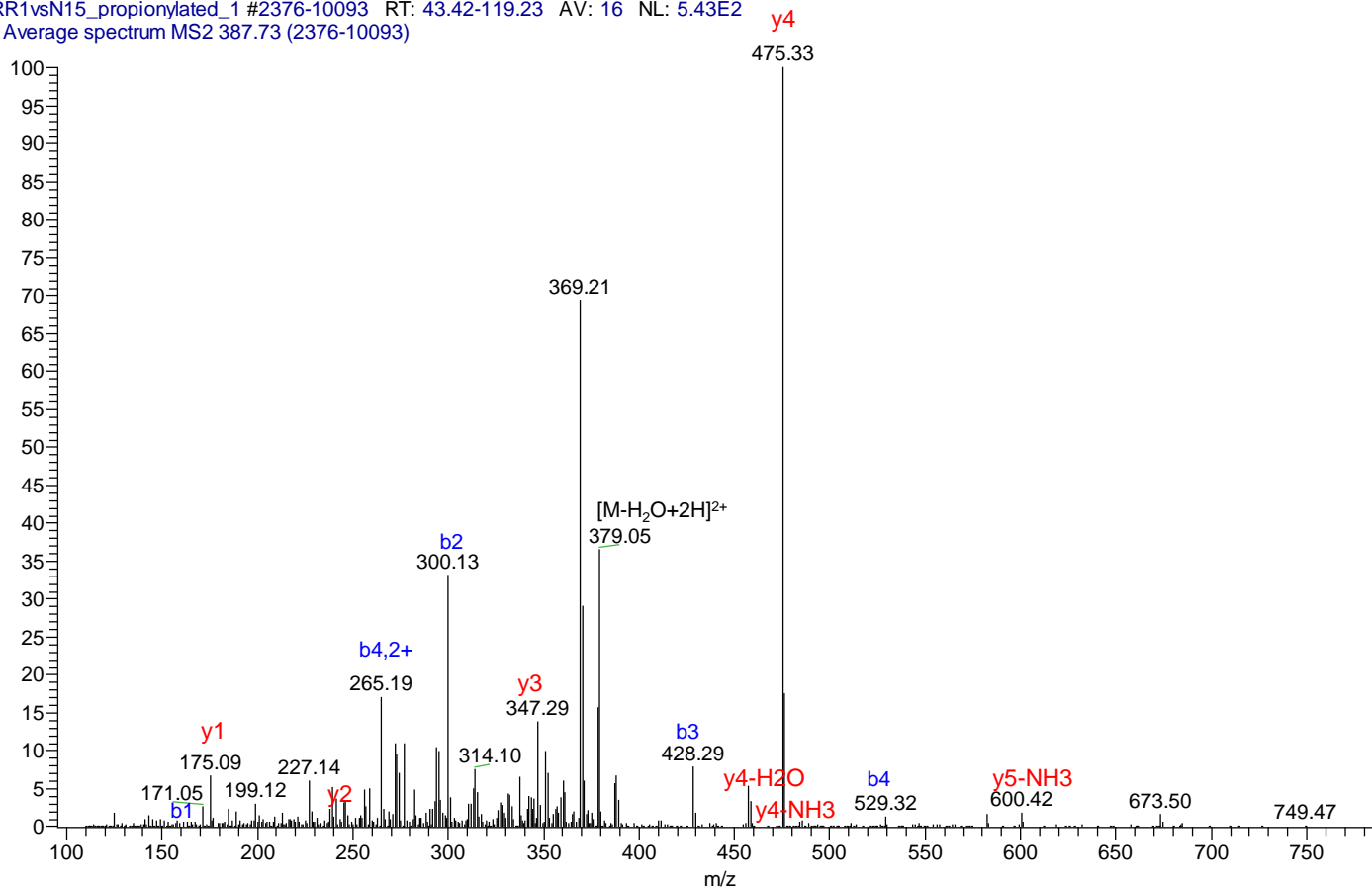


# Figure S1. MS/MS spectra of peptides with PTMs

## H3K4Me1 MS/MS of ${}^3\text{TKQTA R}_8$

TRR1vsN15\_propionylated\_1 #2376-10093 RT: 43.42-119.23 AV: 16 NL: 5.43E2  
T: Average spectrum MS2 387.73 (2376-10093)

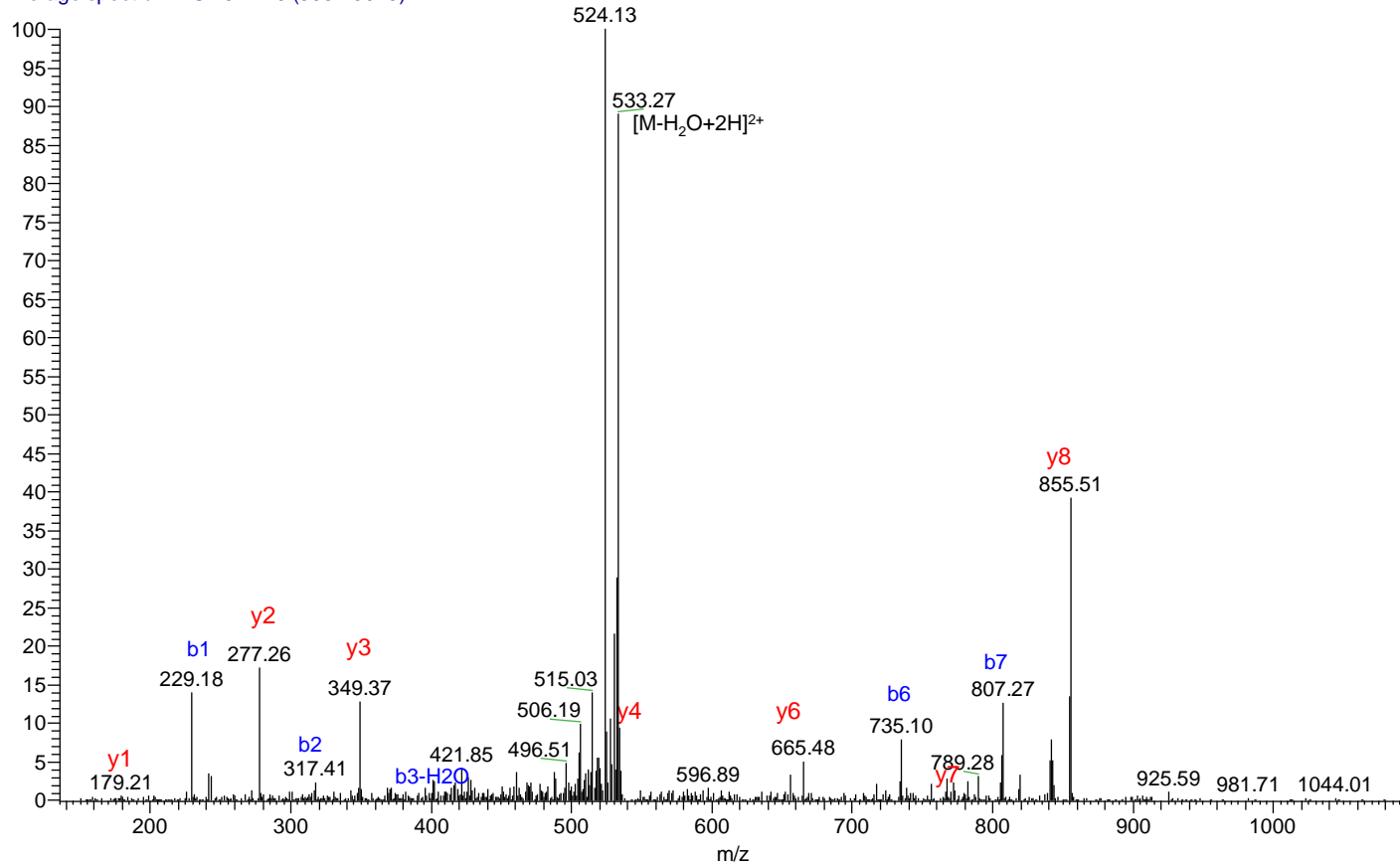


M/Z: 387.7275, Charge: 2+, Mass error: 1ppm, Ions Score: 37; Expect: 0.22

# H3K9Ac

## MS/MS of 9KSTGAKAPR<sub>17</sub>

WT8vsN15\_propionylated\_1 #3062-3073 RT: 53.60-53.73 AV: 3 NL: 1.53E3  
T: Average spectrum MS2 542.29 (3062-3073)

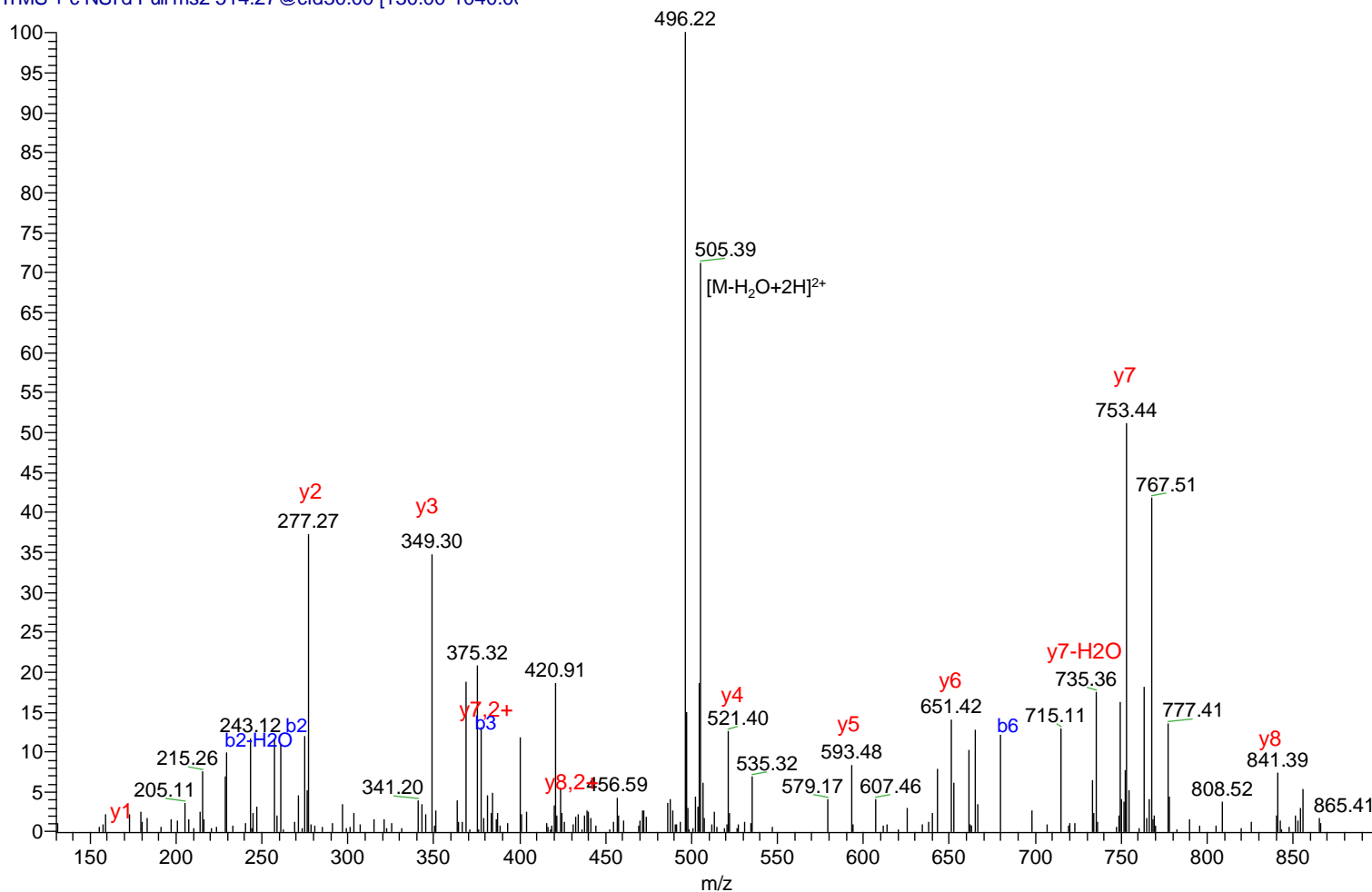


M/Z: 542.2835, Charge: 2+, Mass error: 1ppm, Ions Score: 29; Expect: 2.7

# H3K14Ac

## MS/MS of 9KSTGAKAPR<sub>17</sub>

WT7vsN15\_propionylated\_1 #2412 RT: 45.01 AV: 1 NL: 7.60E2  
T: ITMS + c NSI d Full ms2 514.27@cid30.00 [130.00-1040.0]

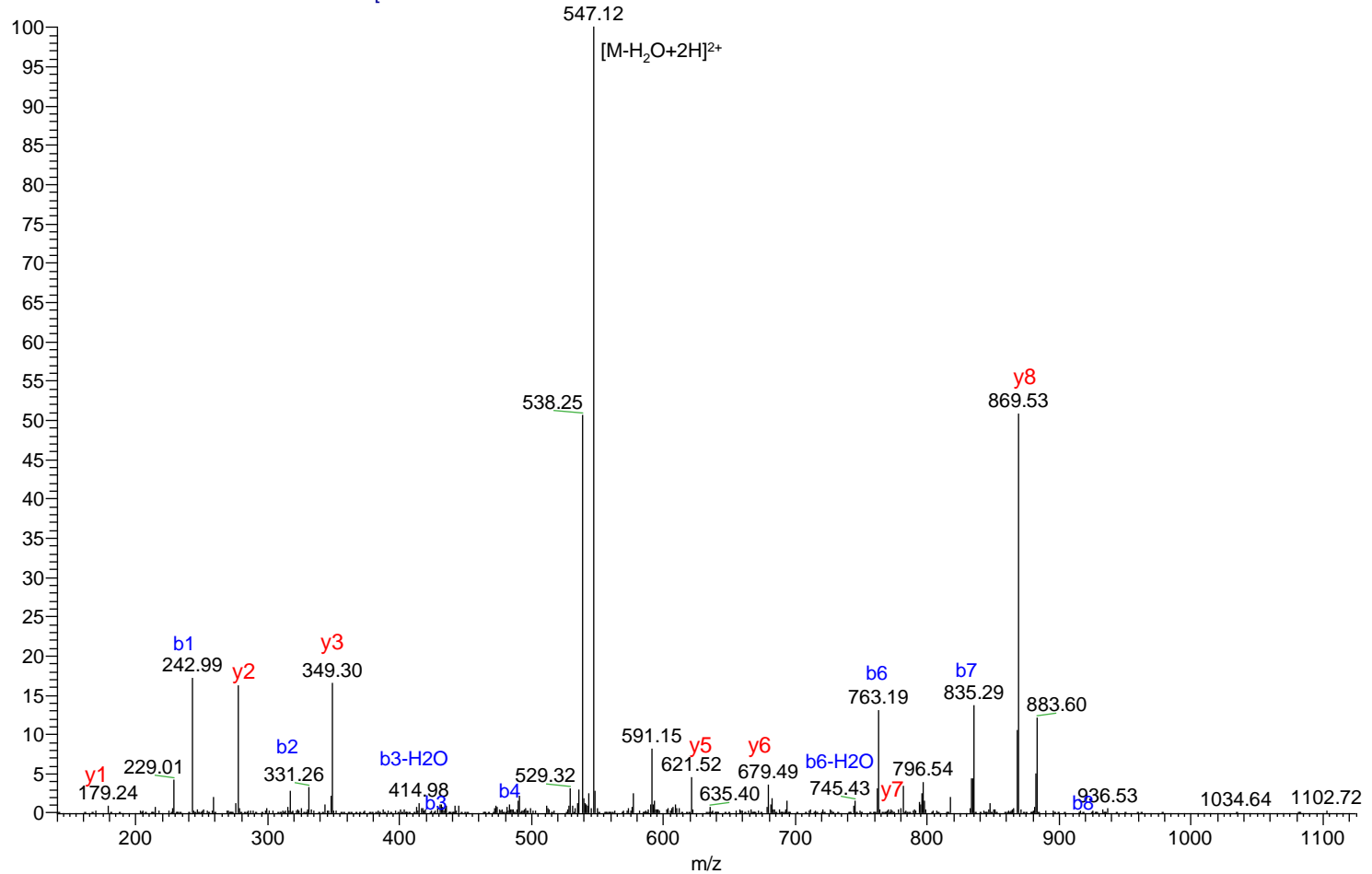


M/Z: 514.2699, Charge: 2+, Mass error: 0ppm, Ions Score: 63; Expect: 0.0014

# H3K14Me1

## MS/MS of 9KSTGAKAPR<sub>17</sub>

TRR1avsN15\_propionylated\_2 #4059 RT: 64.50 AV: 1 NL: 3.50E4  
T: ITMS + c NSI d Full ms2 556.30@cid30.00 [140.00-1125.00]

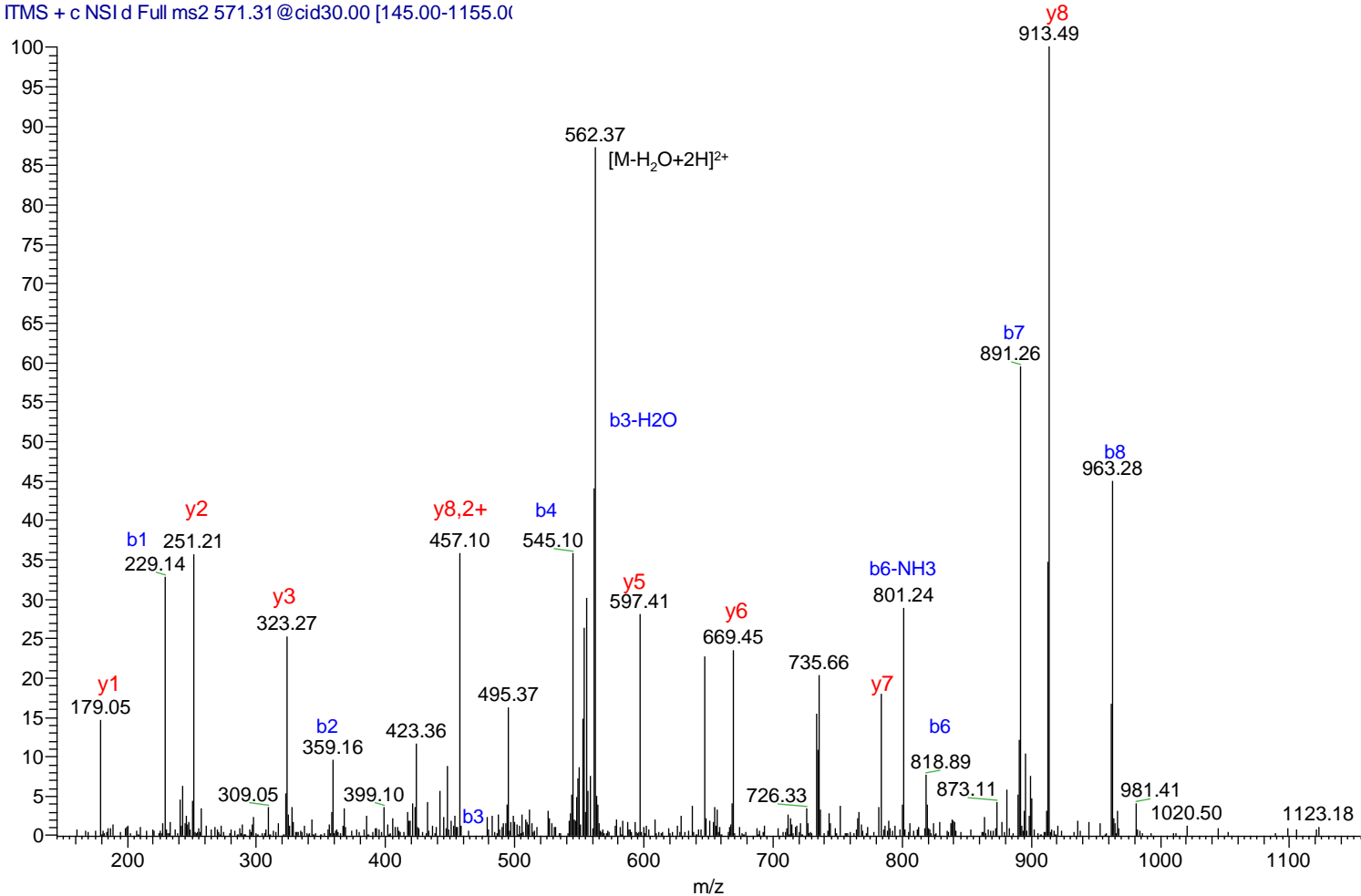


M/Z: 556.2968, Charge: 2+, Mass error: -3ppm, Ions Score: 42; Expect: 0.14

# H3K18Ac

## MS/MS of 18KQLASKAAR<sub>26</sub>

TRR1avsN15\_propionylated\_2 #5382 RT: 76.66 AV: 1 NL: 4.71E3  
T: ITMS + c NSI d Full ms2 571.31 @cid30.00 [145.00-1155.0]

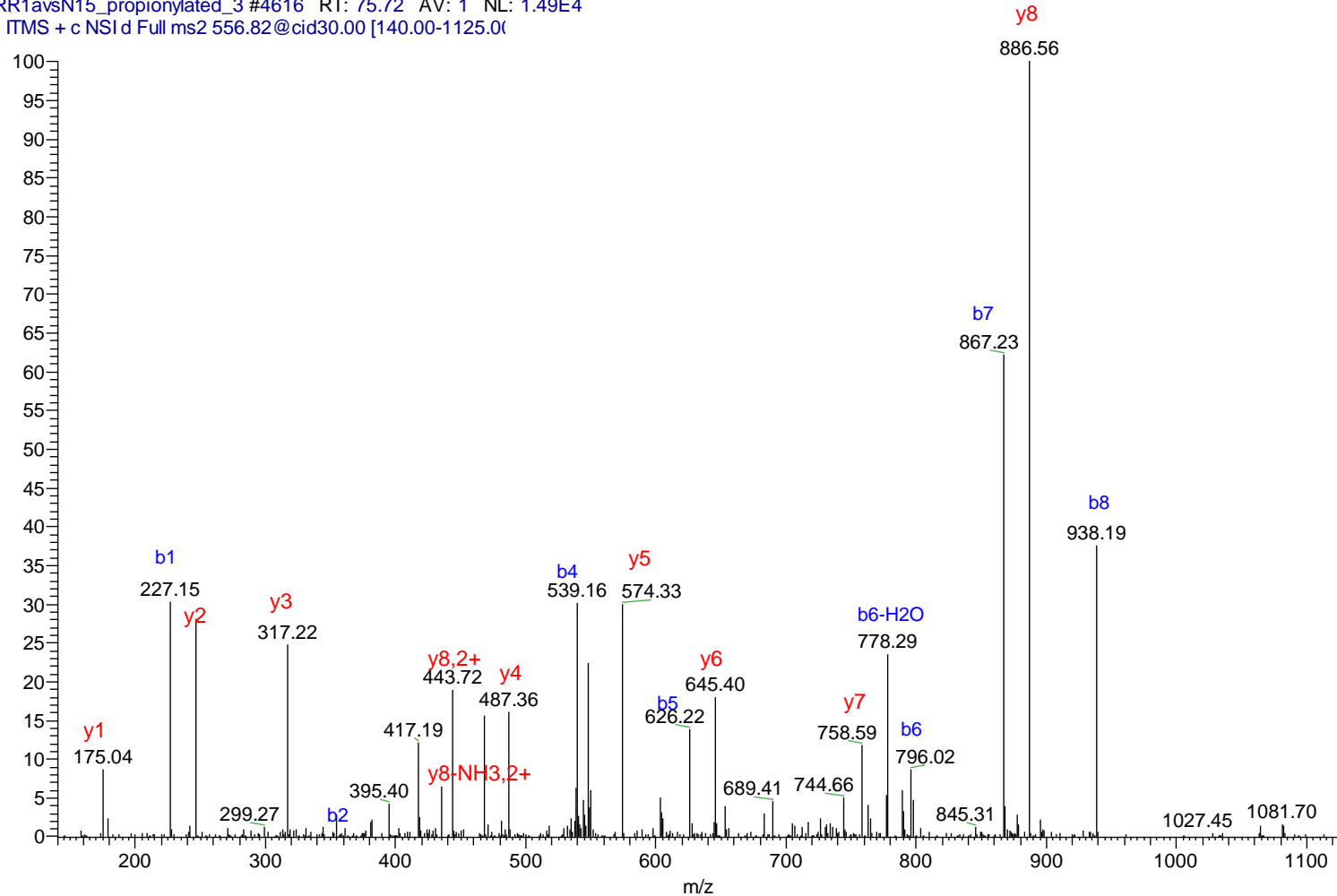


M/Z: 571.3108, Charge: 2+, Mass error: 1ppm, Ions Score: 47; Expect: 0.085

# H3K18AcK23Ac

## MS/MS of 18KQLASKAAR<sub>26</sub>

TRR1avsN15\_propionylated\_3 #4616 RT: 75.72 AV: 1 NL: 1.49E4  
T: ITMS + c NSI d Full ms2 556.82@cid30.00 [140.00-1125.00]

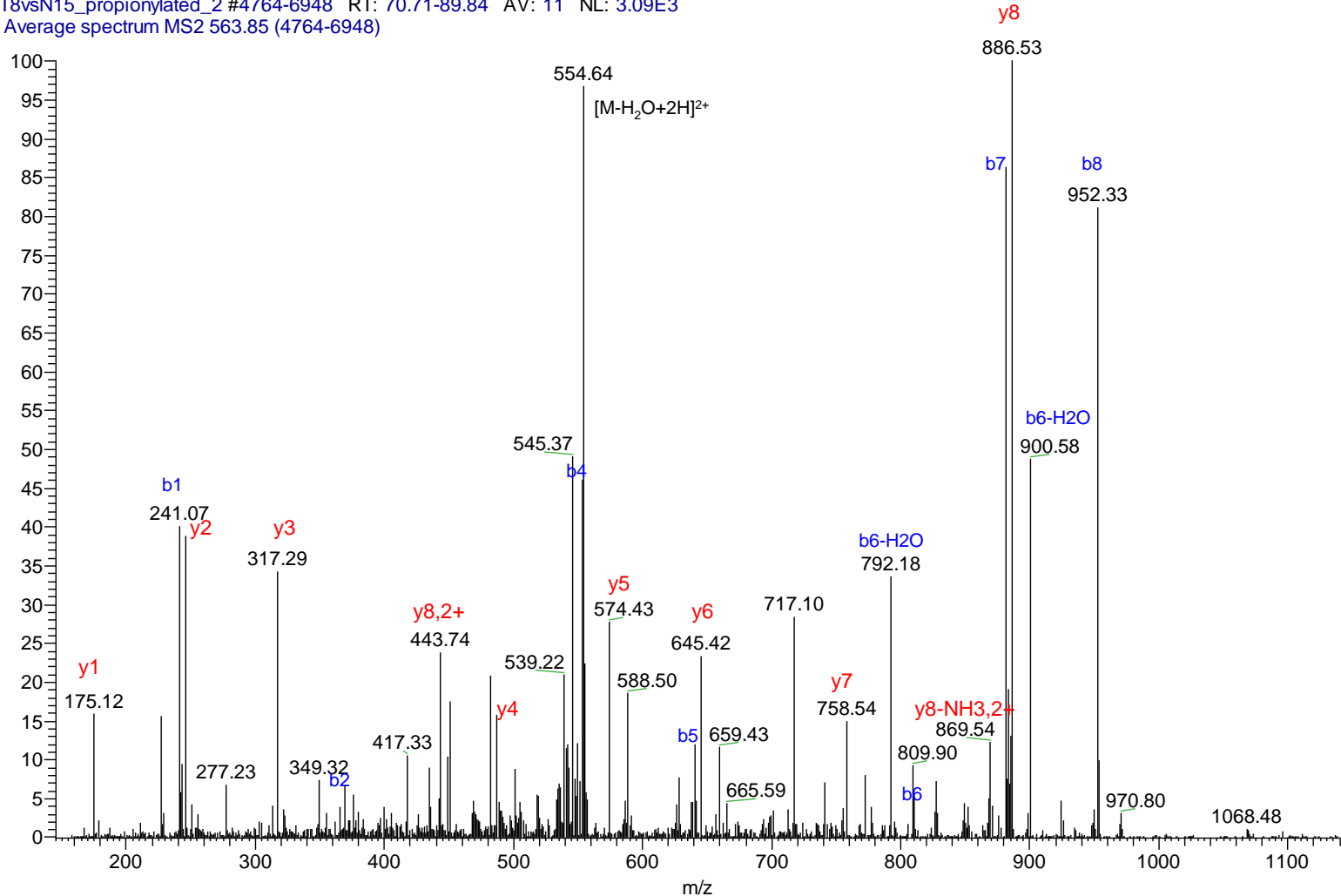


M/Z: 556.8246, Charge: 2+, Mass error: 0ppm, Ions Score: 56; Expect: 0.0039

# H3K23Ac

## MS/MS of 18KQLASKAAR<sub>26</sub>

WT8vsN15\_propionylated\_2 #4764-6948 RT: 70.71-89.84 AV: 11 NL: 3.09E3  
T: Average spectrum MS2 563.85 (4764-6948)

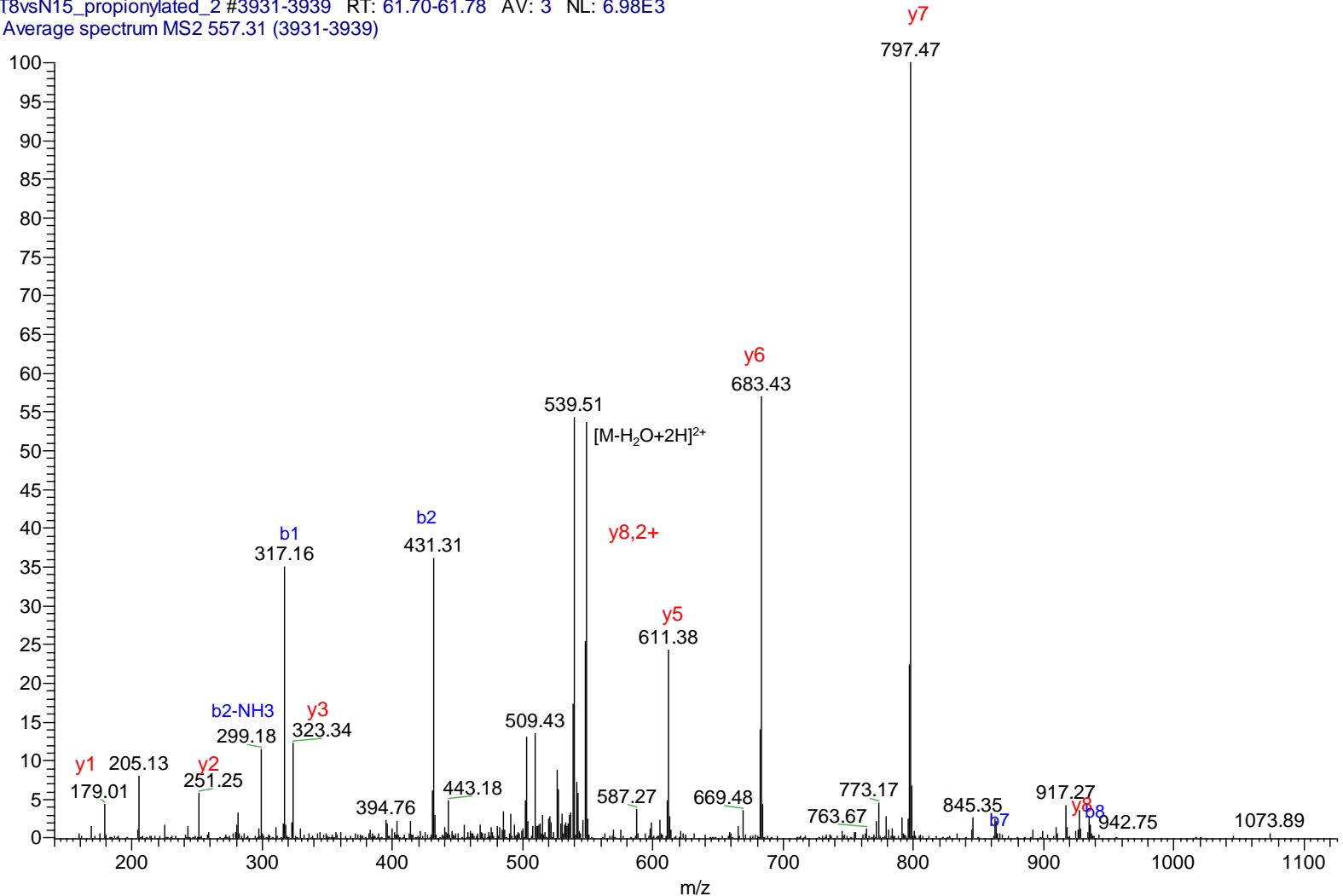


M/Z: 563.8319, Charge: 2+, Mass error: -1ppm, Ions Score: 57; Expect: 0.0039

# H3K23Me1

## MS/MS of 18KQLASKAAR<sub>26</sub>

WT8vsN15\_propionylated\_2 #3931-3939 RT: 61.70-61.78 AV: 3 NL: 6.98E3  
T: Average spectrum MS2 557.31 (3931-3939)



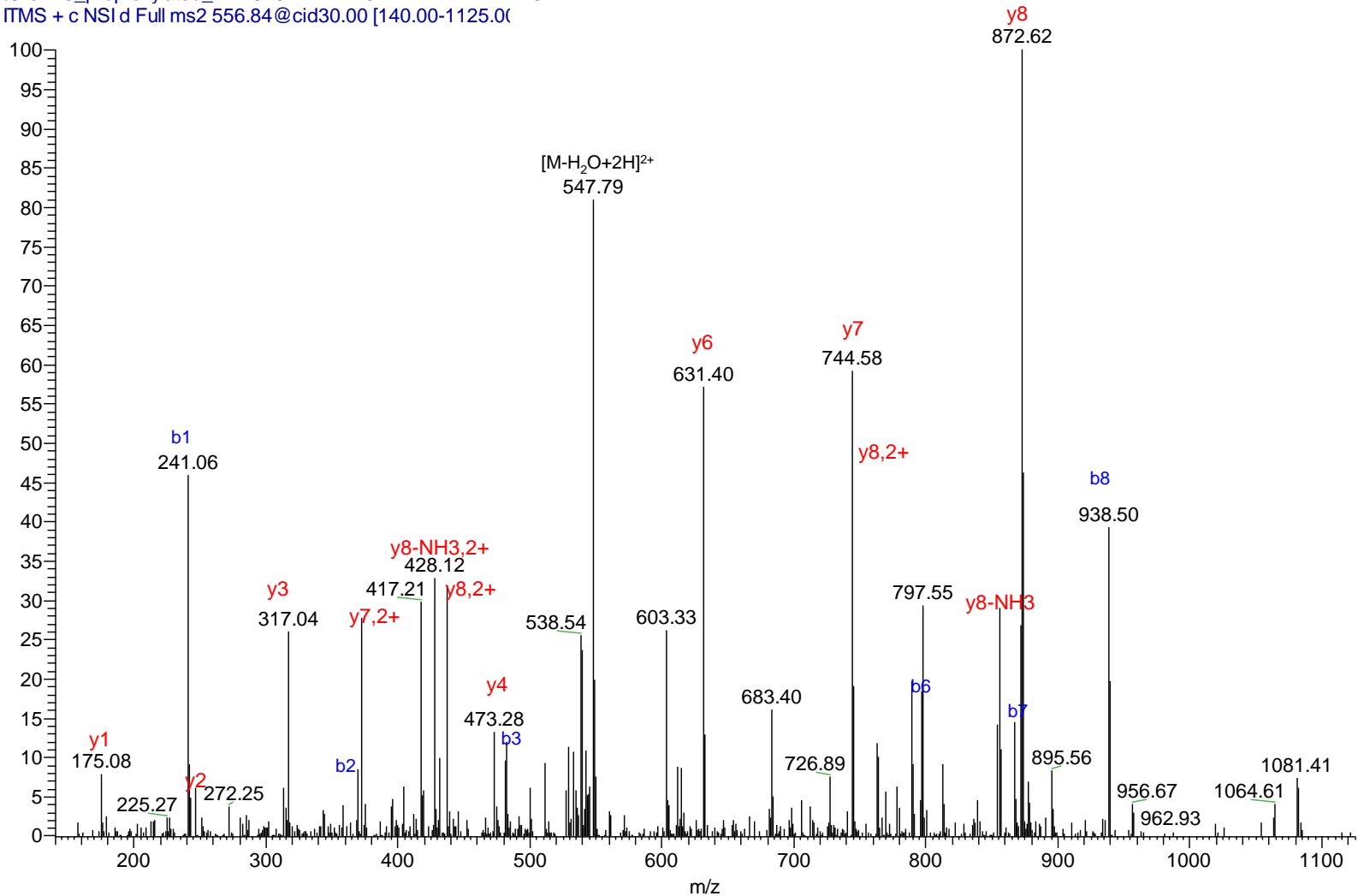
M/Z: 557.3122, Charge: 2+, Mass error: -1ppm, Ions Score: 53; Expect: 0.023



# H3K23Me2

## MS/MS of 18KQLASKAAR<sub>26</sub>

WT8vsN15\_propionylated\_2 #4826 RT: 71.32 AV: 1 NL: 1.73E4  
T: ITMS + c NSI d Full ms2 556.84@cid30.00 [140.00-1125.00]

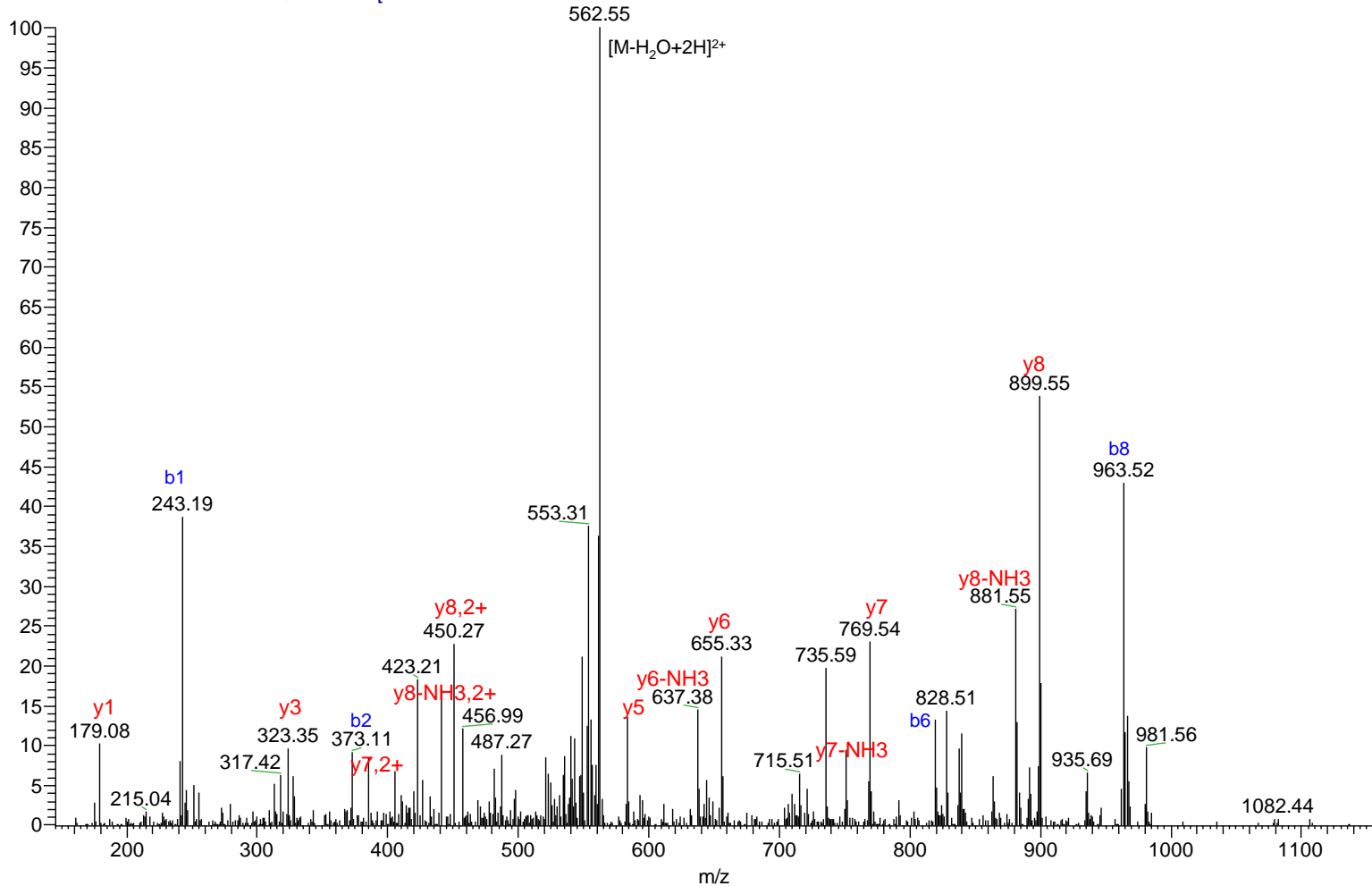


M/Z: 556.8409, Charge: 2+, Mass error: -4ppm, Ions Score: 28; Expect: 4.8

# H3K23Me3

## MS/MS of 18KQLASKAAR<sub>26</sub>

WT7vsN15\_propionylated\_1 #4777 RT: 69.90 AV: 1 NL: 5.75E3  
T: ITMS + c NSI d Full ms2 571.33@cid30.00 [145.00-1155.00]

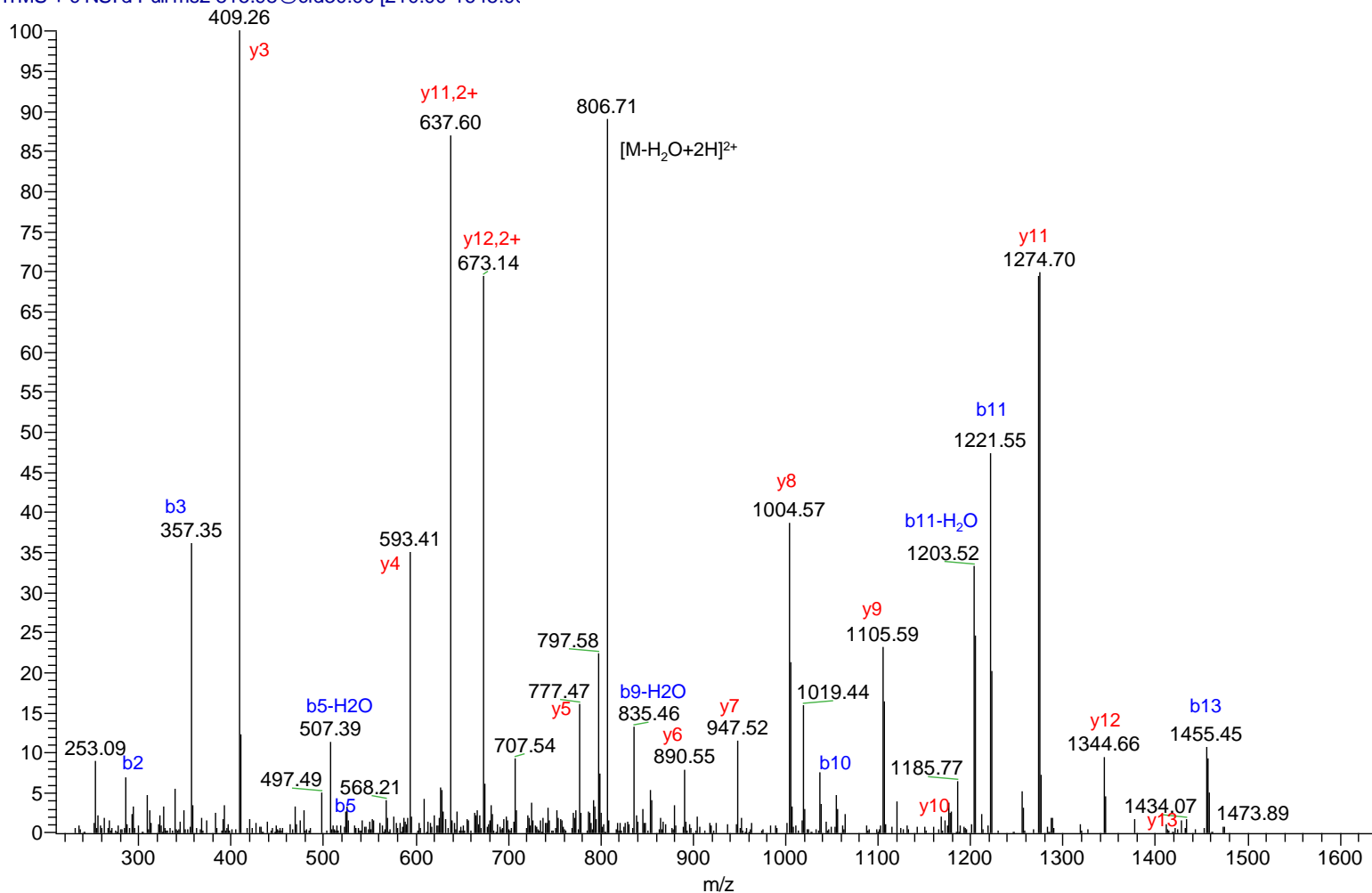


M/Z: 571.3286, Charge: 2+, Mass error: 0ppm, Ions Score: 34; Expect: 2.1

# H3K27Me1

## MS/MS of ${}_{27}KSAPATGGIKKPHR_{40}$

WT7vsN15\_propionylated\_1 #4853 RT: 70.56 AV: 1 NL: 5.22E3  
T: ITMS + c NSI d Full ms2 815.98@cid30.00 [210.00-1645.0]



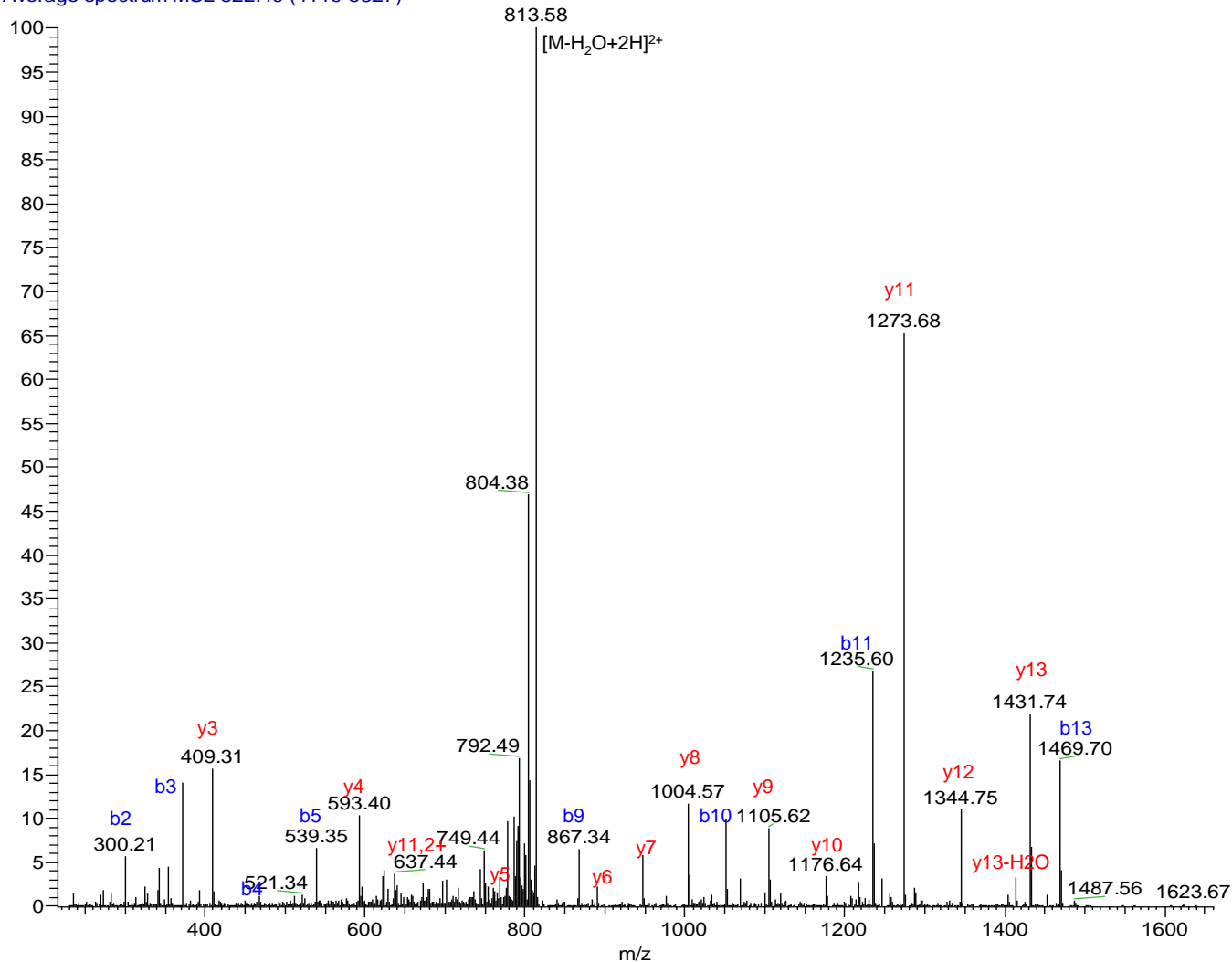
M/Z: 815.476, Charge: 2+, Mass error: 1ppm, Ions Score: 83; Expect: 4.9e-05

# H3K27Me2

## MS/MS of ${}_{27}KSAPATGGIKKPHR_{40}$

WTvsN15\_propionylated\_1 #4119-5827 RT: 62.78-78.37 AV: 19 NL: 6.52E2

T: Average spectrum MS2 822.49 (4119-5827)

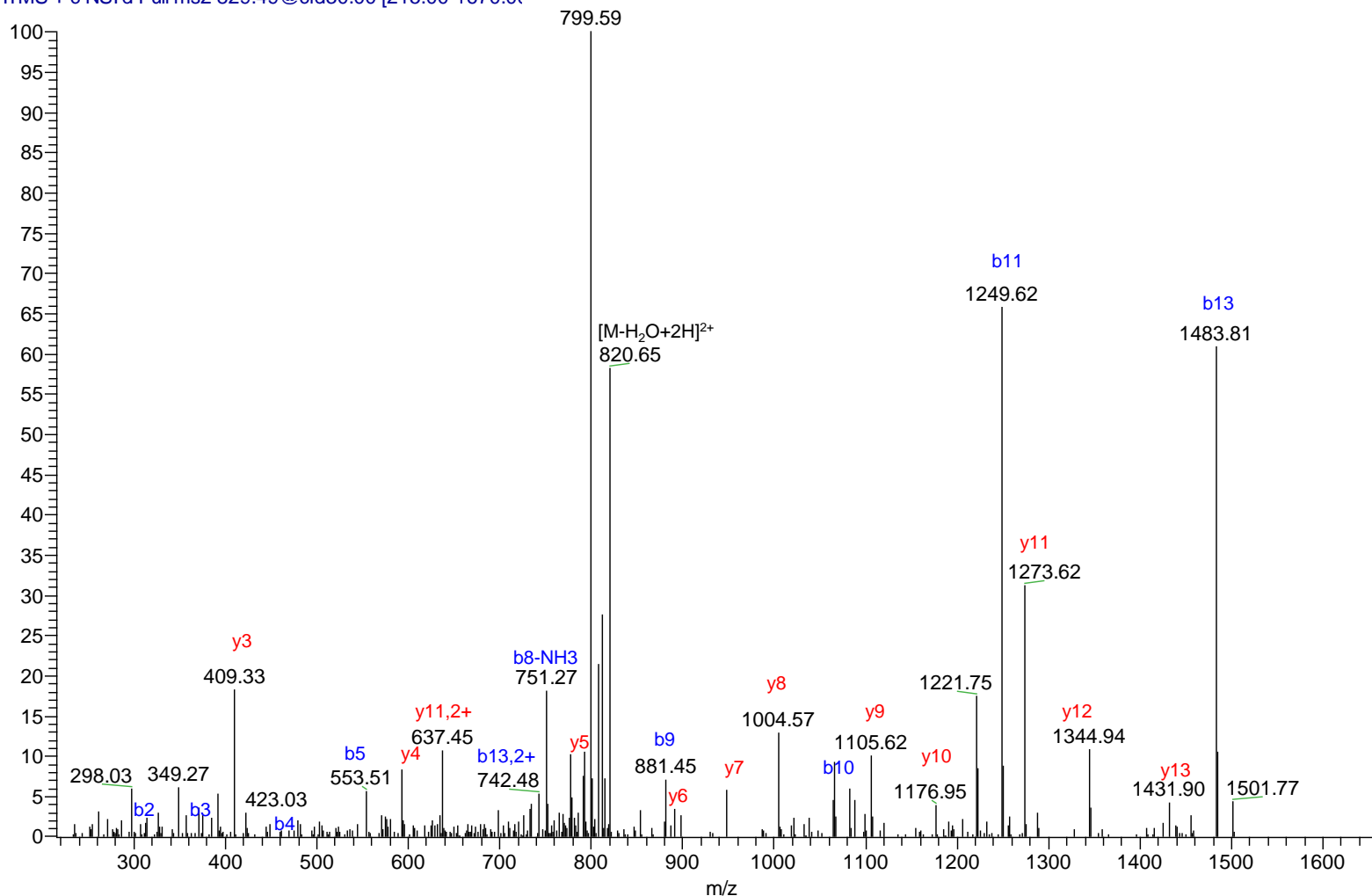


M/Z: 822.4858, Charge: 2+, Mass error: 3ppm, Ions Score: 96; Expect: 2.1e-06

# H3K27Me3

## MS/MS of $_{27}KSAPATGGIKKPHR_{40}$

WTvsN15\_propionylated\_1 #4253 RT: 64.09 AV: 1 NL: 1.99E3  
T: ITMS + c NSI d Full ms2 829.49@cid30.00 [215.00-1670.00]

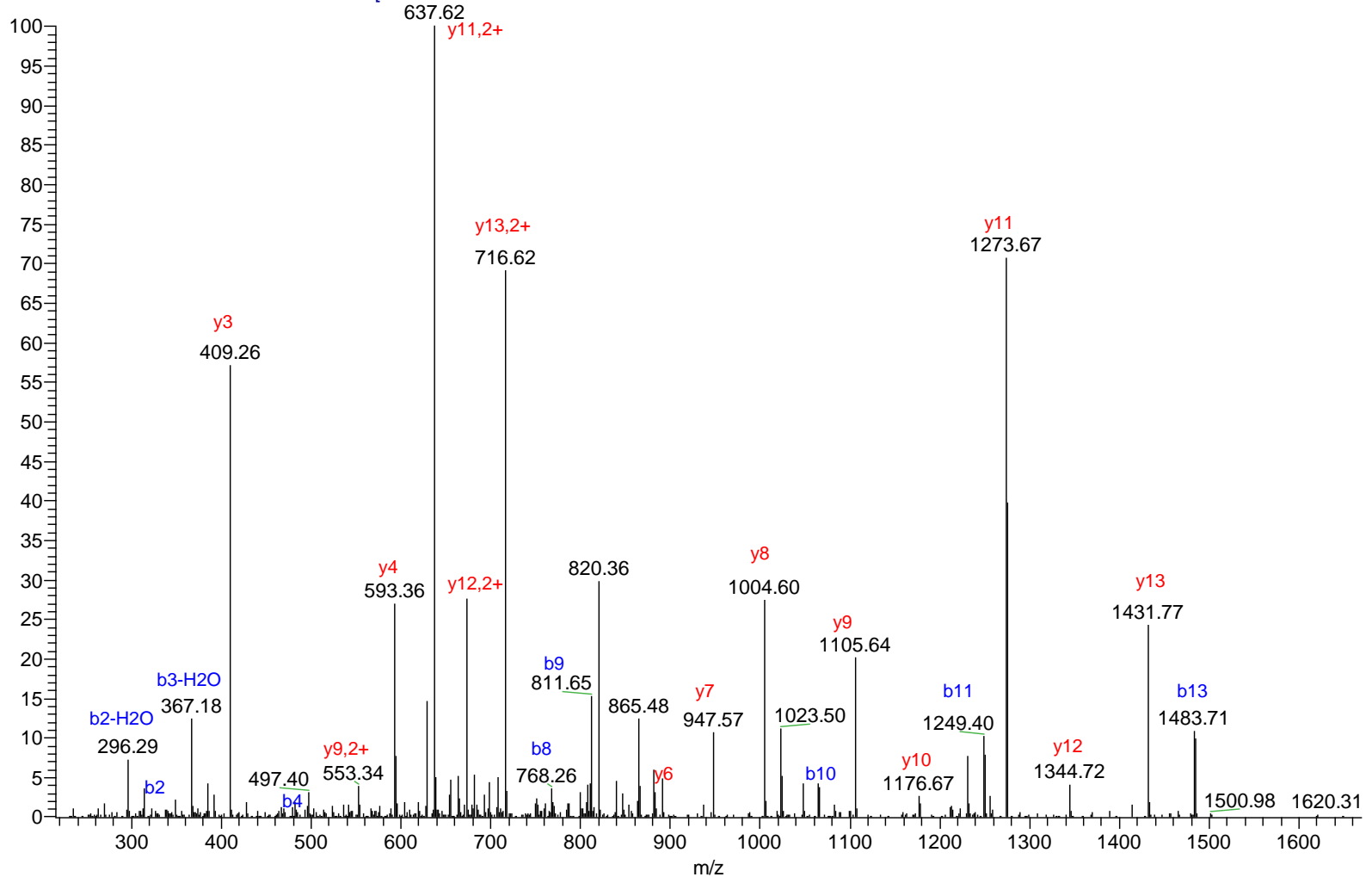


M/Z: 829.4924, Charge: 2+, Mass error: 2ppm, Ions Score: 62; Expect: 0.005

# H3K27Ac

## MS/MS of $_{27}KSAPATGGIKKPHR_{40}$

WTvsN15\_propionylated\_1 #4788 RT: 69.21 AV: 1 NL: 2.69E4  
T: ITMS + c NSI d Full ms2 829.97@cid30.00 [215.00-1670.00]

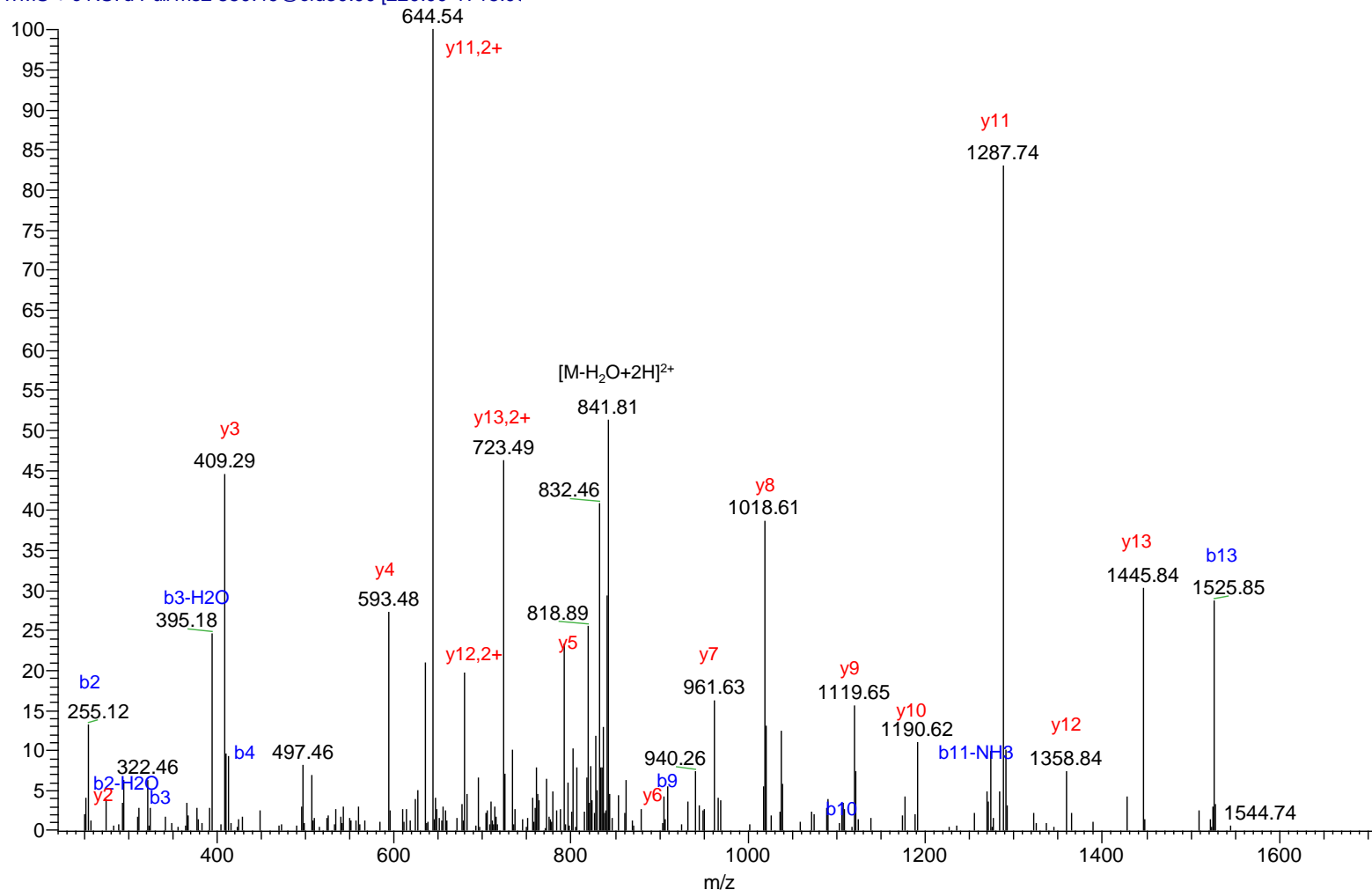


M/Z: 829.474, Charge: 2+, Mass error: 1ppm, Ions Score: 74; Expect:3.8e-04

# H3K27Me1K36Me1

## MS/MS of ${}_{27}KSAPATGGIKKPHR_{40}$

WT8vsN15\_propionylated\_4 #4852 RT: 77.90 AV: 1 NL: 9.57E2  
T: ITMS + c NSI d Full ms2 850.49@cid30.00 [220.00-1715.00]

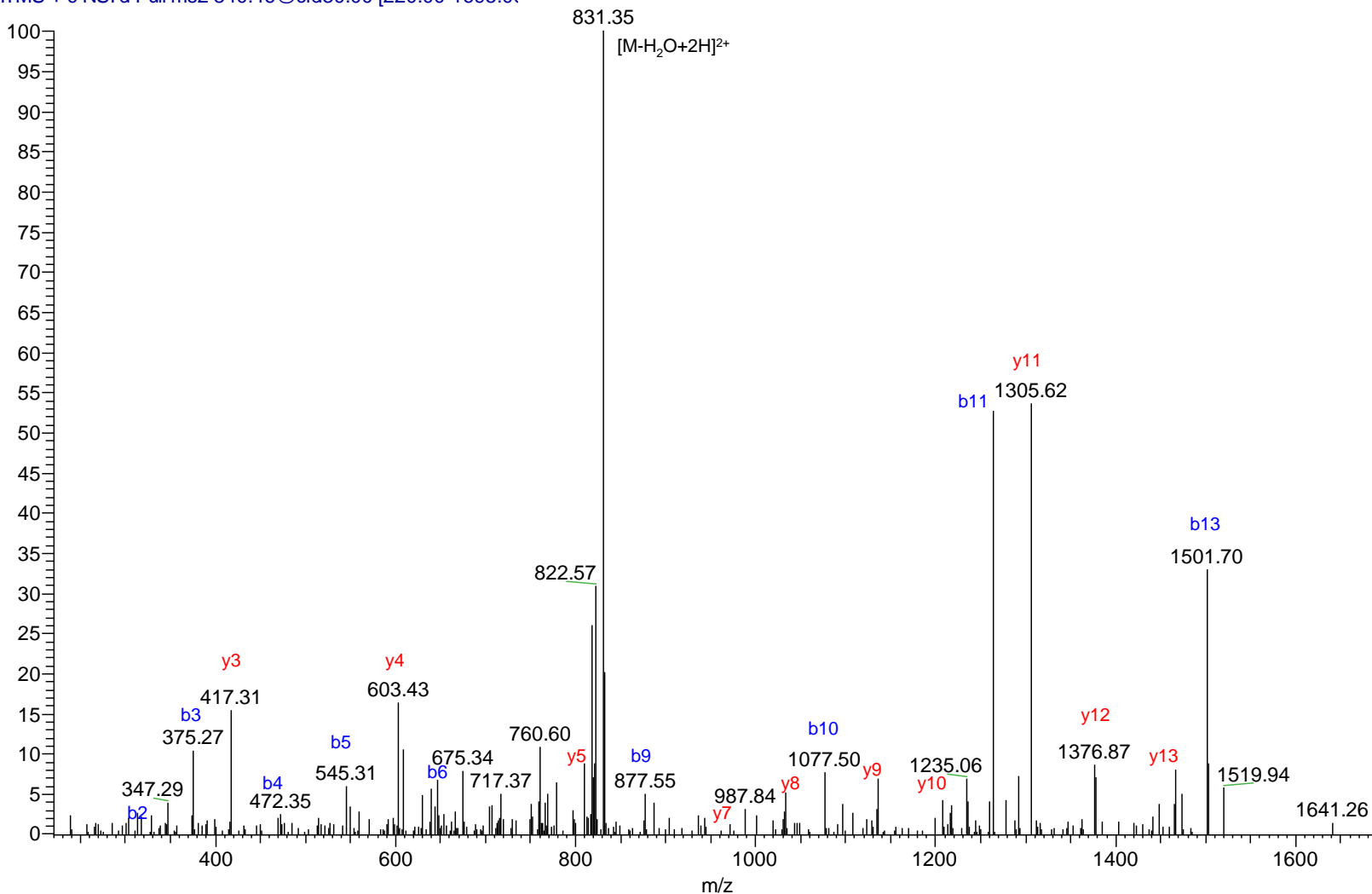


M/Z: 850.4947, Charge: 2+, Mass error: -2ppm, Ions Score: 86; Expect: 2.1e-05

# H3K27Me2K36Me1

## MS/MS of $_{27}$ KSAPATGGIKKPHR $_{40}$

TRR1avsN15\_propionylated\_3 #3747 RT: 68.03 AV: 1 NL: 8.78E2  
T: ITMS + c NSI d Full ms2 840.46@cid30.00 [220.00-1695.0]



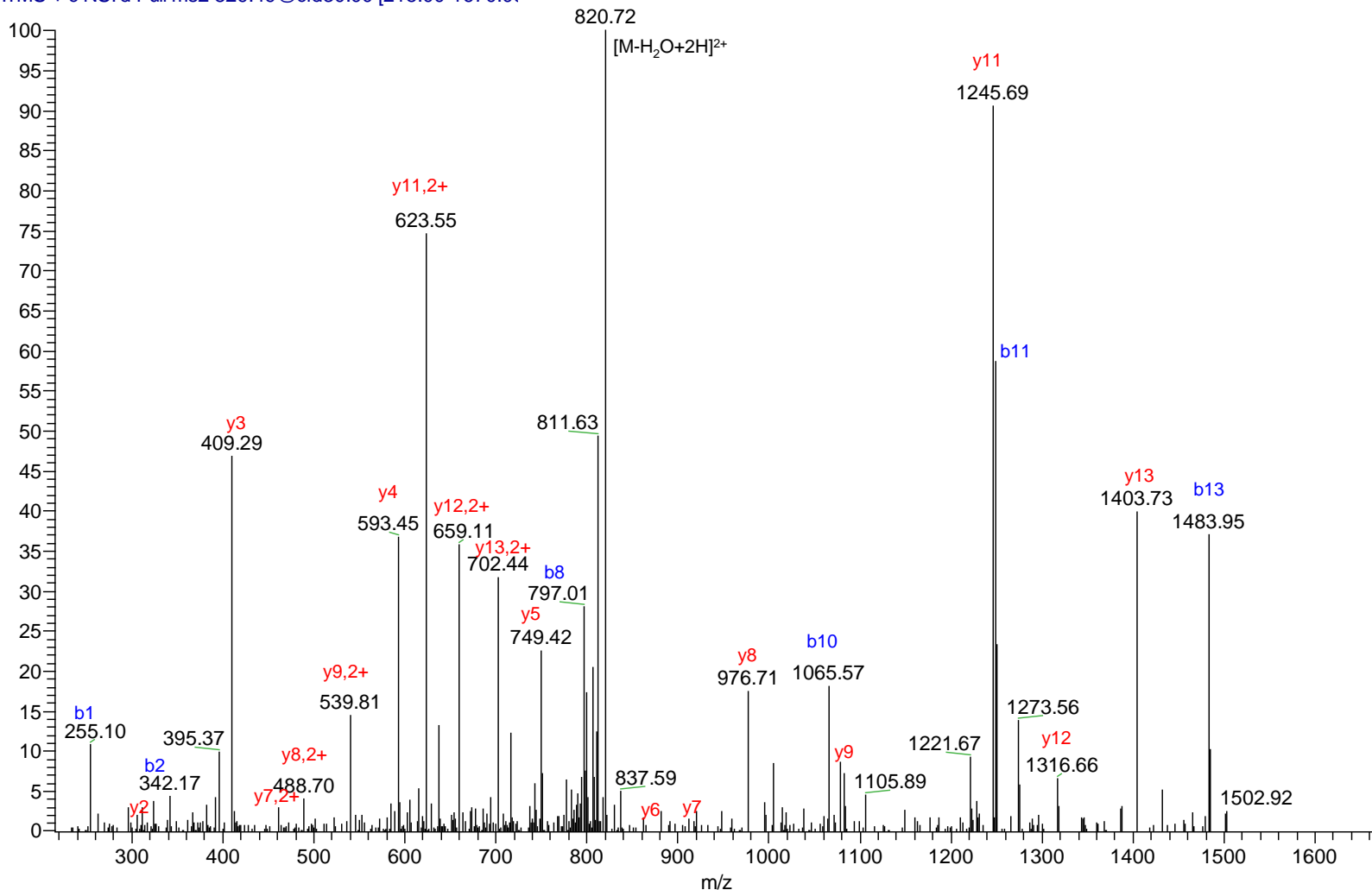
M/Z: 840.4605, Charge: 2+, Mass error: 2ppm, Ions Score: 72; Expect: 4.7e-04



# H3K27Me1K36Me2

## MS/MS of $_{27}KSAPATGGIKKPHR_{40}$

WT8vsN15\_propionylated\_2 #4936 RT: 72.30 AV: 1 NL: 4.70E3  
T: ITMS + c NSI d Full ms2 829.49@cid30.00 [215.00-1670.00]

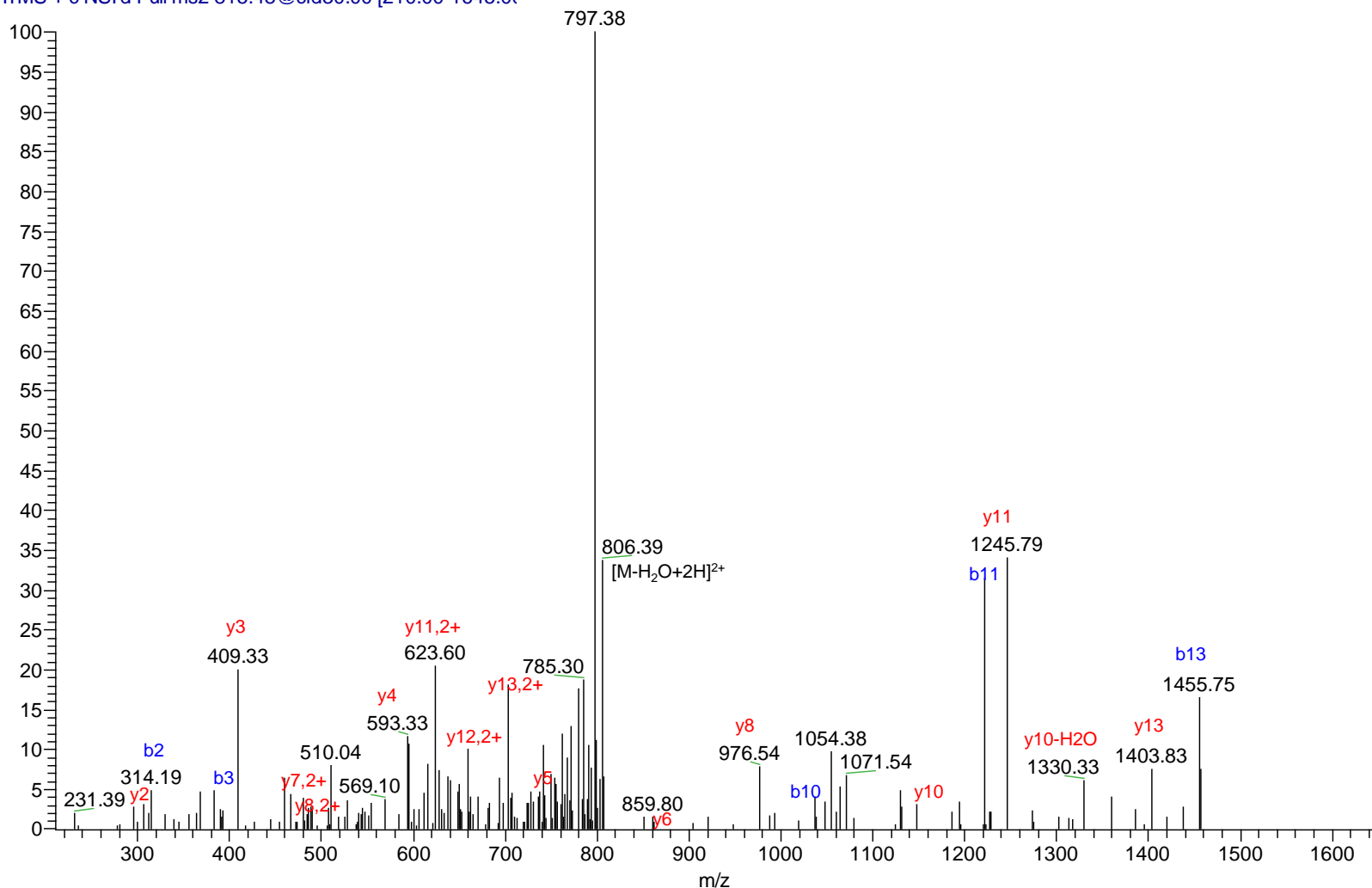


M/Z: 829.4922, Charge: 2+, Mass error: 1ppm, Ions Score: 67; Expect: 0.0015

# H3K27AcK36Me2

## MS/MS of $_{27}$ KSAPATGGIKKPHR $_{40}$

WT8vsN15\_propionylated\_1 #3950 RT: 62.71 AV: 1 NL: 4.39E2  
T: ITMS + c NSI d Full ms2 815.48@cid30.00 [210.00-1645.0]

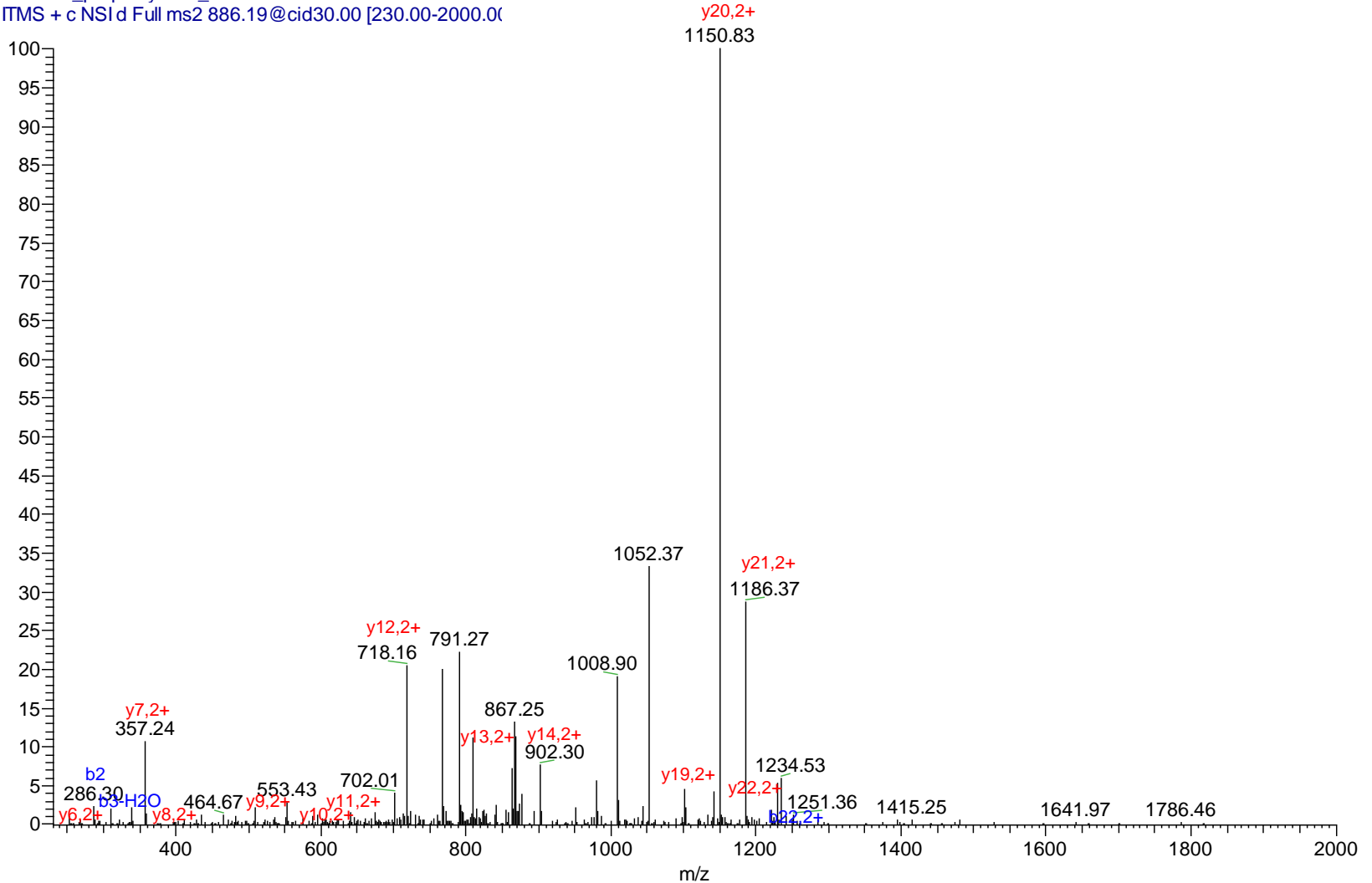


M/Z: 815.4771, Charge: 2+, Mass error: 2ppm, Ions Score: 62; Expect: 0.005

# H3K27Me1R40Me2

## MS/MS of $_{27}KSAPATGGIKKPHRFRPGTVALR_{49}$

WT8vsN15\_propionylated\_2 #6109 RT: 82.67 AV: 1 NL: 8.64E3  
T: ITMS + c NSI d Full ms2 886.19@cid30.00 [230.00-2000.0]

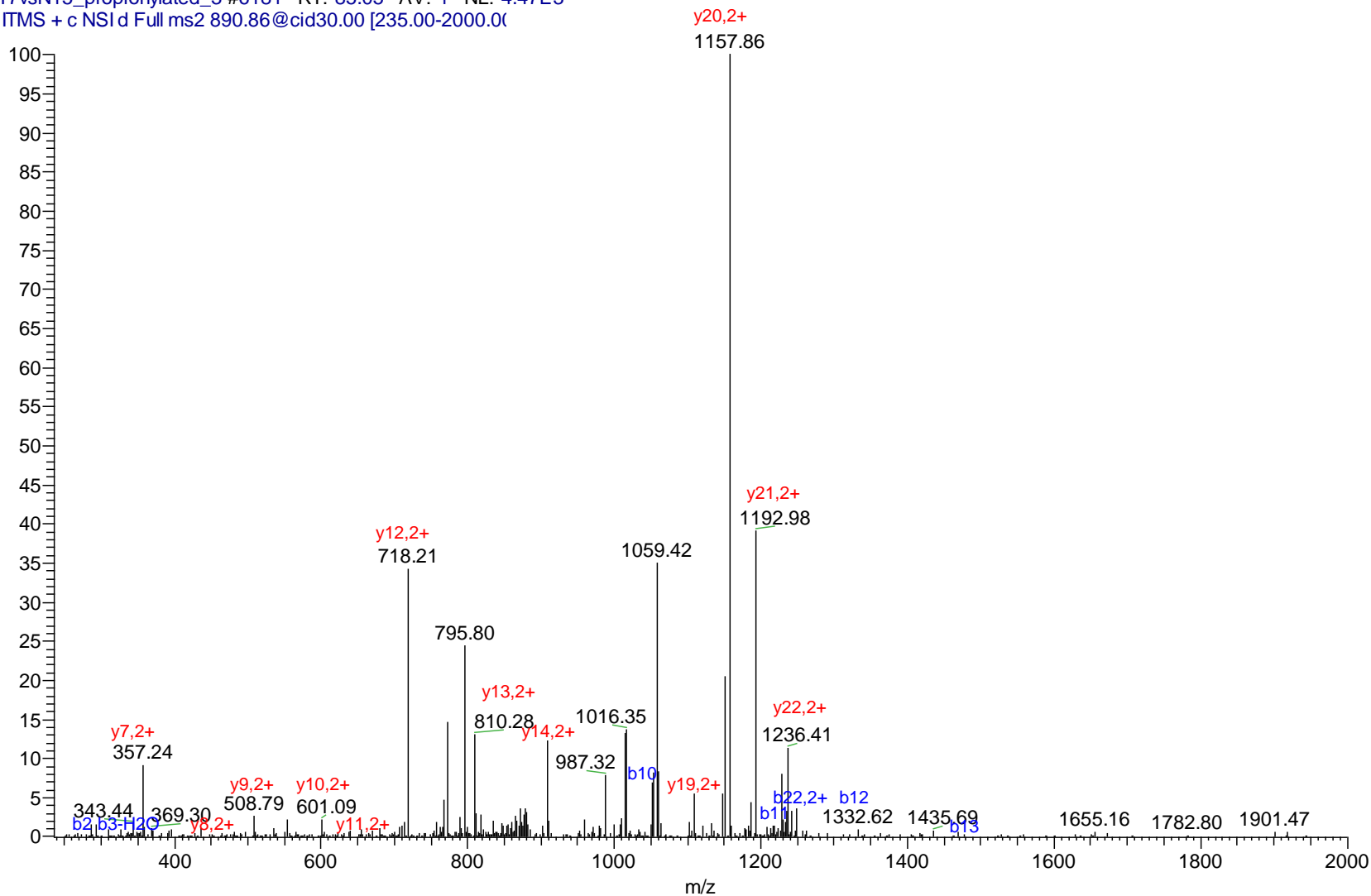


M/Z: 885.8521, Charge: 3+, Mass error: -3ppm, Ions Score: 59; Expect: 0.01

# H3K27Me1K36Me1R40Me2

## MS/MS of $_{27}KSAPATGGIKKPHRFRPGTVALR_{49}$

WT7vsN15\_propionylated\_3 #6181 RT: 85.05 AV: 1 NL: 4.47E3  
T: ITMS + c NSI d Full ms2 890.86@cid30.00 [235.00-2000.00]

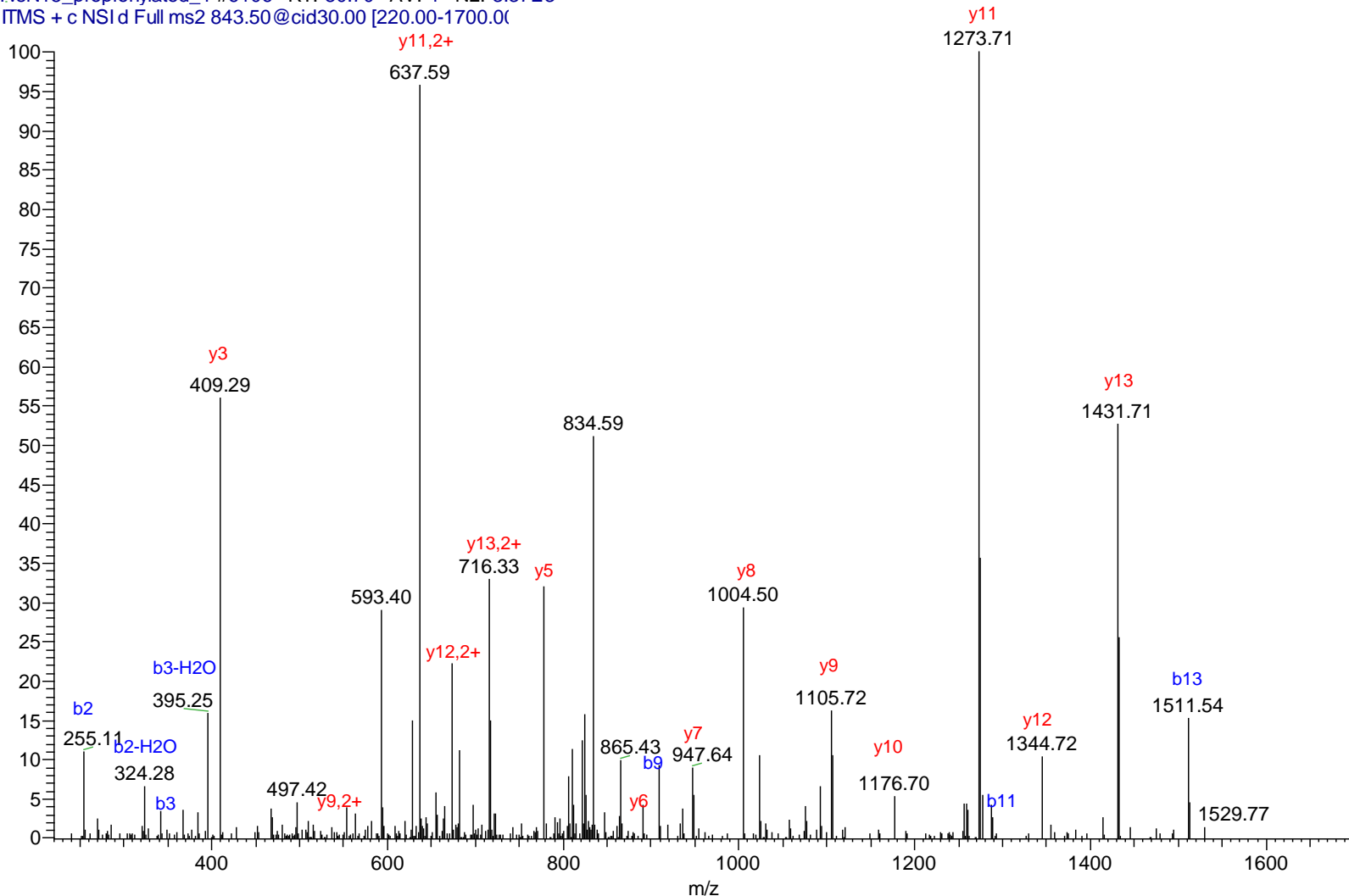


M/Z: 890.5257, Charge: 3+, Mass error: -4ppm, Ions Score: 64; Expect: 0.0026

# H3K27Me1K36Me1K37Me3

## MS/MS of $_{27}KSAPATGGIKKPHR_{40}$

WTvsN15\_propionylated\_1 #6106 RT: 80.70 AV: 1 NL: 3.37E3  
T: ITMS + c NSI d Full ms2 843.50@cid30.00 [220.00-1700.0]

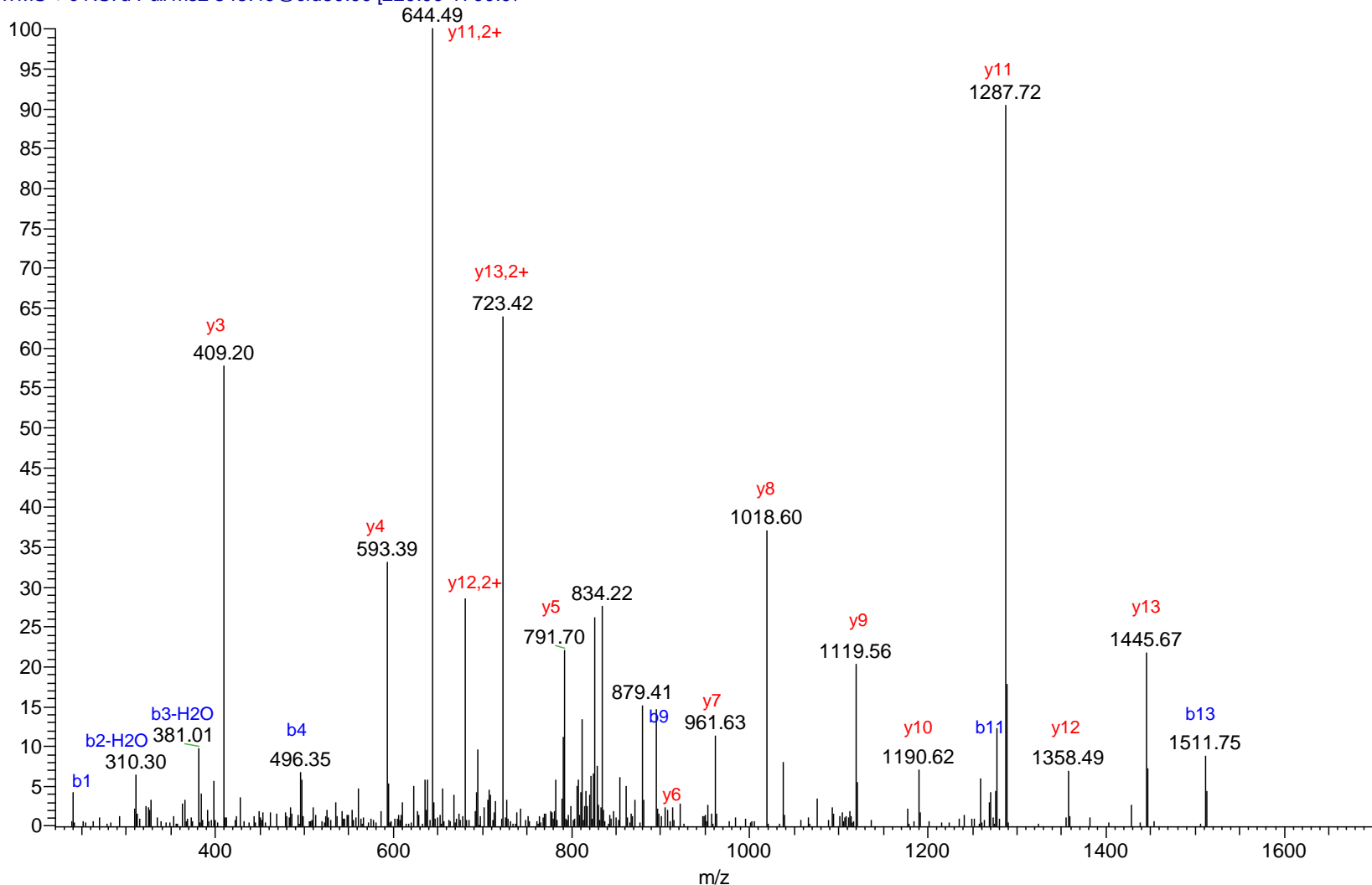


M/Z: 843.5031, Charge: 2+, Mass error: -4ppm, Ions Score: 60; Expect: 0.0072

# H3K36Me1

## MS/MS of 27KSAPATGGIKKPHR40

TRR1bvsN15\_propionylated\_3 #5434 RT: 76.90 AV: 1 NL: 1.79E3  
T: ITMS + c NSI d Full ms2 843.49@cid30.00 [220.00-1700.00]

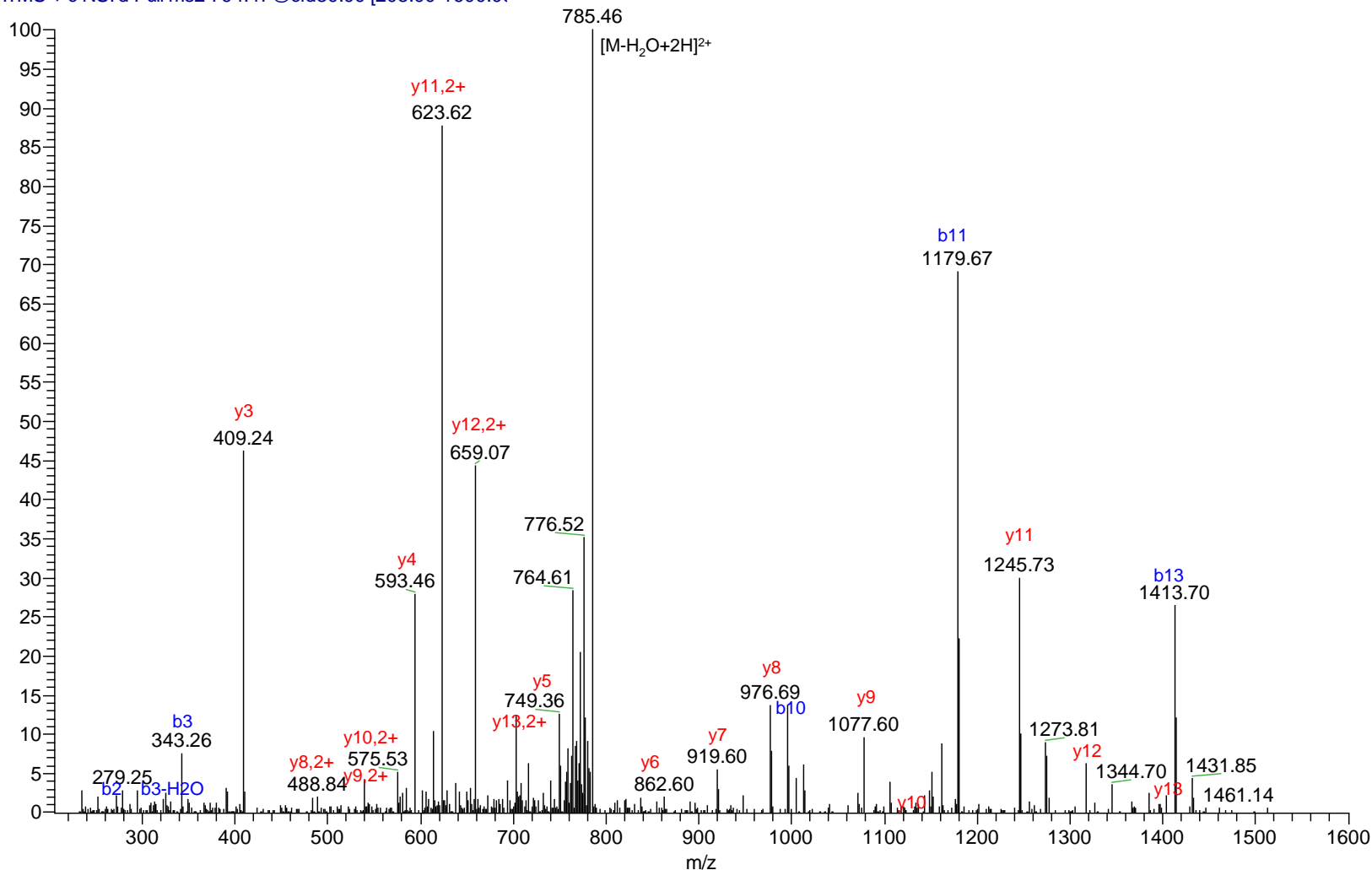


M/Z: 843.4901, Charge: 2+, Mass error: 2ppm, Ions Score: 83; Expect: 4e-05

# H3K36Me2

## MS/MS of 27KSAPATGGIKKPHR40

TRR1avsN15\_propionylated\_1 #4177 RT: 62.68 AV: 1 NL: 2.69E3  
T: ITMS + c NSI d Full ms2 794.47 @cid30.00 [205.00-1600.00]

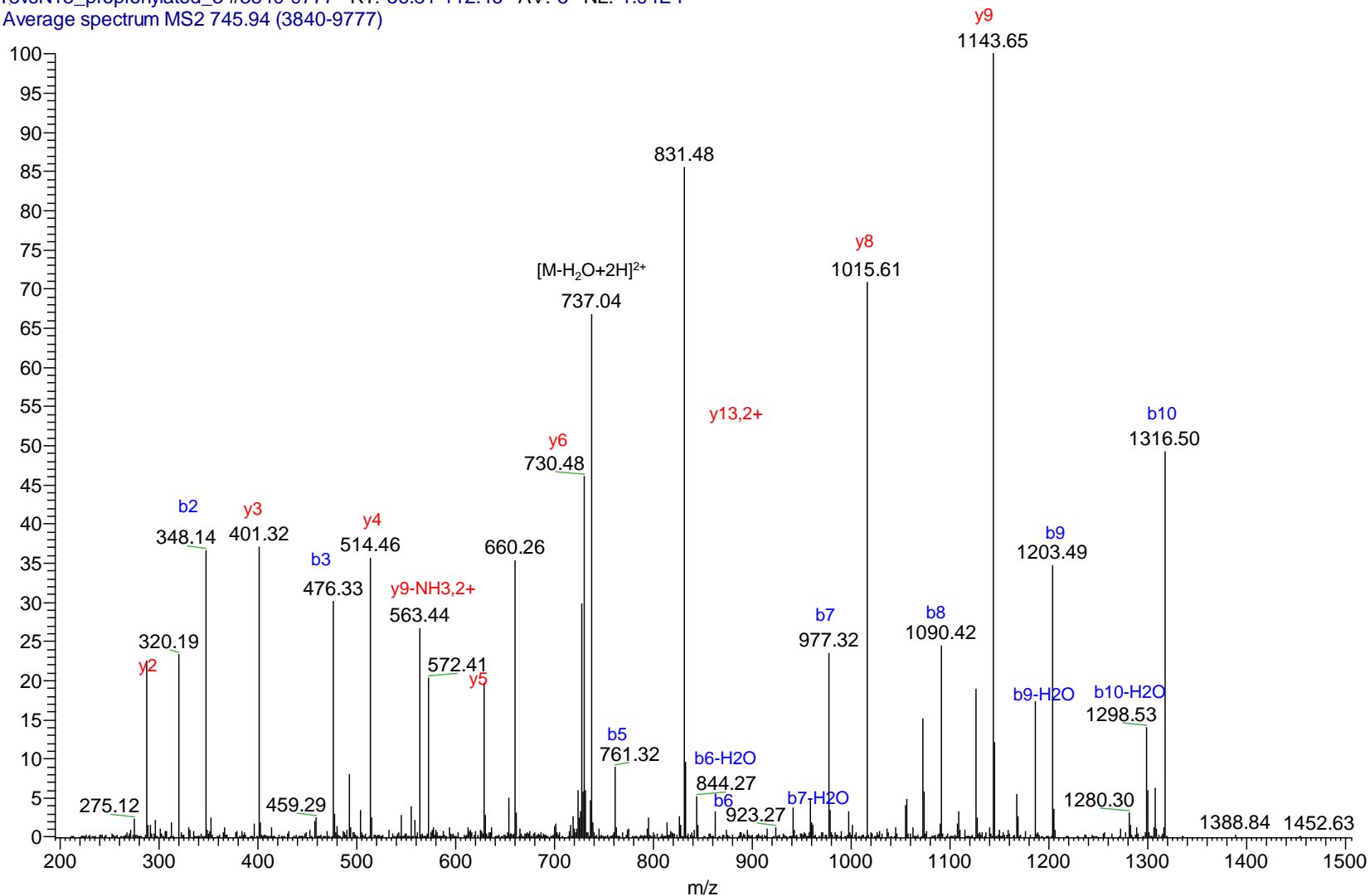


M/Z: 794.4721, Charge: 2+, Mass error: 3ppm, Ions Score: 55; Expect: 0.024

# H3K56Me1

## MS/MS of 53KYQKSTDLLIR63

WT8vsN15\_propionylated\_3 #3840-9777 RT: 56.81-112.46 AV: 5 NL: 1.94E4  
T: Average spectrum MS2 745.94 (3840-9777)



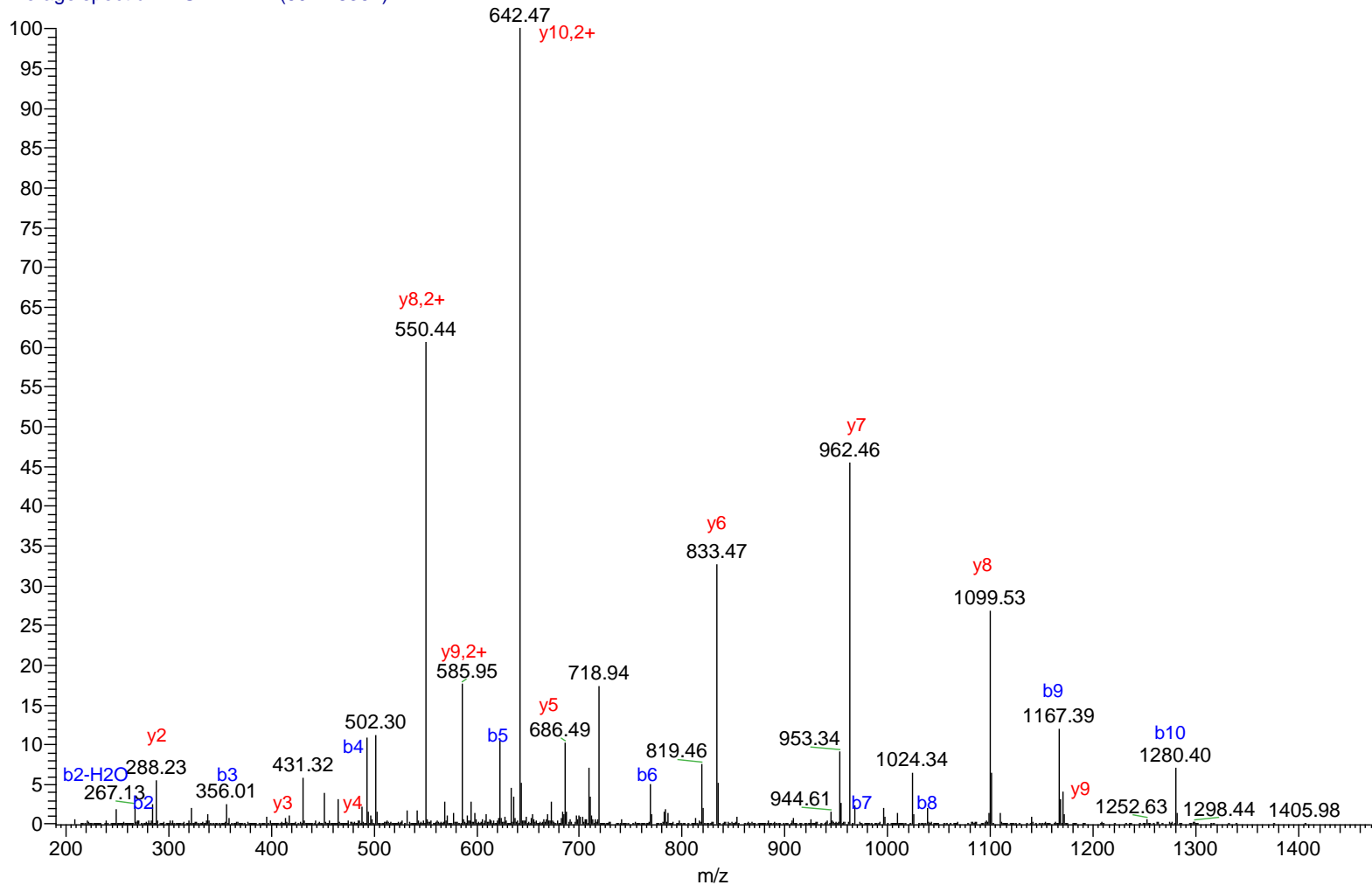
M/Z: 745.9297, Charge: 2+, Mass error: -4ppm, Ions Score: 67; Expect: 3.3e-04



# H3K79Me1

## MS/MS of 73DIAHEFKAELR<sub>83</sub>

WT7vsN15\_propionylated\_3 #6012-8354 RT: 83.64-106.95 AV: 8 NL: 2.62E3  
T: Average spectrum MS2 727.42 (6012-8354)

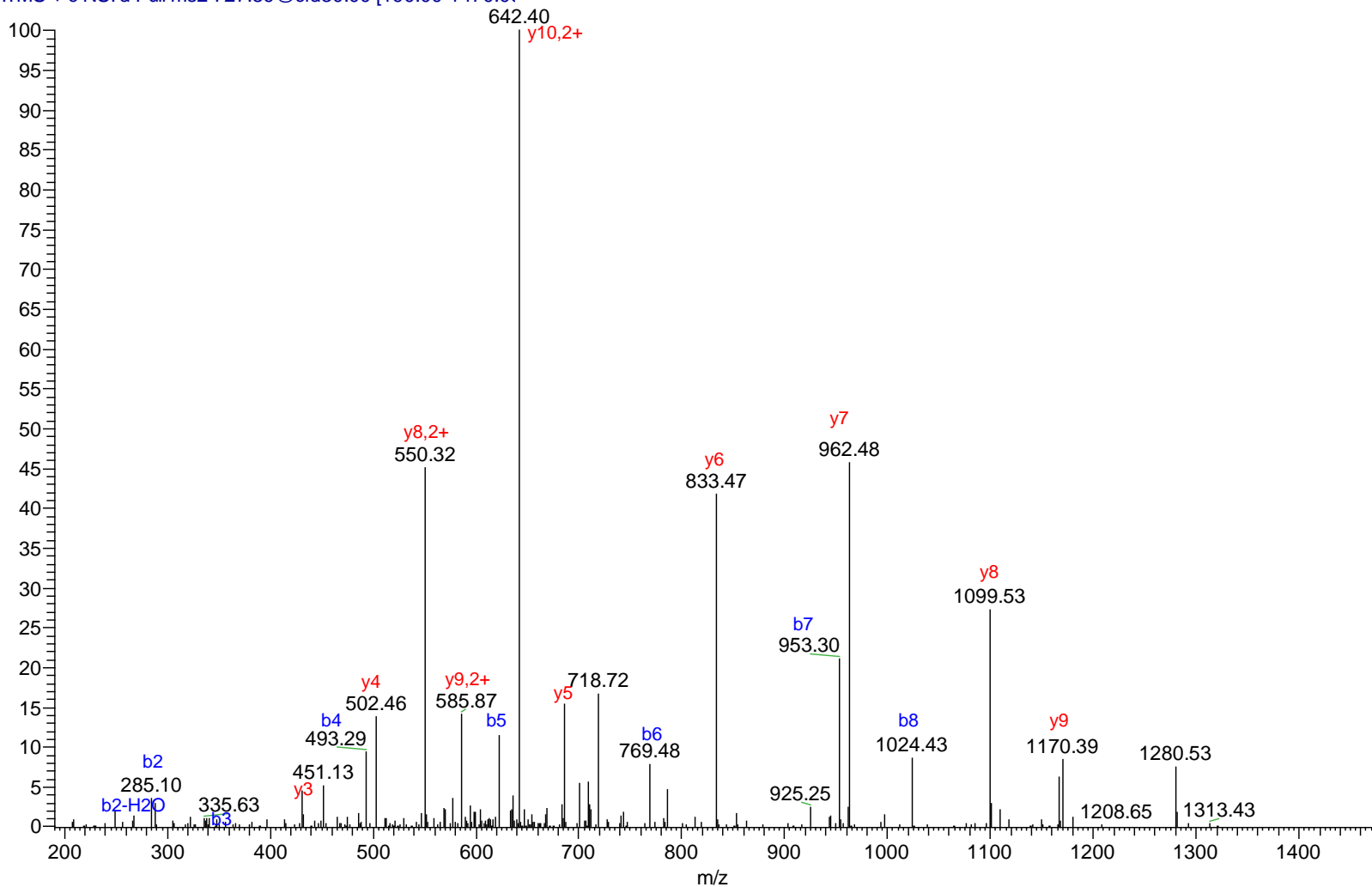


M/Z: 727.8858, Charge: 2+, Mass error: 0ppm, Ions Score: 57; Expect: 0.0017

# H3K79Me1

## MS/MS of 73DIAHEFKAELR<sub>83</sub>

WT8vsN15\_propionylated\_2 #8895 RT: 107.00 AV: 1 NL: 5.31E3  
T: ITMS + c NSI d Full ms2 727.89@cid30.00 [190.00-1470.0]

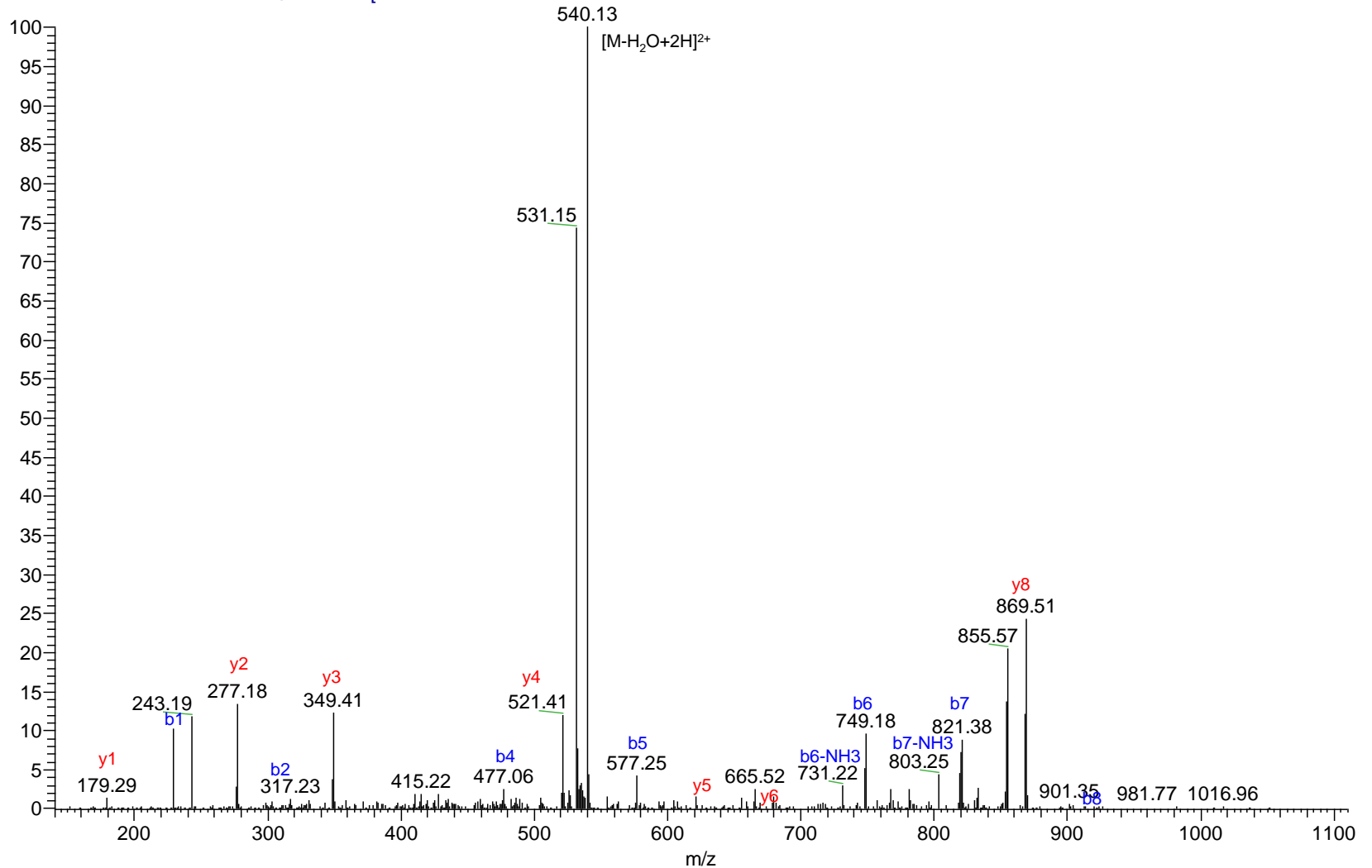


M/Z: 727.8863, Charge: 2+, Mass error: 1ppm, Ions Score: 54; Expect: 0.0036

# H3.3K9AcK14Ac

## MS/MS of 9KSTGVKAPR<sub>17</sub>

WT8vsN15\_propionylated\_1 #3306 RT: 56.33 AV: 1 NL: 7.24E3  
T: ITMS + c NSI d Full ms2 549.29@cid30.00 [140.00-1110.00]

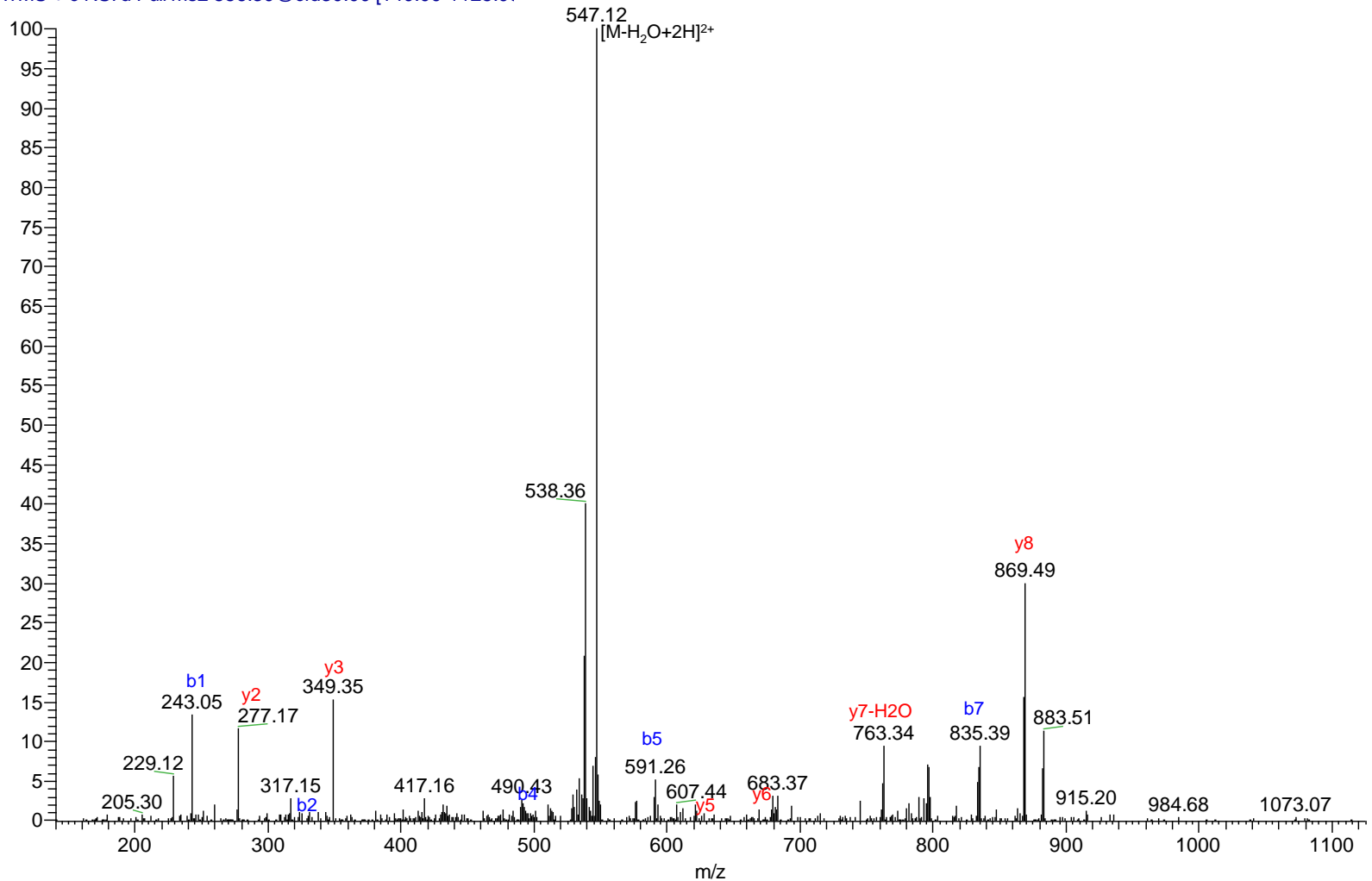


M/Z: 549.2912, Charge: 2+, Mass error: 1ppm, Ions Score: 45; Expect: 0.081

# H3.3K14Ac

## MS/MS of 9KSTGVKAPR<sub>17</sub>

TRR1bvsN15\_propionylated\_1 #4183 RT: 65.58 AV: 1 NL: 1.06E4  
T: ITMS + c NSI d Full ms2 556.30@cid30.00 [140.00-1125.00]

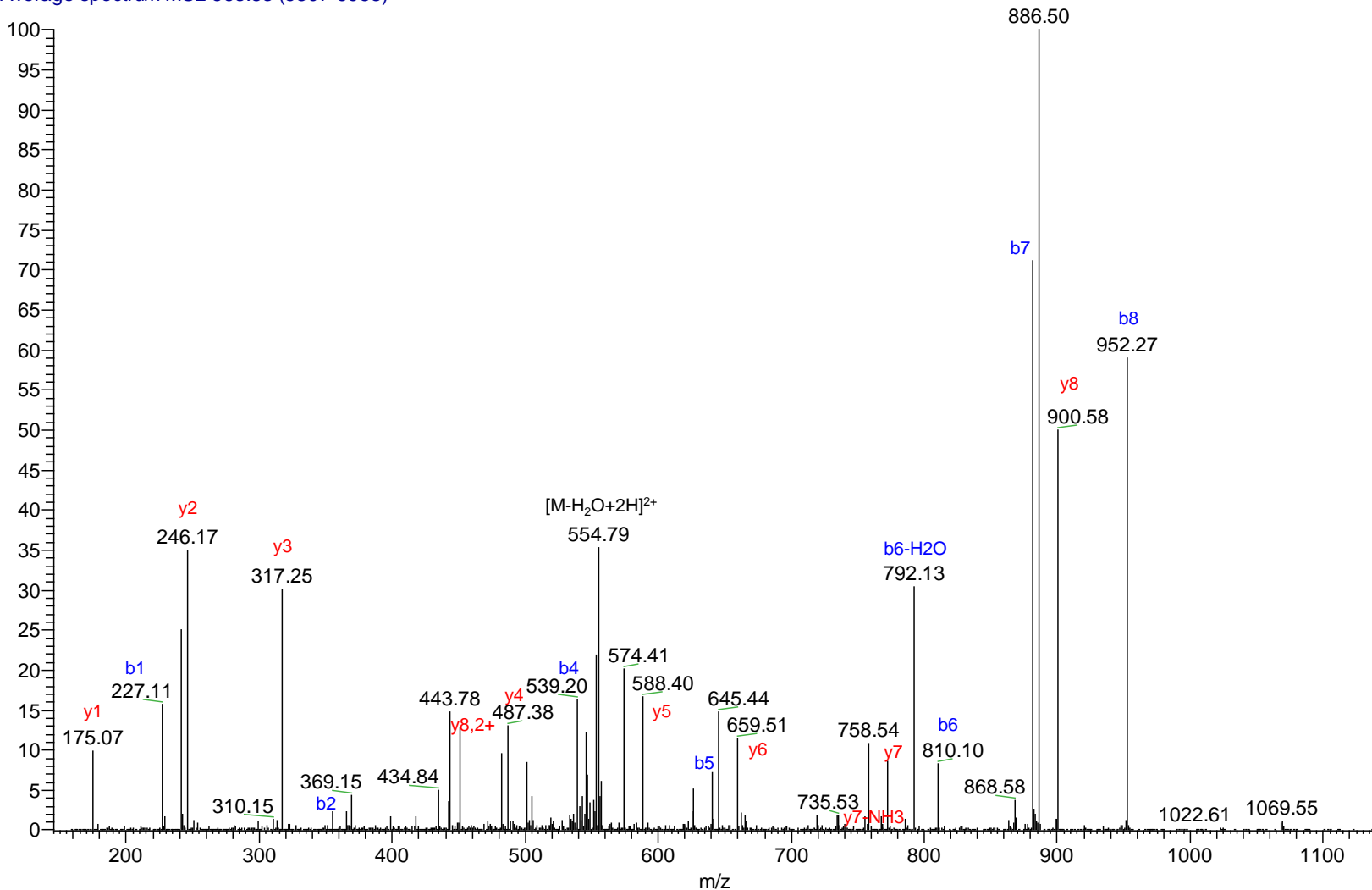


M/Z: 556.2992, Charge: 2+, Mass error: 1ppm, Ions Score: 47; Expect: 0.054

# H3.3K18AcK23Ac

## MS/MS of 18KQLATKAAR<sub>26</sub>

TRR1bvsN15\_propionylated\_1 #5807-6988 RT: 80.80-91.22 AV: 12 NL: 4.00E3  
T: Average spectrum MS2 563.83 (5807-6988)

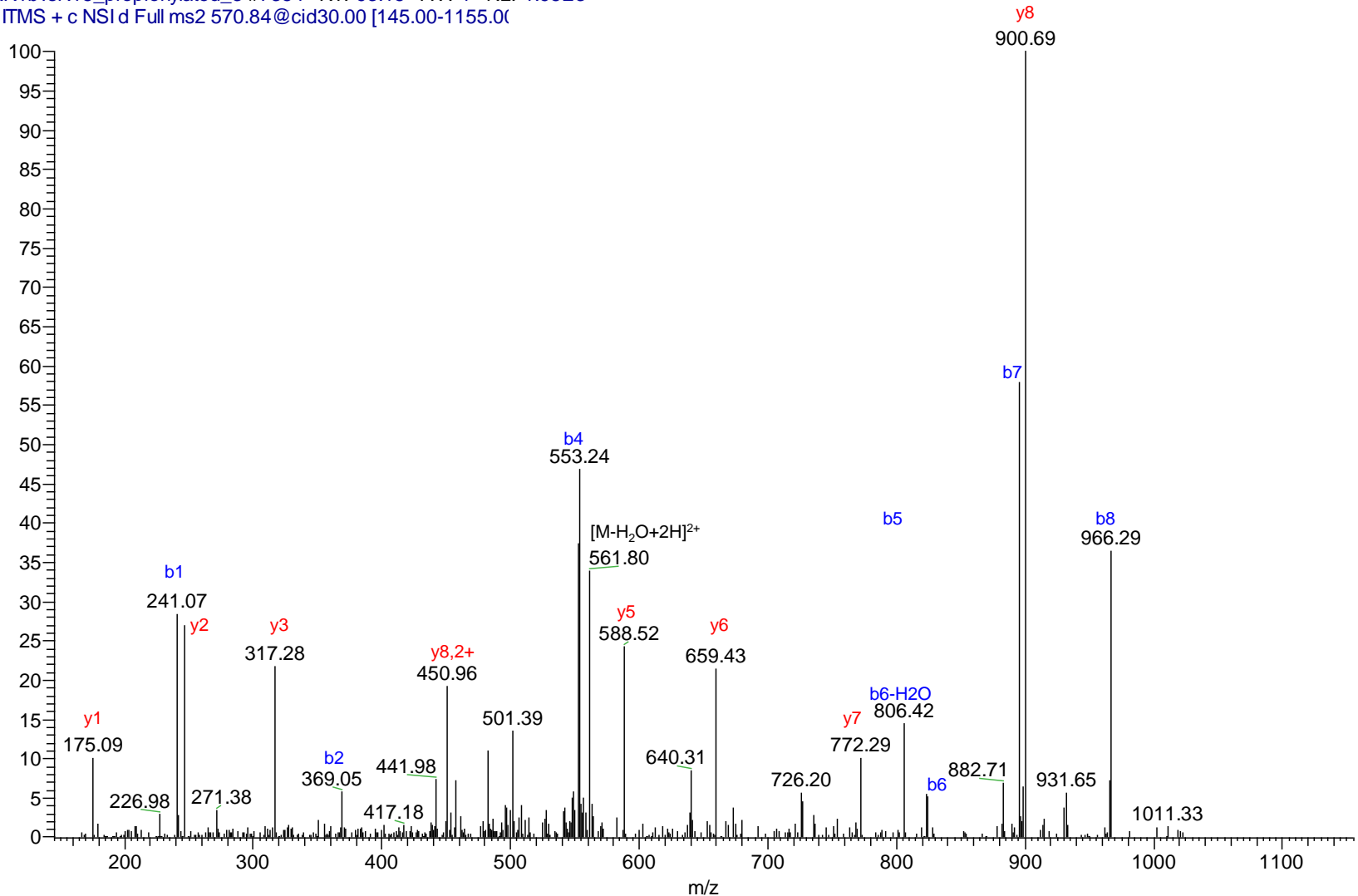


M/Z: 563.8322, Charge: 2+, Mass error: -1ppm, Ions Score: 56; Expect: 0.0045

# H3.3K23Ac

## MS/MS of 18KQLATKAAR<sub>26</sub>

TRR1bvsN15\_propionylated\_3 #7354 RT: 93.15 AV: 1 NL: 1.95E3  
T: ITMS + c NSI d Full ms2 570.84@cid30.00 [145.00-1155.00]

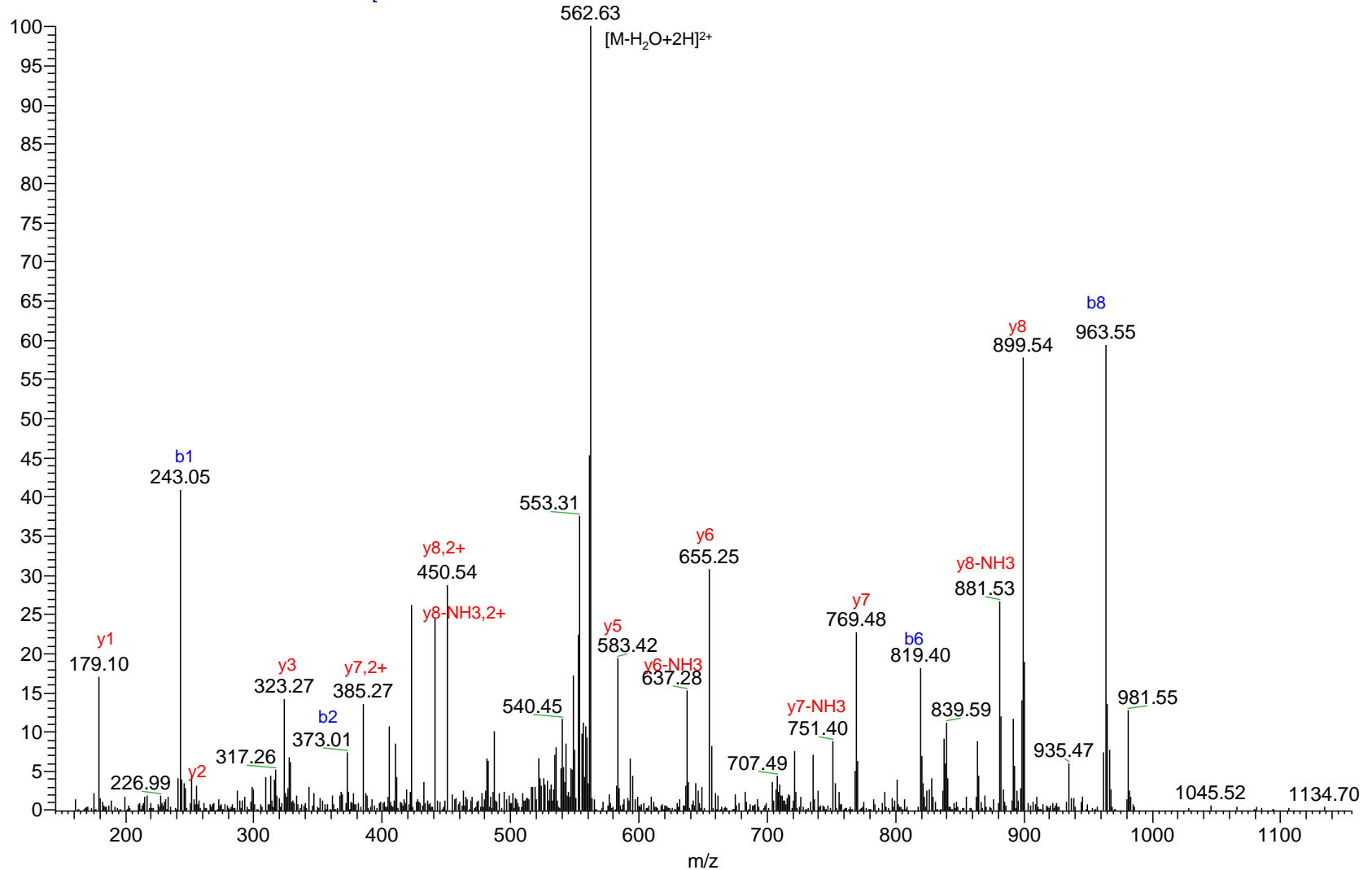


M/Z: 570.8404, Charge: 2+, Mass error: 0ppm, Ions Score: 57; Expect: 0.0046

# H3.3K23Me2

## MS/MS of 18KQLATKAAR<sub>26</sub>

TRR1bvsN15\_propionylated\_3 #5088 RT: 73.60 AV: 1 NL: 4.55E3  
T: ITMS + c NSI d Full ms2 571.33@cid30.00 [145.00-1155.00]

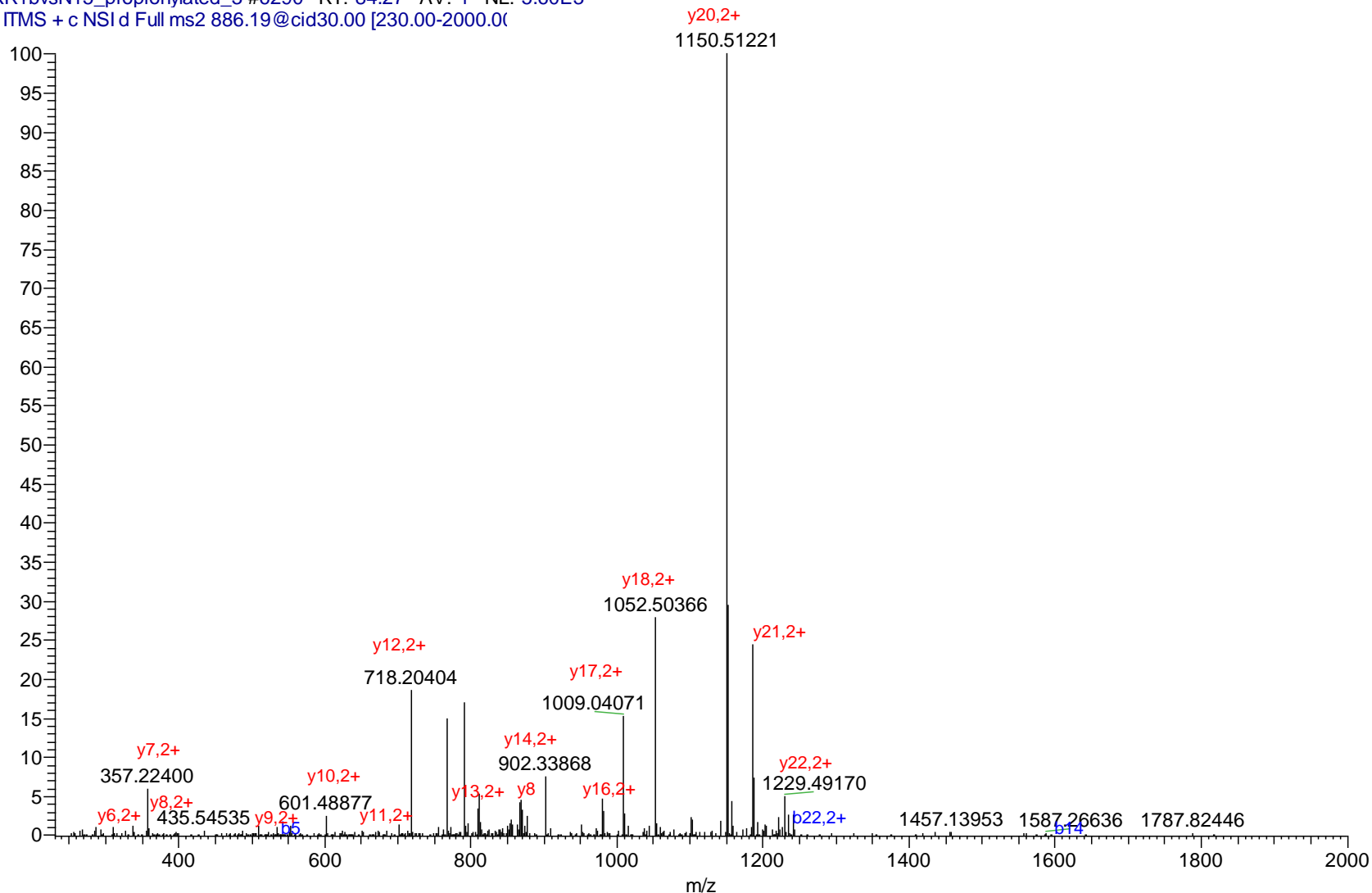


M/Z: 571.329, Charge: 2+, Mass error: 1ppm, Ions Score: 48; Expect: 0.092

# H3.3K27Me1

## MS/MS of 27KSAPVSGGVKKPHKFRPGTVALR<sub>49</sub>

TRR1bvsN15\_propionylated\_3 #6290 RT: 84.27 AV: 1 NL: 5.60E3  
T: ITMS + c NSI d Full ms2 886.19@cid30.00 [230.00-2000.00]



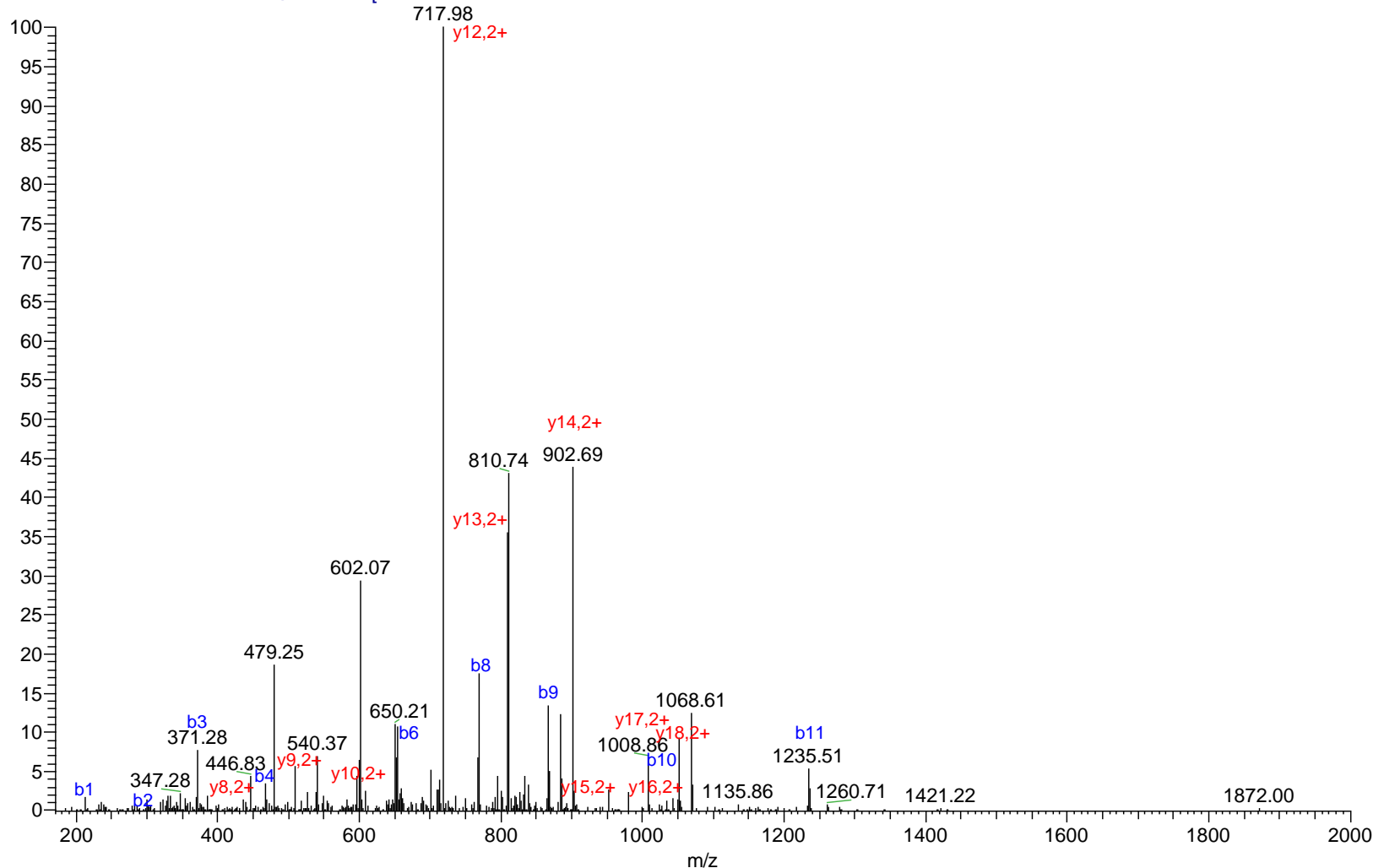
M/Z: 885.8548, Charge: 3+, Mass error: 2ppm, Ions Score: 121; Expect: 5.5e-09



# H3.3K27Me2

## MS/MS of 27KSAPVSGGVKKPHKFRPGTVALR<sub>49</sub>

WT8vsN15\_propionylated\_3 #6091 RT: 79.61 AV: 1 NL: 2.15E4  
T: ITMS + c NSI d Full ms2 668.65@cid30.00 [170.00-2000.0]

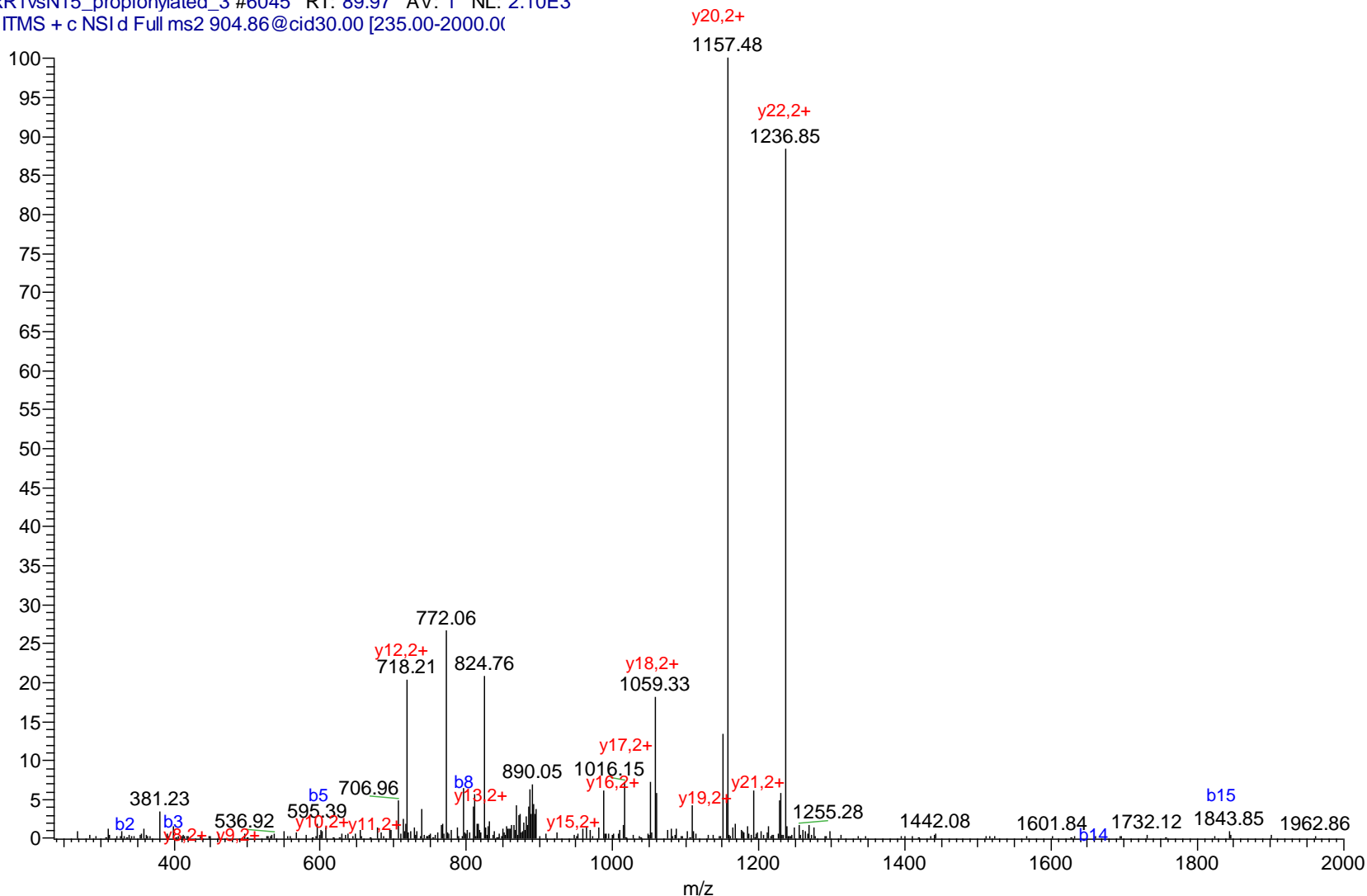


M/Z: 668.1464, Charge: 4+, Mass error: 1ppm, Ions Score: 63; Expect: 0.0031

# H3.3K36Me1

## MS/MS of 27KSAPVSGGVKKPHKFRPGTVALR<sub>49</sub>

TRR1vsN15\_propionylated\_3 #6045 RT: 89.97 AV: 1 NL: 2.10E3  
T: ITMS + c NSI d Full ms2 904.86@cid30.00 [235.00-2000.00]

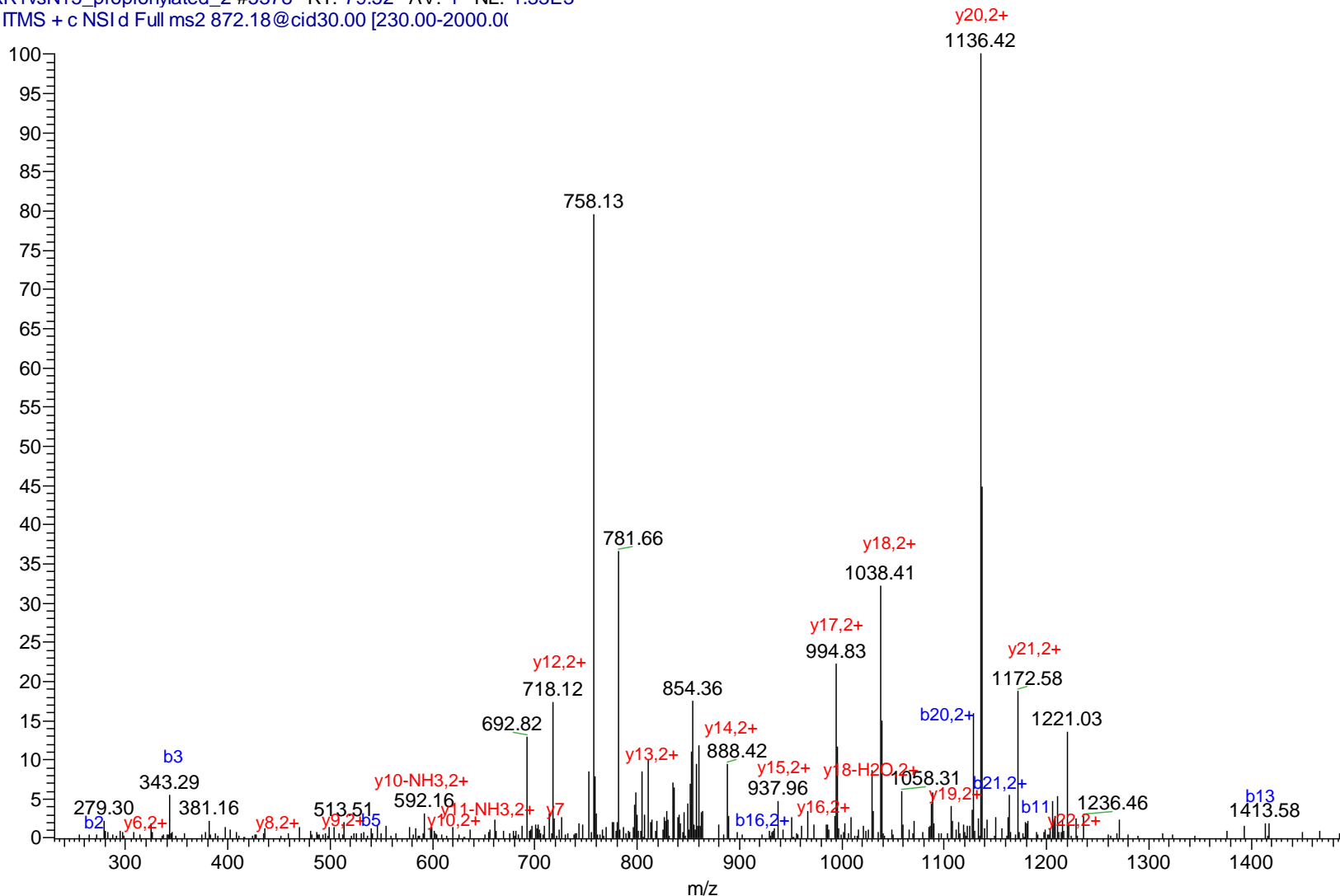


M/Z: 904.5299, Charge: 3+, Mass error: 1ppm, Ions Score: 77; Expect: 1.5e-04

# H3.3K36Me2

## MS/MS of 27KSAPVSGGVKKPHKFRPGTVLR<sub>49</sub>

TRR1vsN15\_propionylated\_2 #5378 RT: 79.52 AV: 1 NL: 1.33E3  
T: ITMS + c NSI d Full ms2 872.18@cid30.00 [230.00-2000.0]

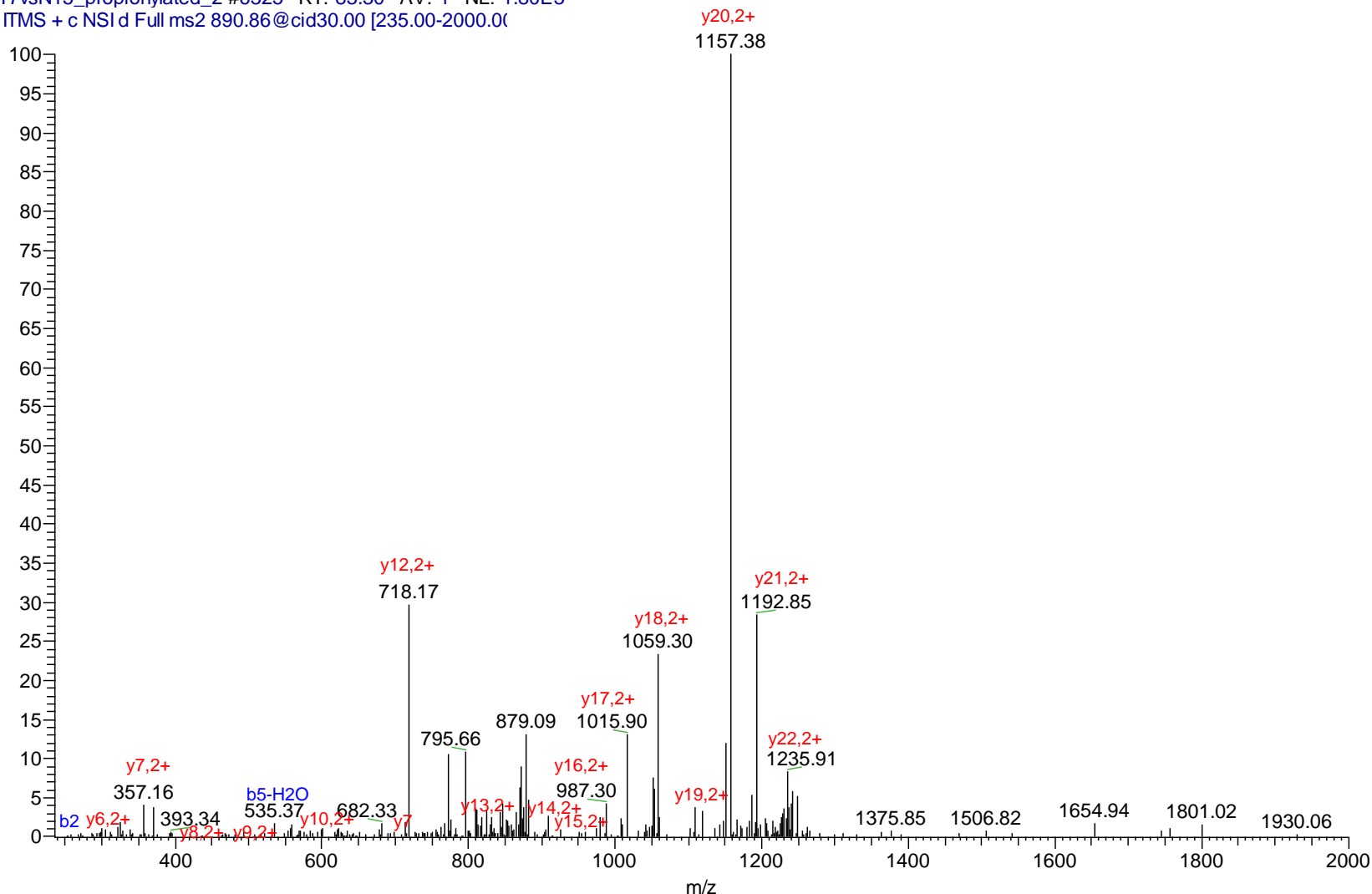


M/Z: 871.8516, Charge: 3+, Mass error: 2ppm, Ions Score: 58; Expect: 0.0086

# H3.3K27Me1K36Me1

## MS/MS of $_{27}KSAPVSGGVKKPHKFRPGTVALR_{49}$

WT7vsN15\_propionylated\_2 #6325 RT: 83.30 AV: 1 NL: 1.80E3  
T: ITMS + c NSI d Full ms2 890.86@cid30.00 [235.00-2000.00]

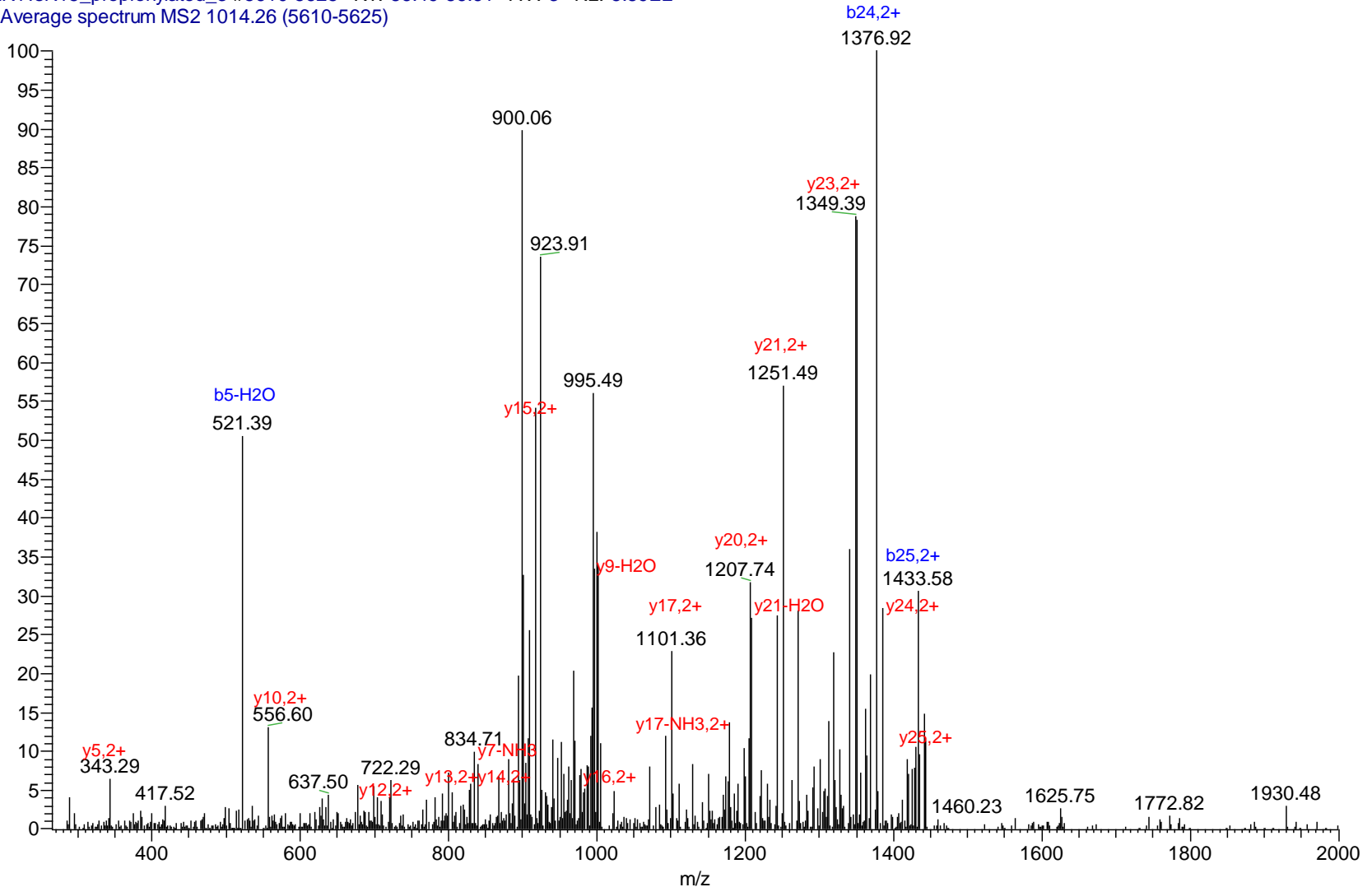


M/Z: 890.5257, Charge: 3+, Mass error: 0ppm, Ions Score: 118; Expect: 9.9e-09

# H3.3K36Me1K37Ac

## MS/MS of 27KSAPVSGGVKKPHKFRPGTVALREIR<sub>49</sub>

TRR1vsN15\_propionylated\_3 #5610-5625 RT: 86.49-86.61 AV: 3 NL: 5.89E2  
T: Average spectrum MS2 1014.26 (5610-5625)

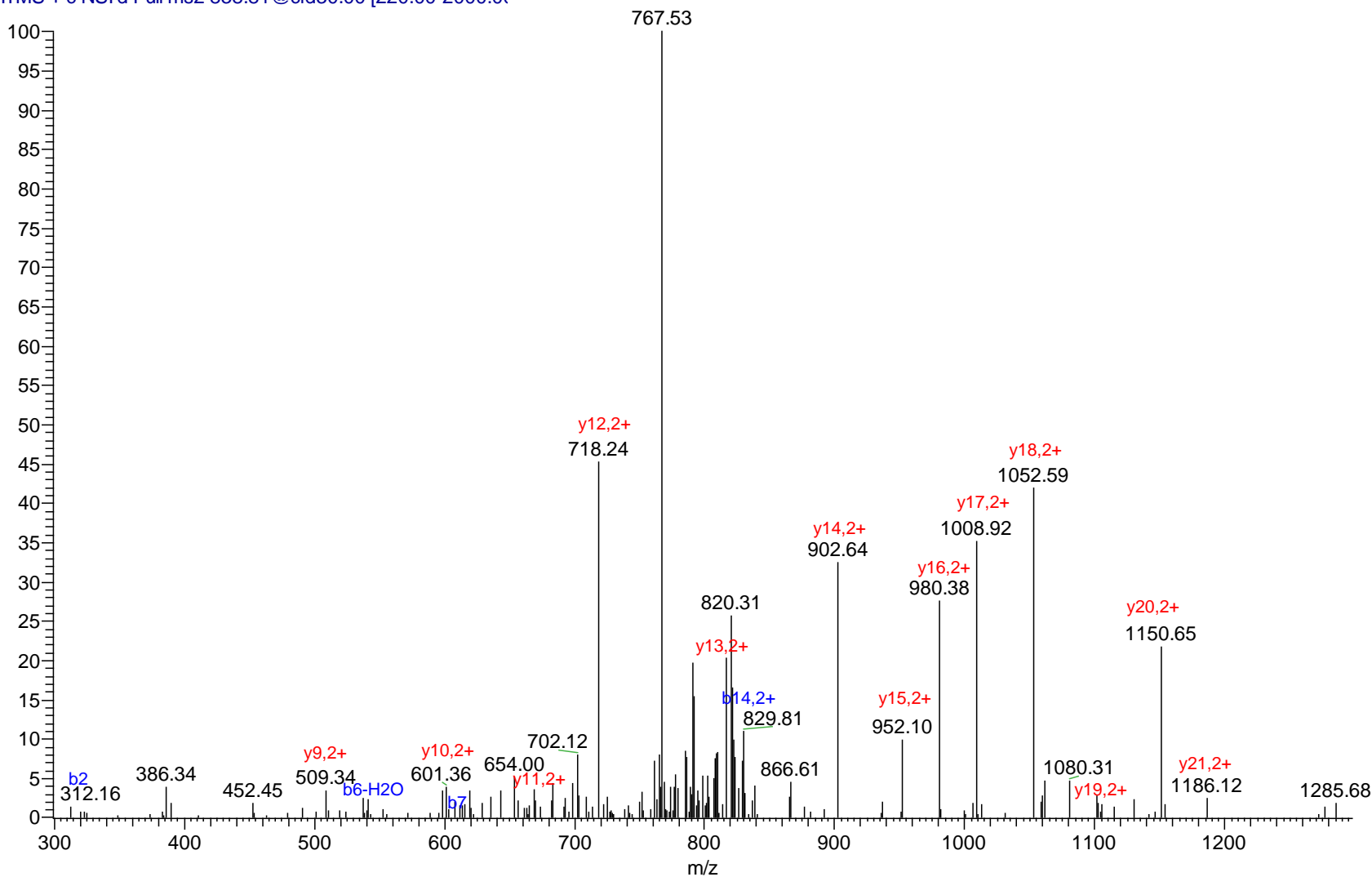


M/Z: 1013.9262, Charge: 3+, Mass error: 2ppm, Ions Score: 52; Expect: 0.048

# H3.3K36AcK37Me1

## MS/MS of $_{28}\text{SAPVSGGVKKPHKFRPGTVALR}_{49}$

WT8vsN15\_propionylated\_2 #6341 RT: 84.68 AV: 1 NL: 1.04E3  
T: ITMS + c NSI d Full ms2 838.81 @cid30.00 [220.00-2000.00]

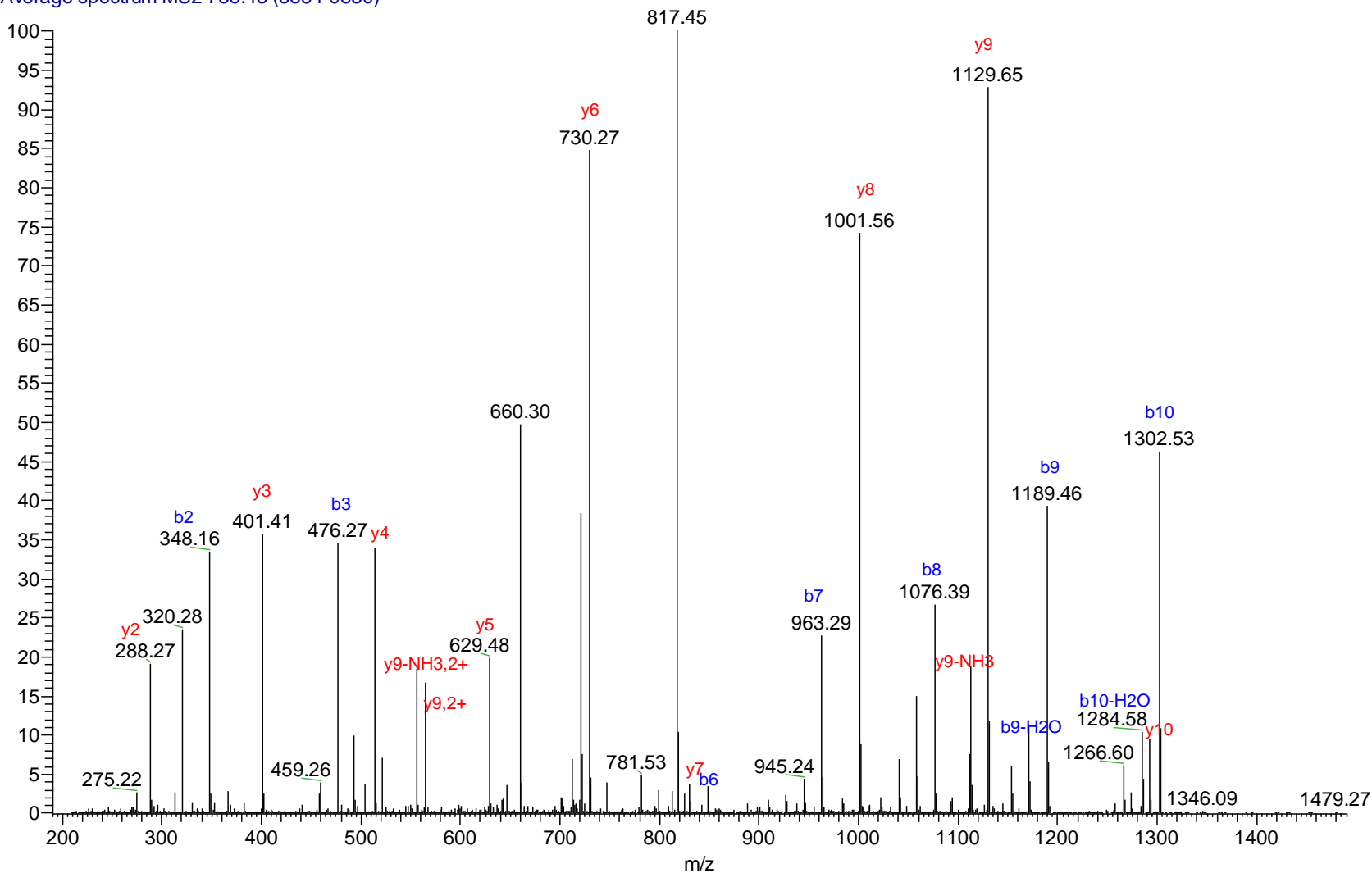


M/Z: 838.4809, Charge: 3+, Mass error: -3ppm, Ions Score: 59; Expect: 0.011

# H3.3K56Ac

## MS/MS of 53KYQKTTDLLIR63

TRR1avsN15\_propionylated\_2 #3384-9830 RT: 58.81-118.05 AV: 40 NL: 1.48E4  
T: Average spectrum MS2 738.43 (3384-9830)

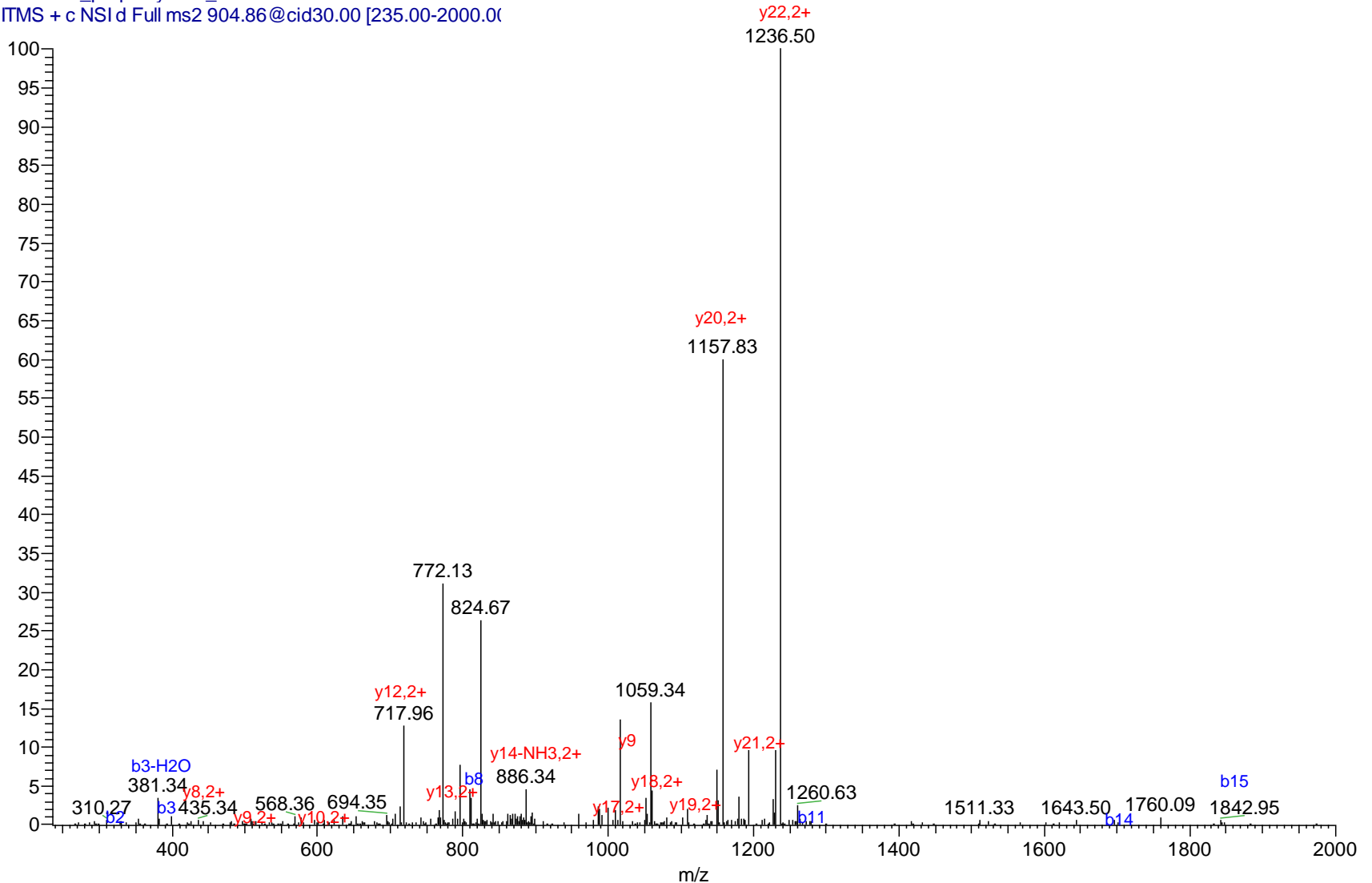


M/Z: 738.9255, Charge: 2+, Mass error: 1ppm, Ions Score: 74; Expect: 7.2e-05

# H3.4K36Ac

## MS/MS of 27KSAPISGGIKKPHKFRPGTVALR<sub>49</sub>

TRR1vsN15\_propionylated\_2 #6563 RT: 89.17 AV: 1 NL: 3.17E3  
T: ITMS + c NSI d Full ms2 904.86@cid30.00 [235.00-2000.00]



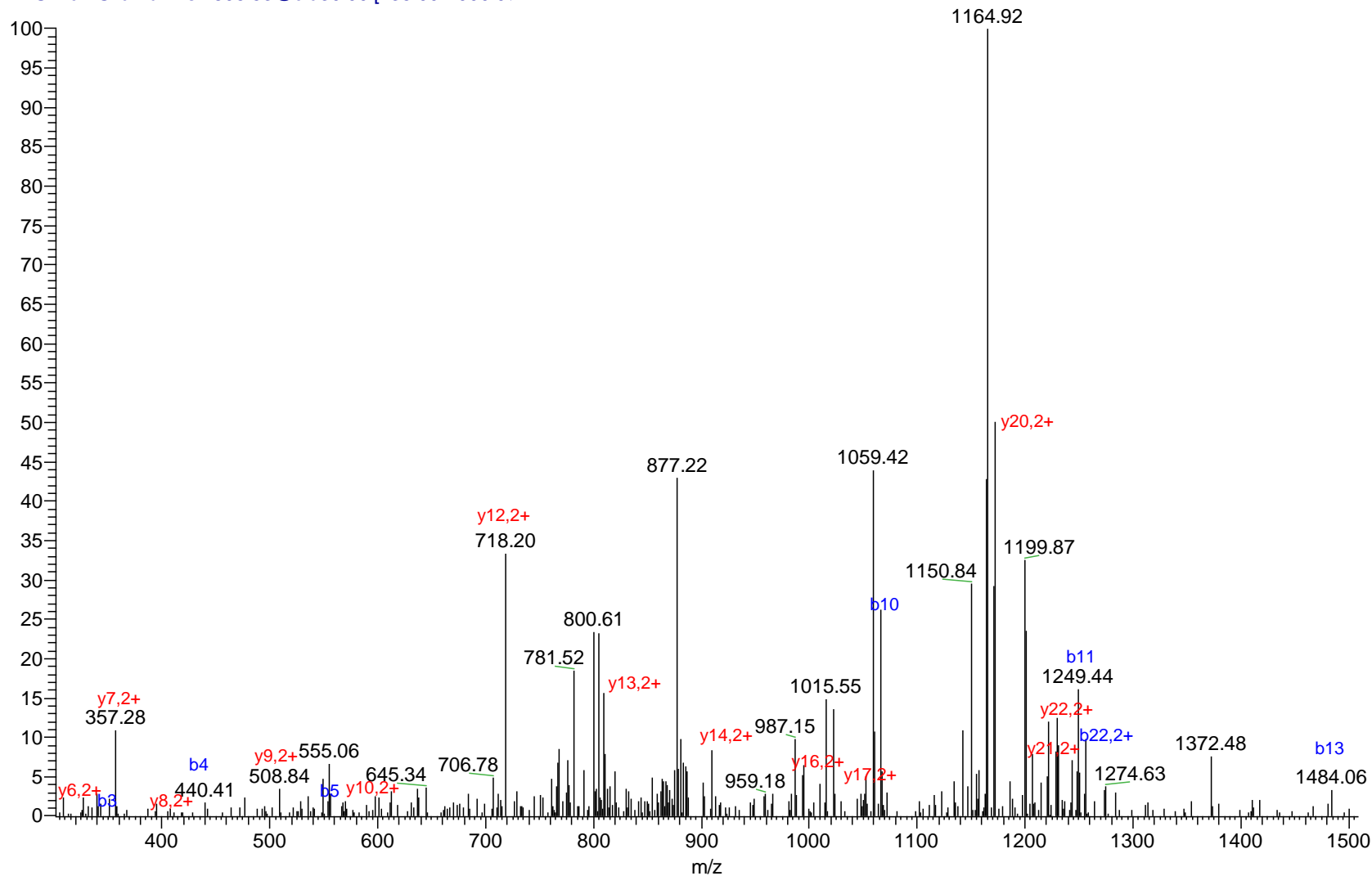
M/Z: 904.5299, Charge: 3+, Mass error: 1ppm, Ions Score: 55; Expect: 0.026



# H3.4K36Me1

## MS/MS of 27KSAPISGGIKKPHKFRPGTVALR<sub>49</sub>

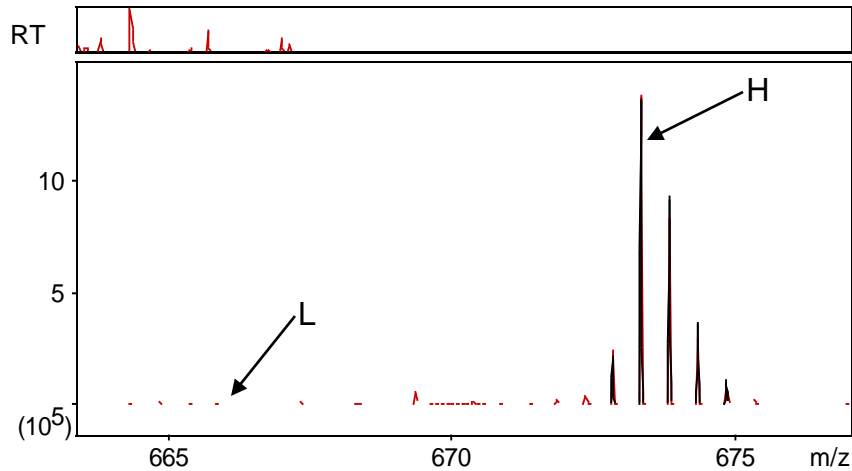
TRR1avsN15\_propionylated\_3 #6018 RT: 86.98 AV: 1 NL: 8.58E2  
T: ITMS + c NSI d Full ms2 895.53@cid30.00 [235.00-2000.00]



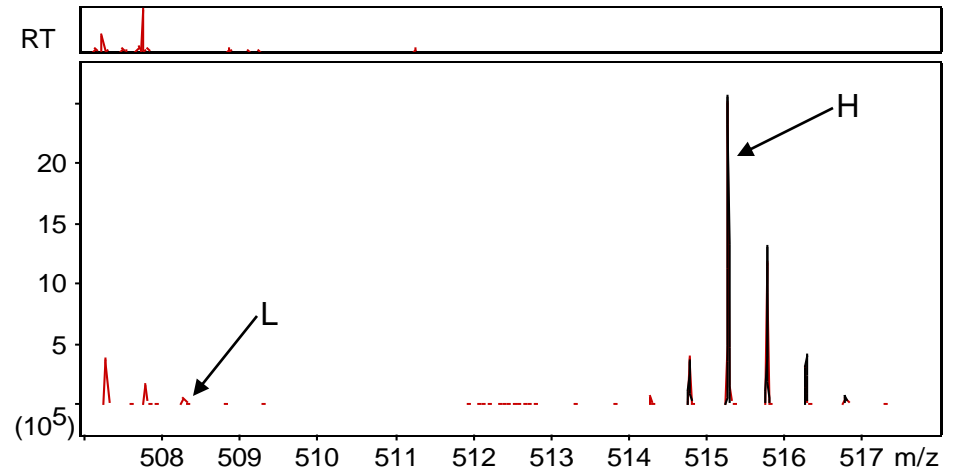
M/Z: 895.1992, Charge: 3+, Mass error: 2ppm, Ions Score: 41; Expect: 0.45

# Figure S2. Labeling efficiencies of four $^{15}\text{N}$ H3 peptides

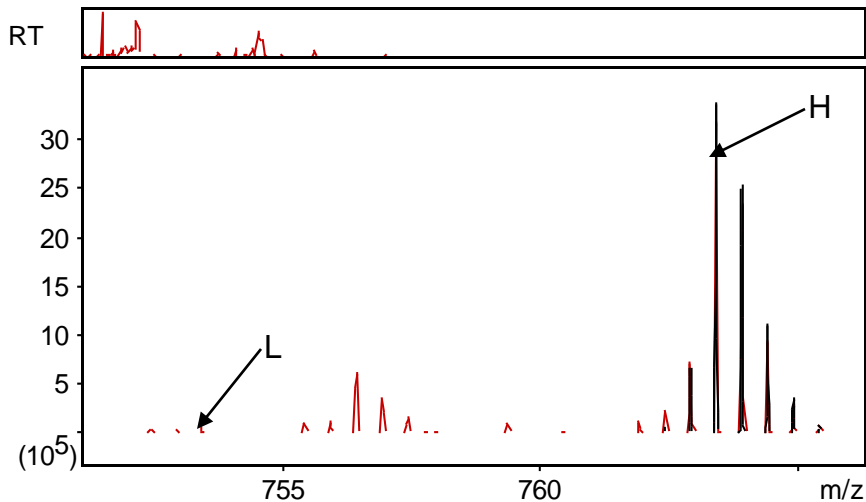
DIAHEFKAELR, Charge:2+, H/L: 99.39%



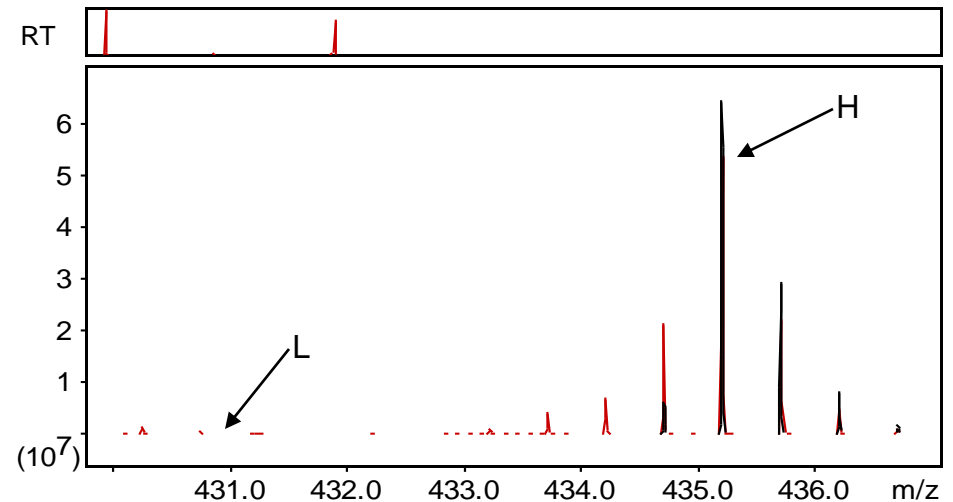
KQLAS<sub>ac</sub>KAAR, Charge:2+, H/L: 99.4%



acKSAPATGGI<sub>me1</sub>KKPHR, Charge:2+, H/L: 99.78%

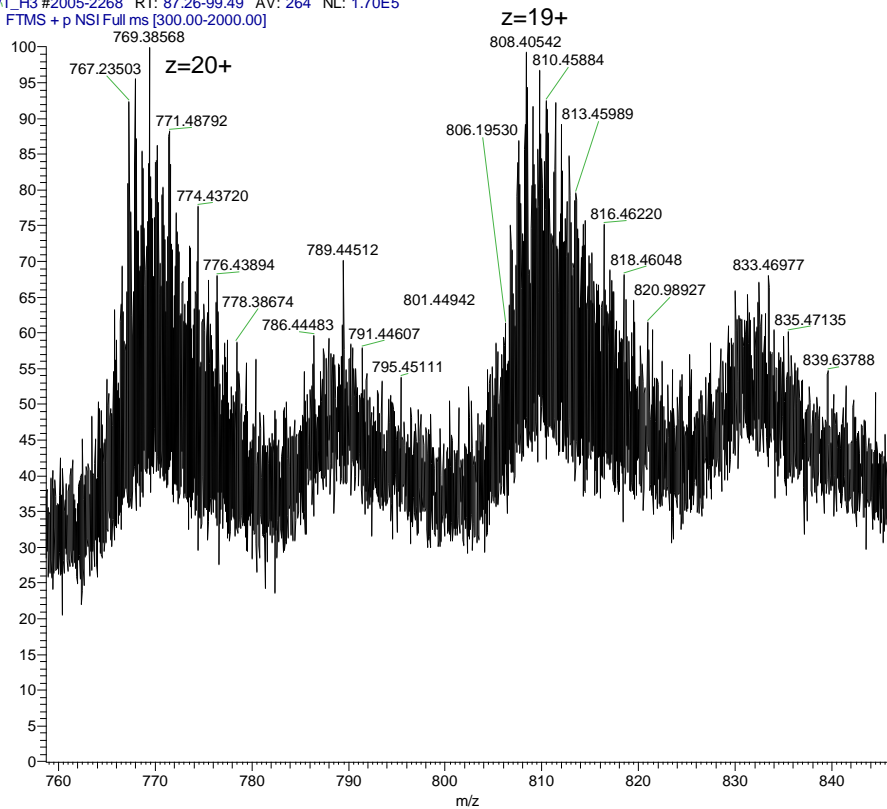


DIAHEFK, Charge:2+, H/L: 99.21%



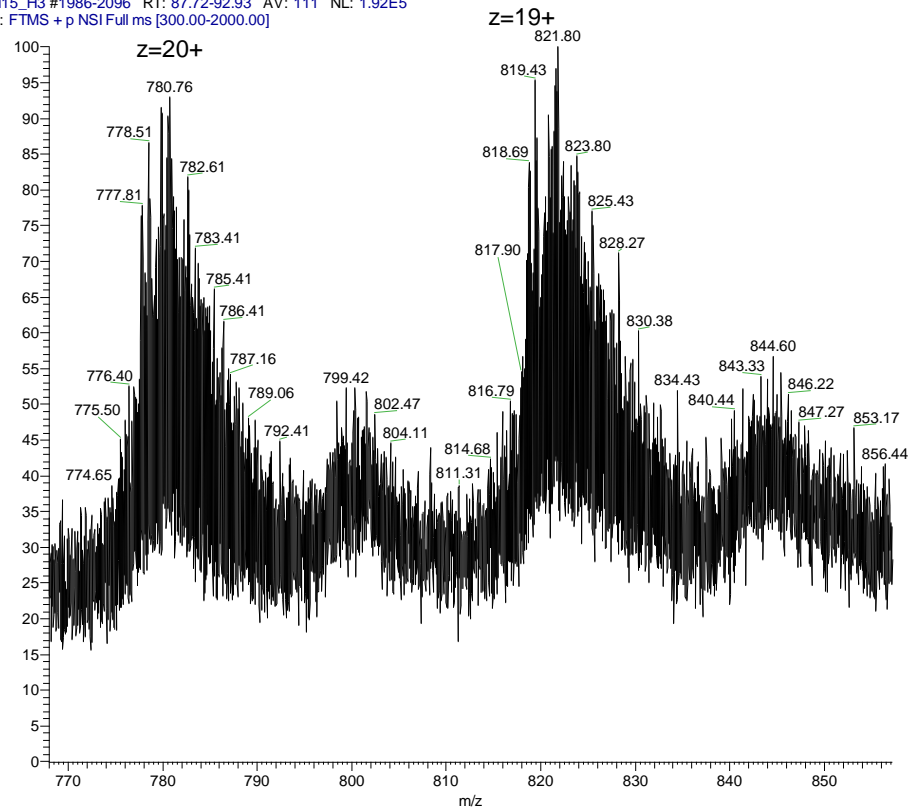
# Figure S3. Mass analysis of WT and $^{15}\text{N}$ intact histone H3

WT\_H3 #2005-2268 RT: 87.26-99.49 AV: 264 NL: 1.70E5  
T: FTMS + p NSI Full ms [300.00-2000.00]



WT H3

N15\_H3 #1986-2096 RT: 87.72-92.93 AV: 111 NL: 1.92E5  
T: FTMS + p NSI Full ms [300.00-2000.00]



$^{15}\text{N}$  H3