Supplementary information 2

Glycoproteomics of a single protein: revealing tens of thousands of Myozyme® glycoforms by hybrid HPLC-MS approaches

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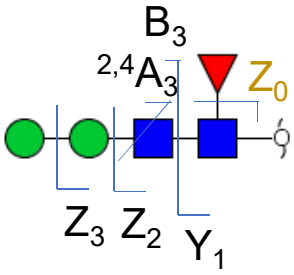
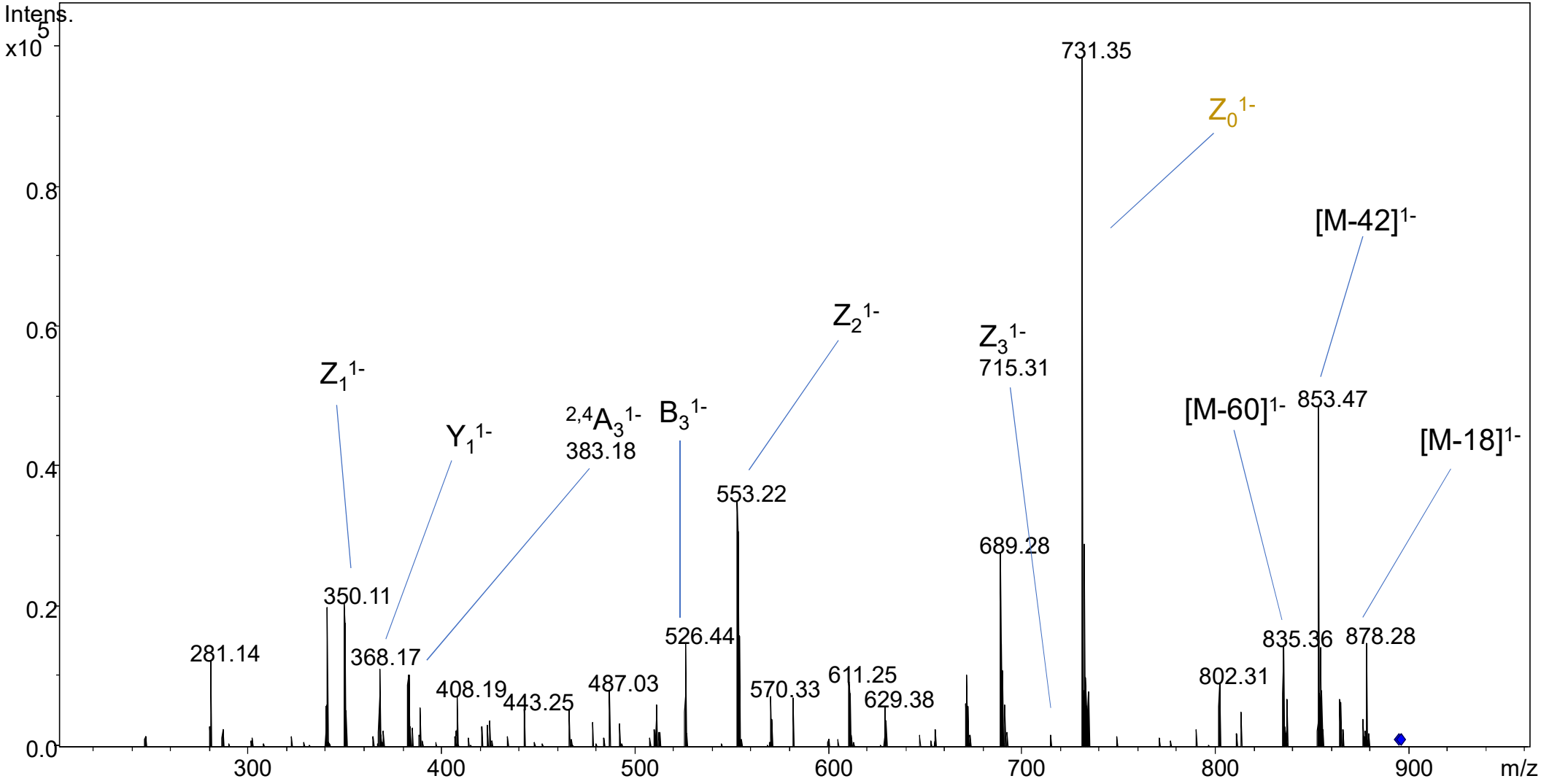
Glycan 1

M2F (H2N2F1)

Monoisotopic mass:	**896.35 Da**	**UniCarb-DB: #2223**
Charge observed:	**1-**	
Theoretical ion:	***m/z* 895.34**	
Observed ion:	***m/z* 895.45**	
Mass deviation:	***m/z* 0.11**	
Retention time:	**58.6 min**	



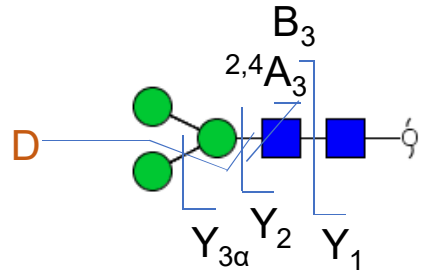


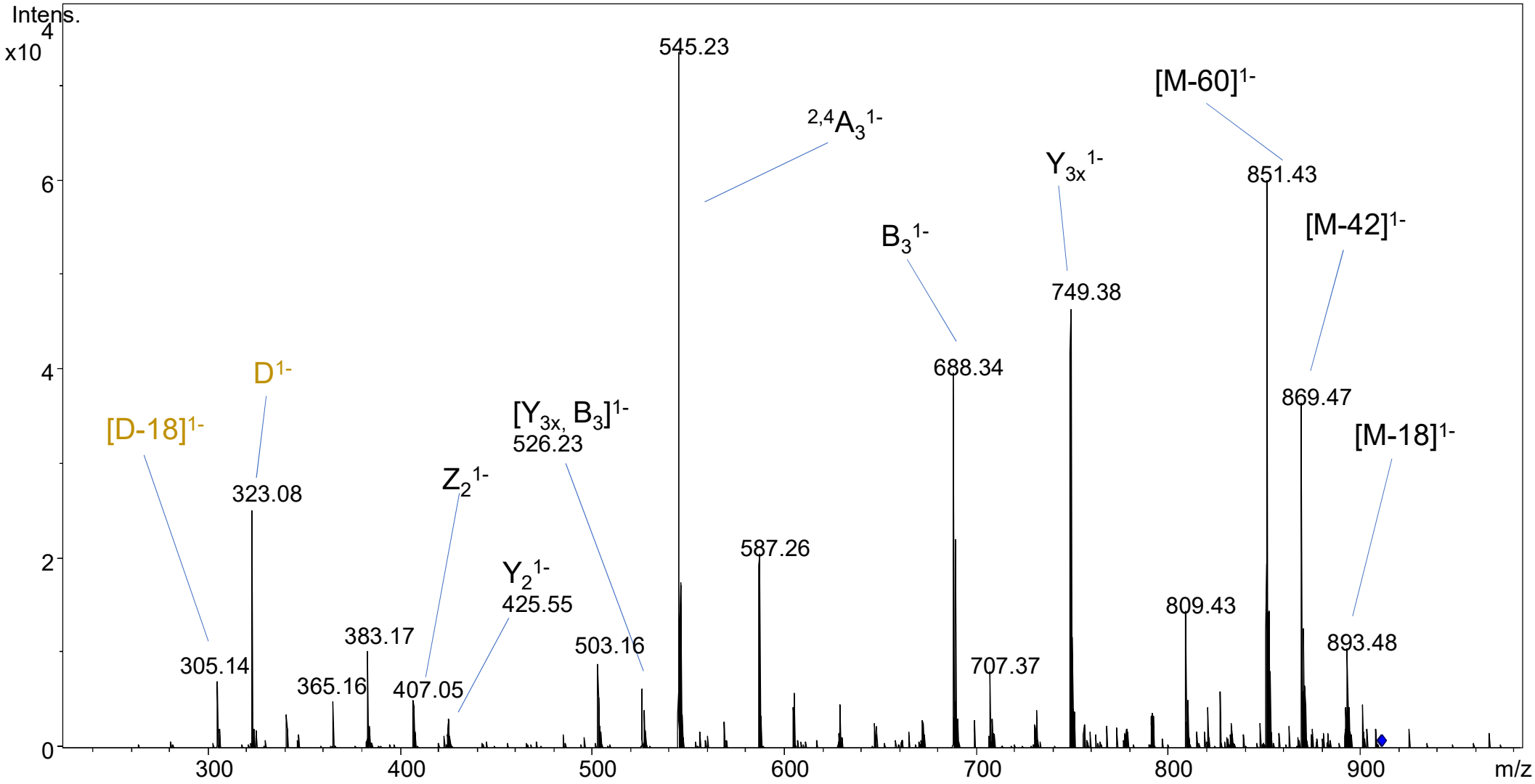
Glycan 2

M3 (H3N2)

Monoisotopic mass:	**912.35 Da**
Charge observed:	**1-**
Theoretical ion:	***m/z* 911.34**
Observed ion:	***m/z* 911.44**
Mass deviation:	***m/z* 0.10**
Retention time:	**55.5 min**

UniCarb-DB: #610

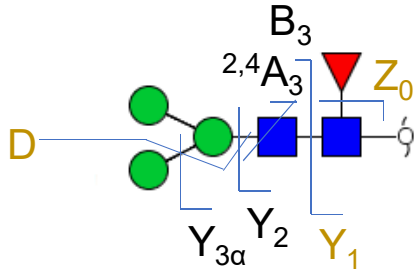


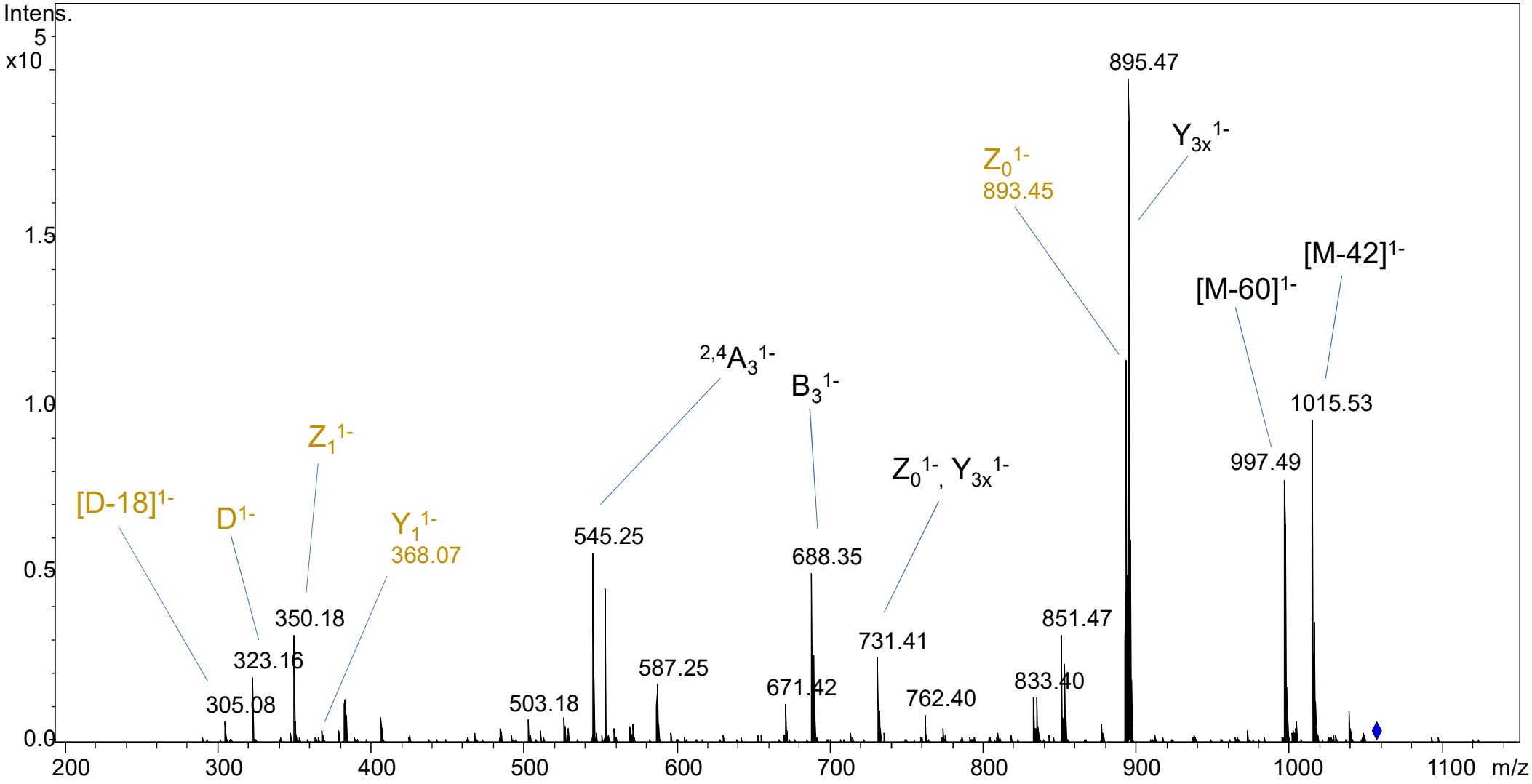


Glycan 3

M3F (H3N2F1)

Monoisotopic mass:	**1058.40 Da**	**UniCarb-DB: #441**
Charge observed:	**1-**	
Theoretical ion:	***m/z* 1057.39**	
Observed ion:	***m/z* 1057.53**	
Mass deviation:	***m/z* 0.14**	
Retention time:	**66.4 min**	

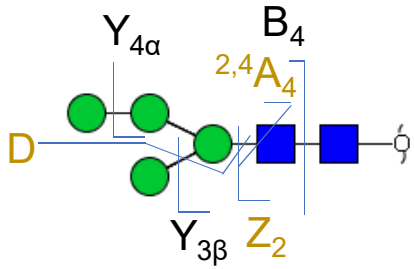


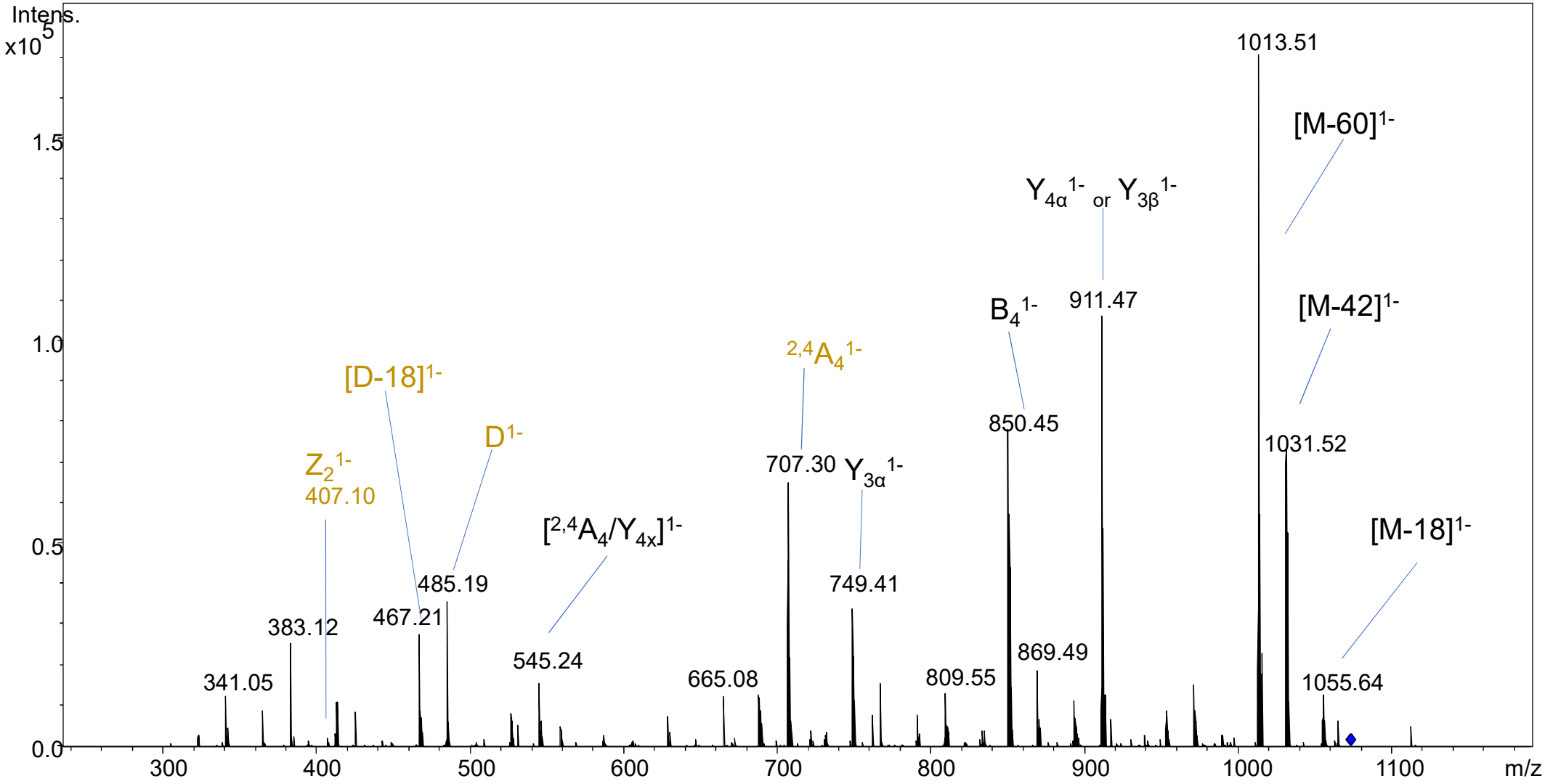


Glycan 4

M4 (H4N2)

Monoisotopic mass:	**1074.40 Da**
Charge observed:	**1-**
Theoretical ion:	***m/z* 1073.39**
Observed ion:	***m/z* 1073.53**
Mass deviation:	***m/z* 0.14**
Retention time:	**52.2 min**



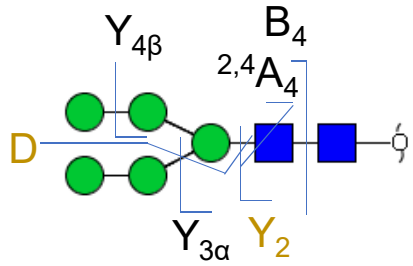


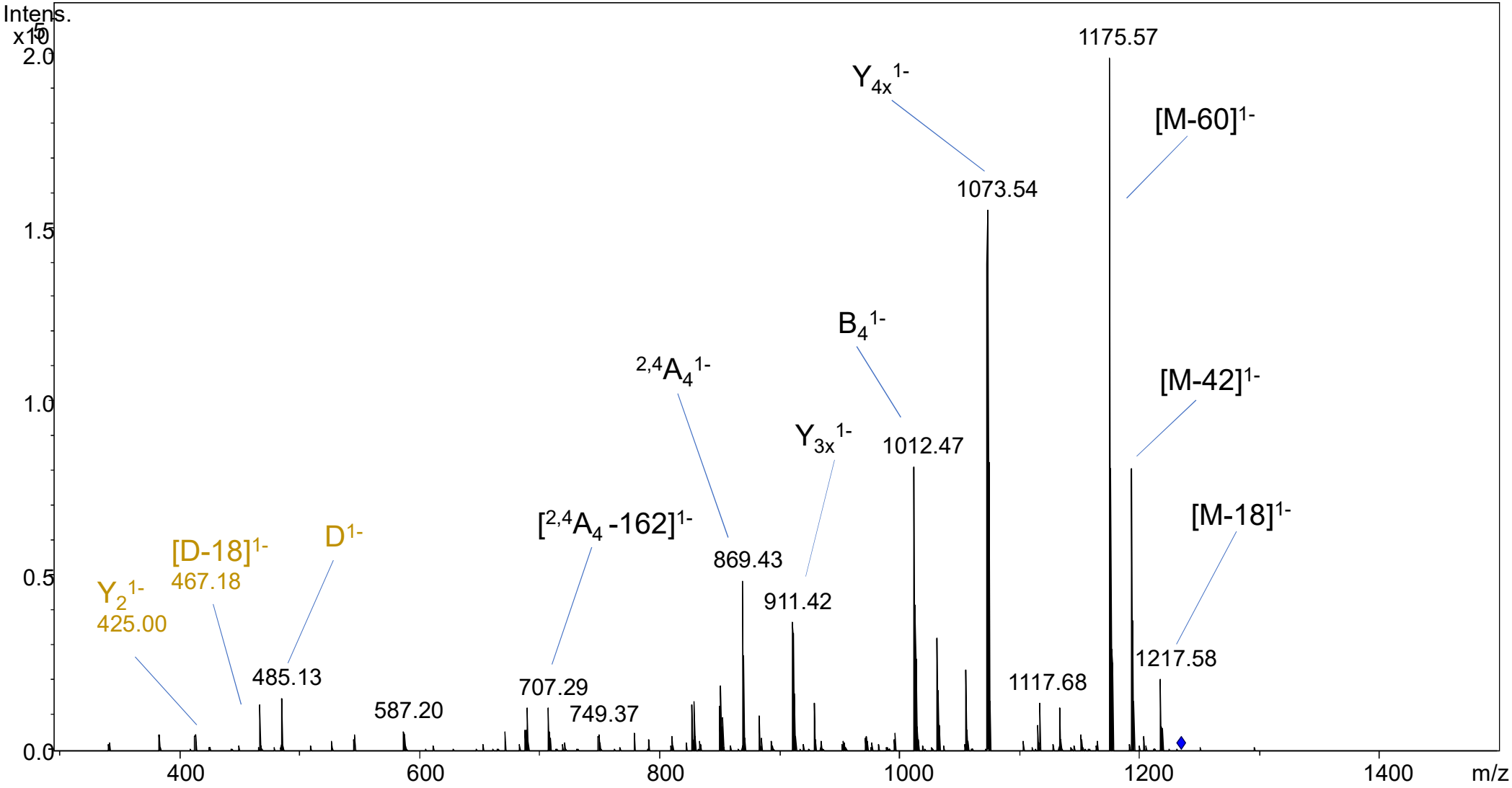
Glycan 5a

M5 (H5N2)

Monoisotopic mass:	**1236.45 Da**
Charge observed:	**1-**
Theoretical ion:	***m/z* 1235.44**
Observed ion:	***m/z* 1235.57**
Mass deviation:	***m/z* 0.13**
Retention time:	**42.6 min**

UniCarb-DB: #2213



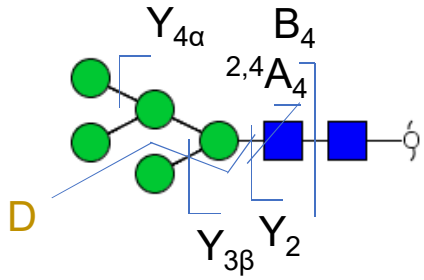


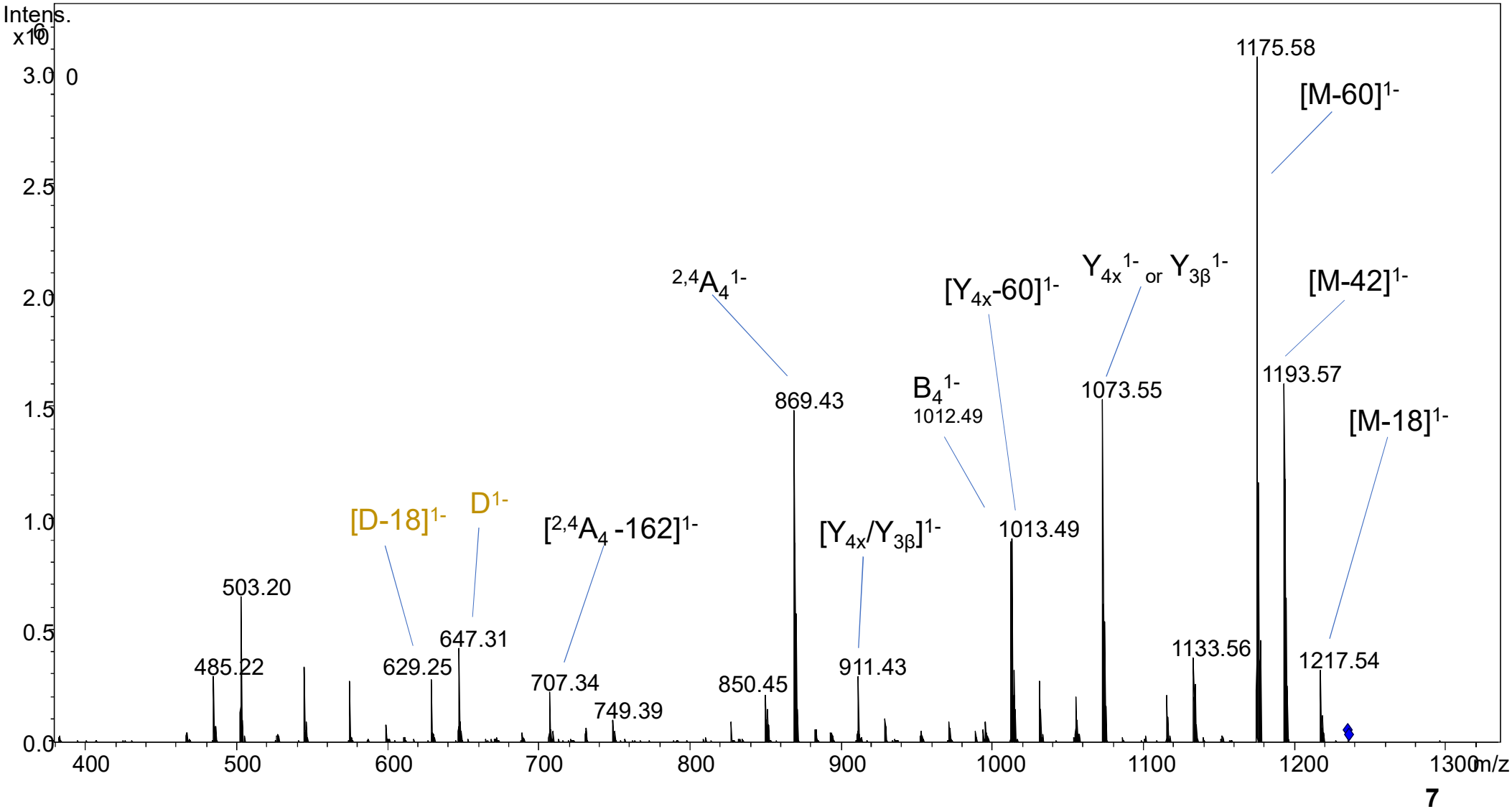
Glycan 5b

M5 (H5N2)

Monoisotopic mass:	**1236.45 Da**
Charge observed:	**1-**
Theoretical ion:	***m/z* 1235.44**
Observed ion:	***m/z* 1235.60**
Mass deviation:	***m/z* 0.16**
Retention time:	**57.6 min**

UniCarb-DB: #395

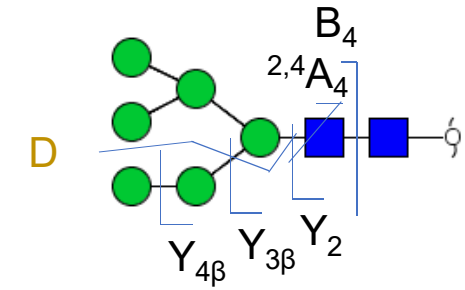


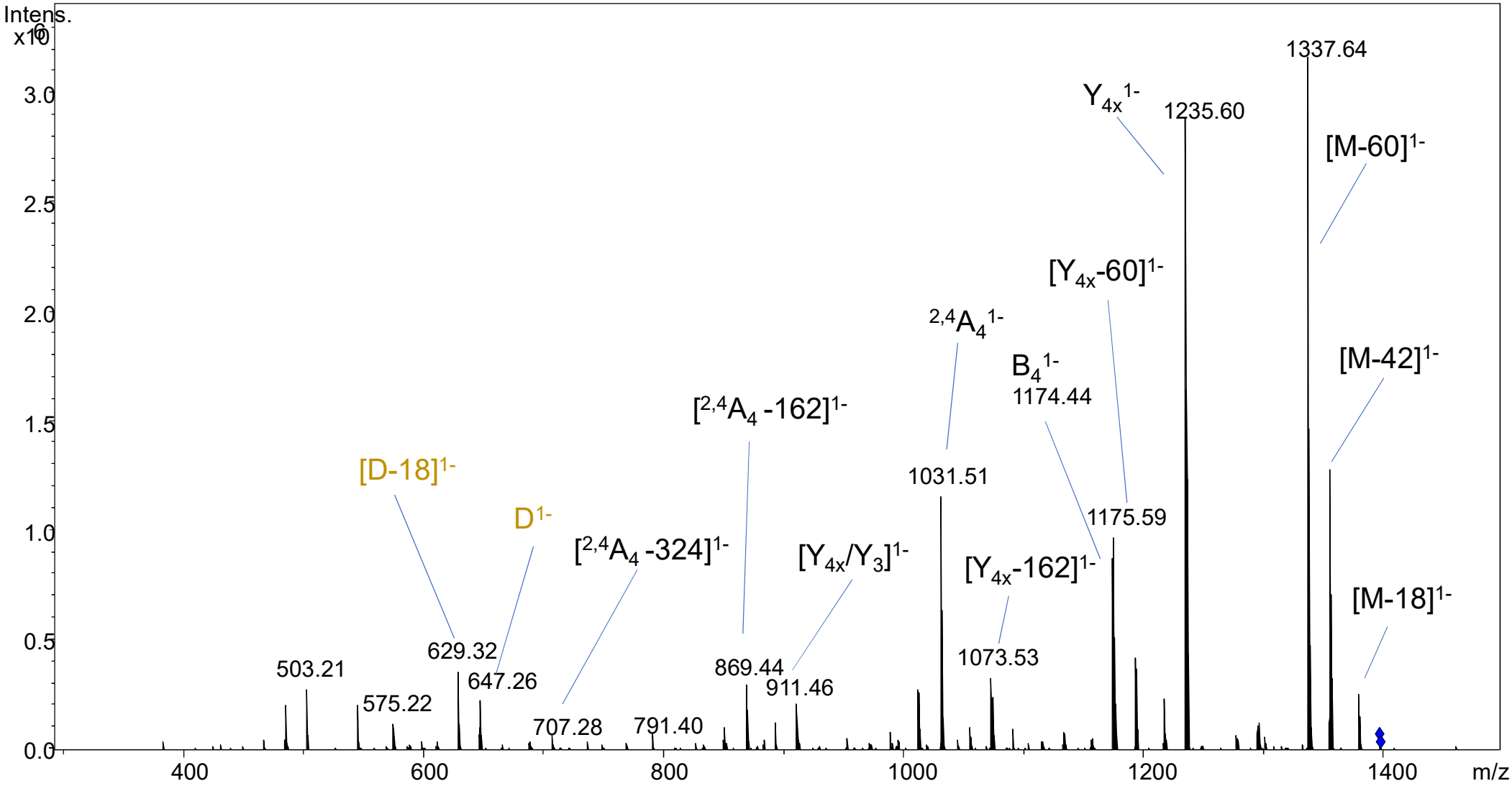


Glycan 6

M6 (H6N2)

Monoisotopic mass:	**1398.51 Da**	**UniCarb-DB: #613**
Charge observed:	**1-**	
Theoretical ion:	***m/z* 1397.50**	
Observed ion:	***m/z* 1397.67**	
Mass deviation:	***m/z* 0.17**	
Retention time:	**48.4 min**	



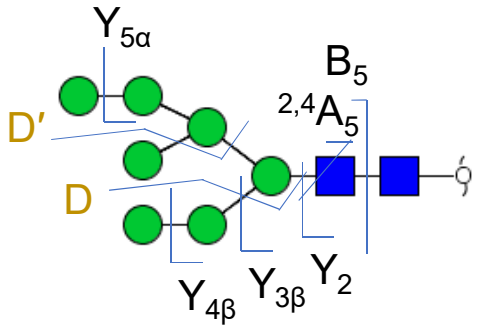


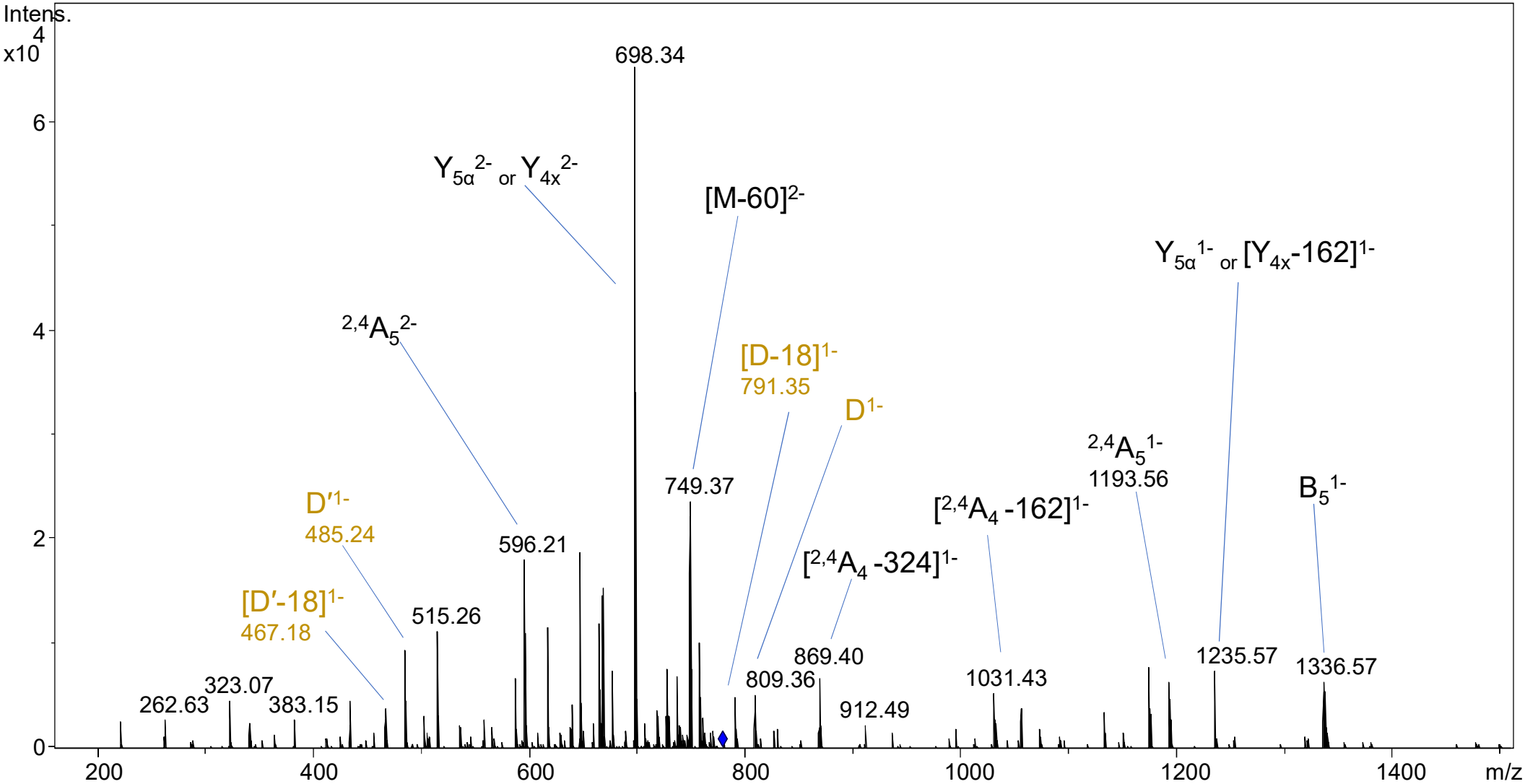
Glycan 7a

M7 (H7N2)

Monoisotopic mass:	**1560.55 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 779.27**
Observed ion:	***m/z* 779.42**
Mass deviation:	***m/z* 0.15**
Retention time:	**46.6 min**

UniCarb-DB: #403



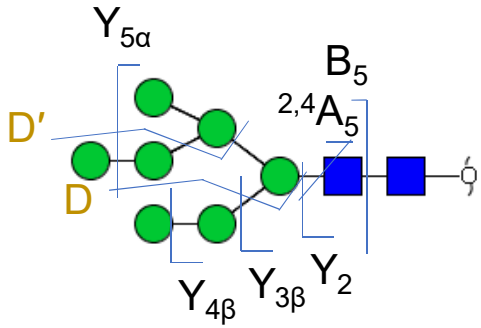


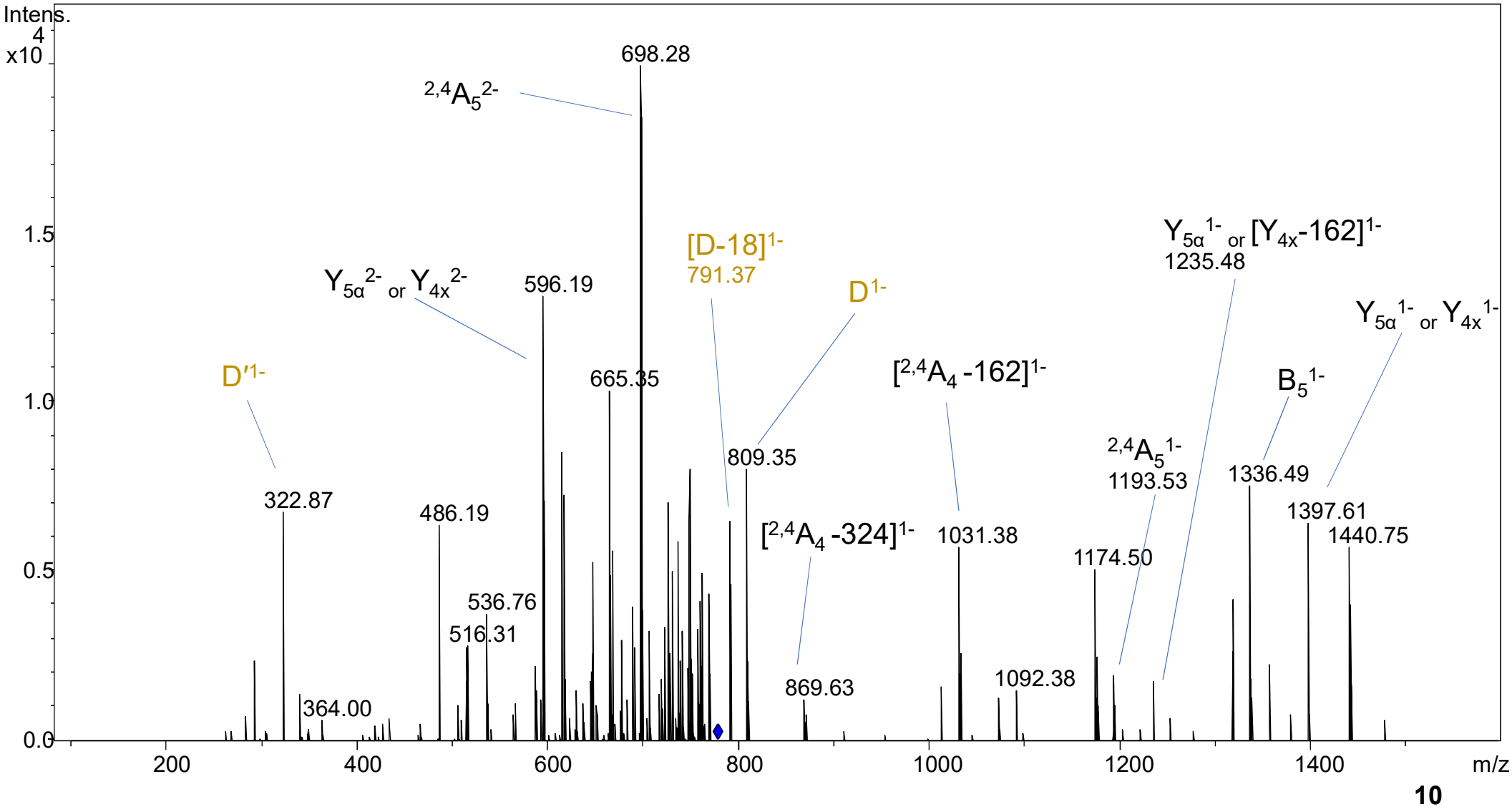
Glycan 7b

M7 (H7N2)

Monoisotopic mass:	**1560.55 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 779.27**
Observed ion:	***m/z* 779.37**
Mass deviation:	***m/z* 0.10**
Retention time:	**48.8 min**

UniCarb-DB: #403

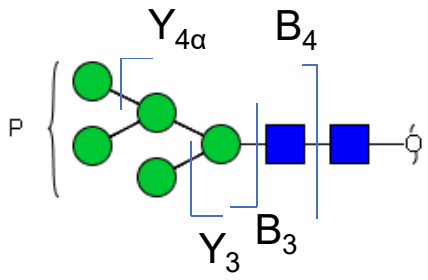


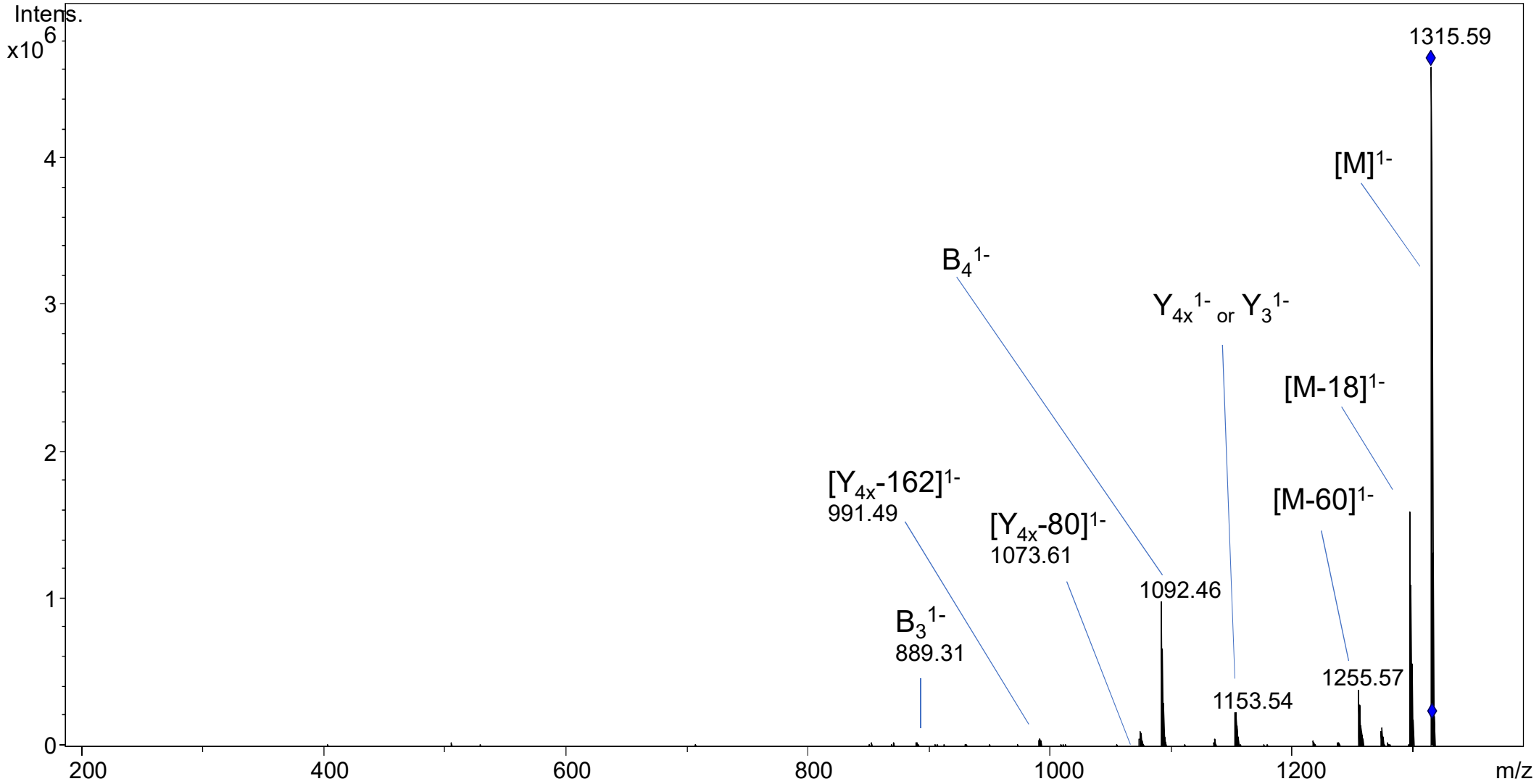


Glycan 8

M5P (H5N2P1)

Monoisotopic mass:	**1316.42 Da**
Charge observed:	**1-**
Theoretical ion:	***m/z* 1315.41**
Observed ion:	***m/z* 1315.58**
Mass deviation:	***m/z* 0.17**
Retention time:	**33.4 min**

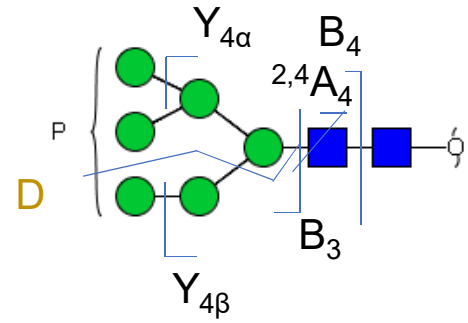


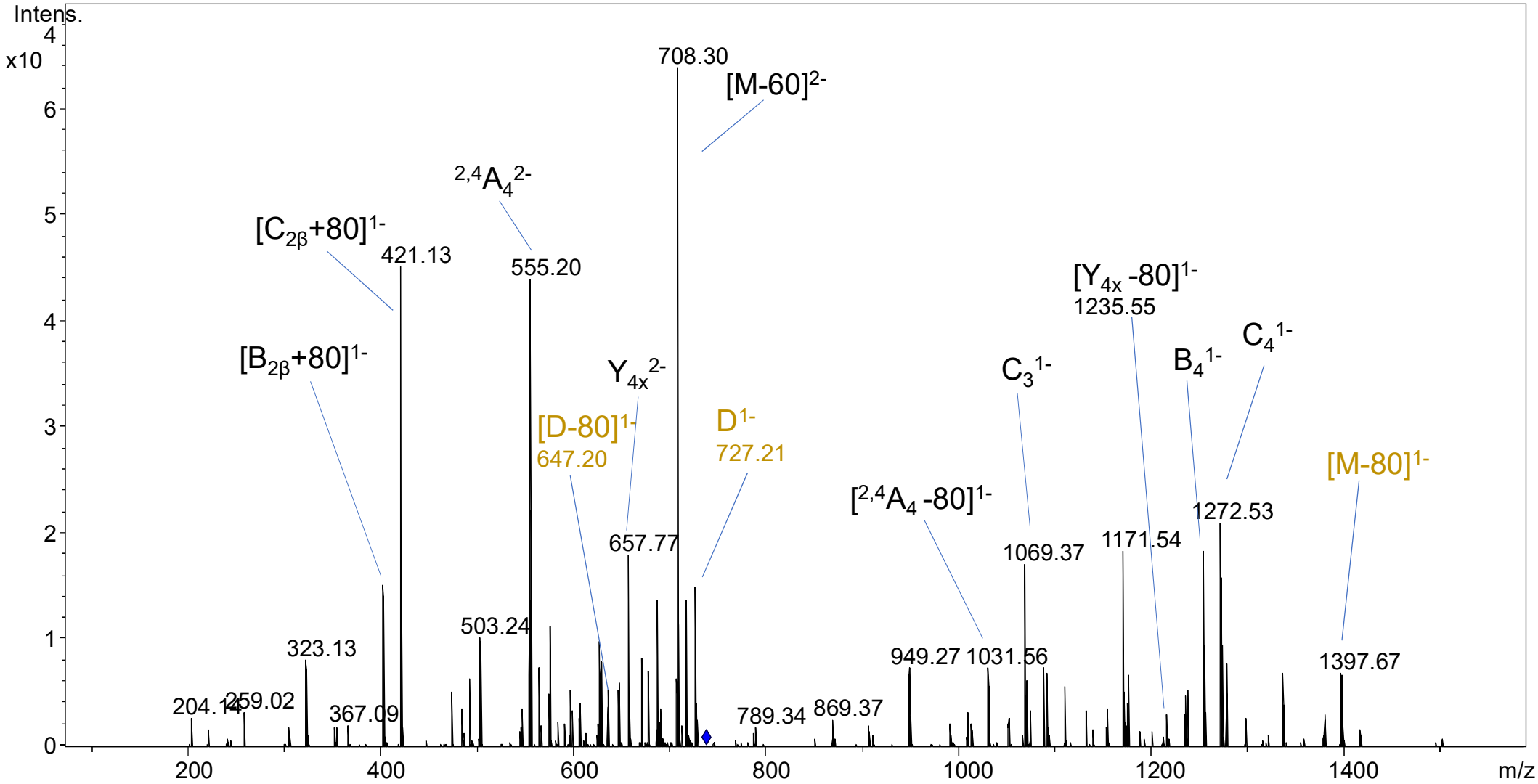


Glycan 9a

M6P (H6N2P1)

Monoisotopic mass:	**1478.47 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 738.23**
Observed ion:	***m/z* 738.34**
Mass deviation:	***m/z* 0.11**
Retention time:	**39.1 min**

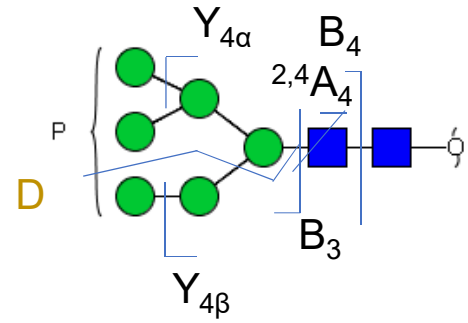


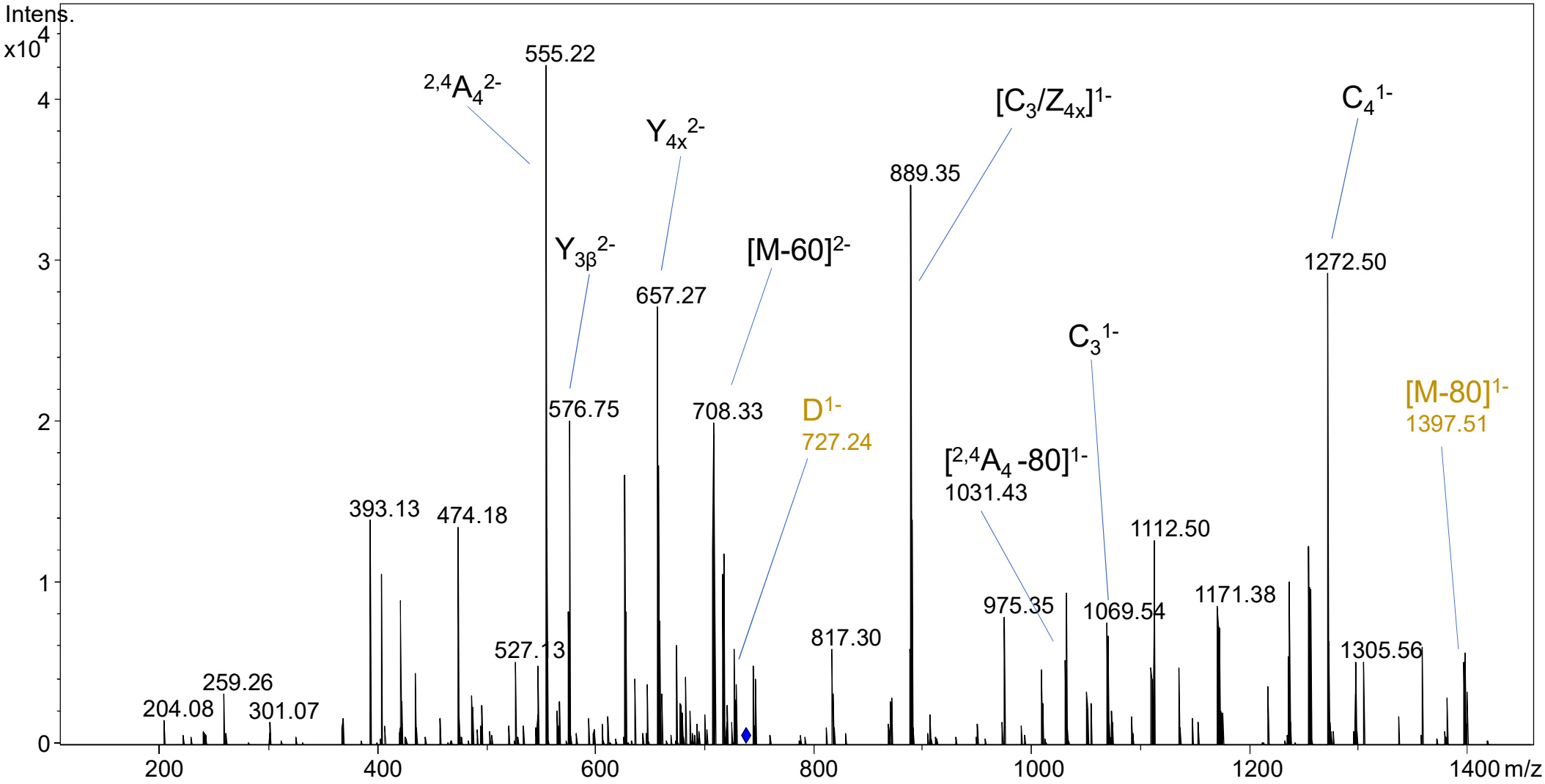


Glycan 9b

M6P (H6N2P1)

Monoisotopic mass:	**1478.47 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 738.23**
Observed ion:	***m/z* 738.33**
Mass deviation:	***m/z* 0.10**
Retention time:	**42.2 min**

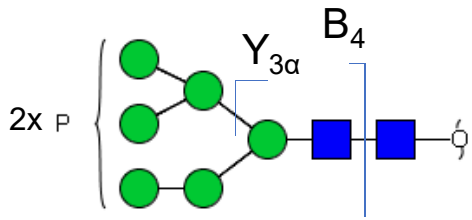


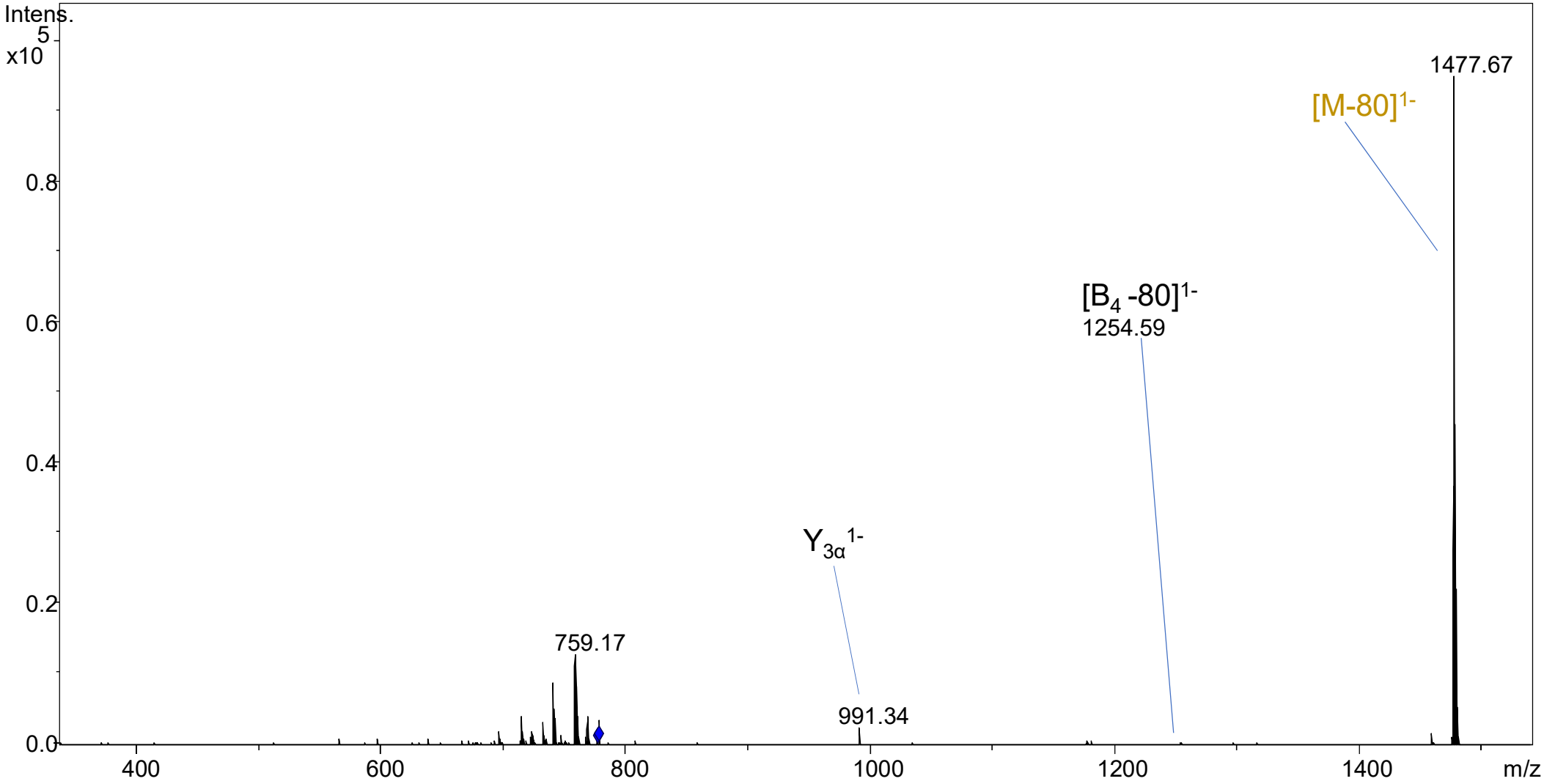


Glycan 10

M6P2 (H6N2P2)

Monoisotopic mass:	**1558.43 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 778.21**
Observed ion:	***m/z* 778.33**
Mass deviation:	***m/z* 0.12**
Retention time:	**30.6 min**

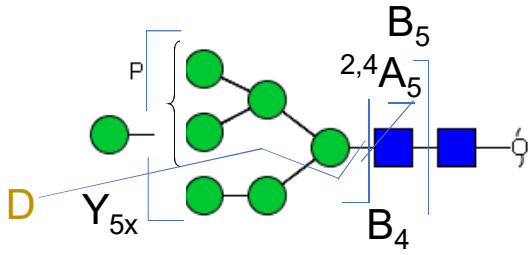


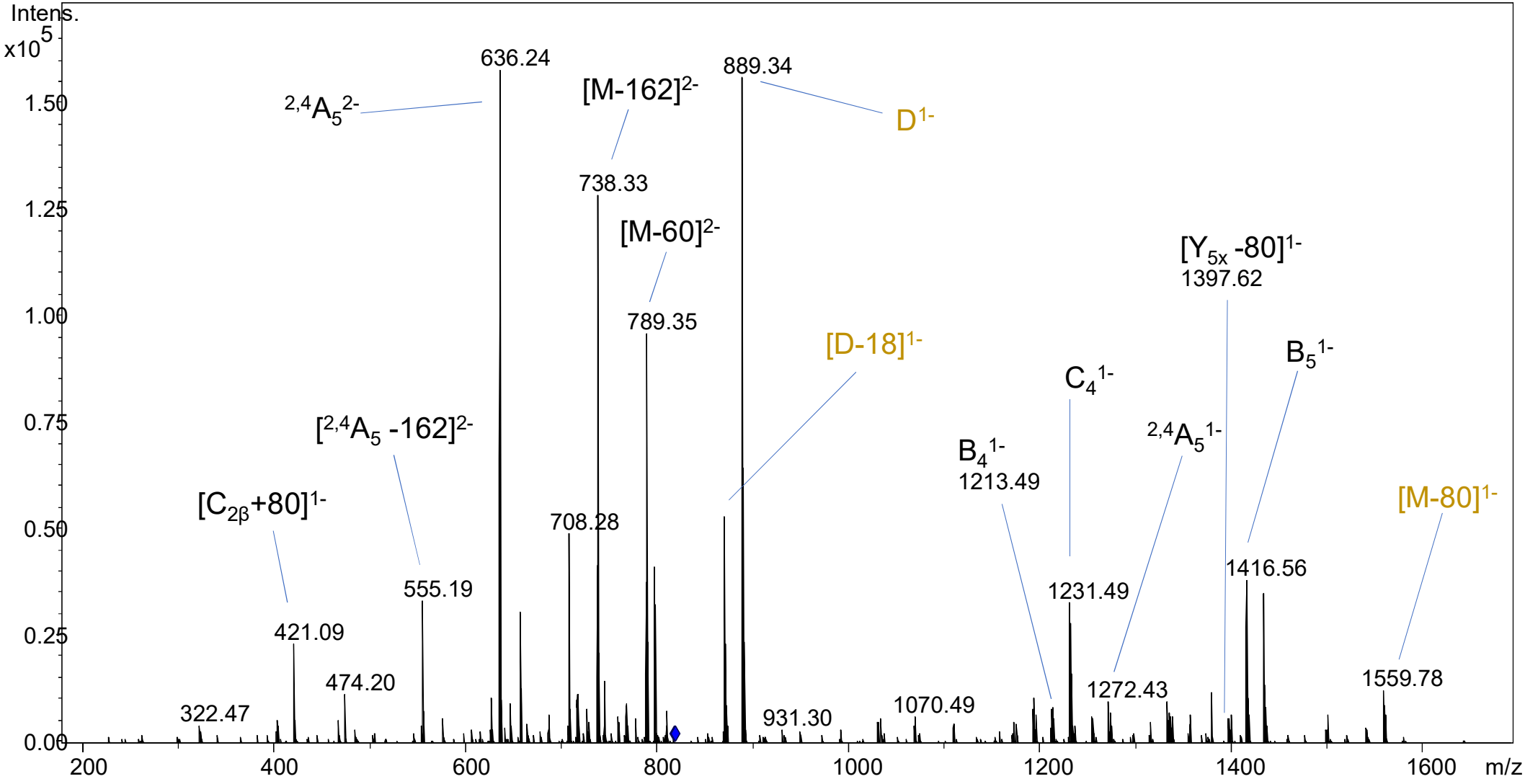


Glycan 11

M7P (H7N2P1)

Monoisotopic mass:	**1640.52 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 819.25**
Observed ion:	***m/z* 819.28**
Mass deviation:	***m/z* 0.03**
Retention time:	**38.7 min**

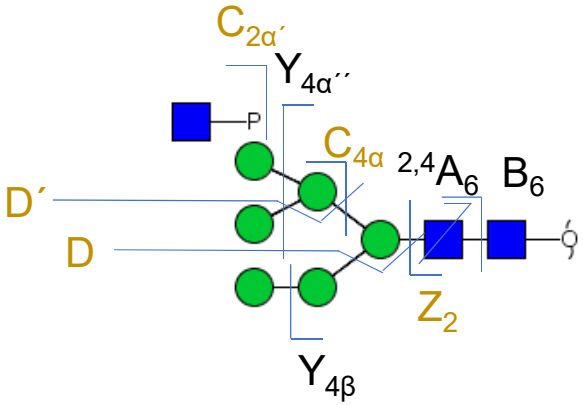


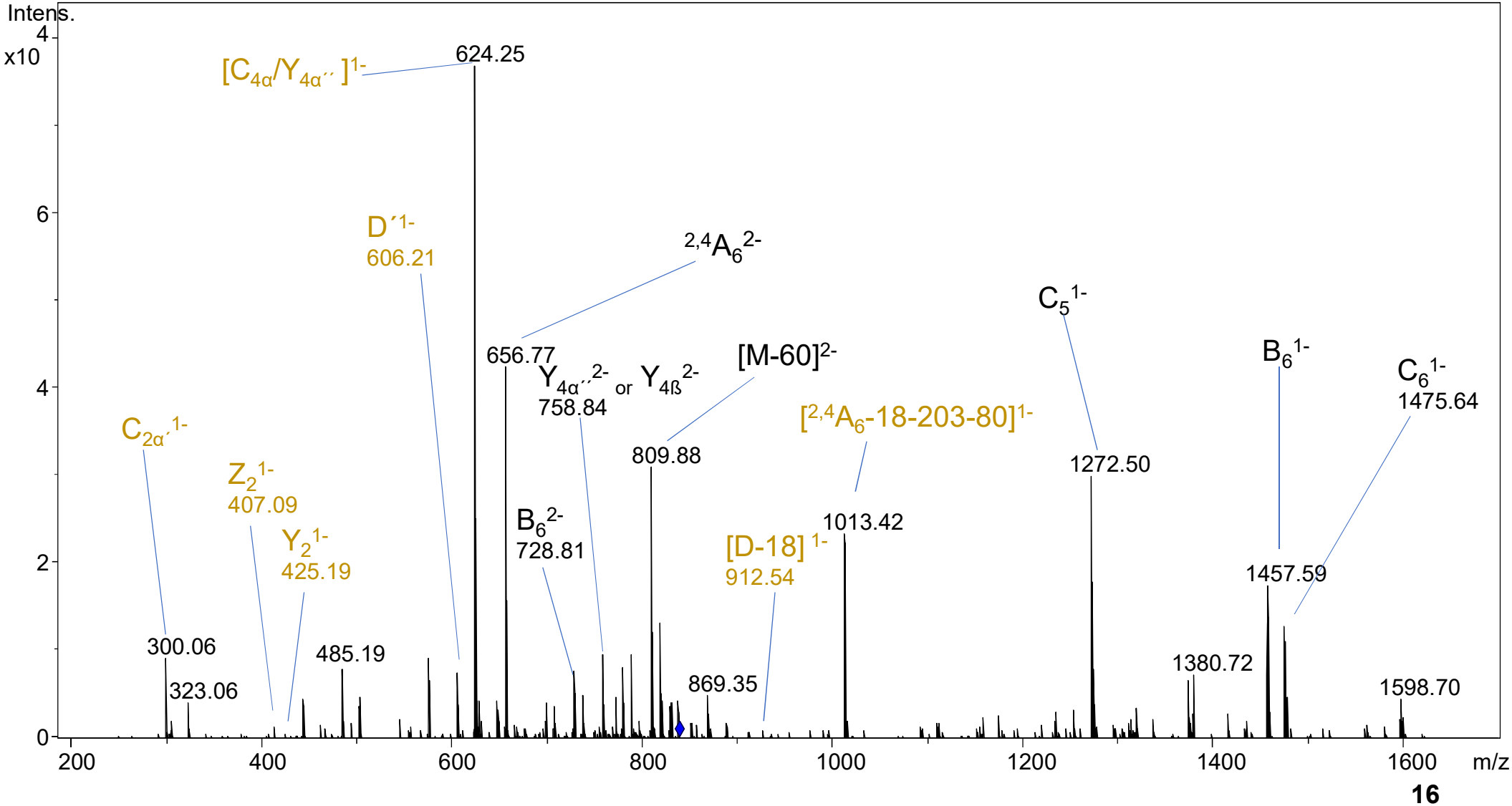


Glycan 12

M6PGlcNAc (H6N3P1)

Monoisotopic mass: 1681.55 Da
Charge observed: 2-
Theoretical ion: *m/z* 839.77
Observed ion: *m/z* 839.90
Mass deviation: *m/z* 0.13
Retention time: 41.9 min
Note: Isomer to glycan 41.

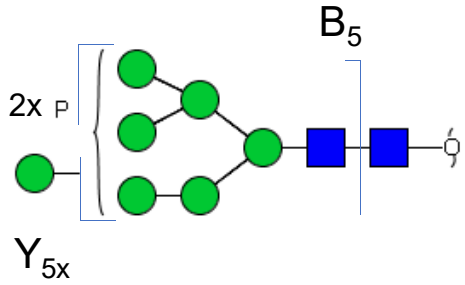


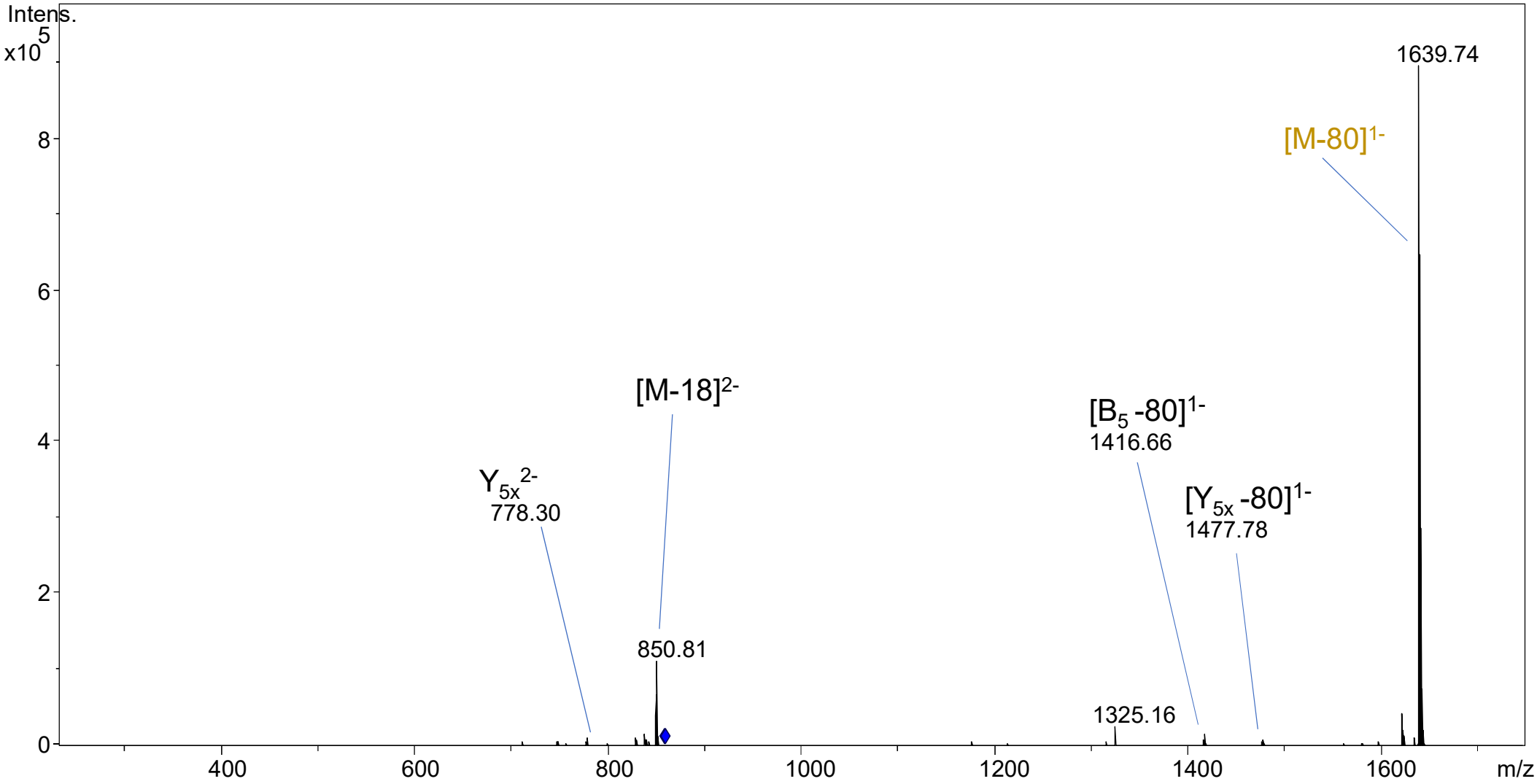


Glycan 13

M7P2
(H7N2P2)

Monoisotopic mass:	**1720.49 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 859.24**
Observed ion:	***m/z* 859.38**
Mass deviation:	***m/z* 0.14**
Retention time:	**31.7 min**

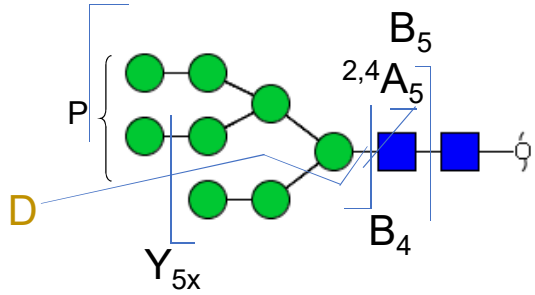


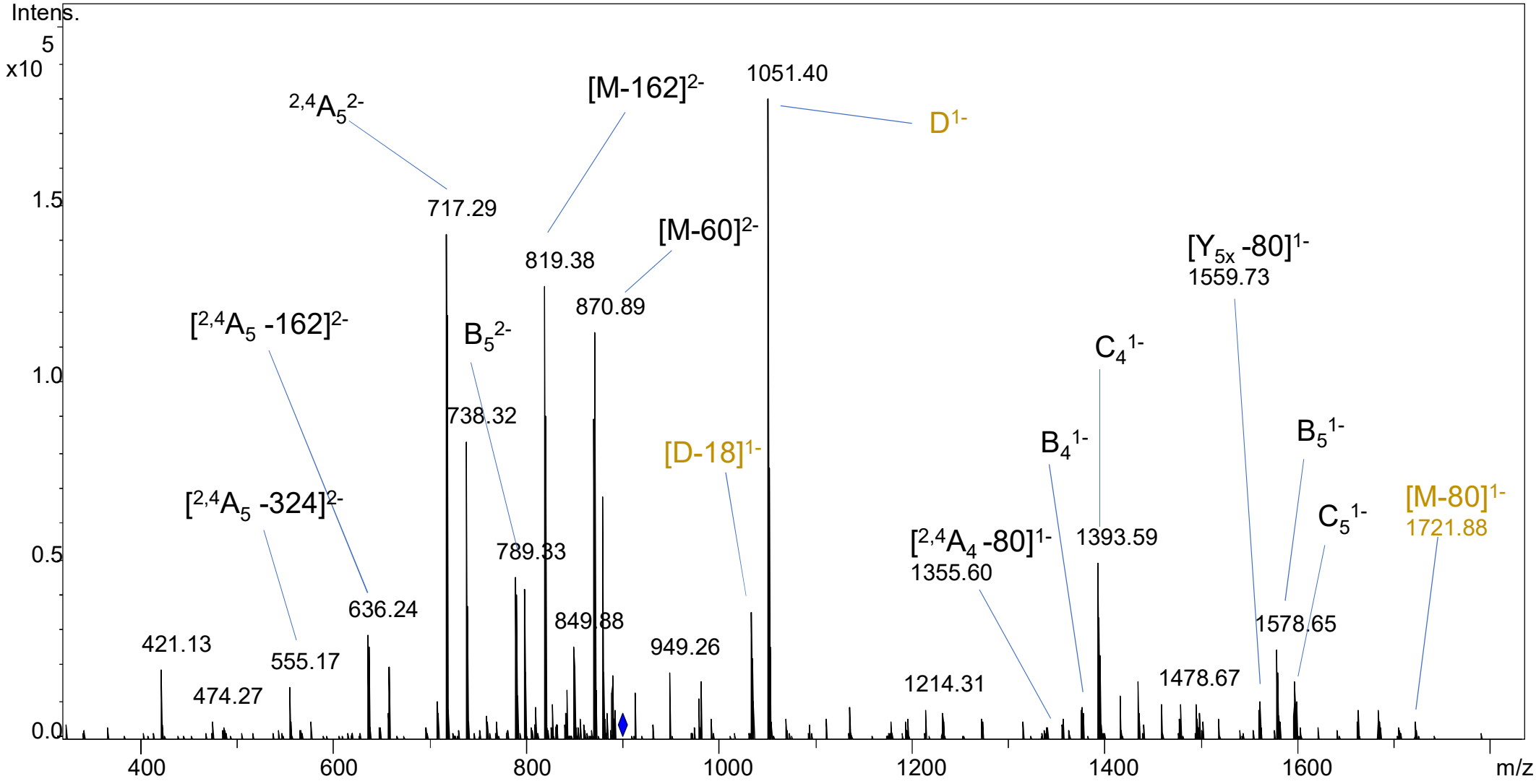


Glycan 14

M8P
(H8N2P1)

Monoisotopic mass:	**1802.57 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 900.28**
Observed ion:	***m/z* 900.40**
Mass deviation:	***m/z* 0.12**
Retention time:	**38.6 min**

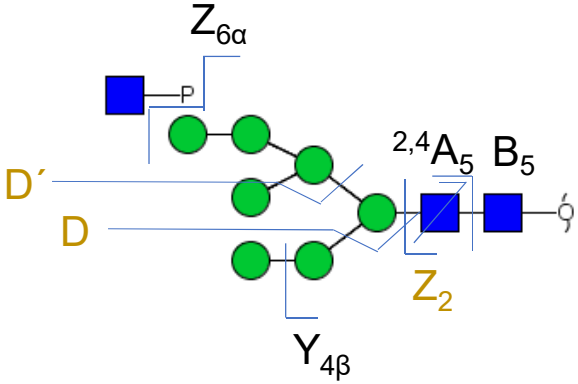


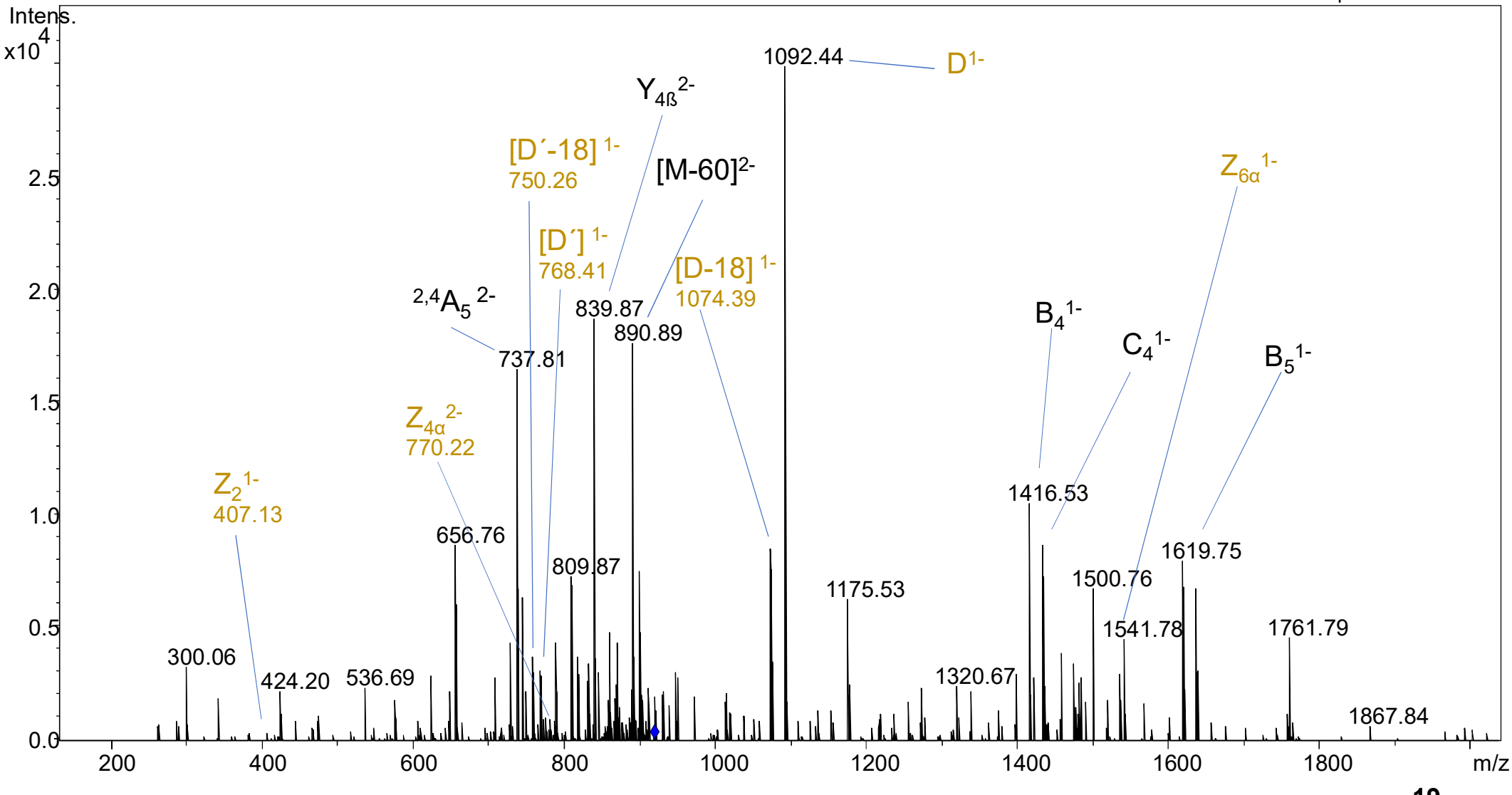


Glycan 15

M7PGlcNAc
(H7N3P1)

Monoisotopic mass: 1843.60 Da
Charge observed: 2-
Theoretical ion: *m/z* 920.79
Observed ion: *m/z* 920.91
Mass deviation: *m/z* 0.12
Retention time: 42.4 min
Note: Isomer to glycan 44.

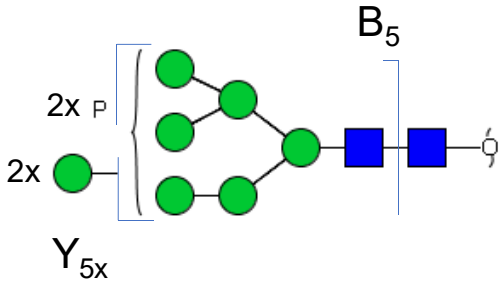


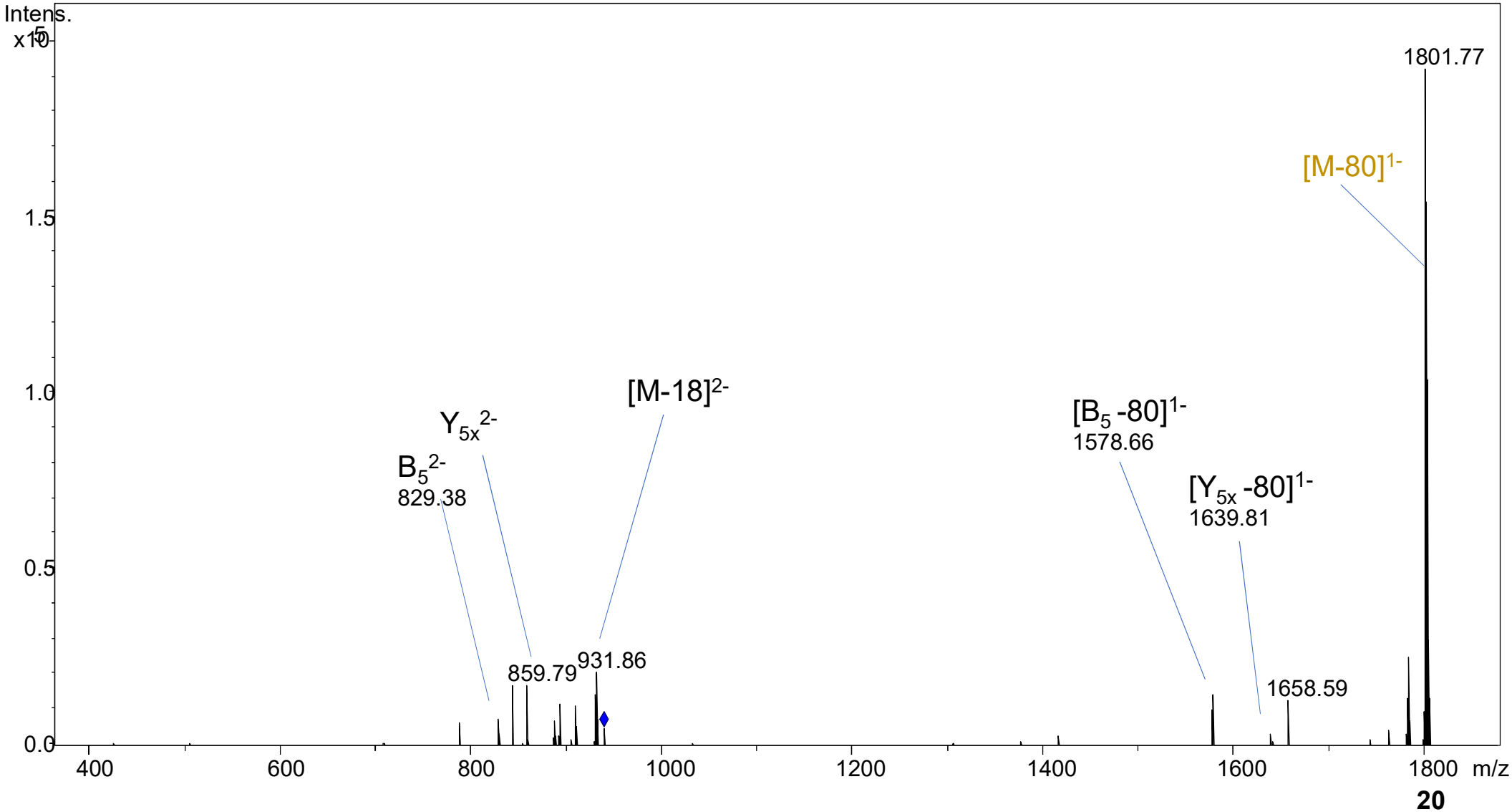


Glycan 16

M8P2
(H8N2P2)

Monoisotopic mass:	**1882.53 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 940.26**
Observed ion:	***m/z* 940.37**
Mass deviation:	***m/z* 0.11**
Retention time:	**31.4 min**

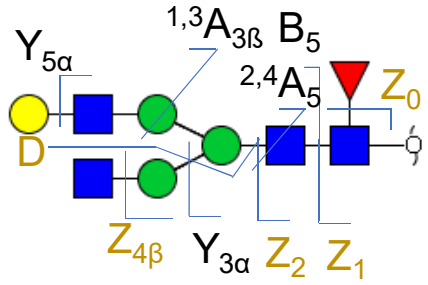


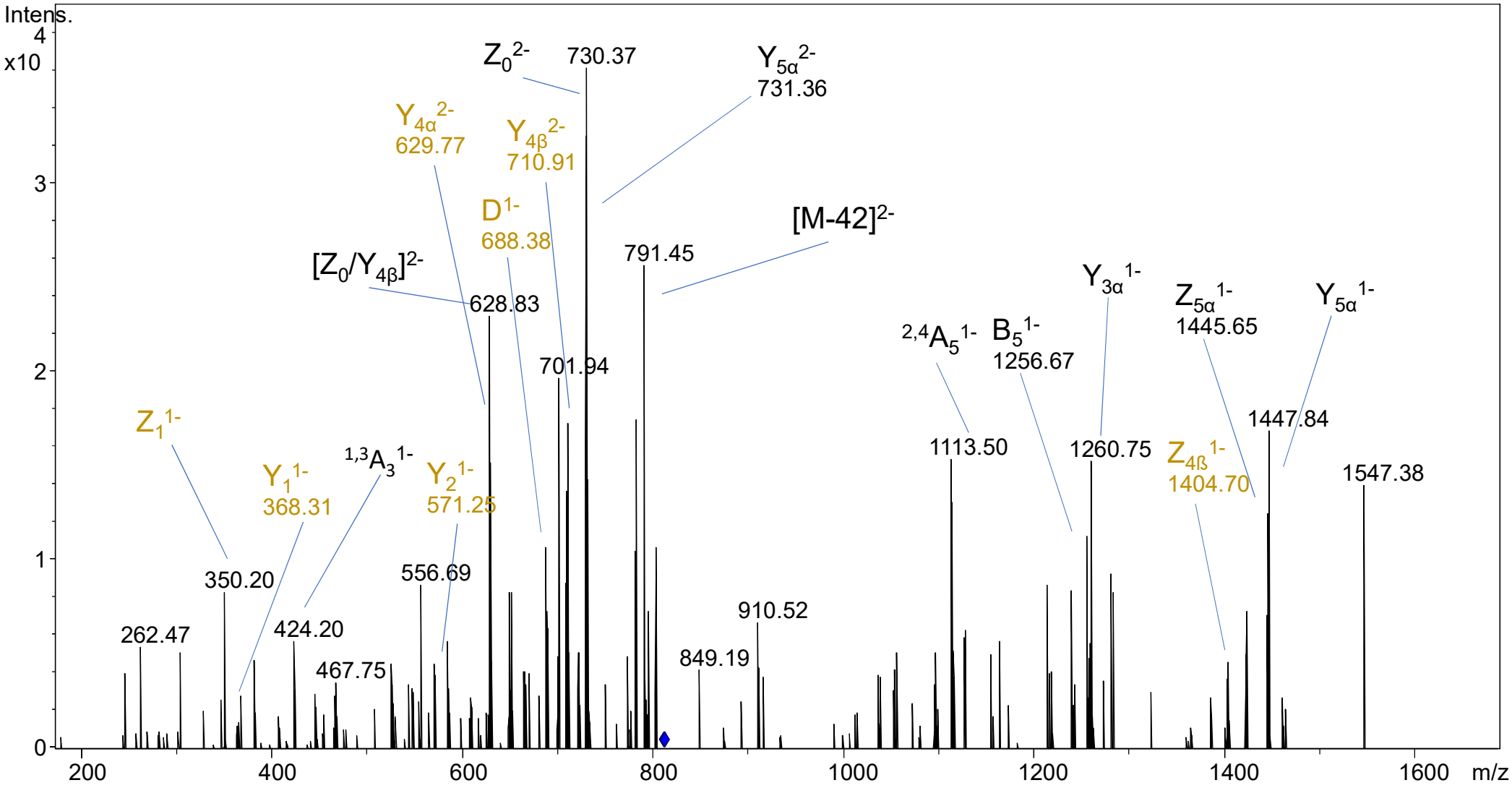


Glycan 17

A2G1F
(H4N4F1)

Monoisotopic mass:	**1626.61 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 812.30**
Observed ion:	***m/z* 812.42**
Mass deviation:	***m/z* 0.12**
Retention time:	**60.2 min**

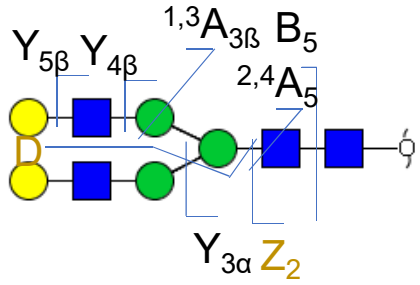


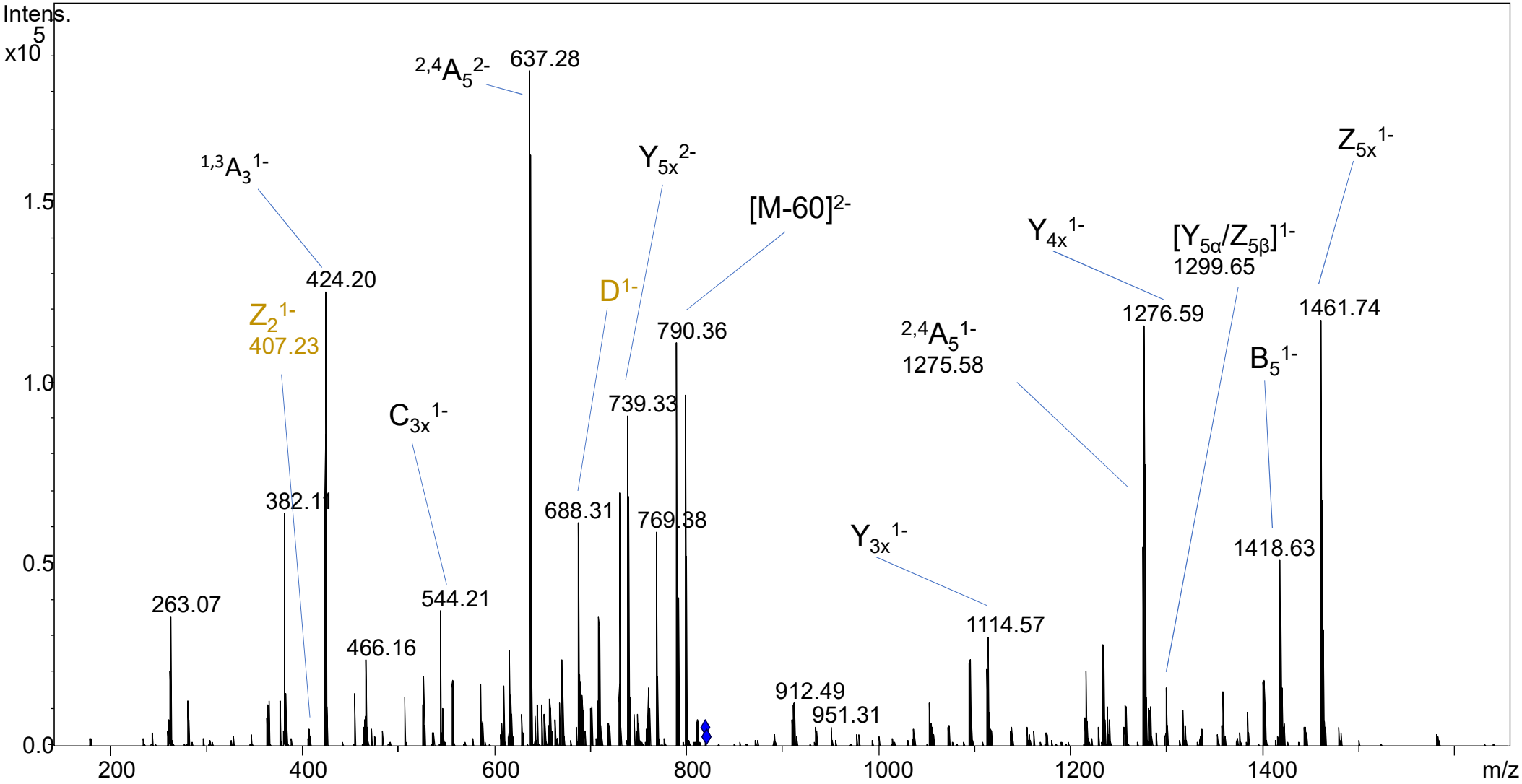


Glycan 18

A2G2
(H5N4)

Monoisotopic mass:	**1642.61 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 820.30**
Observed ion:	***m/z* 820.44**
Mass deviation:	***m/z* 0.14**
Retention time:	**55.9 min**

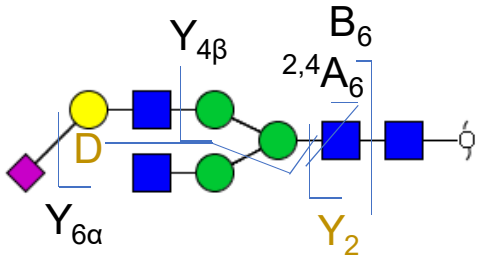


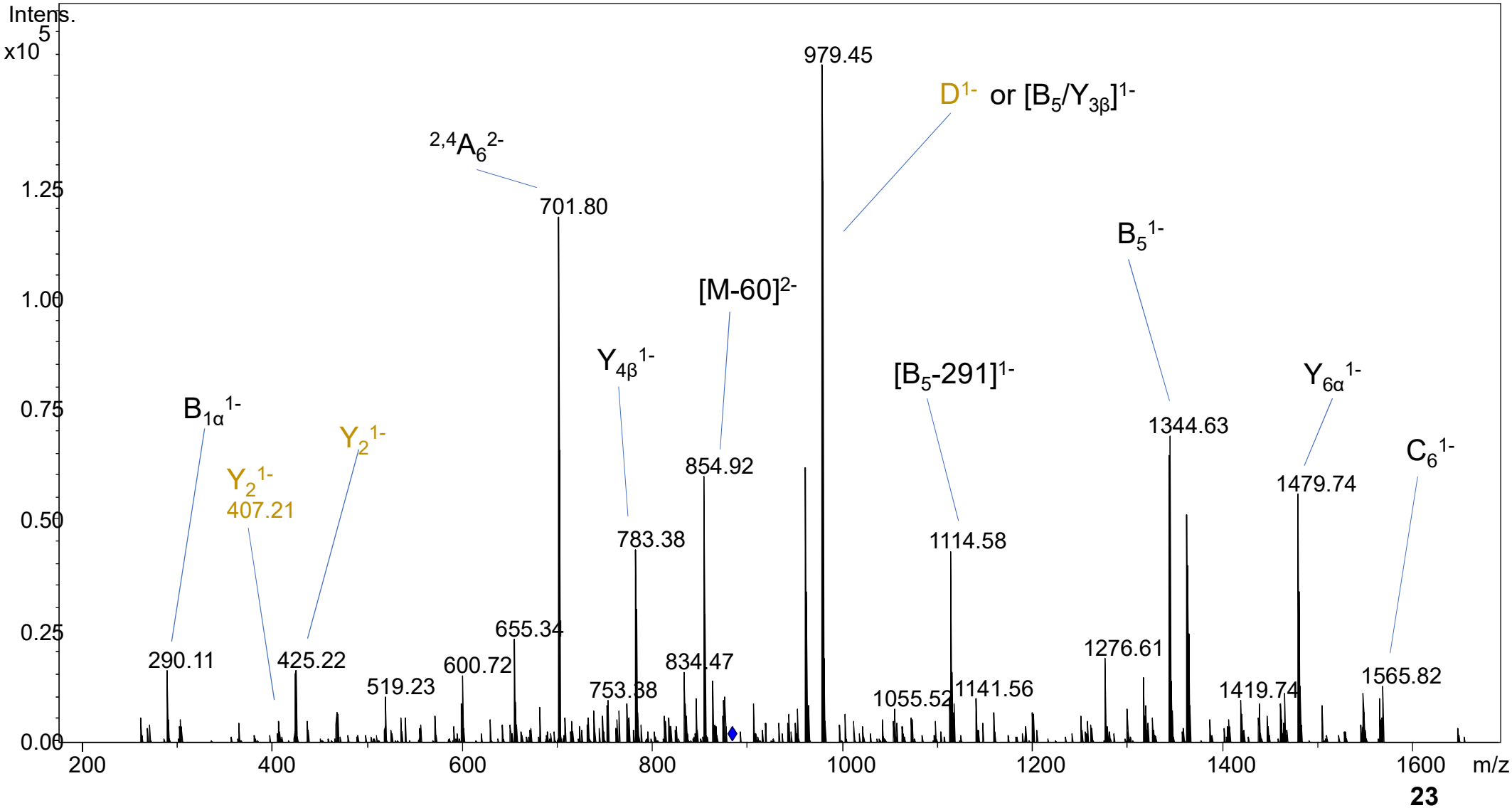


Glycan 19a

A2G0S1
(H4N4S1)

Monoisotopic mass:	**1771.64 Da**
Charge observed:	**1-**
Theoretical ion:	***m/z* 884.82**
Observed ion:	***m/z* 884.95**
Mass deviation:	***m/z* 0.13**
Retention time:	**60.5 min**

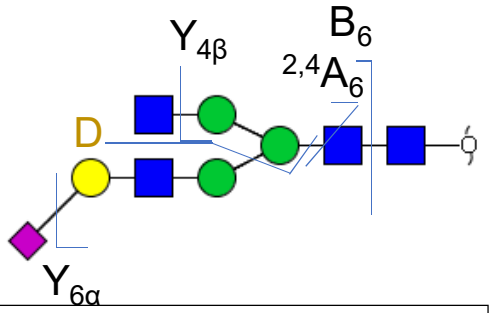


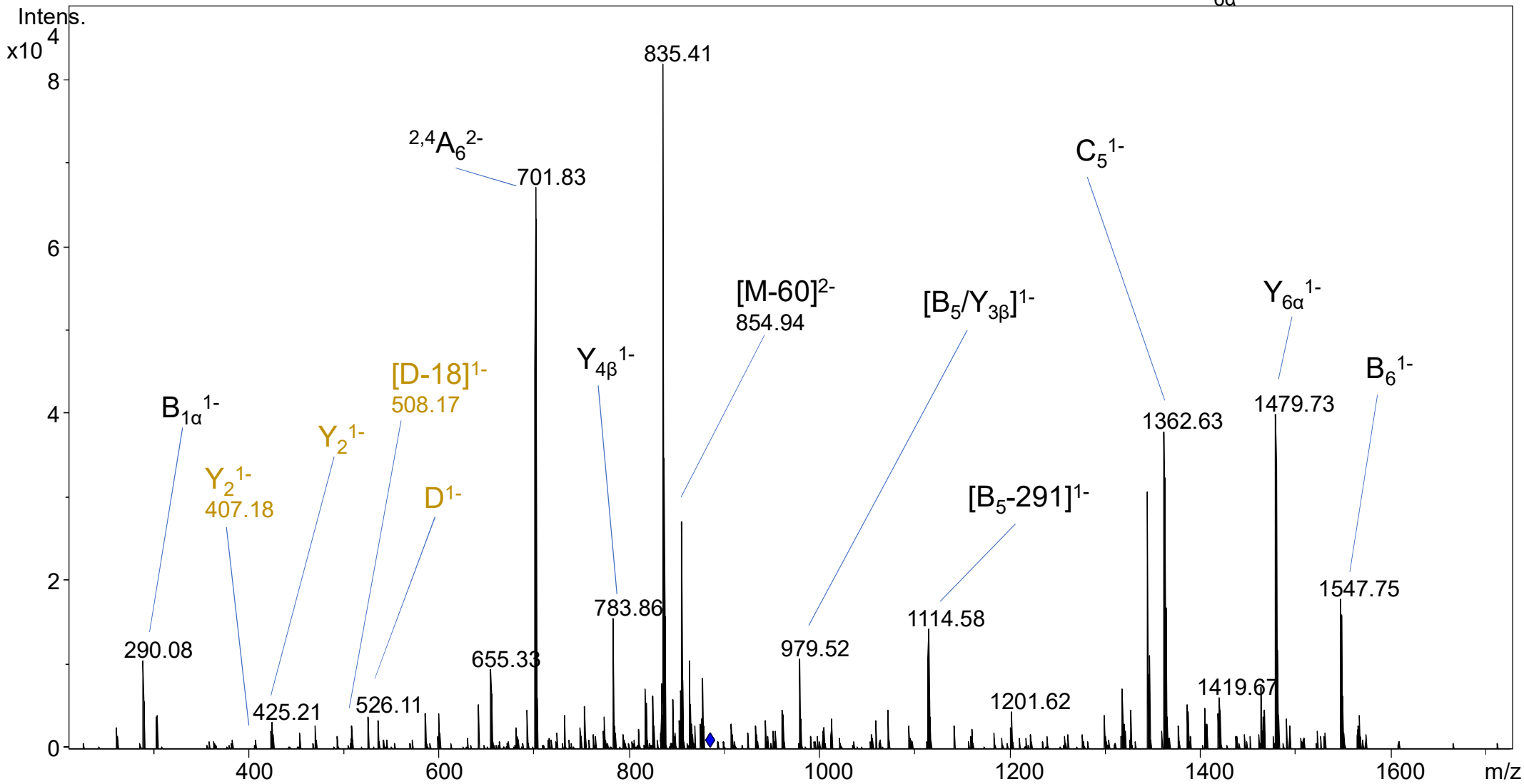


Glycan 19b

A1S1
(H4N4S1)

Monoisotopic mass:	**1771.64 Da**
Charge observed:	**1-**
Theoretical ion:	***m/z* 884.82**
Observed ion:	***m/z* 884.97**
Mass deviation:	***m/z* 0.13**
Retention time:	**61.6 min**

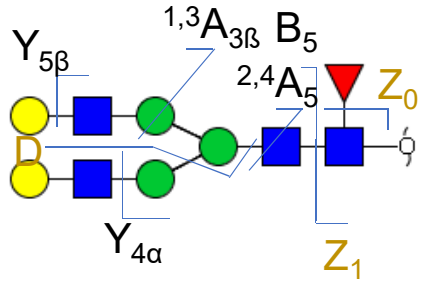


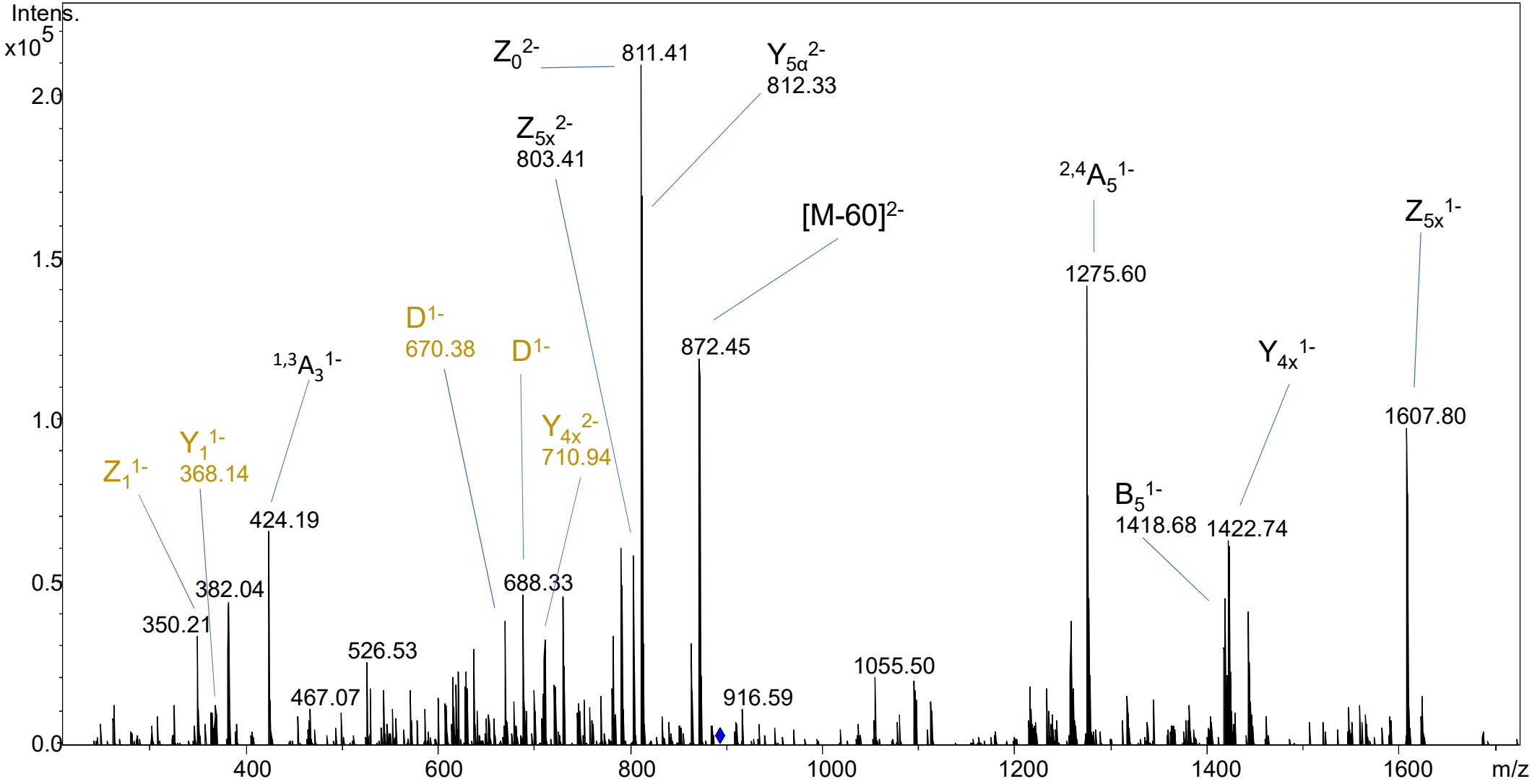


Glycan 20

A2G2F
(H5N4F1)

Monoisotopic mass:	**1788.67 Da**	**UniCarb-DB: #425**
Charge observed:	**2-**	
Theoretical ion:	***m/z* 893.32**	
Observed ion:	***m/z* 893.46**	
Mass deviation:	***m/z* 0.14**	
Retention time:	**63.3 min**	

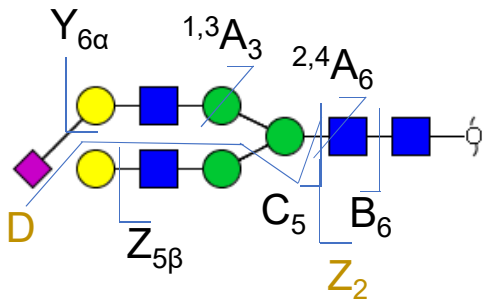


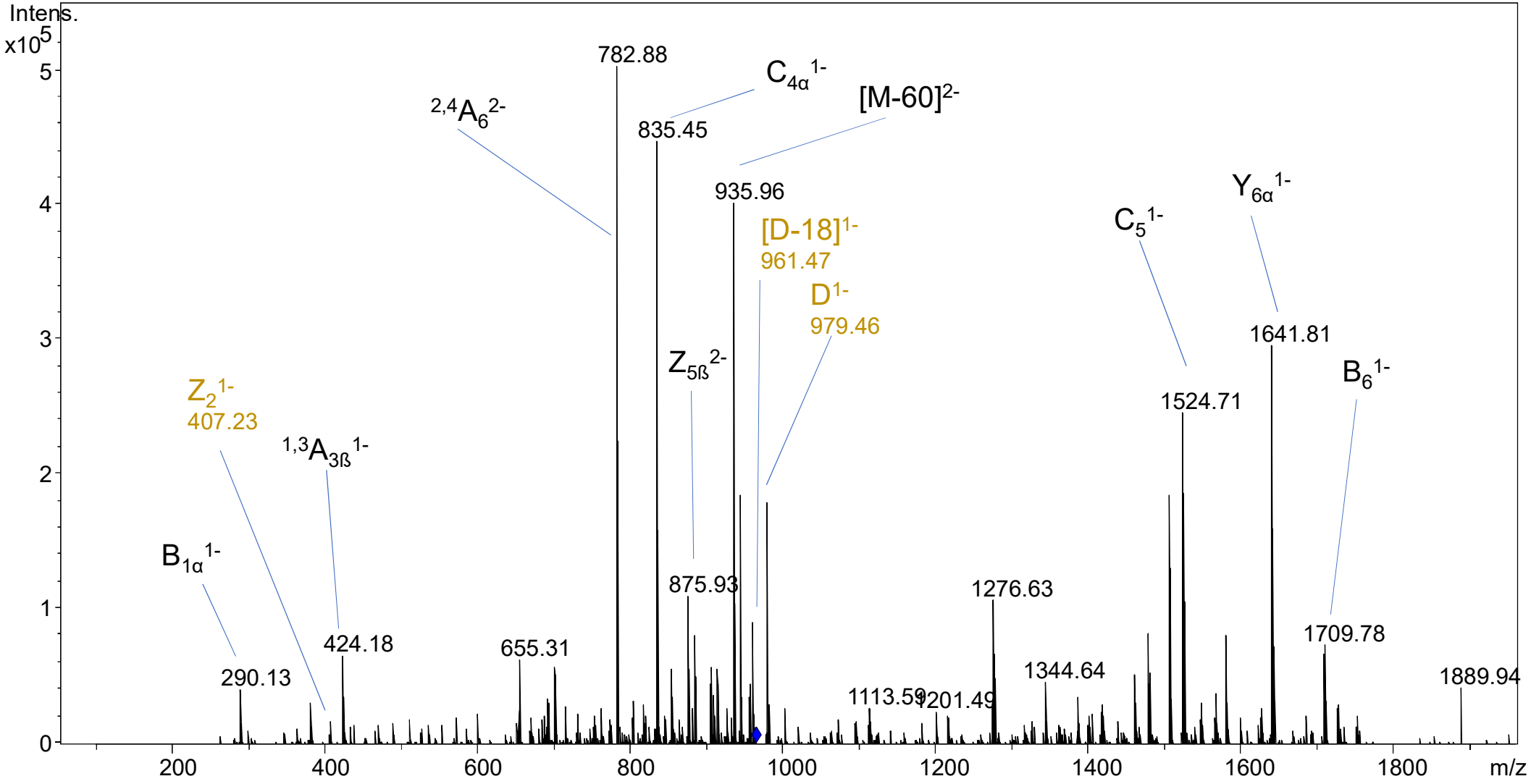


Glycan 21

A2G1S1
(H5N4S1)

Monoisotopic mass:	**1933.70 Da**	**UniCarb-DB: #2338**
Charge observed:	**2-**	
Theoretical ion:	***m/z* 965.84**	
Observed ion:	***m/z* 965.99**	
Mass deviation:	***m/z* 0.15**	
Retention time:	**63.8 min**	

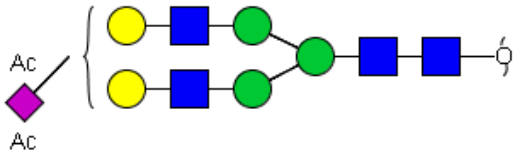


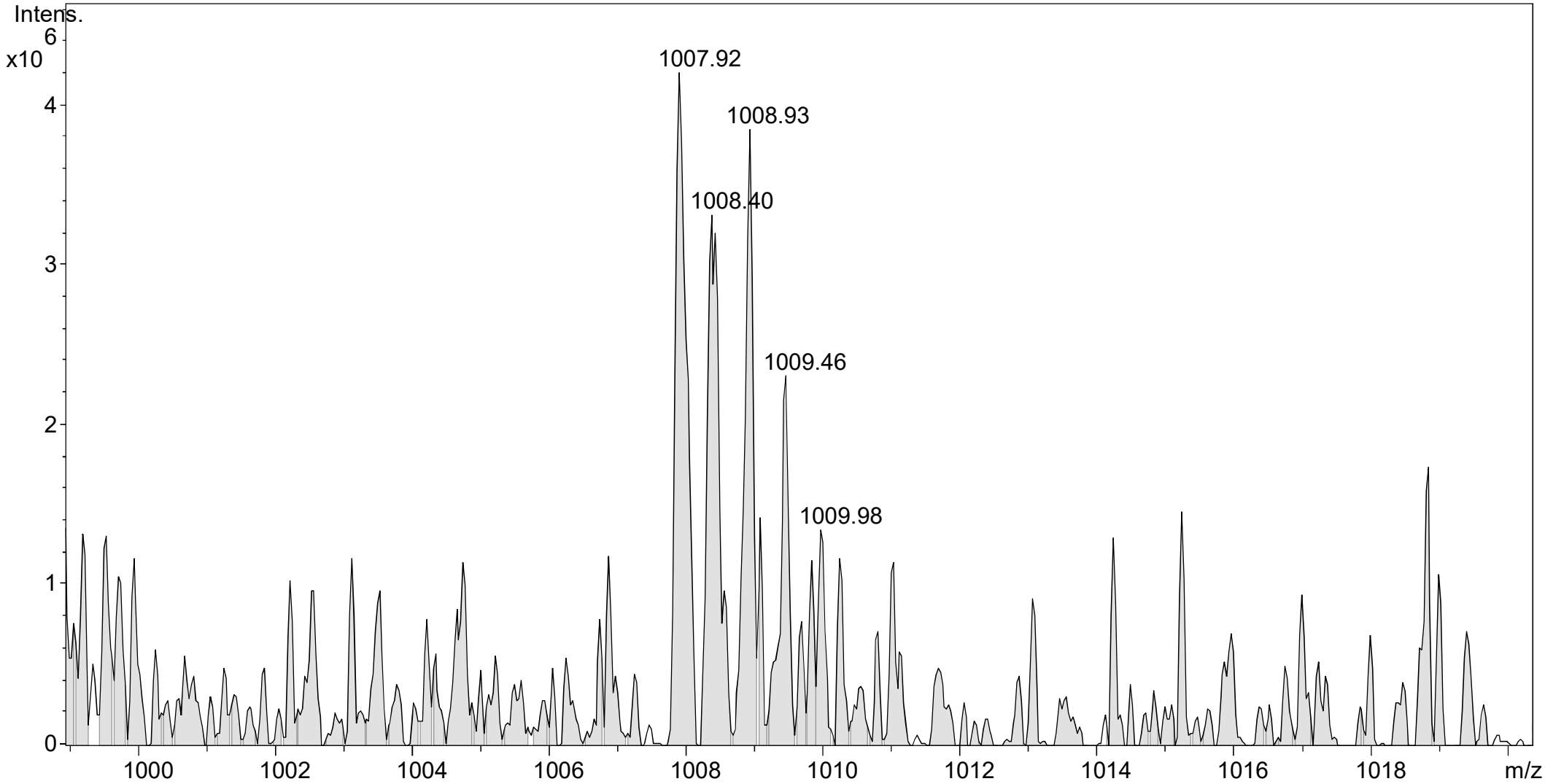


Glycan 22

A2G1S1Ac2
(H5N4S1Ac2)

Monoisotopic mass: **2017.72 Da**
Charge observed: **2-**
Theoretical ion: ***m/z* 1007.86**
Observed ion: ***m/z* 1007.92**
Mass deviation: ***m/z* 0.08**
Retention time: **63.9 min**
Note: No insightful MS2 available.

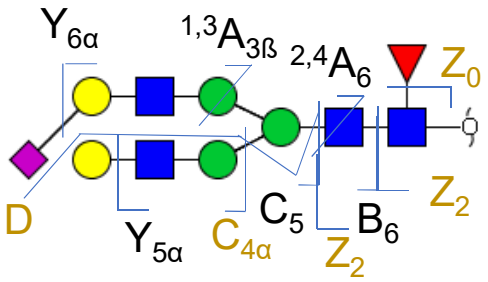


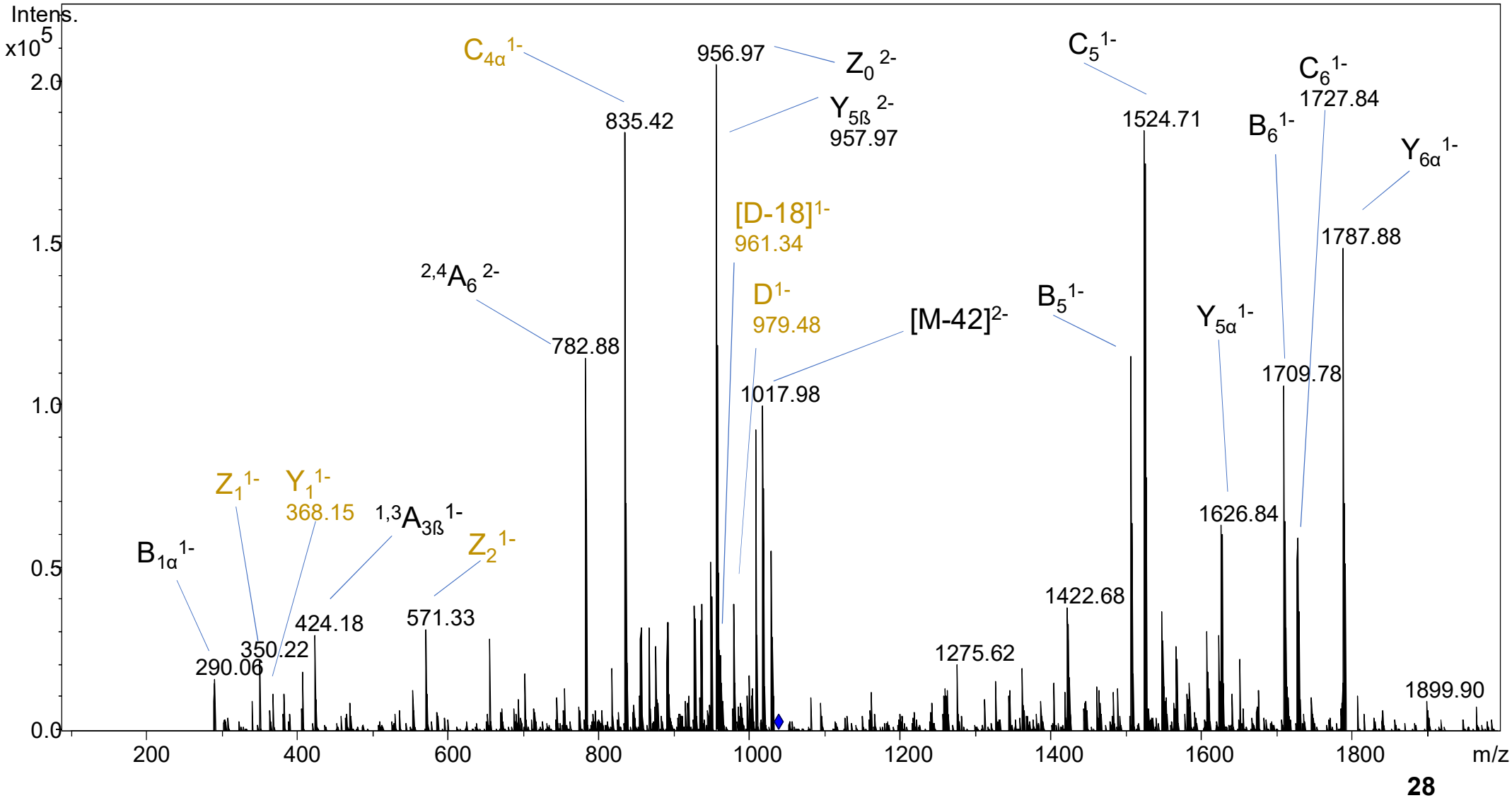


Glycan 23

A2G1S1F
(H5N4F1S1)

Monoisotopic mass:	**2079.76 Da**	**UniCarb-DB: #2364**
Charge observed:	**2-**	
Theoretical ion:	***m/z* 1038.87**	
Observed ion:	***m/z* 1039.00**	
Mass deviation:	***m/z* 0.13**	
Retention time:	**69.6 min**	

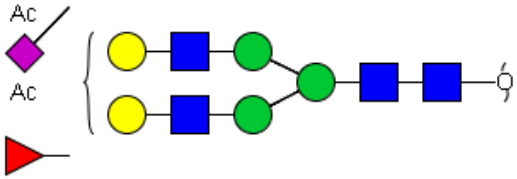


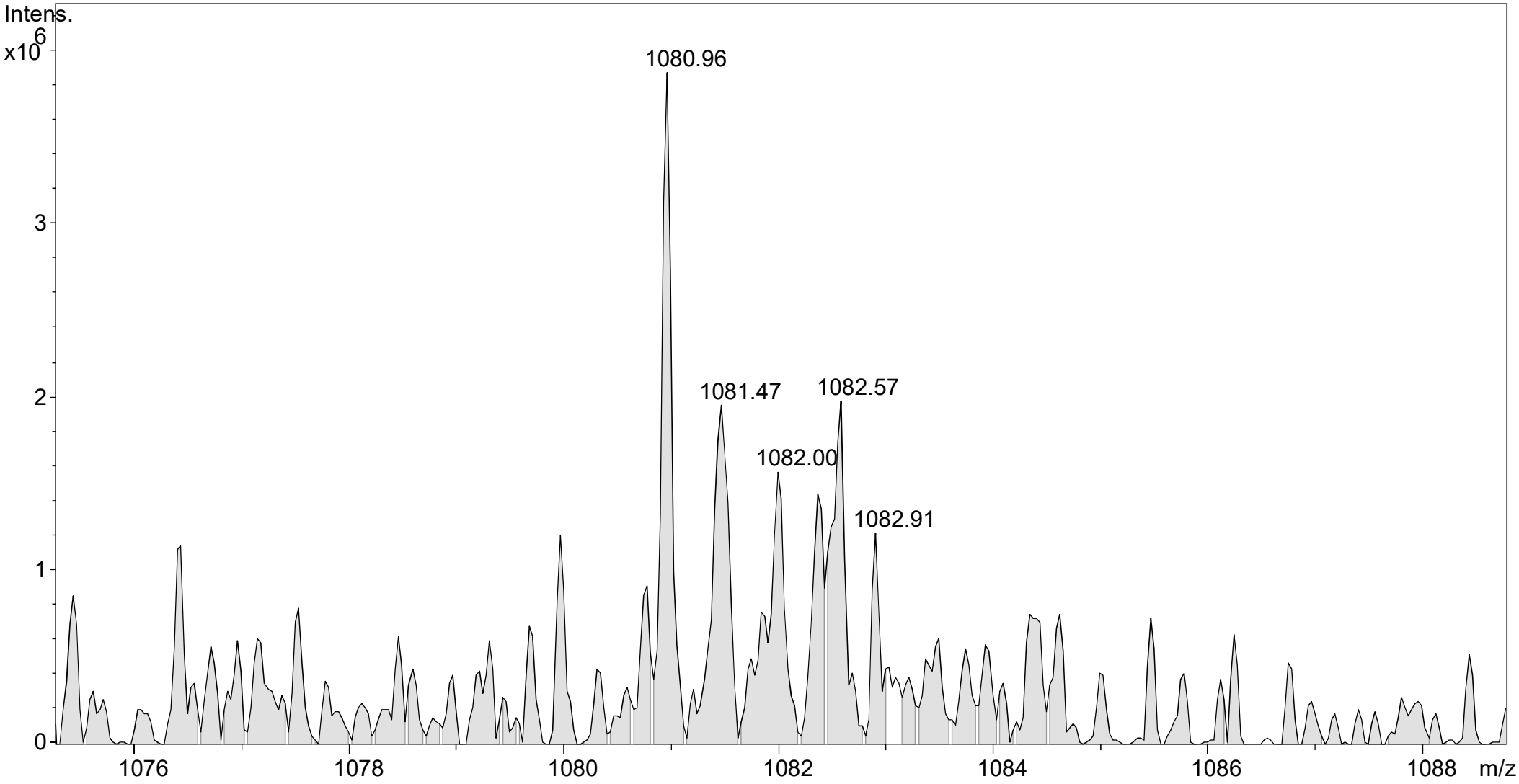


Glycan 24

A2G1S1FAc2
(H5N4F1S1Ac2)

Monoisotopic mass: **2163.78 Da**
Charge observed: **2-**
Theoretical ion: ***m/z* 1080.88**
Observed ion: ***m/z* 1080.96**
Mass deviation: ***m/z* 0.08**
Retention time: **69.3 min**
Note: No insightful MS2 available.

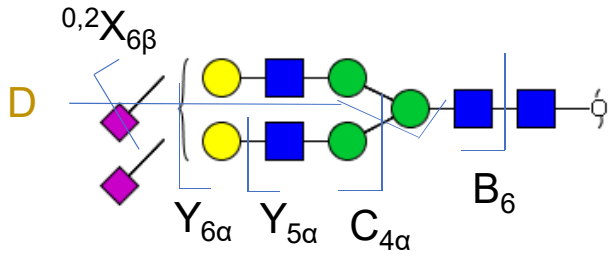


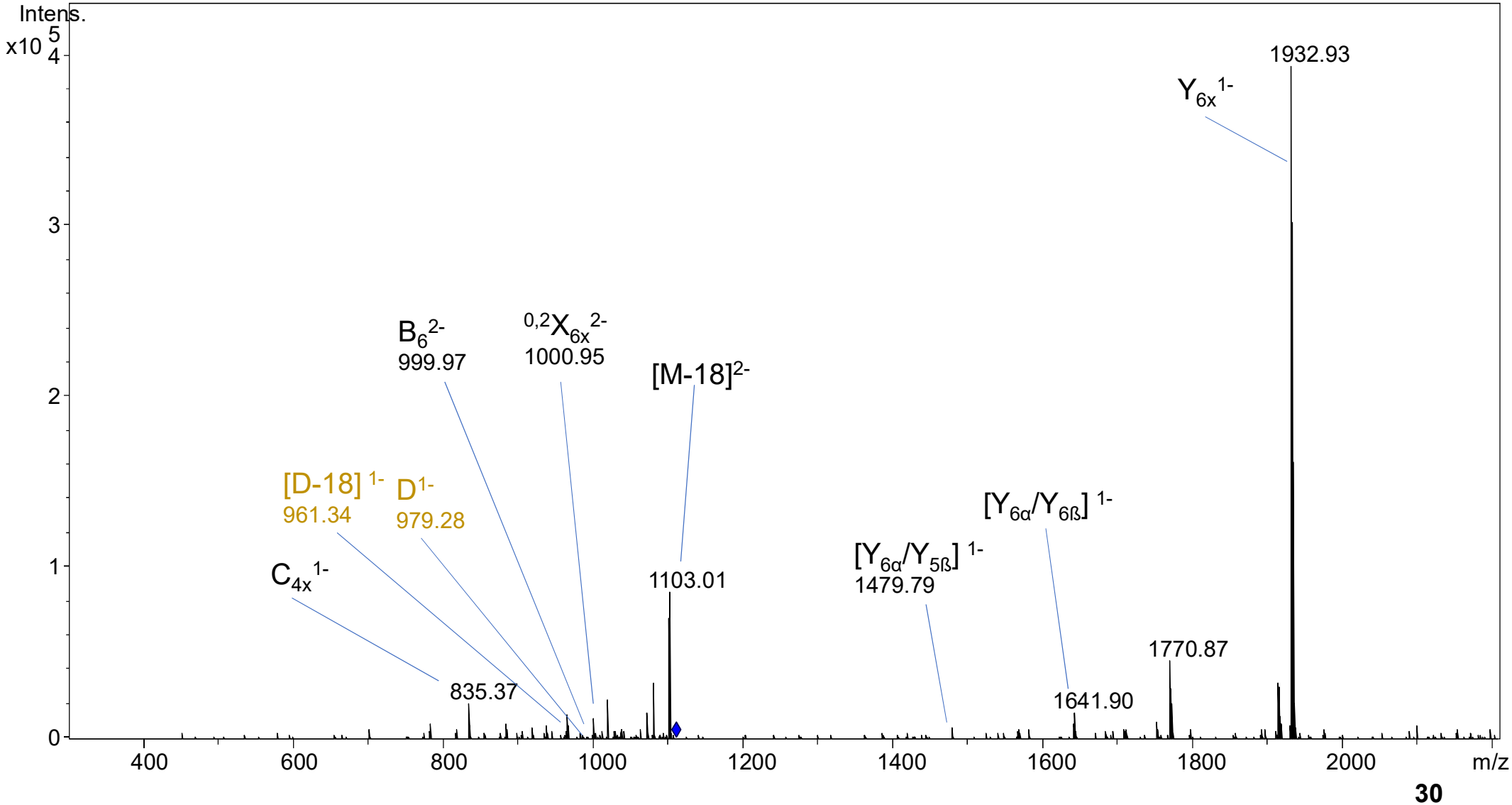


Glycan 25a

A2S2
(H5N4S2)

Monoisotopic mass:	**2224.80 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 1111.42**
Observed ion:	***m/z* 1111.50**
Mass deviation:	***m/z* 0.08**
Retention time:	**62.1 min**

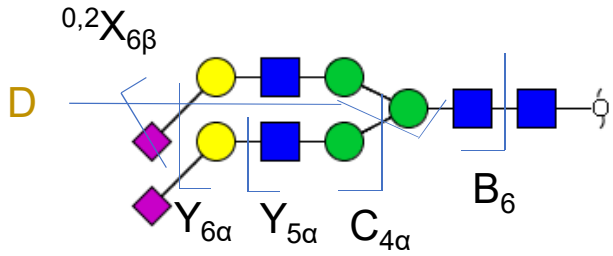


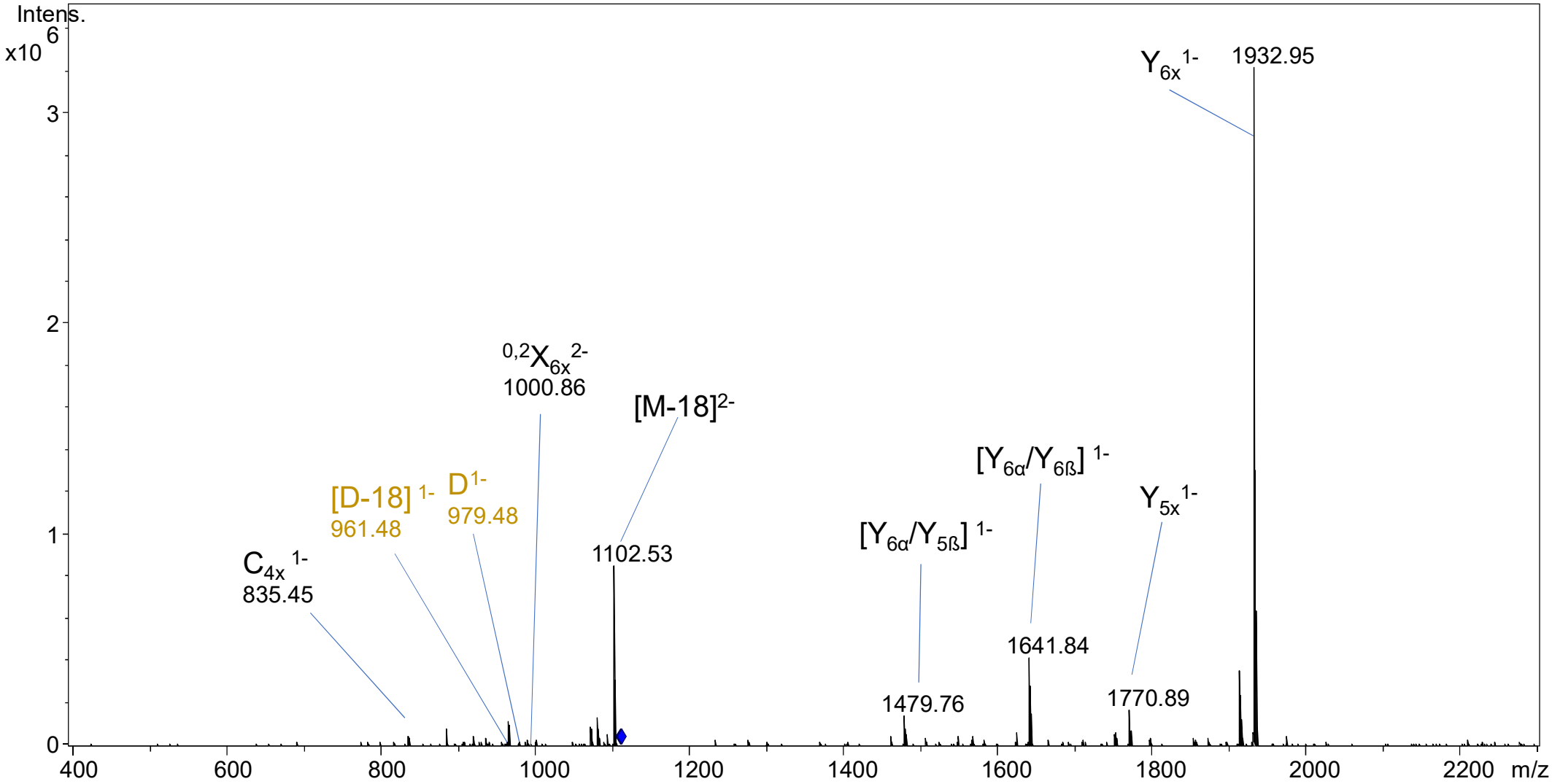


Glycan 25b

A2S2
(H5N4S2)

Monoisotopic mass:	**2224.80 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 1111.42**
Observed ion:	***m/z* 1111.54**
Mass deviation:	***m/z* 0.12**
Retention time:	**70.0 min**

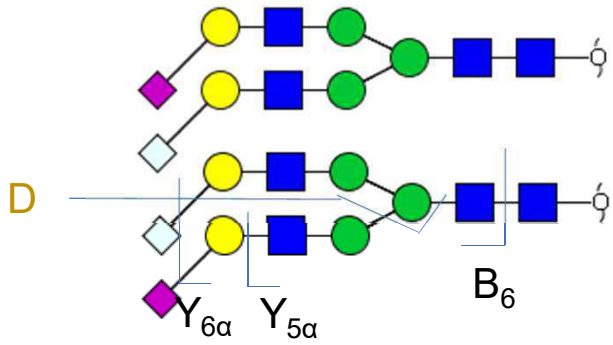


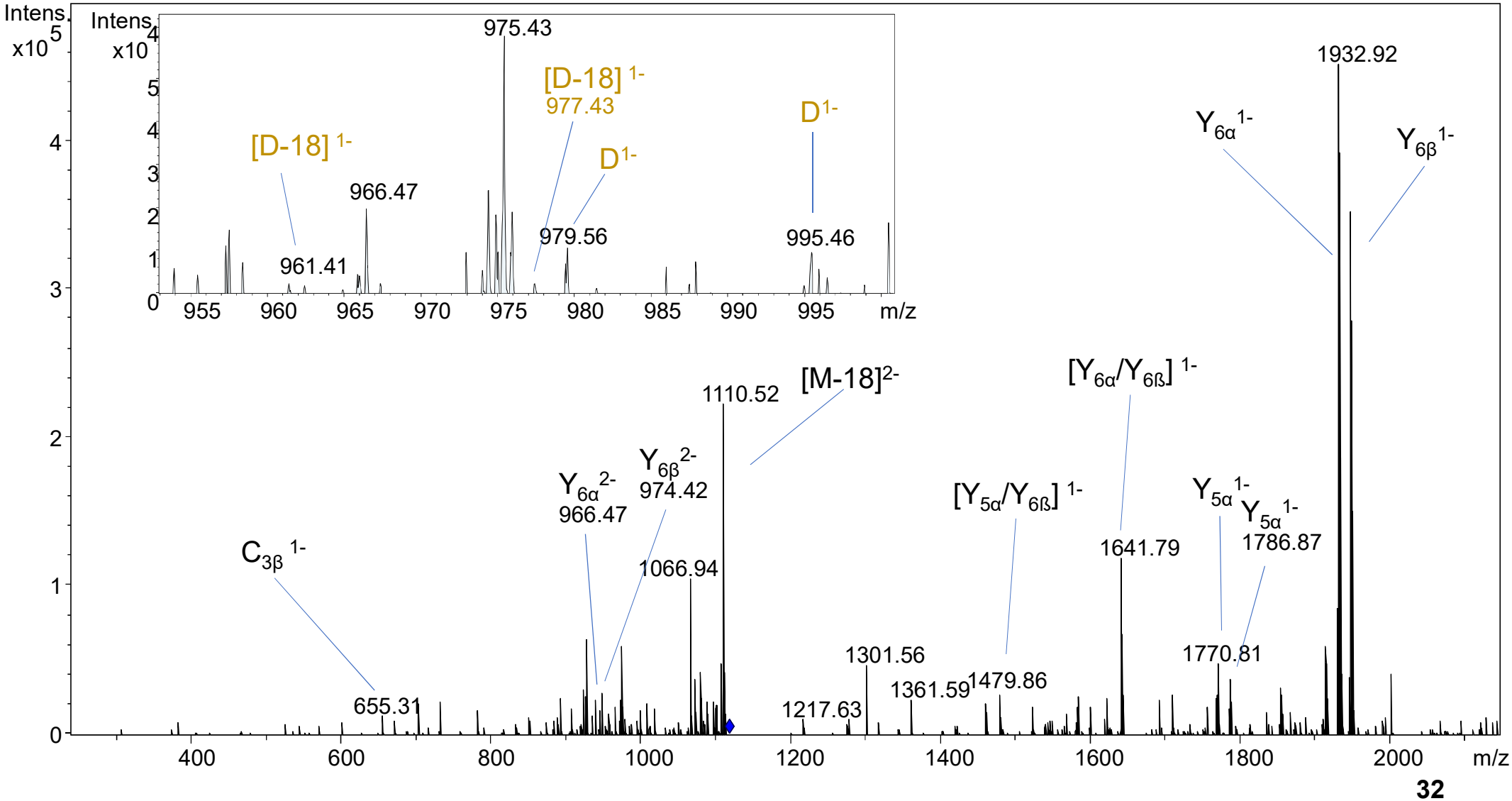


Glycan 26

A2S1Sg1 (H5N4S1G1)

Monoisotopic mass: 2240.79 Da
Charge observed: 2-
Theoretical ion: *m/z* 1119.39
Observed ion: *m/z* 1111.52
Mass deviation: *m/z* 0.13
Retention time: 69.3 min
Note: Isomers comprising the Neu5Ac either on the 3- or 6-arm potentially co-elute, which would explain the presence of both D-ions.

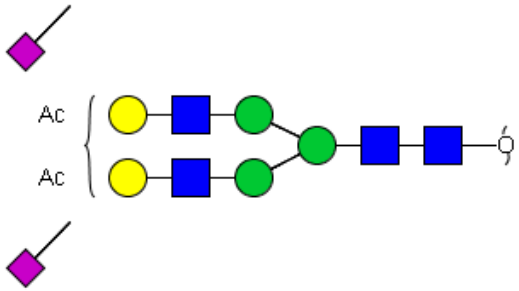


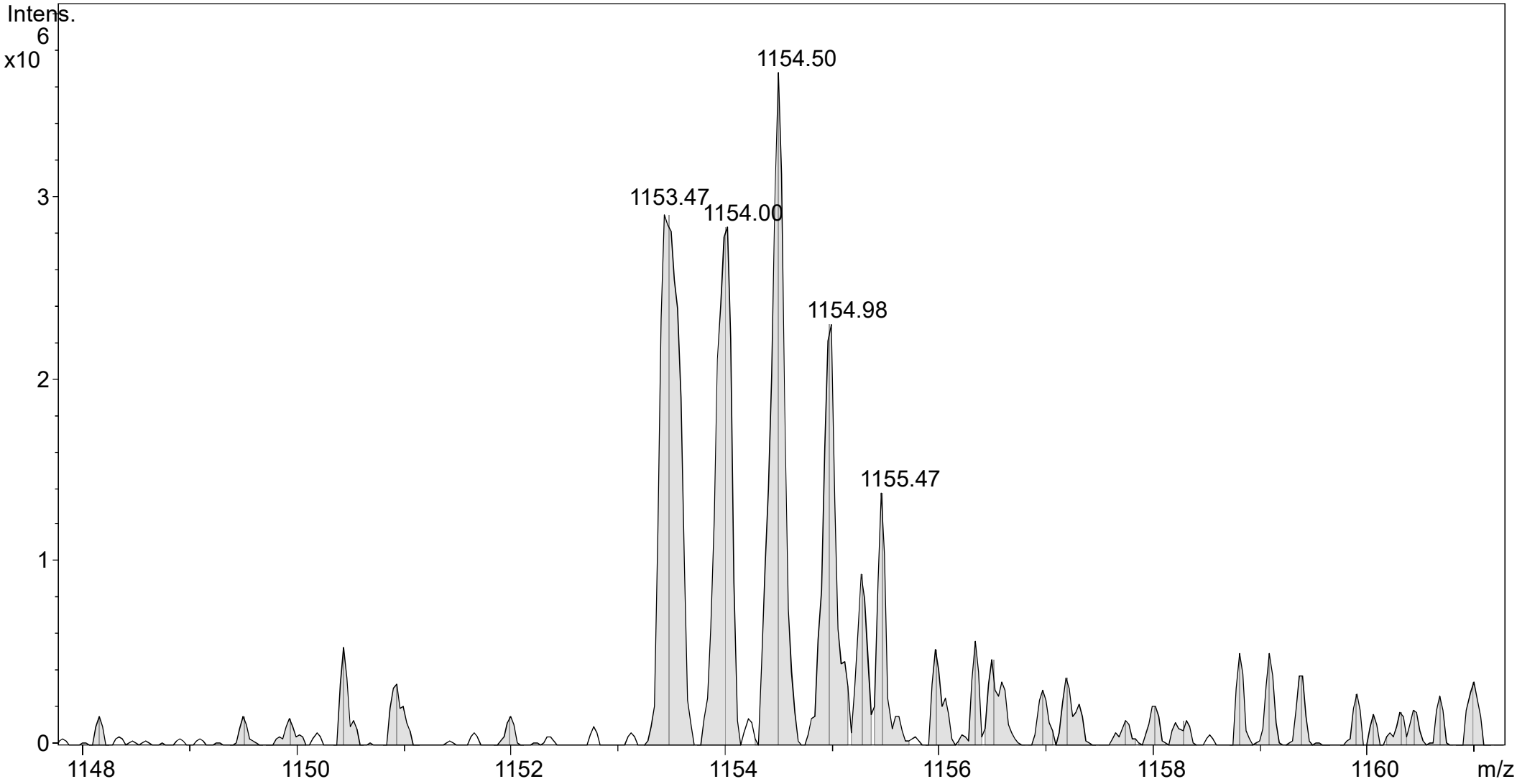


Glycan 27

A2S2Ac2
(H5N4S2Ac2)

Monoisotopic mass:	**2308.81 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 1153.40**
Observed ion:	***m/z* 1153.47**
Mass deviation:	***m/z* 0.07**
Retention time:	**70.1 min**

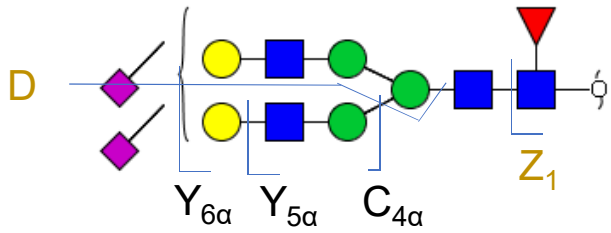


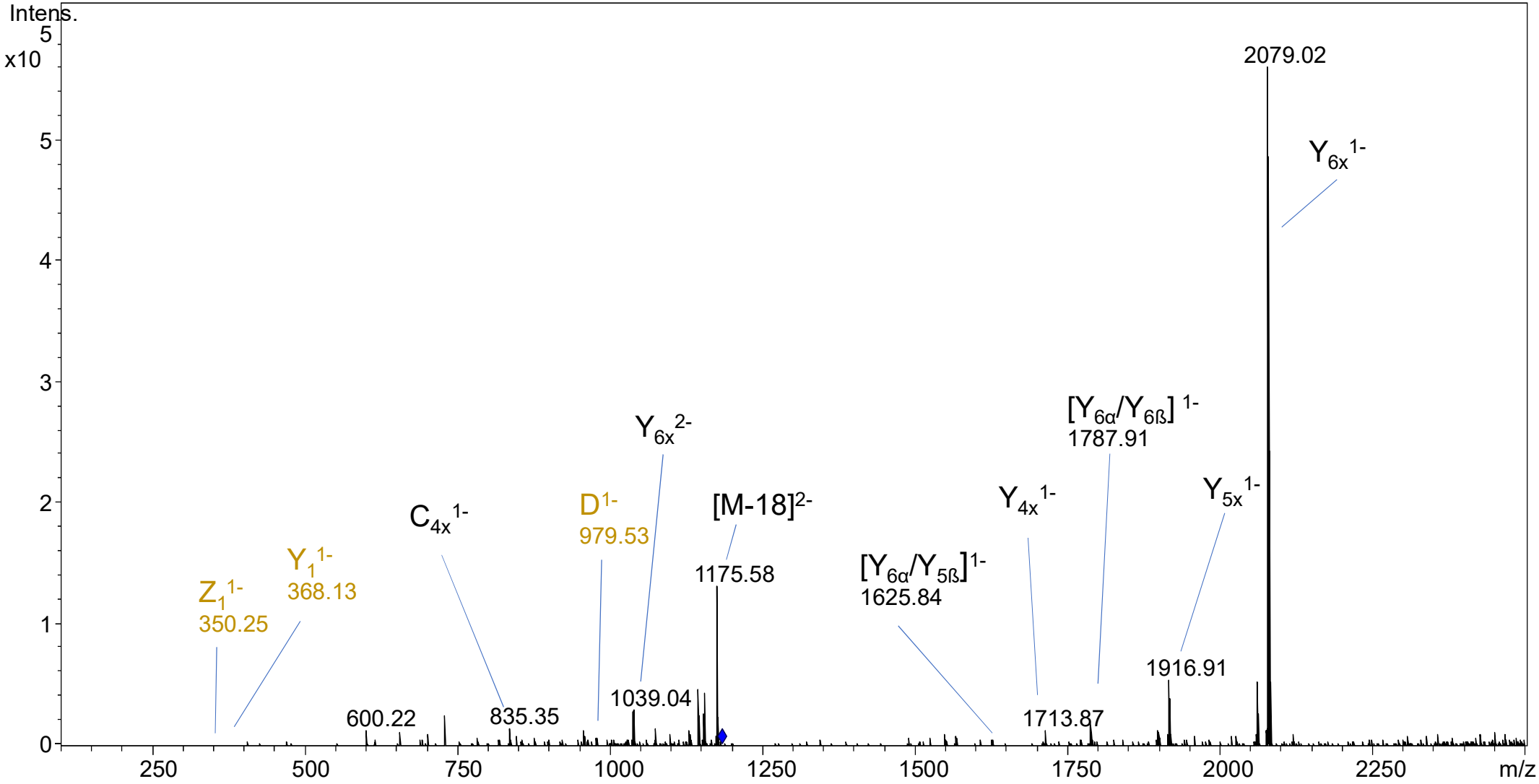


Glycan 28a

A2S2F
(H5N4S2F1)

Monoisotopic mass:	**2370.86 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 1184.42**
Observed ion:	***m/z* 1184.57**
Mass deviation:	***m/z* 0.15**
Retention time:	**67.5 min**



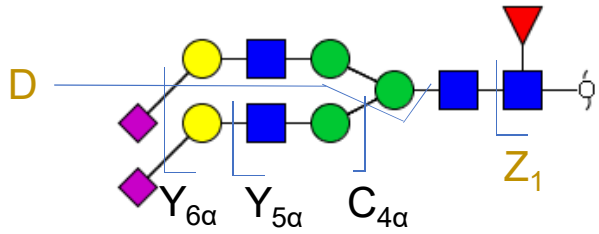


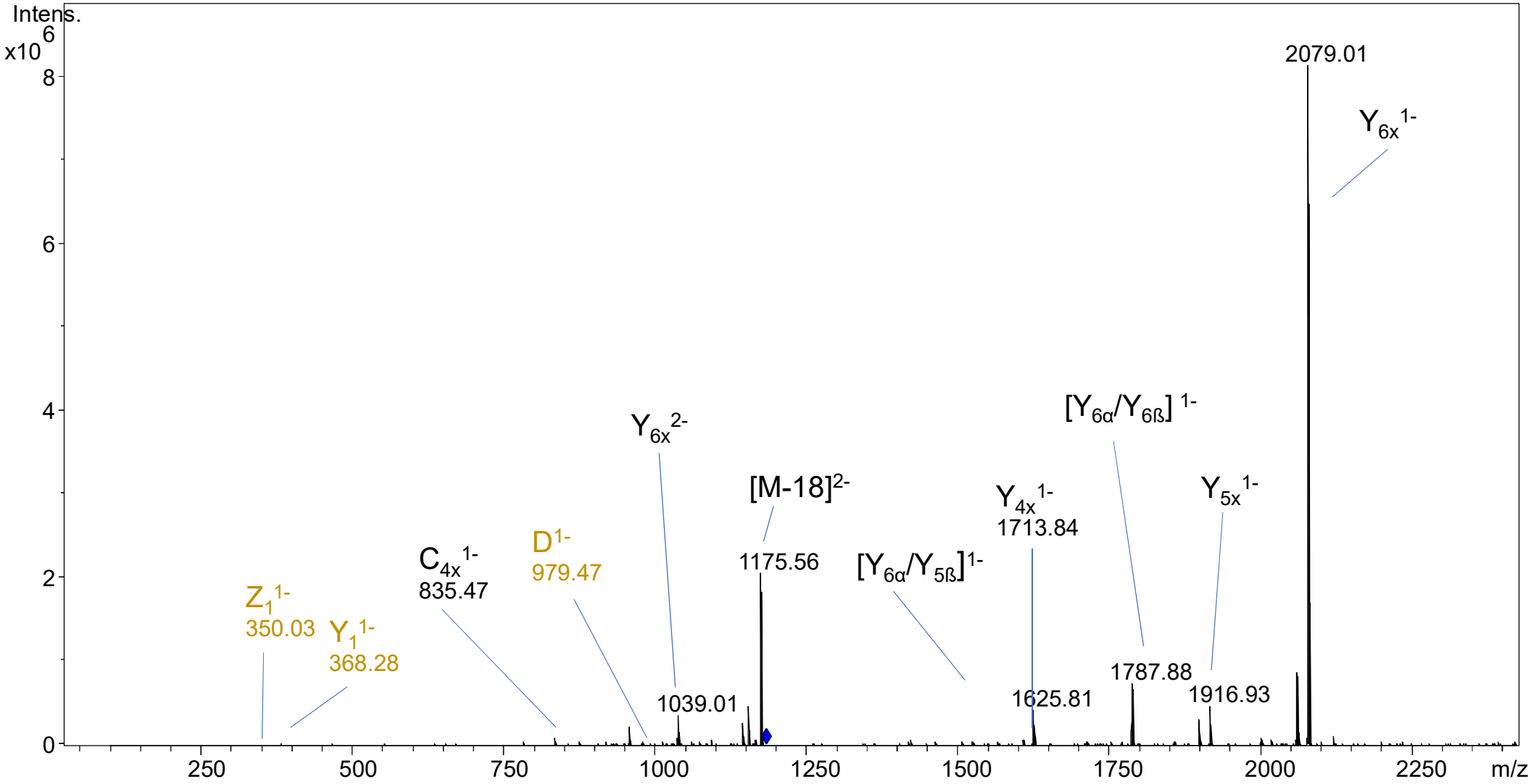
Glycan 28b

A2S2F
(H5N4S2F1)

Monoisotopic mass:	**2370.86 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 1184.42**
Observed ion:	***m/z* 1184.60**
Mass deviation:	***m/z* 0.18**
Retention time:	**74.9 min**

UniCarb-DB: #2563

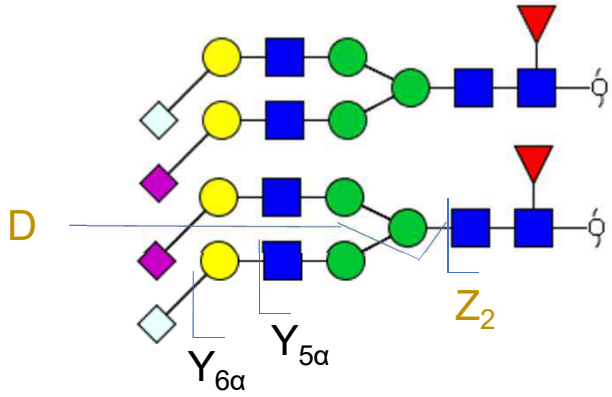


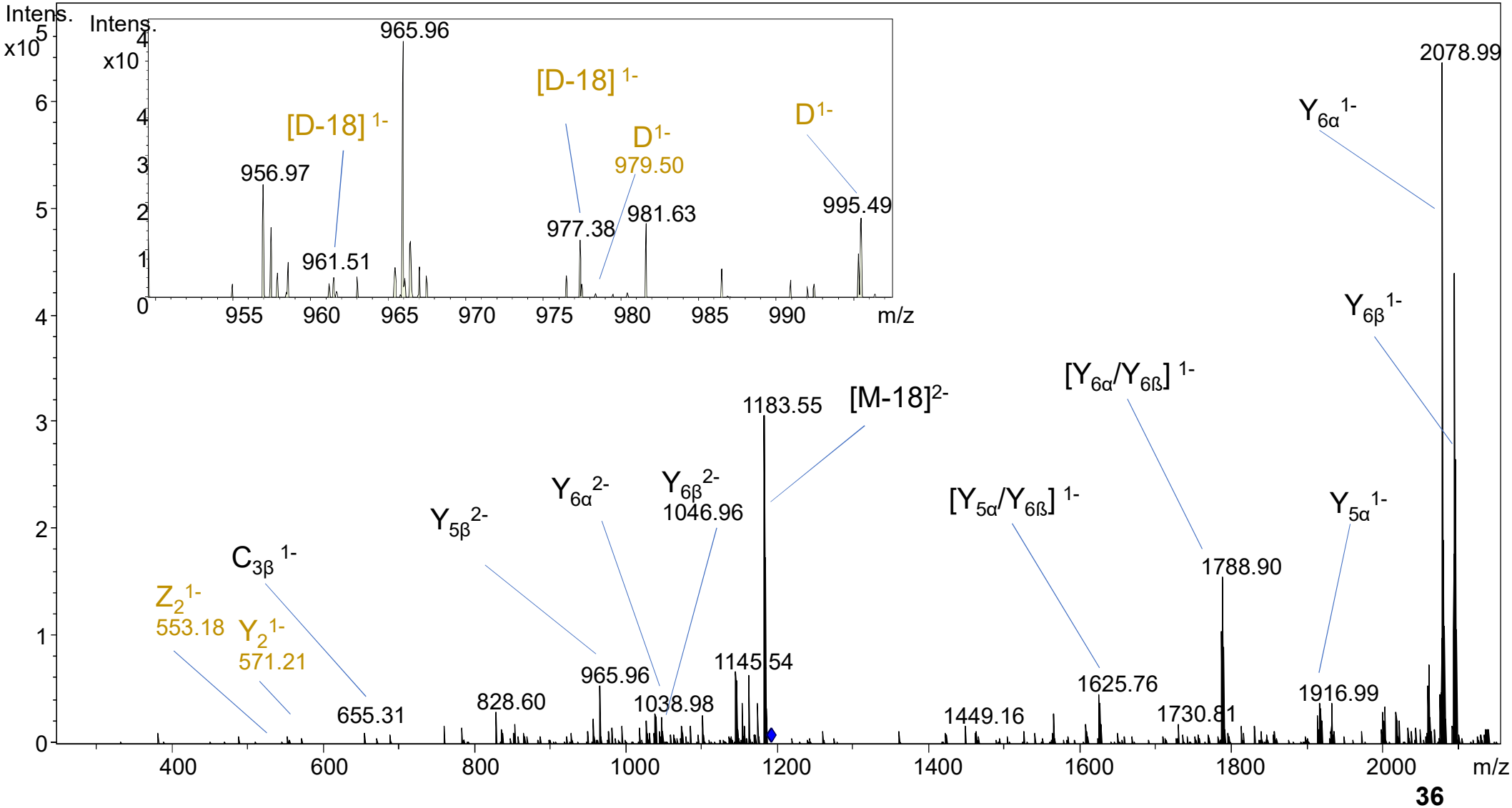


Glycan 29

A2S1Sg1F
(H5N4F1S1G1)

Monoisotopic mass: **2385.85 Da**
Charge observed: **2-**
Theoretical ion: ***m/z* 1192.42**
Observed ion: ***m/z* 1192.56**
Mass deviation: ***m/z* 0.14**
Retention time: **74.4 min**
Note: Isomers comprising the Neu5Ac either on the 3- or 6-arm potentially co-elute, which would explain the presence of both D-ions.

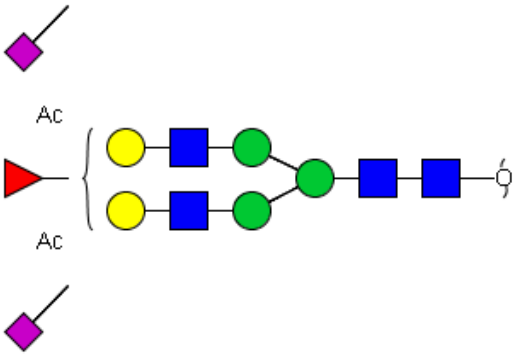


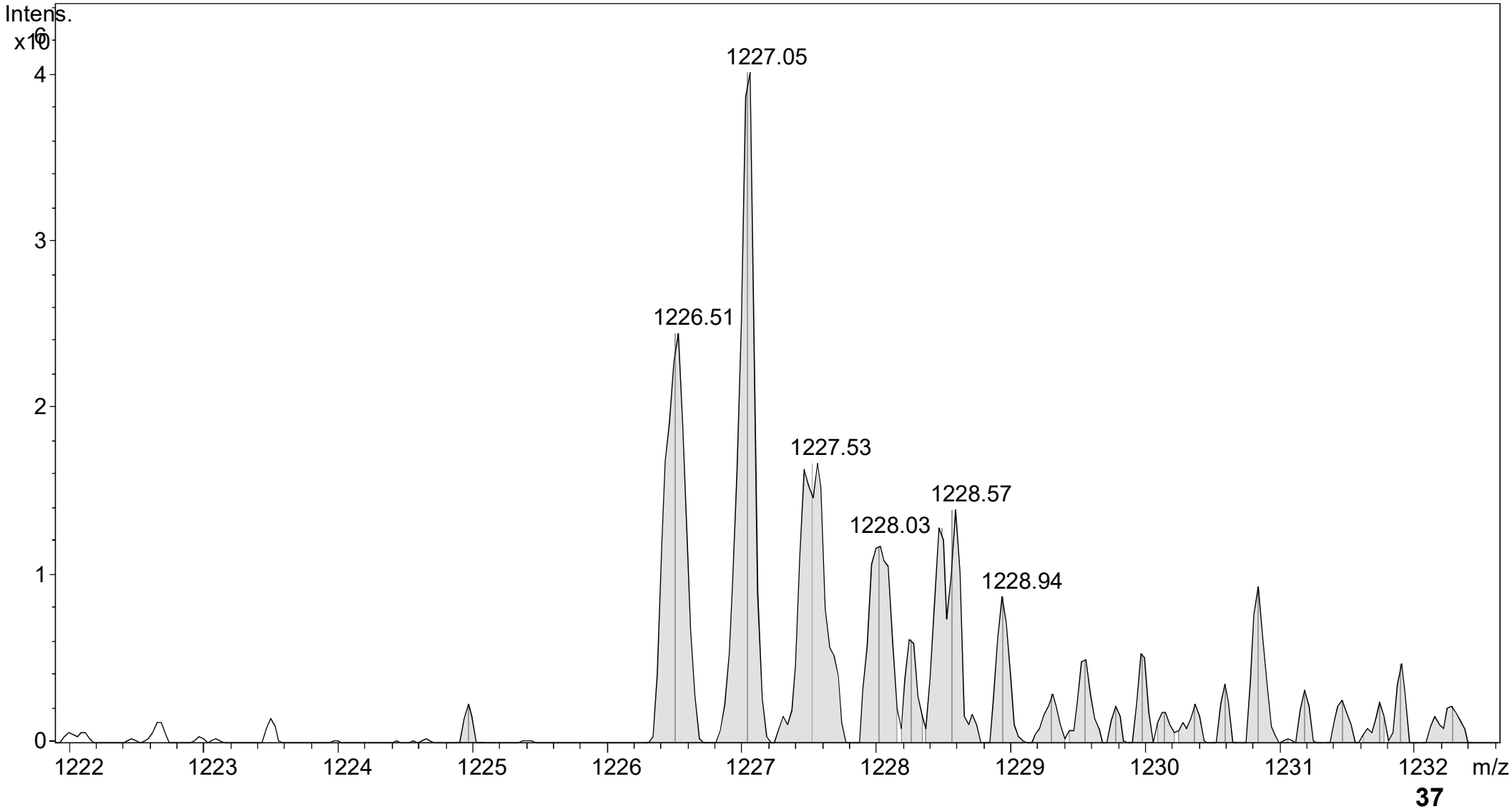


Glycan 30

A2S2Ac2F
(H5N4F1S2Ac2)

Monoisotopic mass:	**2454.87 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 1226.43**
Observed ion:	***m/z* 1226.51**
Mass deviation:	***m/z* 0.08**
Retention time:	**75.5 min**

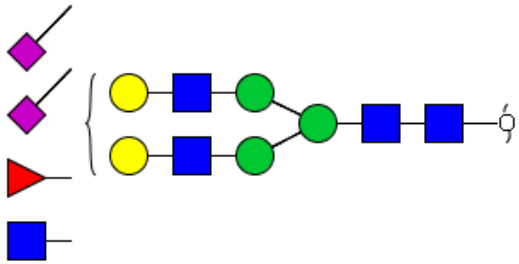


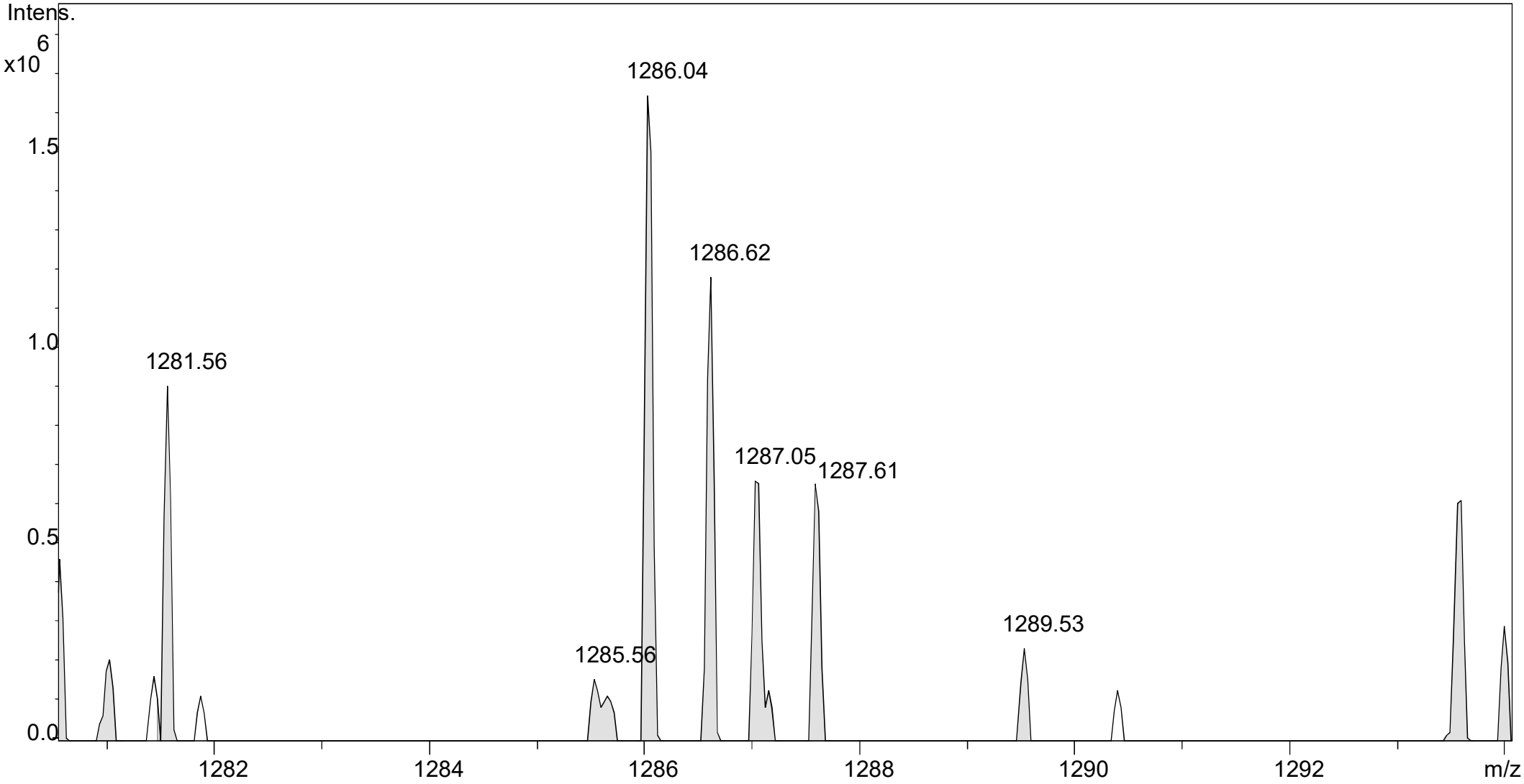


Glycan 31

A3G0S2F
(H5N5F1S2)

Monoisotopic mass: **2573.94 Da**
Charge observed: **2-**
Theoretical ion: ***m/z* 1285.96**
Observed ion: ***m/z* 1286.04**
Mass deviation: ***m/z* 0.09**
Retention time: **73.5 min**
Note: No MS2 was recorded.

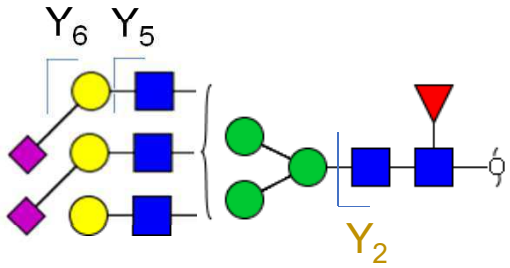


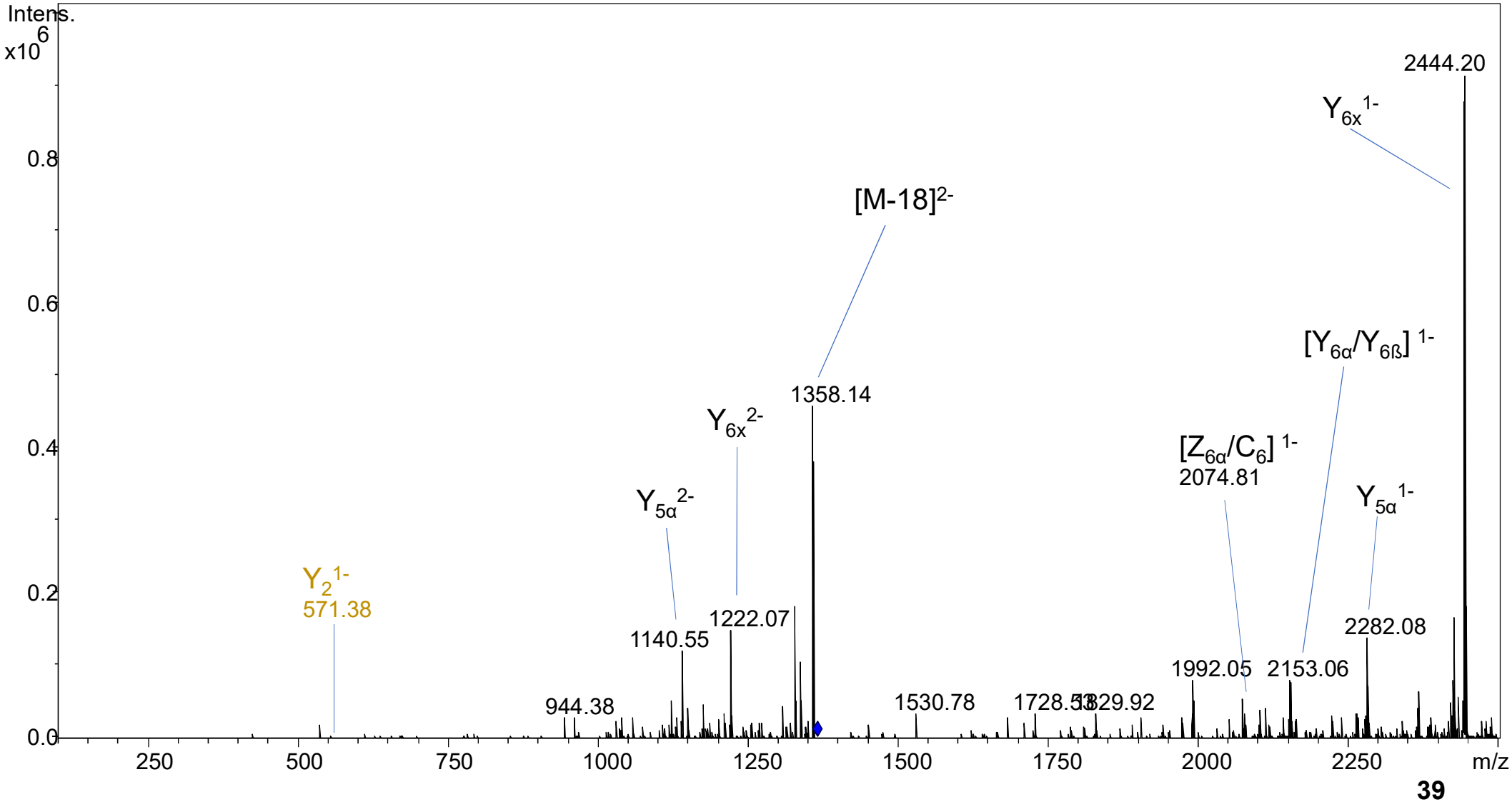


Glycan 32

A3G1S2F
(H6N5F1S2)

Monoisotopic mass:	**2735.98 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 1366.99**
Observed ion:	***m/z* 1367.14**
Mass deviation:	***m/z* 0.15**
Retention time:	**71.8 min**

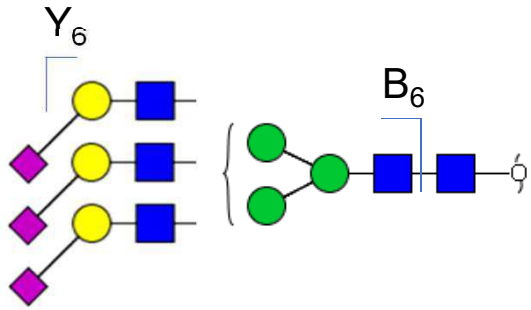


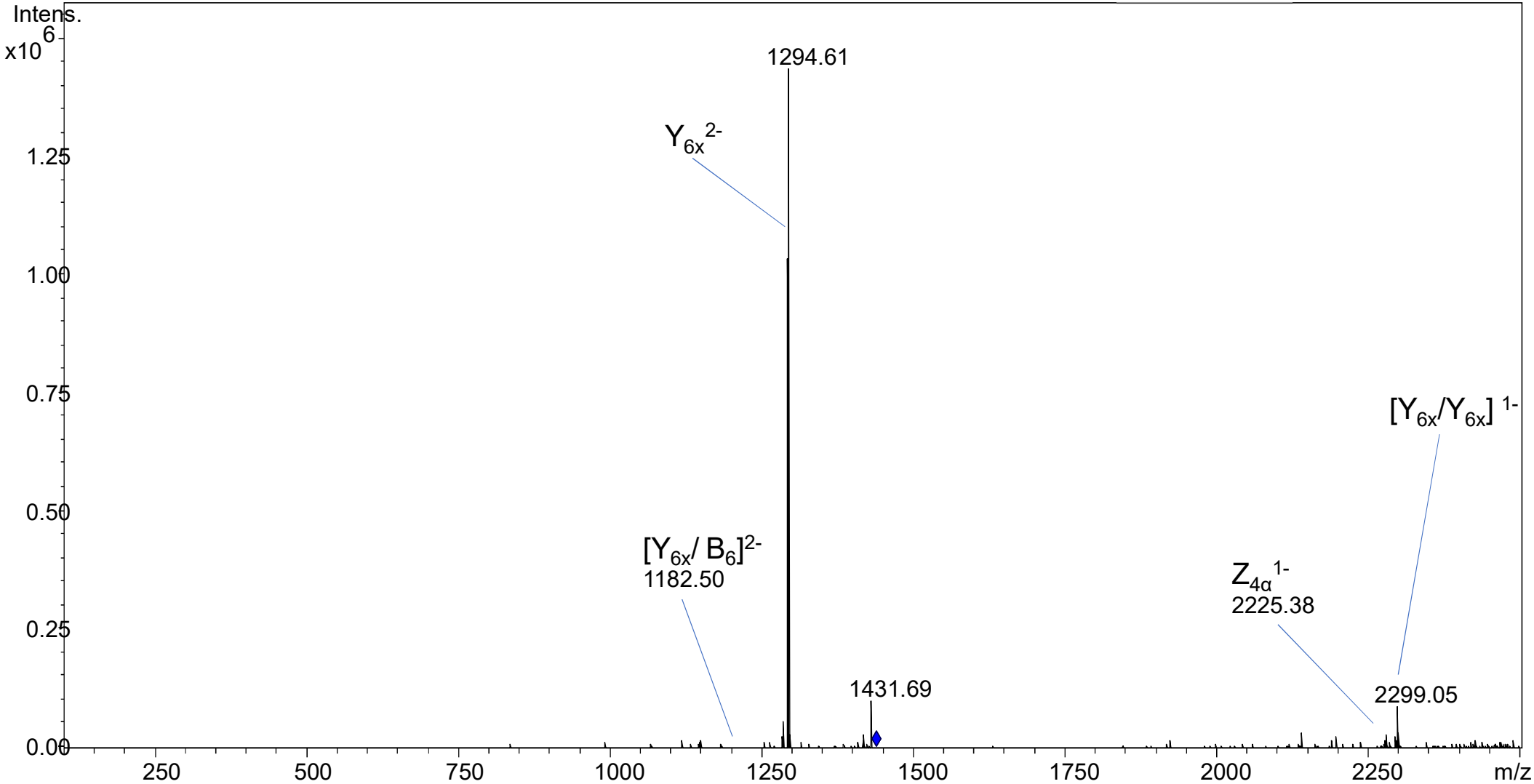


Glycan 33

A3S3
(H6N5S3)

Monoisotopic mass:	**2881.03 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 1439.51**
Observed ion:	***m/z* 1439.68**
Mass deviation:	***m/z* 0.17**
Retention time:	**73.8 min**

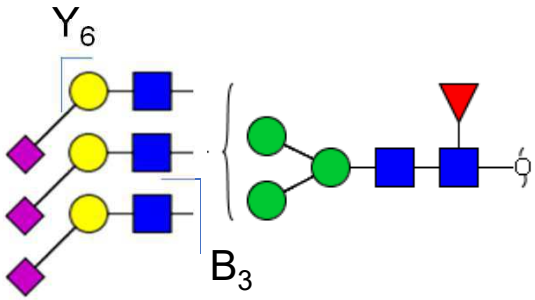


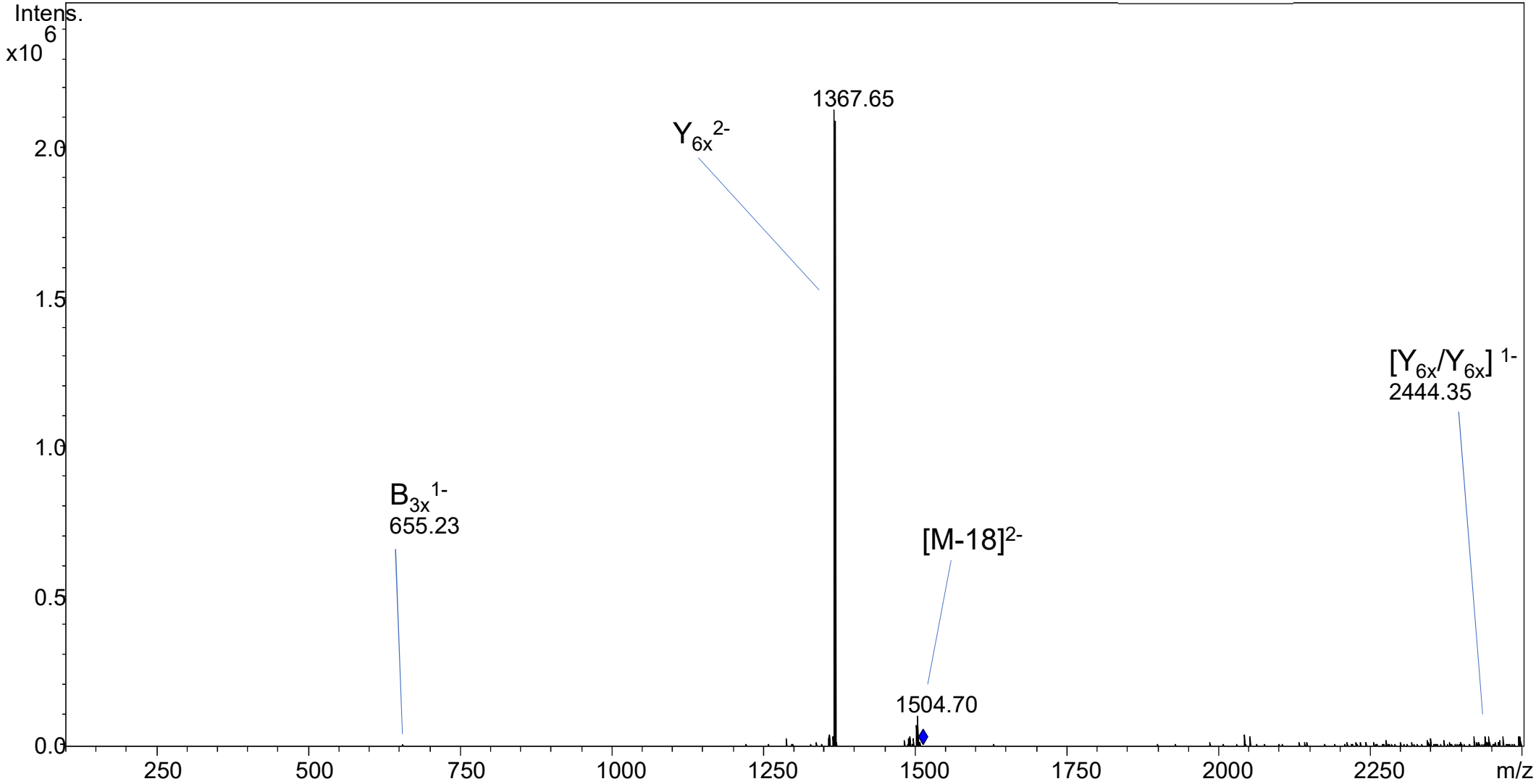


Glycan 34a

A3S3F
(H6N5F1S3)

Monoisotopic mass:	**3027.08 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 1512.54**
Observed ion:	***m/z* 1512.69**
Mass deviation:	***m/z* 0.15**
Retention time:	**76.6 min**

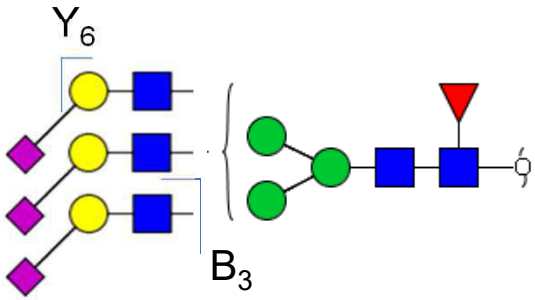


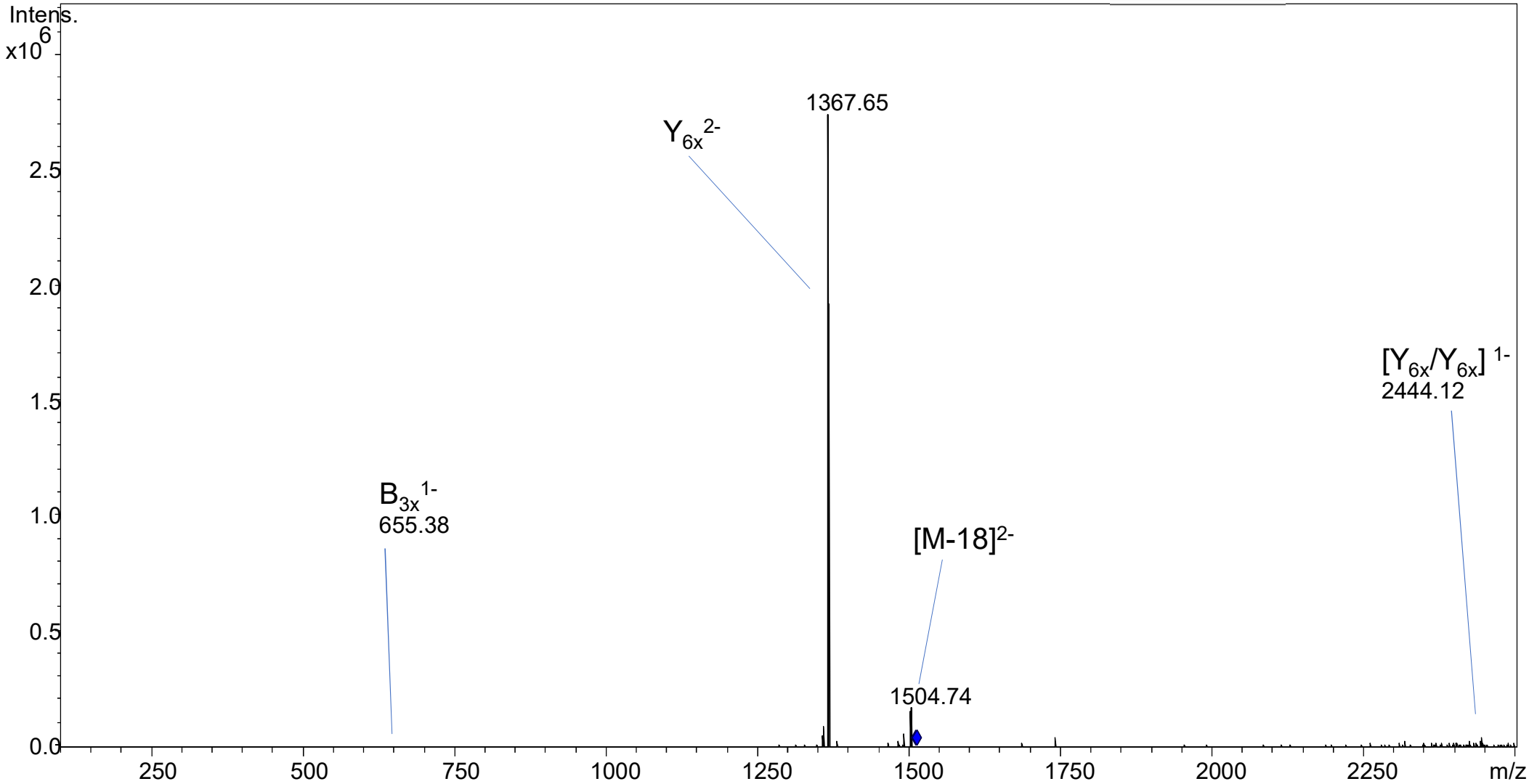


Glycan 34b

A3S3F
(H6N5F1S3)

Monoisotopic mass:	**3027.08 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 1512.54**
Observed ion:	***m/z* 1512.68**
Mass deviation:	***m/z* 0.14**
Retention time:	**78.4 min**

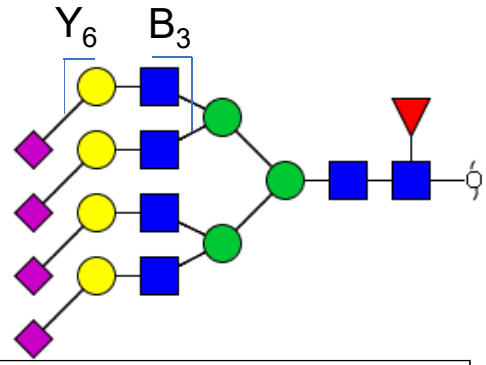


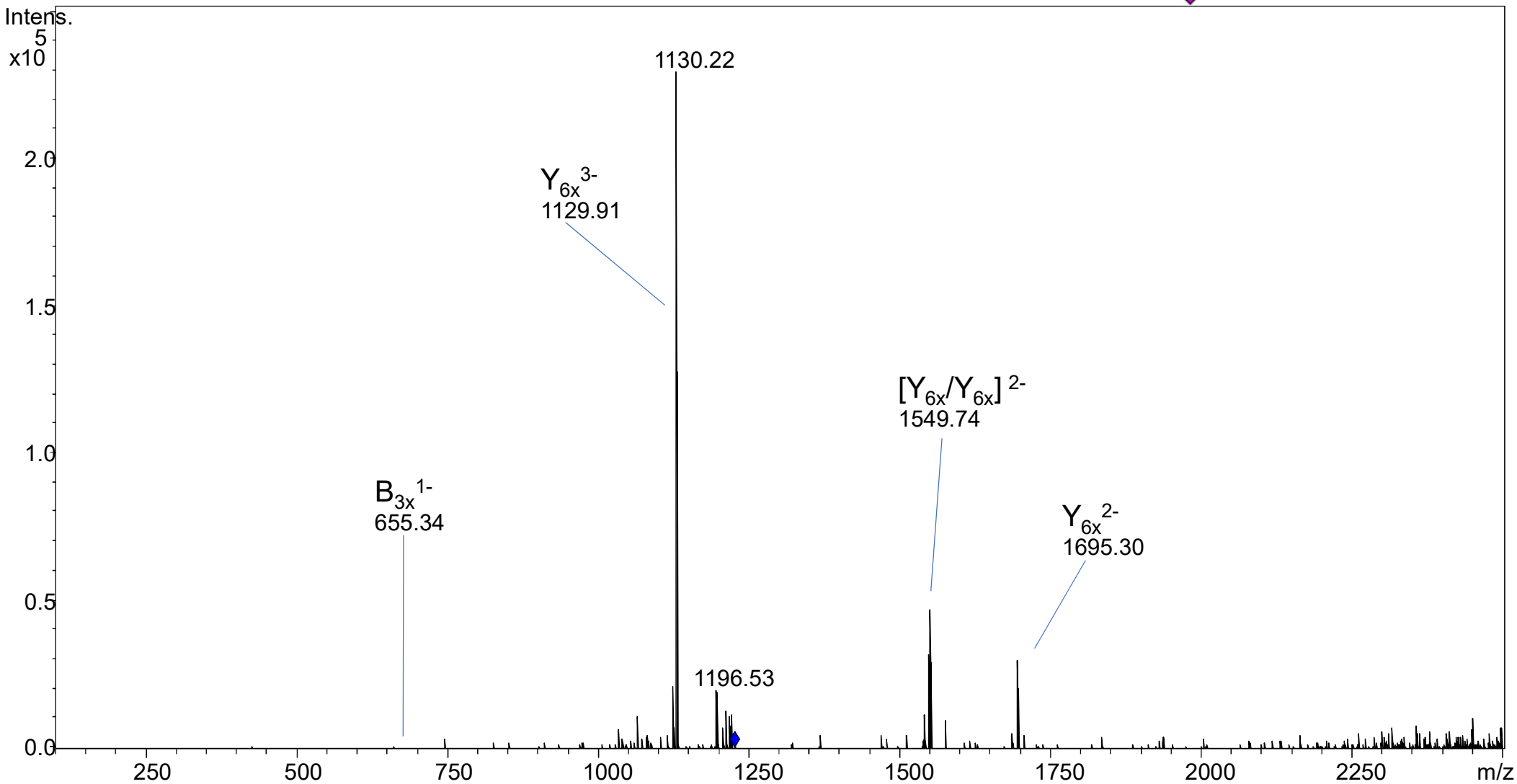


Glycan 35

A4S4F
(H7N6F1S4)

Monoisotopic mass:	**3683.31 Da**
Charge observed:	**3-**
Theoretical ion:	***m/z* 1226.76**
Observed ion:	***m/z* 1226.87**
Mass deviation:	***m/z* 0.11**
Retention time:	**67.8 min**

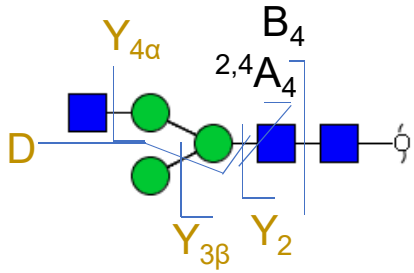


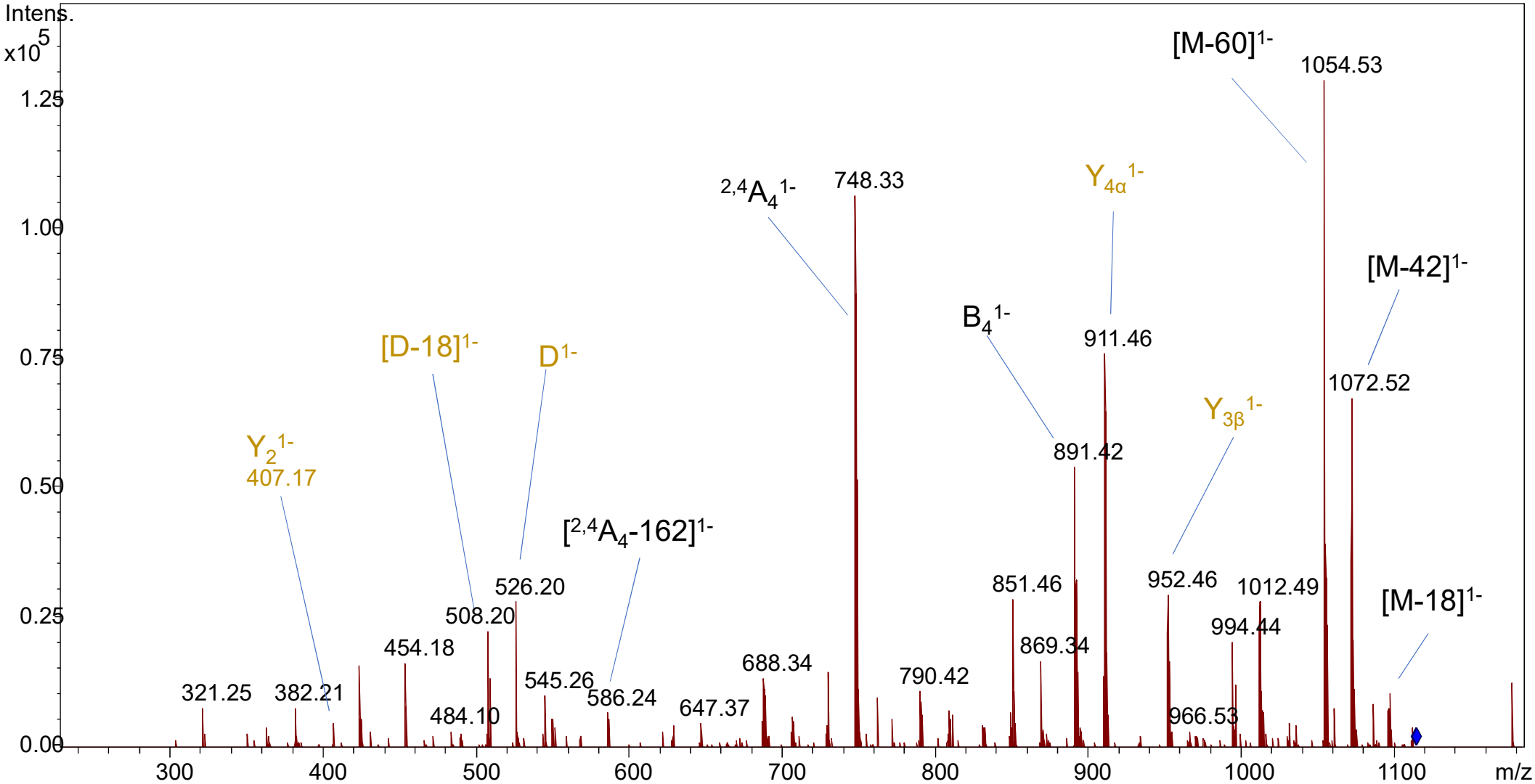


Glycan 36

A1G0
(H3N3)

Monoisotopic mass:	**1115.42 Da**
Charge observed:	**1-**
Theoretical ion:	***m/z* 1114.42**
Observed ion:	***m/z* 1114.55**
Mass deviation:	***m/z* 0.13**
Retention time:	**59.4 min**

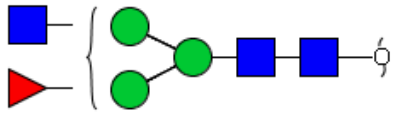


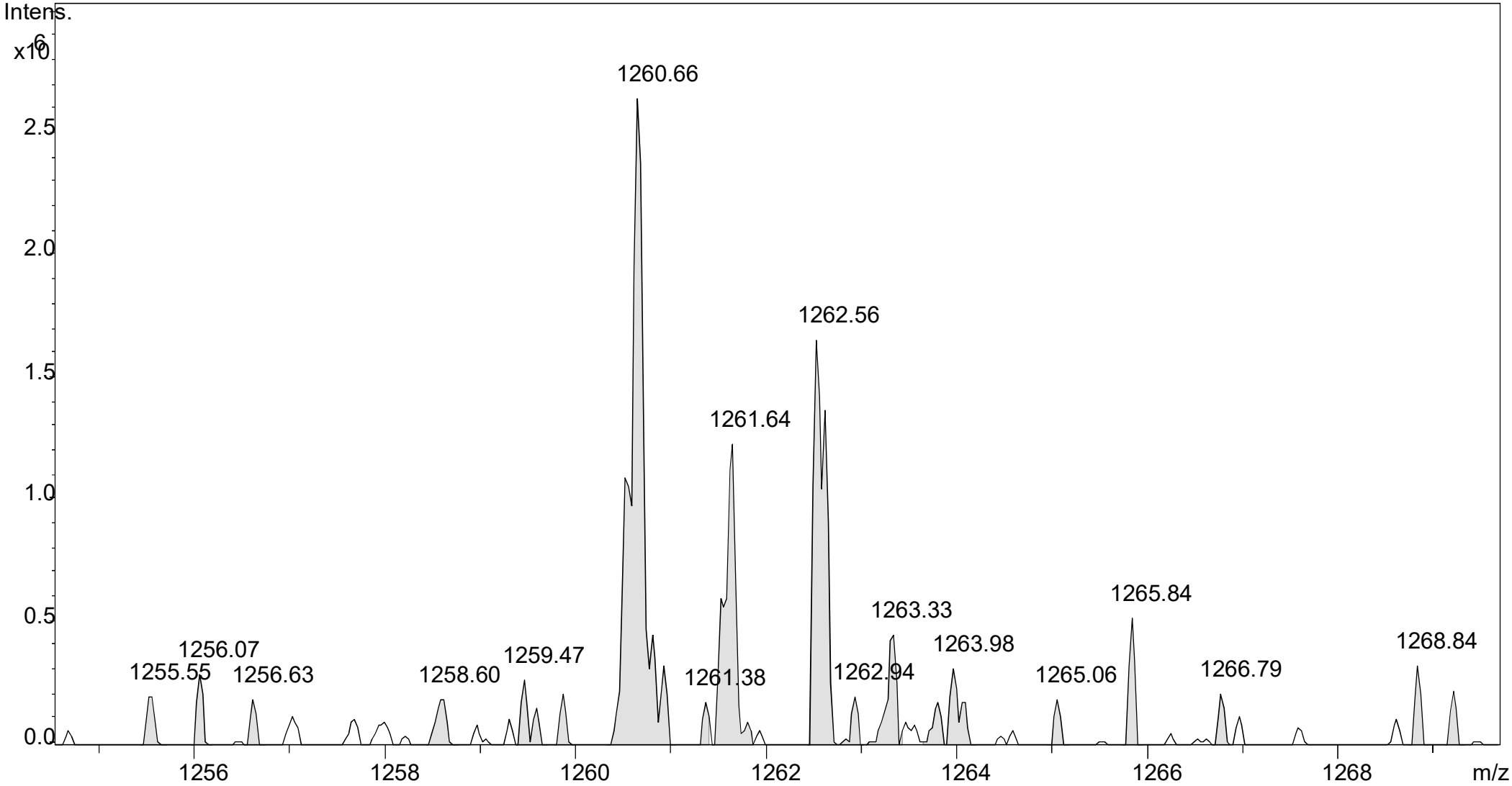


Glycan 37

A1G0F
(H3N3F1)

Monoisotopic mass: 1261.48 Da
Charge observed: 1-
Theoretical ion: *m/z* 1260.47
Observed ion: *m/z* 1260.66
Mass deviation: *m/z* 0.09
Retention time: 69.1 min
Note: No MS2 was recorded.

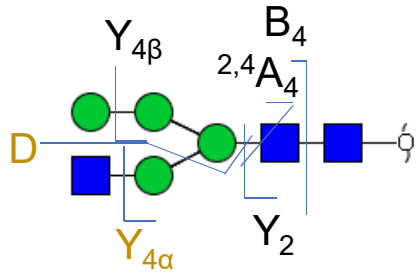


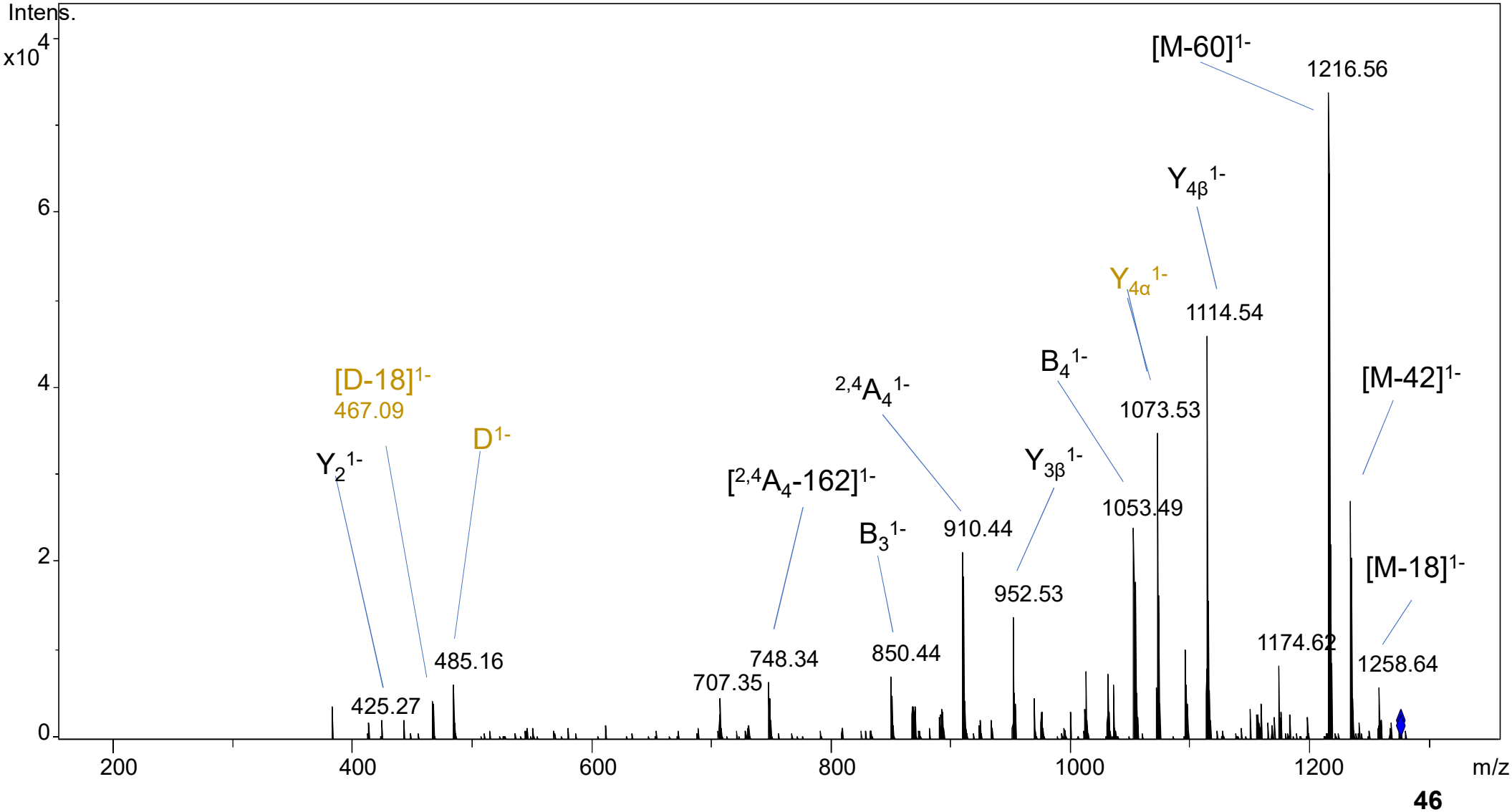


Glycan 38a

A1G0-M4 (H4N3)

Monoisotopic mass:	**1277.47 Da**
Charge observed:	**1-**
Theoretical ion:	***m/z* 1276.47**
Observed ion:	***m/z* 1276.63**
Mass deviation:	***m/z* 0.16**
Retention time:	**43.6 min**

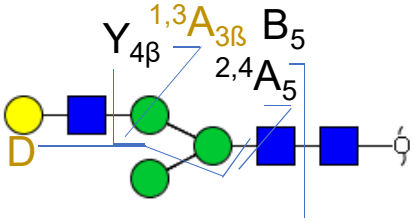


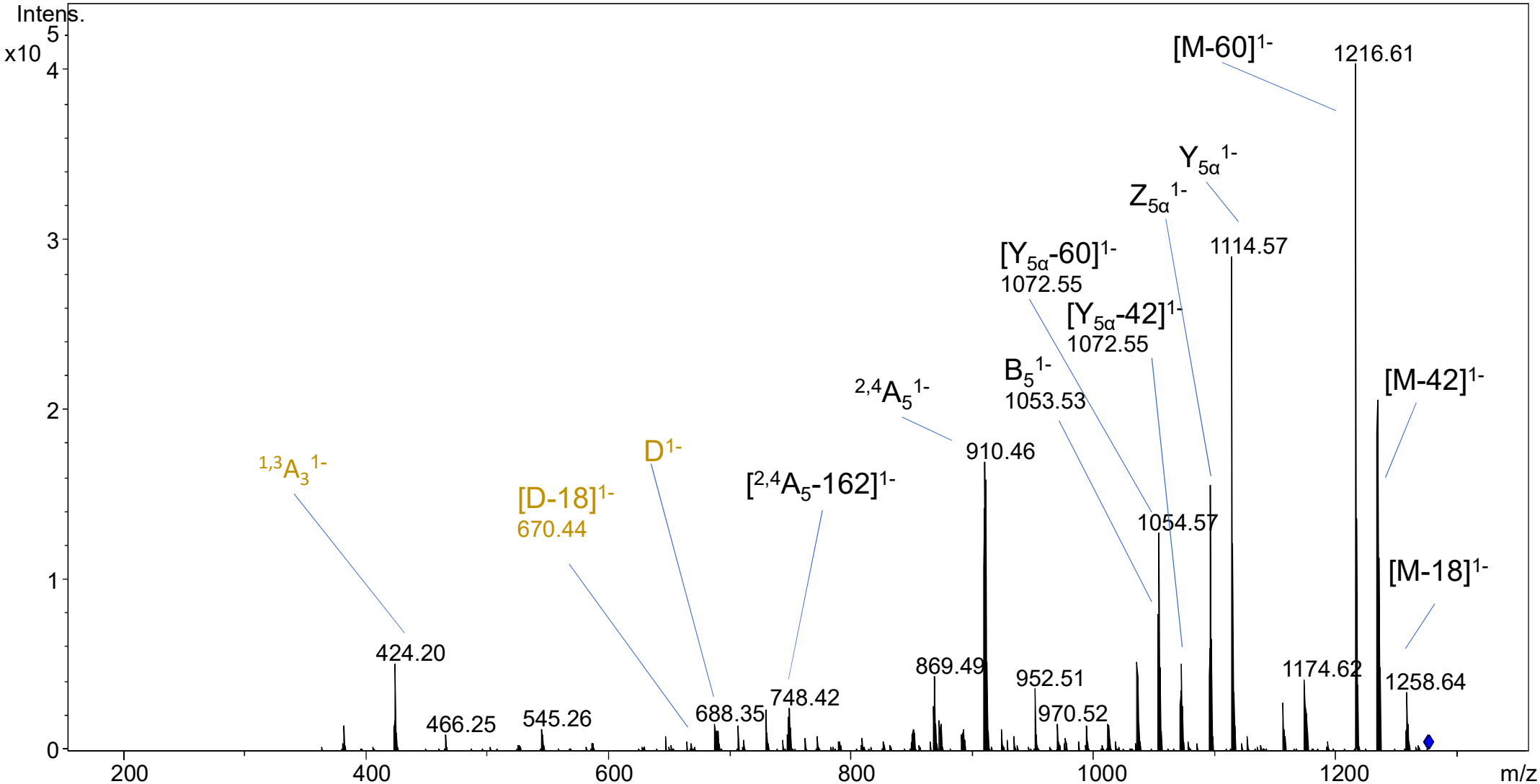


Glycan 38b

A1G1
(H4N3)

Monoisotopic mass:	**1277.47 Da**
Charge observed:	**1-**
Theoretical ion:	***m/z* 1276.47**
Observed ion:	***m/z* 1276.61**
Mass deviation:	***m/z* 0.14**
Retention time:	**50.4 min**

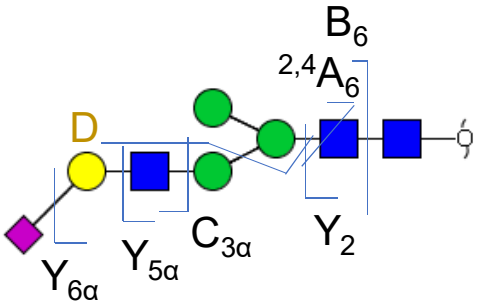


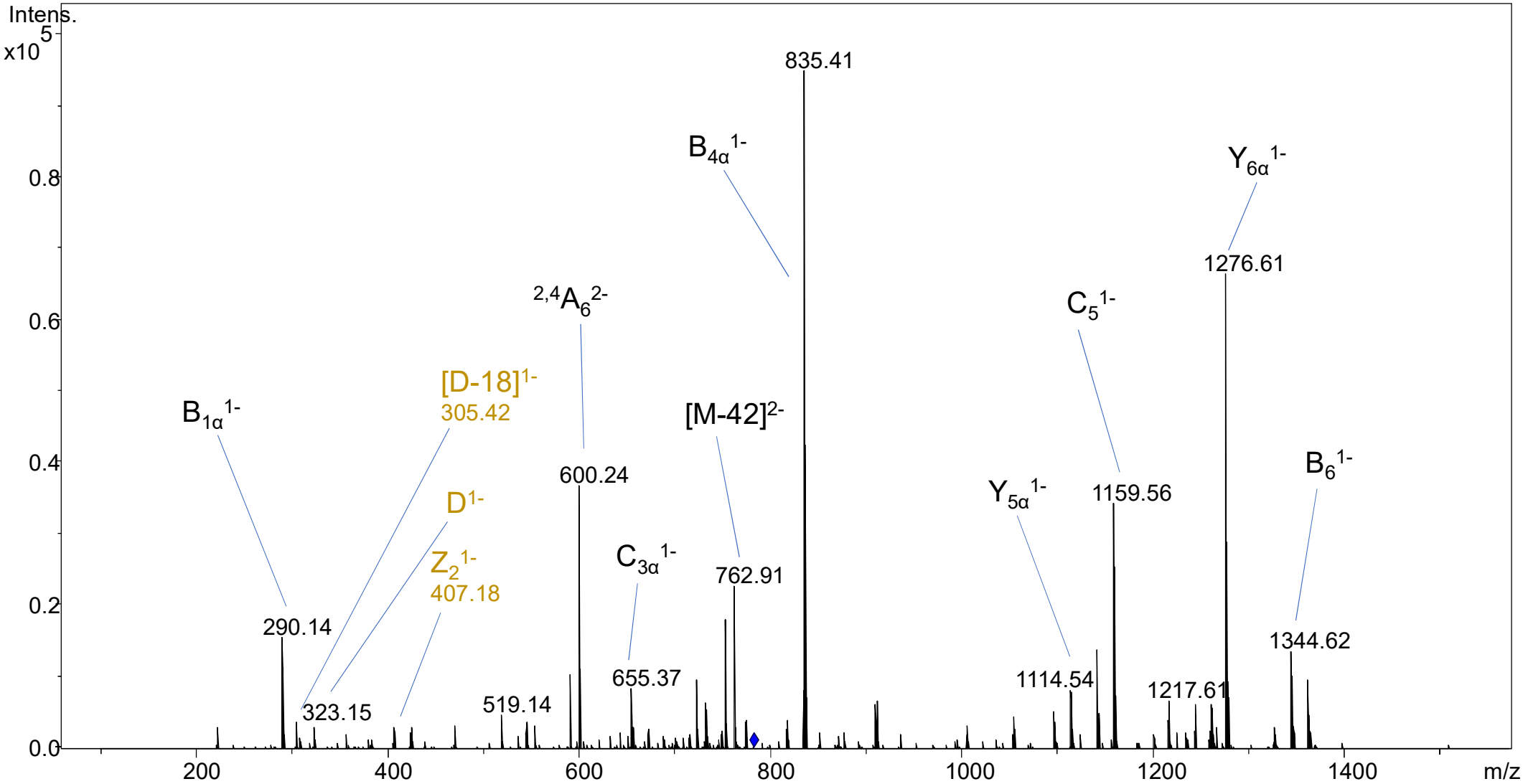


Glycan 39

A1S1
(H4N3S1)

Monoisotopic mass:	**1567.56 Da**
Charge observed:	**1-**
Theoretical ion:	***m/z* 783.28**
Observed ion:	***m/z* 783.39**
Mass deviation:	***m/z* 0.11**
Retention time:	**60.0 min**

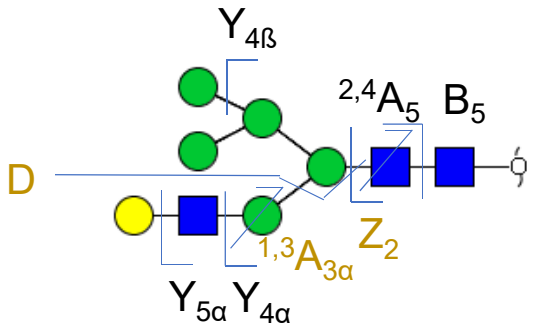


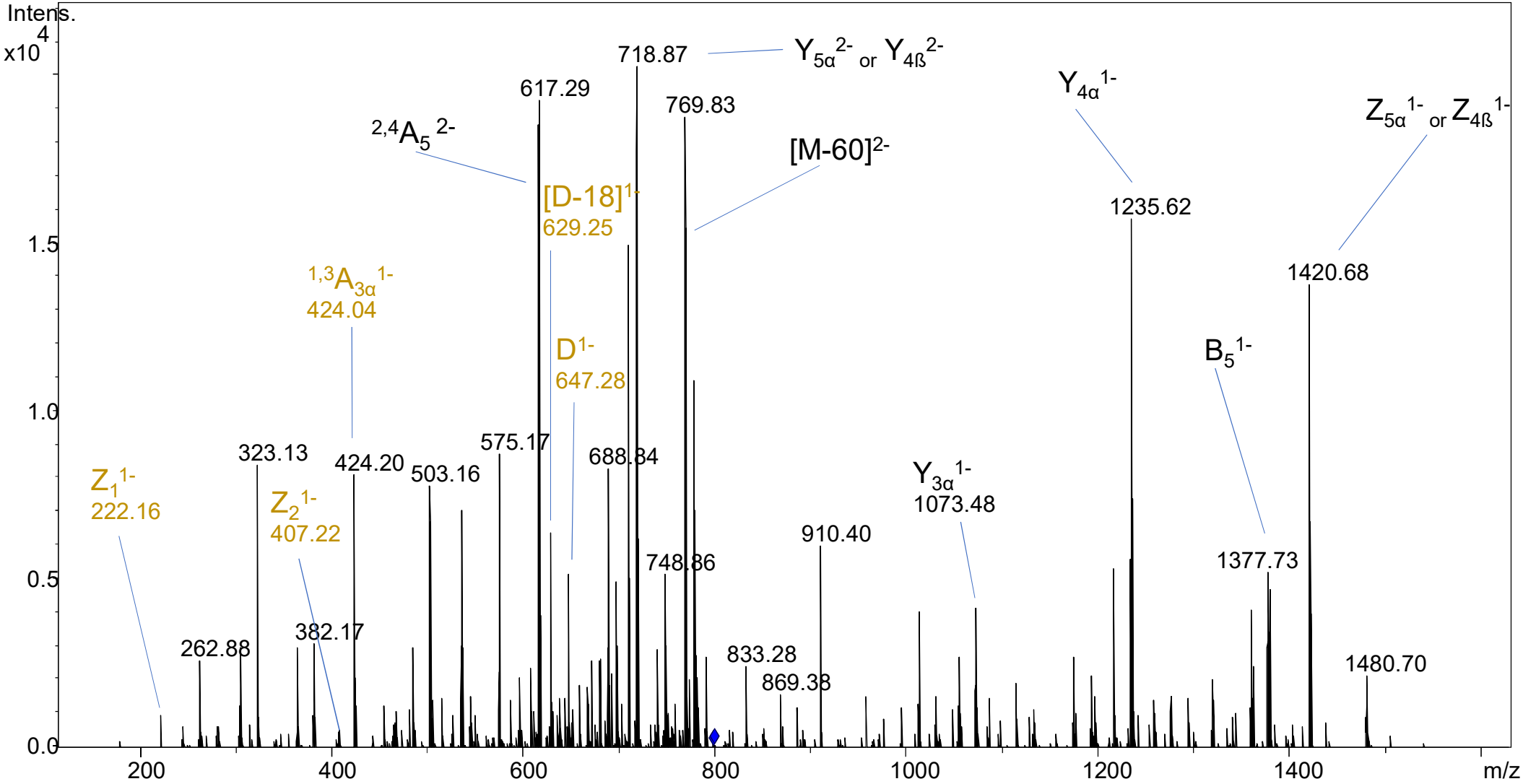


Glycan 40

A1G1-M5 (H6N3)

Monoisotopic mass:	**1601.59 Da**	**UniCarb-DB: #2482**
Charge observed:	**2-**	
Theoretical ion:	***m/z* 799.78**	
Observed ion:	***m/z* 799.88**	
Mass deviation:	***m/z* 0.10**	
Retention time:	**52.8 min**	

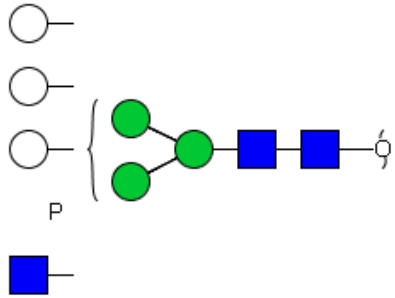


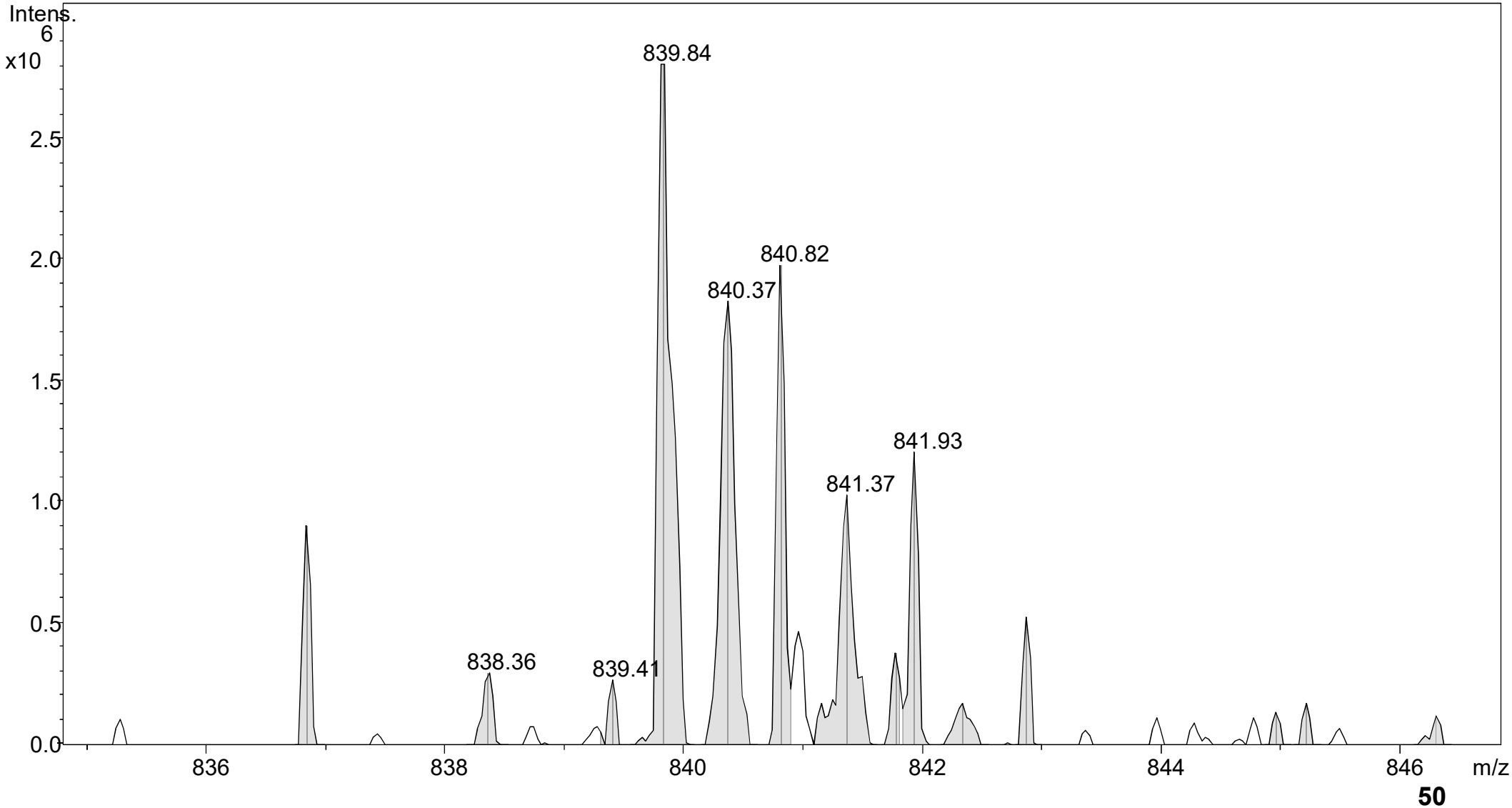


Glycan 41

A1G1-M5P (H6N3P1)

Monoisotopic mass: 1681.55 Da
Charge observed: 2-
Theoretical ion: *m/z* 839.77
Observed ion: *m/z* 839.84
Mass deviation: *m/z* 0.07
Retention time: 39.0 min
Note: Isomer to glycan 12.

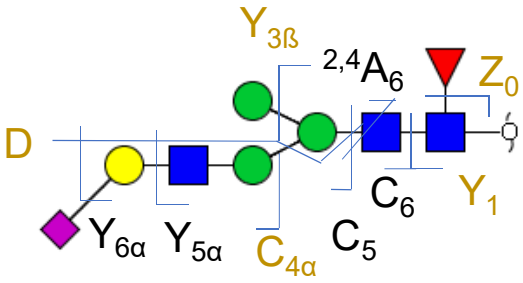


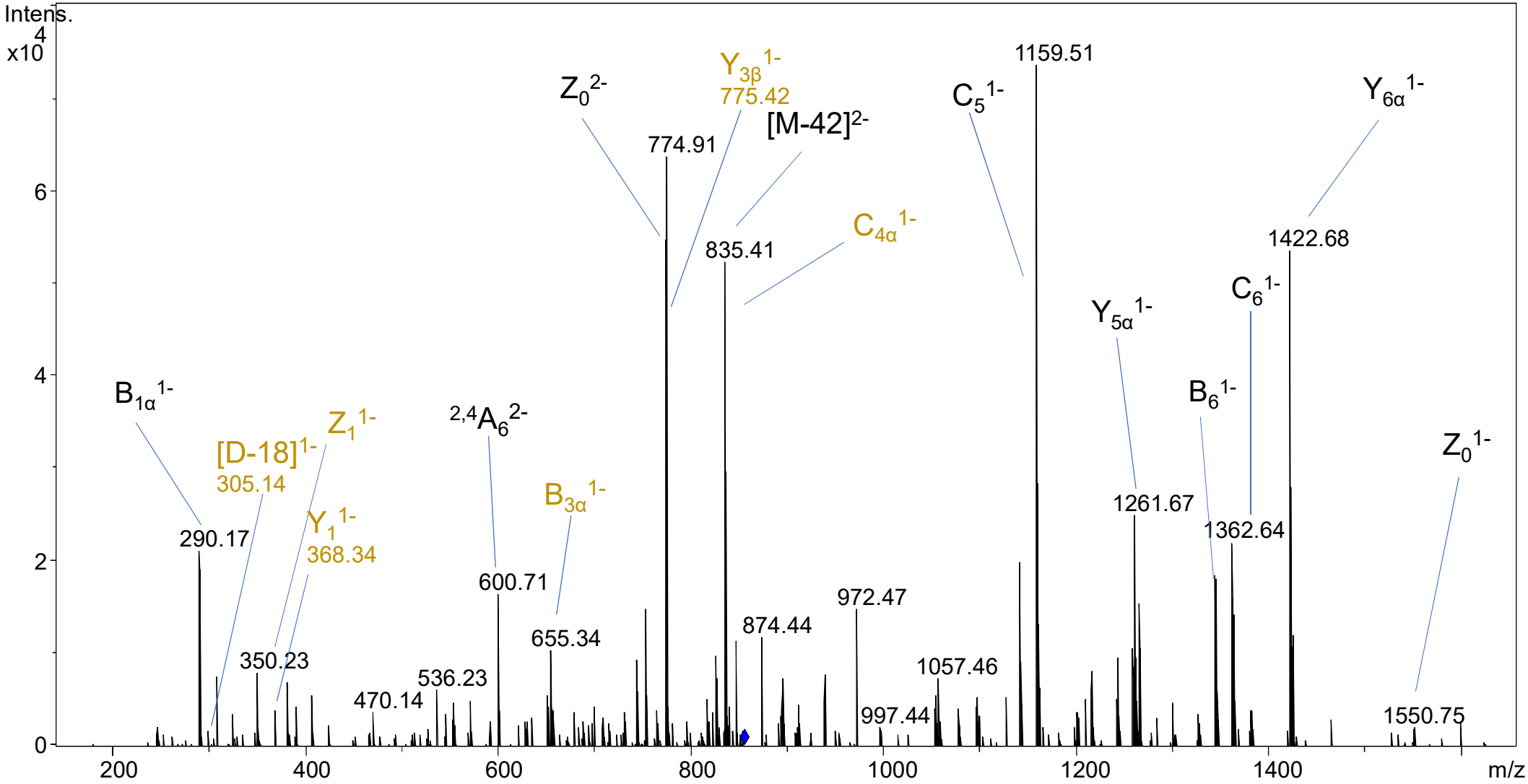


Glycan 42a

A1S1F
(H4N3F1S1)

Monoisotopic mass:	**1714.63 Da**	**UniCarb-DB: #2220**
Charge observed:	**2-**	
Theoretical ion:	***m/z* 856.30**	
Observed ion:	***m/z* 856.43**	
Mass deviation:	***m/z* 0.13**	
Retention time:	**66.6 min**	

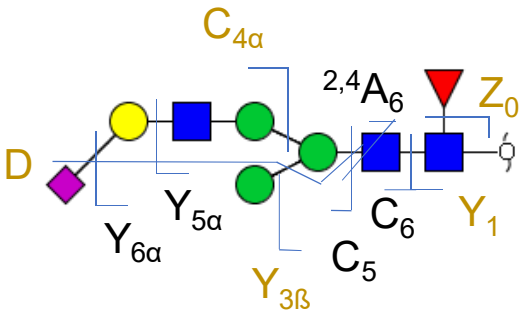


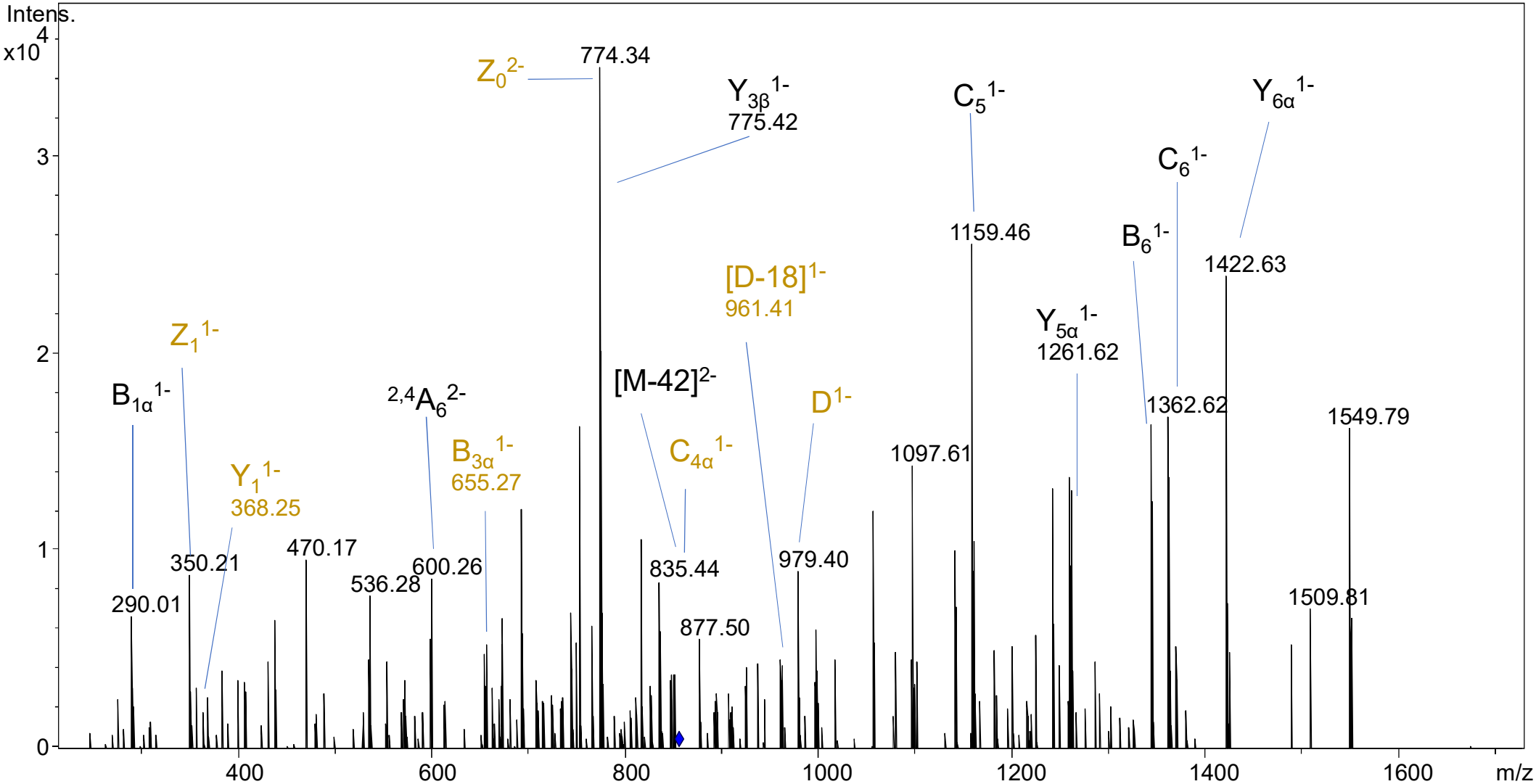


Glycan 42b

A1S1F
(H4N3F1S1)

Monoisotopic mass:	**1714.63 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 856.30**
Observed ion:	***m/z* 856.46**
Mass deviation:	***m/z* 0.16**
Retention time:	**77.1 min**

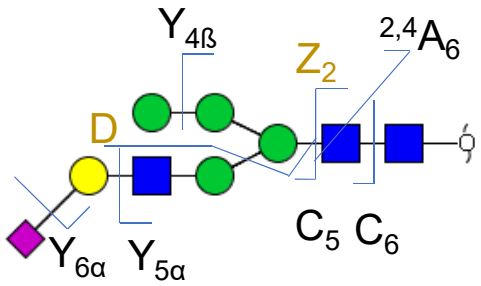


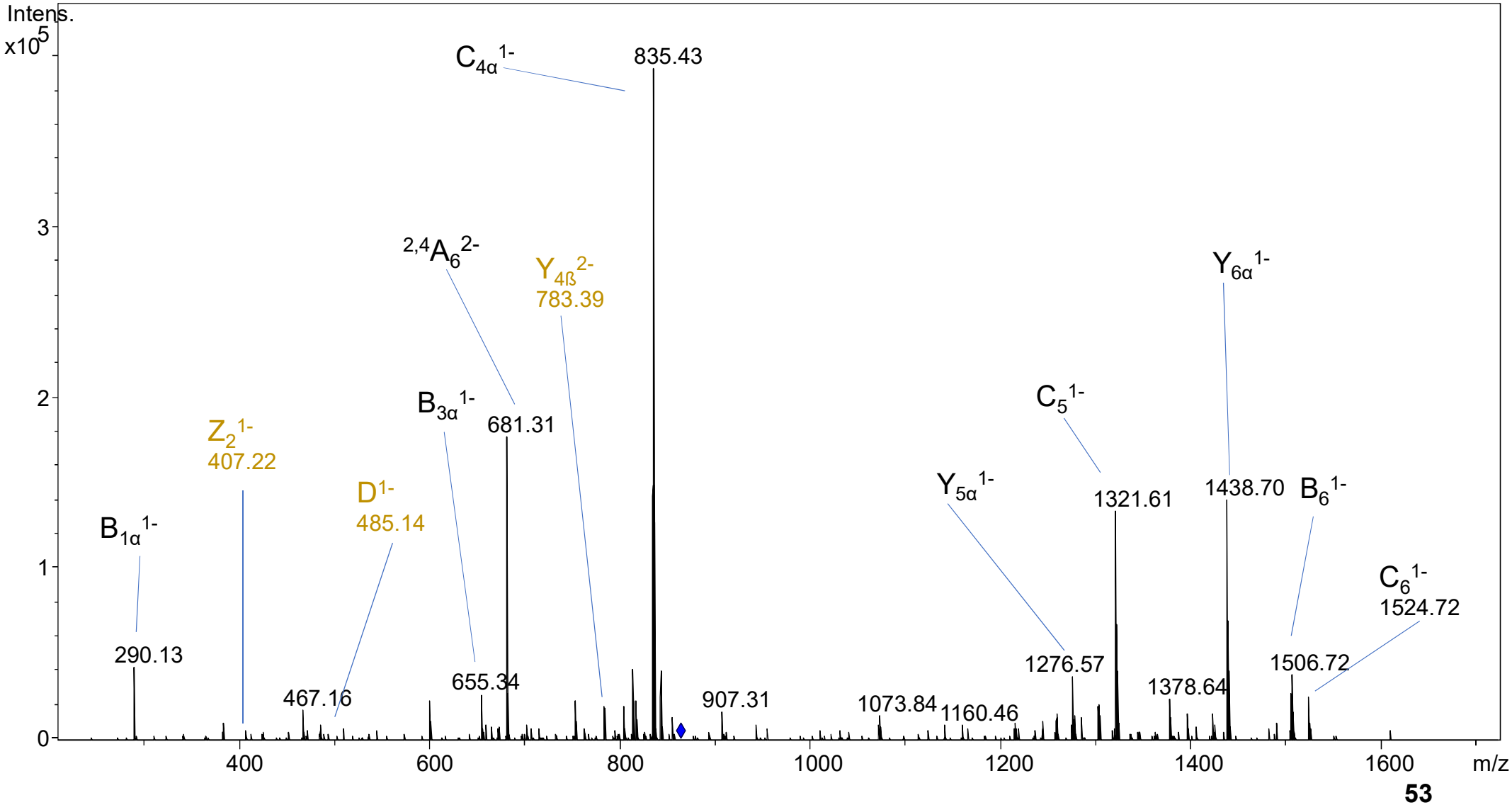


Glycan 43

A1S1-M4
(H5N3S1)

Monoisotopic mass:	**1730.62 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 864.30**
Observed ion:	***m/z* 864.44**
Mass deviation:	***m/z* 0.14**
Retention time:	**58.6 min**

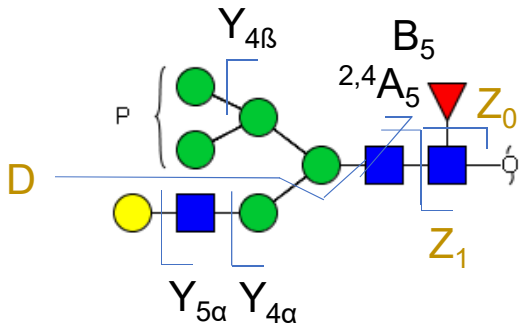


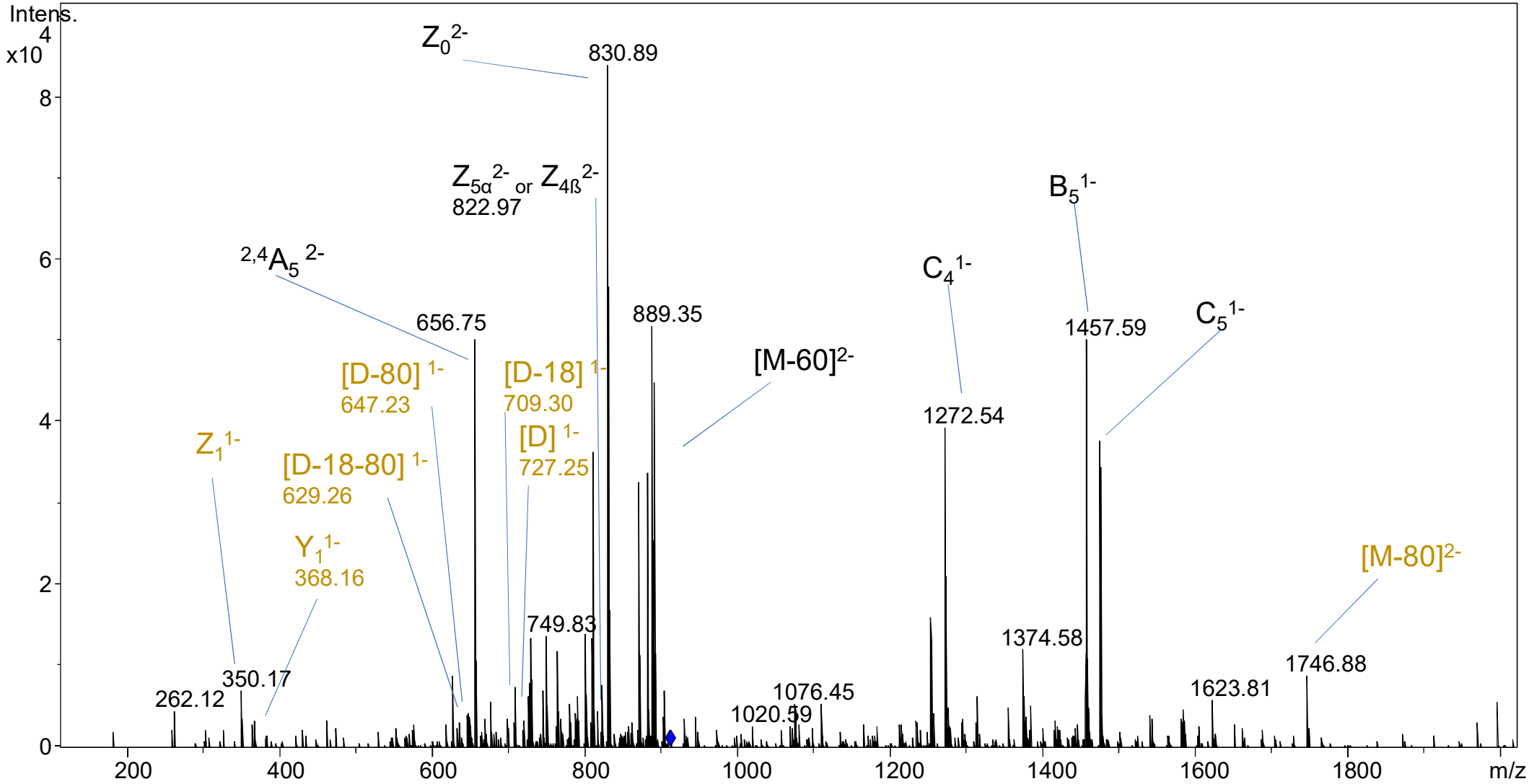


Glycan 44

A1G1F-M5P
(H6N3F1P1)

Monoisotopic mass:	**1827.60 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 912.80**
Observed ion:	***m/z* 912.92**
Mass deviation:	***m/z* 0.12**
Retention time:	**47.0 min**

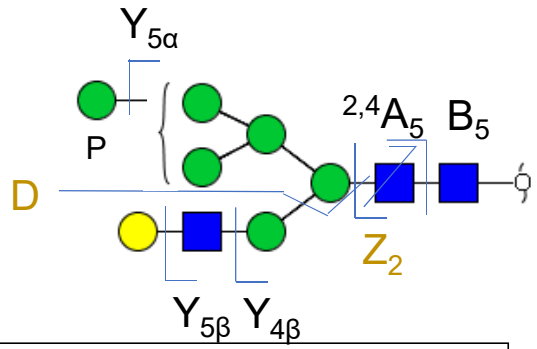


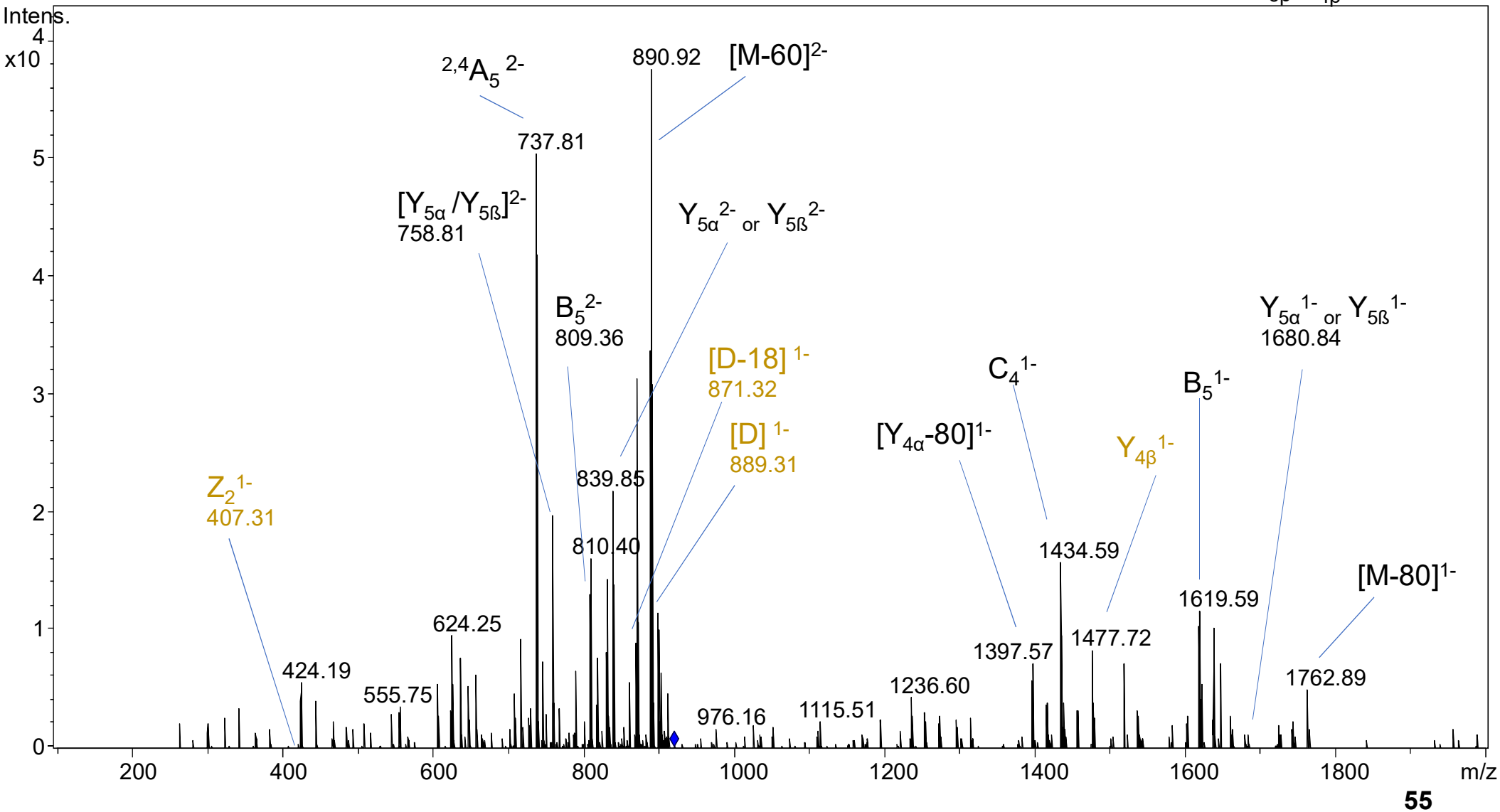


Glycan 45

A1G1-M6P
(H7N3P1)

Monoisotopic mass: 1843.60 Da
Charge observed: 2-
Theoretical ion: *m/z* 920.79
Observed ion: *m/z* 920.93
Mass deviation: *m/z* 0.14
Retention time: 41.4 min
Note: Isomer to glycan 15.

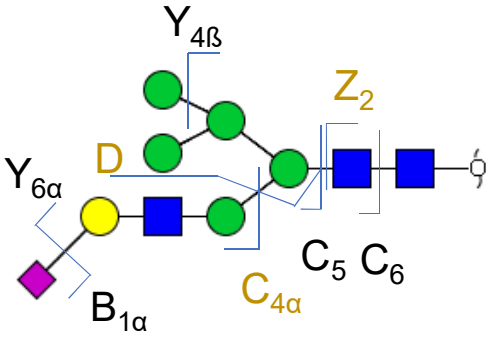


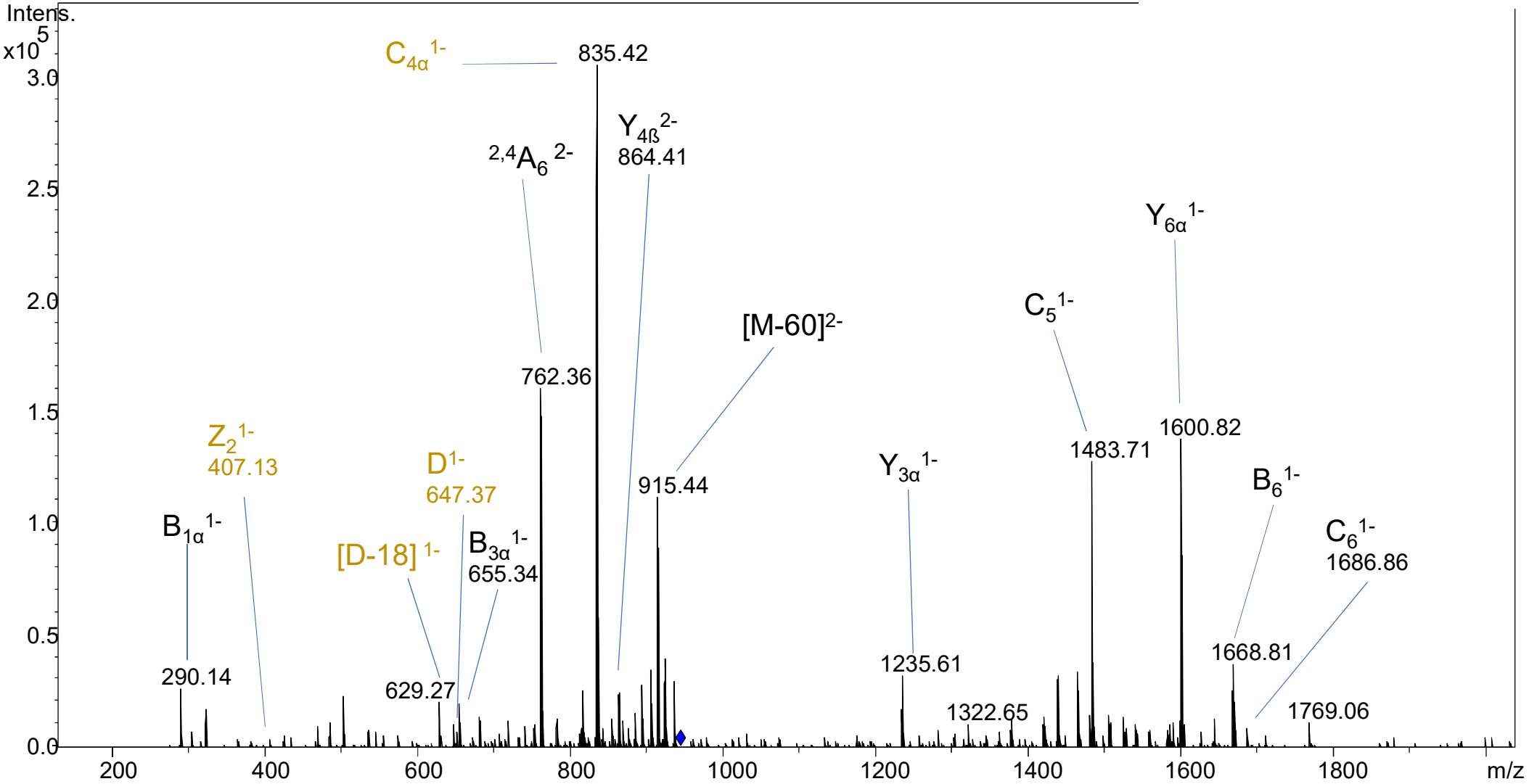


Glycan 46

A1S1-M5
(H6N3S1)

Monoisotopic mass:	**1892.68 Da**	**UniCarb-DB: #2523**
Charge observed:	**2-**	
Theoretical ion:	***m/z* 945.35**	
Observed ion:	***m/z* 945.48**	
Mass deviation:	***m/z* 0.01**	
Retention time:	**61.1 min**	

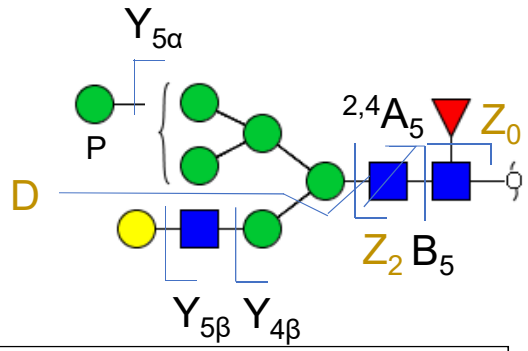


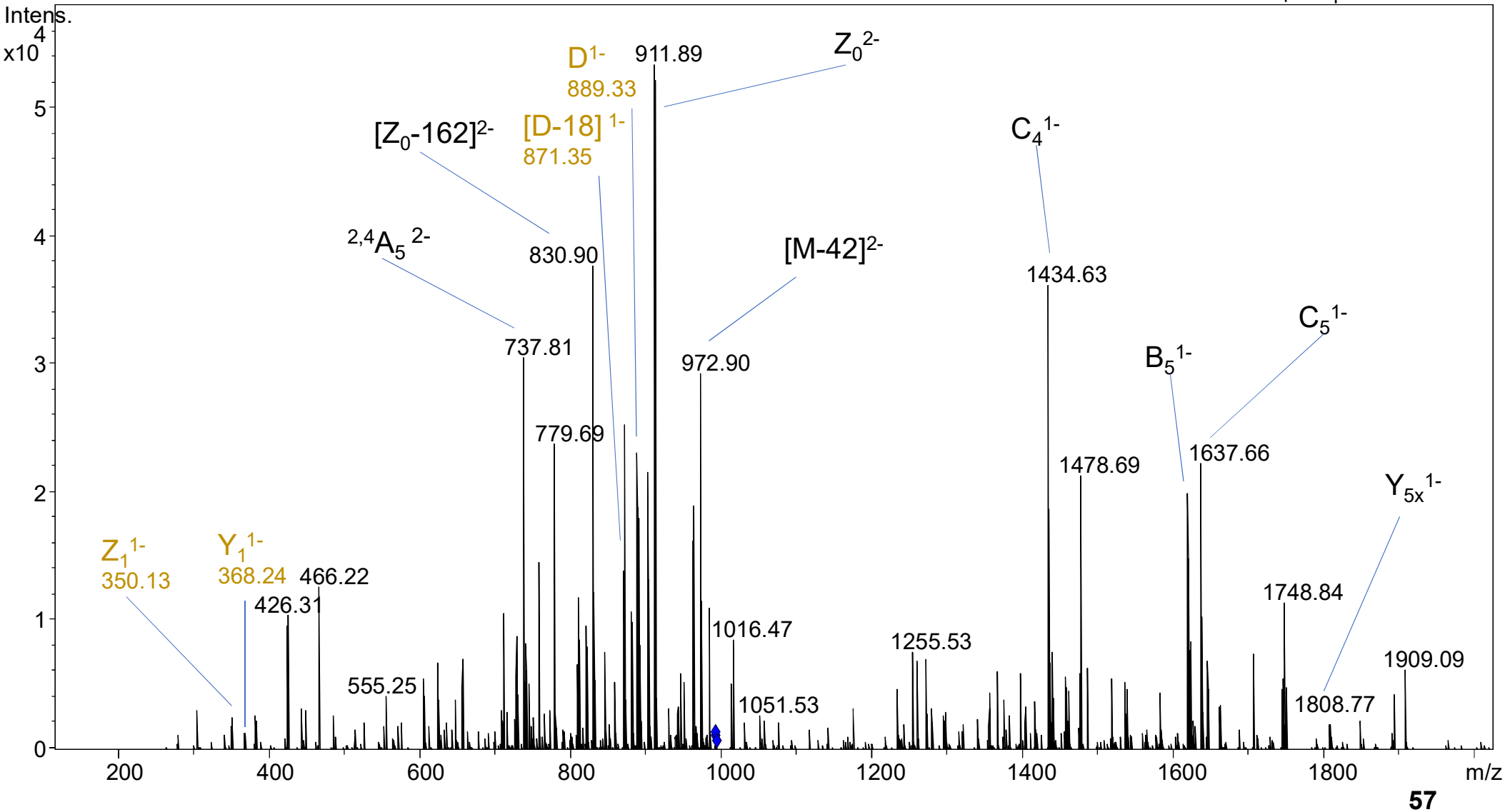


Glycan 47

A1G1F-M6P
(H7N3F1P1)

Monoisotopic mass:	**1989.65 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 993.82**
Observed ion:	***m/z* 993.96**
Mass deviation:	***m/z* 0.14**
Retention time:	**48.7 min**

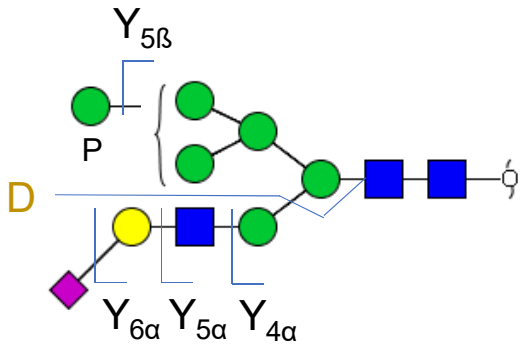


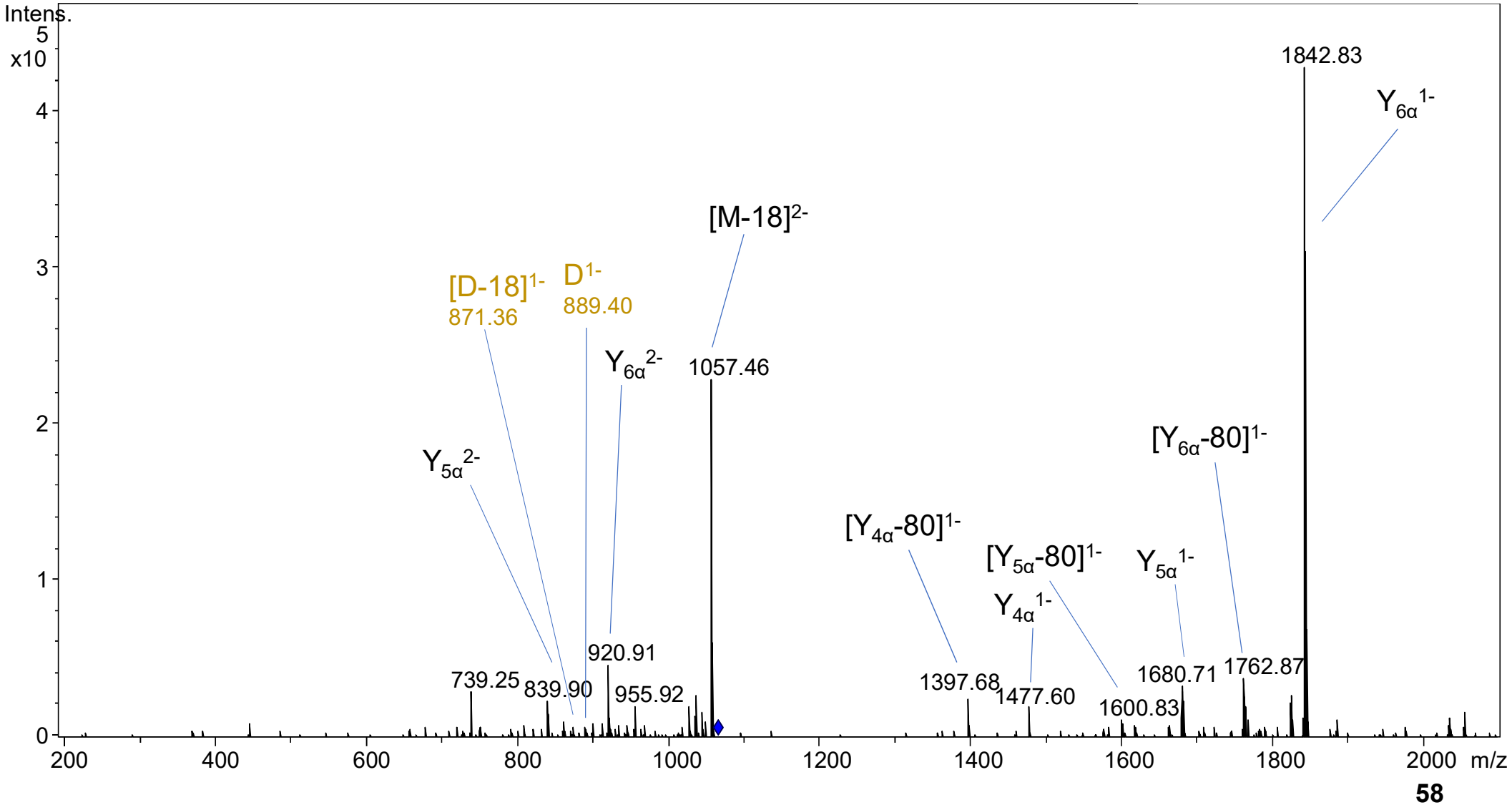


Glycan 48

A1S1-M6P
(H7N3S1P1)

Monoisotopic mass:	**2134.69 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 1066.34**
Observed ion:	***m/z* 1066.48**
Mass deviation:	***m/z* 0.14**
Retention time:	**51.0 min**

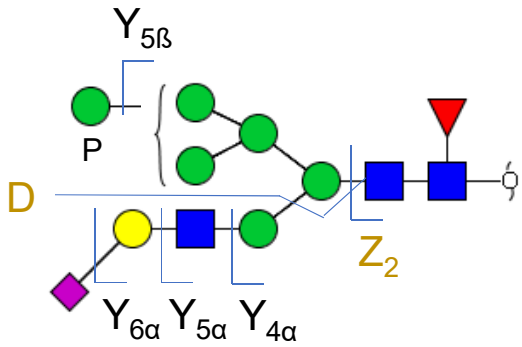


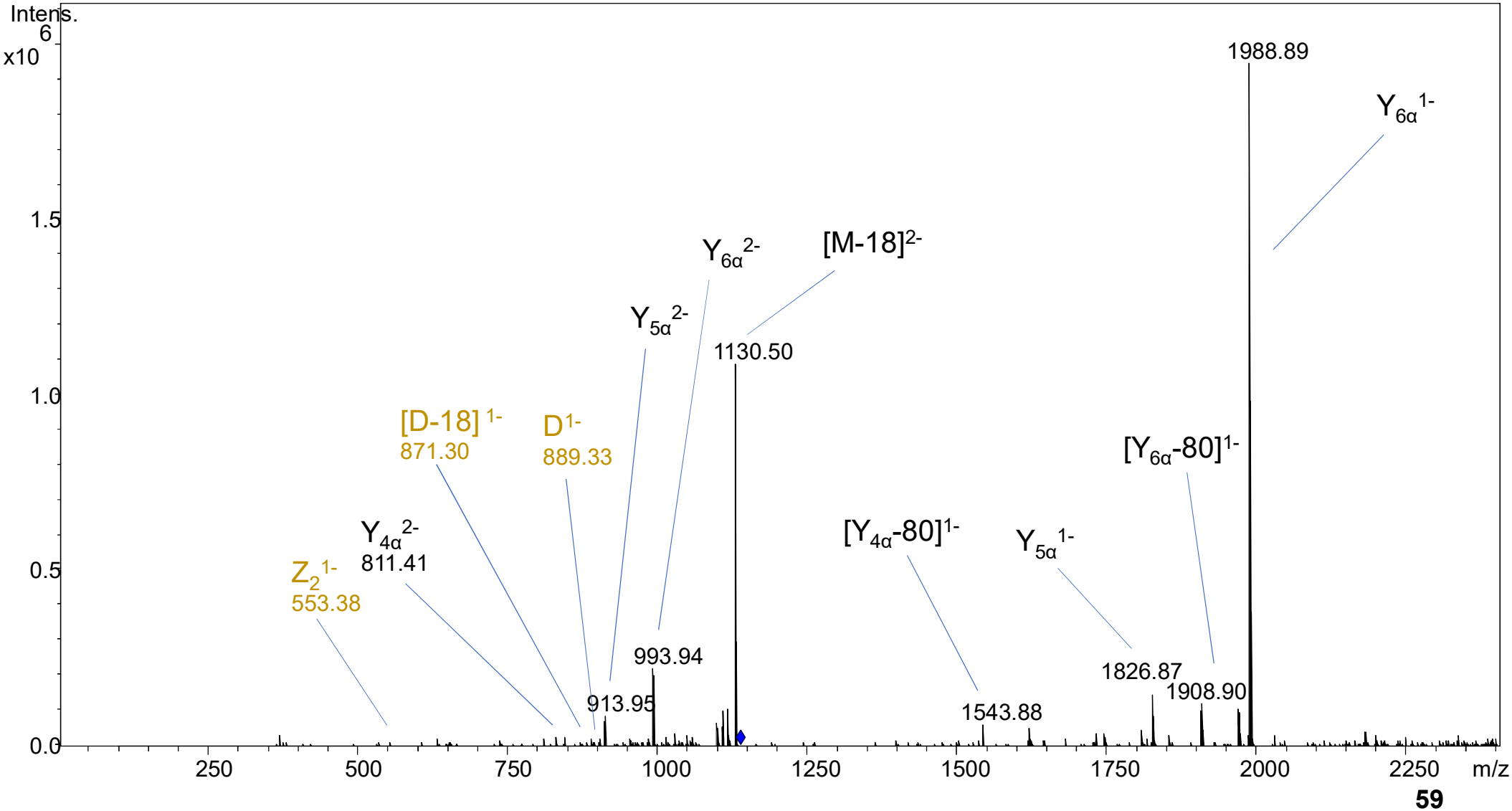


Glycan 49

A1S1F-M6P (H7N3F1S1P1)

Monoisotopic mass:	**2280.75 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 1139.37**
Observed ion:	***m/z* 1139.51**
Mass deviation:	***m/z* 0.13**
Retention time:	**56.5 min**

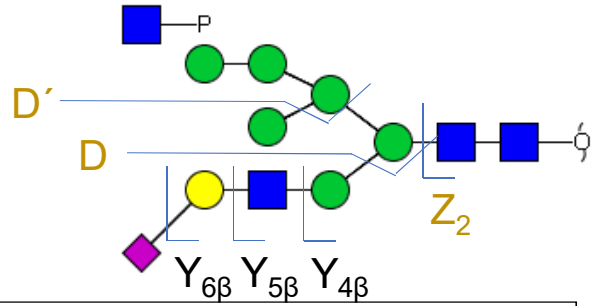


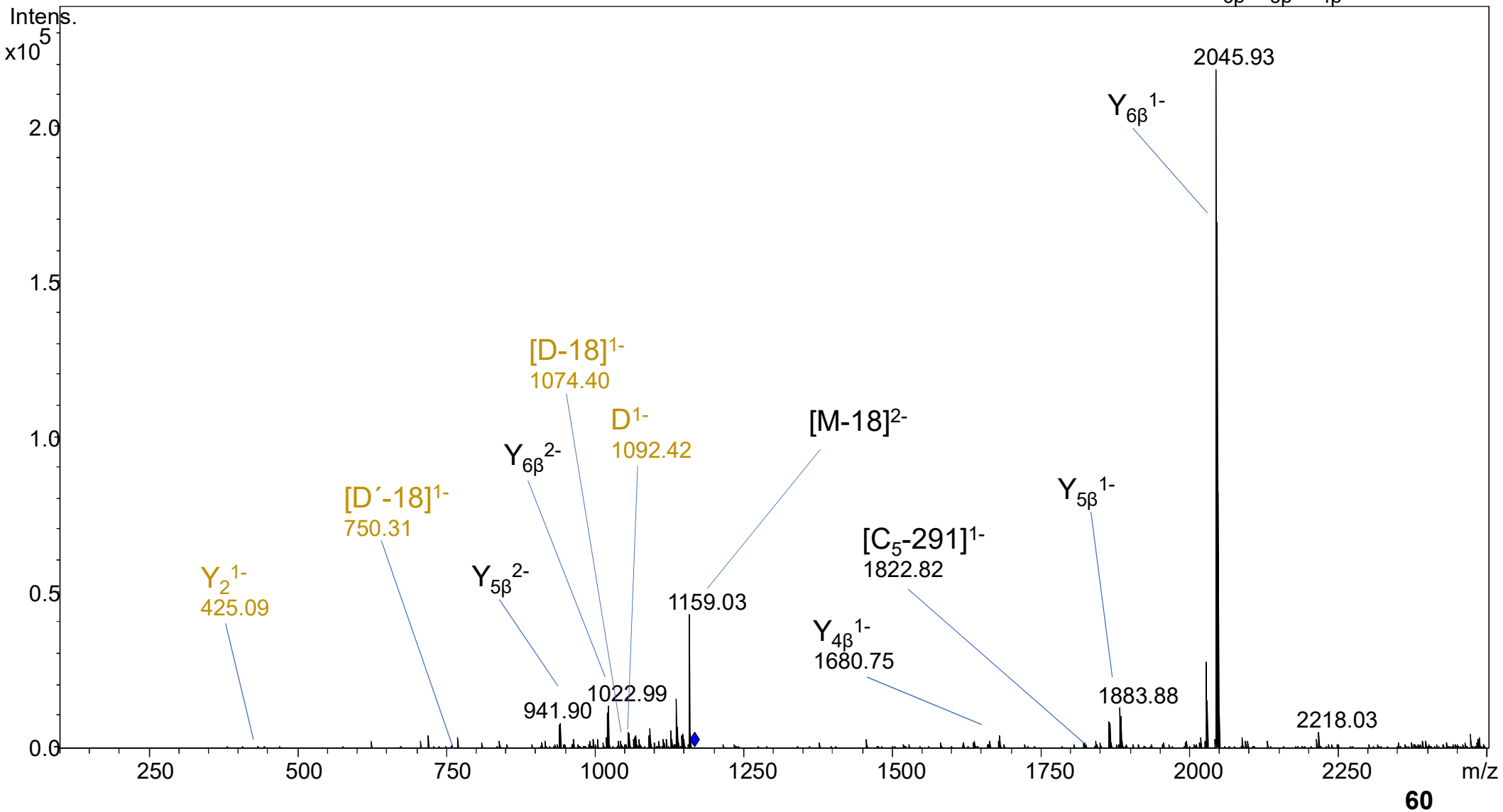


Glycan 50

A1S1-M6PGlcNAc
(H7N4S1P1)

Monoisotopic mass:	**2337.77 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 1167.88**
Observed ion:	***m/z* 1168.00**
Mass deviation:	***m/z* 0.12**
Retention time:	**53.7 min**





Glycan 51

A1S1F-M6PGlcNAc
(H7N4F1S1P1)

Monoisotopic mass:	**2483.83 Da**
Charge observed:	**2-**
Theoretical ion:	***m/z* 1240.90**
Observed ion:	***m/z* 1241.08**
Mass deviation:	***m/z* 0.18**
Retention time:	**58.7 min**