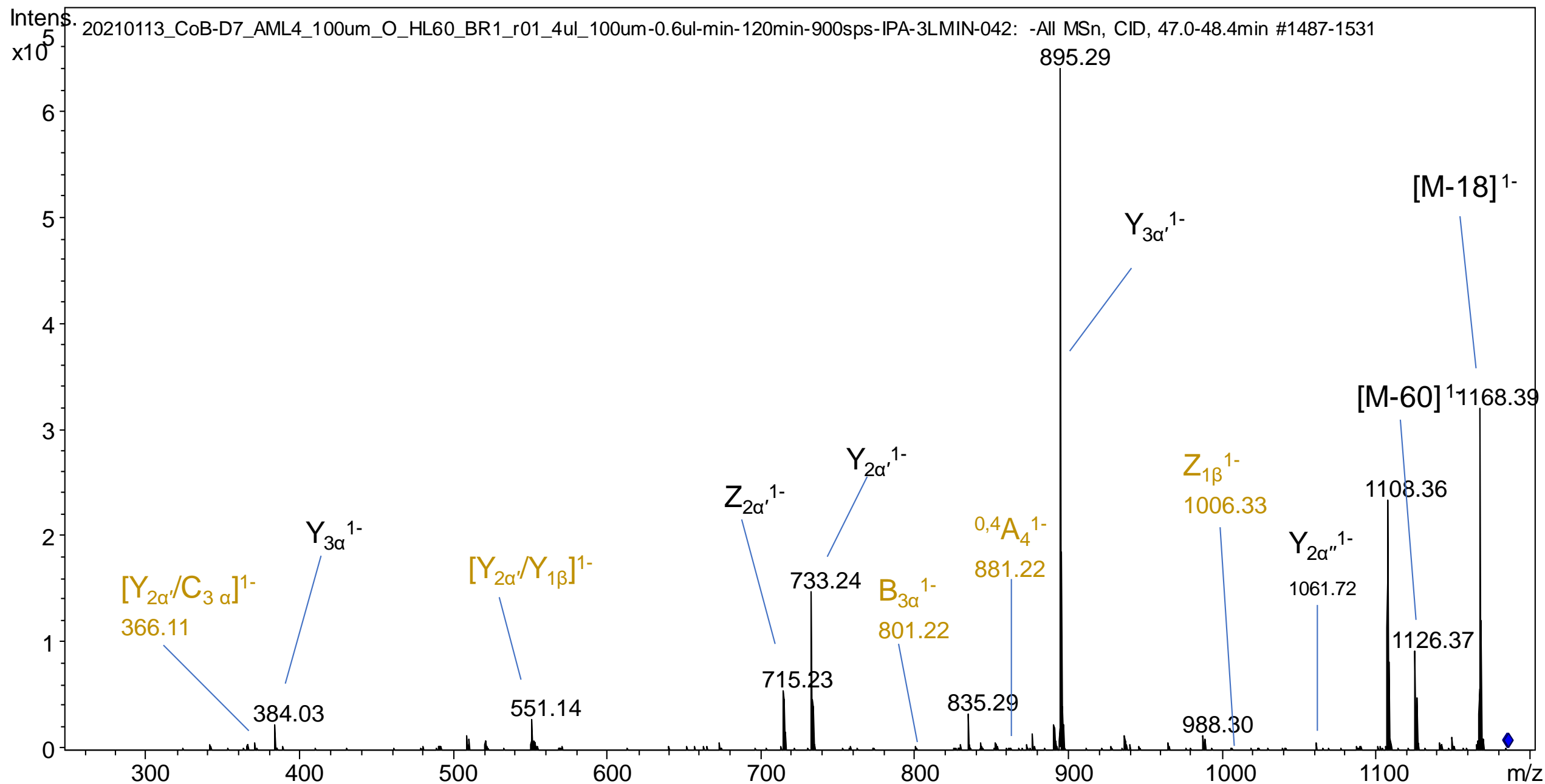


H2N2F1S1

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

| | |
|---------------------------|---------------------------|
| Monoisotopic mass: | 1187.45 Da |
| Charge observed: | 1- |
| Theoretical ion: | <i>m/z</i> 1186.44 |
| Observed ion: | <i>m/z</i> 1186.37 |
| Mass deviation: | <i>m/z</i> 0.07 |
| Retention time: | 47.7 min |

Note: α -2,3 linkage of sialic acids confirmed by neuraminidase S and A treatment; m/z 364 fragment indicates Lewis x



Glycan 12b

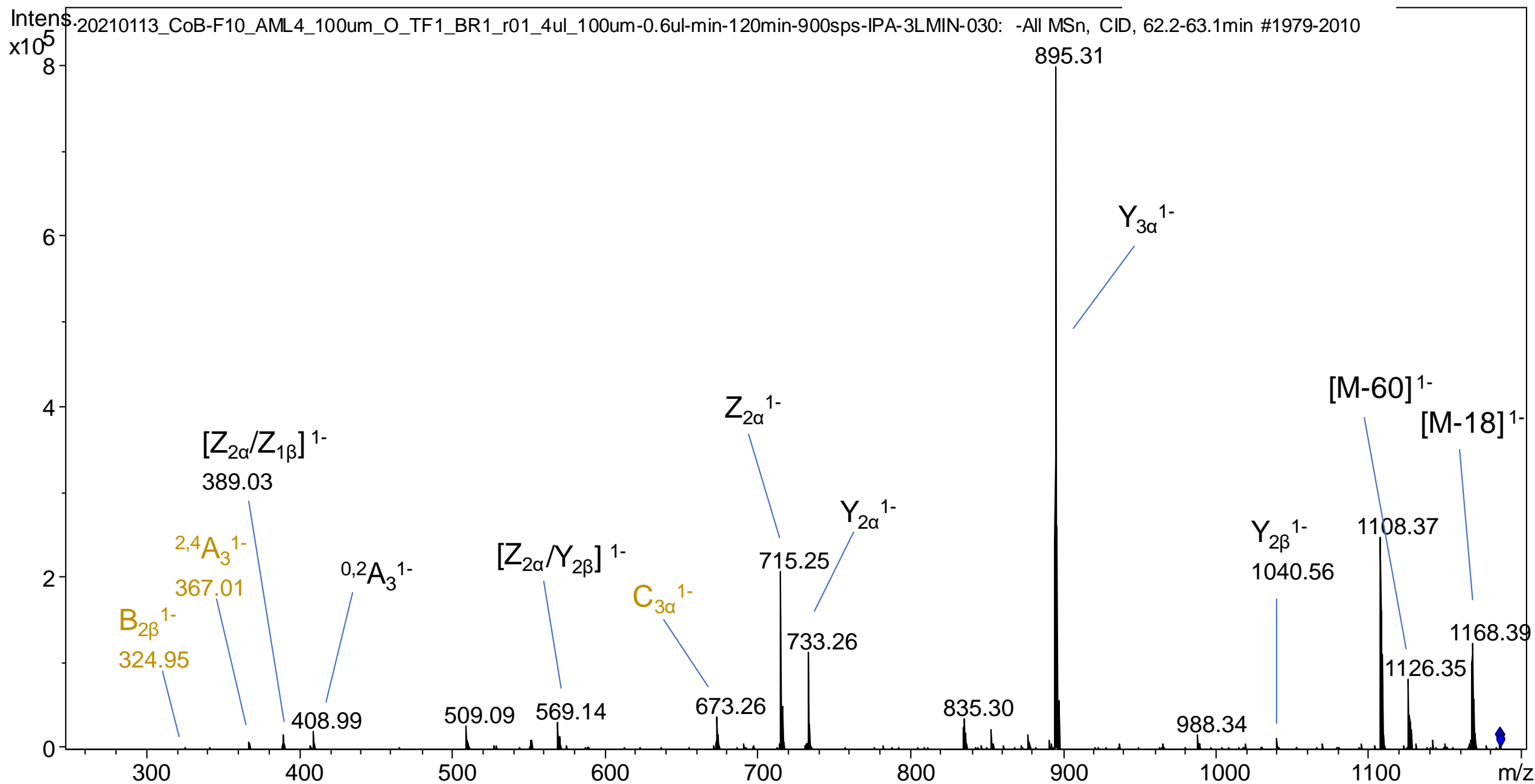
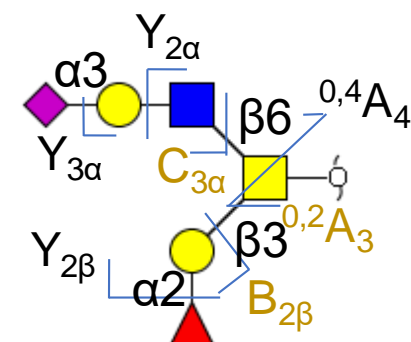
H2N2F1S1

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

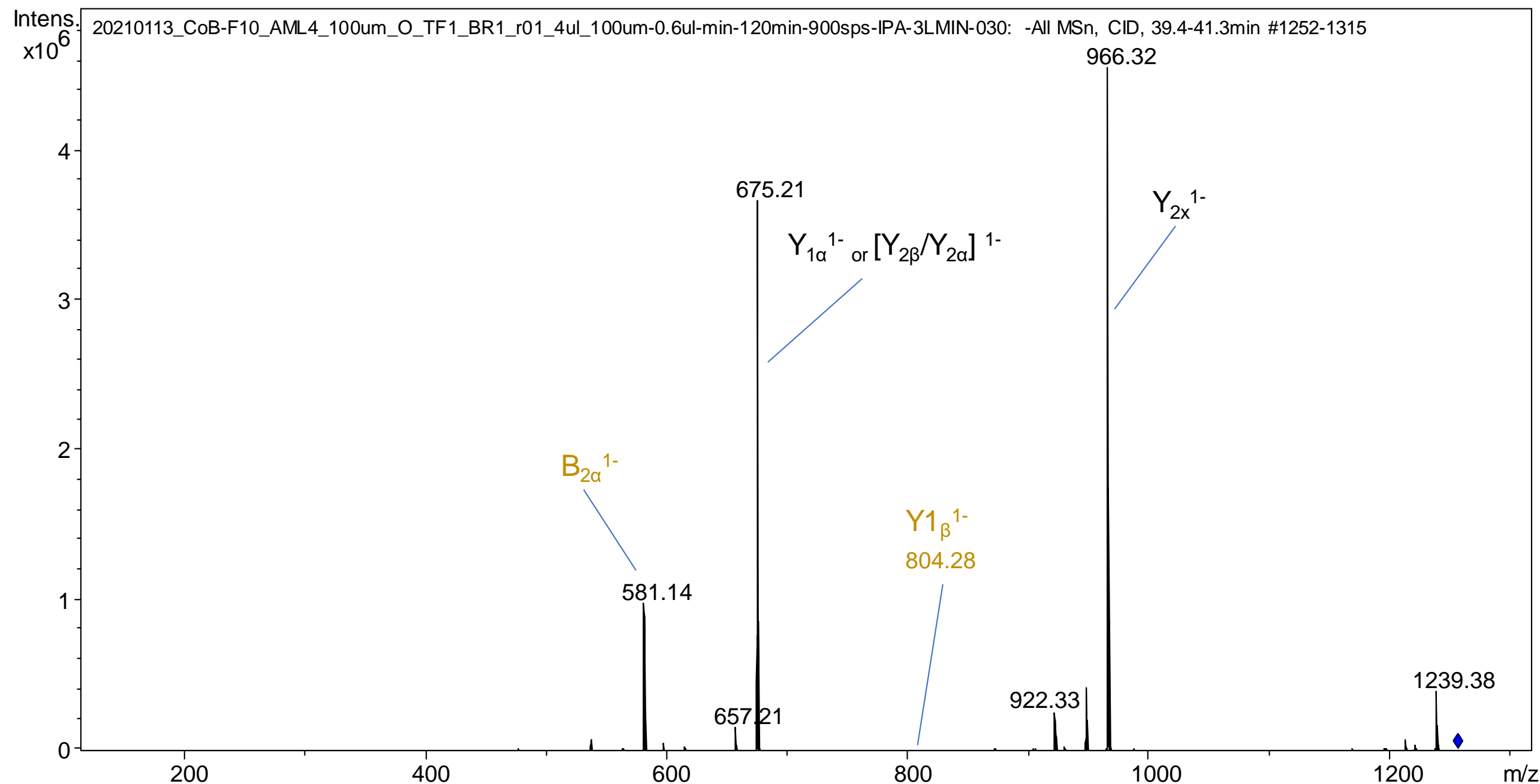
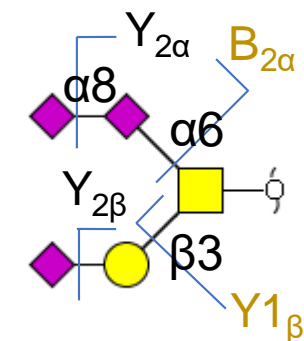


Glycan 13

H1N1S3

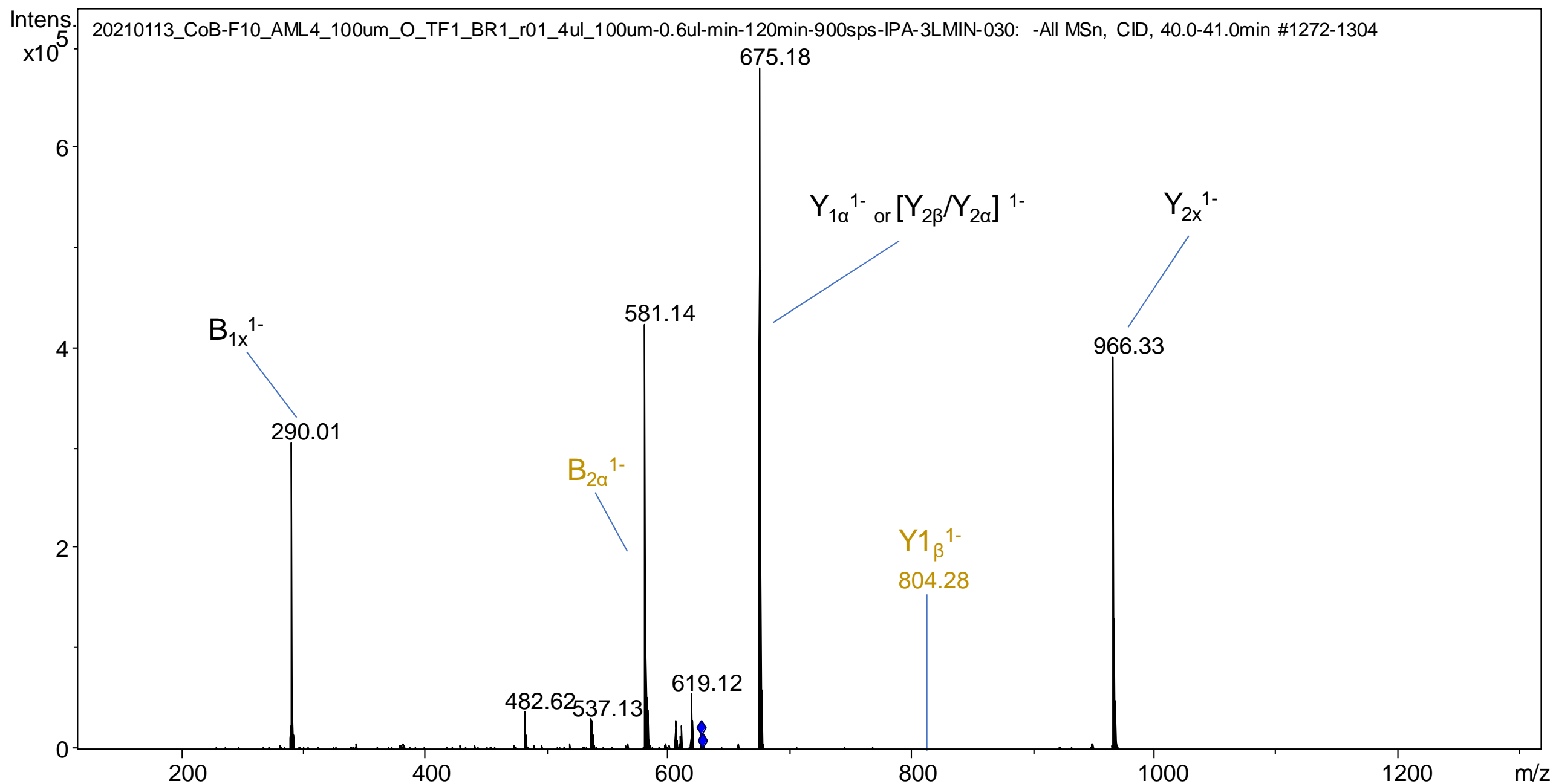
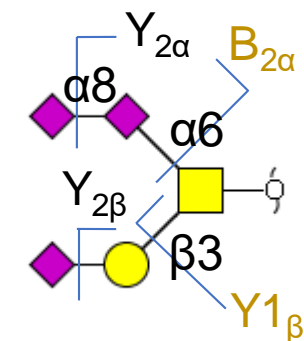
Depicted MS² was obtained from
analysis of cell line: TF-1

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



H1N1S3

Monoisotopic mass: 1258.45 Da
Charge observed: 2-
Theoretical ion: m/z 628.22
Observed ion: m/z 628.15
Mass deviation: m/z 0.05
Retention time: 39.9 min
Note: m/z 581 indicates two sialic acids linked to each other



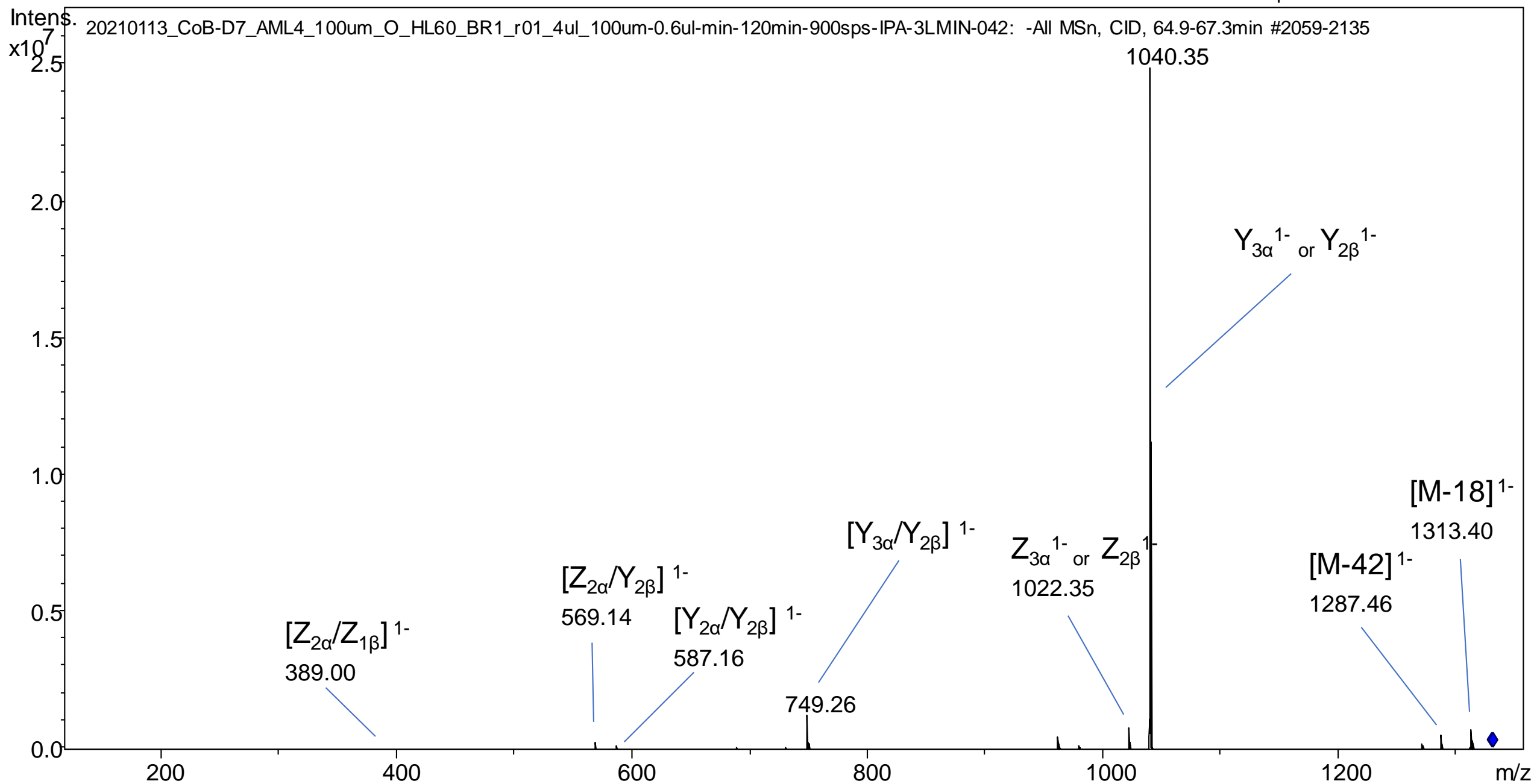
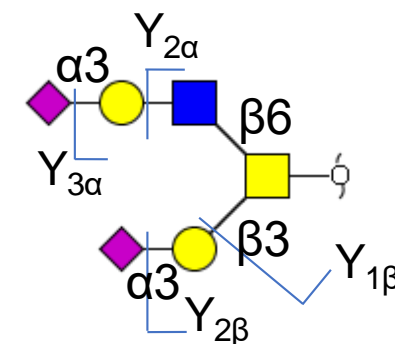
$$\text{H}_2\text{N}_2\text{S}_2$$

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

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

UniCarb-DB: #502

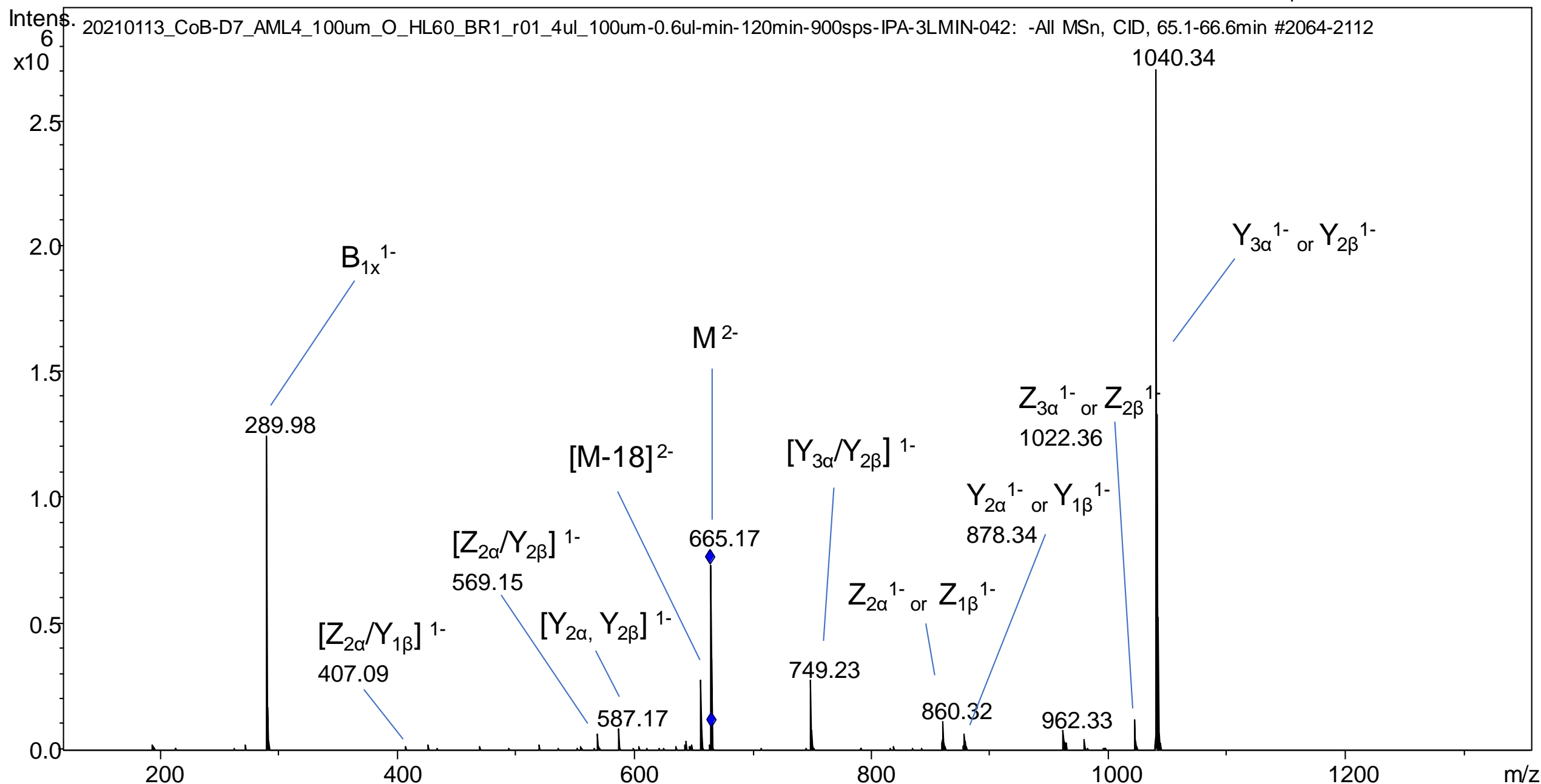
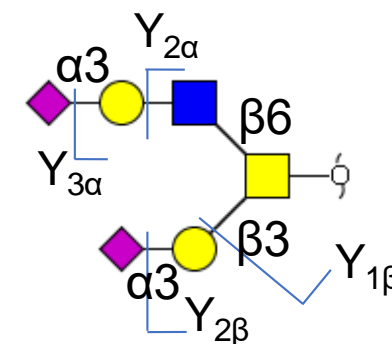
Note:: α -2,3 linkage of both sialic acids confirmed by neuraminidase S and A treatment as no species were detected with α -2,6 as a digested H2N2S1



$$\text{H}_2\text{N}_2\text{S}_2$$

| | |
|---------------------------|--------------------------|
| Monoisotopic mass: | 1332.48 Da |
| Charge observed: | 2- |
| Theoretical ion: | <i>m/z</i> 665.23 |
| Observed ion: | <i>m/z</i> 665.19 |
| Mass deviation: | <i>m/z</i> 0.04 |
| Retention time: | 65.4 min |

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



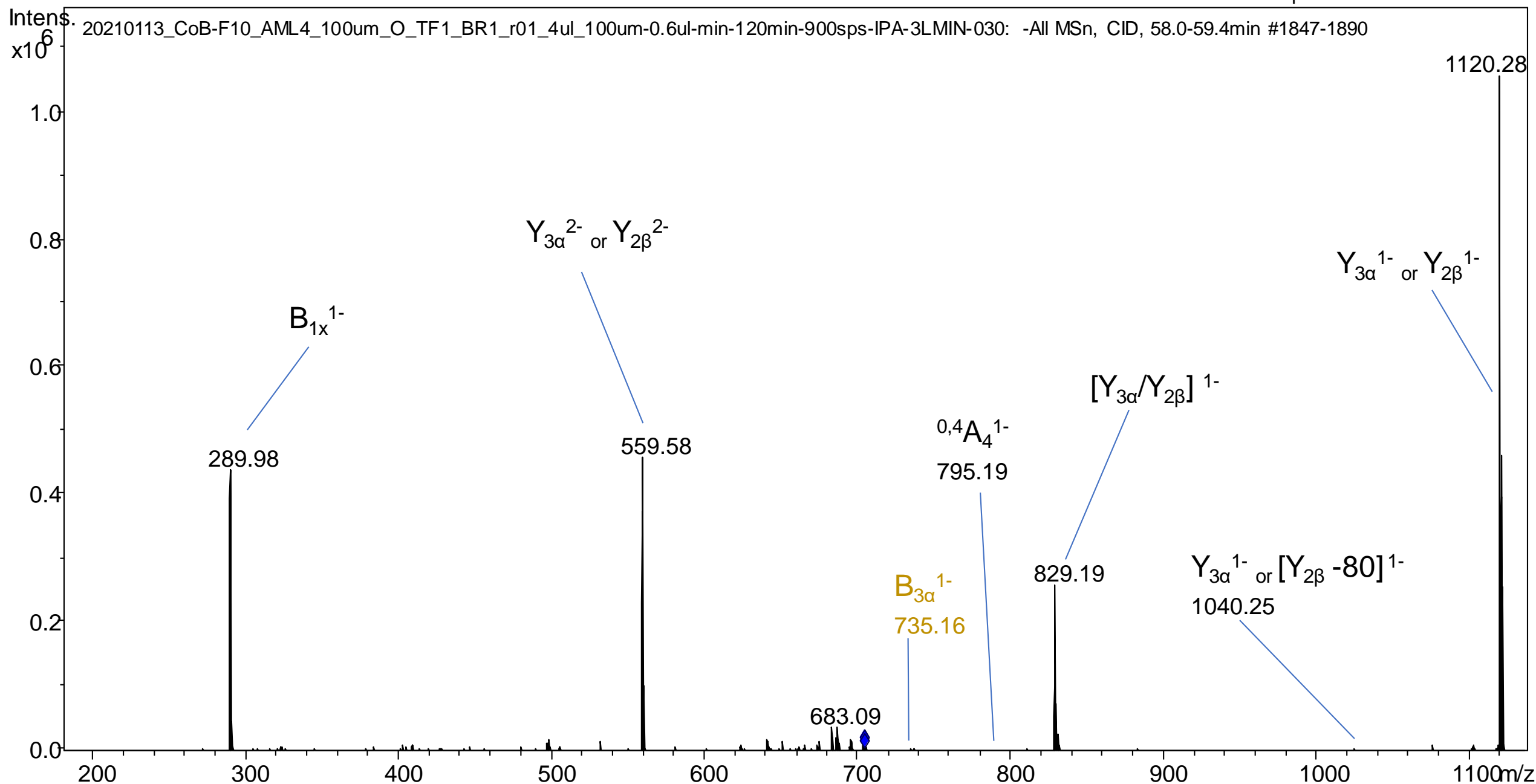
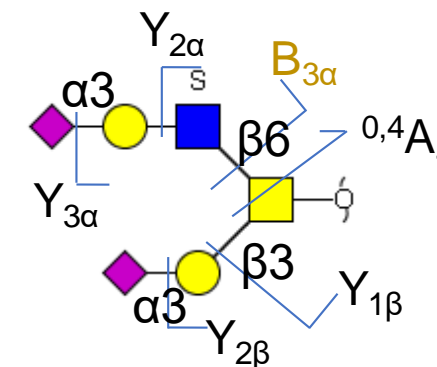
H2N2S2Su1

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

| | |
|---------------------------|--------------------------|
| Monoisotopic mass: | 1412.44 Da |
| Charge observed: | 2- |
| Theoretical ion: | <i>m/z</i> 705.21 |
| Observed ion: | <i>m/z</i> 705.14 |
| Mass deviation: | <i>m/z</i> 0.07 |
| Retention time: | 58.3 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 a digested H2N2S1Su1

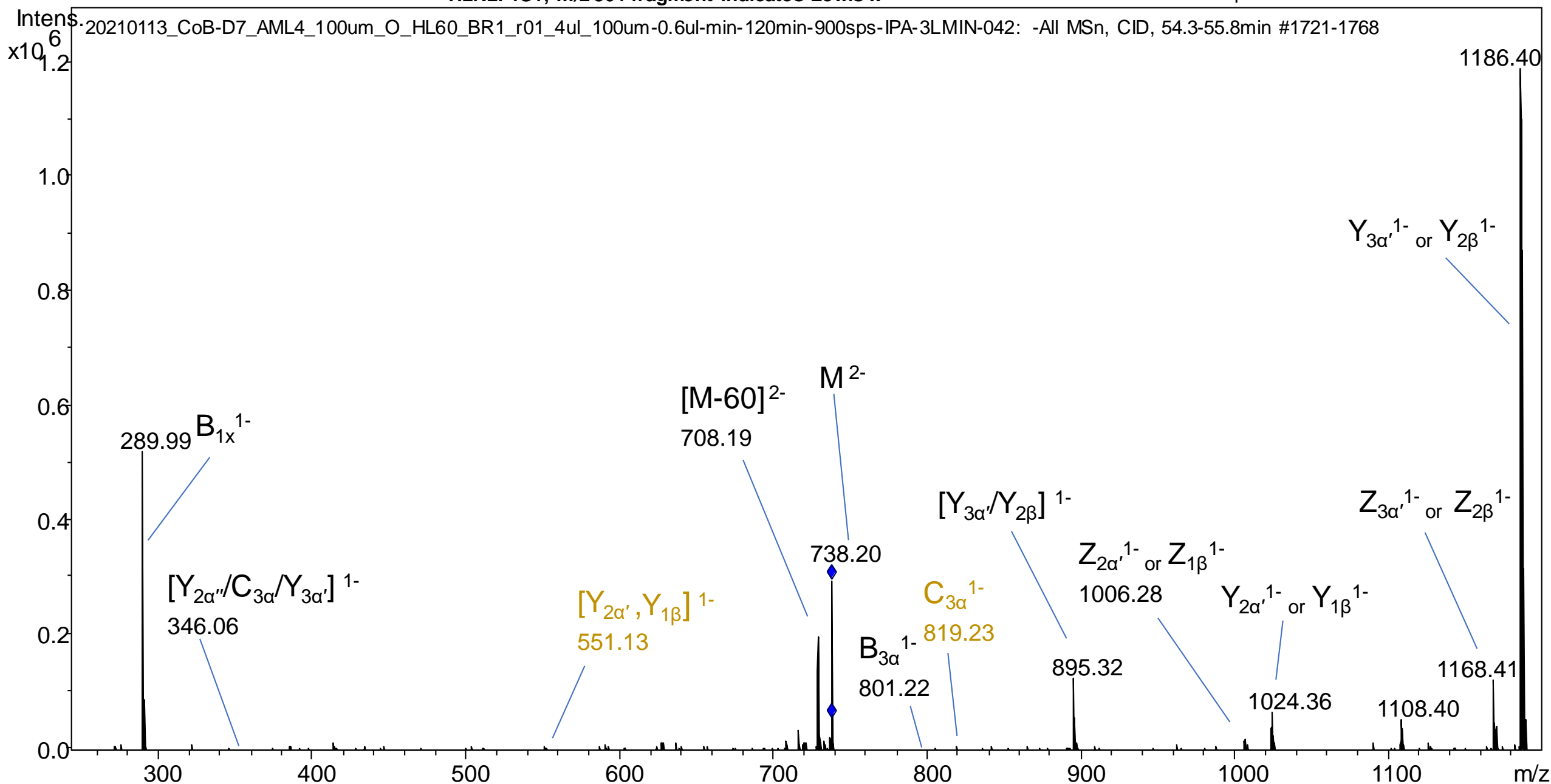
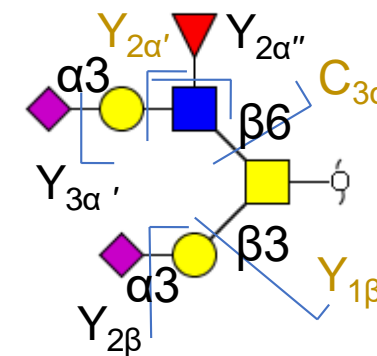
UniCarb-DB: #2650



H2N2F1S2

| | |
|---------------------------|--------------------------|
| Monoisotopic mass: | 1478.54 Da |
| Charge observed: | 2- |
| Theoretical ion: | <i>m/z</i> 738.26 |
| Observed ion: | <i>m/z</i> 738.21 |
| Mass deviation: | <i>m/z</i> 0.05 |
| Retention time: | 54.6 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 a digested H2N2F1S1; m/z 364 fragment indicates Lewis x

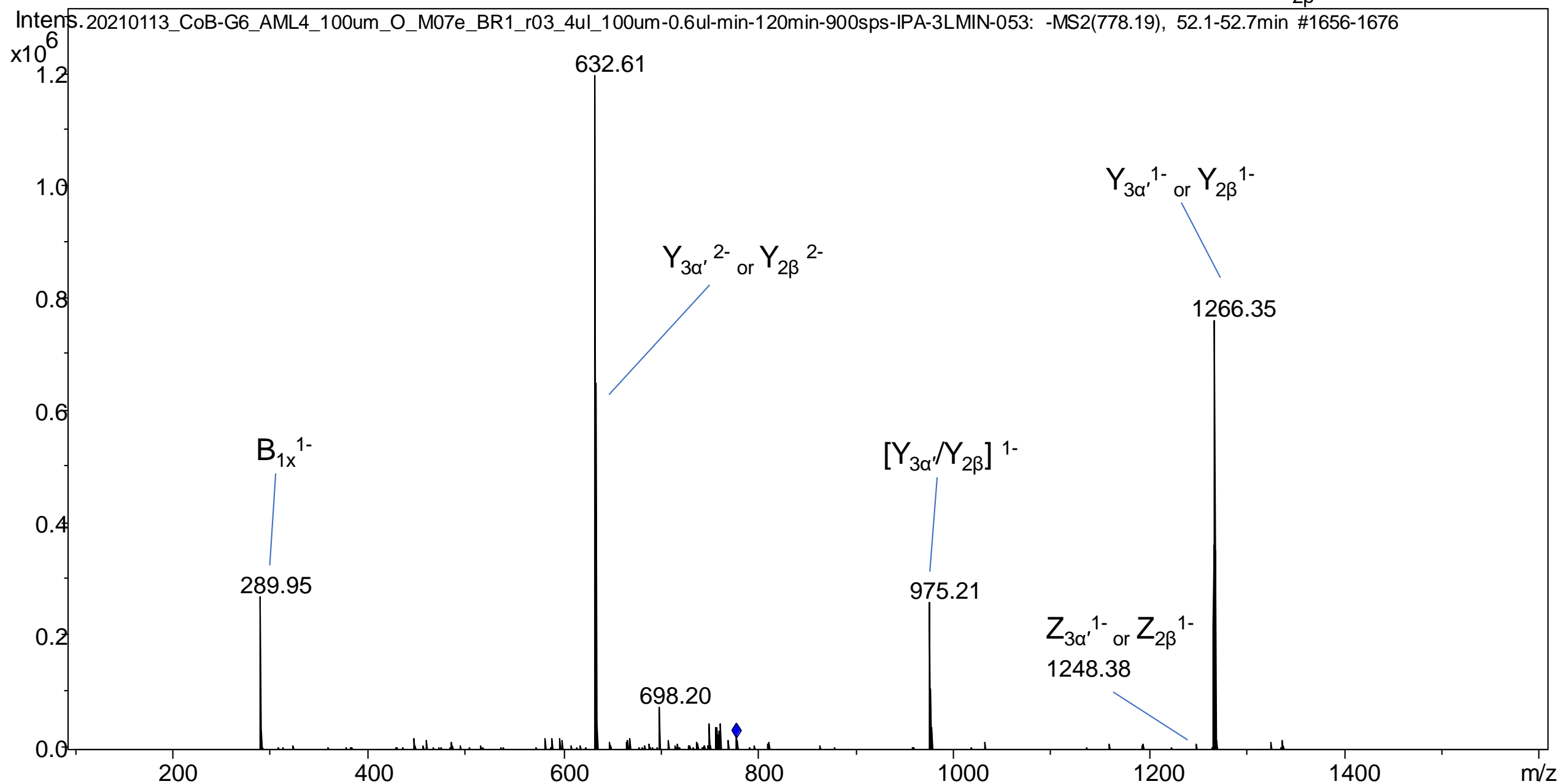
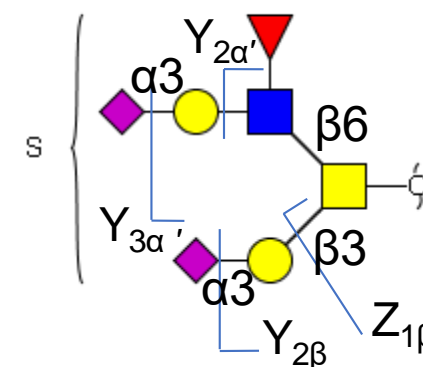


H2N2F1S2Su1

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

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

UniCarb-DB: #2947

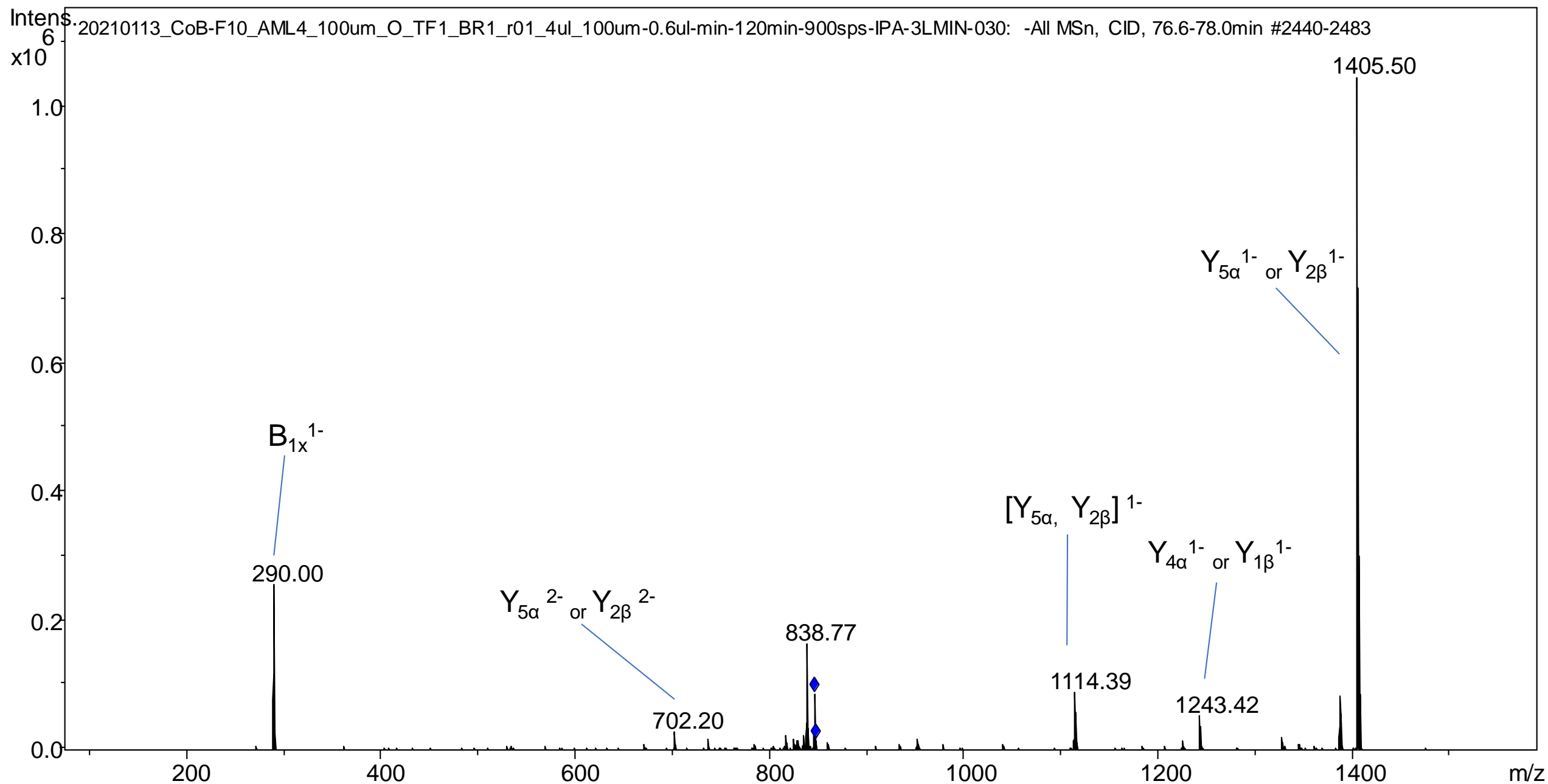
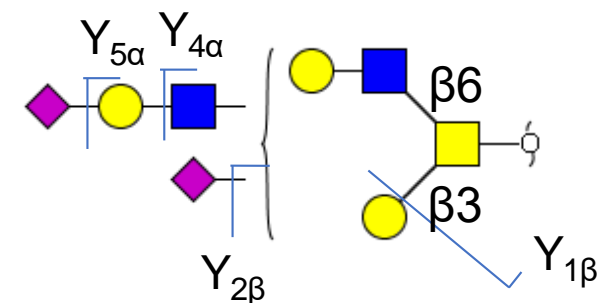


Glycan 18a

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



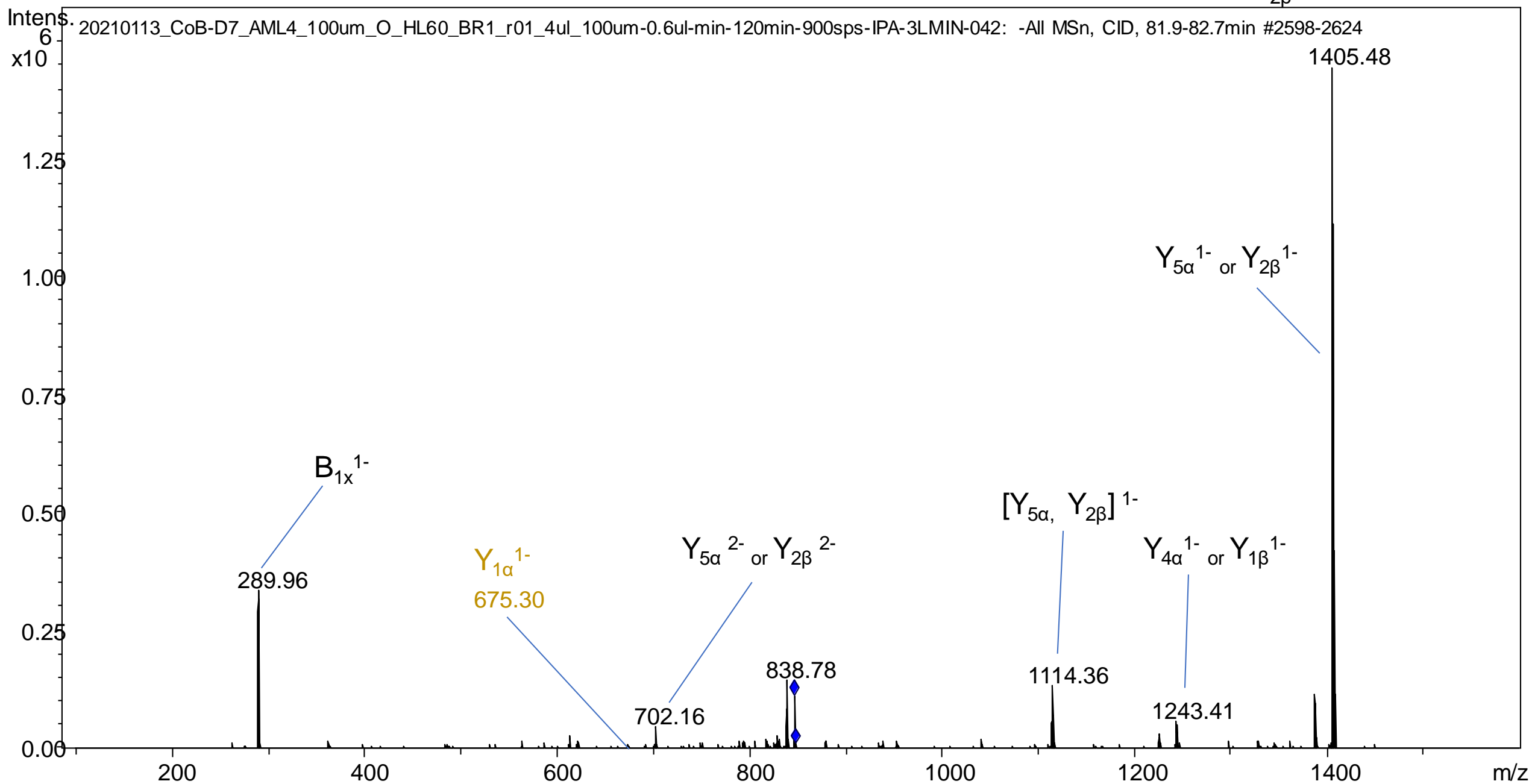
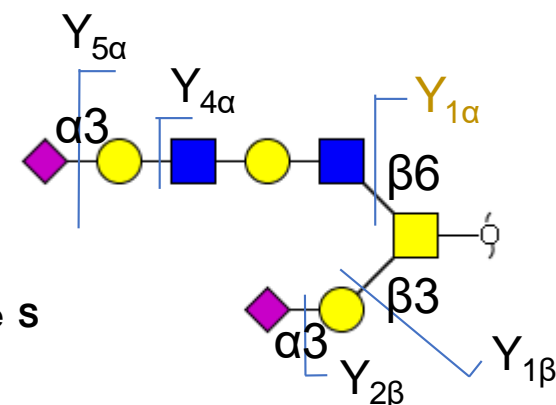
Glycan 18b

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

