Supporting Online Material

Sirt5 Is an NAD-Dependent Protein Lysine Demalonylase and Desuccinylase

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Material and Methods

Reagents and Instrumentation.

Reagents were obtained from Aldrich or Acros in the highest purity available and used as supplied. ^{1}H NMR, ^{13}C NMR and 2D NMR were performed on INOVA 500 spectrometer. NMR data was analyzed by MestReNova (version 5.2.5). LCMS was carried out on a SHIMADZU LC-MS-QP8000α with a Sprite TARGA C18 column (40 × 2.1 mm, 5 μm, Higgins Analytical, Inc., Mountain View, CA) monitoring at 215 and 260 nm. Solvents used in LC-MS were water with 0.1% formic acid and acetonitrile with 0.1% formic acid.

Synthesis of Fmoc-Lys(*t*Bu-malonyl)-OH and Fmoc-Lys(*t*Bu-succinyl)-OH.

Mono-*t*butyl-malonate (480 mg, 3.0 mmol) or mono-*t*butyl-succinate (522.0 mg, 3 mmol) in anhydrous N,-N'-dimethylformamide (DMF, 2.0 mL) was added N-hydroxysuccinimide (334 mg, 2.9 mmol) with stirring at room temperature. Then N,N'-dicyclohexylcarbodiimide (598 mg, 2.9 mmol) in anhydrous DMF (3.0 mL) was added to the reaction. After stirring for 2 h, the reaction mixture was filtered. The filtrate was added to a solution of Fmoc-Lys-OH (736 mg, 2.0 mmol) with N, N-diisopropylethylamine (DIEA, 1.0 mL, 5.8 mmol) in anhydrous DMF (2.0 mL) at room temperature. The resulting reaction mixture was stirred for another 30 min. Then the reaction mixture was added 10 mL water and 6 mL 1 M HCl to adjust pH to 2~3. The mixture was extracted three times by 100 mL ethyl acetate and washed twice with 50 mL brine. The organic layer was dried with anhydrous sodium sulfate. After removal of the solvents *in vacuo*, the residue was purified by silica gel column using 10:1 CH_2Cl_2:CH_3OH to give the desired product in about 90% yield.

Fmoc-Lys(tBu-Malonyl)-OH. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.01 (t, 1H, J = 5.5 Hz), 7.89 (d, 2H, J = 7.0 Hz), 7.73 (dd, 2H, J = 2.0, 7.0 Hz), 7.46 (d, 1H, J = 7.0 Hz), 7.41 (t, 2H, J = 7.5 Hz), 7.33 (t, 2H, J = 7.8 Hz), 4.27 (m, 2H), 4.23 (m, 1H), 3.87 (dt, 1H, J = 4.5, 8.5 Hz), 3.07 (s, 2H), 3.03 (m, 2H), 1.65 (m, 2H), 1.39 (m, 2H), 1.38 (s, 9H), 1.33 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 174.26, 167.29, 165.24, 156.08, 143.91, 143.84, 140.75, 140.73, 127.66, 127.11, 125.33, 120.15, 120.14, 80.40, 80.39, 65.57, 54.10, 46.70, 43.74, 38.51, 30.74, 28.65, 27.70, 23.02. LC-MS (ESI) calcd. for $C_{28}H_{35}N_2O_7$ [M+H^+] 511.3, obsd. 510.8.

Fmoc-Lys(tBu-Succinyl)-OH. ^{1}H NMR (500 MHz, CD_3OD): δ 7.75 (d, 2H, *J* = 8.0 Hz), 7.61 (dd, 2H, *J* = 4.5, 7.5 Hz), 7.35 (t, 2H, *J* = 7.3 Hz), 7.26 (t, 2H, *J* = 7.3 Hz), 4.30 (m, 2H), 4.15 (t, 1H, *J* = 7.0 Hz), 4.04 (dd, 1H, *J* = 4.5, 8.0 Hz), 3.14 (t, 2H, *J* = 7.0 Hz), 2.49 (t, 2H, *J* = 7.0 Hz), 2.38 (t, 2H, *J* = 7.3 Hz), 1.75 (m, 2H), 1.49 (m, 2H), 1.40 (s, 9H), 1.37 (m, 2H). ^{13}C NMR (125 MHz, CD_3OD): δ 180.22, 174.46, 173.72, 158.64, 145.49, 145.28, 142.69, 142.68, 128.91, 128.29, 126.38, 126.35, 121.07, 81.81, 67.97, 57.11, 48.54, 40.37, 33.23, 31.93, 31.87, 30.17, 28.48, 24.32. LC-MS (ESI) calcd. for $C_{29}H_{37}N_2O_7$ [$M+H^+$] 525.3, obsd. 524.8.

Cloning, expression and purification of human Sirtuins

Human Sirt1, 2, 3, 5, and 6 were expressed as previously described (*26*). Sirt4 was not included in this study because of the expression problem. Human *Sirt7* coding sequence was PCR-amplified using primers JT072_SIRT7(1-400)EcoRI5 (5'-AGTCAGGAATTCATGGCAGCCGGGGGTCT-3') and JT073_Sirt7(1-400)XhoI3 (5'-AGTCAGCTCGAGTTACGTCACTTTCTTCCTTTTT-3'). Amplified product was digested with EcoRI and XhoI. The digested PCR product was purified and ligated into the similarly digested expression vector pET28a. C-terminal Flag-tagged *Sirt5* (*Flag-Sirt5*) and truncated *Sirt5(34-302)* were cloned using TOPO and Gateway cloning technology (Invitrogen Corp., Carlsbad, CA) into pDEST-F1 for expression. Sirt7, Flag-Sirt5 and Sirt5(34-302) were expressed in *E. coli* and purified as described (*26*). After purification, Flag-Sirt5 and Sirt5(34-302) were digested by TEV at room temperature for 2 h and purified by HisTrap^{TM} HP Column (GE Healthcare, Piscataway, NJ) and gel filtration on a HiLoad 26/60 Superdex 75 prep grade column (GE Healthcare, Piscataway, NJ). Protein concentrations were determined by Bradford reagent.

Sirt5 X-ray diffraction data collection and structural refinement.

Sirt5-H3K9 thioacetyl peptide and Sirt5-H3K9 succinyl peptide were prepared at a 1:20 protein:peptide molar ratio and incubated for 30~60 min on ice. Crystals were grown by the method of hanging drop vapor diffusion. Sirt5-H3K9 succinyl peptide co-crystals were soaked in 10 mM NAD for 10~120 min before data collection. All the X-ray diffraction data were collected at CHESS (Cornell High Energy Synchrotron

Source) A1 or F1 station. The data were processed using the programs HKL2000 (*27*). The two structures of Sirt5 complexes were solved by molecular replacement using the program Molrep from the CCP4 suite of programs (*28*). The Sirt5-ADPR structure (PDB code: 2B4Y) was served as the searching template. Refinement and model building were performed with REFMAC5 and COOT from CCP4. The X-ray diffraction data collection and structure refinement statistics are shown in Table S5.

Synthesis of acetyl, thioacetyl, malonyl, and succinyl peptides

Acetyl, thioacetyl, malonyl and succinyl peptides were synthesized on Wang resin using standard Fmoc/*t*Bu chemistry *O*-benzotriazol-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate/1-hydroxybenzotriazol (HBTU/HOBt) protocol (*26*). Modified lysine was incorporated using Fmoc-Lys(acetyl)-OH, Fmoc-Lys(thioacetyl)-OH (*29*), Fmoc-Lys(*t*Bu-malonyl)-OH and Fmoc-Lys(*t*Bu-succinyl)-OH. Cleavage from the resin and removal of all protecting groups were done by incubating the resin with trifluoroacetic acid (TFA) containing phenol (5%), thioanisole (5%), ethanedithiol (2.5%), and water (5%) for 2 h. The crude peptides were purified by reverse phase HPLC on BECKMAN COULTER System Gold 125P solvent module & 168 Detector with a TARGA C18 column (250 × 20 mm, 10 μm, Higgins Analytical, Inc., Mountain View, CA) monitoring at 215 nm. Mobile phases used were 0.1% aqueous TFA (solvent A) and 0.1% TFA in acetonitrile (solvent B). Peptides were eluted with a flow rate of 10 mL/min with the following gradient: 0% solvent B for 5 min, then 0 % to 25% solvent B over 25 min. The identity and purity of the peptides were verified by LC-MS. Table S1 and S4 listed the synthetic peptides.

Deacetylation, demalonylation, and desuccinylation activity assay and determination of k_{cat} and K_m.

The deacylase activity of human Sirt1, Sirt2, Sirt3, Sirt5, Sirt6 and Sirt7 were measured by detecting the deacylated peptide from the acyl peptides (Table S1 and S4) using LC-MS. Purified sirtuin was incubated with 0.3 mM acyl peptides, 1.0 mM NAD in 20 mM Tris-HCl buffer (pH 7.5) containing 1 mM DTT in 60 μL reactions for 2 h at 37°C. The reactions were stopped with 60μL 10% TFA and analyzed by LC-MS.

For determination of k_{cat} and K_m, human Sirt1, Sirt2, Sirt3 and Sirt5 were measured by detecting the deacylated peptide from H3K9 acyl peptides using HPLC. Purified sirtuin was incubated with 1.0 mM of

NAD in 20 mM Tris-HCl buffer (pH 7.5) containing acyl peptides (0–750 μM) and 1 mM DTT in 60 μL reactions at 37°C. The reactions were stopped with 100 mM HCl and 160 mM acetic acid, analyzed by HPLC with a reverse phase C18 column (250 × 4.6 mm, 90 A, 10 μm, GraceVydac, Southborough, MA), with a linear gradient of 0% to 20% B for 10 min (1 mL/min). Product quantification was based on the area of absorption monitored at 215 nm, assuming hydrolysis of the acyl group does not affect the absorption. The k_{cat} and K_m values were obtained by curve-fitting the $V_{initial}$/[E] versus [S] plot using KaleidaGraph. For Sirt5 R105M, the observed second order rate constant, k_{obs} (rate/([Sirtuin][NAD])) was detected instead of k_{cat} and K_m because of the very weak deacylation activity. The experiments were done in duplicate.

For comparing the deacetylation, demalonylation and desuccinylation activities of Sirt5 on different peptide backbones (results shown in Table 1), histone H3, GDH and ACS2 peptides with two tryptophan residues at the C-terminal were used to allow better detection and quantification on HPLC. The peptide sequences were listed in Table S4. The determination of k_{cat} and K_m was carried out essentially the same as mentioned above with slight modifications. The reactions were quenched with 60 μL 10% TFA. The chromatography gradient was 0% to 50% B for 20 min (1 mL/min). The peptides were detected and quantified on the LC by the absorption at 280 nm.

Purification of *O*-Ma-ADPR and *O*-Su-ADPR with HPLC and analysis by MS.

Sirt5 or Sirt1 (1 μM) was incubated with 0.5 mM malonyl or succinyl peptides and 1.0 mM NAD in 20 mM Tris-HCl buffer (pH 7.5) with 1 mM DTT in 60 μL reactions for 2 h at 37°C. The reactions were terminated by adding 60 μL 10% TFA. After centrifugation to remove precipitated proteins, the supernatant was analyzed by HPLC using a 50 mM ammonium acetate isocratic system on a Sprite TARGA C18 column (40 × 2.1 mm, 5 μm, Higgins Analytical, Inc.). The product *O*-Ma-ADPR (retention time 1.6 min) and *O*-Su-ADPR (retention time 2.3 min) was collected and the molecular weights were confirmed by MALDI-MS (Fig. S10). The ADPR (retention time 1.2 min) and NAD (retention time 5 min) have also been confirmed by MALDI-MS.

O-Su-ADPR generated from bovine liver mitochondrial peptide mixtures (Fig. S12) was purified as above and analyzed using an Agilent 1100 high-performance liquid chromatographer coupled to an ABI 4000 Q-trap mass spectrometer operating in IDA negative ion mode. Chromatography consisted of an HILIC column (Nest Group, 100 Å, 5 μm, polyhydroxyethyl A, 1 × 150 mm) eluted with a gradient of acetonitrile versus 10 mM ammonium acetate at 0.05 ml/min. This extra LC step was needed because the sample was more complicated than the reactions using only synthetic peptides.

Detection of succinyl lysine from bovine liver mitochondrial proteins using the ^{32}P-NAD assay.

Bovine liver mitochondria was isolated as previously described (*30*). Mitochondria from 5 g bovine liver was lysed for 30 min at 4°C in ice-cold lysis buffer (25 mM Tris-HCl pH 8.0, 50 mM NaCl, 0.1% Triton X-100) containing protease cocktail inhibitor (P8340, Sigma). The supernatants were collected and exchanged to 25 mM Tris-HCl (pH 8.0) with 50 mM NaCl using centrifugal filter (MILLIPORE, Billerica, MA) to remove endogenous NAD. The extracts were stored at -80°C. For trypsin digestion, 1.5 mg of the bovine liver mitochondria proteins or BSA (used as the control) was dissolved in 6 M urea, 60 mM Tris-HCl (pH 8.0), 15 mM DTT in a reaction volume of 450 μL. The solution was heated at 95°C for 15 min and then cooled to room temperature. Then 22.5 μL of 1M iodoacetamide (final concentration ~50 mM) was added and the mixture was incubated at room temperature with gentle mixing for 1 h. Then 3.6 mL of 50 mM Tris-HCl (pH 7.4) with 1 mM $CaCl_2$ was added to the reaction mixture to lower the urea concentration to 0.75 M. Finally, 150 μL of 100 μg/mL modified trypsin (Promega Corporation, Madison, WI) were added and the reaction mixture was incubated at 37 °C for 12 h. After quenching the reaction by adding 65 μL 10% TFA to pH 2~3, the digested peptides were desalted by using Sep-Pak C18 cartridge 1cc/50 mg (Waters Corporation, Milford, MA) and lyophilized.

To detect the acyl-ADPR compounds formed in sirtuin-catalyzed deacylation reactions, reactions were performed in 10 μL solutions with 1 μCi ^{32}P-NAD (ARC Inc., ARP 0141, 800Ci/mmol, 0.125μM), 50 mM Tris-HCl pH 8.0, 150 mM NaCl, 10 mM DTT. The acyl peptide substrates used were 100 μM H3K9 acetyl, malonyl, or succinyl peptide, 2 μg calf thymus histones (Roche Applied Science, Indianapolis, IN), 20 μg

bovine liver mitochondrial peptides, or 20 μg BSA peptides. The reactions were incubated with 1 μM Sirt5 or Sirt1 at 37°C for 1 h. CD38 catalytic domain was used to generate ADPR as a control. A total of 0.5 μL of each reaction were spotted onto silica gel TLC plates and developed with 7:3 ethanol:ammonium bicarbonate (1 M aqueous solution). After development, the plates were air-dried and exposed to a PhosphorImaging screen (GE Healthcare, Piscataway, NJ). The signal was detected using a STORM860 phosphorimager (GE Healthcare, Piscataway, NJ).

Affinity purification of lysine-succinyl peptides and protein identification

Flag-Sirt5 (25 μg) was bound onto 100 μL anti-Flag M2 affinity gel (A2220, Sigma) by incubation at 4°C in NETN buffer (50 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5% NP40) for 2 hr (*31*). The supernatant was removed and the gel was washed three times with NETN buffer. The tryptic mitochondria peptides (~1 mg) obtained above were resolubilized in 0.5 mL NETN buffer and insoluble particles were removed by centrifugation at 10,000 × g for 10 min. Affinity purification was carried out by incubating the peptides with Flag-Sirt5 bound anti-Flag M2 affinity gel at 4°C for 3 h with gentle shaking. The gel was washed three times with 1 mL of NETN buffer and twice with ETN buffer (50 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA). The bound peptides were eluted three times with 100 μL of 0.1% TFA. The elutions were combined and lyophilized. The resulting peptides were cleaned using C18 ZipTips (Millipore, Bedford, Massachusetts) according to the manufacture's instructions, prior to LC-MS/MS analysis performed at the Proteomic and MS Facility of Cornell University. Tandem mass spectra were searched against NCBI-nr database with MASCOT search engine (Matrix Science, London, UK) using acetyl, malonyl, and succinyl lysine as modifications.

Detection of lysine succinylation on CPS1 peptides using the ^{32}P-NAD assay

The CPS1 band was cut from a SDS-PAGE gel of the bovine liver mitochondria lysate. The protein was in-gel digested with trypsin and extracted and desalted as the following. The gel band was washed in 100 μL water for 5 min, followed by 100 μL 100 mM Ammonium bicarbonate : acetonitrile (1:1) for 10 min and finally 50 μL acetonitrile for 5 min. The acetonitrile was then discarded and the gel band was allowed to

dry in the ventilated fume hood for 5-10 min. The gel slice was then rehydrated with 15 μL trypsin solution (10 μg/mL modified trypsin in 1mM HCl) on ice for 30 min. The trypsin solution was topped with 10 μL 50 mM Ammonium bicarbonate with 10% acetonitrile. The digestion reactions were kept at 30°C for 12 h. The resulting solution was acidified with formic acid (1% in final). The trypsin digested peptides were extracted twice with 30 μl of 50% acetonitrile with 0.2% TFA (45 min incubation at room temperature followed by 5 min sonication). The third extraction was done with 30 μl of 90% acetonitrile with 0.2% TFA (5 min). All the extracts were combined and lyophilized. When dried, the peptides were dissolved in 12 μl of 0.1% TFA and desalted by ZipTips (Millipore, Bedford, Massachusetts). The desalted peptides were lyophilized again and reconstituted in water. The GDH peptides from in-gel digestion and the histone peptides from in-solution digestion were prepared as described above. The ^{32}P-NAD assays were carried out as described above. To lower the detection limit, higher concentrations of sirtuins were used. The Sirt5 was used at a final concentration of 52 μM and the Hst2 was used at 24 μM. The sample peptides were used at a concentration of 0.3 μg/μl and the control peptides were at 20 μM.

Generation of Sirt5 deficient mouse line

Sirt5 +/+ and -/- mice were generated at the Institut Clinique de la Souris (Strasbourg, France). Briefly, exon 4 of Sirt5 locus was flanked with loxP sites using standard genetic engineering and gene targeting procedures. The resulting Sirt5 floxed mice were bred with CMV-Cre transgenic mice to generate germline Sirt5 deficient (Sirt5 -/-) mice and control Sirt5 +/+ mice. The absence of Sirt5 mRNA in different tissues of Sirt5 -/- mice was confirmed by Q-RT-PCR analysis and the loss of Sirt5 protein expression was verified by western blot using an anti-Sirt5 antibody (Abcam ab62740)

Preparation of liver lysate from Sirt5 wt and KO mouse

The 29-week-old male Sirt5 +/+ and -/- littermates were fasted overnight (from 6:00 PM to 10:00 AM) and then provided with free access to food for four hours prior to sacrifice. Liver tissues were rapidly removed, snap-frozen with liquid nitrogen, and stored at -80°C for analysis. The liver samples were first broken into small pieces, and then homogenized in 1mL of the lysis buffer (50 mM Tris-HCl pH 8.0, 150

mM NaCl, 2 mM EDTA, 0.1% NP-40, 10% glycerol). The crude lysates were incubated at 4°C on shaker for 30 min then centrifuged at 10 000 g, 4°C for 10 min. The concentration of the lysate was determined by Bradford assay.

CPS1 activity assay

CPS1 activities assay was carried out as described by Fahien and Cohen (*32*). The reaction was initiated by addition of the liver lysates to the rest of the reaction mixture. The reaction mixture contained 50mM Tris-HCl pH 8.0, 2.5 mM phosphoenopyruvate, 0.2 mM NADH, 30 mM NH_4Cl, 100 mM $KHCO_3$, 5 mM ATP, 10 mM MgSO4, 10 mM N-acetylglutamate, 15 U/ml pyruvate kinase / lactate dehydrogenase (SIGMA P0294). The reactions were performed at 37 degree and the decrease in absorbance at 340 nm was monitored. The initial velocity of the reaction was calculated to get the CPS1 activity. The activity assay was done with three pairs of Sirt5 wt and KO mice.

Digestion of selected proteins for nano LC-MS/MS analysis

Commercial GDH (Sigma G2626), malate dehydrogenase (Sigma M2634), citrate synthase (Sigma C3260), and pyruvate dehydrogenase (Sigma P7032) were in-solution digested with trypsin. Typically, 1.0 mg of the protein was dissolved in 6 M guanidine hydrochloride, 150 mM Tris-HCl (pH 8.0), 15 mM DTT in a reaction volume of 400 μL. The solution was incubated at room temperature for 60 min. Then 20 μL of 1M iodoacetamide (final concentration ~50 mM) was added and the mixture was incubated at room temperature with gentle mixing for another 60 min. The excess iodoacetamide was quenched with 40mM DTT. Then 3.6 mL of 50 mM Tris-HCl (pH 7.4) with 1 mM $CaCl_2$ was added to the reaction mixture to lower the guanidine hydrochloride concentration to 0.6 M. Finally, 100 μL of 100 μg/mL modified trypsin (Promega Corporation, Madison, WI) were added and the reaction mixture was incubated at 37 °C for 12 h. After quenching the reaction by adding 65 μL 10% TFA to pH 2~3, the digested peptides were desalted by using Sep-Pak C18 cartridge 1cc/50 mg (Waters Corporation, Milford, MA) and lyophilized prior to nanoLC-MS/MS analysis.

Identification of protein acylation by nanoLC-MS/MS analyses.

The tryptic digest was reconstituted in 50 μL of 2% acetonitrile with 0.5% formic acid and about 200 ng of tryptic digest were injected for nanoLC-ESI-MS/MS analysis. The initial analysis was performed in UltiMatePlus nanoLC (Dionex, Sunnyvale, CA) coupled with to a hybrid triple quadrupole linear ion trap mass spectrometer, 4000 Q Trap from ABI/MDS Sciex (Framingham, MA) equipped with Micro Ion Spray Head II ion source. Most of final analyses were carried out using UltiMate3000 nanoLC (Dionex, Sunnyvale, CA) coupled with a LTQ Orbitrap Velos (Thermo-Fisher Scientific, San Jose, CA) mass spectrometer equipped with "Plug and Play" nano ion source device (CorSolutions LLC, Ithaca, NY).

Tryptic peptides were separated on a PepMap C-18 RP nano column (5 μm, 75 μm i.d. × 150 mm, Dionex), eluted in a 60 to 90-minute gradient of 10% to 40% acetonitrile in 0.1% formic acid at 300 nL/min. For 4000 Q Trap analysis, MS data acquisition was acquired using Analyst 1.4.2 software (AB SCIEX, Framingham, MA) in the positive ion mode for information dependent acquisition (IDA) analysis with nanospray voltage at 1.85 kV and heated interface at 150°C used for all experiments. In IDA analysis, after each survey and an enhanced resolution scan, three highest intensity ions with multiple charge states were selected for tandem MS (MS/MS) with rolling collision energy applied for detected ions based on different charge states and *m/z* values. For Orbitrap analysis, each of tryptic digests was first acquired at CID-based parallel data-dependent acquisition (DDA) mode where FT mass analyzer was used for an MS survey scan followed by MS/MS scans on top 7 most intensity peaks with multiple charged ions above a threshold ion count of 5000 in LTQ mass analyzer. A time-based target acylated peptide inclusion list was then generated based on the preliminary results from CID mode. Finally HCD-based DDA mode with inclusion list was applied for confirmation of acylation modification, where one FT survey scan was followed by 5 MS/MS scan in FT analyzer. MS survey scans and MS/MS scans were acquired at a resolution of 60,000 and 7,500 (fwhm at *m*/*z* 400) respectively. The nano ion source was operated in positive ion mode with a voltage set at 1.5 kV and ion transfer tubing temperature at 225 °C. Either internal calibration using a background ion signal at m/z 445.120025 as a lock mass or external calibration using Ultramark 1621 for FT mass analyzer was performed. The normalized collision energy for CID and HCD were set at 35 % and 38% respectively. All data were acquired under Xcalibur 2.1 operation software (Thermo-Fisher Scientific).

Data analysis. The MS/MS data generated from both 4000 Q Trap and LTQ Orbitrap Velos were submitted to Mascot 2.3 (Matrix Science, Boston, MA) for database searching using in-house licensed Mascot local server and the search was performed to query to SwissProt database (taxonomy: mammal) with

three missed cleavage sites by trypsin allowed. The peptide tolerance was set to 1.2 Da and MS/MS tolerance was set to 0.6 Da for the data from 4000 Q Trap. For Orbitrap data, the peptide tolerance was set to 10 ppm and MS/MS tolerance was set to 0.6 Da (for MS/MS spectra acquired by LTQ) and 0.05 Da (MS/MS spectra acquired by Orbitrap FT mass analyzer). A fixed carbamidomethyl modification of cysteine and several variable modifications on lysine acetylation, malonylation, succinylation, and carbamylation, deamidation of asparagine and glutamine, and methionine oxidation were applied. Only significant scores for the peptides defined by Mascot probability analysis (www.matrixscience.com/help/scoring_help.html#PBM) greater than "identity" were considered for the peptide identification and modification site determinations. All MS/MS spectra for the identified peptides with acylation modifications were manually inspected and validated using both Proteome Discoverer 1.2 and Xcalibur 2.1 software (Thermo-Fisher Scientific, San Jose, CA).

Relative quantitation of acylated peptides by nanoLC-MS/MS analysis

For relatively quantitative analysis of acylated peptides across samples, the peak areas of detected precursor ions at each specific m/z corresponding to the acylated peptides were determined by extracted ion chromatogram (XIC). The XIC of two independent (non-target), tryptic peptides identified from the same proteins in the same LC-MS/MS runs were also used as reference control for normalization of loaded sample digests. Specifically, we acquired nanoLC-MS/MS files in triplicate by Orbitrap at HCD mode as described above for identification of acylated peptides. The peak area of target acylated peptides in one LC-MS/MS data file was first extracted using Xcalibur 2.1 software with mass tolerance at 5ppm and mass precision at 4 decimal. A layout template file was then generated and applied to all LC-MS/MS data files yielding peak area of all peptides of interest. Finally, peak area of each acylated peptide in each protein was normalized to the peak areas of the two reference peptides in each LC/MS/MS data file. The relative standard deviation (RSD) for most acylated peptides in three XIC measurements is less than 20%.

References for Methods

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Table S1. Acetyl peptides used to assay the deacetylase activity of sirtuins.

Protein/lysine residue	Peptide sequence	Predicted mass (m/z)	Observed mass (m/z)
Histone H3 K9 (1-17)	ARTKQTAR**(AcK)**STGGKAPR	928.6 $[M+2H]^{2+}$	928.5 $[M+2H]^{2+}$
Histone H3 K9 (4-15)	KQTAR**(AcK)** STGGKA	1274.7 $[M+H]^{+}$	1273.8 $[M+H]^{+}$
Histone H3 K14 (6-22)	TARKSTGG**(AcK)**APRKQLAT	907.0 $[M+2H]^{2+}$	906.7 $[M+2H]^{2+}$
Histone H4 K5 (1-16)	SGRG**(AcK)**GGKGLGKGGAK	728.9 $[M+2H]^{2+}$	728.8 $[M+2H]^{2+}$
Histone H4 K8 (1-16)	SGRGKGG**(AcK)**GLGKGGAK	728.9 $[M+2H]^{2+}$	728.8 $[M+2H]^{2+}$
Histone H4 K12 (1-16)	SGRGKGGKGLG**(AcK)**GGAK	728.9 $[M+2H]^{2+}$	728.6 $[M+2H]^{2+}$
Histone H4 K16 (8-24)	KGLGKGGA**(AcK)**RHRKVLRD	959.6 $[M+2H]^{2+}$	958.9 $[M+2H]^{2+}$
Histone H4 K91 (80-99)	TVTAMDVVYAL**(AcK)**RQGRTLYG	1142.6 $[M+2H]^{2+}$	1142.2 $M+2H]^{2+}$
Histone H2A K5 (1-17)	SGRG**(AcK)**QGGKARAKAKTR	900.0 $[M+2H]^{2+}$	899.3 $[M+2H]^{2+}$
Histone H2A K13 (1-17)	SGRGKQGGKARA**(AcK)**AKTR	900.0 $[M+2H]^{2+}$	899.8 $[M+2H]^{2+}$
Histone H2B K5 (1-18)	PEPA**(AcK)**SAPAPKKGSKKAV	917.1 $[M+2H]^{2+}$	916.7 $[M+2H]^{2+}$
Histone H2B K12 (1-18)	PEPAKSAPAPK**(AcK)**GSKKAV	917.1 $[M+2H]^{2+}$	916.8 $[M+2H]^{2+}$
Histone H2B K15 (1-18)	PEPAKSAPAPKKGS**(AcK)**KAV	917.1 $[M+2H]^{2+}$	916.4 $[M+2H]^{2+}$
α-tubulin K40 (32-48)	PDGQMPSD**(AcK)**TIGGGDDS	859.9 $[M+2H]^{2+}$	859.4 $[M+2H]^{2+}$
p53 K382 (374-389)	GQSTSRHK**(AcK)**LMFKTEG	939.0 $[M+2H]^{2+}$	938.5 $[M+2H]^{2+}$
Acetyl-CoA synthetase 2 K642 (620-636)	RLPKTRSG**(AcK)**VMRRLLRK	1069.2 $[M+2H]^{2+}$	1068.6 $[M+2H]^{2+}$

Table S2. The kinetic parameters of four human sirtuins on H3 K9 acetyl peptide

Sirtuins	k_{cat} (s^{-1})	K_m **for acetyl peptide** (μM)	k_{cat}/K_m ($s^{-1}M^{-1}$)
Sirt1	0.039 ± 0.001	38 ± 4	1.0×10^3
Sirt2	0.030 ± 0.001	190 ± 14	1.6×10^2
Sirt3	0.012 ± 0.001	50 ± 9	2.4×10^2
Sirt5	ND*	ND (>750)*	2.0

* The k_{cat} and K_m values for Sirt5 could not be determined because the V versus [S] plot was linear (K_m was greater than the highest substrate concentration tested). Thus only k_{cat}/K_m value can be obtained.

The k_{cat} and K_m values were obtained by curve-fitting the $V_{initial}/[E]$ versus [S] plot using KaleidaGraph**.**

Table S3. The kinetic parameters of mutant Sirt5 on H3K9 acetyl and succinyl peptides

		k_{cat} (s^{-1})	K_m for peptide (μM)	k_{cat}/K_m ($s^{-1}M^{-1}$)
Sirt5	**deacetylation**	ND*	ND (> 750)*	2
	desuccinylation	0.029 ± 0.002	41 ± 11	710
Sirt5 H158Y	**deacetylation**	no activity observed	no activity observed	-
	desuccinylation	ND*	ND (> 750)*	75
Sirt5 Y102F	**deacetylation**	ND*	ND (> 750)*	2
	desuccinylation	ND*	ND (> 750)*	397
Sirt5 R105M	**deacetylation**	ND*	ND (> 750)*	0.5
	desuccinylation	ND*	ND (> 750)*	0.9

* The k_{cat} and K_m values cannot be determined because the V versus [S] plot is linear (K_m is much greater than the highest substrate concentration tested, 750 μM). Thus only k_{cat}/K_m value can be obtained.

Table S4. Acetyl, thioacetyl, malonyl, and succinyl peptides used for Sirt5 crystallization and kinetics studies.

Protein/lysine residue	**Peptide sequence**	**Predicted mass (m/z)**	**Observed mass $[M+H]^+$ (m/z)**
Histone H3 K9 (4-15)	KQTAR**(TacK)**STGGKA	1290.7 $[M+H]^+$	1290.0 $[M+H]^+$
Histone H3 K9 (4-15)	KQTAR**(MaK)**STGGKA	1318.7 $[M+H]^+$	1317.7 $[M+H]^+$
Histone H3 K9 (4-15)	KQTAR**(SuK)**STGGKA	1332.7 $[M+H]^+$	1331.3 $[M+H]^+$
Histone H3 K9 (4-13)WW	KQTAR**(AcK)**STGGWW	724.4 $[M+2H]^{2+}$	724.7 $[M+2H]^{2+}$
Histone H3 K9 (4-13)WW	KQTAR**(MaK)**STGGWW	746.4 $[M+2H]^{2+}$	746.7 $[M+2H]^{2+}$
Histone H3 K9 (4-13)WW	KQTAR**(SuK)**STGGWW	753.4 $[M+2H]^{2+}$	753.7 $[M+2H]^{2+}$
GDH K503 (498-509)WW	SGASE(**AcK**)DIVHSGWW	800.9 $[M+2H]^{2+}$	801.2 $[M+2H]^{2+}$
GDH K503 (498-509)WW	SGASE(**MaK**)DIVHSGWW	822.9 $[M+2H]^{2+}$	823.1 $[M+2H]^{2+}$
GDH K503 (498-509)WW	SGASE(**SuK**)DIVHSGWW	829.9 $[M+2H]^{2+}$	830.2 $[M+2H]^{2+}$
ACS2 K628 (623-632)WW	KTRSG**(AcK)**VMRRWW	816.9 $[M+2H]^{2+}$	817.1 $[M+2H]^{2+}$
ACS2 K628 (623-632)WW	KTRSG**(MaK)**VMRRWW	838.9 $[M+2H]^{2+}$	839.1 $[M+2H]^{2+}$
ACS2 K628 (623-632)WW	KTRSG**(SuK)**VMRRWW	845.9 $[M+2H]^{2+}$	846.4 $[M+2H]^{2+}$

Table S5. Data Collection and Refinement Statistics.

	Sirt5-Thioacetyl H3K9	Sirt5-Succinyl H3K9-NAD
Data collection		
Space group	P212121	P212121
Cell dimensions		
a, *b*, *c* (Å)	52.54, 67.88, 156.75	52.69, 69.41, 156.32
α, β, γ (°)	90, 90, 90	90, 90, 90
Resolution (Å)	50-2.00	50-1.55
R_{sym} or R_{merge} (%)	9.0 (34.4)	6.6 (39.8)
$I / \sigma I$	38.65 (4.23)	42.26 (5.15)
Completeness (%)	99.9 (99.8)	99.7 (98.5)
Redundancy	6.8 (5.3)	7.1 (5.7)
Refinement		
Resolution (Å)	30-2.00	30-1.55
No. reflections	39228	79514
R_{work} / R_{free} (%)	21.52/25.80	14.26/19.09
No. of protein atoms	8170	8170
No. of ligand/ion molecules		
Thioacetyl H3K9	2	---
Succinyl H3K9	---	2
CHES	2	---
NAD	---	2
Zn	2	2
No. of water	187	732
R.m.s deviations		
Bond lengths (Å)	0.0256	0.0230
Bond angles (°)	1.774	2.031

Numbers showed in the parentheses are for the highest resolution shell.

Table S6. Acetyl, malonyl, and succinyl GDH peptides identified by LC-MS/MS.

	Peptide sequence and modification
1	77-GASIVEDK(acetyl)LVEDLK-90
2	77-GASIVEDK(succinyl)LVEDLK-90
3	85-LVEDLK(acetyl)TR-92
4	108-IIK(acetyl)PCNHVLSLSFPIR-123
5	108-IIK(succinyl)PCNHVLSLSFPIR-123
6	152-YSTDVSVDEVK(acetyl)ALASLMTYK-171
7	152-YSTDVSVDEVK(acetyl)ALASLM(ox)TYK-171
8	152-YSTDVSVDEVK(succinyl)ALASLMTYK-171
9	172-CAVVDVPFGGAK(acetyl)AGVK-187
10	188-INPK(acetyl)NYTDNELEK-200
11	353-LQHGTILGFPK(acetyl)AK-363
12	353-LQHGTILGFPK(succinyl)AK-363
13	364-AK(acetyl)IYEGSILEVDCDILIPAASEK-386
14	366-IYEGSILEVDCDILIPAASEK(acetyl)QLTK-390
15	398-AK(acetyl)IIAEGANGPTTPEADK-415
16	398-AK(acetyl)IIAEGANGPTTPEADKIFLER-420
17	400- IIAEGANGPTTPEADK(acetyl)IFLER-420
18	400- IIAEGANGPTTPEADK(succinyl)IFLER-420
19	454-LTFK(acetyl)YER-460
20	454-LTFK(malonyl)YER-460
21	454-LTFK(succinyl)YER-460
22	478-FGK(acetyl)HGGTIPIVPTAEFQDR-496
23	497-ISGASEK(acetyl)DIVHSGLAYTMER-516
24	497-ISGASEK(malonyl)DIVHSGLAYTMER-516
25	497-ISGASEK(succinyl)DIVHSGLAYTMER-516
26	497-ISGASEK(succinyl)DIVHSGLAYTM(ox)ER-516
27	524-TAMK(acetyl)YNLGLDLR-535
28	524-TAM(ox)K(acetyl)YNLGLDLR-535
29	524-TAMK(malonyl)YNLGLDLR-535
30	524-TAMK(succinyl)YNLGLDLR-535
31	524-TAM(ox)K(succinyl)YNLGLDLR-535
32	536-TAAYVNAIEK(acetyl)VFR-548
33	536-TAAYVNAIEK(succinyl)VFR-548

Table S7. Acetyl, malonyl, and succinyl peptides identified on other metabolic enzymes

Proteins		Modified peptides
Malate dehydrogenase, mitochondrial	1	177-ANAFVAELK(acetyl)GLDPAR-191
	2	230-IQEAGTEVVK(succinyl)AK-241
	3	230-IQEAGTEVVK(malonyl)AK-241
	4	297-KGIEK(succinyl)NLGIGK-307
	5	298-GIEK(succinyl)NLGIGK-307
	6	302-NLGIGK(acetyl)ISPFEEK-314
	7	308-ISPFEEK(acetyl)M(ox)IAEAIPELK-324
	8	325-ASIK(acetyl)KGEEFVK-335
	9	325-ASIKK(malonyl)GEEFVK-335
	10	325-ASIK(succinyl)KGEEFVK-335
Citrate synthase, mitochondrial	1	74-GMK(acetyl)GLVYETSVLDPDEGIR-92
	2	74-GMK(succinyl)GLVYETSVLDPDEGIR-92
	3	74-GMK(succinyl)GLVYETSVLDPDEGIRFR-94

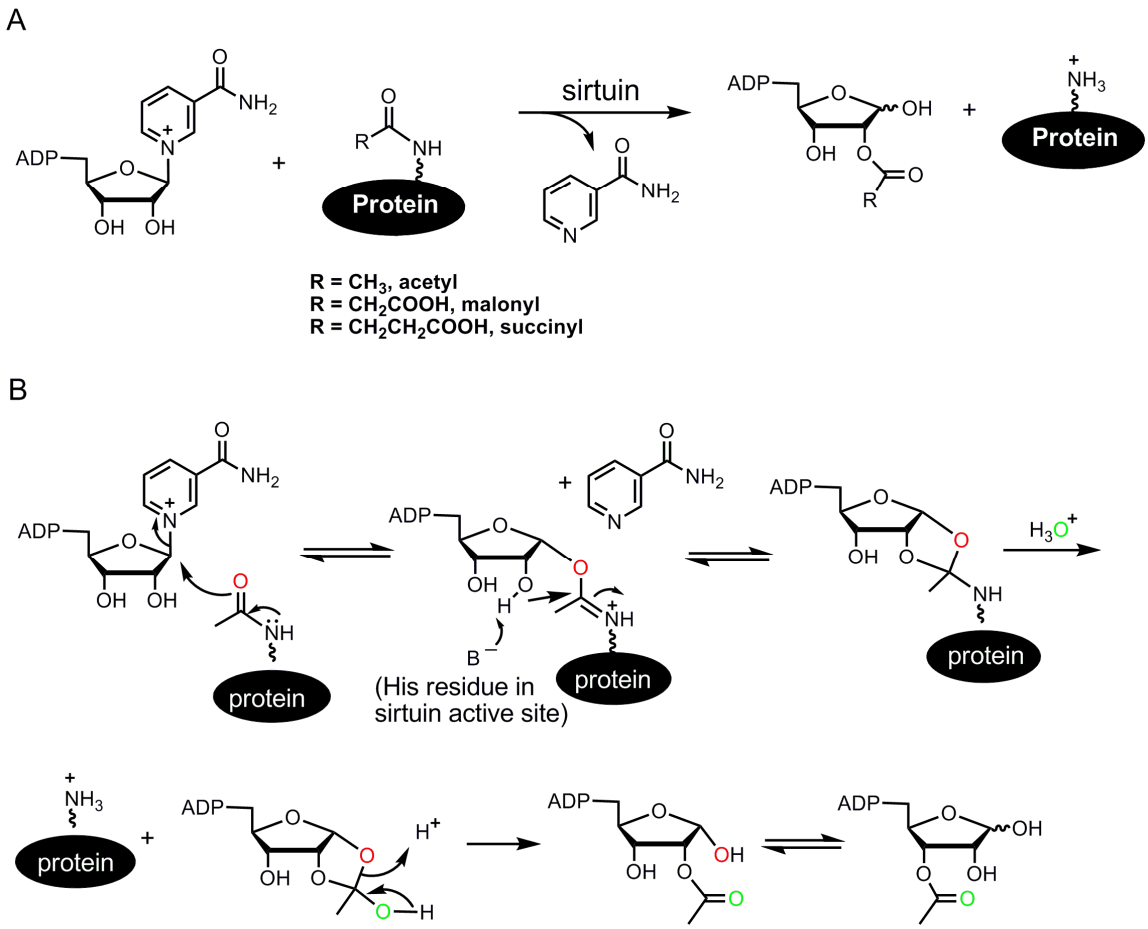
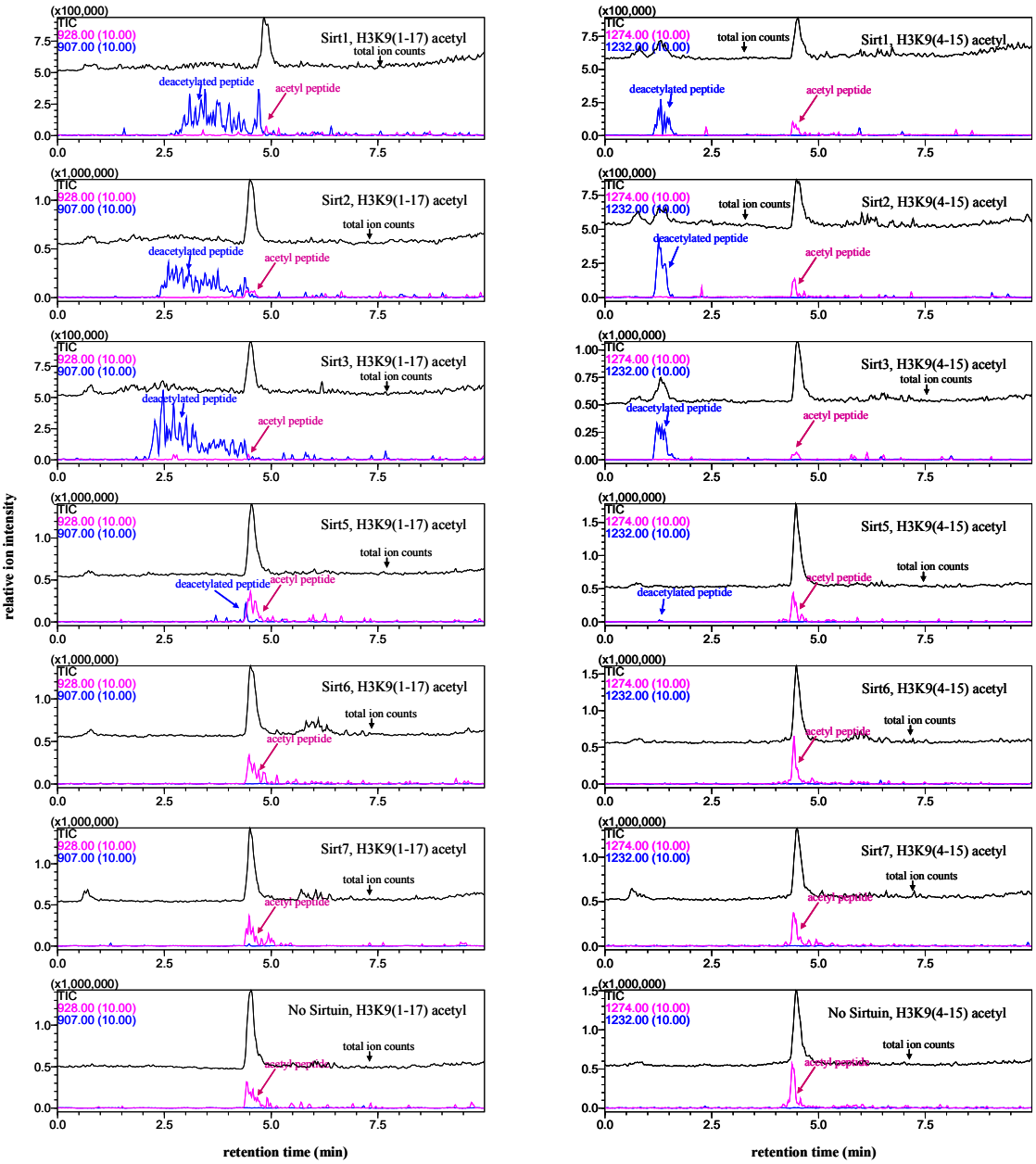
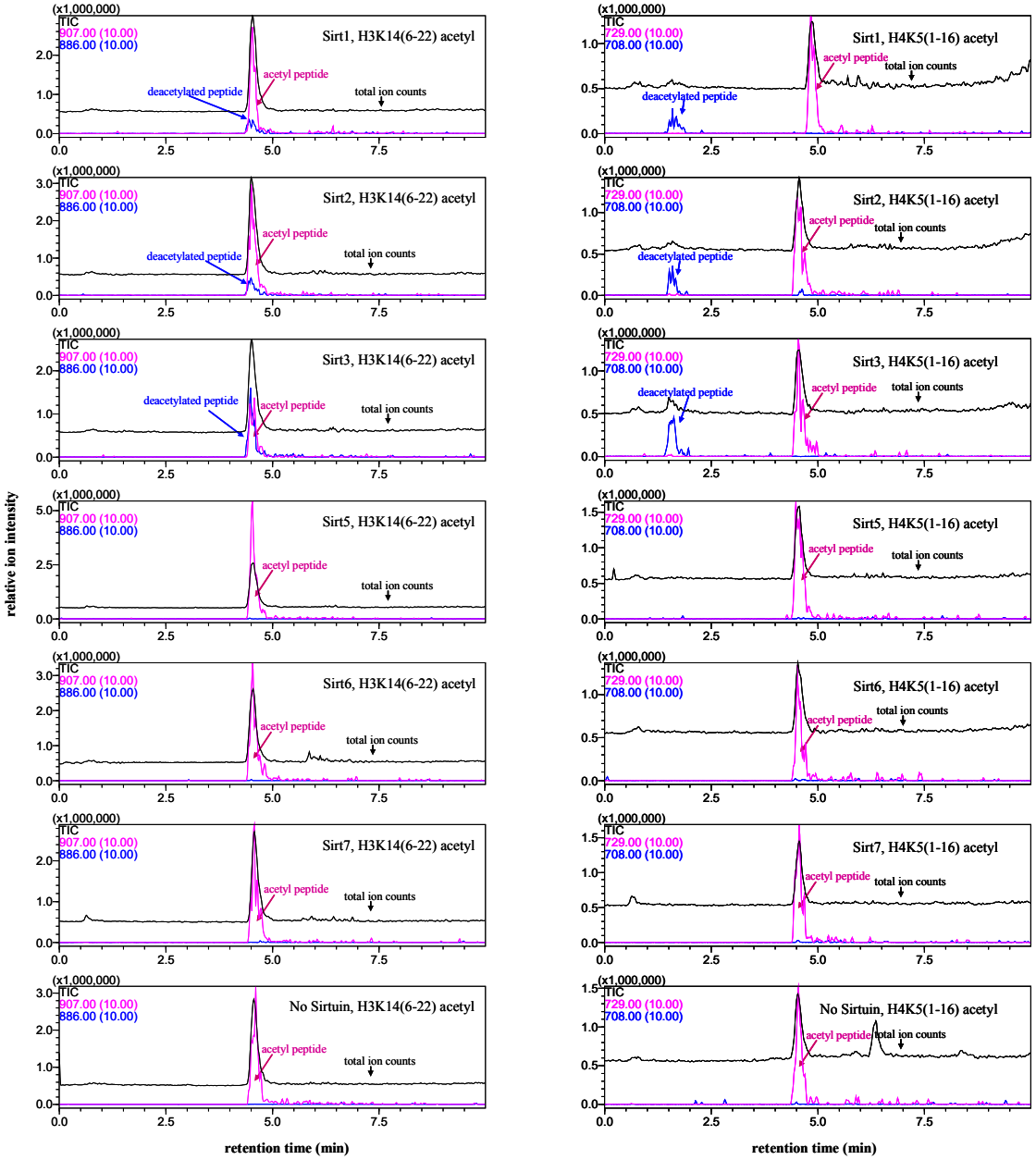
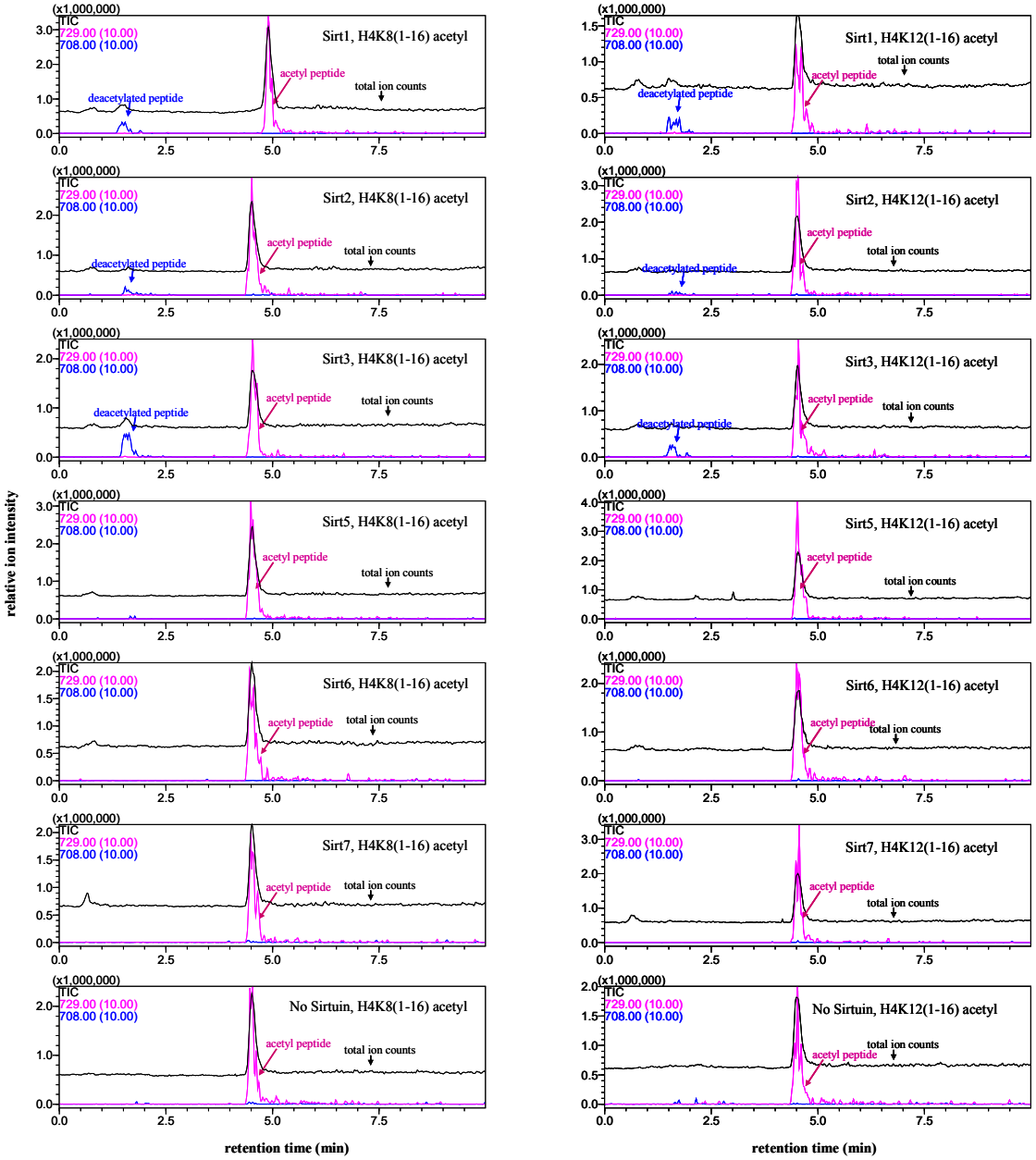
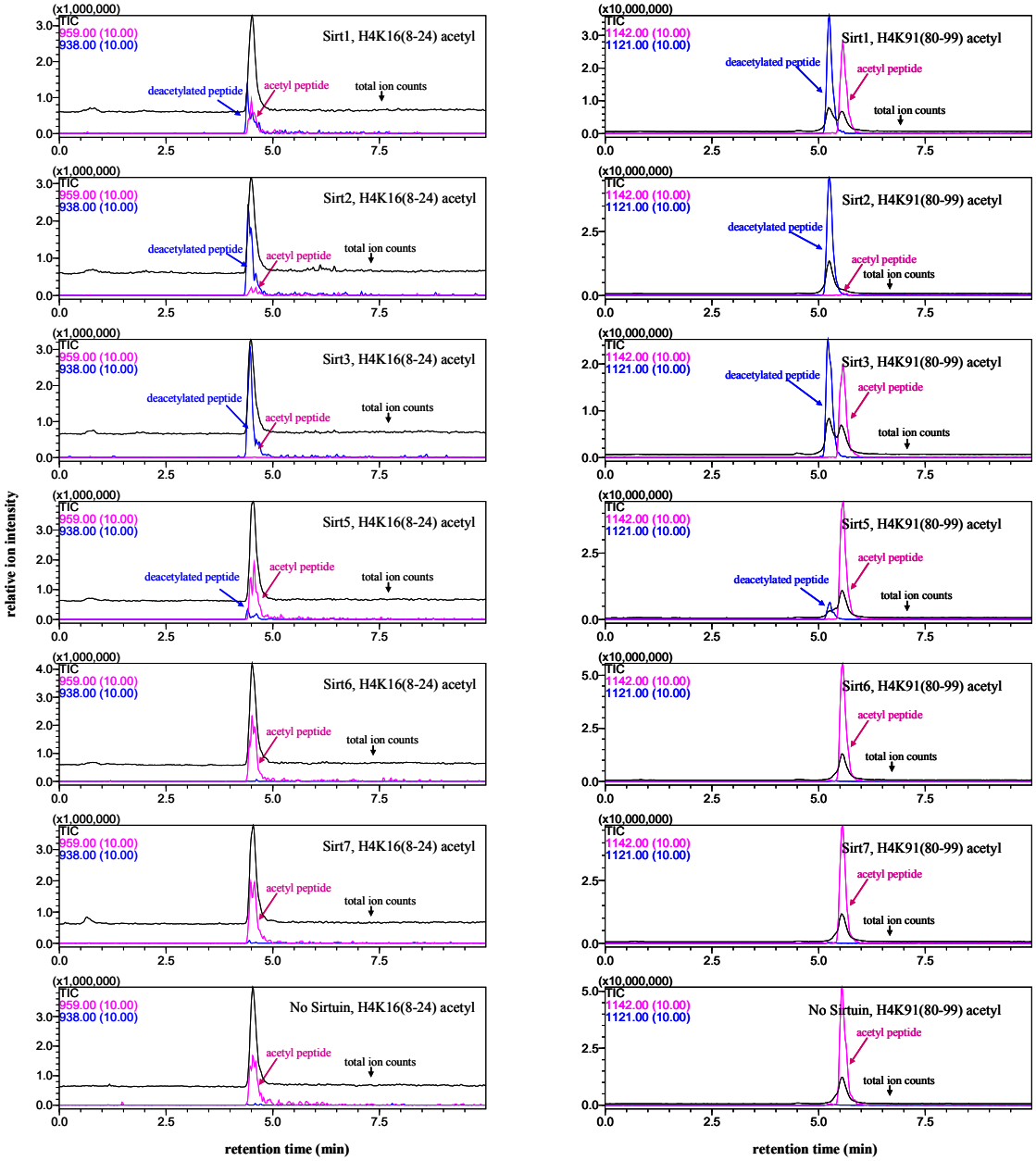


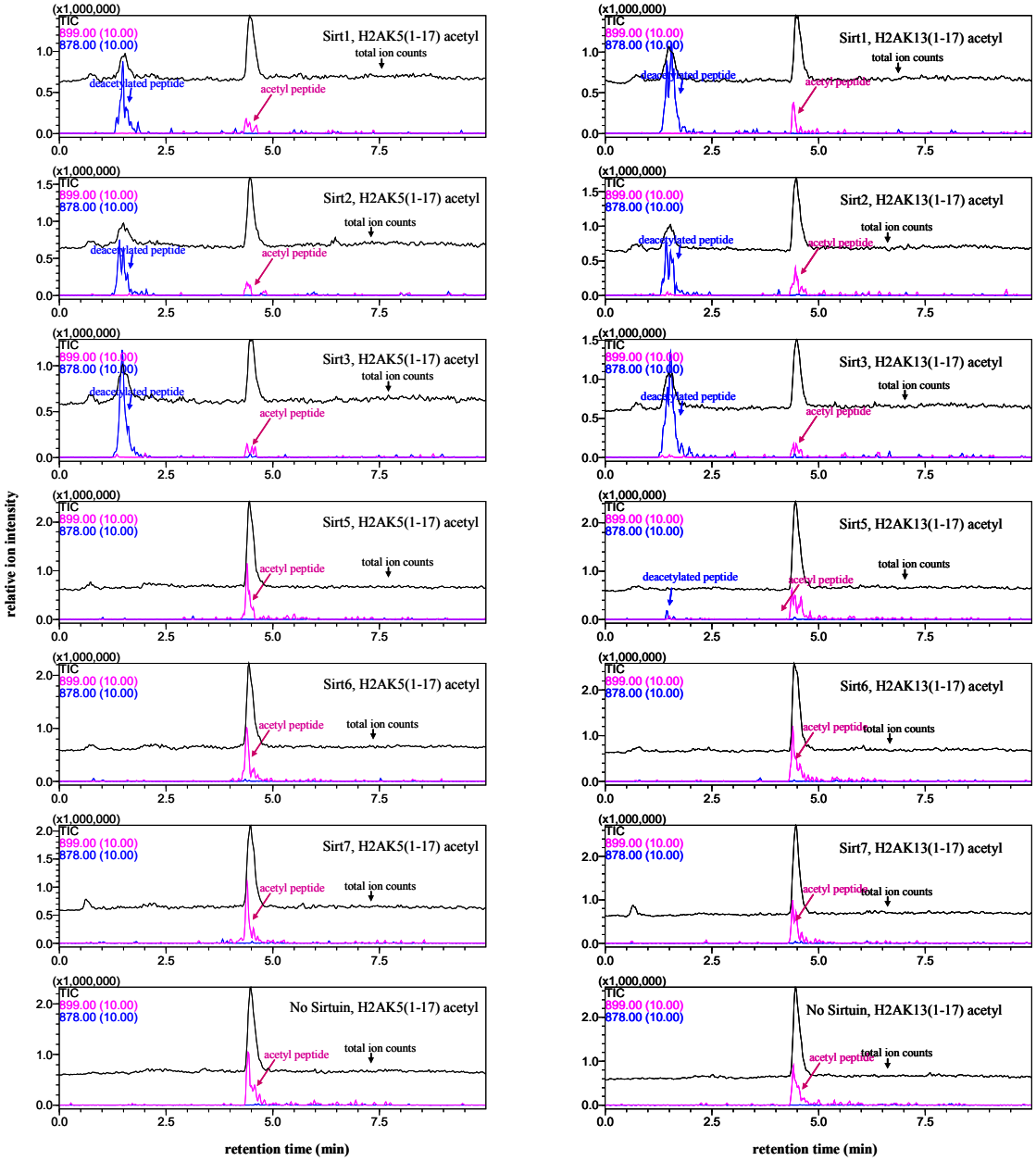
Fig. S1. (**A**) The sirtuins catalyzed NAD-dependent deacylation reaction is shown, and the reaction generates *O*-acyl-ADP-ribose. (**B**) Mechanism of sirtuin-catalyzed NAD-dependent deacetylation. Some of the oxygen atoms were colored differently to indicate where they come from.

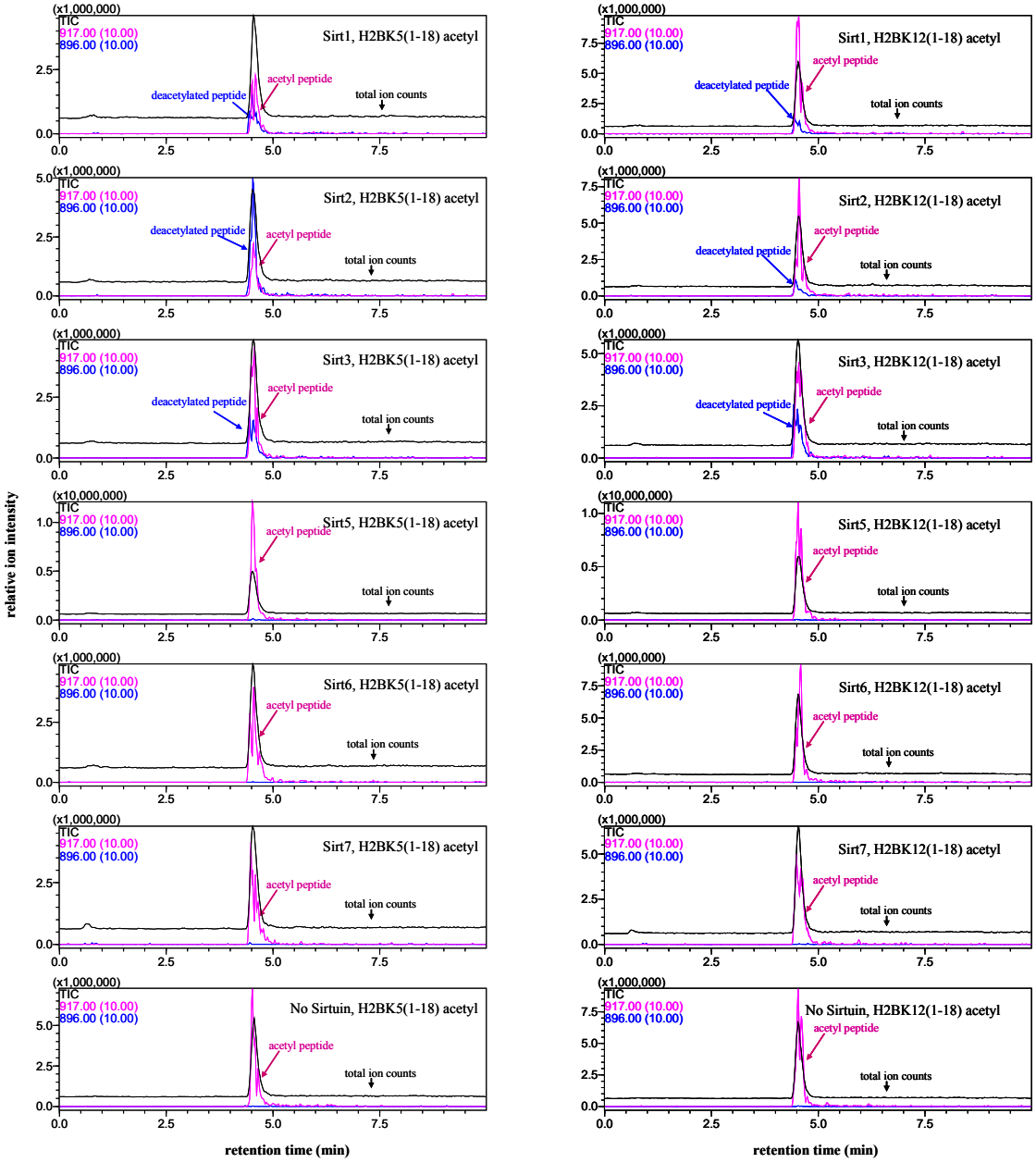

relative ion intensity
retention time (min)
retention time (min)
Sirt1, H3K9(1-17) acetyl
Sirt2, H3K9(1-17) acetyl
Sirt3, H3K9(1-17) acetyl
Sirt5, H3K9(1-17) acetyl
Sirt6, H3K9(1-17) acetyl
Sirt7, H3K9(1-17) acetyl
No Sirtuin, H3K9(1-17) acetyl
Sirt1, H3K9(4-15) acetyl
Sirt2, H3K9(4-15) acetyl
Sirt3, H3K9(4-15) acetyl
Sirt5, H3K9(4-15) acetyl
Sirt6, H3K9(4-15) acetyl
Sirt7, H3K9(4-15) acetyl
No Sirtuin, H3K9(4-15) acetyl
TIC
928.00 (10.00)
907.00 (10.00)
1274.00 (10.00)
1232.00 (10.00)
total ion counts
deacetylated peptide
acetyl peptide

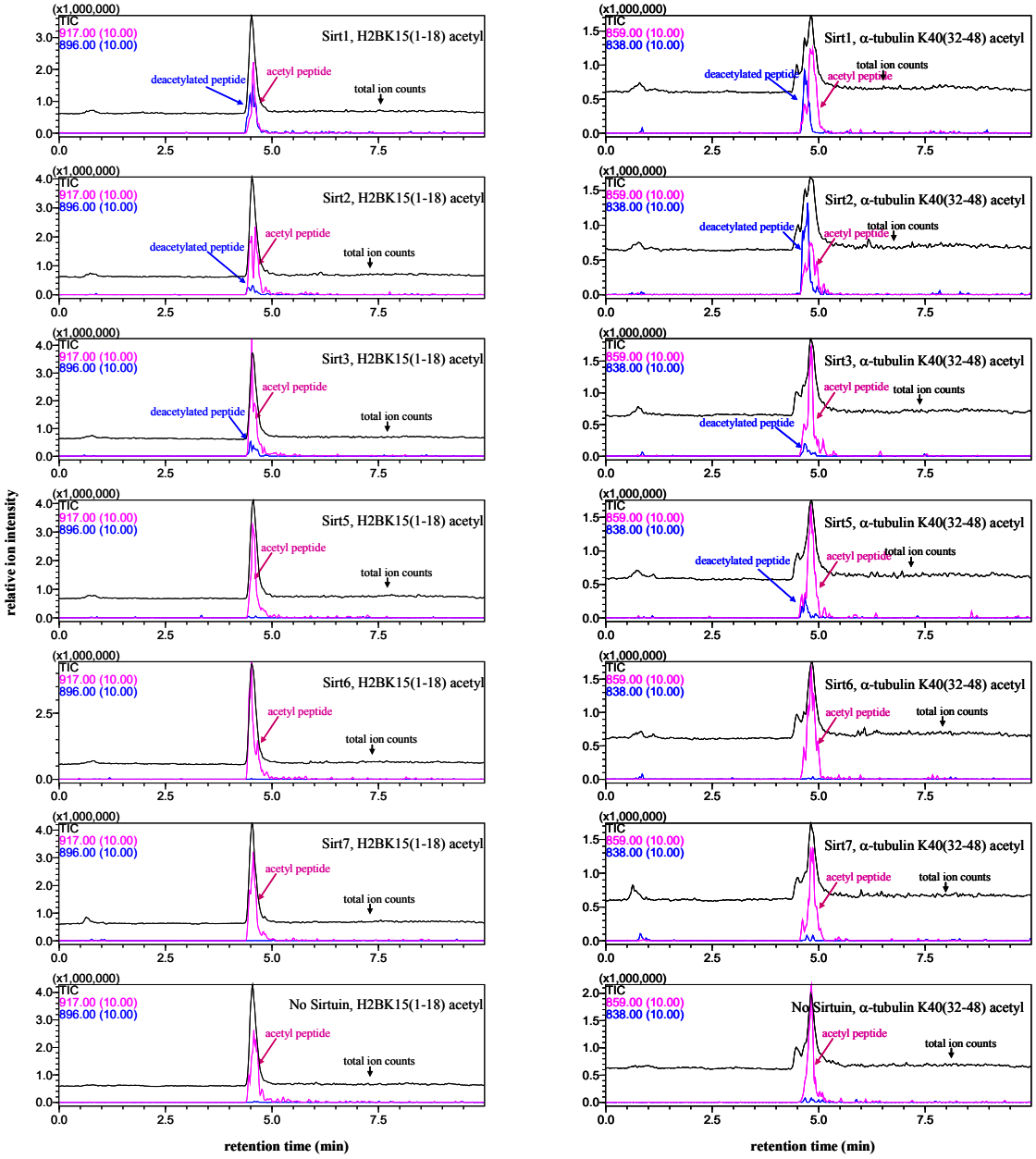

relative ion intensity
retention time (min)
(x1,000,000)
TIC
907.00 (10.00)
886.00 (10.00)
729.00 (10.00)
708.00 (10.00)
Sirt1, H3K14(6-22) acetyl
Sirt2, H3K14(6-22) acetyl
Sirt3, H3K14(6-22) acetyl
Sirt5, H3K14(6-22) acetyl
Sirt6, H3K14(6-22) acetyl
Sirt7, H3K14(6-22) acetyl
No Sirtuin, H3K14(6-22) acetyl
Sirt1, H4K5(1-16) acetyl
Sirt2, H4K5(1-16) acetyl
Sirt3, H4K5(1-16) acetyl
Sirt5, H4K5(1-16) acetyl
Sirt6, H4K5(1-16) acetyl
Sirt7, H4K5(1-16) acetyl
No Sirtuin, H4K5(1-16) acetyl
deacetylated peptide
acetyl peptide
total ion counts


(x1,000,000)
TIC
729.00 (10.00)
708.00 (10.00)
Sirt1, H4K8(1-16) acetyl
Sirt1, H4K12(1-16) acetyl
Sirt2, H4K8(1-16) acetyl
Sirt2, H4K12(1-16) acetyl
Sirt3, H4K8(1-16) acetyl
Sirt3, H4K12(1-16) acetyl
Sirt5, H4K8(1-16) acetyl
Sirt5, H4K12(1-16) acetyl
Sirt6, H4K8(1-16) acetyl
Sirt6, H4K12(1-16) acetyl
Sirt7, H4K8(1-16) acetyl
Sirt7, H4K12(1-16) acetyl
No Sirtuin, H4K8(1-16) acetyl
No Sirtuin, H4K12(1-16) acetyl
deacetylated peptide
acetyl peptide
total ion counts
relative ion intensity
retention time (min)


(x1,000,000)
TIC
959.00 (10.00)
938.00 (10.00)
Sirt1, H4K16(8-24) acetyl
deacetylated peptide
acetyl peptide
total ion counts
Sirt2, H4K16(8-24) acetyl
Sirt3, H4K16(8-24) acetyl
Sirt5, H4K16(8-24) acetyl
Sirt6, H4K16(8-24) acetyl
Sirt7, H4K16(8-24) acetyl
No Sirtuin, H4K16(8-24) acetyl
(x10,000,000)
1142.00 (10.00)
1121.00 (10.00)
Sirt1, H4K91(80-99) acetyl
Sirt2, H4K91(80-99) acetyl
Sirt3, H4K91(80-99) acetyl
Sirt5, H4K91(80-99) acetyl
Sirt6, H4K91(80-99) acetyl
Sirt7, H4K91(80-99) acetyl
No Sirtuin, H4K91(80-99) acetyl
relative ion intensity
retention time (min)


(x1,000,000)
TIC
899.00 (10.00)
878.00 (10.00)
Sirt1, H2AK5(1-17) acetyl
Sirt1, H2AK13(1-17) acetyl
Sirt2, H2AK5(1-17) acetyl
Sirt2, H2AK13(1-17) acetyl
Sirt3, H2AK5(1-17) acetyl
Sirt3, H2AK13(1-17) acetyl
Sirt5, H2AK5(1-17) acetyl
Sirt5, H2AK13(1-17) acetyl
Sirt6, H2AK5(1-17) acetyl
Sirt6, H2AK13(1-17) acetyl
Sirt7, H2AK5(1-17) acetyl
Sirt7, H2AK13(1-17) acetyl
No Sirtuin, H2AK5(1-17) acetyl
No Sirtuin, H2AK13(1-17) acetyl
deacetylated peptide
acetyl peptide
total ion counts
relative ion intensity
retention time (min)


relative ion intensity
retention time (min)
Sirt1, H2BK5(1-18) acetyl
Sirt2, H2BK5(1-18) acetyl
Sirt3, H2BK5(1-18) acetyl
Sirt5, H2BK5(1-18) acetyl
Sirt6, H2BK5(1-18) acetyl
Sirt7, H2BK5(1-18) acetyl
No Sirtuin, H2BK5(1-18) acetyl
Sirt1, H2BK12(1-18) acetyl
Sirt2, H2BK12(1-18) acetyl
Sirt3, H2BK12(1-18) acetyl
Sirt5, H2BK12(1-18) acetyl
Sirt6, H2BK12(1-18) acetyl
Sirt7, H2BK12(1-18) acetyl
No Sirtuin, H2BK12(1-18) acetyl
TIC
917.00 (10.00)
896.00 (10.00)
deacetylated peptide
acetyl peptide
total ion counts


(x1,000,000)
TIC
917.00 (10.00)
896.00 (10.00)
Sirt1, H2BK15(1-18) acetyl
deacetylated peptide
acetyl peptide
total ion counts
Sirt2, H2BK15(1-18) acetyl
Sirt3, H2BK15(1-18) acetyl
Sirt5, H2BK15(1-18) acetyl
Sirt6, H2BK15(1-18) acetyl
Sirt7, H2BK15(1-18) acetyl
No Sirtuin, H2BK15(1-18) acetyl
859.00 (10.00)
838.00 (10.00)
Sirt1, α-tubulin K40(32-48) acetyl
Sirt2, α-tubulin K40(32-48) acetyl
Sirt3, α-tubulin K40(32-48) acetyl
Sirt5, α-tubulin K40(32-48) acetyl
Sirt6, α-tubulin K40(32-48) acetyl
Sirt7, α-tubulin K40(32-48) acetyl
No Sirtuin, α-tubulin K40(32-48) acetyl
relative ion intensity
retention time (min)

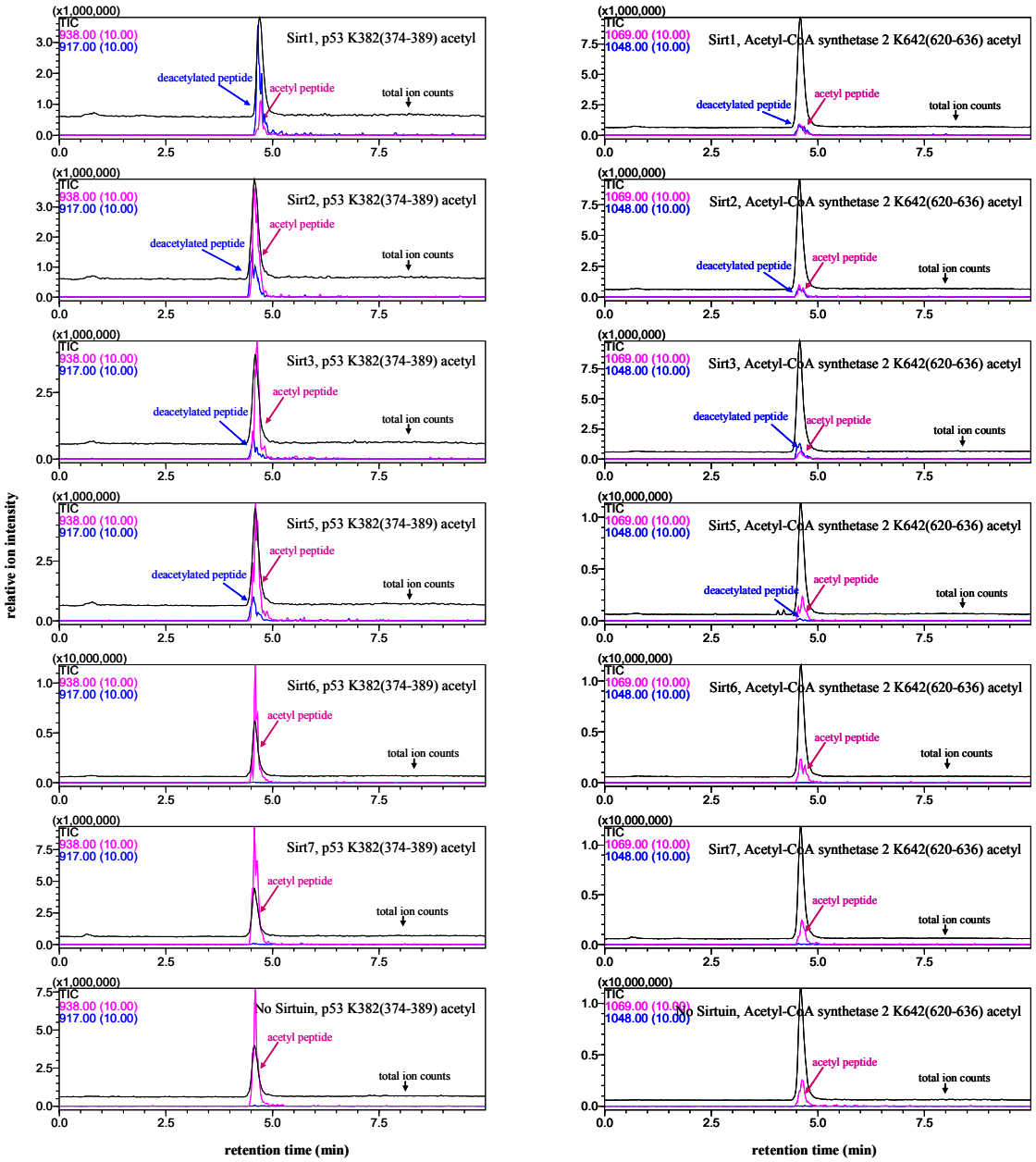


Fig. S2. Sirt1, Sirt2, Sirt3, Sirt5, Sirt6, and Sirt7-catalyzed hydrolysis of different acetyl lysine peptides. Purified sirtuins were incubated with 0.3 mM acetyl peptides, 1.0 mM NAD in 20 mM Tris-HCl buffer (pH 7.5) containing 1 mM DTT in 60 μL reactions for 2 h at 37°C. The reactions were stopped with 60 μL 10% TFA. After removing the precipitated protein, reaction mixture was then analyzed by LC-MS. Shown here are the MS traces from the LC-MS analyses for the reactions. Pink traces show the ion intensities (10x magnified) for the masses of the acetyl peptides and blue traces show the ion intensities (10x magnified) for the mass of the deacetylated peptides. Black traces show the intensity for the total ion counts (TIC). All 16 peptides (Table S1) could be deacetylated by Sirt1-3, while only 8 peptides (H3K9(1-17), H3K9(4-15),

H4K16(8-22), H4K91(80-99), H2AK13(1-17), α-tubulin K40(32-48), p53 K382(374-389), and Acetyl-CoA synthetase 2 K642(620-636)) could be deacetylated slowly by Sirt5. No activity was detected for Sirt6 and Sirt7.

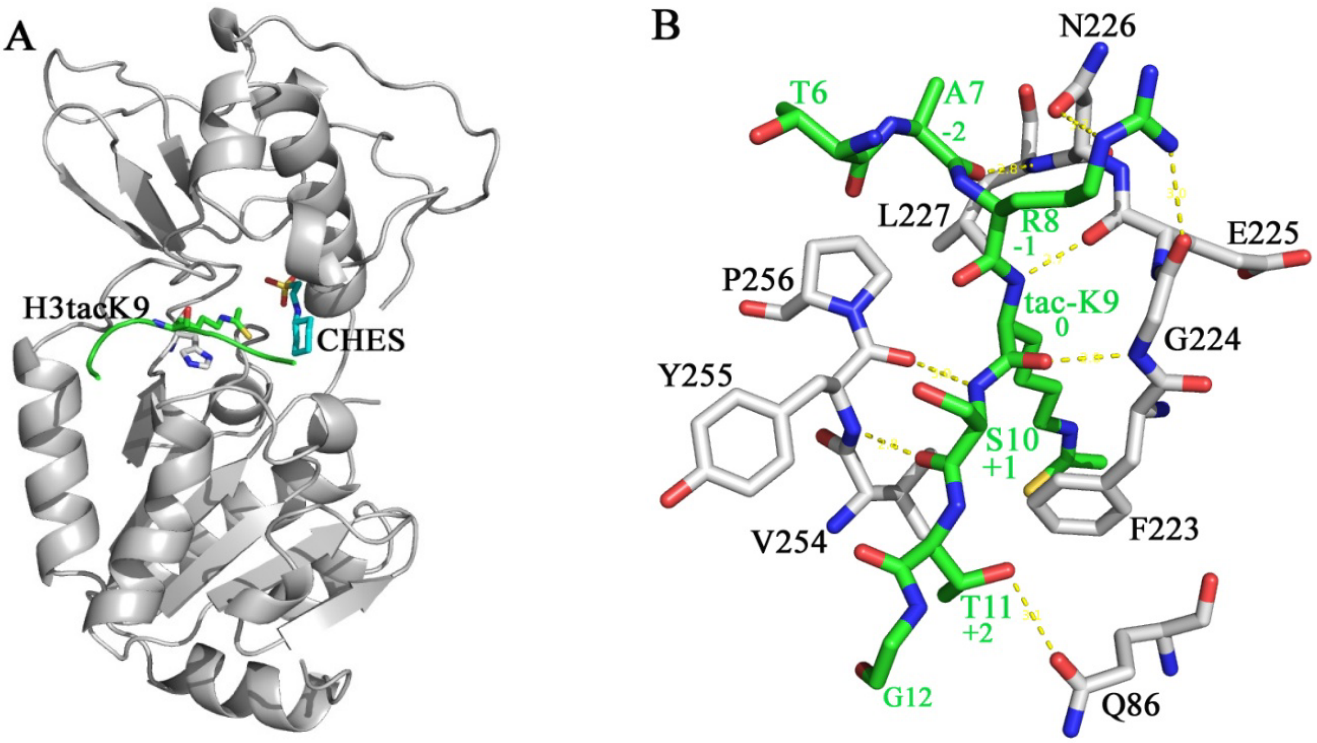
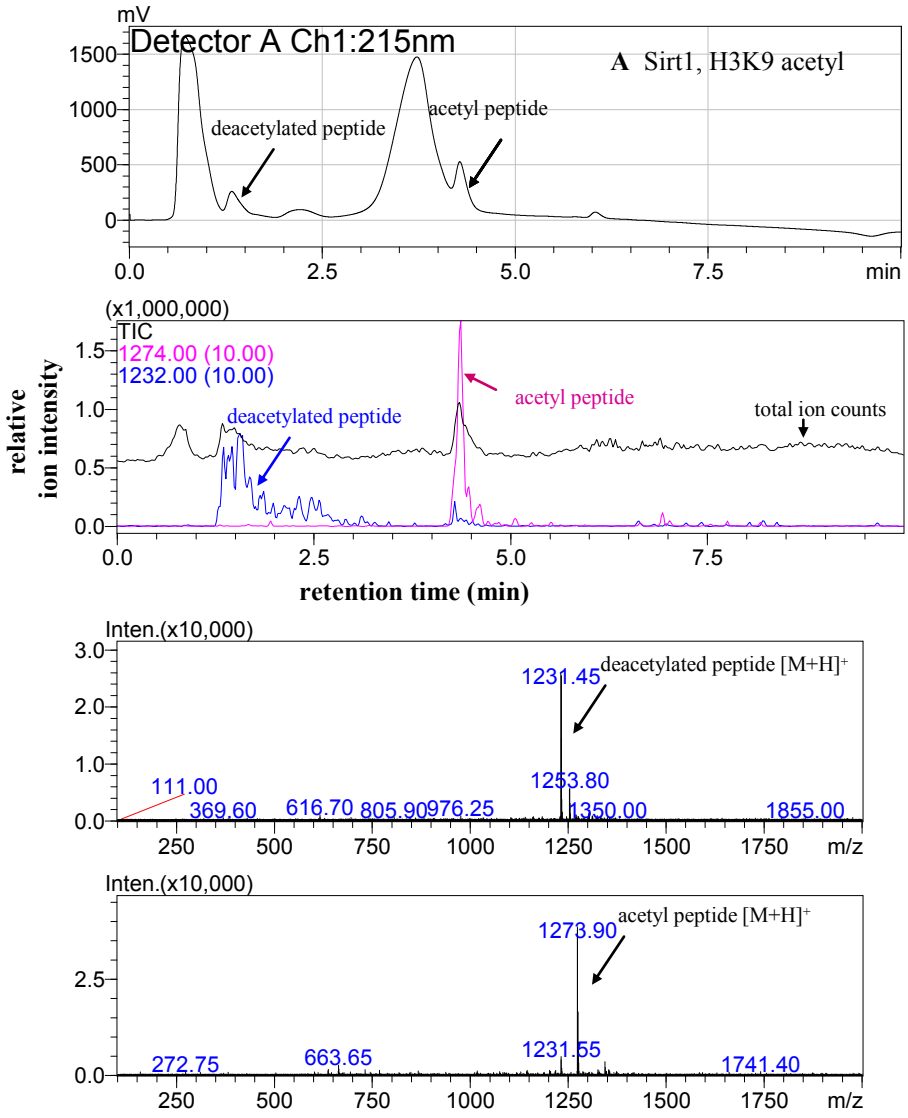
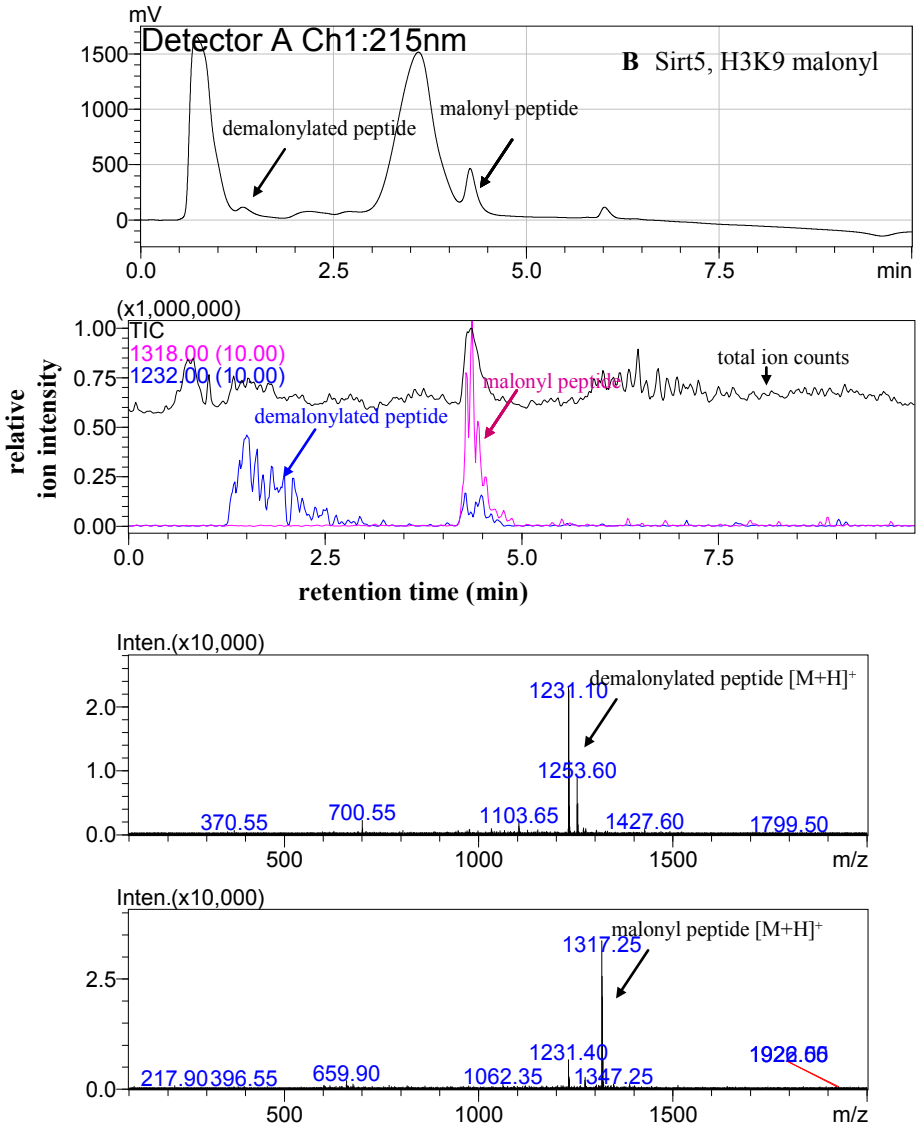


Fig. S3. (A) Overall structure of Sirt5 in complex with an H3K9 thioacetyl peptide. The thioacetyl lysine side chain (green) and the CHES molecule (cyan) are shown in stick (cyan). (B) H-bonding interactions (dashed yellow lines) between thioacetyl peptide (green) and Sirt5 (light grey).


mV
Detector A Ch1:215nm
A Sirt1, H3K9 acetyl
deacetylated peptide
acetyl peptide
min
(x1,000,000)
TIC
1274.00 (10.00)
1232.00 (10.00)
relative ion intensity
deacetylated peptide
acetyl peptide
total ion counts
retention time (min)
Inten.(x10,000)
deacetylated peptide [M+H]+
1231.45
111.00
369.60
616.70
805.90
976.25
1253.80
1350.00
1855.00
m/z
Inten.(x10,000)
acetyl peptide [M+H]+
1273.90
272.75
663.65
1231.55
1741.40
m/z


mV
Detector A Ch1:215nm
B Sirt5, H3K9 malonyl
demalonylated peptide
malonyl peptide
1500
1000
500
0
0.0
2.5
5.0
7.5
min
(x1,000,000)
TIC
1318.00 (10.00)
1232.00 (10.00)
malonyl peptide
total ion counts
demalonylated peptide
relative ion intensity
1.00
0.75
0.50
0.25
0.00
retention time (min)
Inten.(x10,000)
demalonylated peptide [M+H]+
1231.10
1253.60
370.55
700.55
1103.65
1427.60
1799.50
2.0
1.0
0.0
500
1000
1500
m/z
Inten.(x10,000)
malonyl peptide [M+H]+
1317.25
1231.40
217.90
396.55
659.90
1062.35
1347.25
2.5
0.0

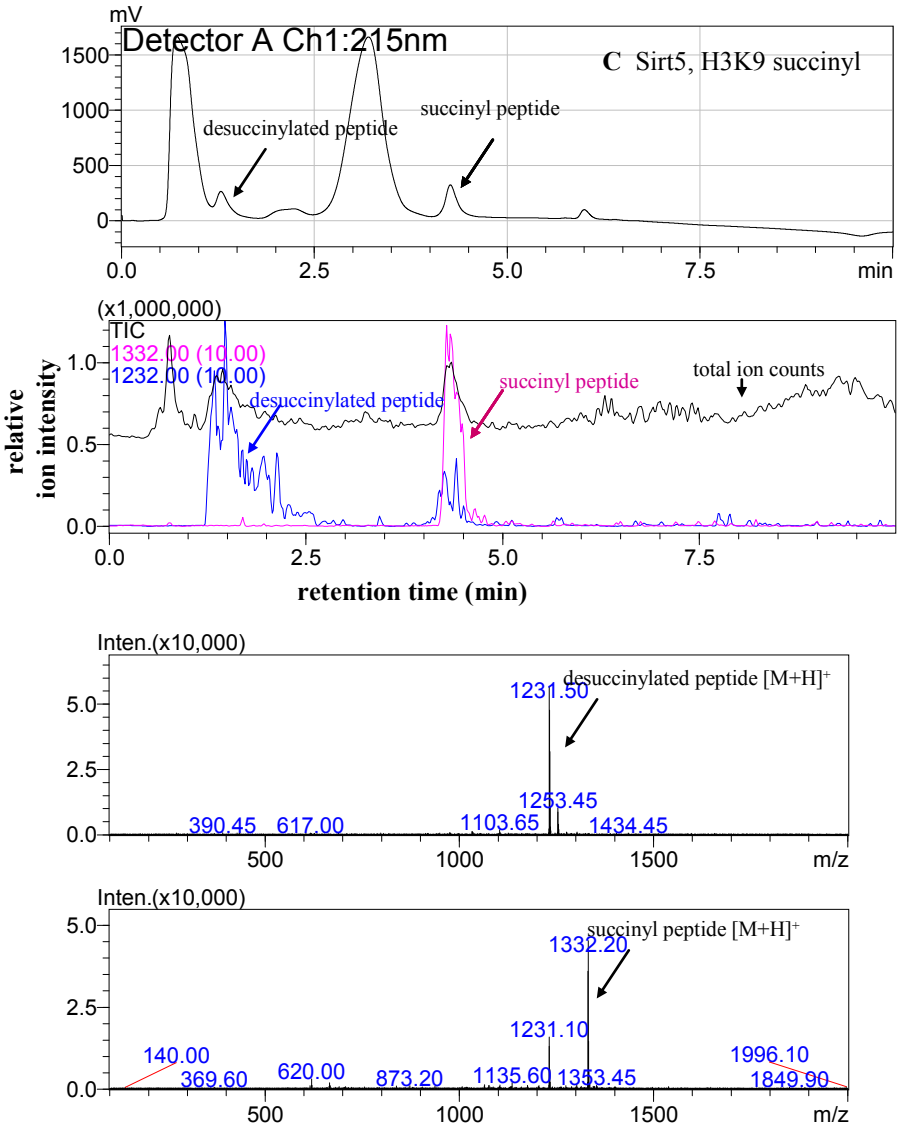


Fig. S4. Sirt1 and Sirt5 catalyzed-hydrolysis of different acyl peptides monitored using LC-MS. For each reaction, the LC trace, MS trace, deacylated peptide mass spectra and acyl peptide mass spectra are shown. LC traces detect the UV-Vis absorption at 215 nm of compounds coming off the column, and MS traces detect the masses of the compounds coming off the column. In the MS trace, pink traces show the ion intensities (10x magnified) for the masses of the acyl peptides (acetyl, m/z 1274.0; malonyl, m/z 1318.0; succinyl, m/z 1332.0) and blue traces show the ion intensities (10x magnified) for the mass of the deacylated peptide (m/z 1232.0). Black traces show the ion intensity for all masses from 100-2000 (total ion counts or TIC). With Sirt1, the deacetylated product was detected when H3K9 acetyl peptide (A) was used as the substrate. With Sirt5, the demalonylated (B) and desuccinylated (C) products were detected.

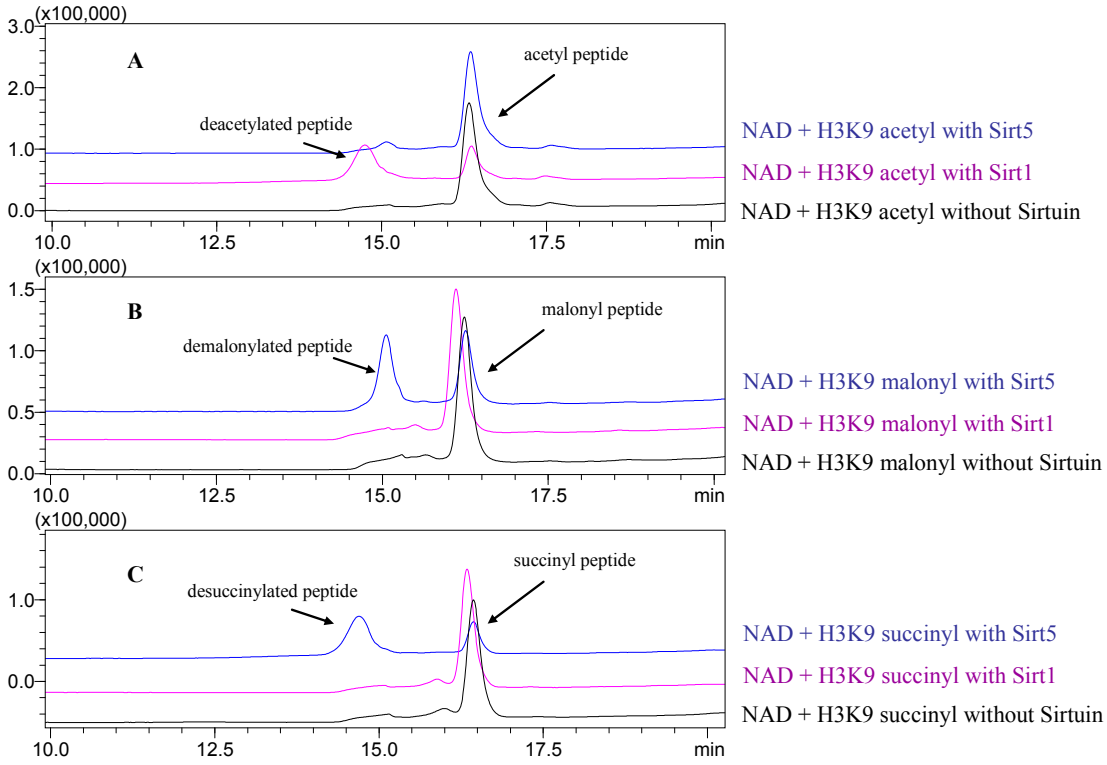


Fig. S5. Sirt1 and Sirt5-catalyzed deacetylation, demalonylation and desuccinylation of H3 K9 peptides were examined by HPLC (monitored at 215 nm). In Fig. S2 and S4, LC-MS was used to monitor the reactions. However, in the LC traces, the acyl peptide and deacylated peptide peaks overlapped with other peaks. Here, a longer HPLC column was used to better separate the acyl peptide and deacylated peptide. The results further confirmed that Sirt1 had better deacetylase activity, while Sirt5 had better demalonylase and desuccinylase activity.

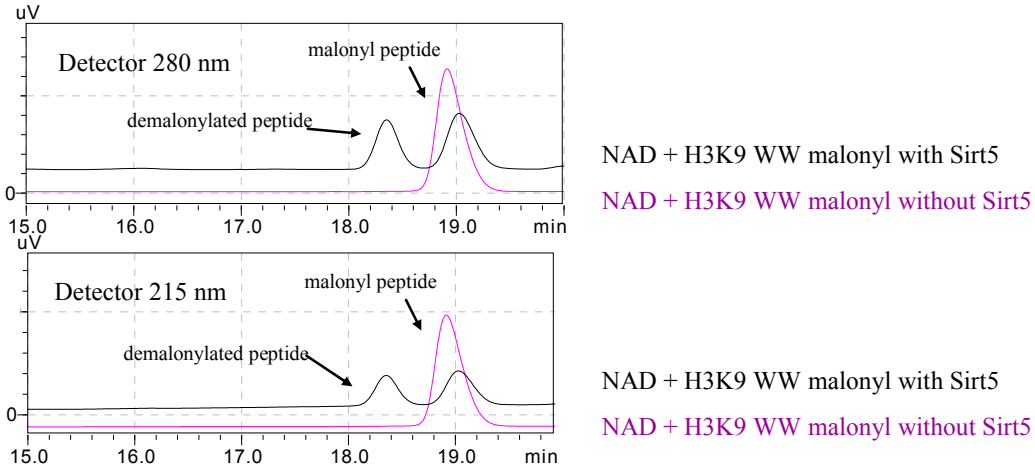


Fig. S6. HPLC traces for the demalonylation reactions with H3K9 WW peptide were monitored at 280 nm and 215 nm. The pink traces are controls showing the peptides without Sirt5. The black traces are the reactions with Sirt5. The new peak at 18.3 min is the demalonylated product.

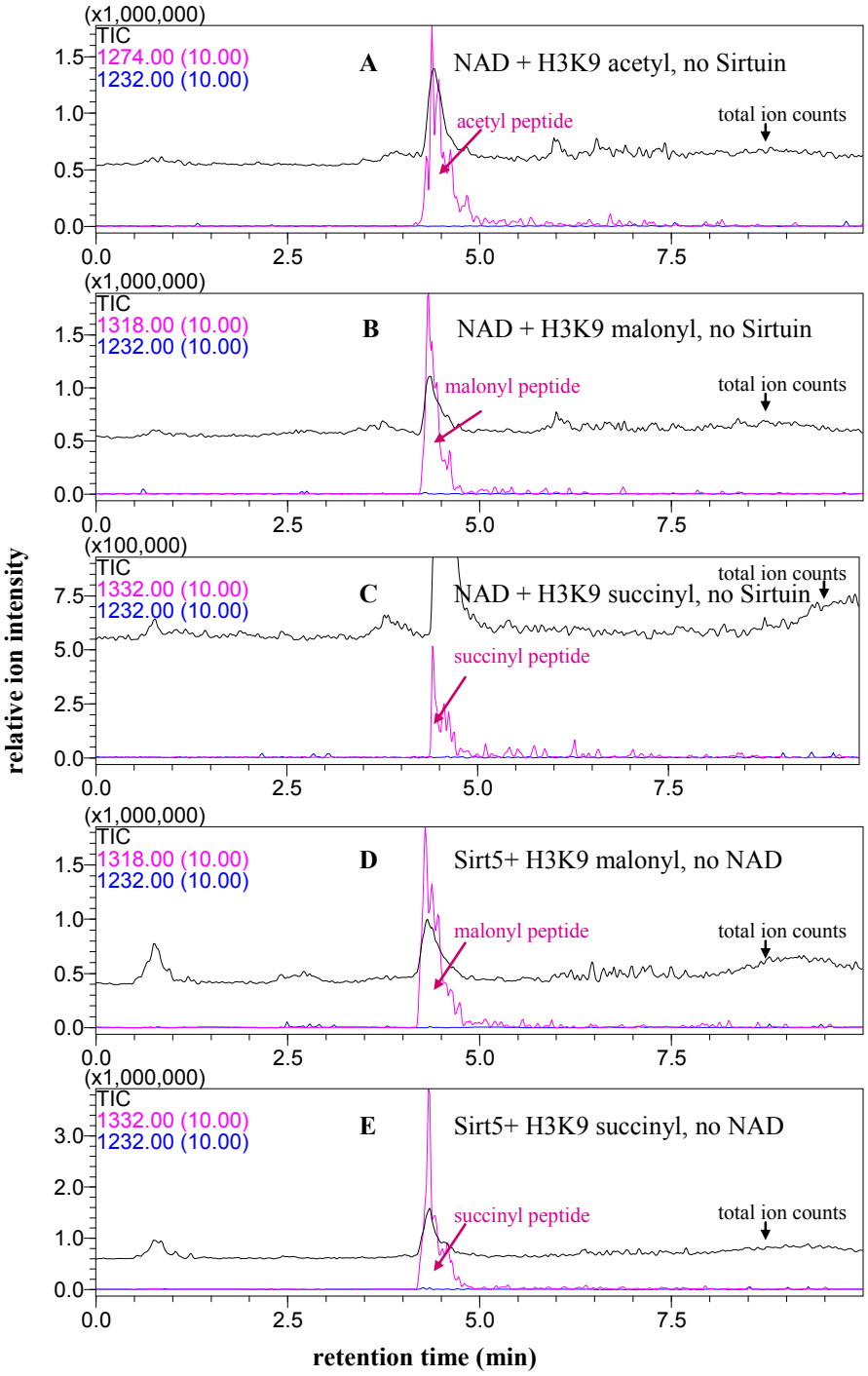


Fig. S7. In the absence of Sirt5 or NAD, malonyl and succinyl peptides could not be hydrolyzed. Shown here are the MS traces from the LC-MS analyses for the reactions. Pink traces show the ion intensities (10x magnified) for the masses of the acyl peptides (acetyl peptide, m/z 1274.0; malonyl peptide, m/z 1318.0; succinyl peptide, m/z 1332.0) and blue traces show the ion intensities (10x magnified) for the mass of the deacylated peptide (m/z 1232.0). Black traces show the intensity for total ion counts (TIC). There was no deacylated peptide generated without sirtuin (A, B, C) or NAD (D and E).

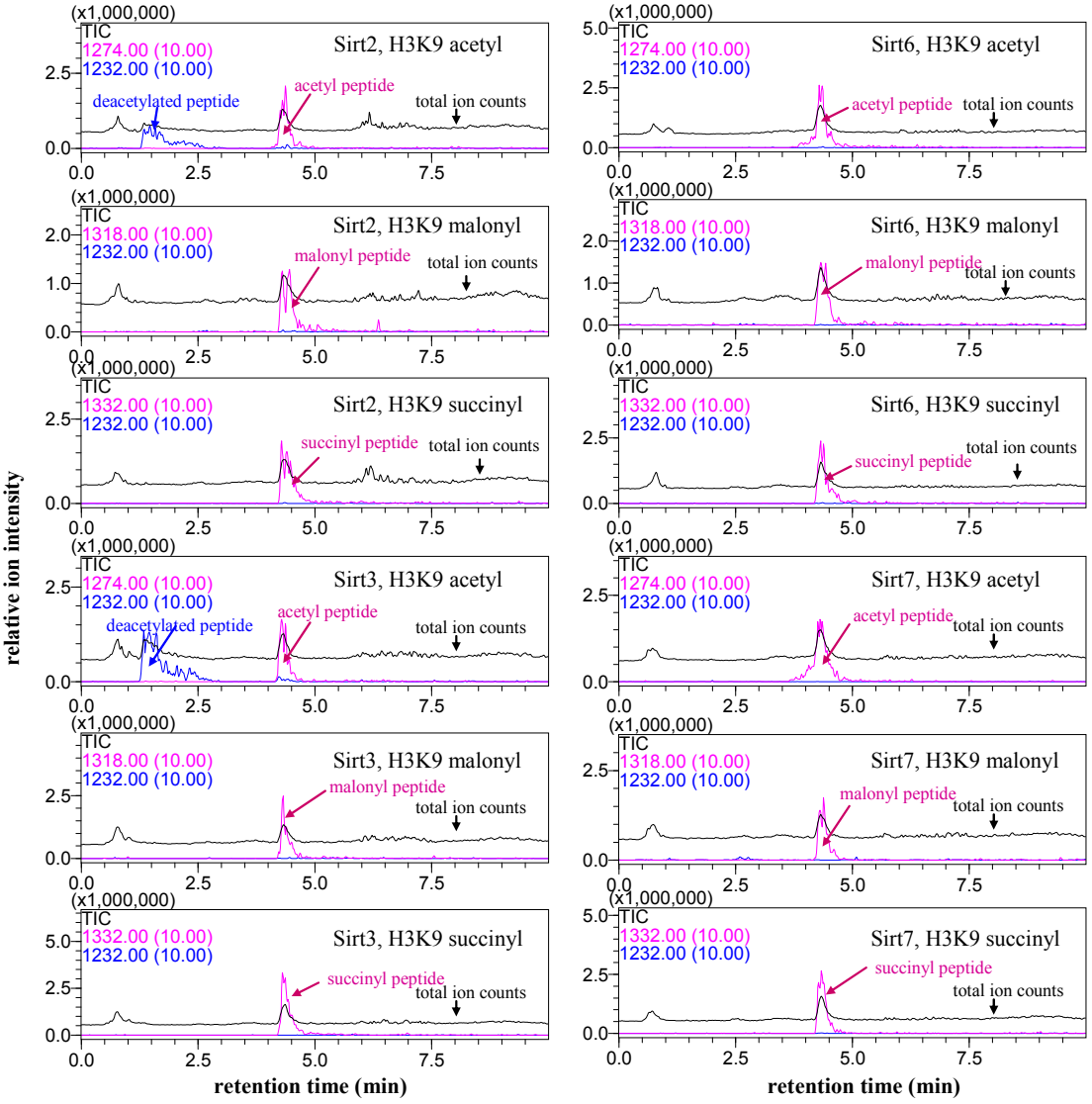


Fig. S8. Sirt2, Sirt3, Sirt6, and Sirt7 did not catalyze the hydrolysis of malonyl and succinyl lysine. Shown here are the MS traces from the LC-MS analyses of the reactions. Pink traces show the ion intensities (10x magnified) for the masses of the acyl peptides (acetyl peptide, m/z 1274.0; malonyl peptide, m/z 1318.0; succinyl peptide, m/z 1332.0) and blue traces show the ion intensities (10x magnified) for the mass of the deacylated peptide (m/z 1232.0). Black traces show the intensity for total ion counts (TIC). Sirt2 and Sirt3 only displayed deacetylation activity, while no activity was detected for Sirt6 and Sirt7.

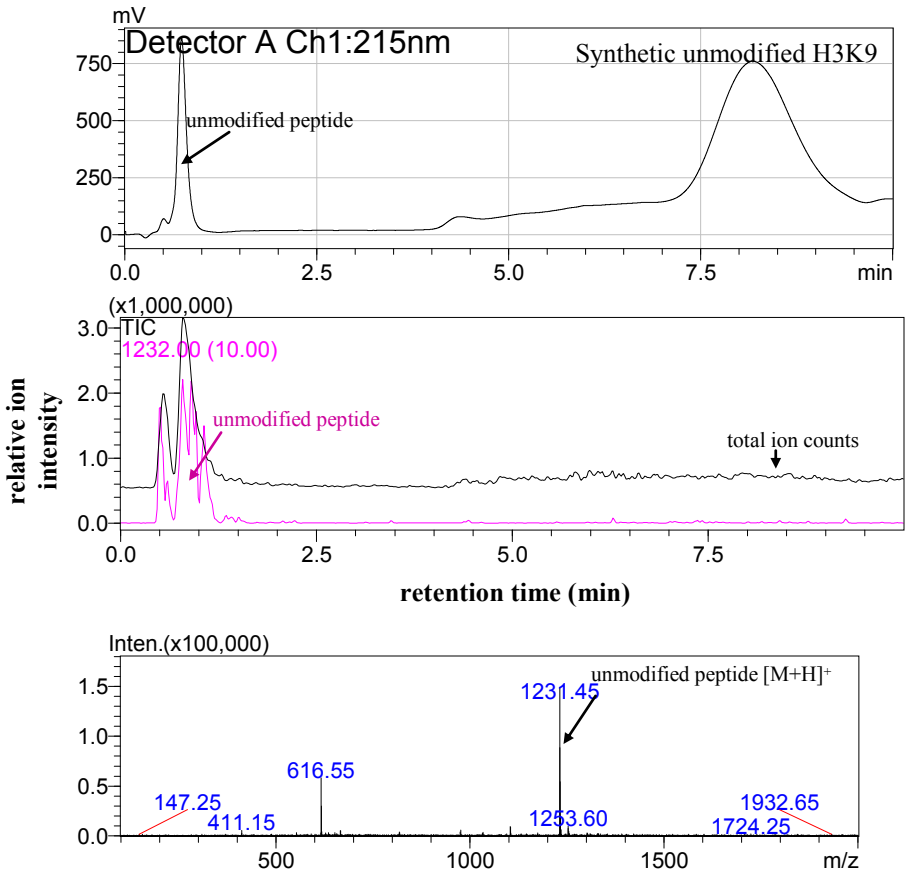
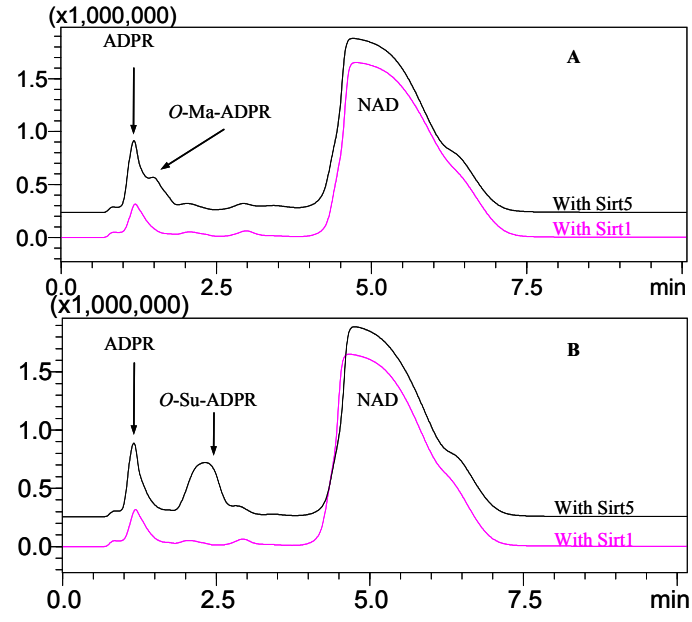
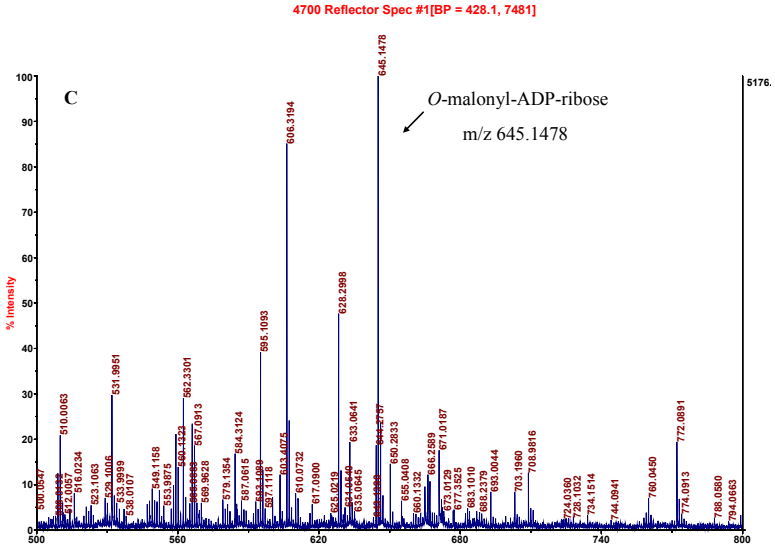
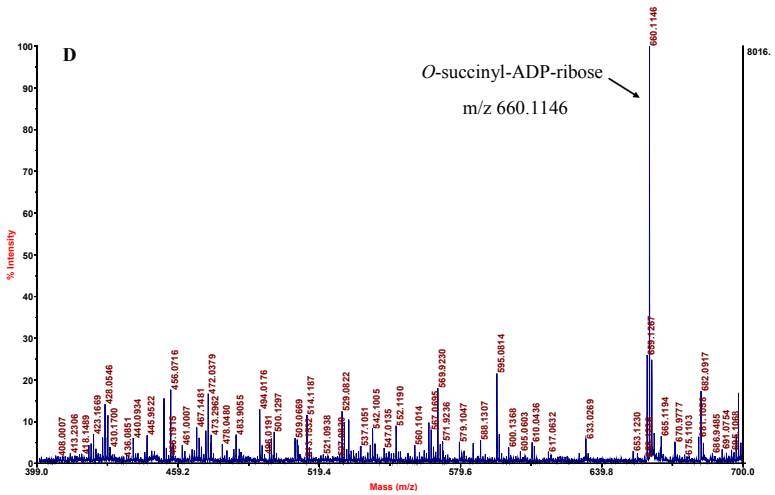


Fig. S9. LC-MS of synthetic unmodified H3K9 peptide. The LC trace, MS trace, and mass spectra are shown from top to bottom. LC traces detect the UV-Vis absorption at 215 nm of compounds coming off the column, and MS traces detect the masses of the compounds coming off the column. Pink traces show the ion intensities (10x magnified) for the masses of the unmodified peptide (m/z 1232.0). Black traces show the ion intensity for all masses from 100-2000 (total ion counts or TIC). The demalonylated and desuccinylated peptides (Fig. S4) had identical masses to the synthetic unmodified H3K9 peptide.


(x1,000,000)
ADPR
A
O-Ma-ADPR
NAD
With Sirt5
With Sirt1
1.5
1.0
0.5
0.0
0.0
2.5
5.0
7.5
min
(x1,000,000)
ADPR
B
O-Su-ADPR
NAD
With Sirt5
With Sirt1
1.5
1.0
0.5
0.0
0.0
2.5
5.0
7.5
min


4700 Reflector Spec #1[BP = 428.1, 7481]
C
O-malonyl-ADP-ribose
m/z 645.1478
% Intensity
Mass (m/z)
645.1478
606.3194
628.2998
531.9951
695.1093
562.2301
510.0063
633.0641
603.4075
650.2833
772.0891
708.9916
703.1960
760.0450
774.0913
744.0941
734.1514
788.0580
794.0663
500
560
620
680
740
800


4700 Reflector Spec #1[BP = 660.1, 8017]
D
O-succinyl-ADP-ribose
m/z 660.1146
% Intensity
Mass (m/z)
660.1146
595.0814
689.1267
456.0716
428.0546
494.0176
472.0379
682.0917
399.0
459.2
519.4
579.6
639.8
700.0

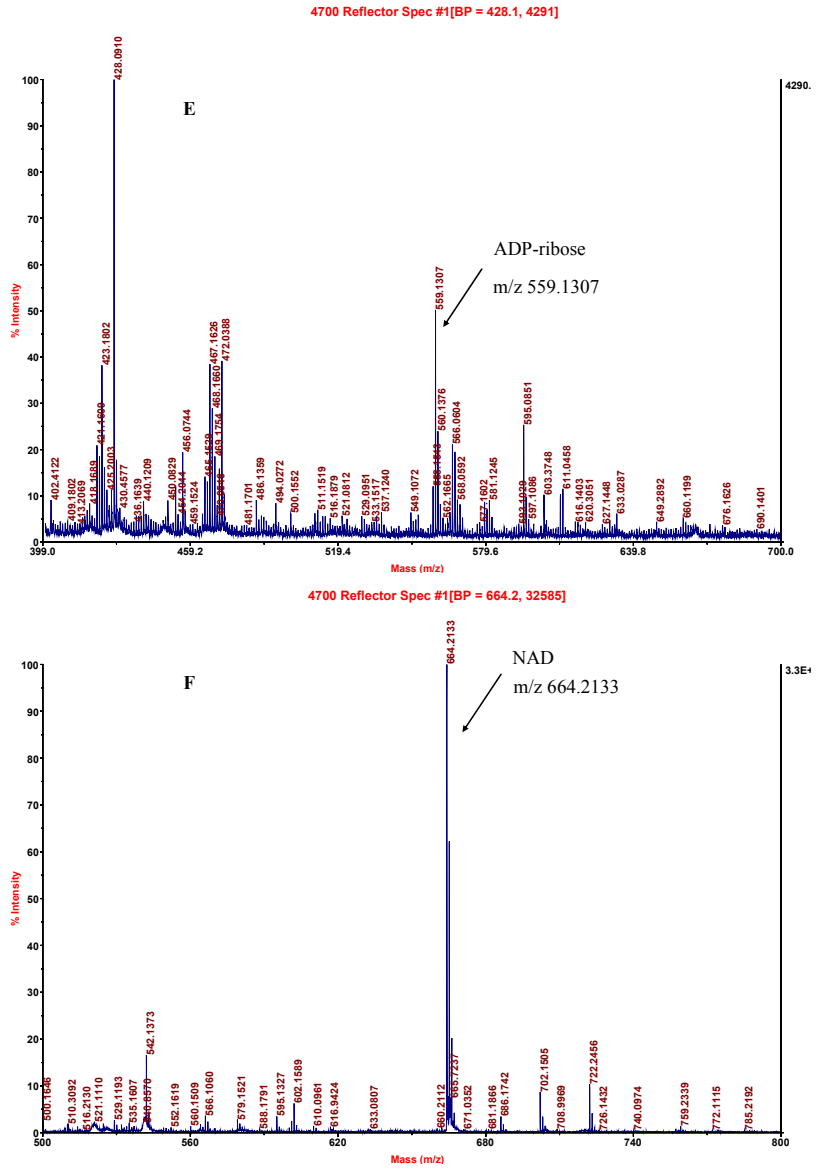


Fig. S10. HPLC purification of *O*-malony-ADPR (**A**) and *O*-succinyl-ADPR (**B**) and MS confirmation (**C** and **D**). The black traces in A and B were the HPLC traces (detected at 260 nm) for Sirt5-catalyzed demalonylation and desuccinylation of the H3K9 acyl peptides. Incubating the peptides with Sirt1 did not generate the same products (pink traces). The *O*-malony-ADPR (**C**) and *O*-succinyl-ADPR (**D**) as well as ADPR (**E**) and NAD (**F**) were further analyzed by MALDI-MS. The formation of *O*-malonyl-ADPR and *O*-succinyl-ADPR suggested that the mechanism of Sirt5-catalyzed reactions was the same as the deacetylation mechanism of Class I sirtuins.

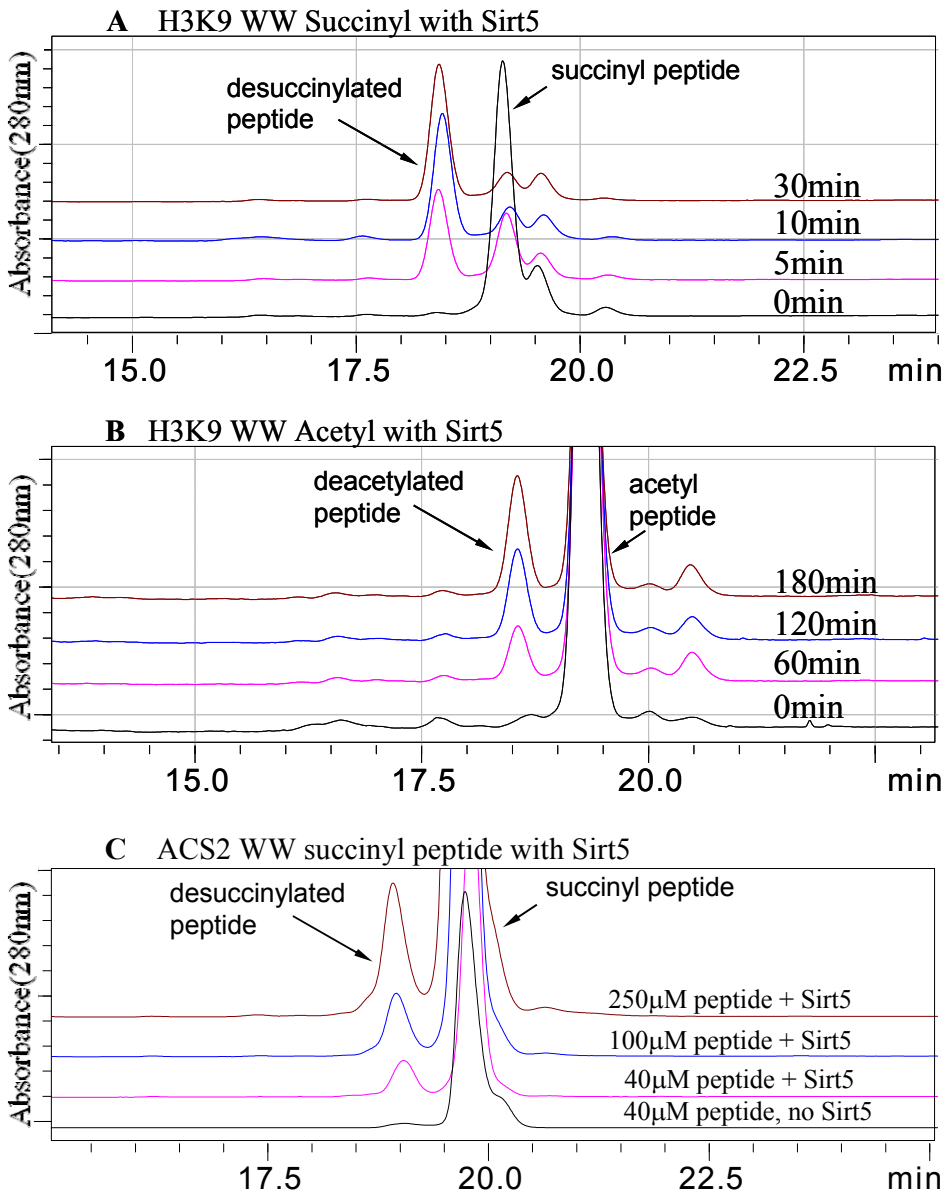


Fig. S11. Time courses and sample HPLC traces from kinetic studies for Sirt5-catalyzed deacylation reactions. (**A**) Time course of Sirt5-catalyzed desuccinylation reaction using the H3K9 WW succinyl peptide. The desuccinylated peptide increased over time. The reaction was monitored at 280 nm. Most substrates were converted to products after 10 min. (**B**) Time course of Sirt5-catalyzed deacetylation reaction using the H3K9 WW acetyl peptide. The deacetylated peptide increased over time. The reaction was monitored at 280 nm. A very small percentage of substrate was converted to product even after 180 min. (**C**) Sample HPLC traces from Sirt5 kinetics studies.

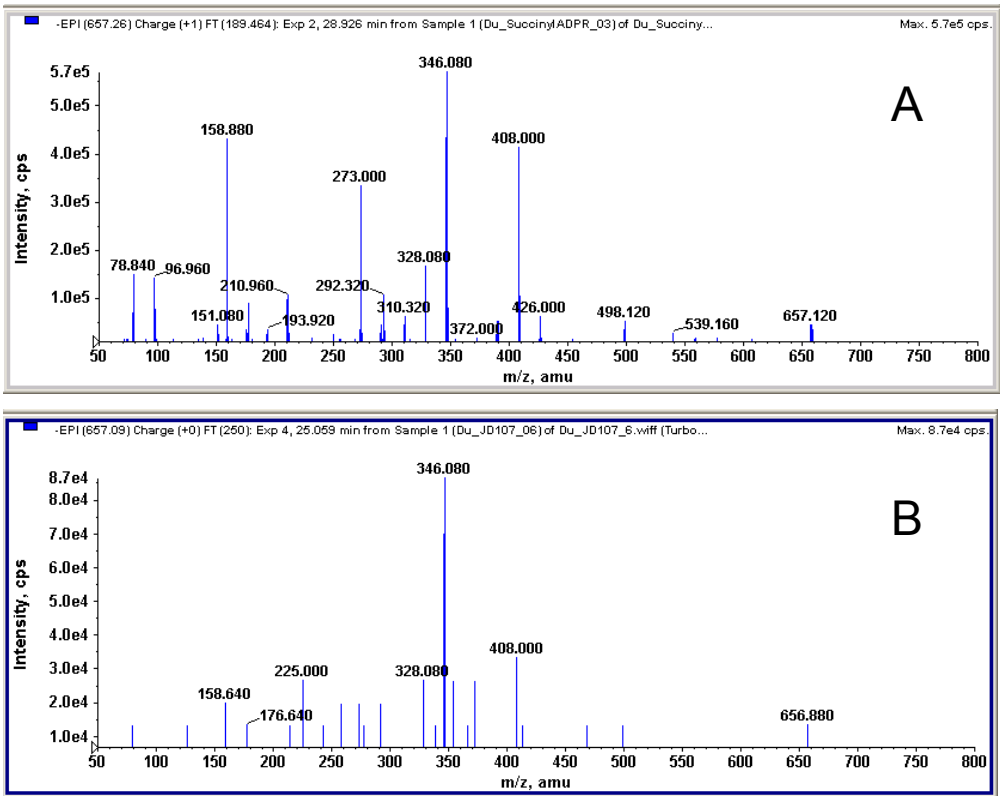
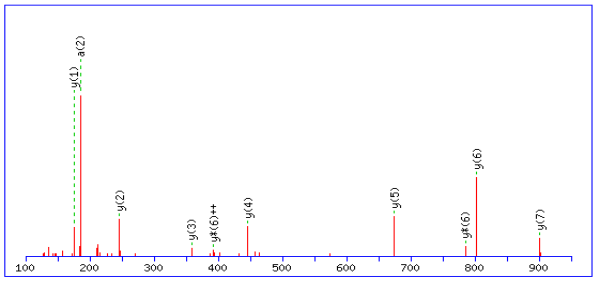


Fig. S12. The MS/MS spectra of (A) Sirt5 and succinyl H3K9 peptide derived *O*-succinyl-ADP-ribose (m/z 657, $[M-2H]^-$) and (B) Sirt5 and mitochondria lysate peptide mixtures derived *O*-succinyl-ADP-ribose (m/z 657, $[M-2H]^-$).



A

HMGCS2_BOVIN

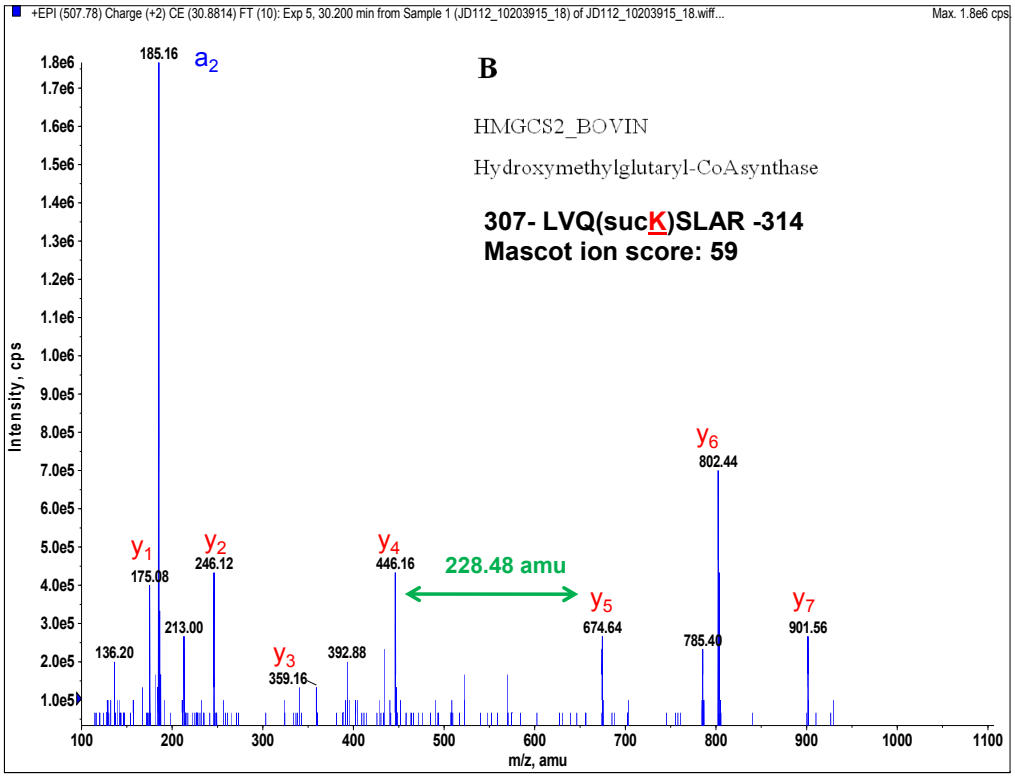
Hydroxymethylglutaryl-CoAsynthase

Peptide sequence (307-314)

LVQ(SucK)SLAR

Monoisotopic mass of neutral peptide Mr(calc): 1013.5869
Variable modifications:
K4 : Succinyl (K)
Ions Score: 59 **Expect:** 0.0016
Matches (Bold Red): 10/76 fragment ions using 15 most intense peaks

#	a	a^{++}	a*	a^{*++}	b	b^{++}	b*	b^{*++}	Seq.	y	y^{++}	y*	y^{*++}	#
1	86.0964	43.5519			114.0913	57.5493			L					8
2	**185.1648**	93.0861			213.1598	107.0835			V	**901.5101**	451.2587	884.4836	442.7454	7
3	313.2234	157.1153	296.1969	148.6021	341.2183	171.1128	324.1918	162.5995	Q	**802.4417**	401.7245	**785.4152**	**393.2112**	6
4	541.3344	271.1708	524.3079	262.6576	569.3293	285.1683	552.3028	276.6550	K	**674.3832**	337.6952	657.3566	329.1819	5
5	628.3664	314.6869	611.3399	306.1736	656.3614	328.6843	639.3348	320.1710	S	**446.2722**	223.6397	429.2456	215.1264	4
6	741.4505	371.2289	724.4240	362.7156	769.4454	385.2264	752.4189	376.7131	L	**359.2401**	180.1237	342.2136	171.6104	3
7	812.4876	406.7474	795.4611	398.2342	840.4825	420.7449	823.4560	412.2316	A	**246.1561**	123.5817	229.1295	115.0684	2
8									R	**175.1190**	88.0631	158.0924	79.5498	1

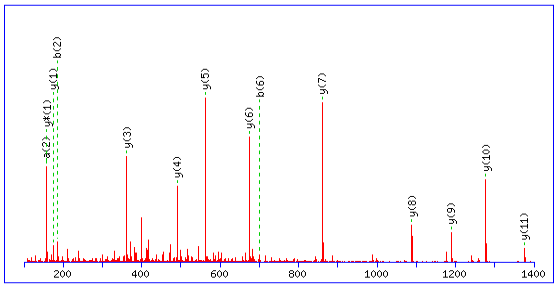


B

HMGCS2_BOVIN

Hydroxymethylglutaryl-CoAsynthase

307- LVQ(sucK)SLAR -314
Mascot ion score: 59



C

THTR_BOVIN

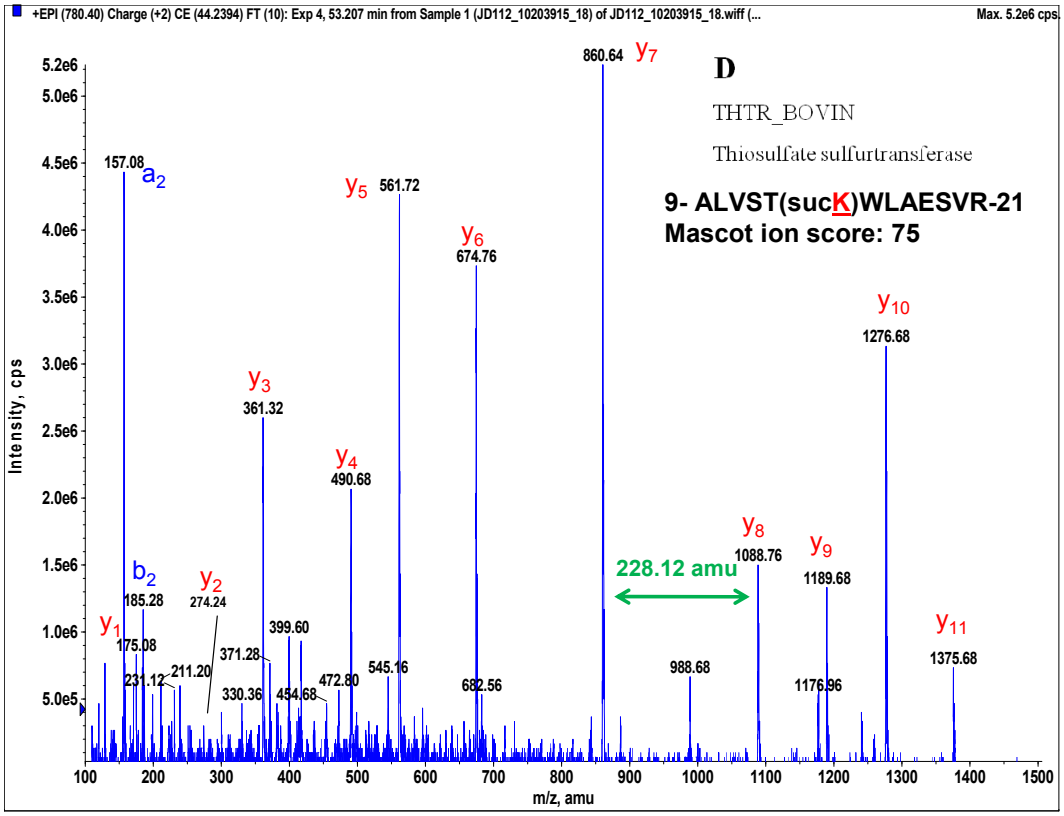
Thiosulfate sulfurtransferase

Peptide sequence (9-21)

ALVST(SucK)WLAESVR

Monoisotopic mass of neutral peptide Mr(calc): 1558.8355
Variable modifications:
K6 : Succinyl (K)
Ions Score: 75 Expect: 1.8e-05
Matches (Bold Red): 14/124 fragment ions using 24 most intense peaks

#	a	a^{++}	a*	a^{*++}	b	b^{++}	b*	b^{*++}	Seq.	y	y^{++}	y*	y^{*++}	#
1	44.0495	22.5284			72.0444	36.5258			A					13
2	**157.1335**	79.0704			**185.1285**	93.0679			L	1488.8057	744.9065	1471.7791	736.3932	12
3	256.2020	128.6046			284.1969	142.6021			V	**1375.7216**	688.3644	1358.6951	679.8512	11
4	343.2340	172.1206			371.2289	186.1181			S	**1276.6532**	638.8302	1259.6266	630.3170	10
5	444.2817	222.6445			472.2766	236.6419			T	**1189.6212**	595.3142	1172.5946	586.8009	9
6	672.3927	336.7000	655.3661	328.1867	**700.3876**	350.6974	683.3610	342.1842	K	**1088.5735**	544.7904	1071.5469	536.2771	8
7	858.4720	429.7396	841.4454	421.2264	886.4669	443.7371	869.4403	435.2238	W	**860.4625**	430.7349	843.4359	422.2216	7
8	971.5560	486.2817	954.5295	477.7684	999.5510	500.2791	982.5244	491.7658	L	**674.3832**	337.6952	657.3566	329.1819	6
9	1042.5932	521.8002	1025.5666	513.2869	1070.5881	535.7977	1053.5615	527.2844	A	**561.2991**	281.1532	544.2726	272.6399	5
10	1171.6357	586.3215	1154.6092	577.8082	1199.6307	600.3190	1182.6041	591.8057	E	**490.2620**	245.6346	473.2354	237.1214	4
11	1258.6678	629.8375	1241.6412	621.3242	1286.6627	643.8350	1269.6361	635.3217	S	**361.2194**	181.1133	344.1928	172.6001	3
12	1357.7362	679.3717	1340.7096	670.8585	1385.7311	693.3692	1368.7046	684.8559	V	274.1874	137.5973	257.1608	129.0840	2
13									R	**175.1190**	88.0631	**158.0924**	79.5498	1

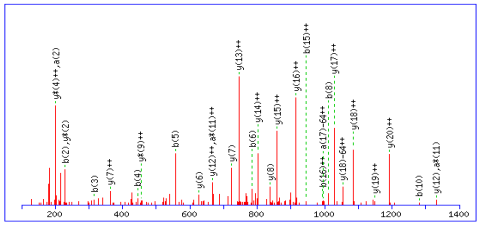


D

THTR_BOVIN

Thiosulfate sulfurtransferase

9- ALVST(sucK)WLAESVR-21
Mascot ion score: 75



E

AATM_BOVIN

Aspartate aminotransferase

Peptide sequence (304-325)

VESQL(SucK)ILIRPMYSNPPINGAR

Monoisotopic mass of neutral peptide Mr(calc): 2611.3792
Variable modifications:
K6 : Succinyl (K)
M12 : Oxidation (M), with neutral losses 0.0000(shown in table), 63.9983
Ions Score: 66 Expect: 0.00032
Matches (Bold Red): 31/364 fragment ions using 43 most intense peaks

#	a	a++	a*	a*++	b	b++	b*	b*++	Seq.	y	y++	y*	y*++	#
1	72.0808	36.5440			100.0757	50.5415			V					22
2	201.1234	101.0653			229.1183	115.0628			E	2513.3181	1257.1627	2496.2915	1248.6494	21
3	288.1554	144.5813			316.1503	158.5788			S	2384.2755	1192.6414	2367.2489	1184.1281	20
4	416.2140	208.6106	399.1874	200.0974	444.2089	222.6081	427.1823	214.0948	Q	2297.2434	1149.1254	2280.2169	1140.6121	19
5	529.2980	265.1527	512.2715	256.6394	557.2930	279.1501	540.2664	270.6368	L	2169.1849	1085.0961	2152.1583	1076.5828	18
6	757.4090	379.2082	740.3825	370.6949	785.4040	393.2056	768.3774	384.6923	K	2056.1008	1028.5540	2039.0743	1020.0408	17
7	870.4931	435.7502	853.4666	427.2369	898.4880	449.7476	881.4615	441.2344	I	1827.9898	914.4985	1810.9633	905.9853	16
8	983.5772	492.2922	966.5506	483.7789	1011.5721	506.2897	994.5455	497.7764	L	1714.9057	857.9565	1697.8792	849.4432	15
9	1096.6612	548.8343	1079.6347	540.3210	1124.6561	562.8317	1107.6296	554.3184	I	1601.8217	801.4145	1584.7951	792.9012	14
10	1252.7623	626.8848	1235.7358	618.3715	1280.7573	640.8823	1263.7307	632.3690	R	1488.7376	744.8724	1471.7111	736.3592	13
11	1349.8151	675.4112	1332.7886	666.8979	1377.8100	689.4086	1360.7835	680.8954	P	1332.6365	666.8219	1315.6100	658.3086	12
12	1496.8505	748.9289	1479.8240	740.4156	1524.8454	762.9264	1507.8189	754.4131	M	1235.5837	618.2955	1218.5572	609.7822	11
13	1659.9138	830.4606	1642.8873	821.9473	1687.9088	844.4580	1670.8822	835.9447	Y	1088.5483	544.7778	1071.5218	536.2645	10
14	1746.9459	873.9766	1729.9193	865.4633	1774.9408	887.9740	1757.9142	879.4608	S	925.4850	463.2461	908.4585	454.7329	9
15	1860.9888	930.9980	1843.9622	922.4848	1888.9837	944.9955	1871.9572	936.4822	N	838.4530	419.7301	821.4264	411.2169	8
16	1958.0416	979.5244	1941.0150	971.0111	1986.0365	993.5219	1969.0099	985.0086	P	724.4100	362.7087	707.3835	354.1954	7
17	2055.0943	1028.0508	2038.0678	1019.5375	2083.0892	1042.0483	2066.0627	1033.5350	P	627.3573	314.1823	610.3307	305.6690	6
18	2168.1784	1084.5928	2151.1518	1076.0796	2196.1733	1098.5903	2179.1468	1090.0770	I	530.3045	265.6559	513.2780	257.1426	5
19	2282.2213	1141.6143	2265.1948	1133.1010	2310.2162	1155.6118	2293.1897	1147.0985	N	417.2205	209.1139	400.1939	200.6006	4
20	2339.2428	1170.1250	2322.2162	1161.6118	2367.2377	1184.1225	2350.2111	1175.6092	G	303.1775	152.0924	286.1510	143.5791	3
21	2410.2799	1205.6436	2393.2533	1197.1303	2438.2748	1219.6410	2421.2483	1211.1278	A	246.1561	123.5817	229.1295	115.0684	2
22									R	175.1190	88.0631	158.0924	79.5498	1

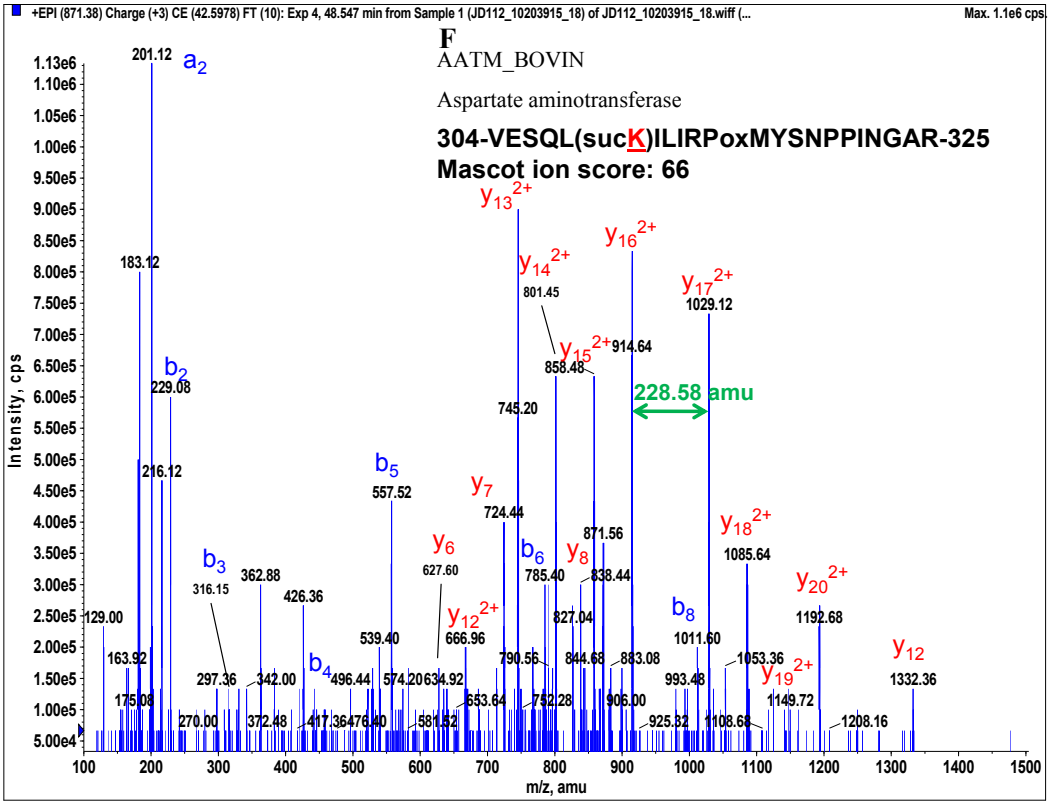


Fig. S13. MS/MS identification of succinyl peptides that were pulled out by Sirt5-FLAG affinity purification. (A and B) HMG-CoA synthase 2 (HMGCS2); (C and D) thiosulfate sulfurtransferase; (E and F) aspartate aminotransferase.

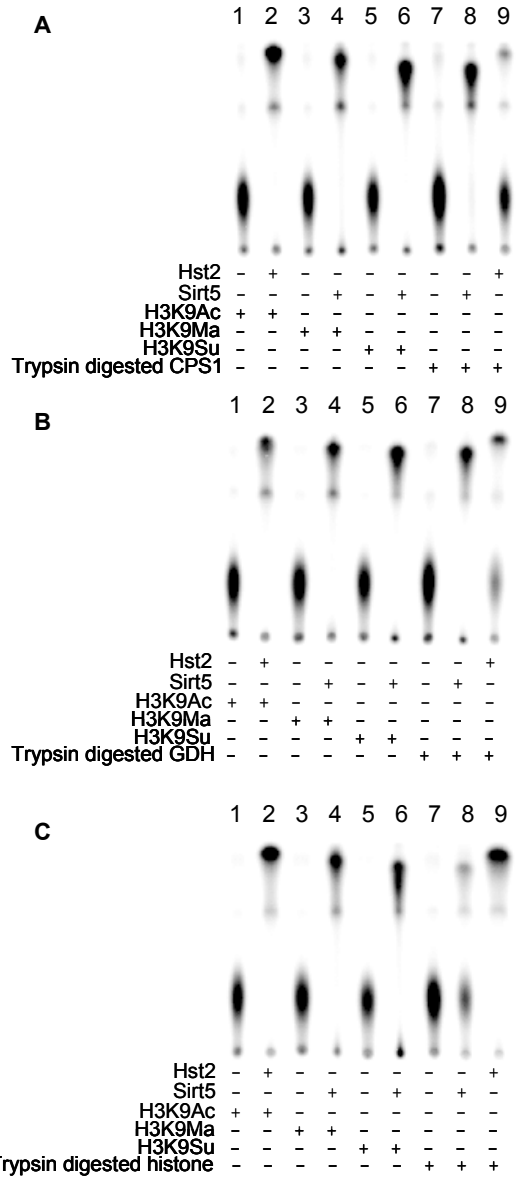
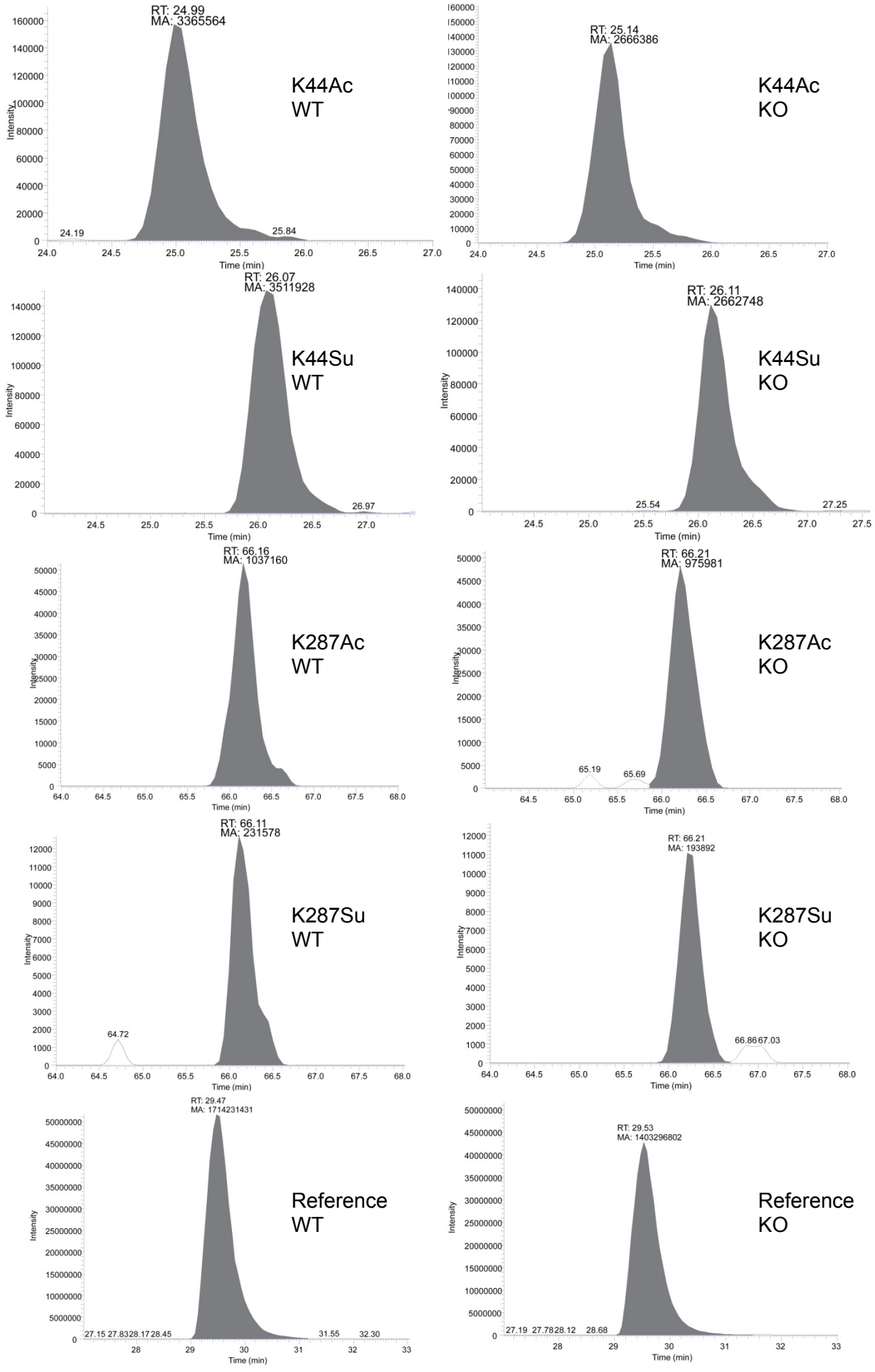


Fig. S14. Succinyl lysine modification could be detected in CPS1 and GDH peptides. Different proteins were digested by trypsin and then incubated with ^{32}P-NAD and either Sirt5 or Hst2. For each panel, lane 1, 3 and 5 were negative controls without sirtuins. Lane 2, 4 and 6 were positive controls showing the positions of *O*-acetyl-, *O*-malonyl- and *O*-succinyl-ADP ribose on the TLC plate, respectively. Lane 7 was trypsin digested peptides without any sirtuin. Lane 8 and 9 were trypsin digested peptides treated with Sirt5 and Hst2 respectively. Lane 8 in all 3 samples showed the formation of *O*-Su-ADPR while lane 9 showed the formation of *O*-Ac-ADPR. The results showed that both CPS1 and GDH contain succinyl lysine modification. In contrast, very little succinyl lysine modification was detected on histone peptides.

Fig. S15. Tyr and Arg residues are conserved in most Class III sirtuins. The conservation of specific sequences motifs within the 14 Class III sirtuins were aligned and the conserved Tyr102 and Arg105 (Sirt5 numbering) residues are shaded.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MA: 3365564
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WT
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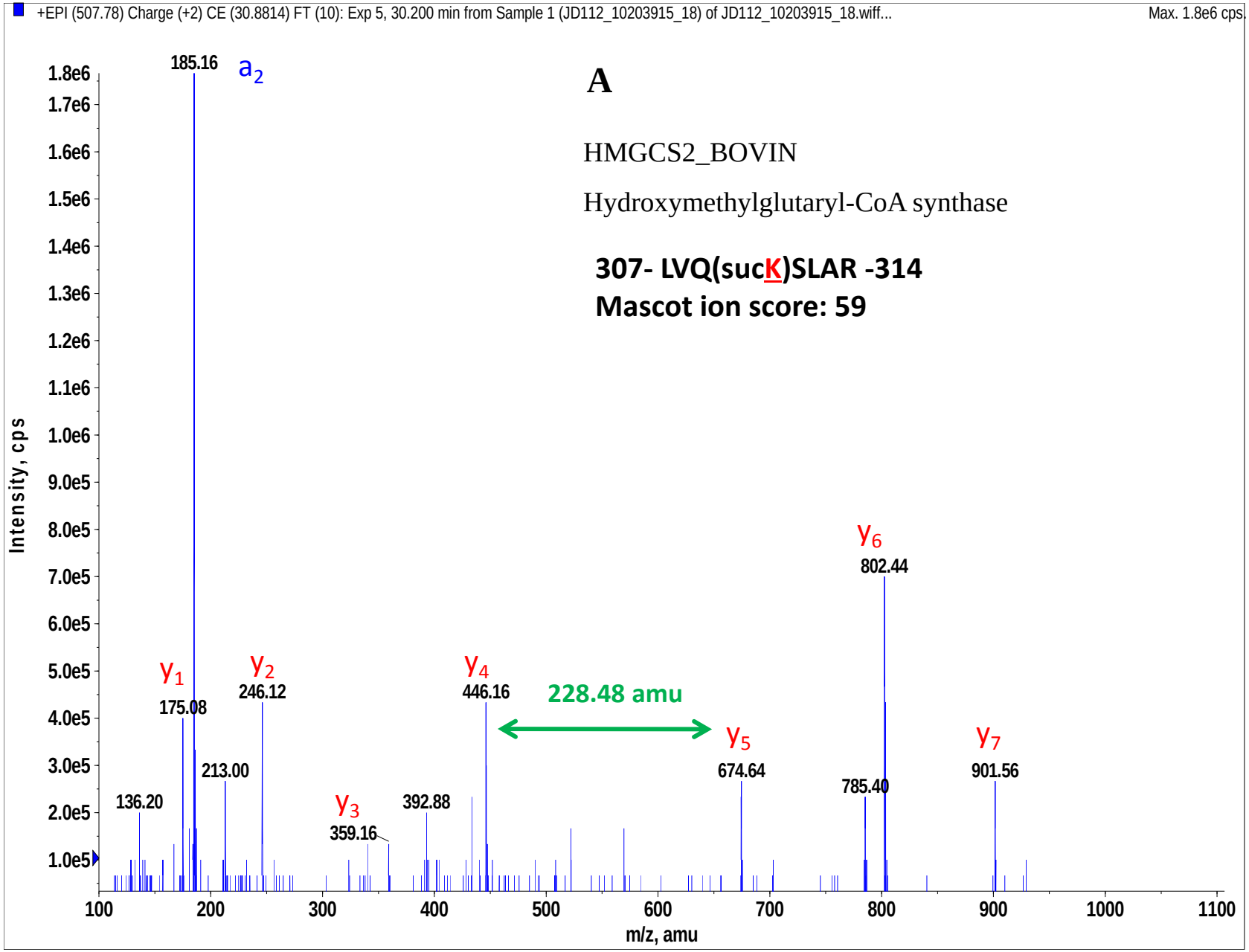
K44Ac
KO
RT: 26.07
MA: 3511928
K44Su
WT
RT: 26.11
MA: 2662748
K44Su
KO
RT: 66.16
MA: 1037160
K287Ac
WT
RT: 66.21
MA: 975981
K287Ac
KO
RT: 66.11
MA: 231578
K287Su
WT
RT: 66.21
MA: 193892
K287Su
KO
RT: 29.47
MA: 1714231431
Reference
WT
RT: 29.53
MA: 1403296802
Reference
KO
Intensity
Time (min)

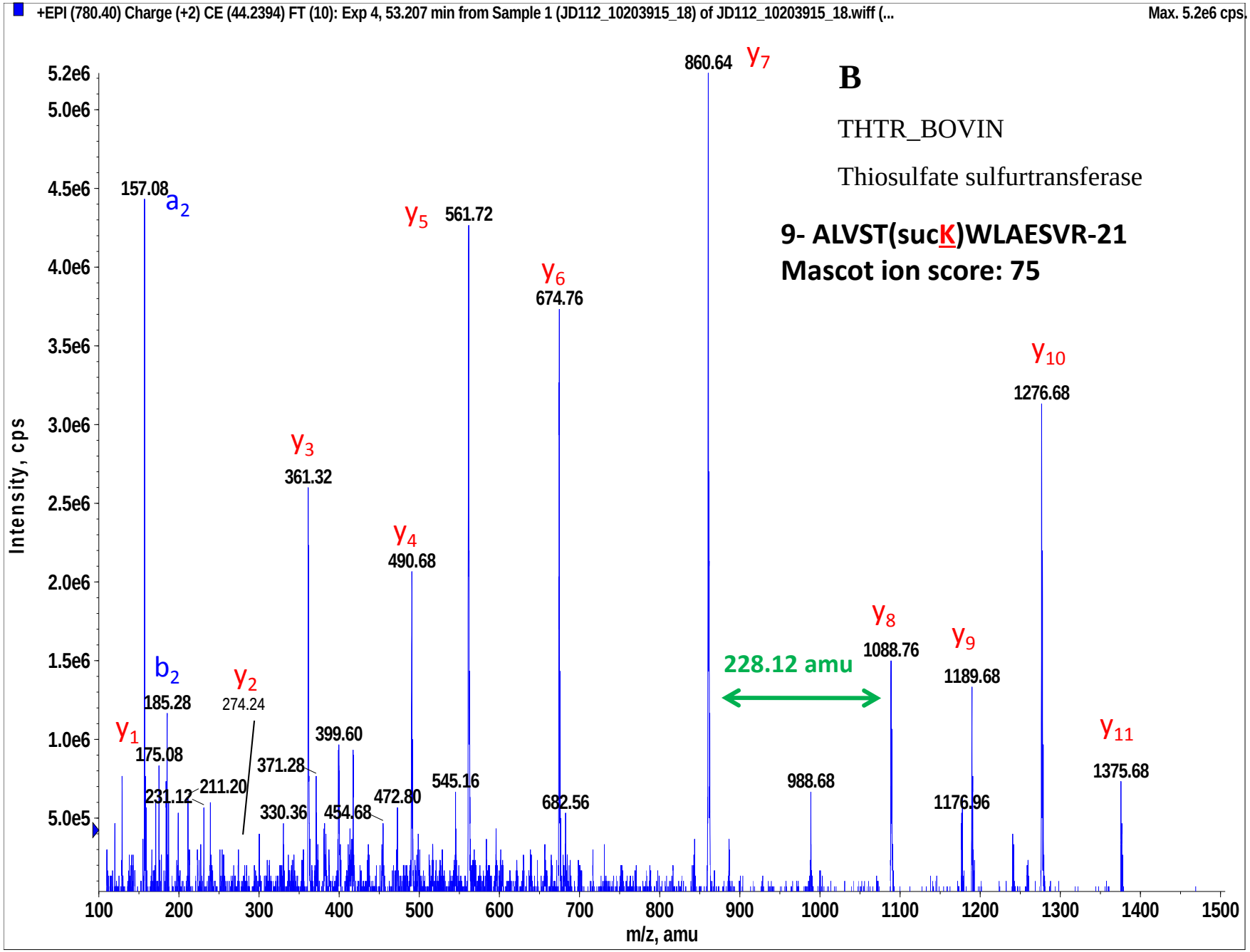
Fig. S16. Relative quantitation analysis of CPS1 K44 and K287 acetylation level and succinylation level from Sirt5 KO and WT mice. Relative quantitation was achieved by extracted ion chromatograms (XICs) for peak areas of CPS1 K44 acetyl and succinyl peptides and K287 acetyl and succinyl peptides from Sirt5 KO and WT mice. A reference peptide was also included to make sure that the amounts of CPS1 peptides injected in different runs were similar.

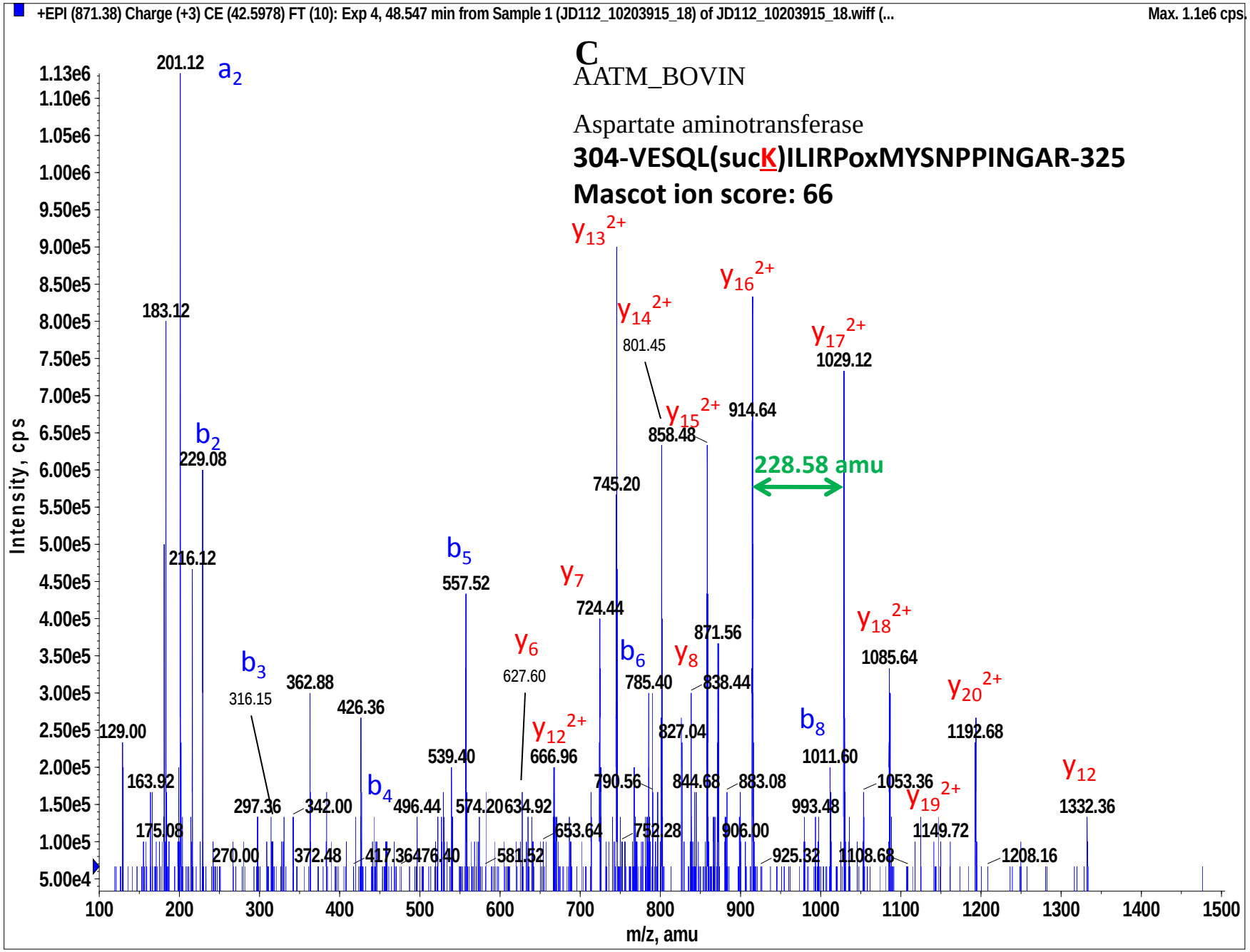
Fig. S17. MS/MS spectra for the identified acyl peptides

Succinyl peptides identified from mitochondria by LC-MS/MS.

Proteins		**Modified peptides**
HMGCS2	1	307-LVQK(succinyl)SLAR-314
Thiosulfate sulfurtransferase	1	9-ALVSTK(succinyl)WLAESVR-21
Aspartate aminotransferase	1	304-VESQLK(succinyl)ILIRPMYSNPPINGAR-325

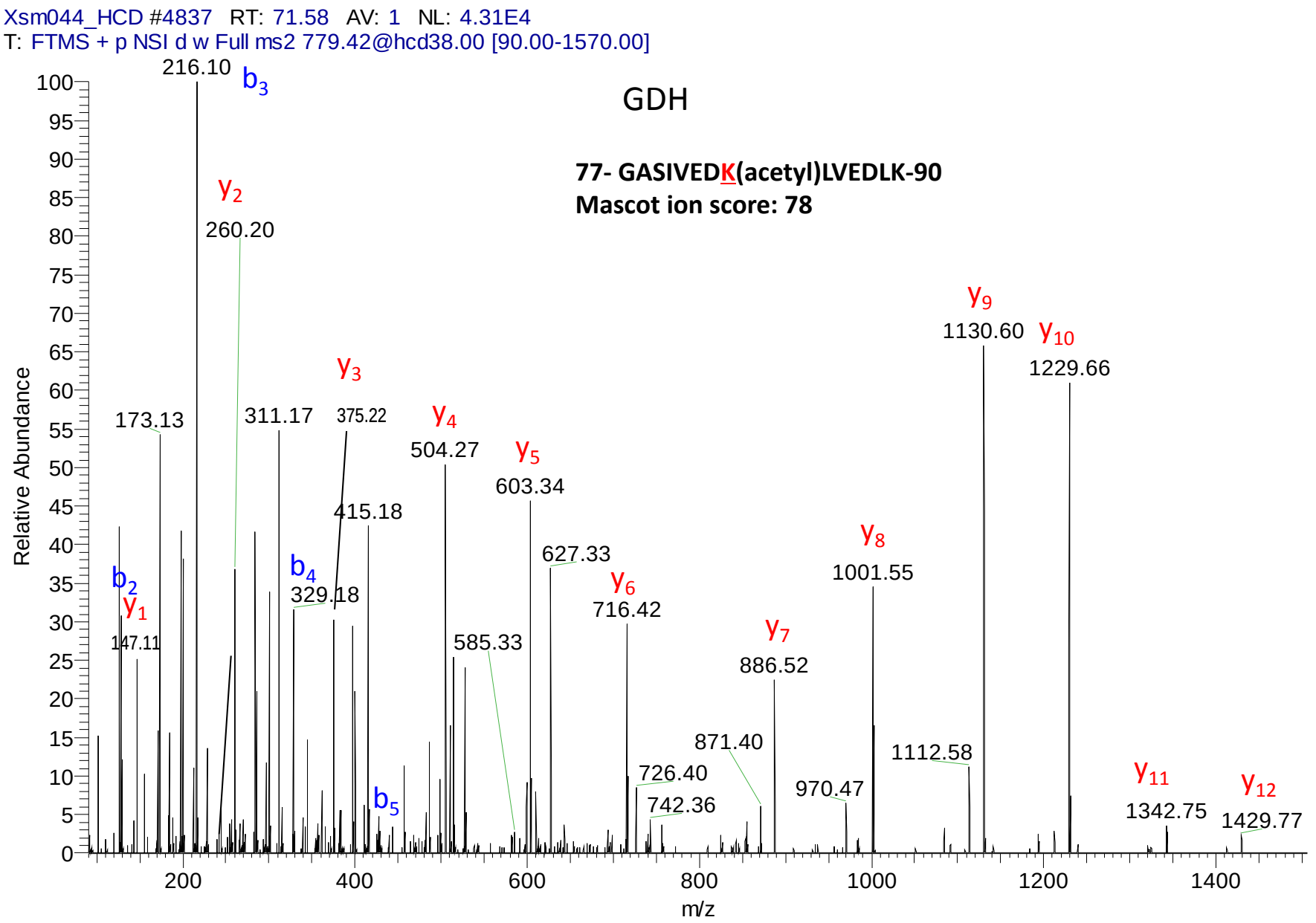

+EPI (507.78) Charge (+2) CE (30.8814) FT (10): Exp 5, 30.200 min from Sample 1 (JD112_10203915_18) of JD112_10203915_18.wiff...
Max. 1.8e6 cps.
A
HMGCS2_BOVIN
Hydroxymethylglutaryl-CoA synthase
307- LVQ(sucK)SLAR -314
Mascot ion score: 59
Intensity, cps
m/z, amu
185.16
a2
y1
175.08
y2
246.12
y3
359.16
y4
446.16
228.48 amu
y5
674.64
y6
802.44
y7
901.56
136.20
213.00
392.88
785.40

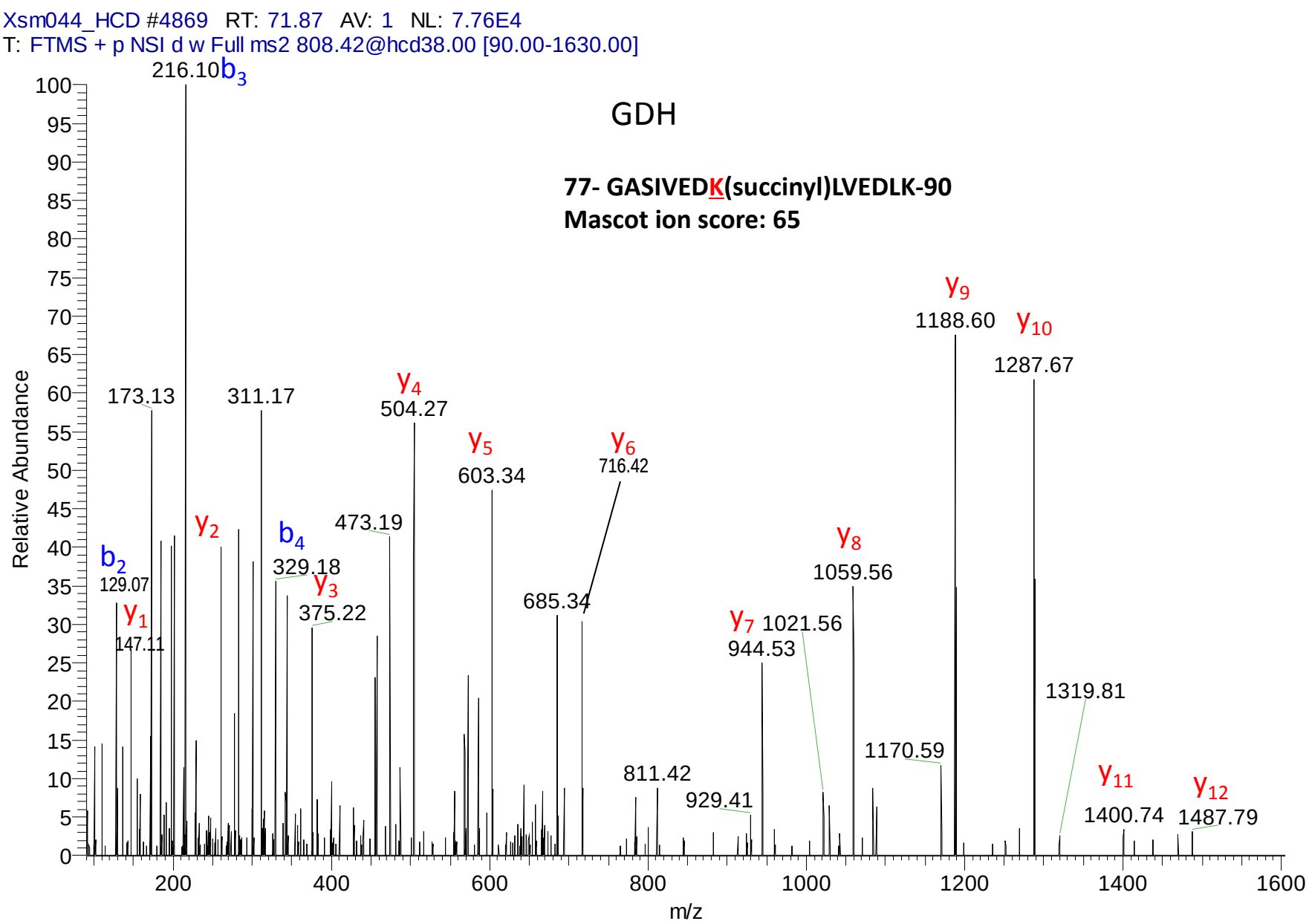

+EPI (780.40) Charge (+2) CE (44.2394) FT (10): Exp 4, 53.207 min from Sample 1 (JD112_10203915_18) of JD112_10203915_18.wiff (...
Max. 5.2e6 cps.
B
THTR_BOVIN
Thiosulfate sulfurtransferase
9- ALVST(sucK)WLAESVR-21
Mascot ion score: 75
228.12 amu
Intensity, cps
m/z, amu
860.64 y7
561.72 y5
674.76 y6
1276.68 y10
361.32 y3
490.68 y4
1088.76 y8
1189.68 y9
1375.68 y11
157.08 a2
185.28 b2
274.24 y2
175.08 y1
231.12
211.20
371.28
399.60
330.36
454.68
472.80
545.16
682.56
988.68
1176.96

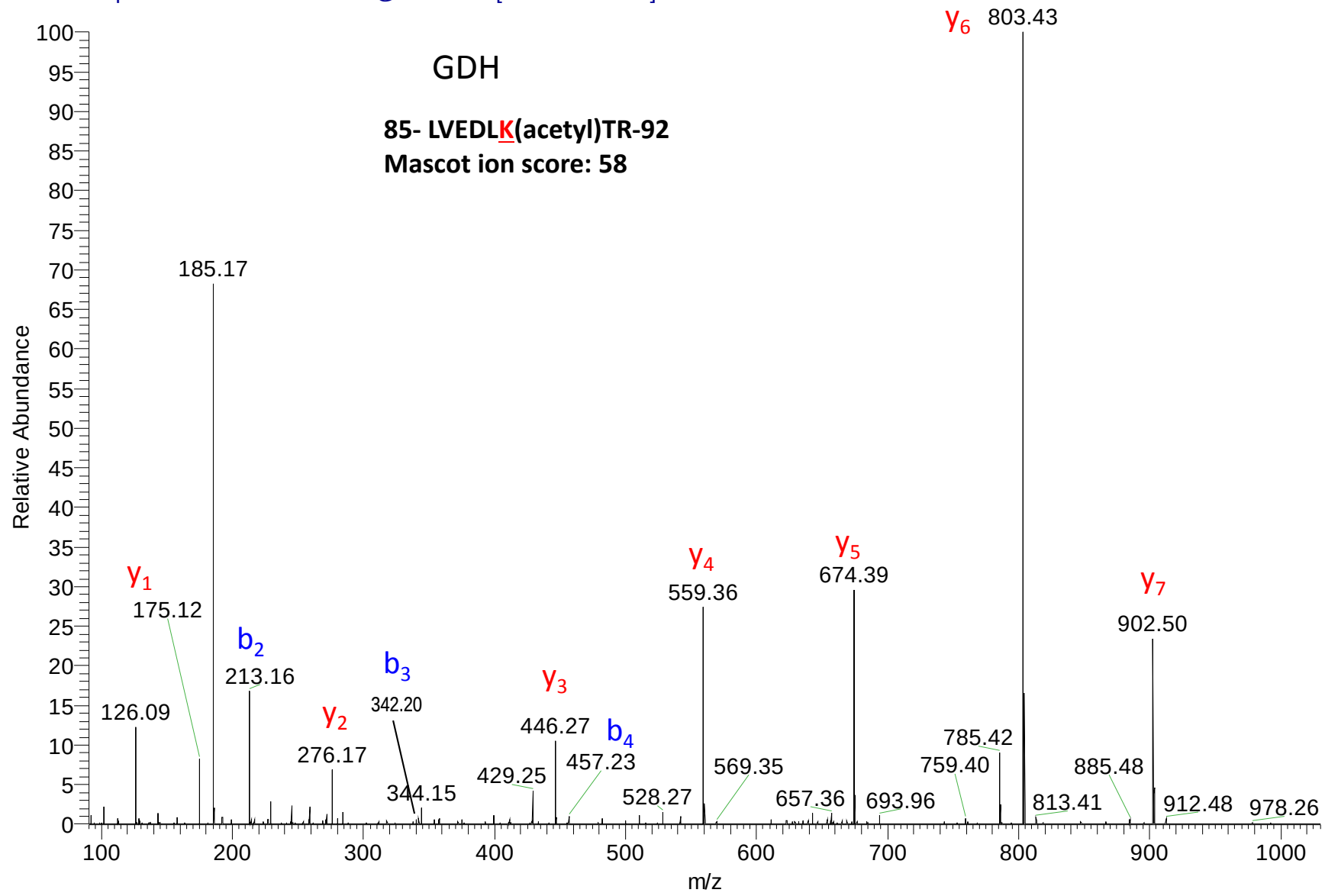

+EPI (871.38) Charge (+3) CE (42.5978) FT (10): Exp 4, 48.547 min from Sample 1 (JD112_10203915_18) of JD112_10203915_18.wiff (...
Max. 1.1e6 cps.
C
AATM_BOVIN
Aspartate aminotransferase
304-VESQL(sucK)ILIRPoxMYSNPPINGAR-325
Mascot ion score: 66
Intensity, cps
m/z, amu
228.58 amu
a2
b2
b3
b4
b5
b6
b8
y6
y7
y8
y12
y12 2+
y13 2+
y14 2+
y15 2+
y16 2+
y17 2+
y18 2+
y19 2+
y20 2+
201.12
183.12
229.08
216.12
316.15
362.88
426.36
557.52
627.60
724.44
745.20
785.40
801.45
858.48
871.56
914.64
1029.12
1085.64
1192.68
1332.36
1011.60
1053.36
1149.72
1208.16
925.32
1108.68
993.48
906.00
883.08
844.68
838.44
827.04
790.56
752.28
653.64
666.96
634.92
581.52
574.20
539.40
496.44
476.40
417.36
372.48
342.00
297.36
270.00
175.08
163.92
129.00

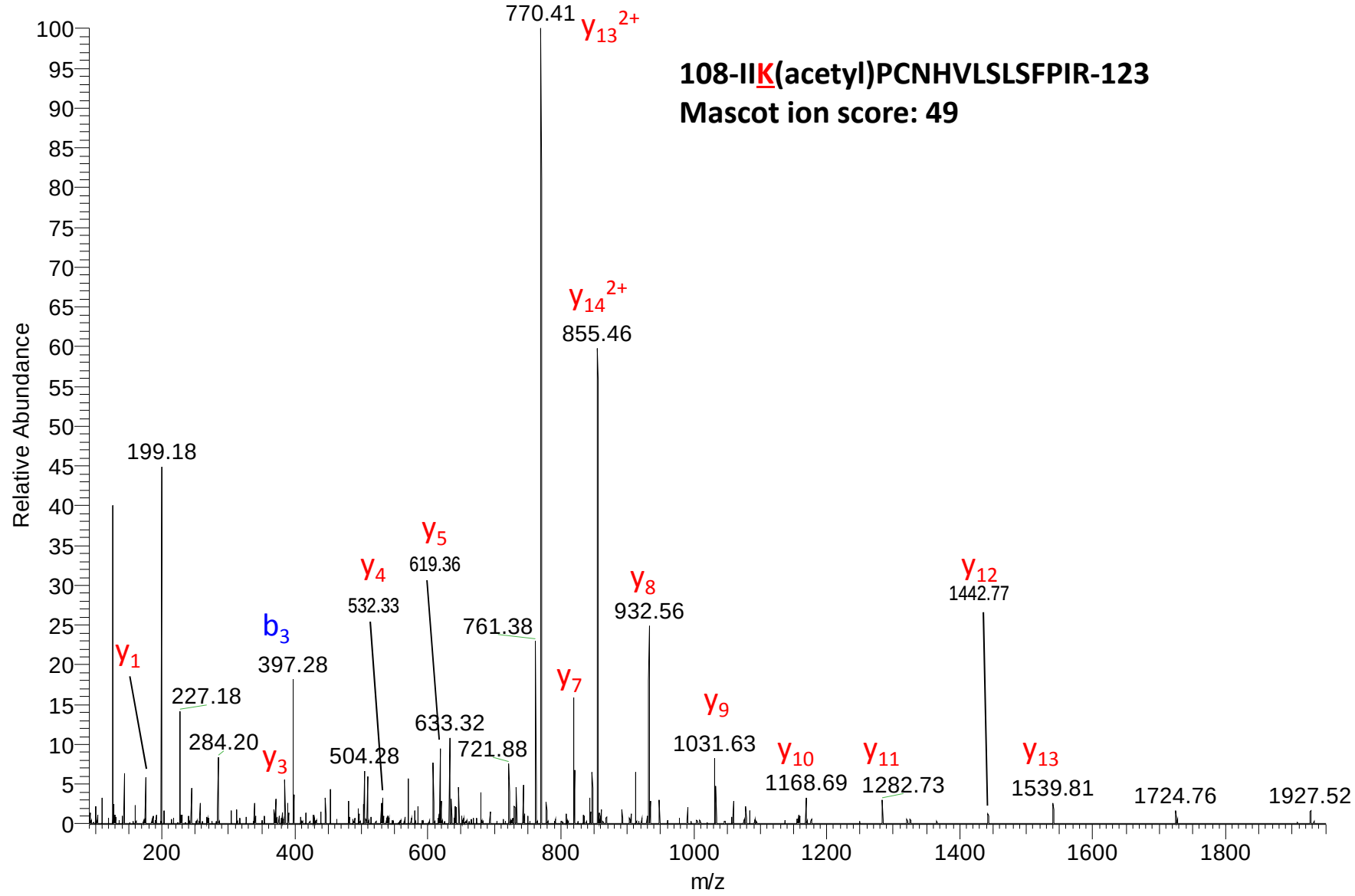
Acetyl, malonyl, and succinyl GDH peptides identified by LC-MS/MS.

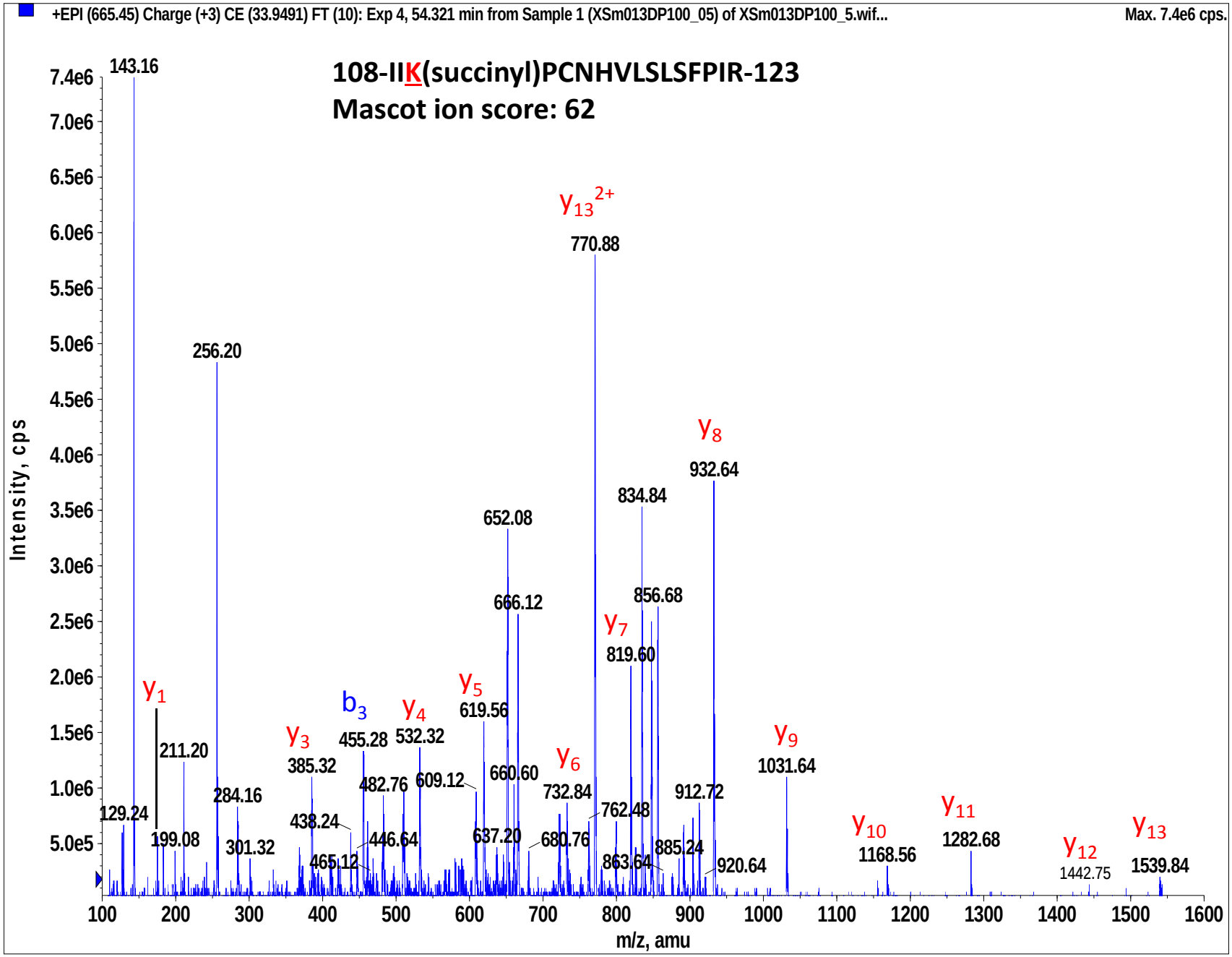
	Peptide sequence and modification
1	77-GASIVEDK(acetyl)LVEDLK-90
2	77-GASIVEDK(succinyl)LVEDLK-90
3	85-LVEDLK(acetyl)TR-92
4	108-IIK(acetyl)PCNHVLSLSFPIR-123
5	108-IIK(succinyl)PCNHVLSLSFPIR-123
6	152-YSTDVSVDEVK(acetyl)ALASLMTYK-171
7	152-YSTDVSVDEVK(acetyl)ALASLM(ox)TYK-171
8	152-YSTDVSVDEVK(succinyl)ALASLMTYK-171
9	172-CAVVDVPFGGAK(acetyl)AGVK-187
10	188-INPK(acetyl)NYTDNELEK-200
11	353-LQHGTILGFPK(acetyl)AK-363
12	353-LQHGTILGFPK(succinyl)AK-363
13	364-AK(acetyl)IYEGSILEVDCDILIPAASEK-386
14	366-IYEGSILEVDCDILIPAASEK(acetyl)QLTK-390
15	398-AK(acetyl)IIAEGANGPTTPEADK-415
16	398-AK(acetyl)IIAEGANGPTTPEADKIFLER-420
17	400- IIAEGANGPTTPEADK(acetyl)IFLER-420
18	400- IIAEGANGPTTPEADK(succinyl)IFLER-420
19	454-LTFK(acetyl)YER-460
20	454-LTFK(malonyl)YER-460
21	454-LTFK(succinyl)YER-460
22	478-FGK(acetyl)HGGTIPIVPTAEFQDR-496
23	497-ISGASEK(acetyl)DIVHSGLAYTMER-516
24	497-ISGASEK(malonyl)DIVHSGLAYTMER-516
25	497-ISGASEK(succinyl)DIVHSGLAYTMER-516
26	497-ISGASEK(succinyl)DIVHSGLAYTM(ox)ER-516
27	524-TAMK(acetyl)YNLGLDLR-535
28	524-TAM(ox)K(acetyl)YNLGLDLR-535
29	524-TAMK(malonyl)YNLGLDLR-535
30	524-TAMK(succinyl)YNLGLDLR-535
31	524-TAM(ox)K(succinyl)YNLGLDLR-535
32	536-TAAYVNAIEK(acetyl)VFR-548
33	536-TAAYVNAIEK(succinyl)VFR-548


Xsm044_HCD #4837 RT: 71.58 AV: 1 NL: 4.31E4
T: FTMS + p NSI d w Full ms2 779.42@hcd38.00 [90.00-1570.00]
GDH
77- GASIVEDK(acetyl)LVEDLK-90
Mascot ion score: 78
Relative Abundance
m/z
216.10
b3
y2
260.20
y3
375.22
y4
504.27
y5
603.34
y9
1130.60
y10
1229.66
173.13
311.17
415.18
627.33
b2
y1
147.11
b4
329.18
y6
716.42
y8
1001.55
585.33
y7
886.52
871.40
726.40
742.36
970.47
1112.58
b5
y11
1342.75
y12
1429.77


Xsm044_HCD #4869 RT: 71.87 AV: 1 NL: 7.76E4
T: FTMS + p NSI d w Full ms2 808.42@hcd38.00 [90.00-1630.00]
GDH
77- GASIVEDK(succinyl)LVEDLK-90
Mascot ion score: 65
Relative Abundance
m/z
216.10 b3
173.13
311.17
y4 504.27
y5 603.34
y6 716.42
473.19
y2
b4 329.18
b2 129.07
y3 375.22
y1 147.11
685.34
y9 1188.60
y10 1287.67
y8 1059.56
y7 1021.56 944.53
1319.81
1170.59
811.42
929.41
y11 1400.74
y12 1487.79


Xsm044_HCD #1307 RT: 40.89 AV: 1 NL: 1.80E6
T: FTMS + p NSI d w Full ms2 508.29@hcd38.00 [90.00-1030.00]
GDH
85- LVEDLK(acetyl)TR-92
Mascot ion score: 58
Relative Abundance
m/z
y6 803.43
185.17
y1 175.12
b2 213.16
b3 342.20
y2 276.17
126.09
y3 446.27
b4 457.23
429.25
344.15
y4 559.36
528.27
y5 674.39
569.35
657.36
693.96
785.42
759.40
y7 902.50
885.48
813.41
912.48
978.26


Xsm044_HCD #4816 RT: 71.39 AV: 1 NL: 8.56E4
T: FTMS + p NSI d w Full ms2 646.37@hcd38.00 [90.00-1950.00]
108-IIK(acetyl)PCNHVLSLSFPIR-123
Mascot ion score: 49
Relative Abundance
m/z
770.41
y13 2+
y14 2+
855.46
199.18
y5
619.36
y4
532.33
y8
932.56
y12
1442.77
b3
397.28
761.38
y1
y7
227.18
y9
1031.63
633.32
284.20
y3
504.28
721.88
y10
1168.69
y11
1282.73
y13
1539.81
1724.76
1927.52


+EPI (665.45) Charge (+3) CE (33.9491) FT (10): Exp 4, 54.321 min from Sample 1 (XSm013DP100_05) of XSm013DP100_5.wif...
Max. 7.4e6 cps.
108-IIK(succinyl)PCNHVLSLSFPIR-123
Mascot ion score: 62
Intensity, cps
m/z, amu
y13 2+
770.88
y8
932.64
834.84
652.08
856.68
666.12
y7
819.60
y5
619.56
y1
b3
y4
532.32
455.28
y3
385.32
y6
732.84
y9
1031.64
660.60
609.12
482.76
762.48
912.72
211.20
284.16
438.24
446.64
637.20
680.76
885.24
863.64
920.64
y10
1168.56
y11
1282.68
y12
1442.75
y13
1539.84
143.16
256.20
129.24
199.08
301.32
465.12
7.4e6
7.0e6
6.5e6
6.0e6
5.5e6
5.0e6
4.5e6
4.0e6
3.5e6
3.0e6
2.5e6
2.0e6
1.5e6
1.0e6
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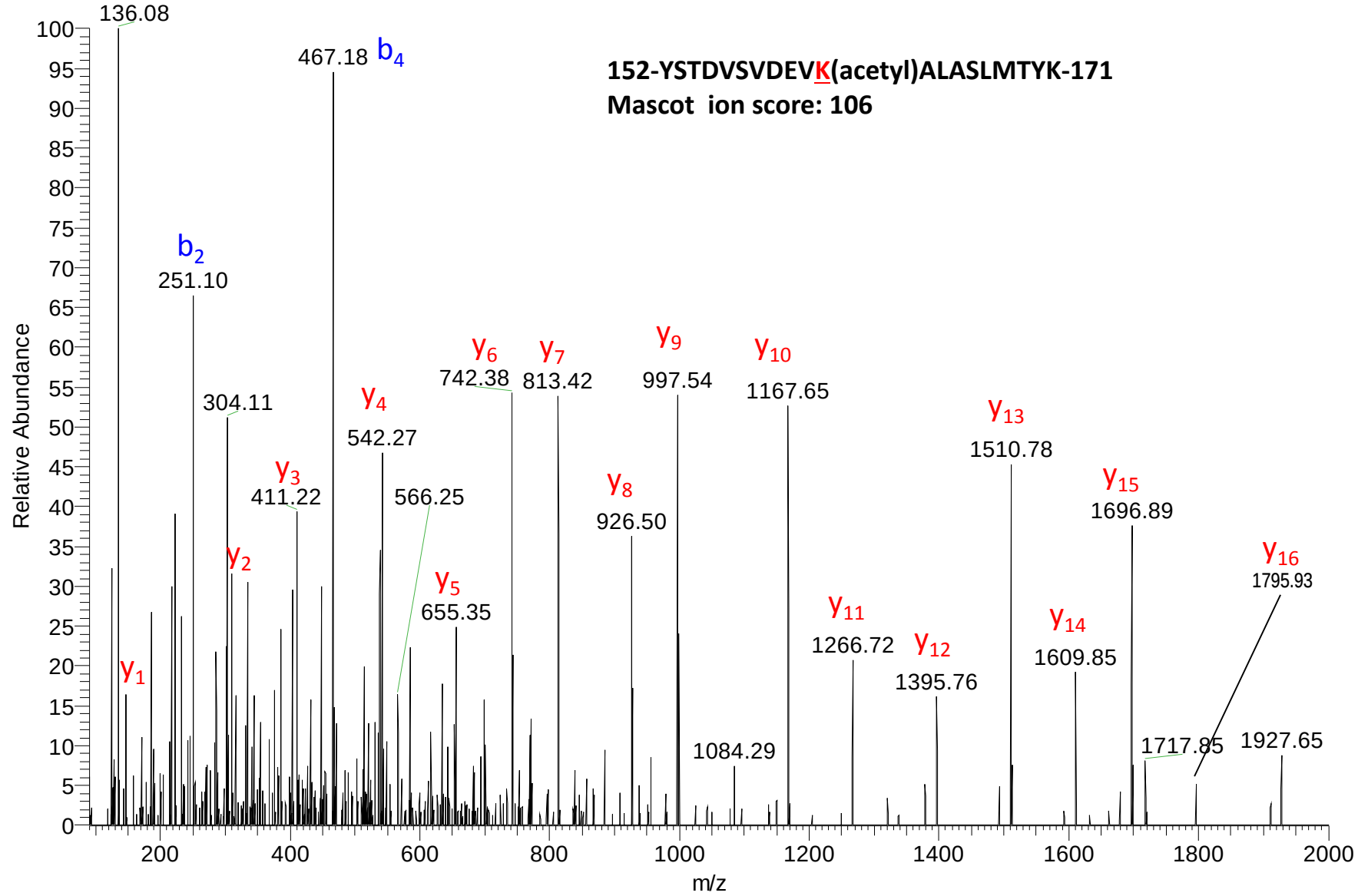
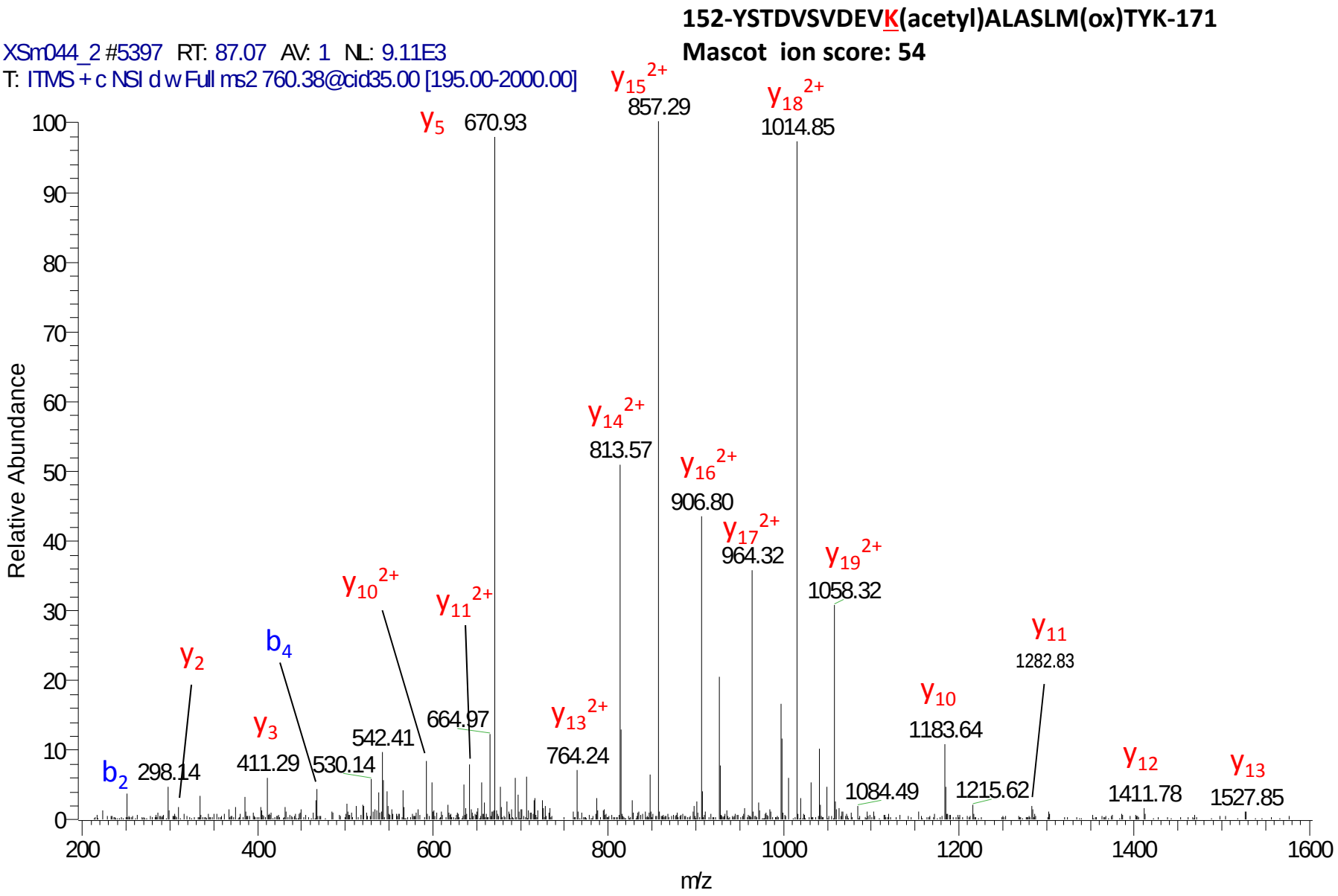
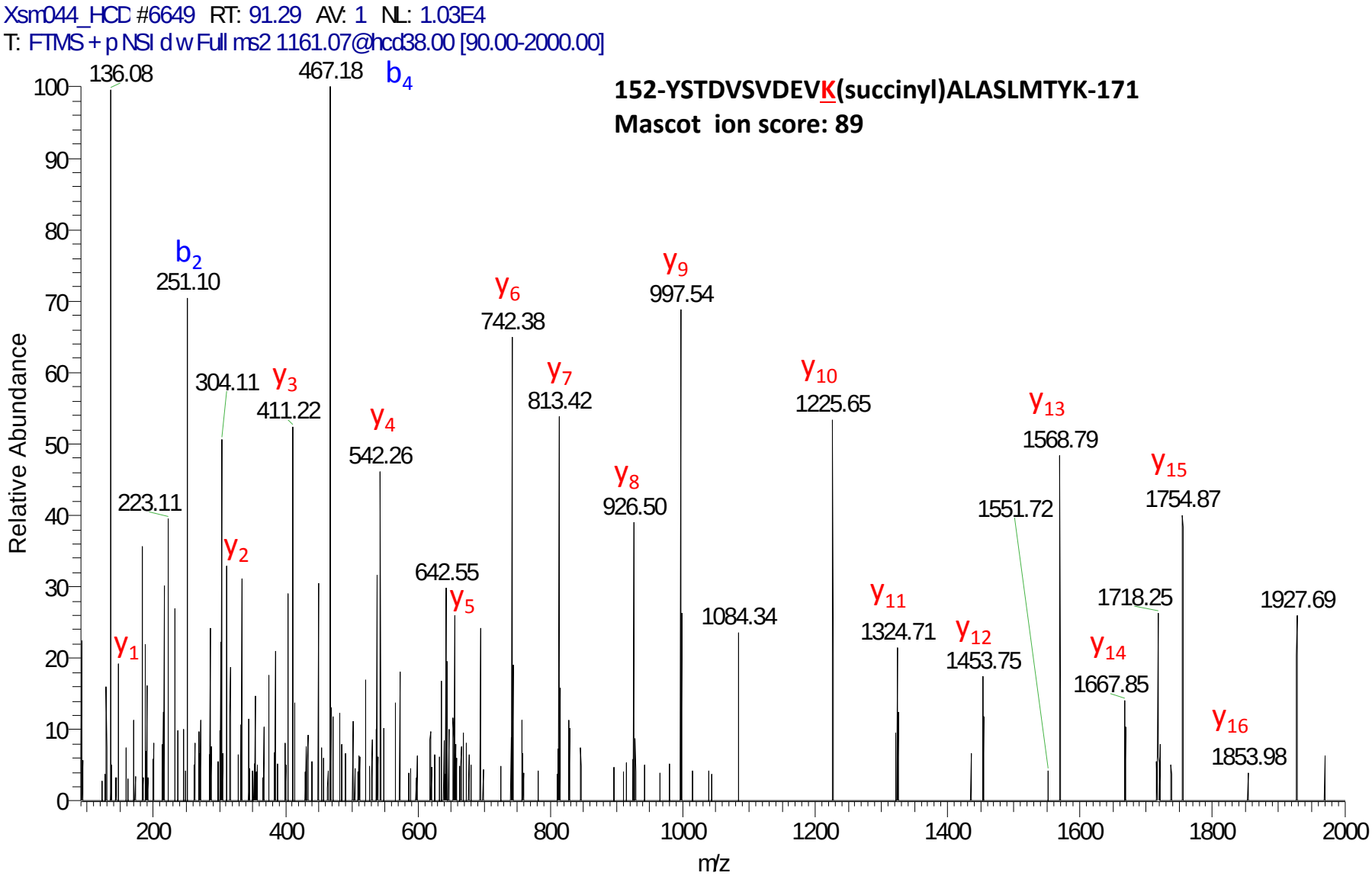
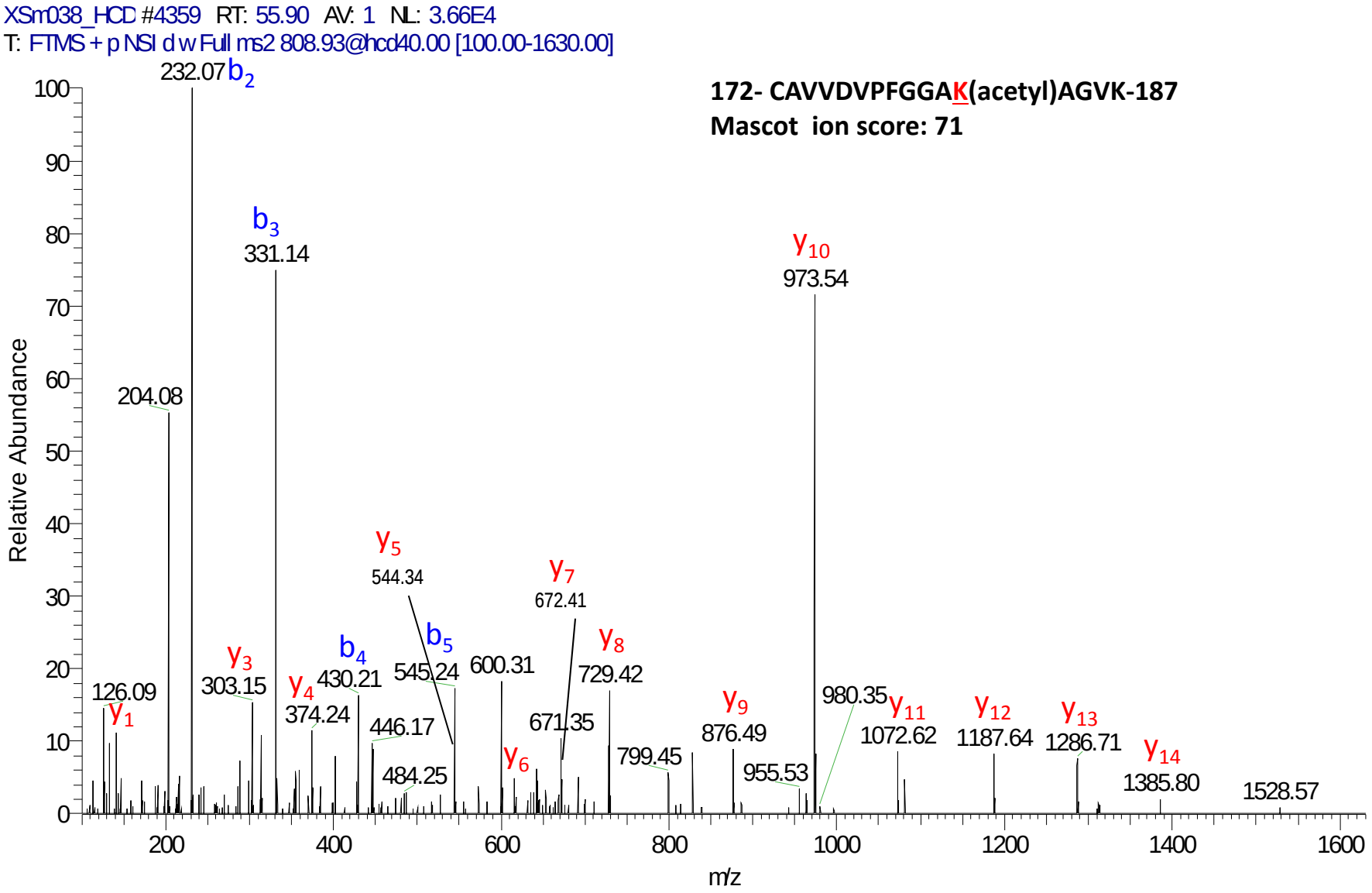
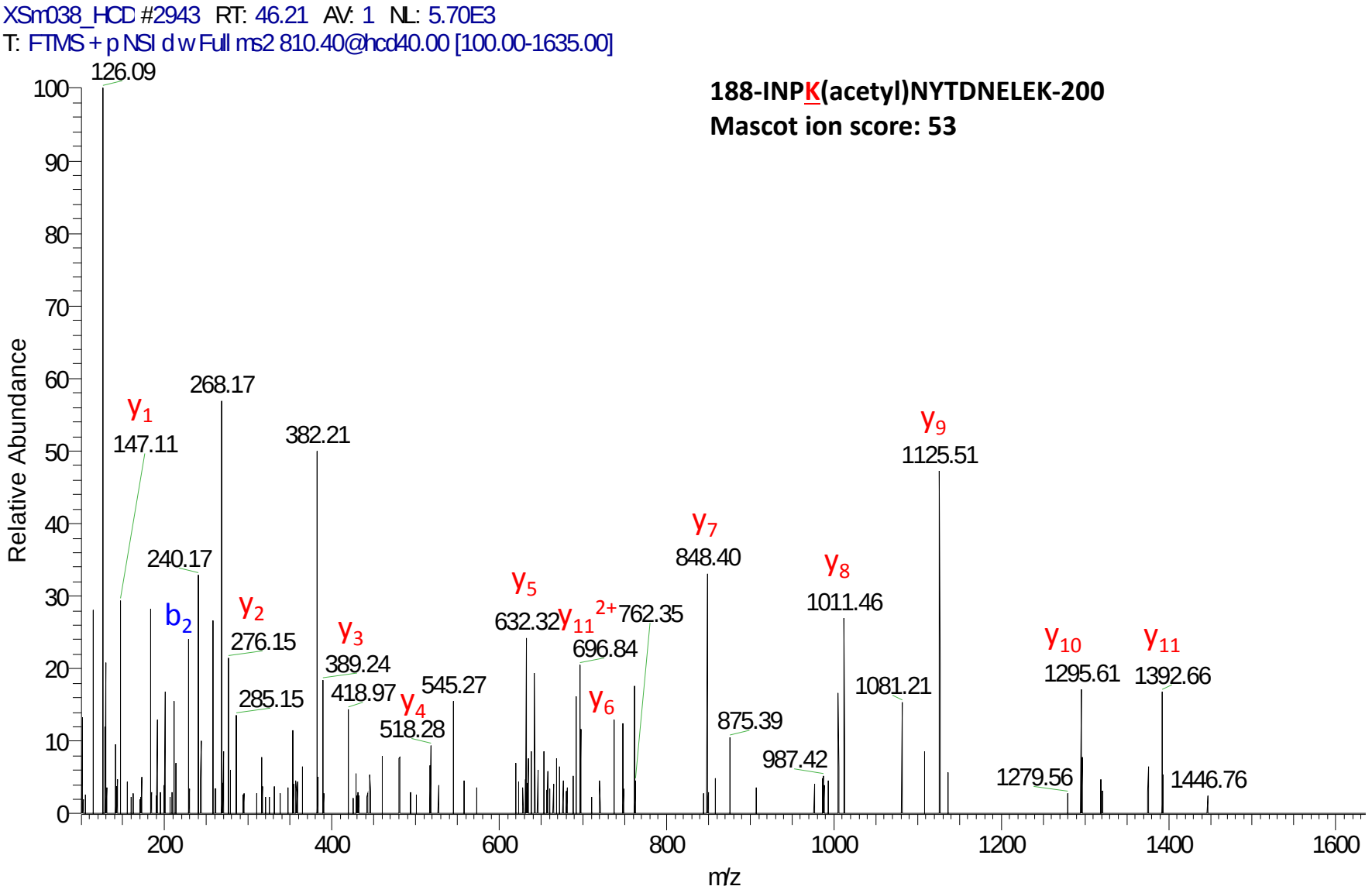
800
900
1000
1100
1200
1300
1400
1500
1600

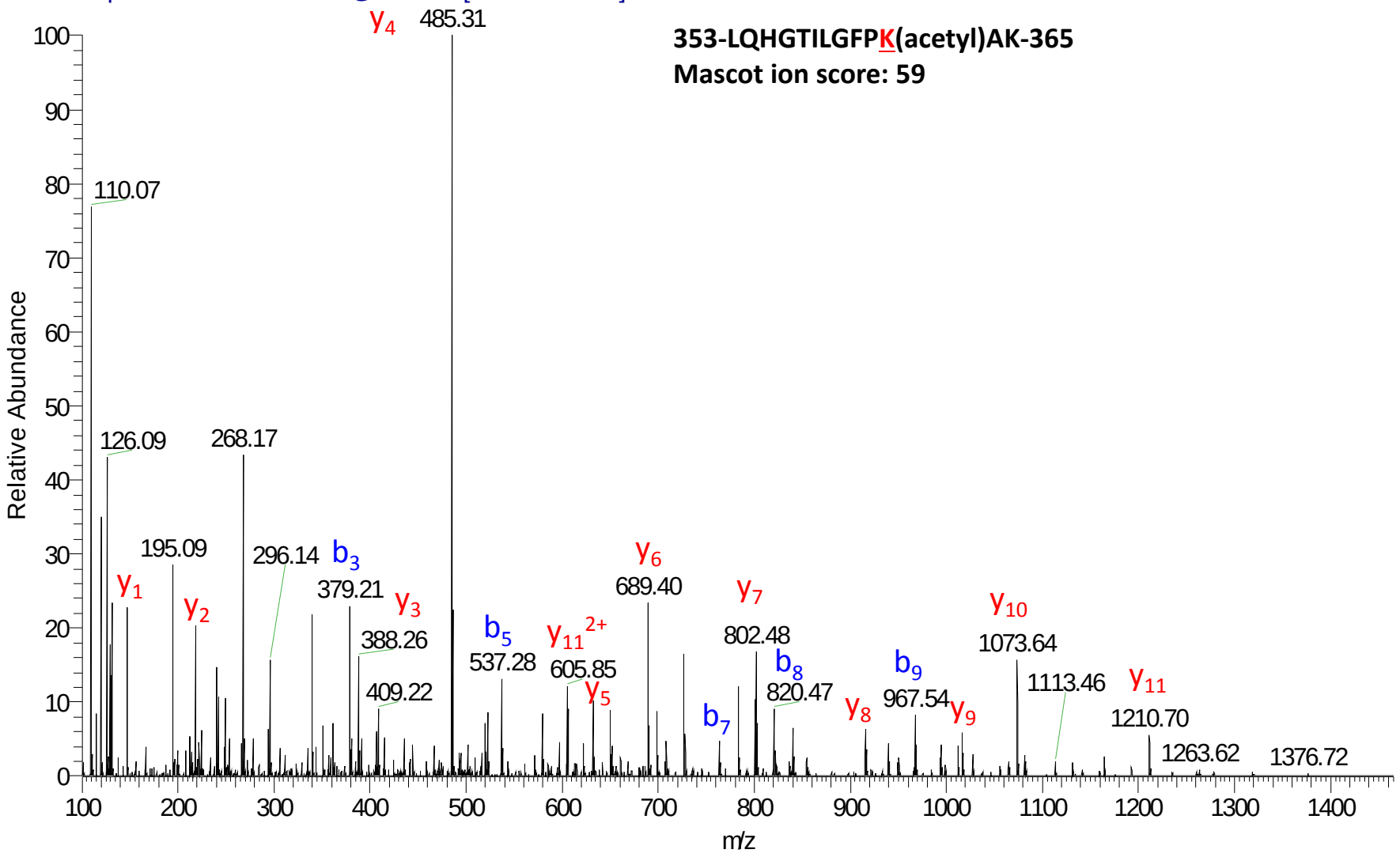

Xsm044_HCD #6643 RT: 91.20 AV: 1 NL: 6.01E3
T: FTMS + p NSI d w Full ms2 1132.07@hcd38.00 [90.00-2000.00]
152-YSTDVSVDEVK(acetyl)ALASLMTYK-171
Mascot ion score: 106
Relative Abundance
m/z
136.08
467.18 b4
b2 251.10
304.11
y4 542.27
y3 411.22
566.25
y2
y5 655.35
y1
y6 742.38
y7 813.42
y9 997.54
y10 1167.65
y8 926.50
1084.29
y11 1266.72
y12 1395.76
y13 1510.78
y14 1609.85
y15 1696.89
1717.85
y16 1795.93
1927.65

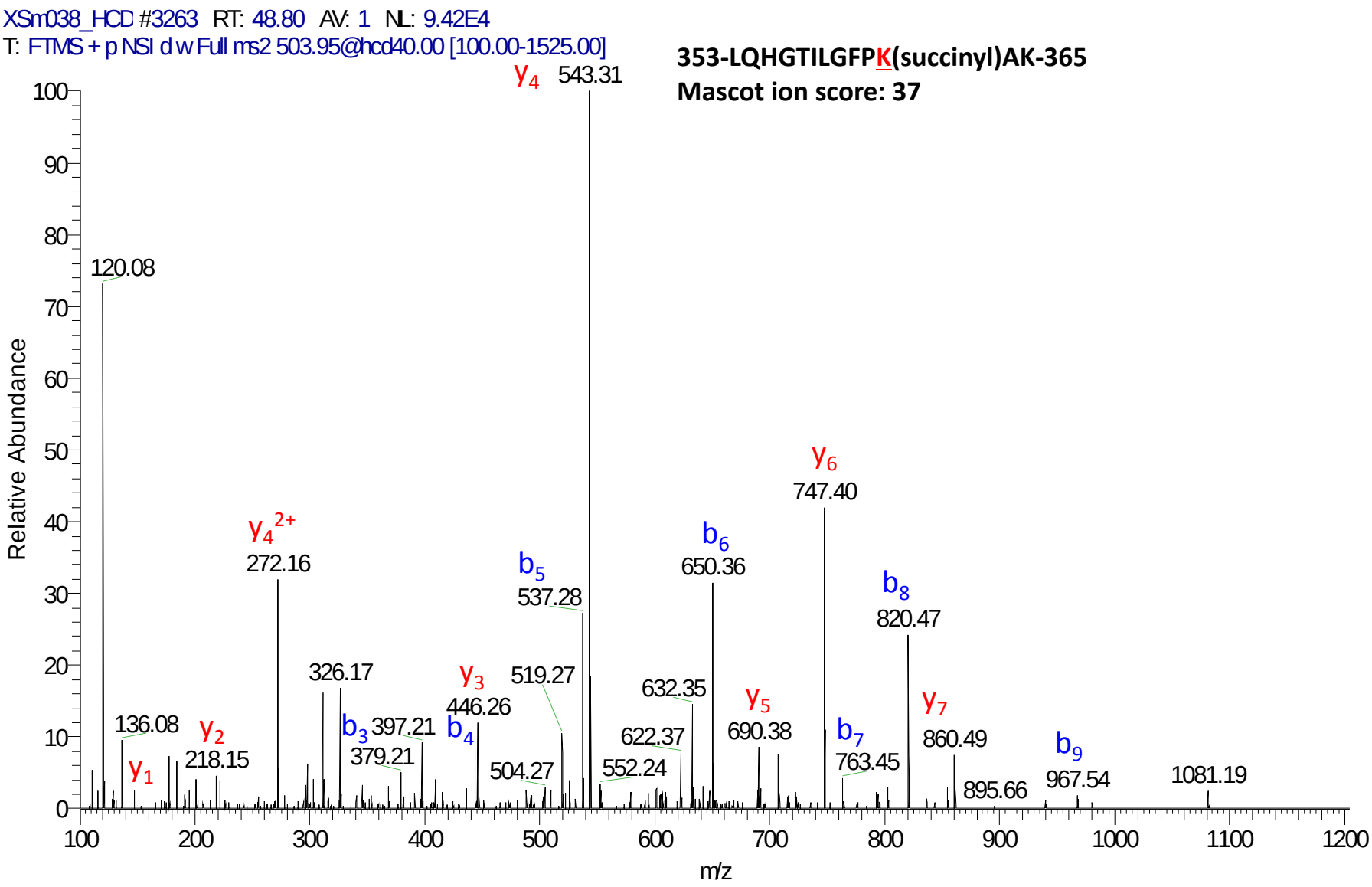

152-YSTDVSVDEVK(acetyl)ALASLM(ox)TYK-171
Mascot ion score: 54
XSm044_2 #5397 RT: 87.07 AV: 1 NL: 9.11E3
T: ITMS + c NSI d w Full ms2 760.38@cid35.00 [195.00-2000.00]
Relative Abundance
m/z
y5 670.93
y15 2+ 857.29
y18 2+ 1014.85
y14 2+ 813.57
y16 2+ 906.80
y17 2+ 964.32
y19 2+ 1058.32
y11 1282.83
y10 1183.64
y12 1411.78
y13 1527.85
y10 2+
y11 2+
y2
b4
y3
b2 298.14
411.29
530.14
542.41
664.97
y13 2+ 764.24
1084.49
1215.62

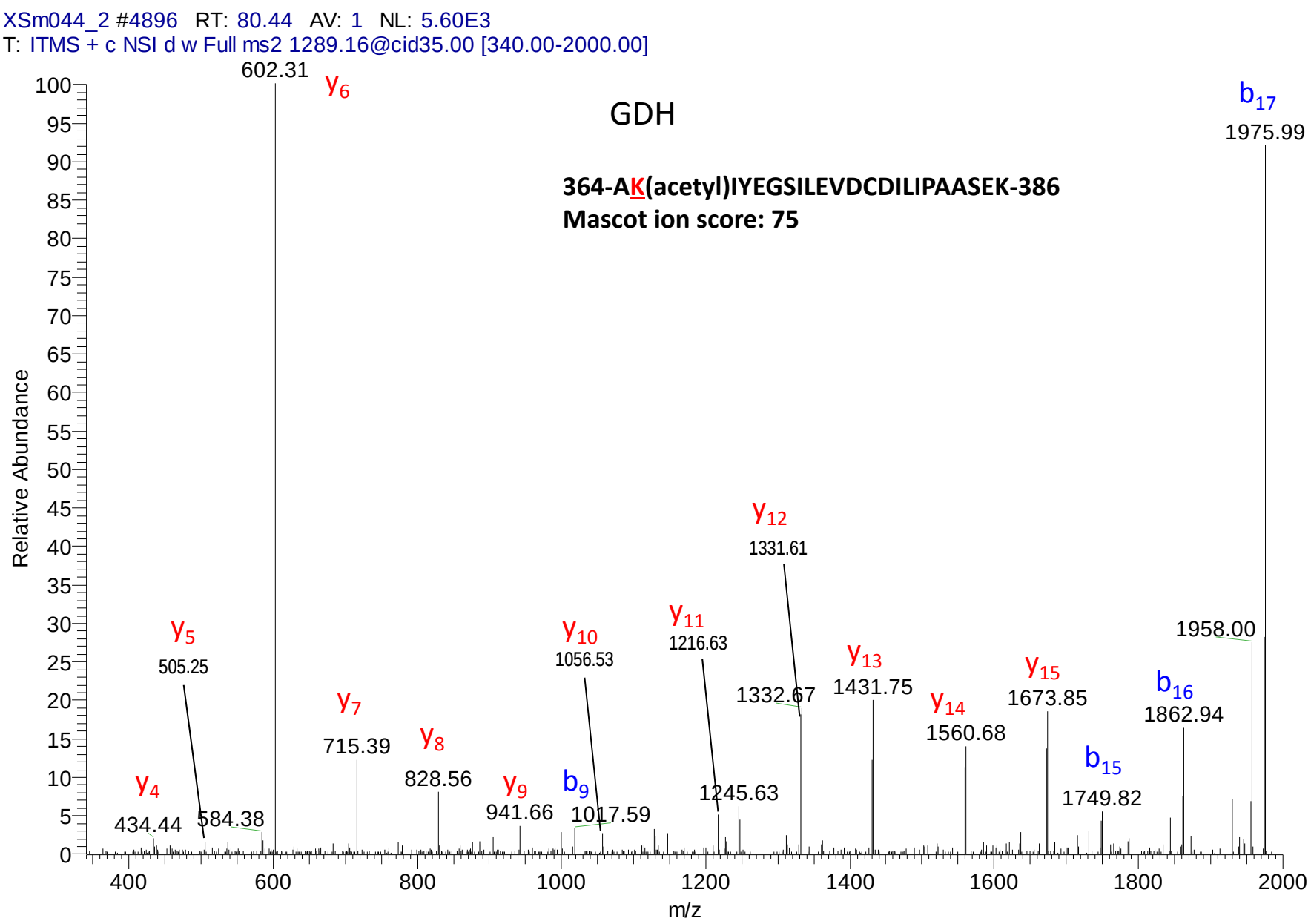

Xsm044_HCD #6649 RT: 91.29 AV: 1 NL: 1.03E4
T: FTMS + p NSI d w Full ms2 1161.07@hcd38.00 [90.00-2000.00]
152-YSTDVSVDEVK(succinyl)ALASLMTYK-171
Mascot ion score: 89
Relative Abundance
m/z
136.08
467.18
b4
b2
251.10
304.11
y3
411.22
y4
542.26
y6
742.38
y7
813.42
y9
997.54
y10
1225.65
y13
1568.79
y15
1754.87
223.11
y2
642.55
y5
y8
926.50
1084.34
y11
1324.71
y12
1453.75
1551.72
1718.25
y14
1667.85
1927.69
y16
1853.98
y1

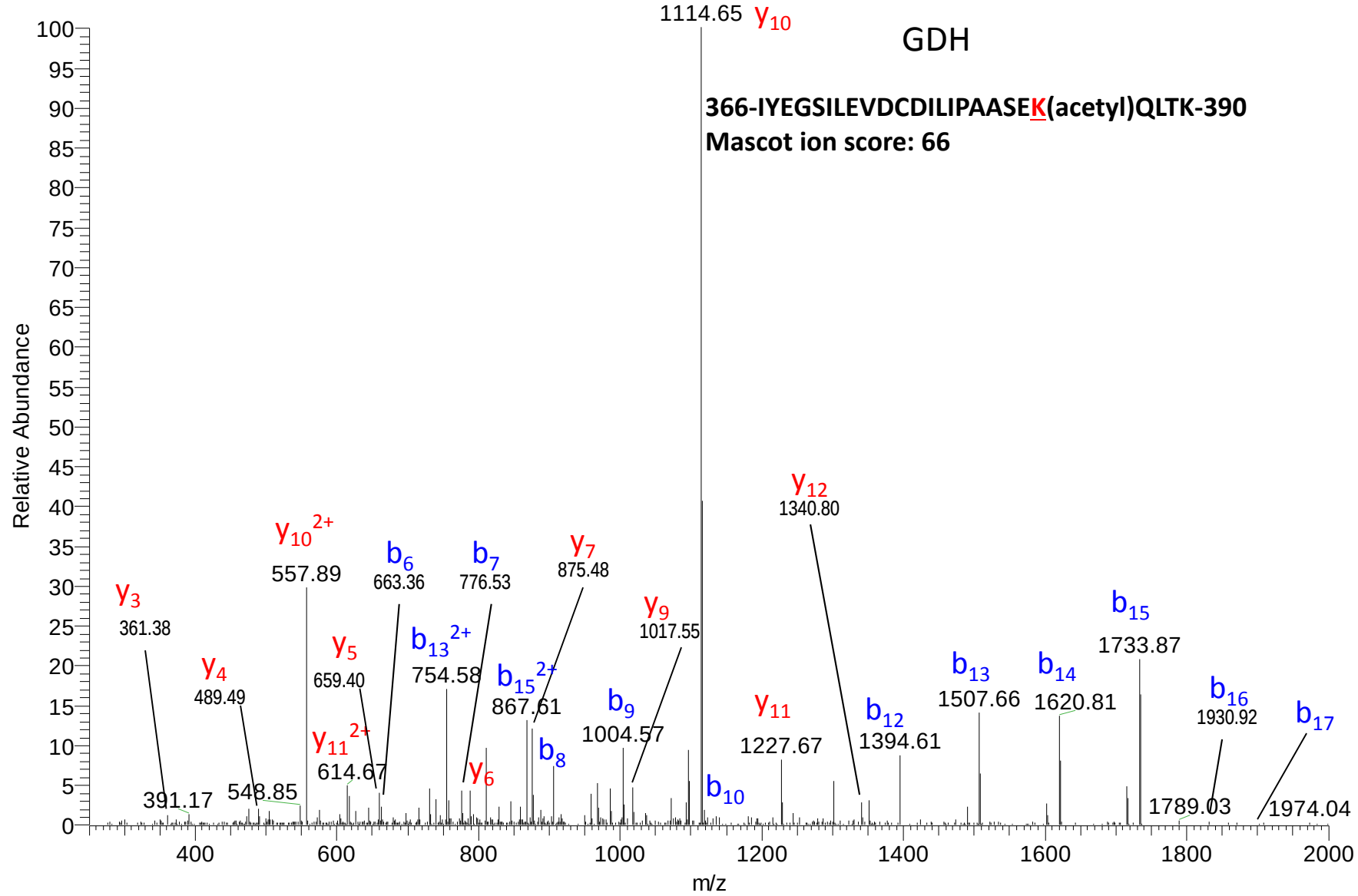

XSm038_HCD #4359 RT: 55.90 AV: 1 NL: 3.66E4
T: FTMS + p NSI d w Full ms2 808.93@hcd40.00 [100.00-1630.00]
172- CAVVDVPFGGAK(acetyl)AGVK-187
Mascot ion score: 71
Relative Abundance
m/z
232.07 b2
b3 331.14
y10 973.54
204.08
y5 544.34
y7 672.41
b4 430.21
b5 545.24
600.31
y8 729.42
y3 303.15
y4 374.24
126.09 y1
446.17
671.35
y6
484.25
799.45
y9 876.49
980.35
y11 1072.62
y12 1187.64
y13 1286.71
y14 1385.80
955.53
1528.57

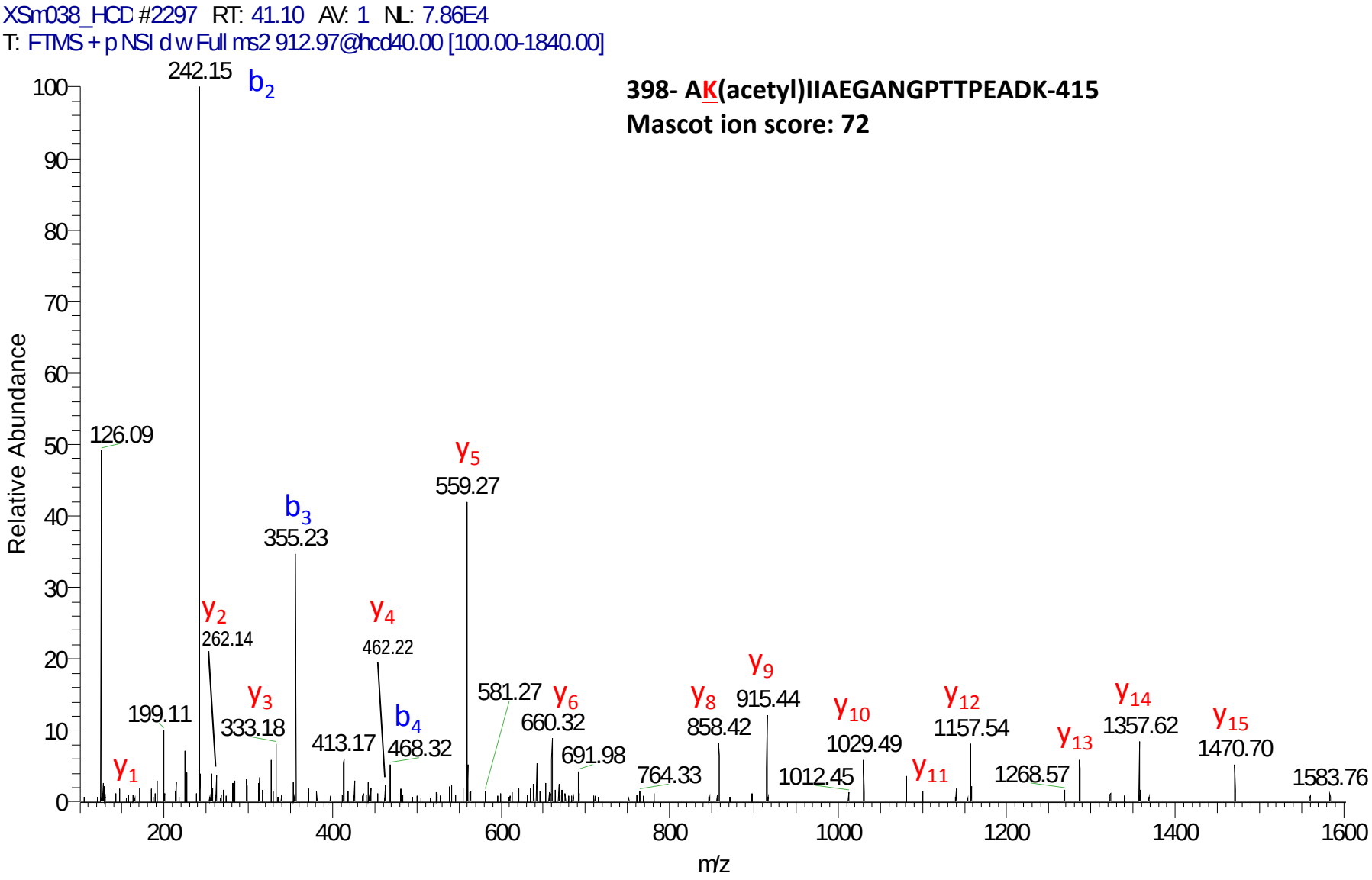

XSm038_HCD #2943 RT: 46.21 AV: 1 NL: 5.70E3
T: FTMS + p NSI d w Full ms2 810.40@hcd40.00 [100.00-1635.00]
188-INPK(acetyl)NYTDNELEK-200
Mascot ion score: 53
Relative Abundance
m/z
126.09
147.11
268.17
382.21
240.17
276.15
285.15
389.24
418.97
518.28
545.27
632.32
696.84
762.35
848.40
875.39
987.42
1011.46
1081.21
1125.51
1279.56
1295.61
1392.66
1446.76
y1
b2
y2
y3
y4
y5
y11 2+
y6
y7
y8
y9
y10
y11

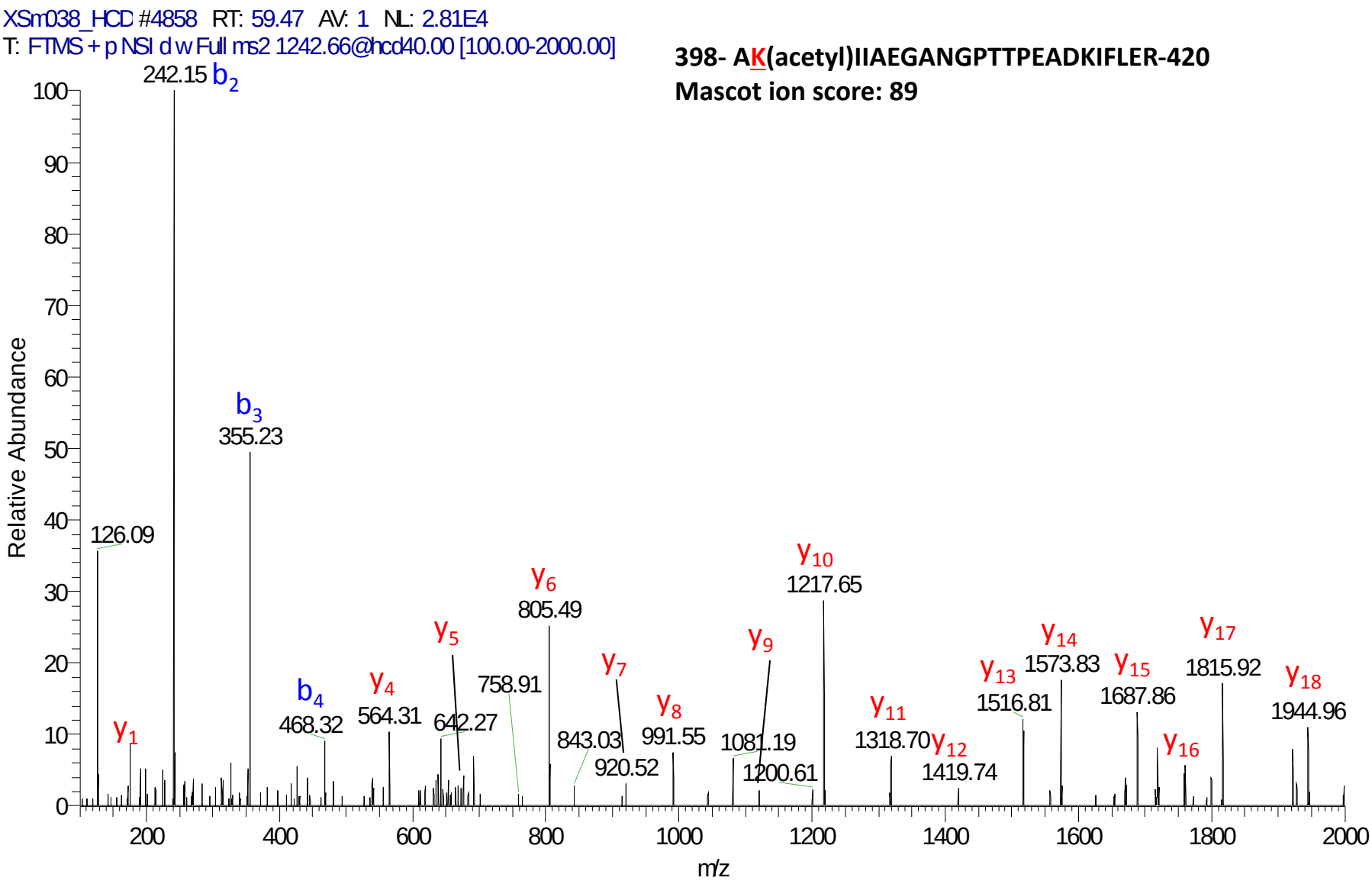

XSm038_HCD #3168 RT: 48.11 AV: 1 NL: 3.13E4
T: FTMS + p NSI d w Full ms2 726.42@hcd40.00 [100.00-1465.00]
353-LQHGTILGFPK(acetyl)AK-365
Mascot ion score: 59
Relative Abundance
m/z
y4 485.31
110.07
126.09
268.17
195.09
296.14
b3
379.21
y1
y2
y3
388.26
409.22
b5
537.28
y11 2+
605.85
y5
y6
689.40
y7
802.48
b8
820.47
b7
y8
b9
967.54
y9
y10
1073.64
1113.46
y11
1210.70
1263.62
1376.72

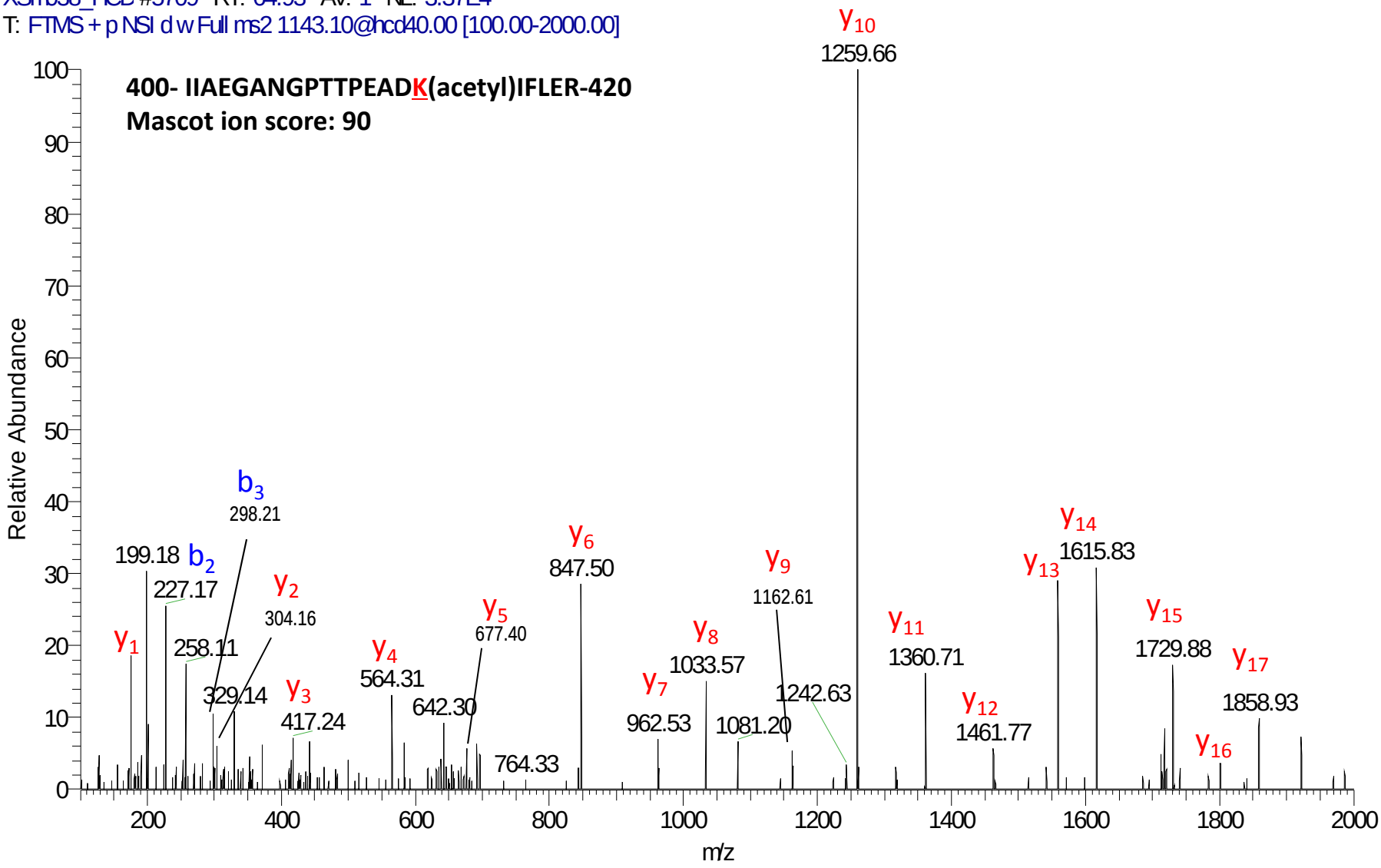

XSm038_HCD #3263 RT: 48.80 AV: 1 NL: 9.42E4
T: FTMS + p NSI d w Full ms2 503.95@hcd40.00 [100.00-1525.00]
353-LQHGTILGFPK(succinyl)AK-365
Mascot ion score: 37
Relative Abundance
m/z
y4 543.31
120.08
y4 2+ 272.16
326.17
b5 537.28
y3 446.26
519.27
b3 397.21
b4
379.21
136.08
y2 218.15
y1
504.27
552.24
622.37
632.35
b6 650.36
y5 690.38
y6 747.40
b7 763.45
b8 820.47
y7 860.49
895.66
b9 967.54
1081.19

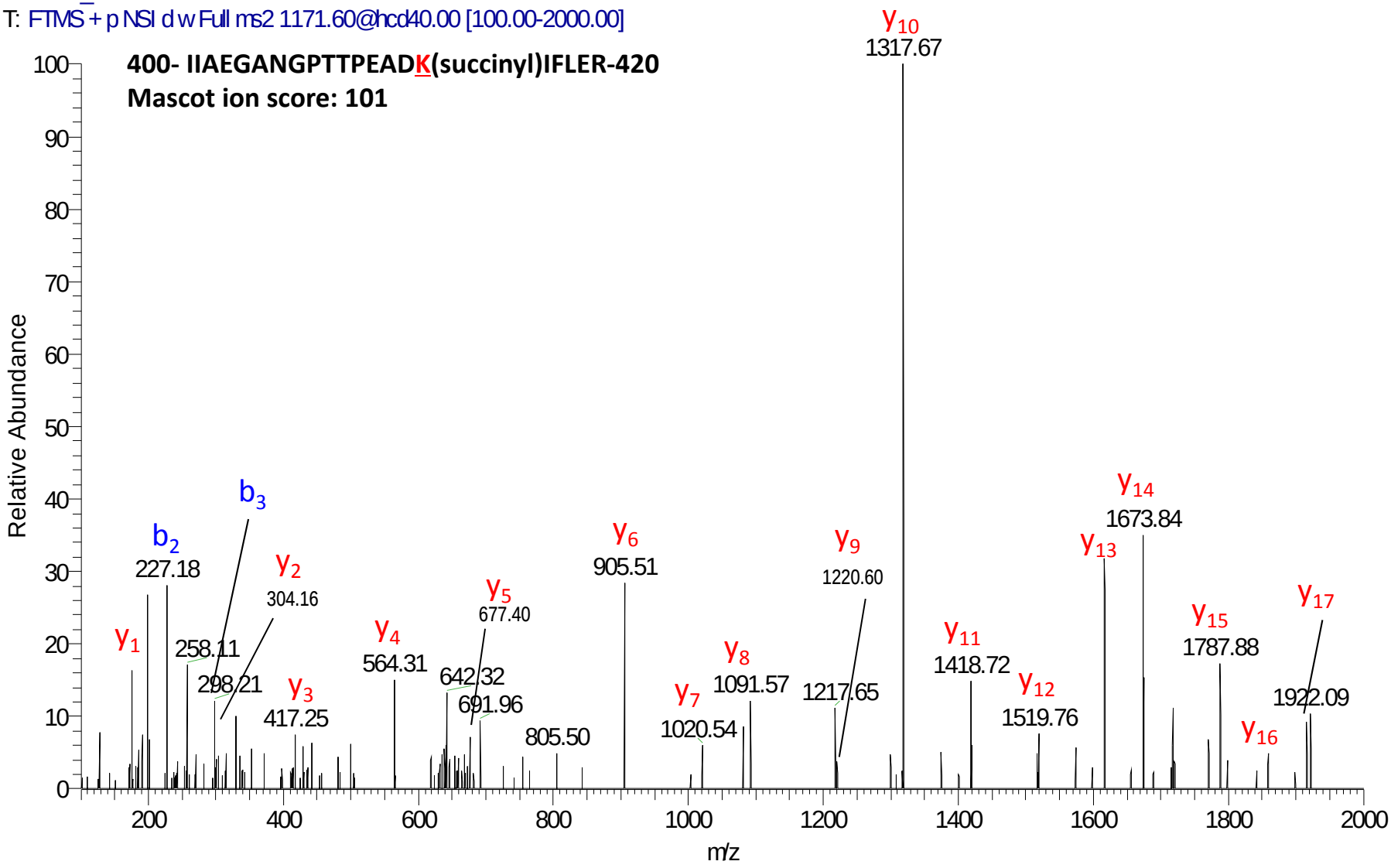

XSm044_2 #4896 RT: 80.44 AV: 1 NL: 5.60E3
T: ITMS + c NSI d w Full ms2 1289.16@cid35.00 [340.00-2000.00]
GDH
364-AK(acetyl)IYEGSILEVDCDILIPAASEK-386
Mascot ion score: 75
Relative Abundance
m/z
602.31
y6
b17
1975.99
y5
505.25
y7
715.39
y8
828.56
y4
434.44
584.38
y9
941.66
b9
1017.59
y10
1056.53
y11
1216.63
1245.63
y12
1331.61
1332.67
y13
1431.75
y14
1560.68
y15
1673.85
b15
1749.82
b16
1862.94
1958.00

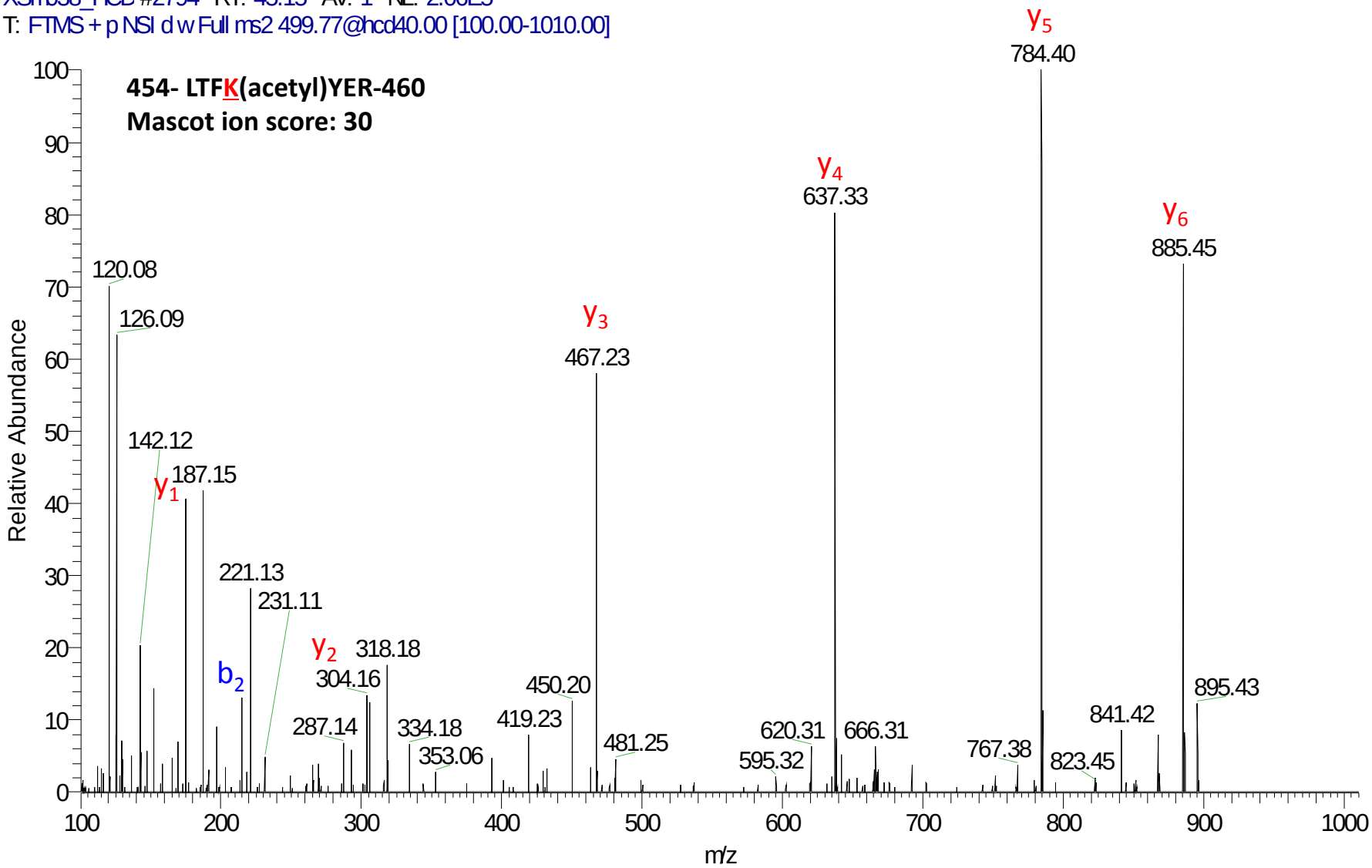

XSm044_2 #5034 RT: 82.13 AV: 1 NL: 2.32E3
T: ITMS + c NSI d w Full ms2 950.16@cid35.00 [250.00-2000.00]
GDH
366-IYEGSILEVDCDILIPAASEK(acetyl)QLTK-390
Mascot ion score: 66
Relative Abundance
m/z
1114.65 y10
y12 1340.80
y10 2+ 557.89
b6 663.36
b7 776.53
y7 875.48
y3 361.38
y9 1017.55
b15 1733.87
y5 659.40
b13 2+ 754.58
b15 2+ 867.61
b13 1507.66
b14 1620.81
b16 1930.92
b17
y4 489.49
b9 1004.57
y11 1227.67
b12 1394.61
y11 2+ 614.67
b8
y6
391.17
548.85
b10
1789.03
1974.04

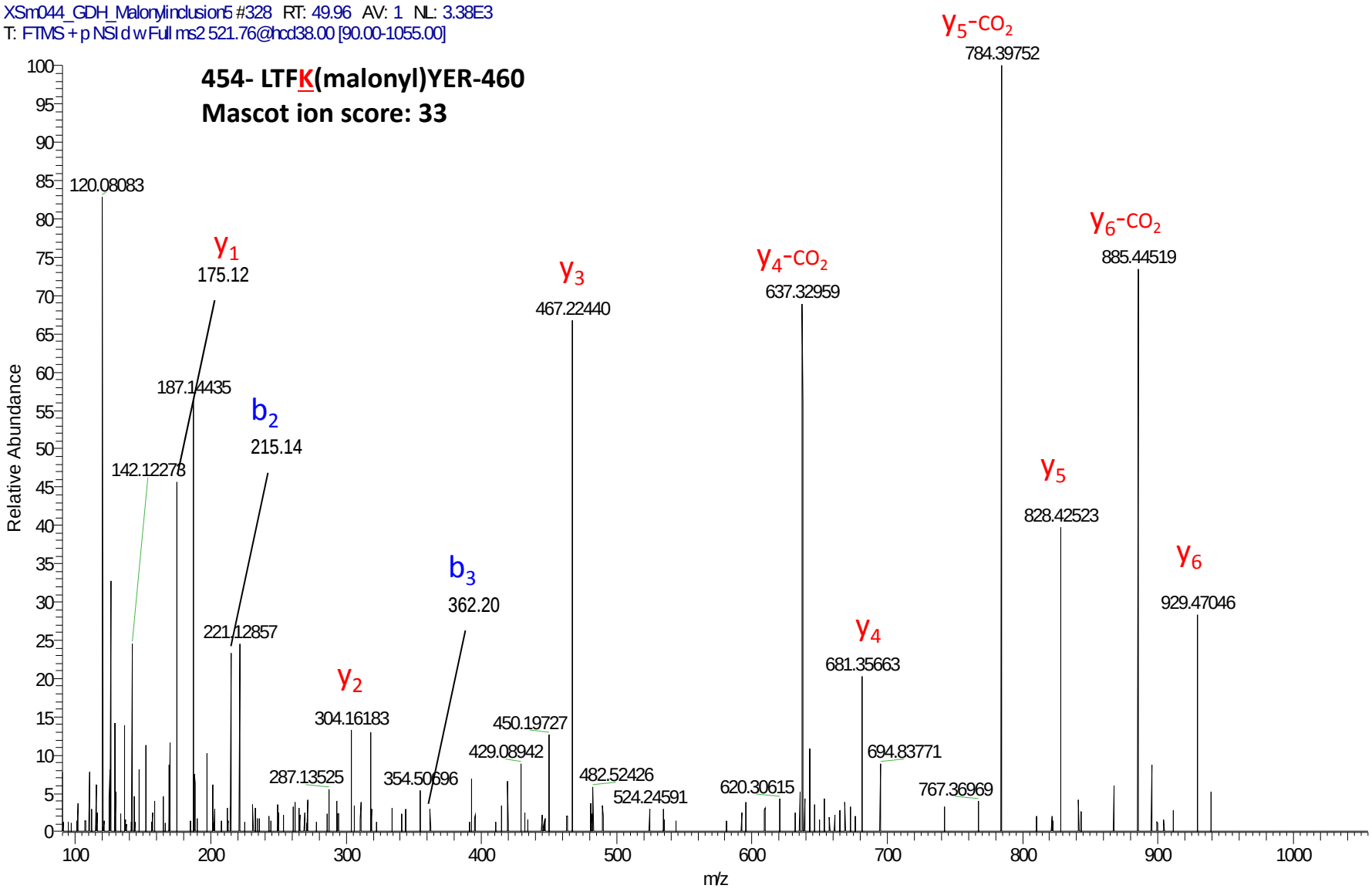

XSm038_HCD #2297 RT: 41.10 AV: 1 NL: 7.86E4
T: FTMS + p NSI d w Full ms2 912.97@hcd40.00 [100.00-1840.00]
398- AK(acetyl)IIAEGANGPTTPEADK-415
Mascot ion score: 72
Relative Abundance
m/z
242.15
b2
126.09
y5
559.27
b3
355.23
y2
262.14
y4
462.22
y3
333.18
199.11
b4
468.32
413.17
581.27
y6
660.32
691.98
764.33
y9
915.44
y8
858.42
y10
1029.49
1012.45
y12
1157.54
y11
y13
1268.57
y14
1357.62
y15
1470.70
1583.76

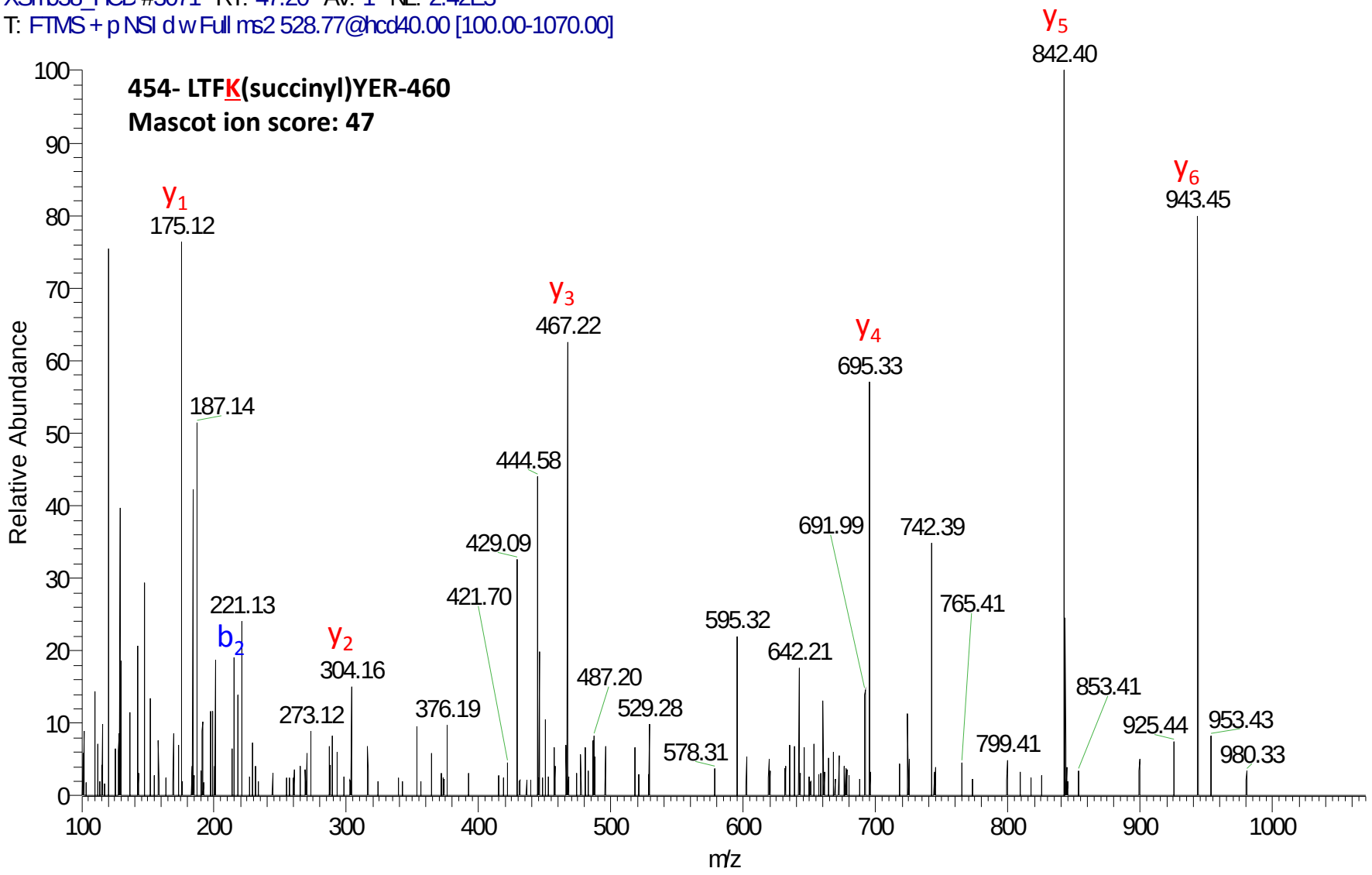

XSm038_HCD #4858 RT: 59.47 AV: 1 NL: 2.81E4
T: FTMS + p NSI d w Full ms2 1242.66@hcd40.00 [100.00-2000.00]
398- AK(acetyl)IIAEGANGPTTPEADKIFLER-420
Mascot ion score: 89
Relative Abundance
m/z
242.15 b2
b3 355.23
126.09
y1
b4 468.32
y4 564.31
y5
642.27
758.91
y6 805.49
y7
843.03
920.52
y8 991.55
y9
1081.19
1200.61
y10 1217.65
y11 1318.70
y12 1419.74
y13 1516.81
y14 1573.83
y15 1687.86
y16
y17 1815.92
y18 1944.96

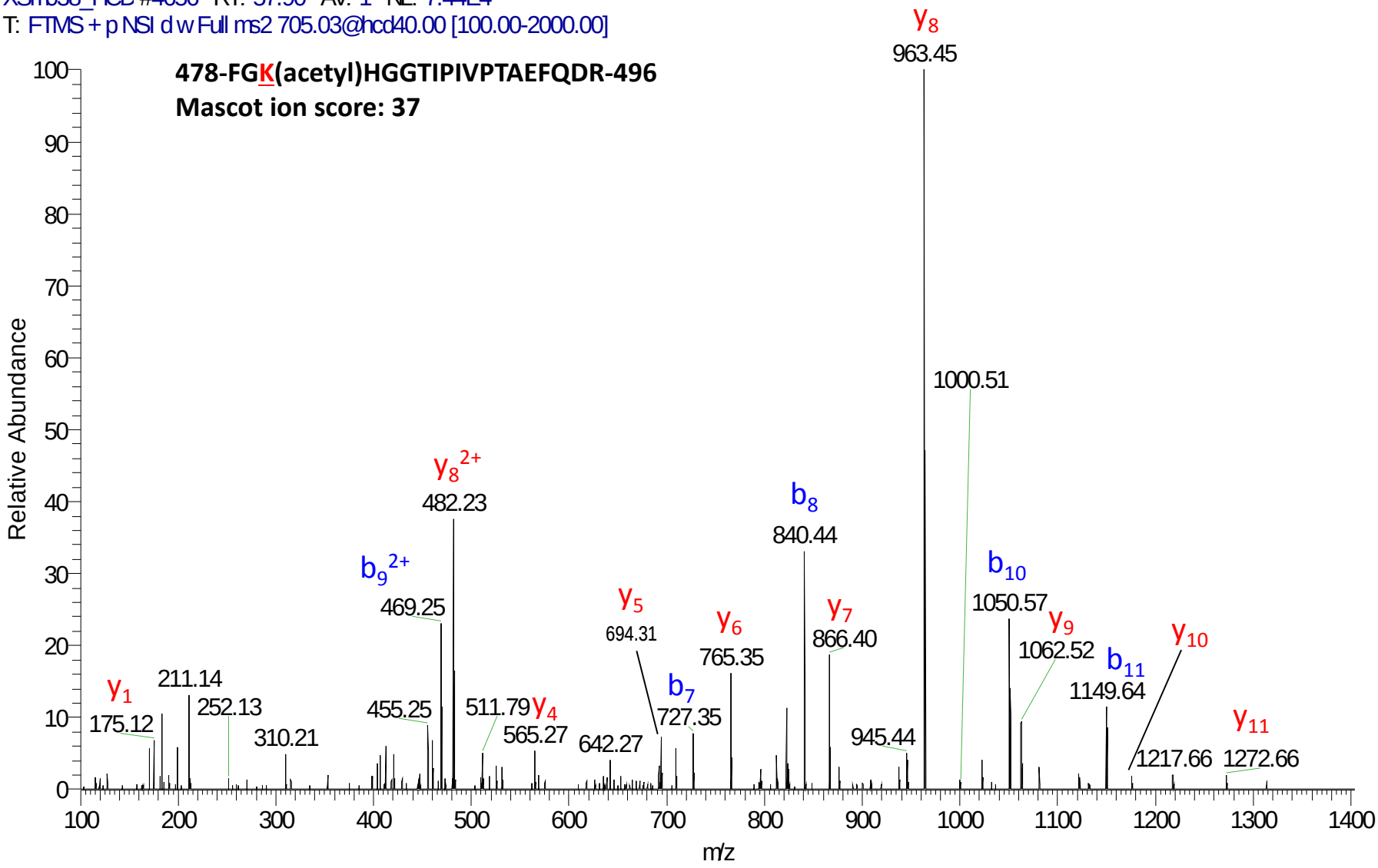

XSm038_HCD #5709 RT: 64.93 AV: 1 NL: 3.37E4
T: FTMS + p NSI d w Full ms2 1143.10@hcd40.00 [100.00-2000.00]
400- IIAEGANGPTTPEADK(acetyl)IFLER-420
Mascot ion score: 90
Relative Abundance
m/z
y10 1259.66
b3 298.21
199.18 b2 227.17
y2 304.16
y1
258.11
329.14
y3 417.24
y4 564.31
642.30
y5 677.40
764.33
y6 847.50
962.53
y7
y8 1033.57
1081.20
y9 1162.61
1242.63
y11 1360.71
y12 1461.77
y13
y14 1615.83
y15 1729.88
y16
y17 1858.93

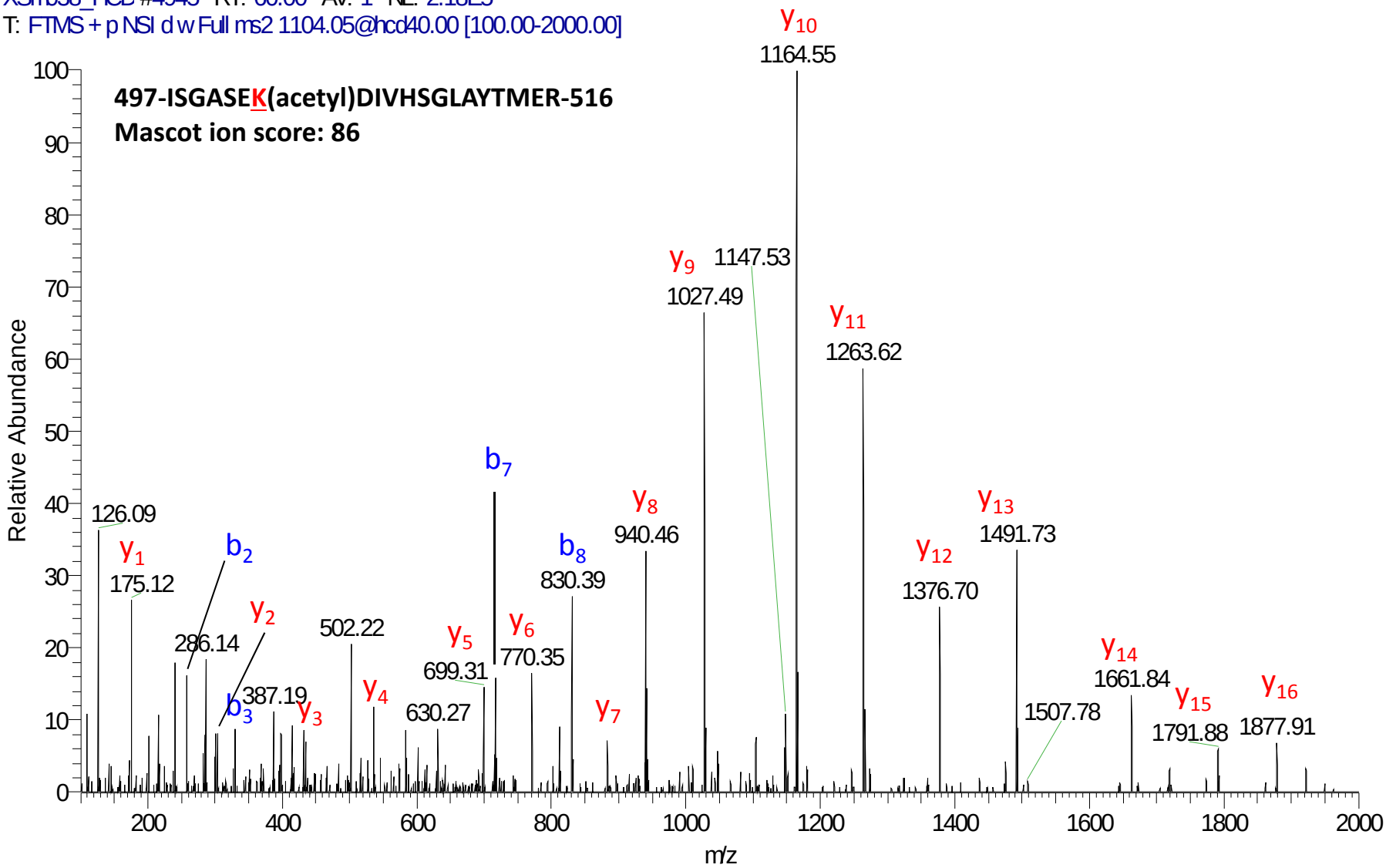

XSm038_HCD #5814 RT: 65.64 AV: 1 NL: 9.89E3
T: FTMS + p NSI d w Full ms2 1171.60@hcd40.00 [100.00-2000.00]
400- IIAEGANGPTTPEADK(succinyl)IFLER-420
Mascot ion score: 101
Relative Abundance
m/z
y10 1317.67
y14 1673.84
y13
y15 1787.88
y17 1922.09
y16
y11 1418.72
y12 1519.76
y9 1220.60
y6 905.51
y8 1091.57
y7 1020.54
y5 677.40
y4 564.31
y2 304.16
b3
b2 227.18
y1
258.11
298.21
y3 417.25
642.32
691.96
805.50
1217.65

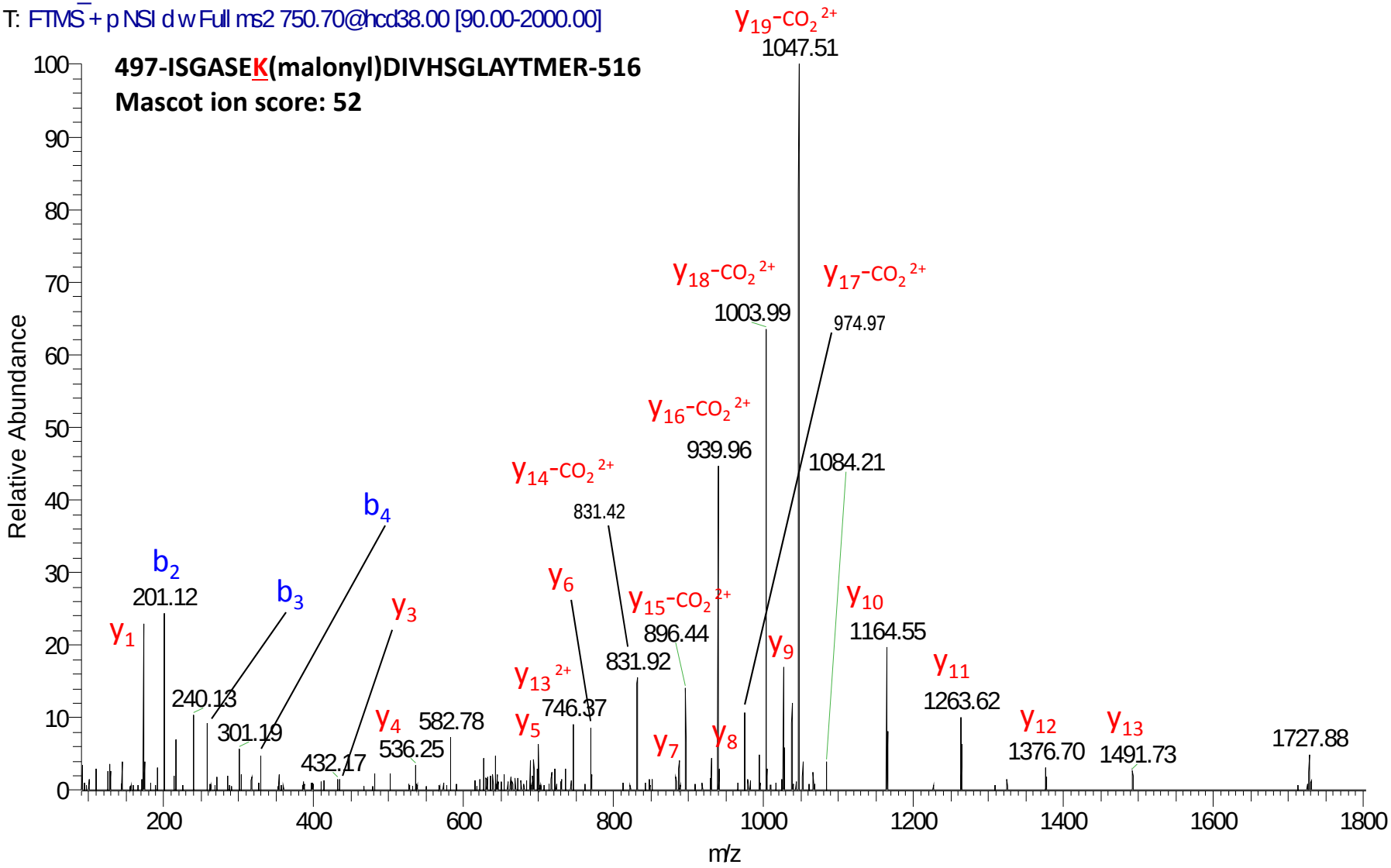

XSm038_HCD #2794 RT: 45.15 AV: 1 NL: 2.06E5
T: FTMS + p NSI d w Full ms2 499.77@hcd40.00 [100.00-1010.00]
454- LTFK(acetyl)YER-460
Mascot ion score: 30
Relative Abundance
m/z
y5 784.40
y4 637.33
y6 885.45
y3 467.23
120.08
126.09
142.12
y1
187.15
221.13
231.11
y2 318.18
304.16
b2
287.14
334.18
353.06
450.20
419.23
481.25
620.31
595.32
666.31
767.38
823.45
841.42
895.43

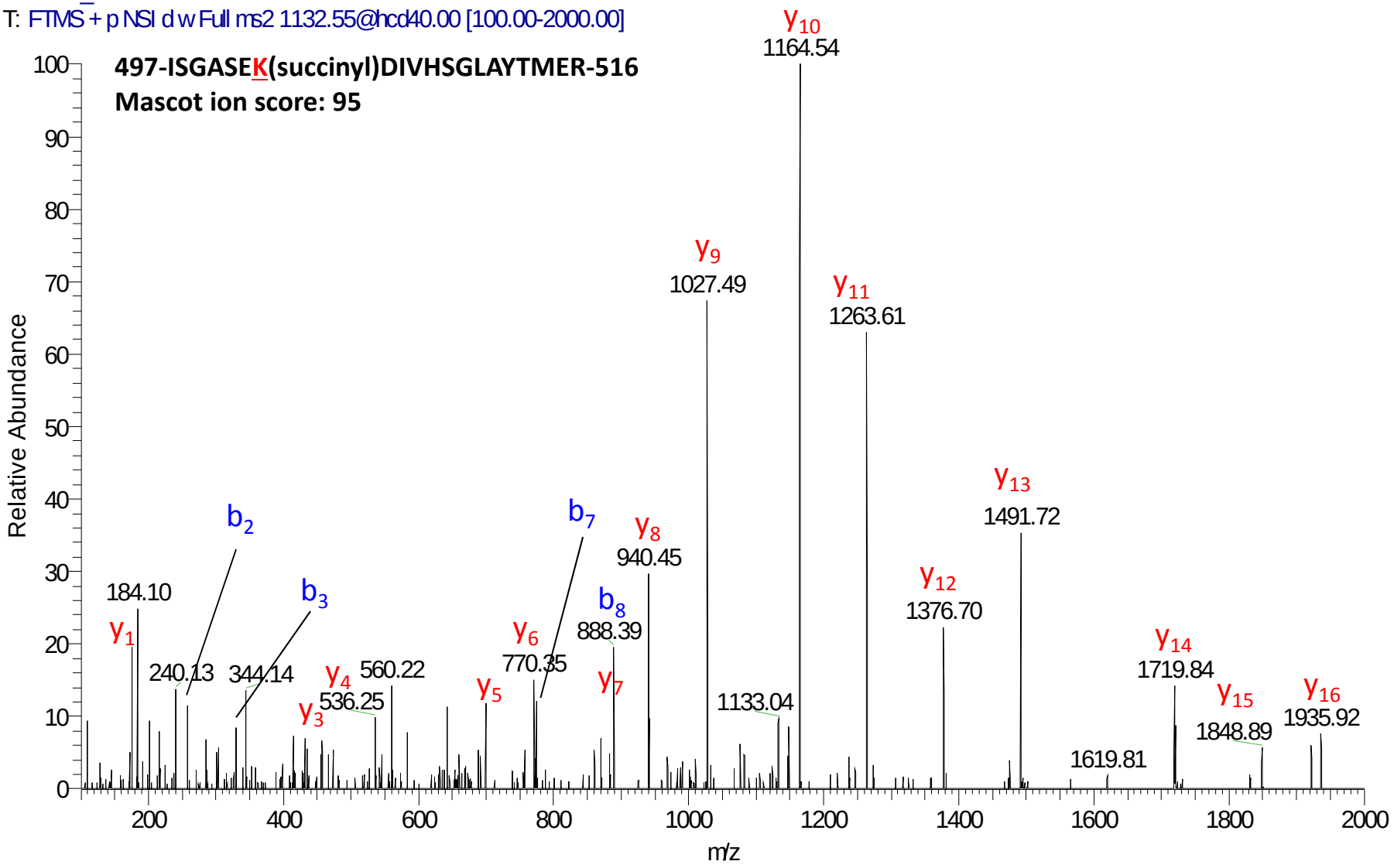

XSm044_GDH_Malonylinclusion5 #328 RT: 49.96 AV: 1 NL: 3.38E3
T: FTMS + p NSI d w Full ms2 521.76@hcd38.00 [90.00-1055.00]
454- LTFK(malonyl)YER-460
Mascot ion score: 33
Relative Abundance
m/z
120.08083
y1
175.12
187.14435
142.12278
b2
215.14
221.12857
y2
304.16183
287.13525
b3
362.20
354.50696
450.19727
429.08942
482.52426
524.24591
y3
467.22440
y4-CO2
637.32959
620.30615
y4
681.35663
694.83771
767.36969
y5-CO2
784.39752
y5
828.42523
y6-CO2
885.44519
y6
929.47046

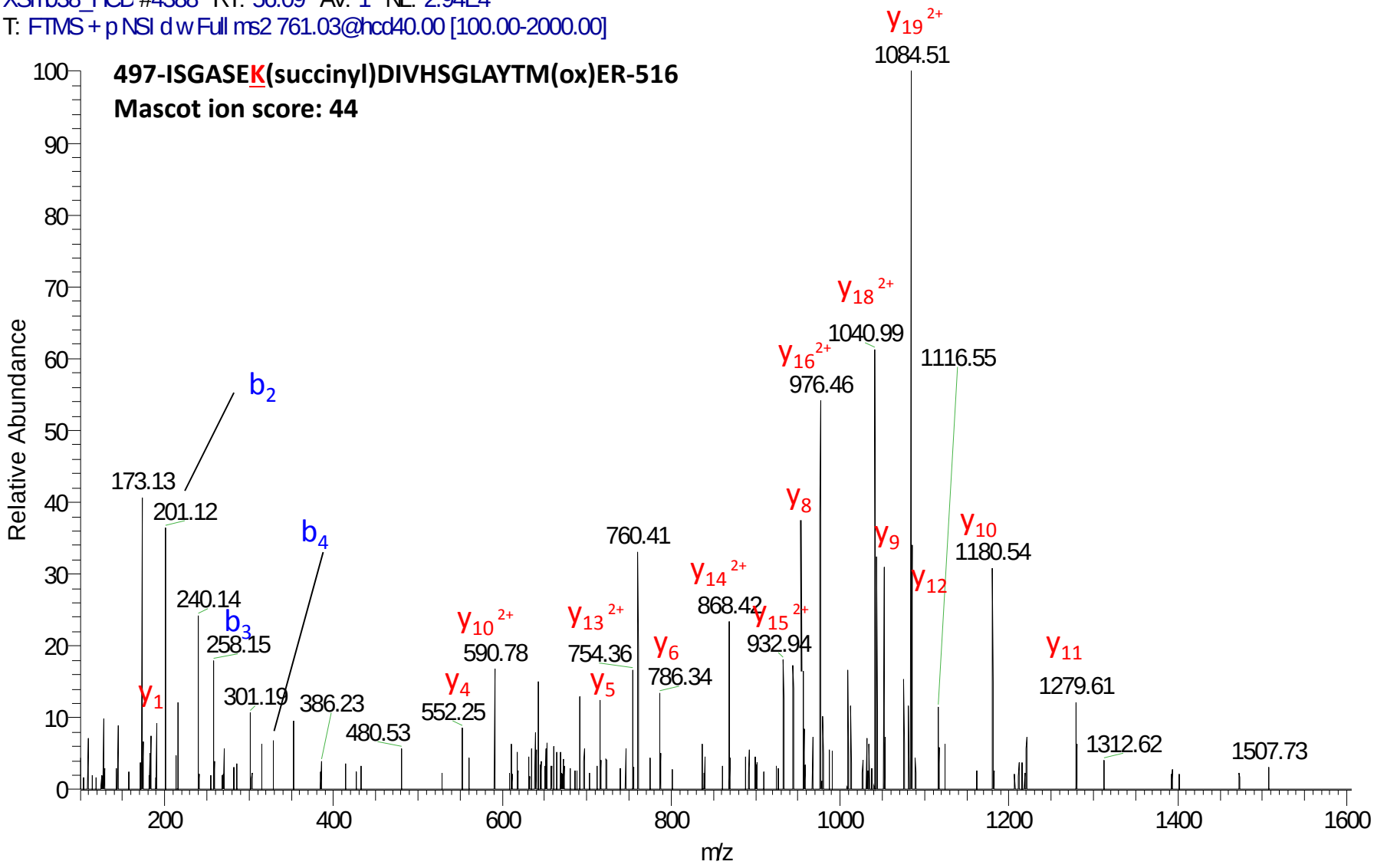

XSm038_HCD #3071 RT: 47.20 AV: 1 NL: 2.42E3
T: FTMS + p NSI d w Full ms2 528.77@hcd40.00 [100.00-1070.00]
454- LTFK(succinyl)YER-460
Mascot ion score: 47
y5 842.40
y6 943.45
y1 175.12
y3 467.22
y4 695.33
187.14
444.58
691.99
742.39
429.09
421.70
765.41
221.13
b2
y2 304.16
595.32
642.21
487.20
853.41
529.28
273.12
376.19
925.44
953.43
578.31
799.41
980.33
Relative Abundance
m/z

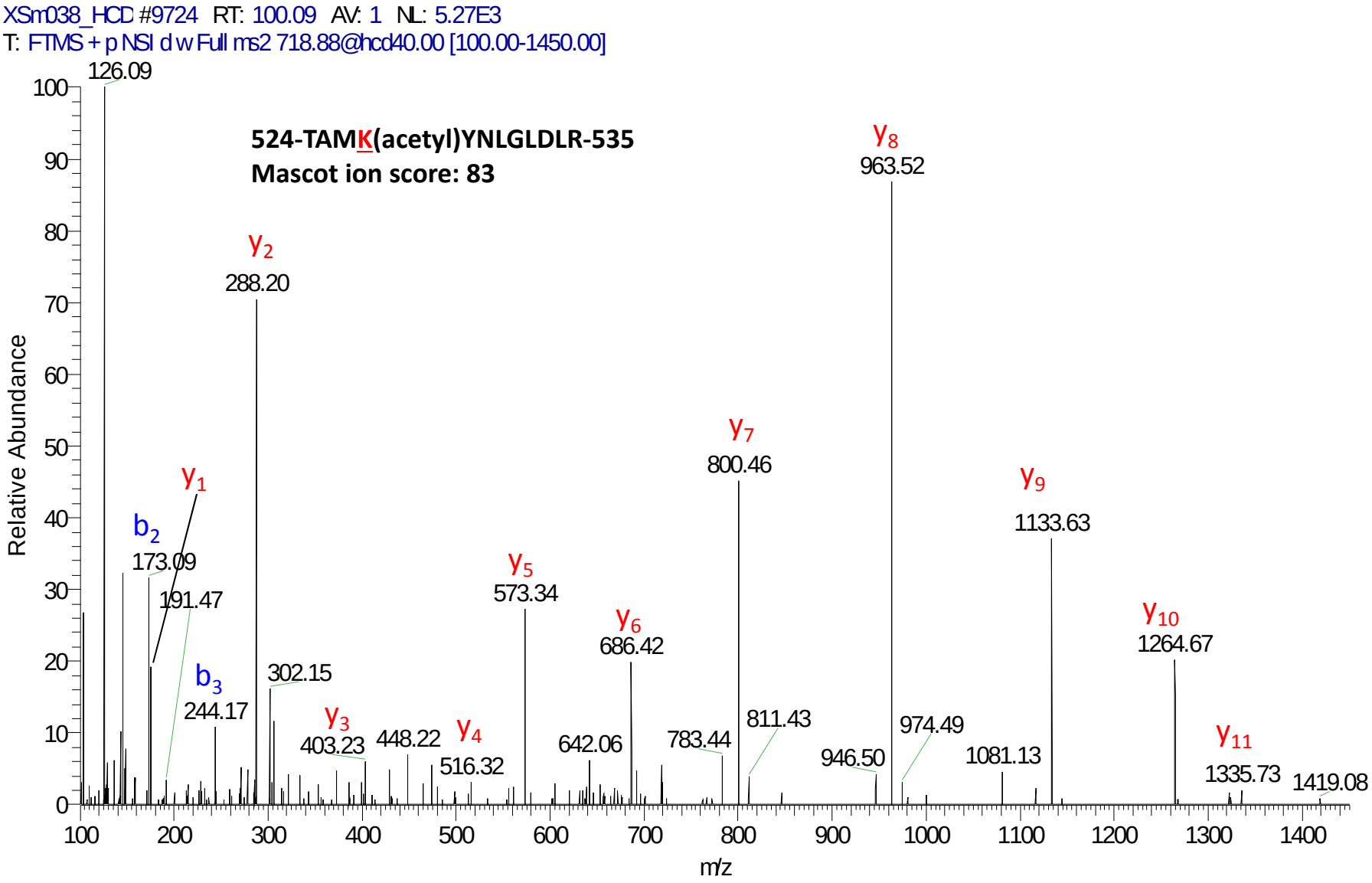

XSm038_HCD #4656 RT: 57.90 AV: 1 NL: 7.44E4
T: FTMS + p NSI d w Full ms2 705.03@hcd40.00 [100.00-2000.00]
478-FGK(acetyl)HGGTIPIVPTAEFQDR-496
Mascot ion score: 37
Relative Abundance
m/z
y8 963.45
1000.51
y8 2+ 482.23
b8 840.44
b10 1050.57
b9 2+ 469.25
y5 694.31
y6 765.35
y7 866.40
y9 1062.52
y10
b11 1149.64
b7 727.35
y1 175.12
211.14
252.13
310.21
455.25
511.79
y4 565.27
642.27
945.44
1217.66
y11 1272.66

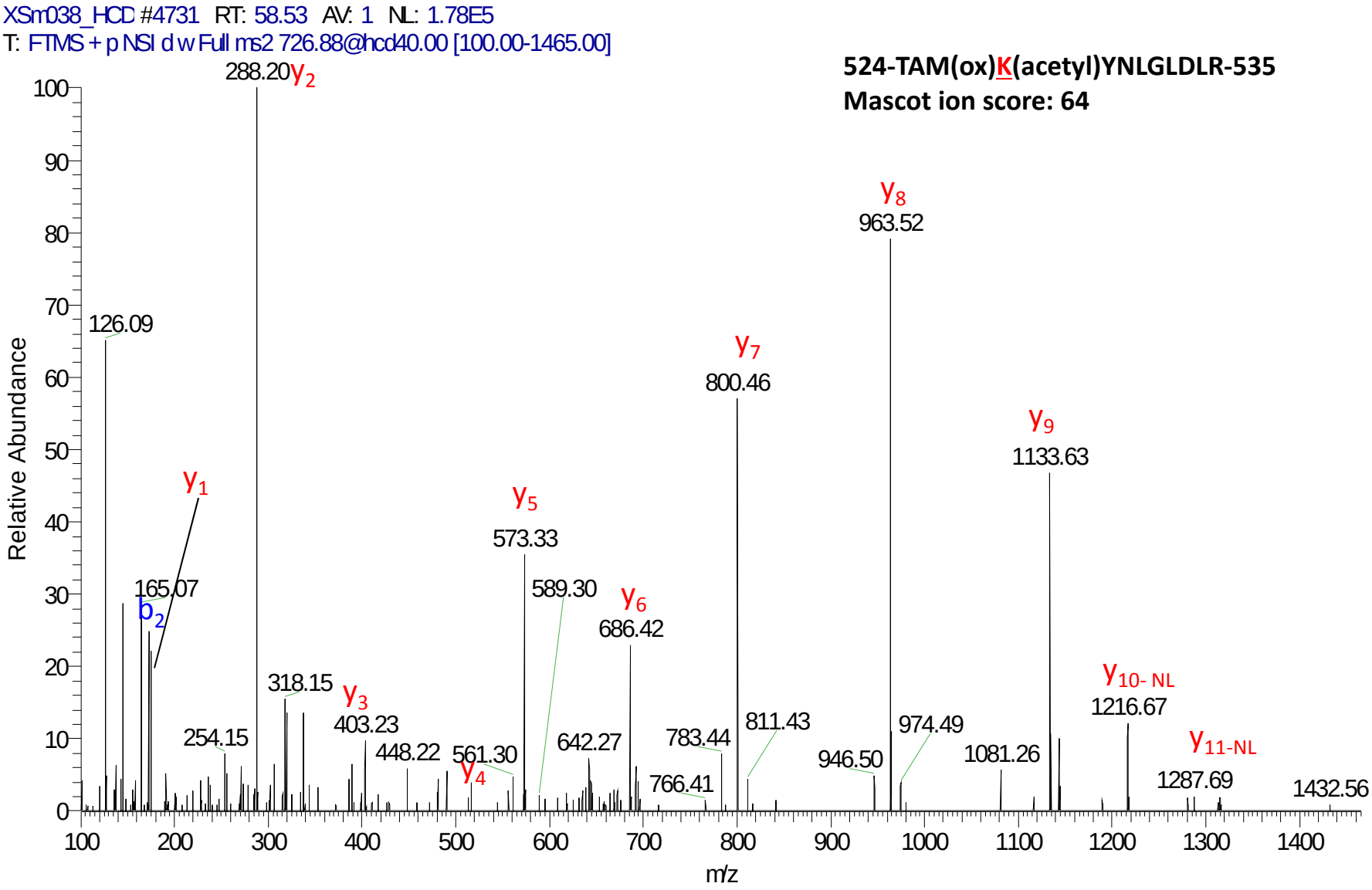

XSm038_HCD #4945 RT: 60.00 AV: 1 NL: 2.18E5
T: FTMS + p NSI d w Full ms2 1104.05@hcd40.00 [100.00-2000.00]
497-ISGASEK(acetyl)DIVHSGLAYTMER-516
Mascot ion score: 86
Relative Abundance
m/z
y10 1164.55
y9 1027.49
1147.53
y11 1263.62
b7
y8 940.46
b8 830.39
y13 1491.73
y12 1376.70
y14 1661.84
y15 1791.88
y16 1877.91
1507.78
126.09
y1 175.12
b2
y2
286.14
502.22
y5 699.31
y6 770.35
b3 387.19
y3
y4
630.27
y7

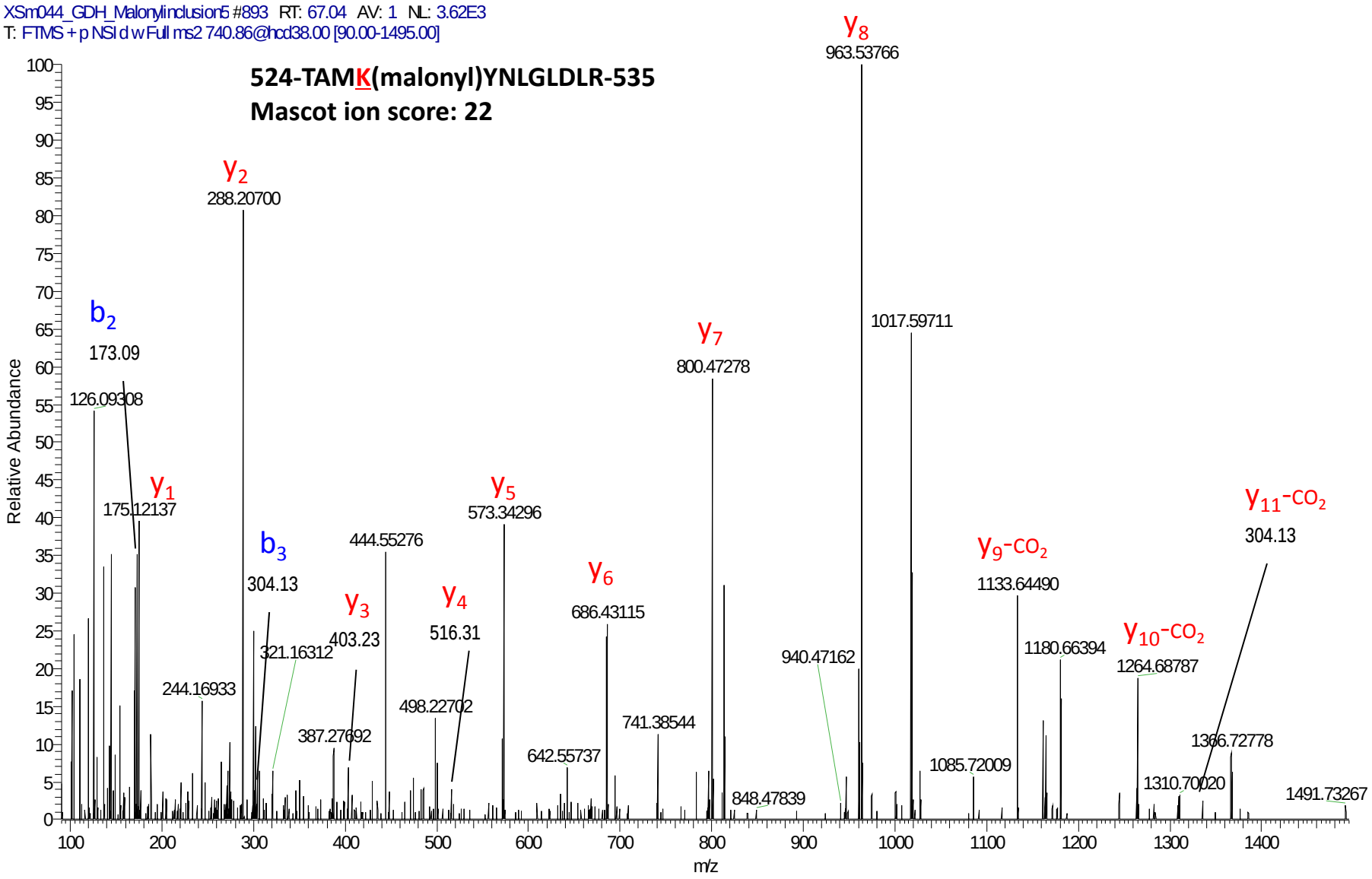

Xsm044_HCD #4002 RT: 64.47 AV: 1 NL: 1.36E5
T: FTMS + p NSI d w Full ms2 750.70@hcd38.00 [90.00-2000.00]
497-ISGASEK(malonyl)DIVHSGLAYTMER-516
Mascot ion score: 52
Relative Abundance
m/z
y19-CO2 2+
1047.51
y18-CO2 2+
1003.99
y17-CO2 2+
974.97
y16-CO2 2+
939.96
1084.21
y14-CO2 2+
831.42
b4
b2
201.12
b3
y3
y6
y15-CO2 2+
896.44
y10
1164.55
y1
831.92
y9
y11
1263.62
y13 2+
746.37
240.13
y4
582.78
y5
y7
y8
y12
1376.70
y13
1491.73
301.19
536.25
432.17
1727.88


XSm038_HCD #5015 RT: 60.41 AV: 1 NL: 4.52E4
T: FTMS + p NSI d w Full ms2 1132.55@hcd40.00 [100.00-2000.00]
497-ISGASEK(succinyl)DIVHSGLAYTMER-516
Mascot ion score: 95
Relative Abundance
m/z
y10 1164.54
y9 1027.49
y11 1263.61
y13 1491.72
y12 1376.70
y14 1719.84
y15 1848.89
y16 1935.92
b2
b3
b7
y8 940.45
b8 888.39
y1
y6 770.35
y4 560.22
y3 536.25
y5
y7
184.10
240.13
344.14
1133.04
1619.81


XSm038_HCD #4388 RT: 56.09 AV: 1 NL: 2.94E4
T: FTMS + p NSI d w Full ms2 761.03@hcd40.00 [100.00-2000.00]
497-ISGASEK(succinyl)DIVHSGLAYTM(ox)ER-516
Mascot ion score: 44
Relative Abundance
m/z
y19 2+ 1084.51
y18 2+ 1040.99
y16 2+ 976.46
1116.55
b2
173.13
201.12
b4
240.14
b3 258.15
y1
301.19
386.23
480.53
y4 552.25
y10 2+ 590.78
y13 2+ 754.36
760.41
y5
y6 786.34
y14 2+ 868.42
y15 2+ 932.94
y8
y9
y12
y10 1180.54
y11 1279.61
1312.62
1507.73


XSm038_HCD #9724 RT: 100.09 AV: 1 NL: 5.27E3
T: FTMS + p NSI d w Full ms2 718.88@hcd40.00 [100.00-1450.00]
524-TAMK(acetyl)YNLGLDLR-535
Mascot ion score: 83
Relative Abundance
m/z
126.09
y2 288.20
y8 963.52
y7 800.46
y1
b2 173.09
191.47
y9 1133.63
y5 573.34
y6 686.42
y10 1264.67
b3 244.17
302.15
y3 403.23
448.22
y4 516.32
642.06
783.44
811.43
946.50
974.49
1081.13
y11 1335.73
1419.08


XSm038_HCD #4731 RT: 58.53 AV: 1 NL: 1.78E5
T: FTMS + p NSI d w Full ms2 726.88@hcd40.00 [100.00-1465.00]
524-TAM(ox)K(acetyl)YNLGLDLR-535
Mascot ion score: 64
Relative Abundance
m/z
288.20 y2
963.52 y8
800.46 y7
1133.63 y9
126.09
y1
165.07 b2
573.33 y5
589.30
686.42 y6
318.15
403.23 y3
254.15
448.22
561.30 y4
642.27
783.44
766.41
811.43
946.50
974.49
1081.26
1216.67 y10- NL
1287.69 y11-NL
1432.56


XSm044_GDH_Malonylinclusion5 #893 RT: 67.04 AV: 1 NL: 3.62E3
T: FTMS + p NSI d w Full ms2 740.86@hcd38.00 [90.00-1495.00]
524-TAMK(malonyl)YNLGLDLR-535
Mascot ion score: 22
Relative Abundance
m/z
y8 963.53766
y2 288.20700
b2 173.09
126.09308
y1 175.12137
b3 304.13
y3 403.23
444.55276
y4 516.31
y5 573.34296
y6 686.43115
y7 800.47278
1017.59711
940.47162
y9-CO2 1133.64490
1180.66394
y10-CO2 1264.68787
y11-CO2 304.13
1366.72778
1310.70020
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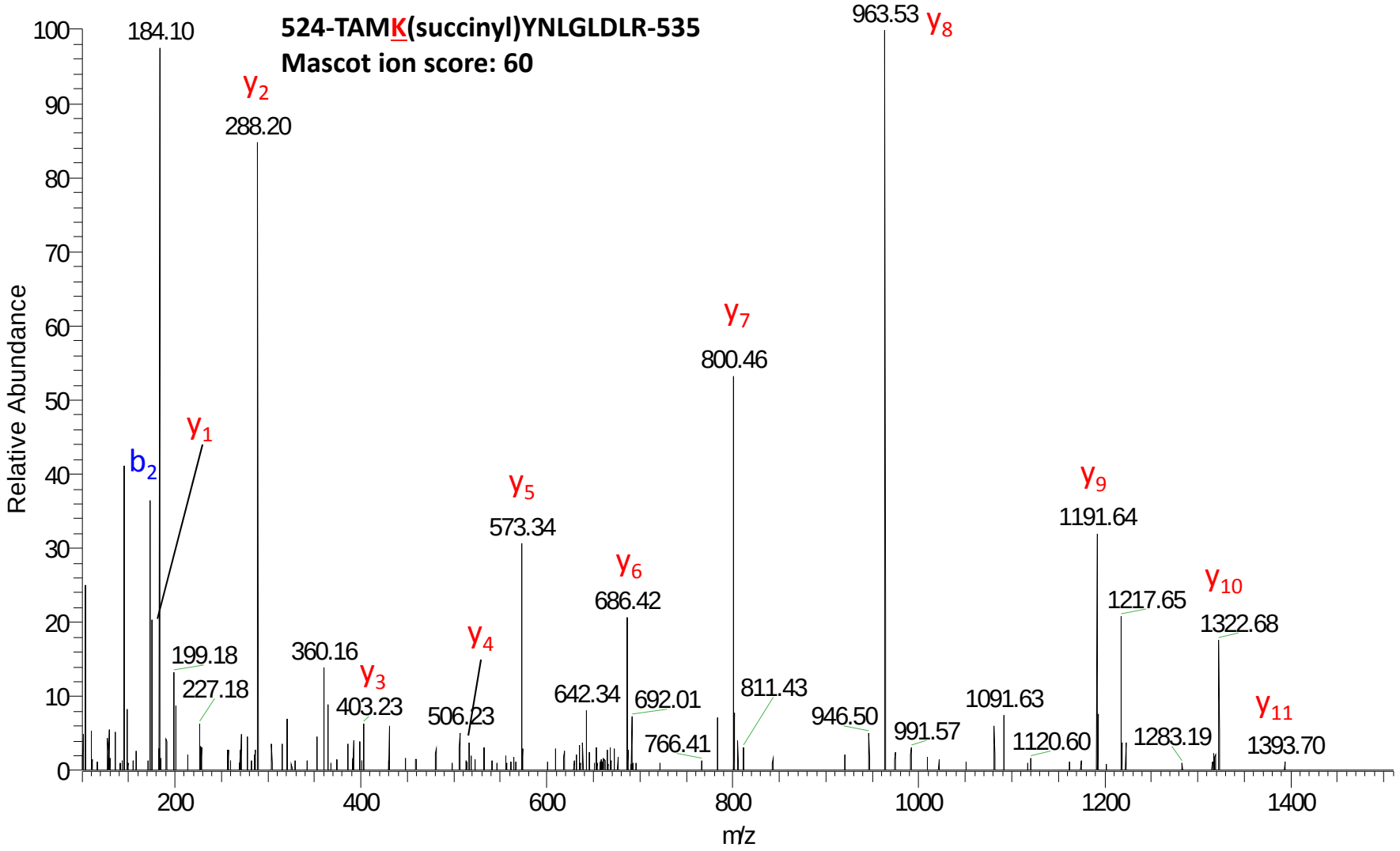
XSm038_HCD #5620 RT: 64.27 AV: 1 NL: 2.57E5
T: FTMS + p NSI d w Full ms2 747.88@hcd40.00 [100.00-1510.00]
524-TAMK(succinyl)YNLGLDLR-535
Mascot ion score: 60
Relative Abundance
m/z
184.10
288.20
963.53
800.46
573.34
686.42
1191.64
1217.65
1322.68
199.18
227.18
360.16
403.23
506.23
642.34
692.01
766.41
811.43
946.50
991.57
1091.63
1120.60
1283.19
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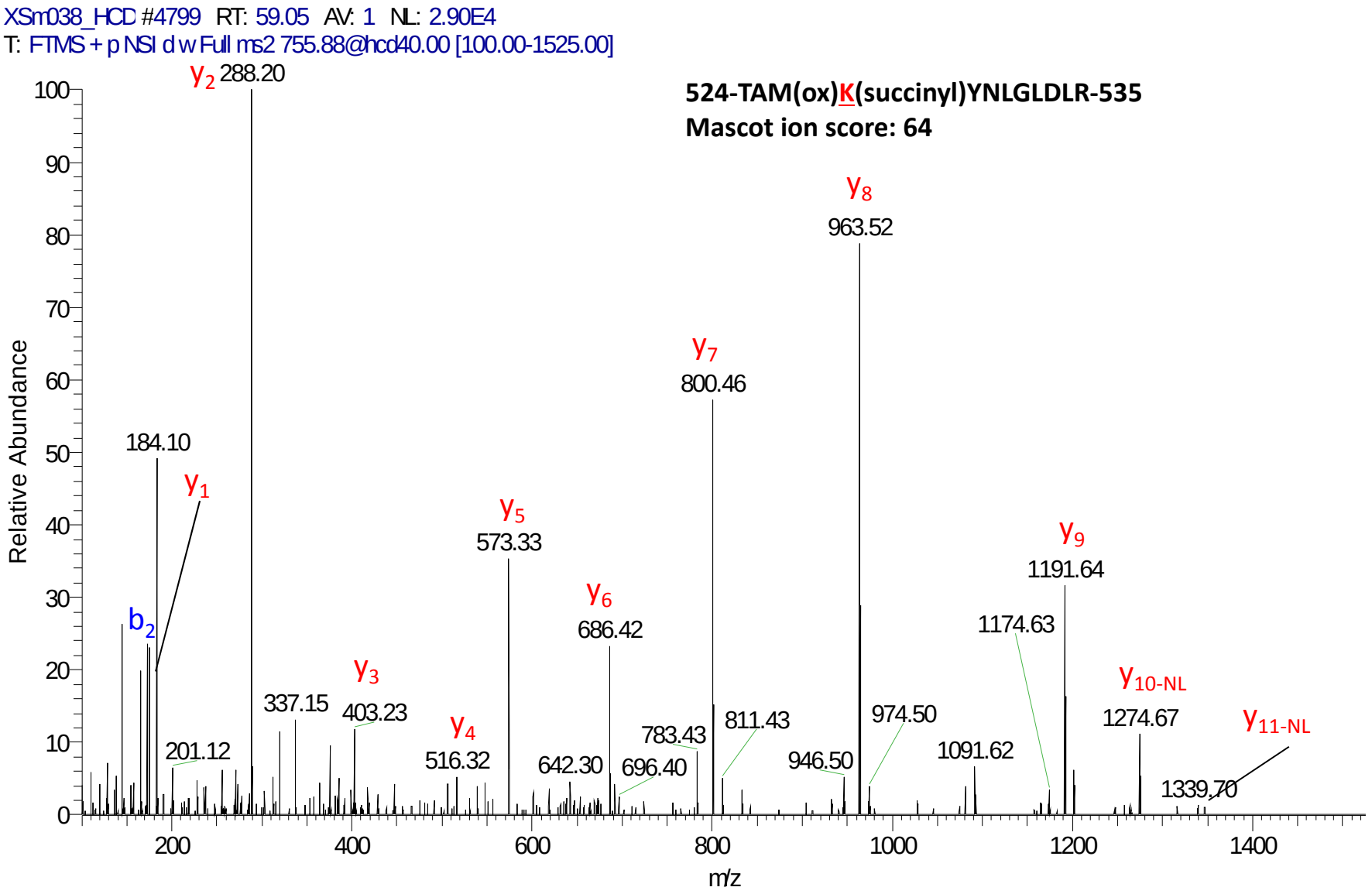
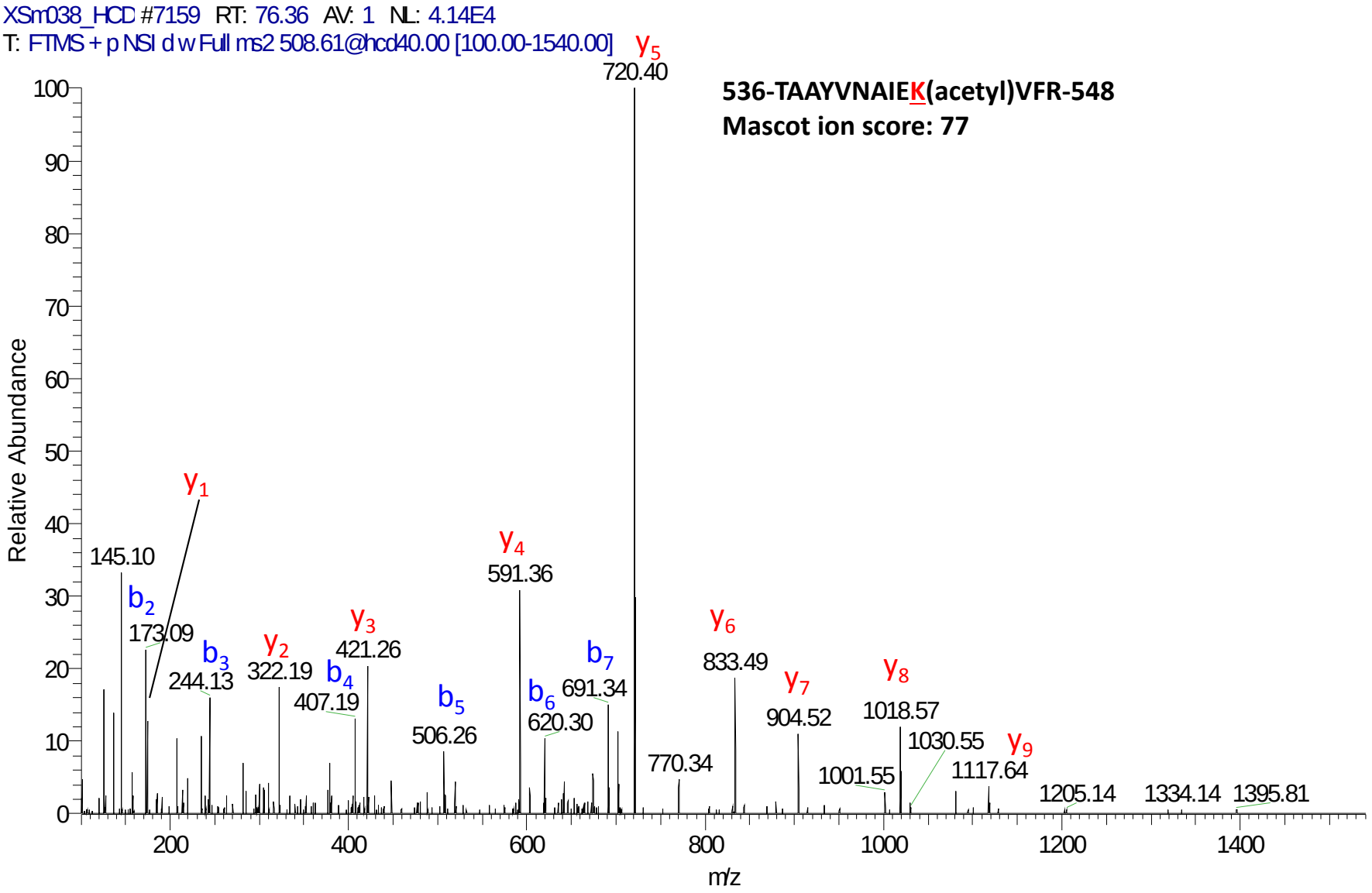
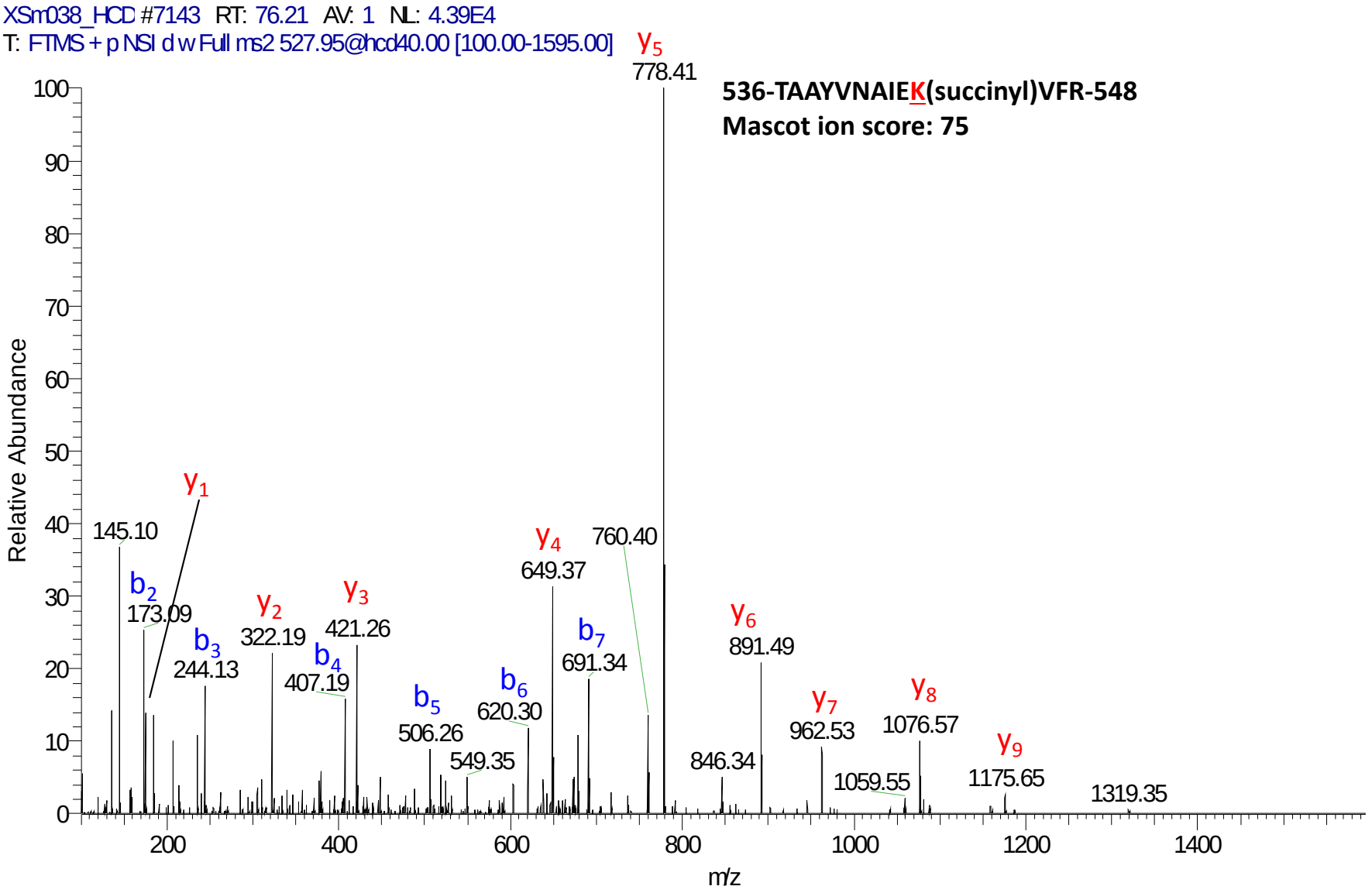
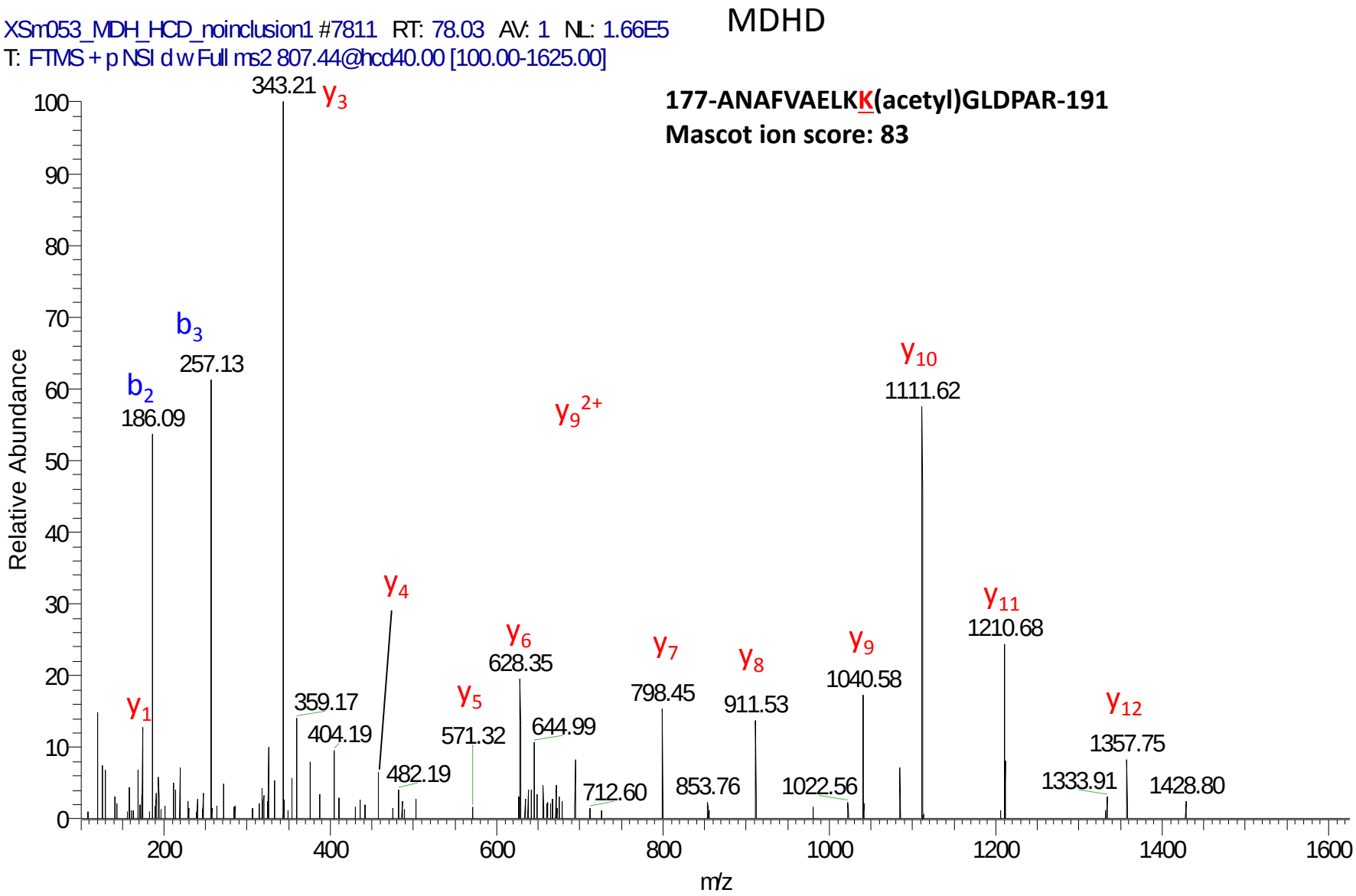

XSm038_HCD #4799 RT: 59.05 AV: 1 NL: 2.90E4
T: FTMS + p NSI d w Full ms2 755.88@hcd40.00 [100.00-1525.00]
524-TAM(ox)K(succinyl)YNLGLDLR-535
Mascot ion score: 64
Relative Abundance
m/z
y2 288.20
y8 963.52
y7 800.46
184.10
y1
y5 573.33
y9 1191.64
y6 686.42
1174.63
b2
y3
y10-NL 1274.67
337.15
403.23
y4
811.43
974.50
783.43
y11-NL
201.12
516.32
642.30
696.40
946.50
1091.62
1339.70

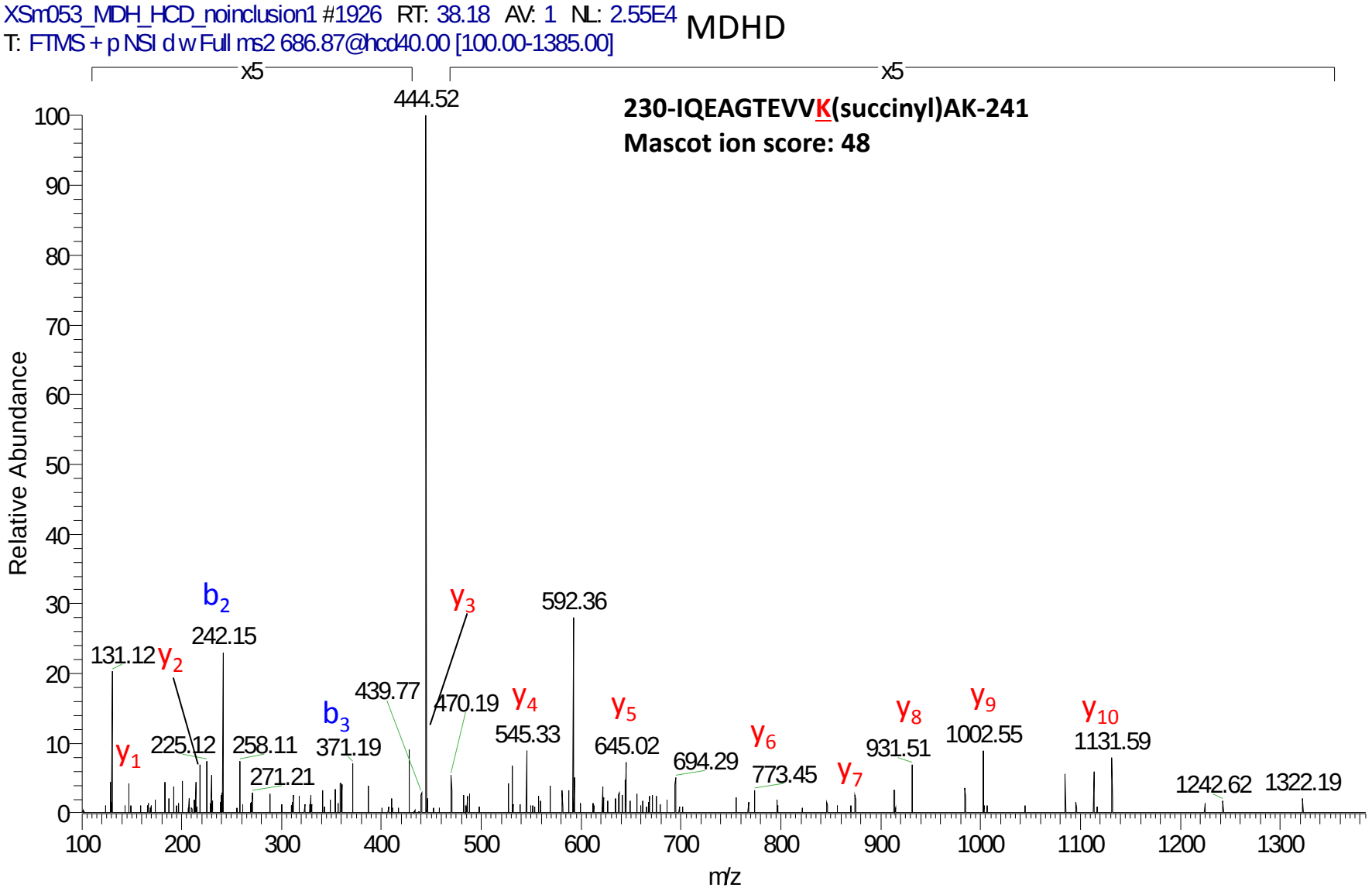

XSm038_HCD #7159 RT: 76.36 AV: 1 NL: 4.14E4
T: FTMS + p NSI d w Full ms2 508.61@hcd40.00 [100.00-1540.00]
536-TAAYVNAIEK(acetyl)VFR-548
Mascot ion score: 77
Relative Abundance
m/z
y5 720.40
y1
145.10
b2 173.09
b3 244.13
y2 322.19
y3 421.26
b4 407.19
b5 506.26
y4 591.36
b6 620.30
b7 691.34
770.34
y6 833.49
y7 904.52
y8 1018.57
1001.55
1030.55
y9 1117.64
1205.14
1334.14
1395.81

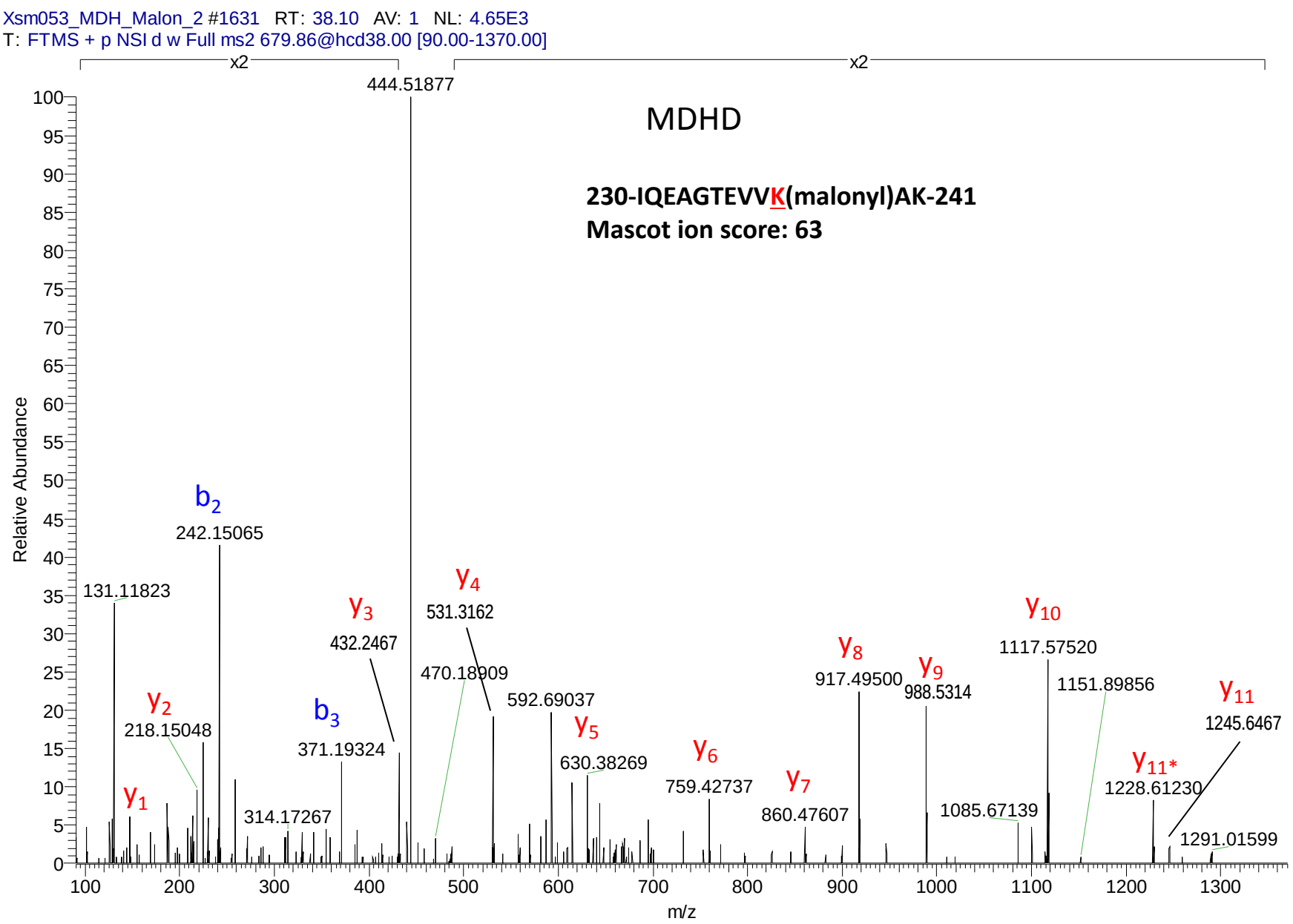

XSm038_HCD #7143 RT: 76.21 AV: 1 NL: 4.39E4
T: FTMS + p NSI d w Full ms2 527.95@hcd40.00 [100.00-1595.00]
536-TAAYVNAIEK(succinyl)VFR-548
Mascot ion score: 75
Relative Abundance
m/z
y5 778.41
y1
145.10
b2 173.09
b3 244.13
y2 322.19
y3 421.26
b4 407.19
b5 506.26
549.35
b6 620.30
y4 649.37
760.40
b7 691.34
y6 891.49
846.34
y7 962.53
1059.55
y8 1076.57
y9 1175.65
1319.35

Acetyl, malonyl, and succinyl peptides identified on other metabolic enzymes

Malate dehydrogenase, mitochondrial	1	177-ANAFVAELK(acetyl)GLDPAR-191
	2	230-IQEAGTEVVK(succinyl)AK-241
	3	230-IQEAGTEVVK(malonyl)AK-241
	4	297-KGIEK(succinyl)NLGIGK-307
	5	298-GIEK(succinyl)NLGIGK-307
	6	302-NLGIGK(acetyl)ISPFEEK-314
	7	308-ISPFEEK(acetyl)M(ox)IAEAIPELK-324
	8	325-ASIK(acetyl)KGEEFVK-335
	9	325-ASIKK(malonyl)GEEFVK-335
	10	325-ASIK(succinyl)KGEEFVK-335
Citrate synthase, mitochondrial	1	74-(acetyl)GLVYETSVLDPDEGIR-92
	2	74-(succinyl)GLVYETSVLDPDEGIR-92
	3	74-GMK(succinyl)GLVYETSVLDPDEGIRFR-94


MDHD
XSm053_MDH_HCD_noinclusion1 #7811 RT: 78.03 AV: 1 NL: 1.66E5
T: FTMS + p NSI d w Full ms2 807.44@hcd40.00 [100.00-1625.00]
177-ANAFVAELKK(acetyl)GLDPAR-191
Mascot ion score: 83
Relative Abundance
m/z
343.21 y3
b3 257.13
b2 186.09
y1
359.17
404.19
y4
482.19
y5 571.32
y6 628.35
644.99
712.60
y7 798.45
853.76
y8 911.53
1022.56
y9 1040.58
y9 2+
y10 1111.62
y11 1210.68
y12 1357.75
1333.91
1428.80

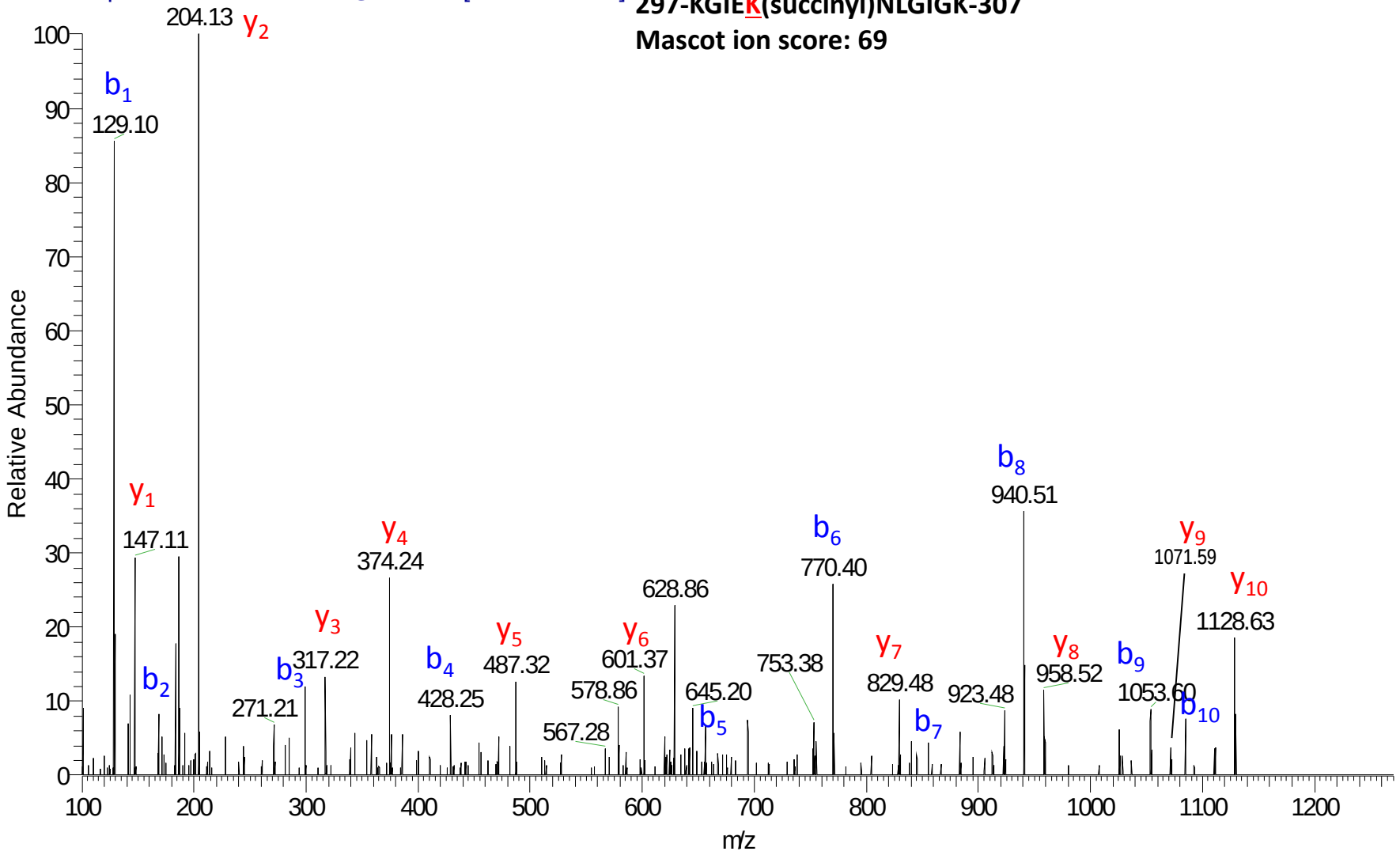

XSm053_MDH_HCD_noinclusion1 #1926 RT: 38.18 AV: 1 NL: 2.55E4
T: FTMS + p NSI d w Full ms2 686.87@hcd40.00 [100.00-1385.00]
MDHD
x5
x5
230-IQEAGTEVVK(succinyl)AK-241
Mascot ion score: 48
Relative Abundance
m/z
444.52
592.36
131.12
242.15
225.12
258.11
271.21
371.19
439.77
470.19
545.33
645.02
694.29
773.45
931.51
1002.55
1131.59
1242.62
1322.19
y1
y2
b2
b3
y3
y4
y5
y6
y7
y8
y9
y10


Xsm053_MDH_Malon_2 #1631 RT: 38.10 AV: 1 NL: 4.65E3
T: FTMS + p NSI d w Full ms2 679.86@hcd38.00 [90.00-1370.00]
x2
x2
444.51877
MDHD
230-IQEAGTEVVK(malonyl)AK-241
Mascot ion score: 63
Relative Abundance
m/z
b2
242.15065
131.11823
y3
432.2467
y4
531.3162
470.18909
y2
218.15048
b3
371.19324
592.69037
y5
630.38269
y1
314.17267
y6
759.42737
y7
860.47607
y8
917.49500
y9
988.5314
y10
1117.57520
1151.89856
y11
1245.6467
y11*
1228.61230
1085.67139
1291.01599

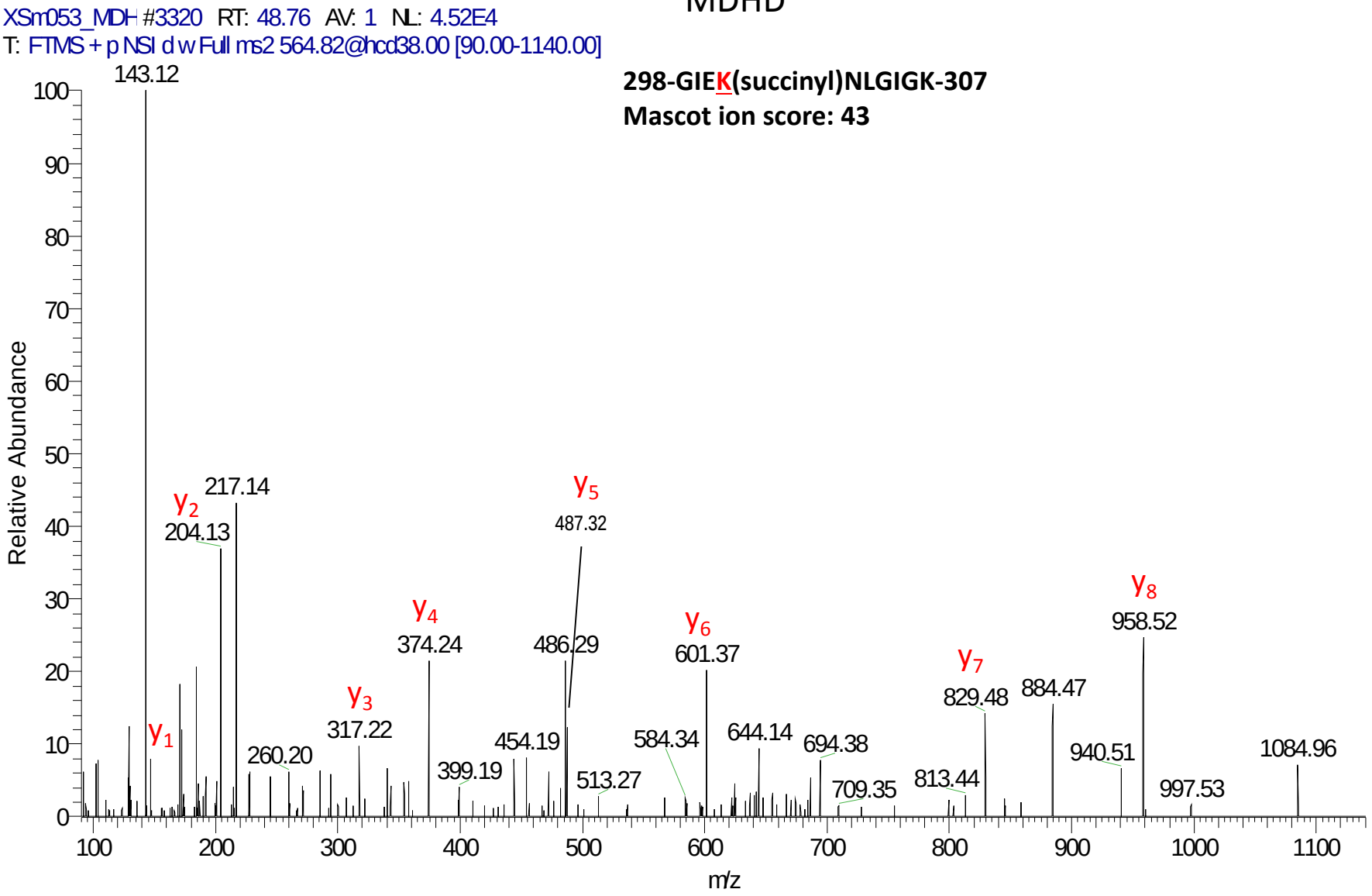
MDHD

XSm053_MDH_HCD_noinclusion1 #2859 RT: 45.19 AV: 1 NL: 4.36E4
T: FTMS + p NSI d w Full ms2 628.86@hcd40.00 [100.00-1270.00]

297-KGIE<u>K</u>(succinyl)NLGIGK-307
Mascot ion score: 69



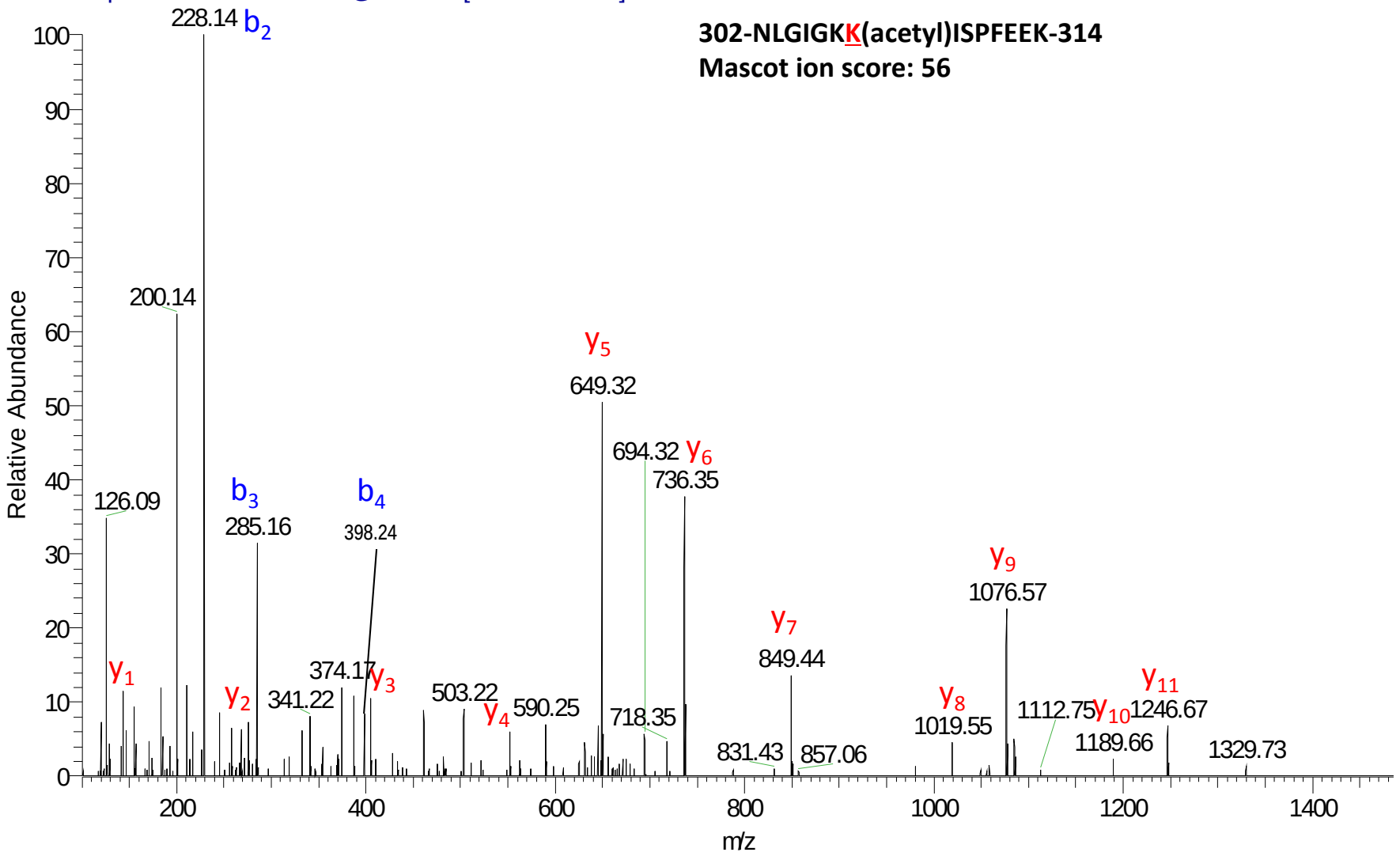
MDHD

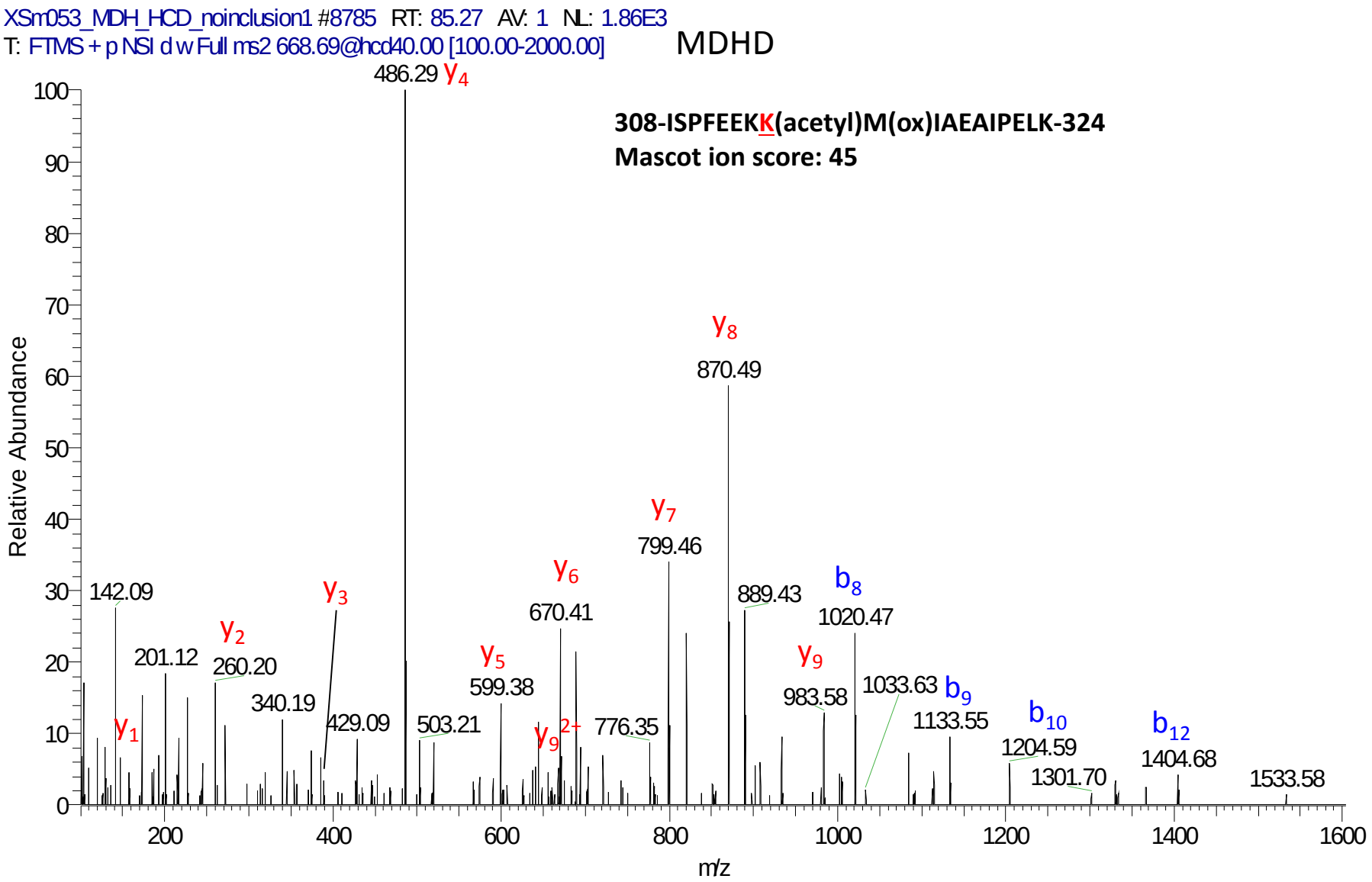

XSm053_MDH #3320 RT: 48.76 AV: 1 NL: 4.52E4
T: FTMS + p NSI d w Full ms2 564.82@hcd38.00 [90.00-1140.00]
298-GIEK(succinyl)NLGIGK-307
Mascot ion score: 43
Relative Abundance
m/z
143.12
217.14
y2
204.13
y5
487.32
y4
374.24
y3
317.22
y1
y6
601.37
y7
829.48
y8
958.52
884.47
940.51
1084.96
997.53
486.29
454.19
584.34
644.14
694.38
709.35
813.44
260.20
399.19
513.27

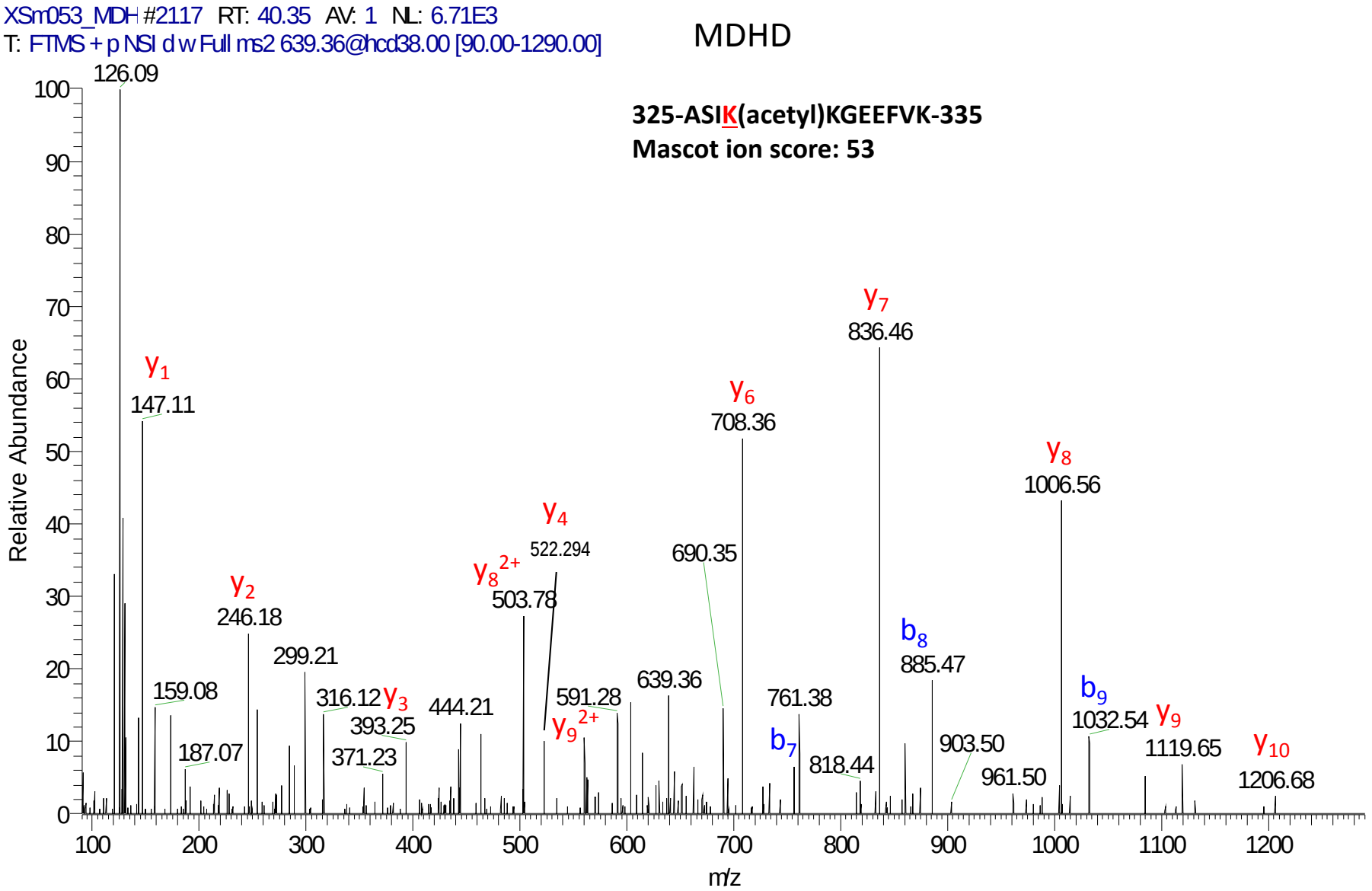
MDHD

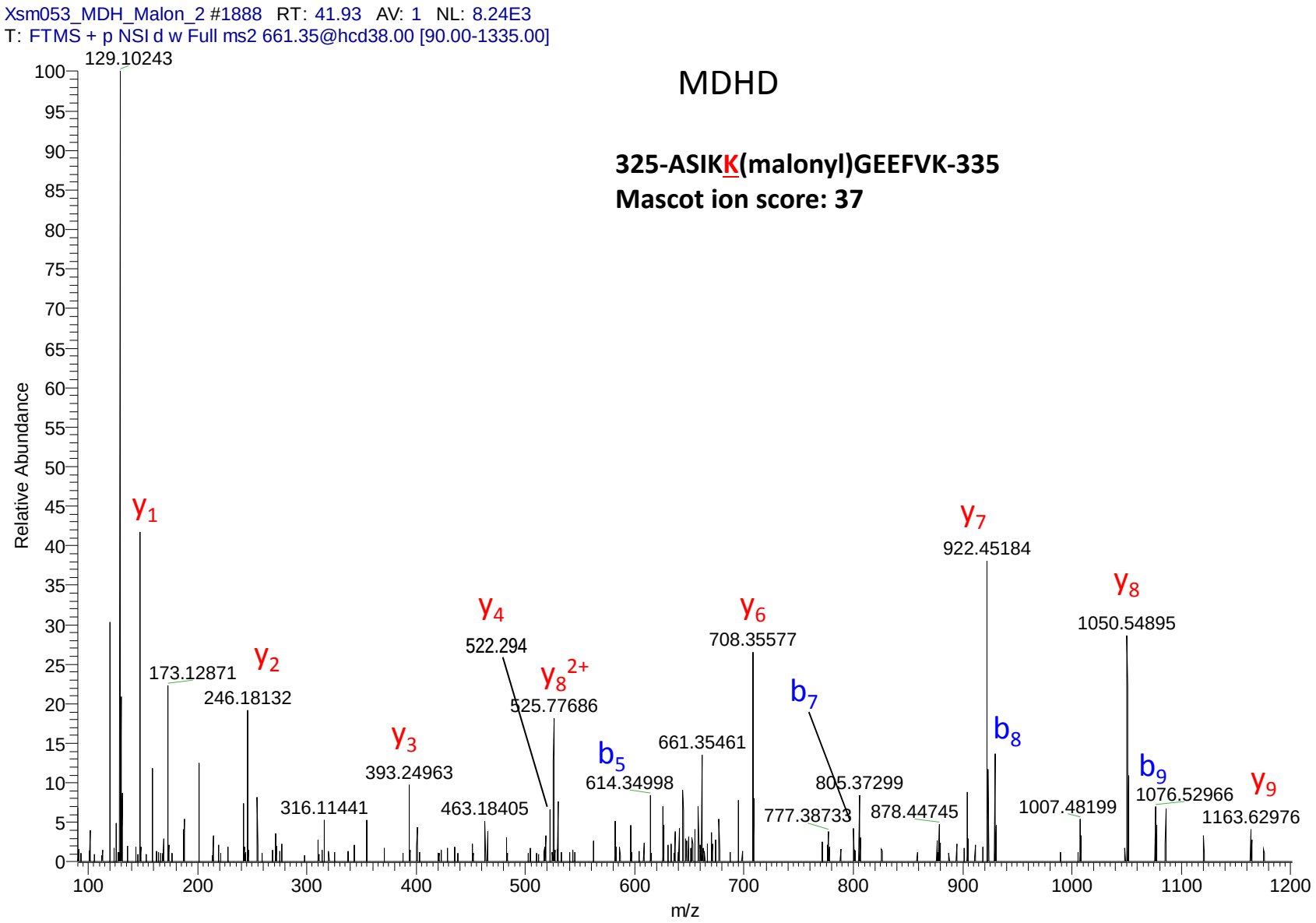
XSm053_MDH_HCD_noinclusion1 #6106 RT: 66.77 AV: 1 NL: 1.67E5
T: FTMS + p NSI d w Full ms2 737.40@hcd40.00 [100.00-1485.00]

302-NLGIGKK(acetyl)ISPFEEK-314
Mascot ion score: 56

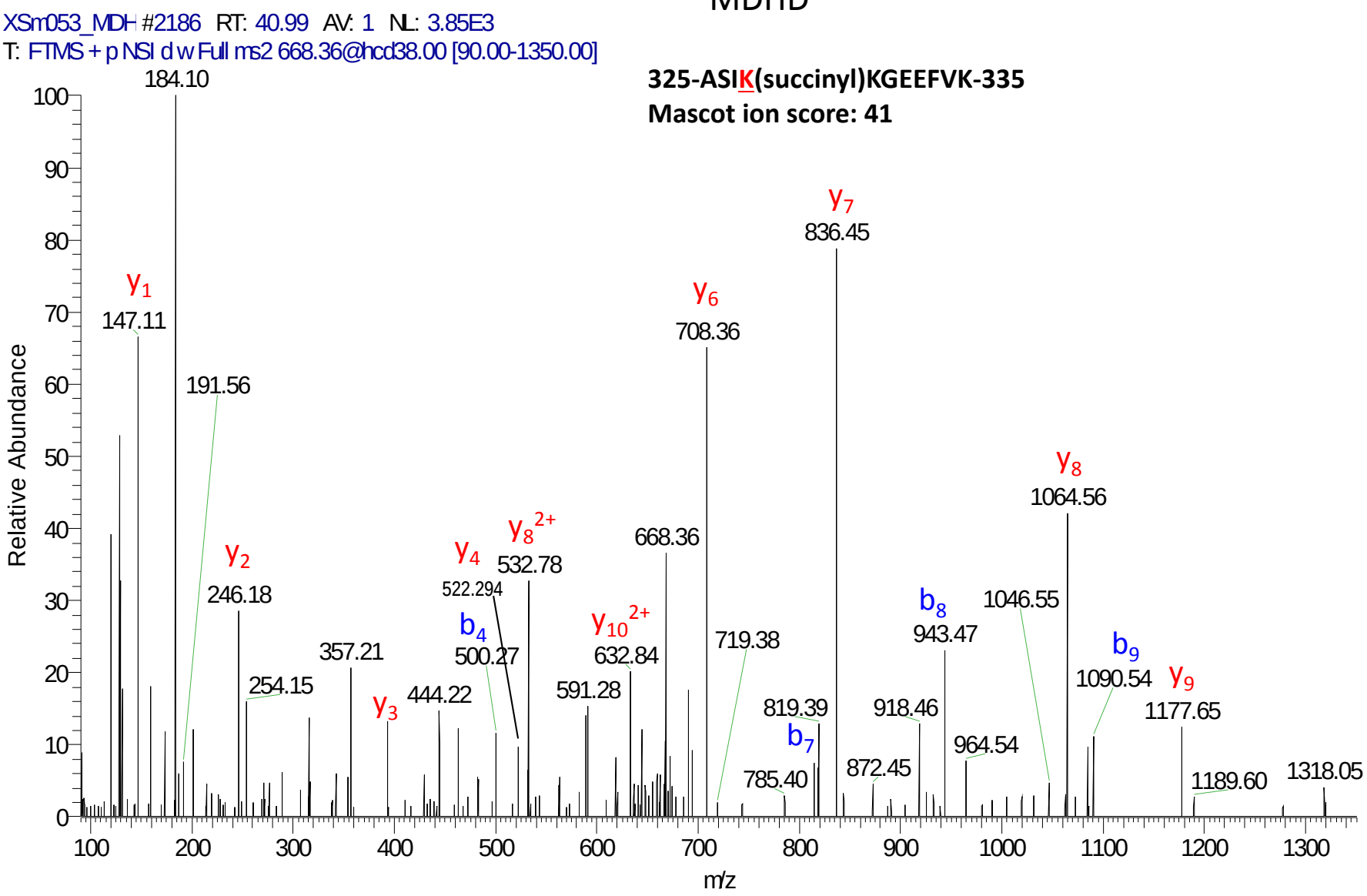


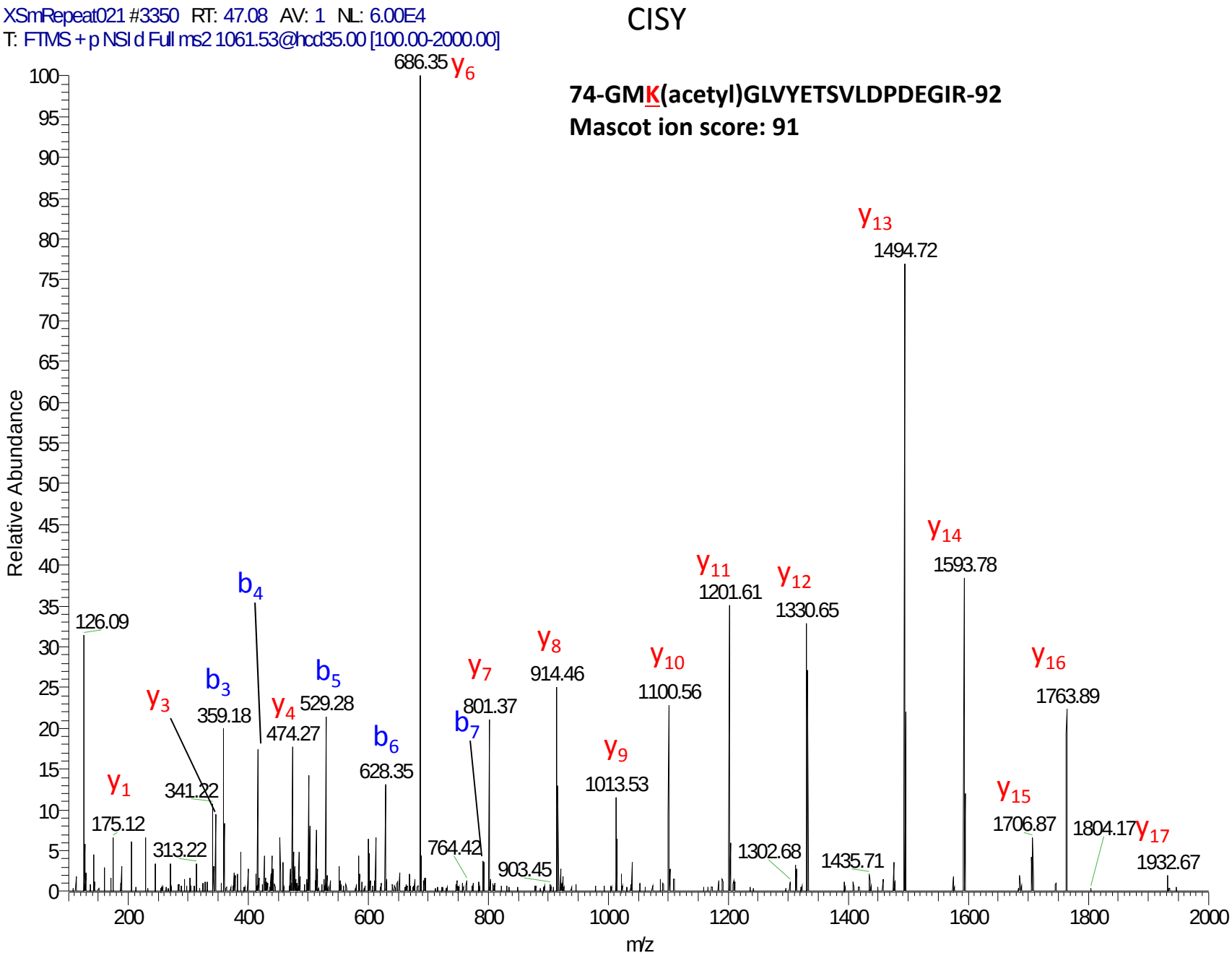

XSm053_MDH_HCD_noinclusion1 #8785 RT: 85.27 AV: 1 NL: 1.86E3
T: FTMS + p NSI d w Full ms2 668.69@hcd40.00 [100.00-2000.00]
MDHD
308-ISPFEEKK(acetyl)M(ox)IAEAIPELK-324
Mascot ion score: 45
Relative Abundance
m/z
486.29 y4
870.49 y8
799.46 y7
670.41 y6
599.38 y5
340.19
260.20 y2
201.12
142.09
y1
429.09 y3
503.21
y9 2+
776.35
889.43
983.58 y9
1020.47 b8
1033.63
1133.55 b9
1204.59 b10
1301.70
1404.68 b12
1533.58
0
10
20
30
40
50
60
70
80
90
100
200
400
600
800
1000
1200
1400
1600

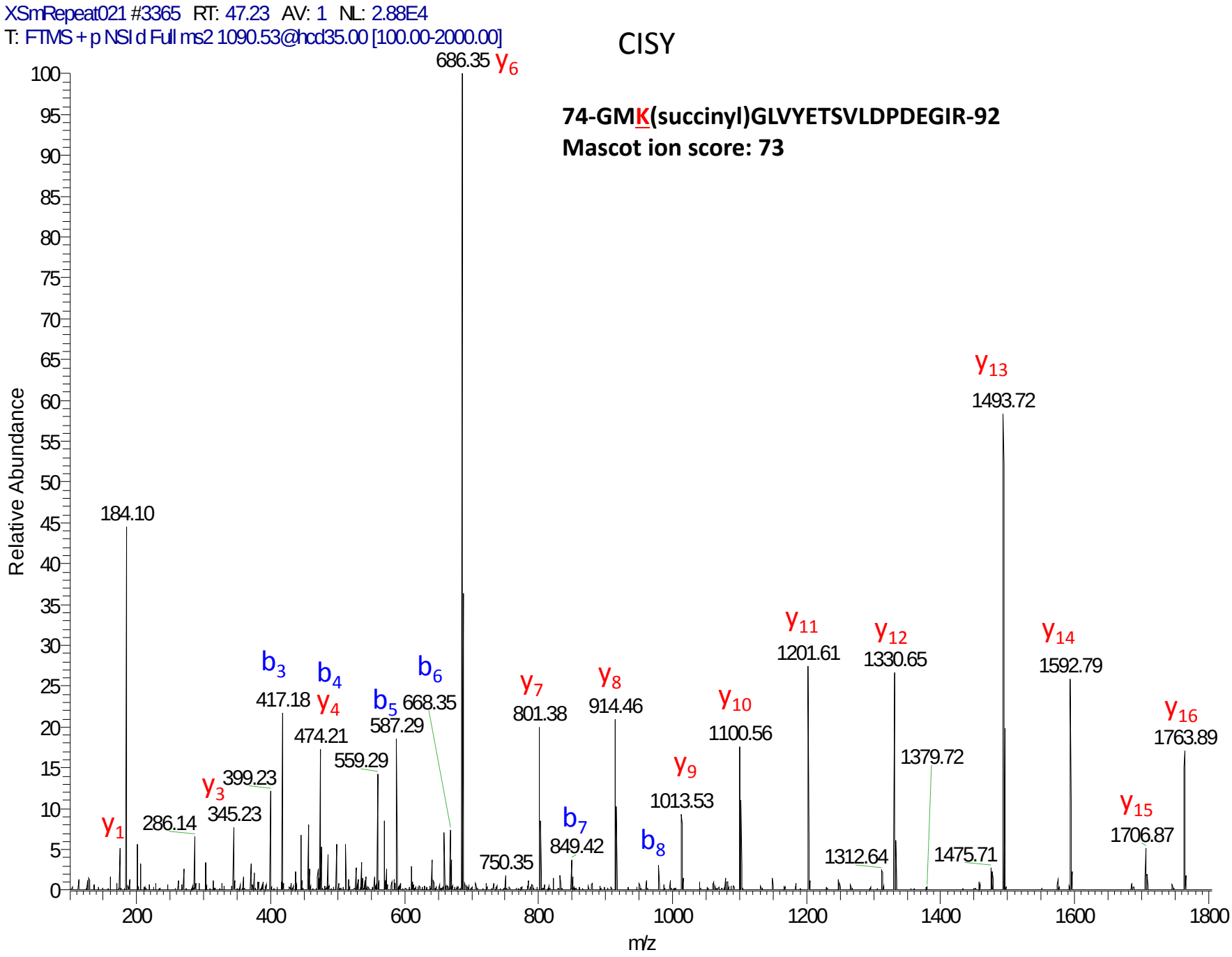

XSm053_MDH #2117 RT: 40.35 AV: 1 NL: 6.71E3
T: FTMS + p NSI d w Full ms2 639.36@hcd38.00 [90.00-1290.00]
MDHD
325-ASIK(acetyl)KGEEFVK-335
Mascot ion score: 53
Relative Abundance
m/z
126.09
y1 147.11
y2 246.18
299.21
159.08
316.12 y3
393.25
187.07
371.23
444.21
y4 522.294
y8 2+ 503.78
y9 2+
591.28
639.36
690.35
y6 708.36
761.38
b7
818.44
y7 836.46
b8 885.47
903.50
961.50
y8 1006.56
b9 1032.54
y9 1119.65
y10 1206.68

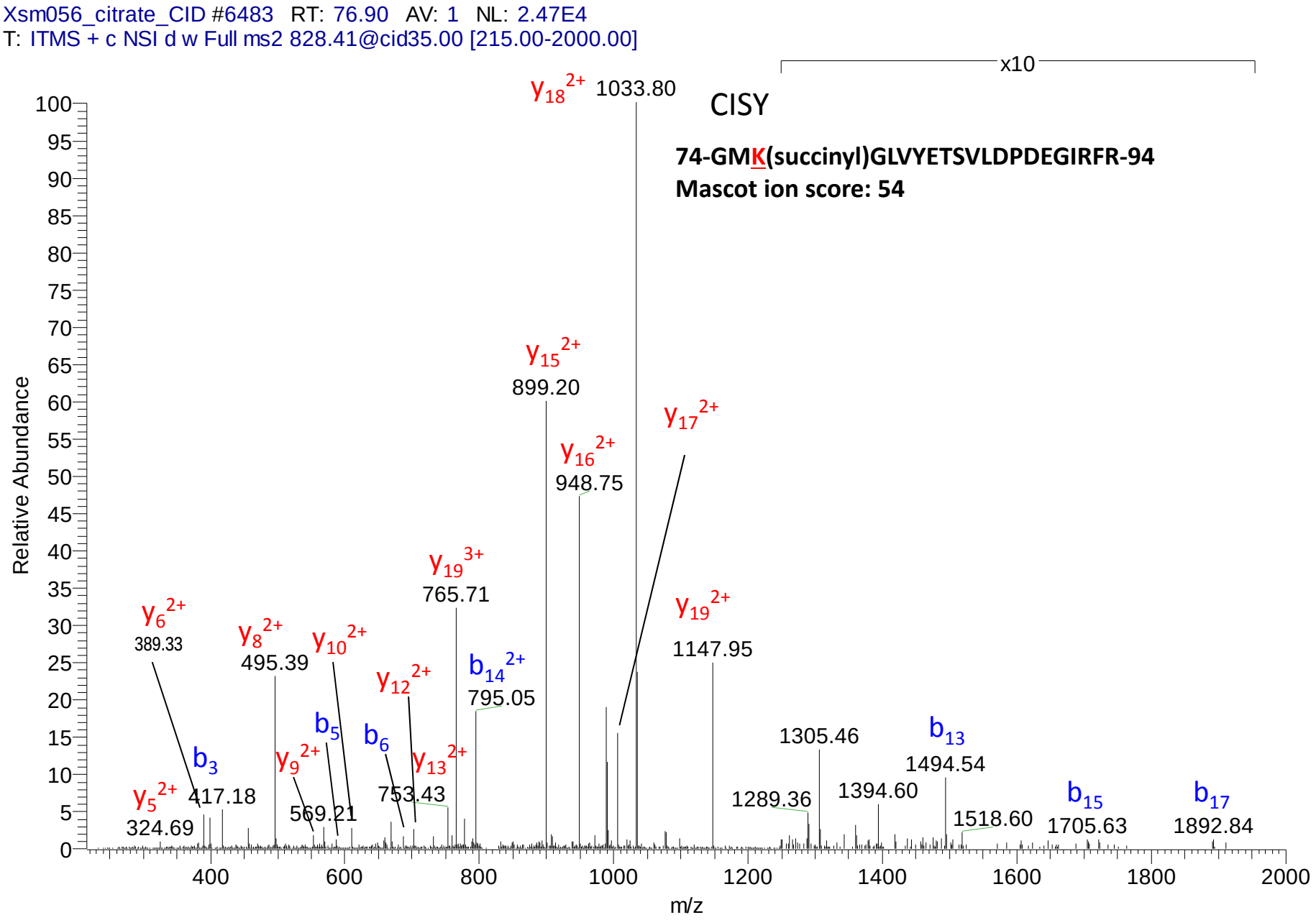

Xsm053_MDH_Malon_2 #1888 RT: 41.93 AV: 1 NL: 8.24E3
T: FTMS + p NSI d w Full ms2 661.35@hcd38.00 [90.00-1335.00]
MDHD
325-ASIKK(malonyl)GEEFVK-335
Mascot ion score: 37
Relative Abundance
m/z
129.10243
y1
y2
173.12871
246.18132
y3
393.24963
316.11441
y4
522.294
463.18405
y8 2+
525.77686
b5
614.34998
661.35461
y6
708.35577
b7
777.38733
805.37299
878.44745
y7
922.45184
b8
1007.48199
y8
1050.54895
b9
1076.52966
y9
1163.62976

MDHD


XSm053_MDH #2186 RT: 40.99 AV: 1 NL: 3.85E3
T: FTMS + p NSI d w Full ms2 668.36@hcd38.00 [90.00-1350.00]
325-ASIK(succinyl)KGEEFVK-335
Mascot ion score: 41
Relative Abundance
m/z
184.10
y1
147.11
191.56
y2
246.18
254.15
357.21
y3
444.22
y4
522.294
y8 2+
532.78
b4
500.27
591.28
y10 2+
632.84
668.36
y6
708.36
719.38
y7
836.45
819.39
b7
785.40
872.45
b8
943.47
918.46
964.54
1046.55
y8
1064.56
b9
1090.54
y9
1177.65
1189.60
1318.05

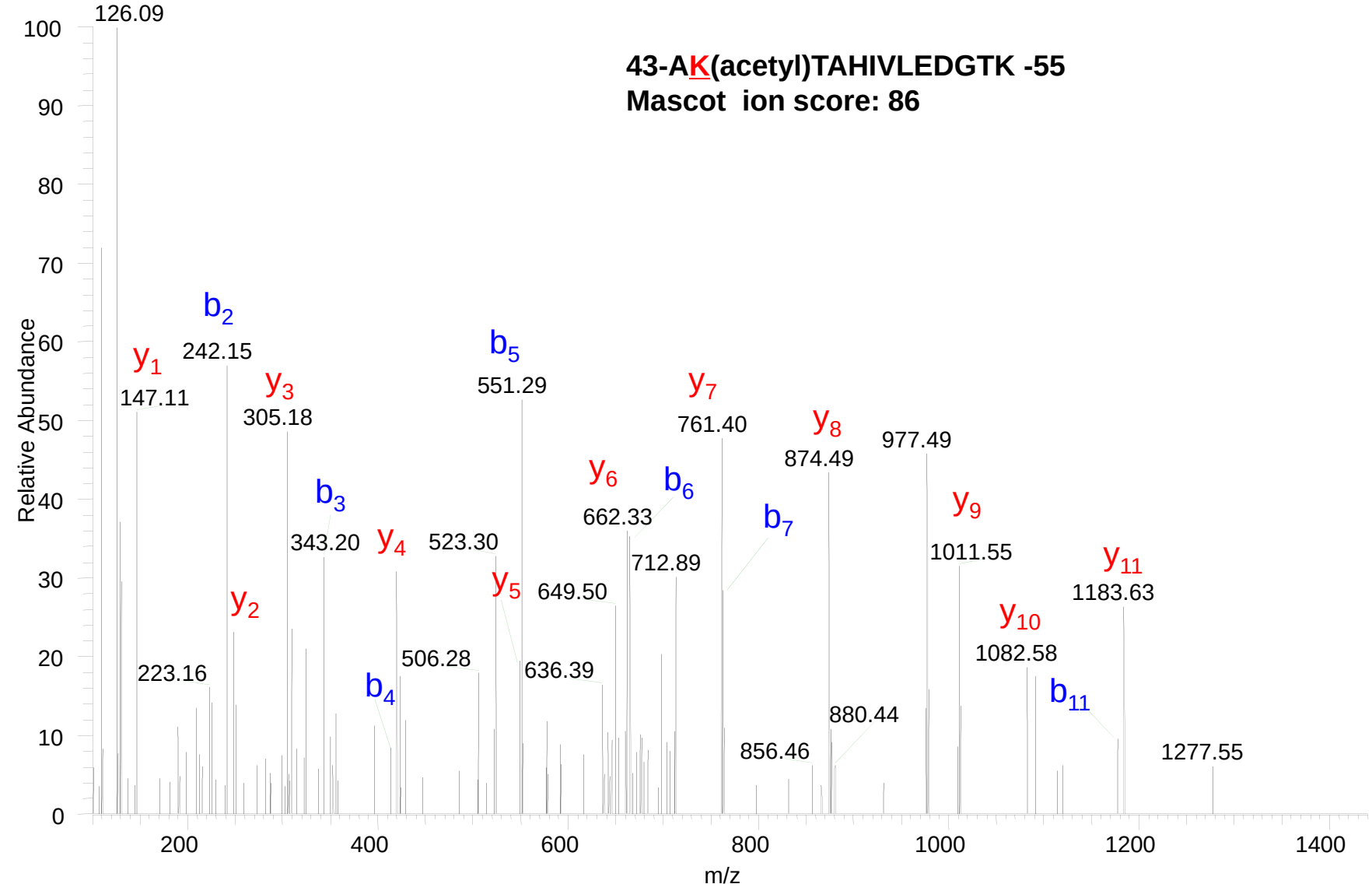

XSmRepeat021 #3350 RT: 47.08 AV: 1 NL: 6.00E4
T: FTMS + p NSI d Full ms2 1061.53@hcd35.00 [100.00-2000.00]
CISY
74-GMK(acetyl)GLVYETSVLDPDEGIR-92
Mascot ion score: 91
Relative Abundance
m/z
686.35 y6
y13 1494.72
y14 1593.78
y11 1201.61
y12 1330.65
y16 1763.89
y8 914.46
y10 1100.56
y7 801.37
b4
b5 529.28
b3 359.18
y4 474.27
y3
b6 628.35
b7
y9 1013.53
y1 175.12
341.22
313.22
764.42
903.45
1302.68
1435.71
y15 1706.87
1804.17
y17 1932.67
126.09


XSmRepeat021 #3365 RT: 47.23 AV: 1 NL: 2.88E4
T: FTMS + p NSI d Full ms2 1090.53@hcd35.00 [100.00-2000.00]
CISY
74-GMK(succinyl)GLVYETSVLDPDEGIR-92
Mascot ion score: 73
Relative Abundance
m/z
686.35 y6
184.10
y1
286.14
y3 399.23
345.23
b3 417.18
y4 474.21
b4
b5 587.29
559.29
b6 668.35
750.35
y7 801.38
b7 849.42
y8 914.46
b8
y9 1013.53
y10 1100.56
y11 1201.61
1312.64
y12 1330.65
1379.72
1475.71
y13 1493.72
y14 1592.79
y15 1706.87
y16 1763.89


Xsm056_citrate_CID #6483 RT: 76.90 AV: 1 NL: 2.47E4
T: ITMS + c NSI d w Full ms2 828.41@cid35.00 [215.00-2000.00]
x10
CISY
74-GMK(succinyl)GLVYETSVLDPDEGIRFR-94
Mascot ion score: 54
Relative Abundance
m/z
y18 2+ 1033.80
y15 2+ 899.20
y16 2+ 948.75
y17 2+
y19 3+ 765.71
y19 2+ 1147.95
y6 2+ 389.33
y8 2+ 495.39
y10 2+
y12 2+
b14 2+ 795.05
b5
b6
b3
y9 2+
y13 2+
y5 2+ 324.69
417.18
569.21
753.43
1305.46
b13 1494.54
1289.36
1394.60
1518.60
b15 1705.63
b17 1892.84

Acetyl and succinyl peptides identified from CPS1 immunoprecipitated from Sirt5 KO mouse

1	**43-AK(acetyl)TAHIVLEDGTK -55**
2	**43-AK(Succinyl)TAHIVLEDGTK -55**
3	**287-K(acetyl)EPLFGISTGNIITGLAAGAK-307**
4	**287-K(succinyl)EPLFGISTGNIITGLAAGAK-307**
5	**1282-VMIGESIDEK(acetyl)R-1292**
6	**1282-VMIGESIDEK(succinyl)R-1292**


XSm070_CPSI_Sirt5KO_110730130949 #1682 RT: 25.23 AV: 1 NL: 8.13E3
T: FTMS + p NSI d w Full ms2 712.89@hcd38.00 [100.00-1440.00]
43-AK(acetyl)TAHIVLEDGTK -55
Mascot ion score: 86
Relative Abundance
m/z
126.09
147.11
242.15
305.18
343.20
223.16
506.28
523.30
551.29
636.39
649.50
662.33
712.89
761.40
856.46
874.49
880.44
977.49
1011.55
1082.58
1183.63
1277.55
b2
y1
y3
b3
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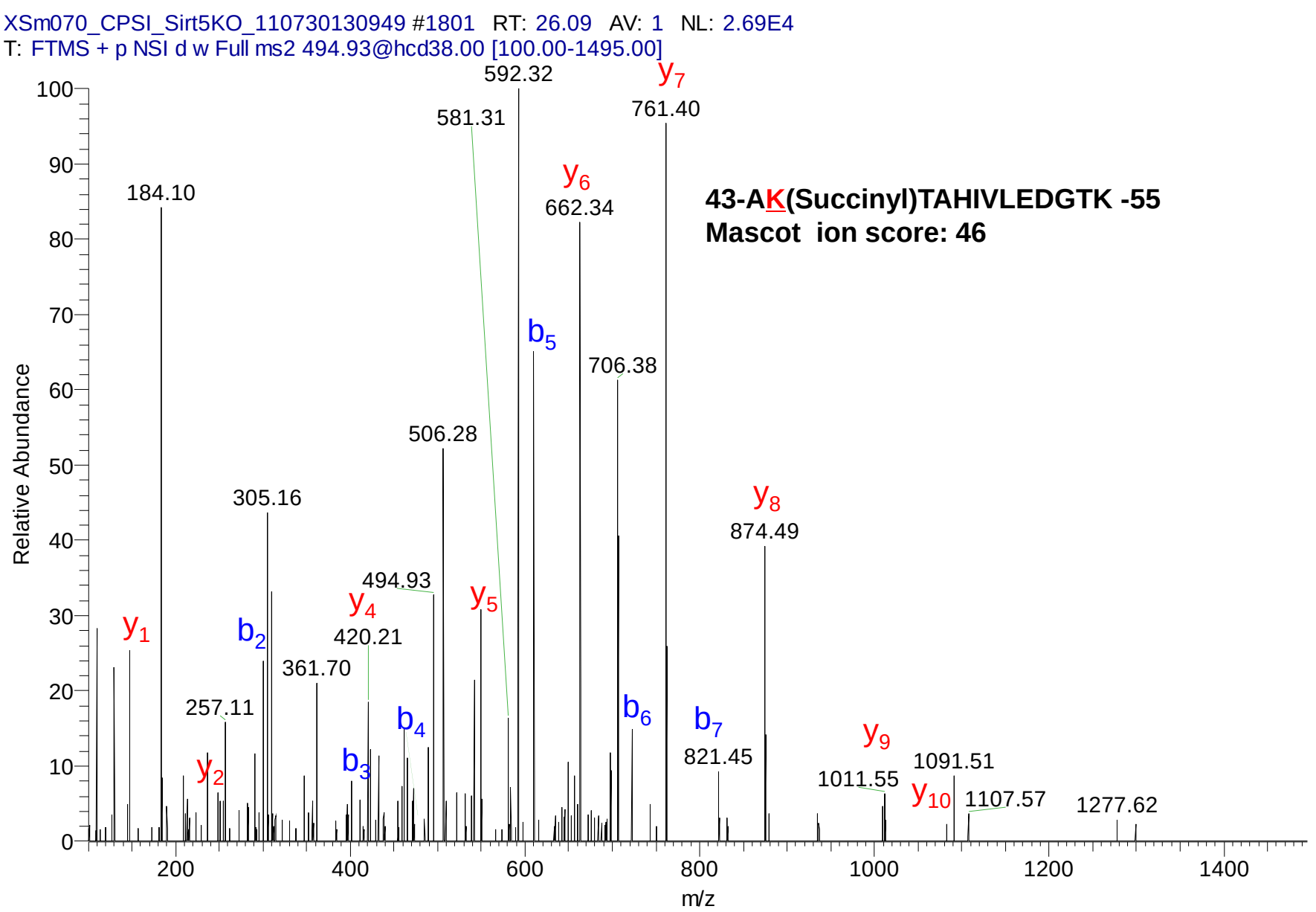
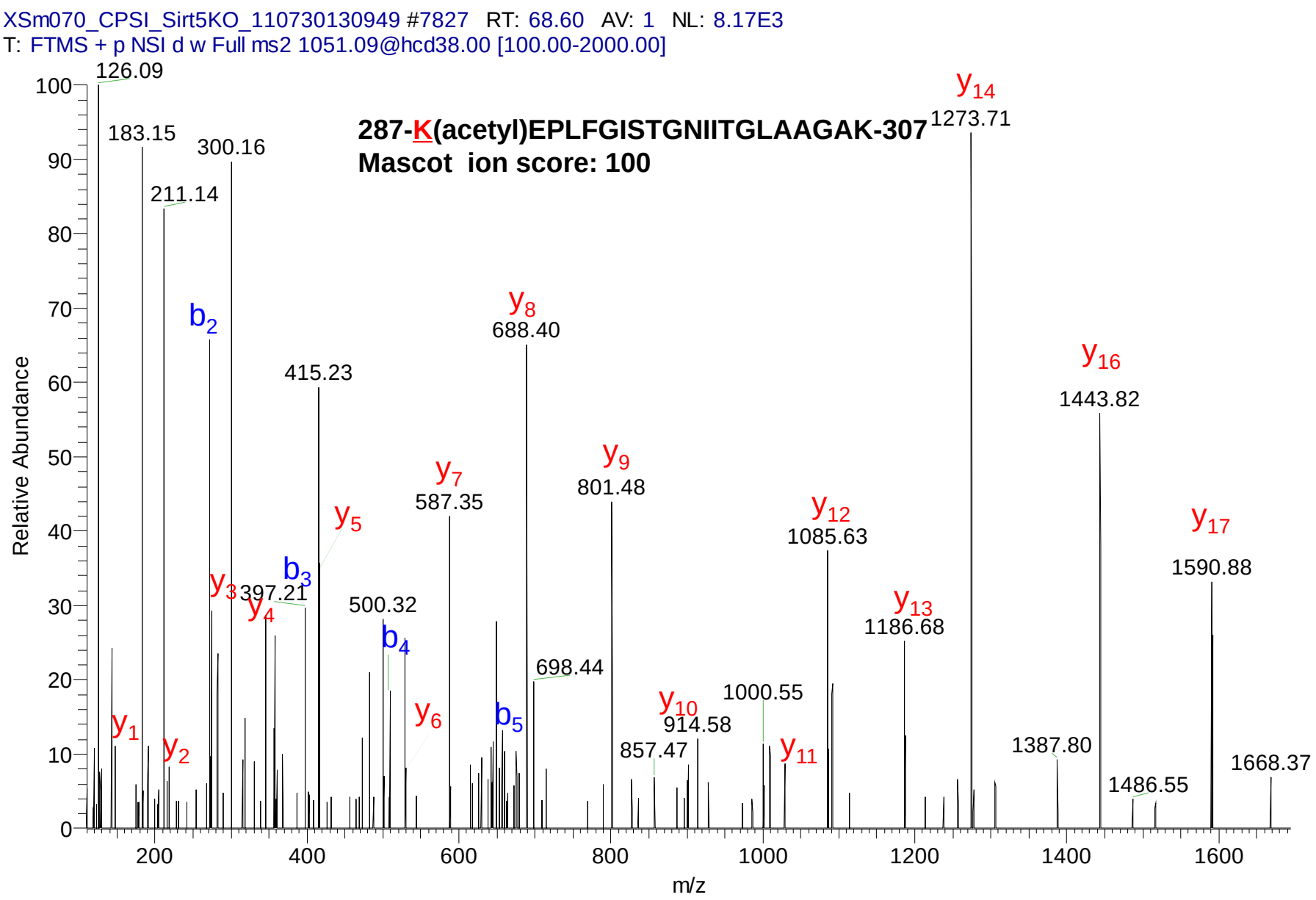
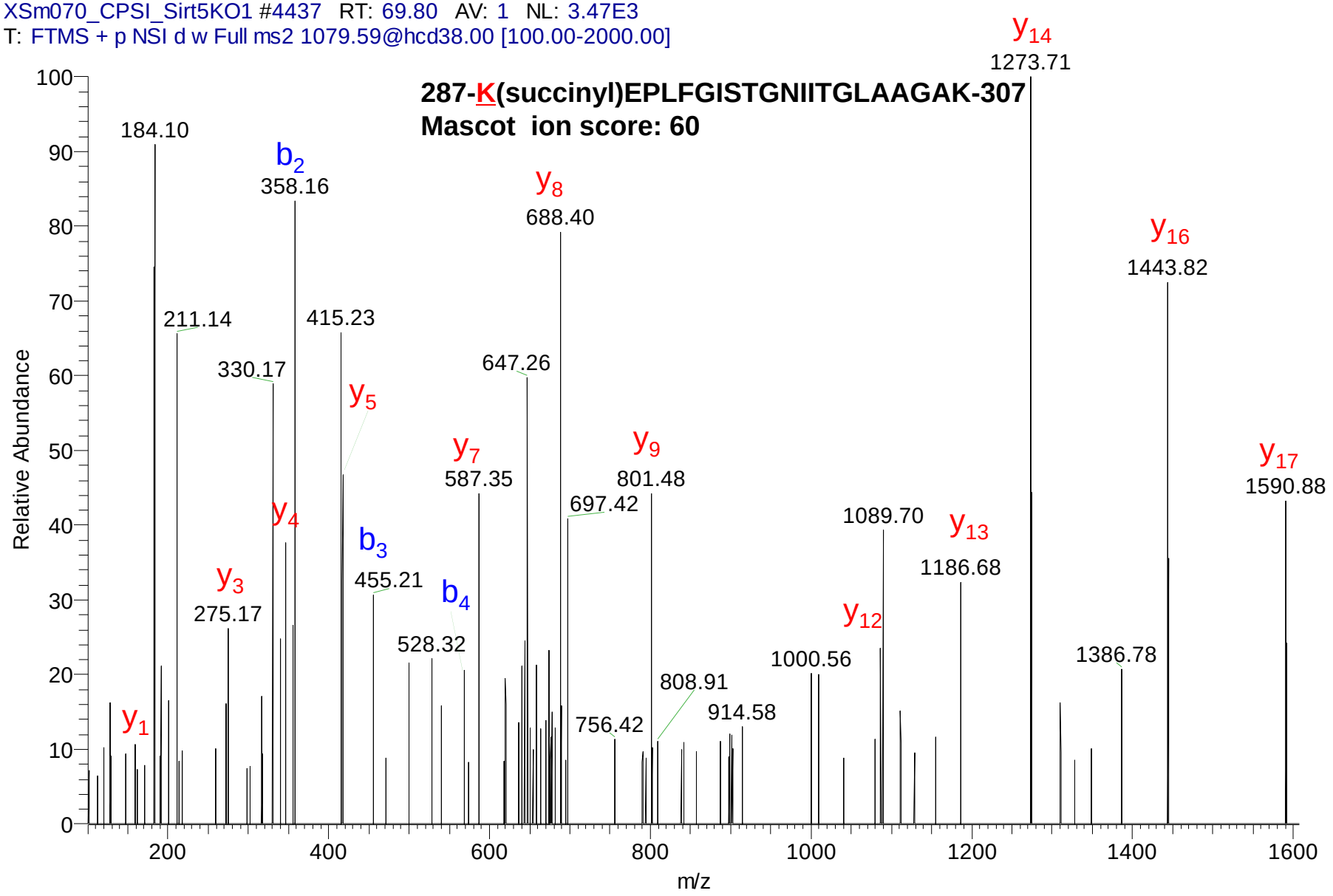
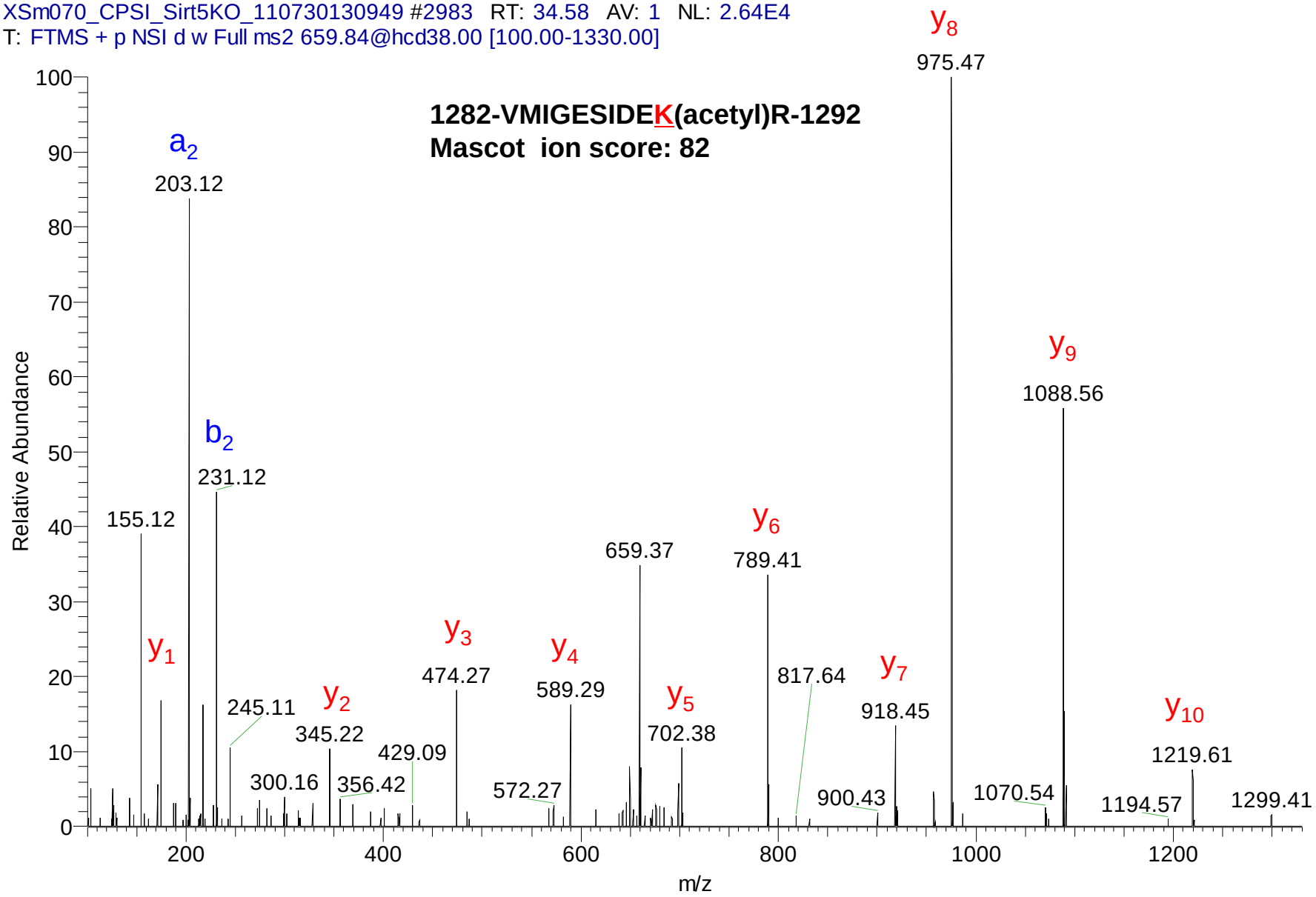
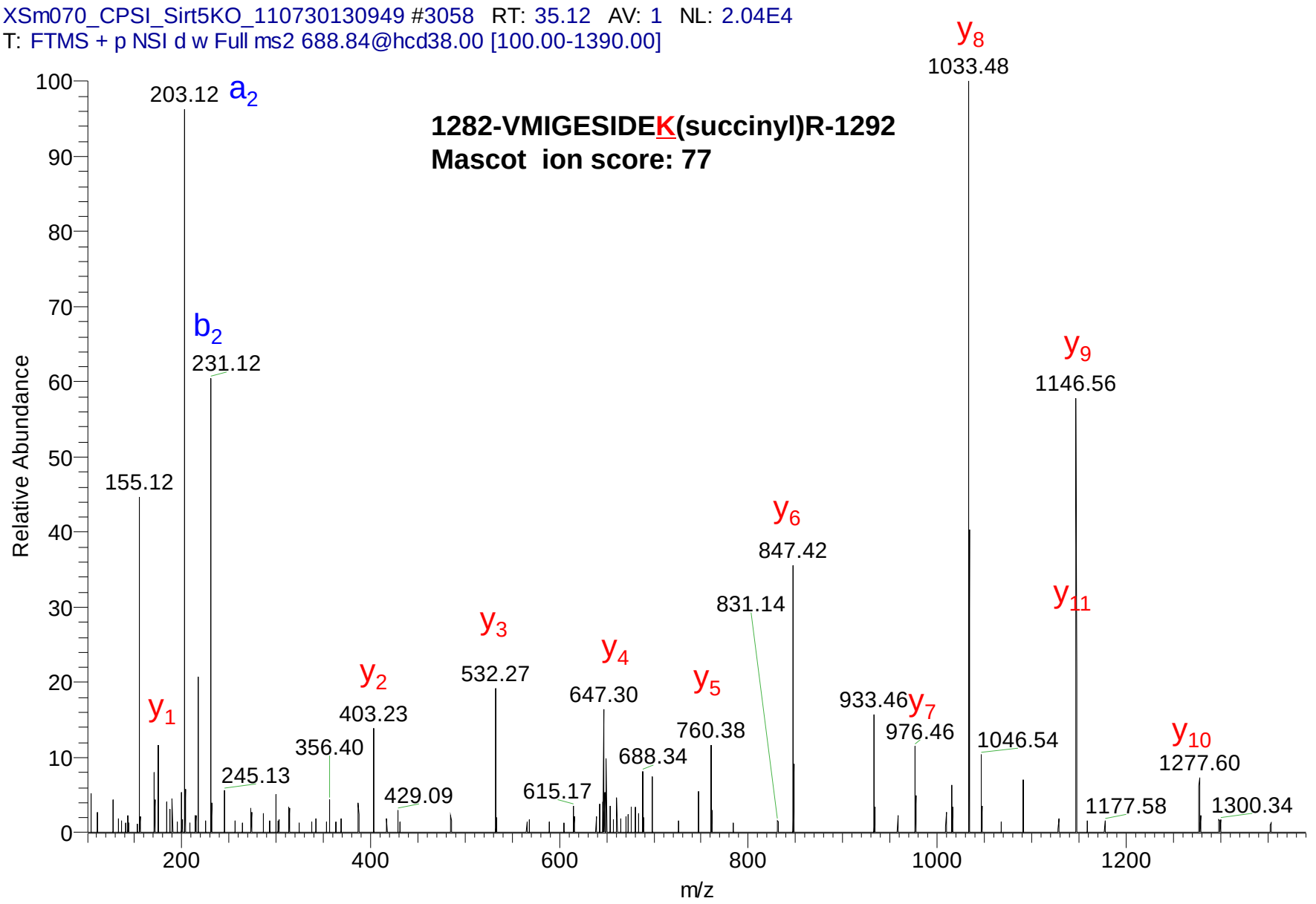
y6
b6
y7
b7
y8
y9
y10
b11
y11


XSm070_CPSI_Sirt5KO_110730130949 #1801 RT: 26.09 AV: 1 NL: 2.69E4
T: FTMS + p NSI d w Full ms2 494.93@hcd38.00 [100.00-1495.00]
43-AK(Succinyl)TAHIVLEDGTK -55
Mascot ion score: 46
Relative Abundance
m/z
592.32
y7
761.40
581.31
y6
662.34
184.10
b5
706.38
506.28
305.16
y8
874.49
494.93
y4
y5
y1
b2
420.21
361.70
257.11
b4
b6
b7
821.45
y9
1011.55
1091.51
y10
1107.57
1277.62
b3
y2


XSm070_CPSI_Sirt5KO_110730130949 #7827 RT: 68.60 AV: 1 NL: 8.17E3
T: FTMS + p NSI d w Full ms2 1051.09@hcd38.00 [100.00-2000.00]
287-K(acetyl)EPLFGISTGNIITGLAAGAK-307
Mascot ion score: 100
Relative Abundance
m/z
126.09
183.15
300.16
211.14
415.23
688.40
1273.71
1443.82
801.48
587.35
1085.63
1590.88
1186.68
397.21
500.32
698.44
1000.55
914.58
857.47
1387.80
1486.55
1668.37
y1
y2
y3
y4
y5
y6
y7
y8
y9
y10
y11
y12
y13
y14
y16
y17
b2
b3
b4
b5


XSm070_CPSI_Sirt5KO1 #4437 RT: 69.80 AV: 1 NL: 3.47E3
T: FTMS + p NSI d w Full ms2 1079.59@hcd38.00 [100.00-2000.00]
287-K(succinyl)EPLFGISTGNIITGLAAGAK-307
Mascot ion score: 60
Relative Abundance
m/z
y14 1273.71
y16 1443.82
y17 1590.88
y8 688.40
b2 358.16
y9 801.48
y7 587.35
y5
y4
b3 455.21
b4
y3 275.17
y1
y13 1186.68
y12
1089.70
1000.56
1386.78
808.91
914.58
756.42
647.26
697.42
528.32
415.23
330.17
211.14
184.10


XSm070_CPSI_Sirt5KO_110730130949 #2983 RT: 34.58 AV: 1 NL: 2.64E4
T: FTMS + p NSI d w Full ms2 659.84@hcd38.00 [100.00-1330.00]
1282-VMIGESIDEK(acetyl)R-1292
Mascot ion score: 82
Relative Abundance
m/z
y8 975.47
y9 1088.56
a2 203.12
b2 231.12
155.12
y1
y2 345.22
y3 474.27
y4 589.29
659.37
y5 702.38
y6 789.41
817.64
y7 918.45
y10 1219.61
245.11
300.16
356.42
429.09
572.27
900.43
1070.54
1194.57
1299.41


XSm070_CPSI_Sirt5KO_110730130949 #3058 RT: 35.12 AV: 1 NL: 2.04E4
T: FTMS + p NSI d w Full ms2 688.84@hcd38.00 [100.00-1390.00]
1282-VMIGESIDEK(succinyl)R-1292
Mascot ion score: 77
Relative Abundance
m/z
203.12 a2
231.12 b2
155.12
y1
y2
403.23
356.40
245.13
429.09
y3
532.27
615.17
y4
647.30
688.34
y5
760.38
y6
847.42
831.14
933.46
y7
976.46
1033.48 y8
1046.54
y9
1146.56
y11
1177.58
y10
1277.60
1300.34