Supplementary information

Serine is a new target residue for endogenous ADP-ribosylation on histones

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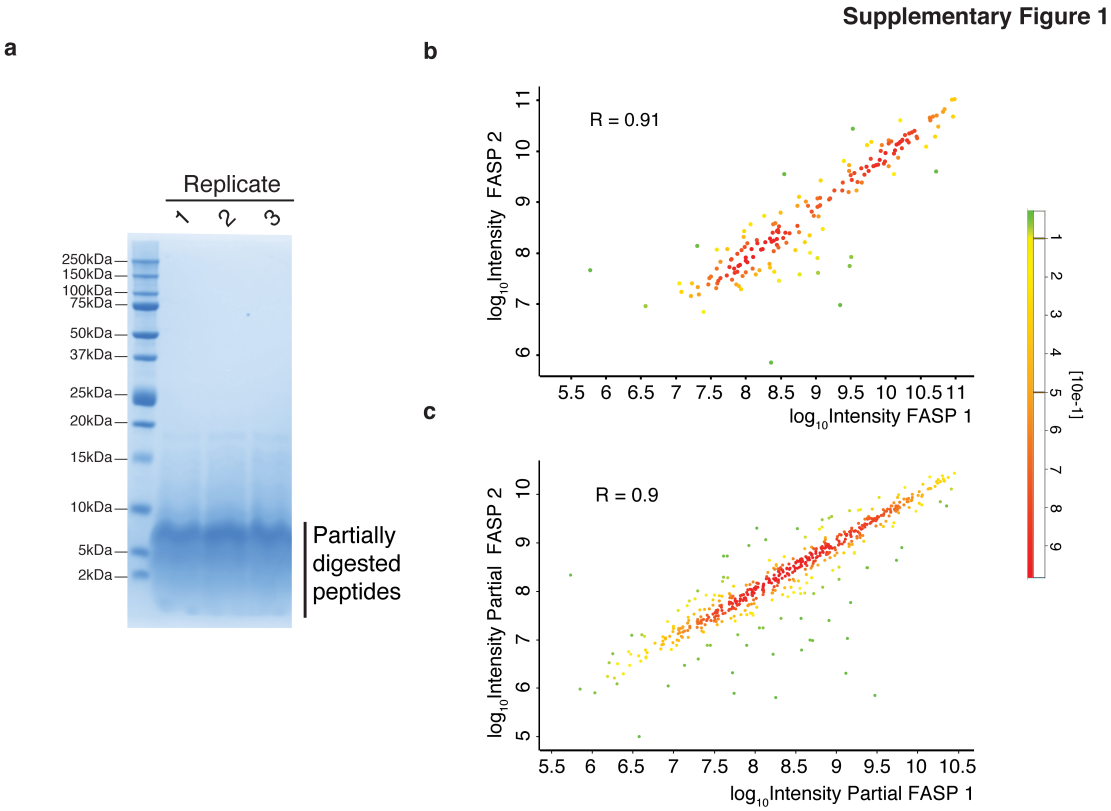
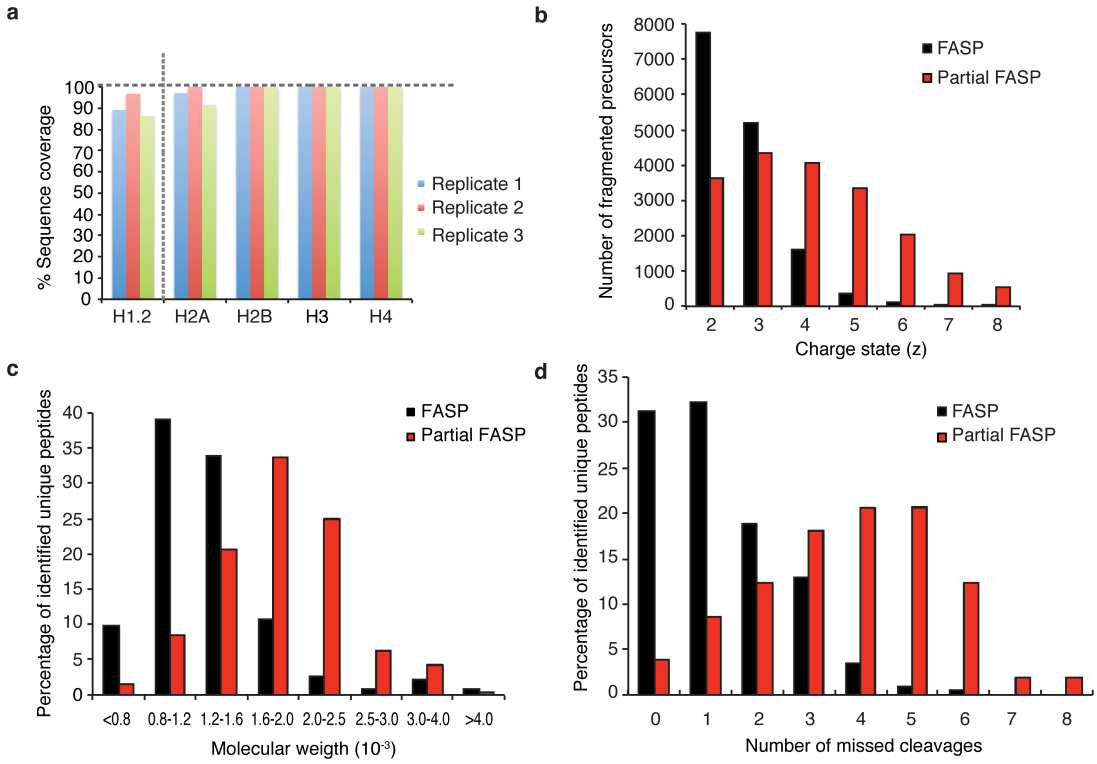
Supplementary results

Supplementary Figures

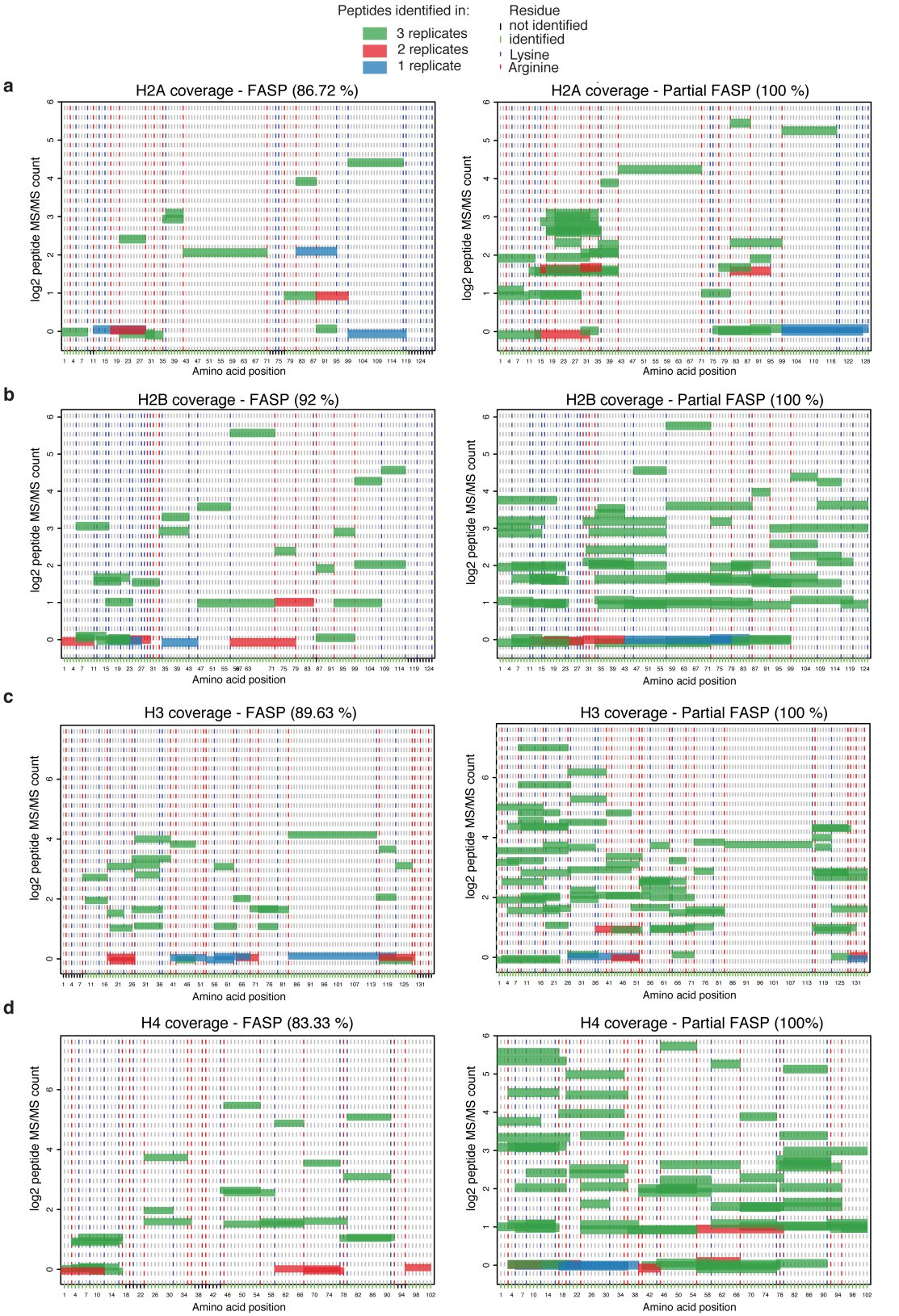


Supplementary Figure 1. Reproducibility of the Partial FASP method. (a) Triplicate digestion of histones with the Partial FASP method. Eluting peptides were resolved on SDS-PAGE and stained with Coomassie. **(b-c)** Correlation of the $\log_{10}$ intensities of unique peptides identified in replicate experiments of histones processed by the FASP **(b)** or the Partial FASP **(c)** method. Color-coding indicates density.

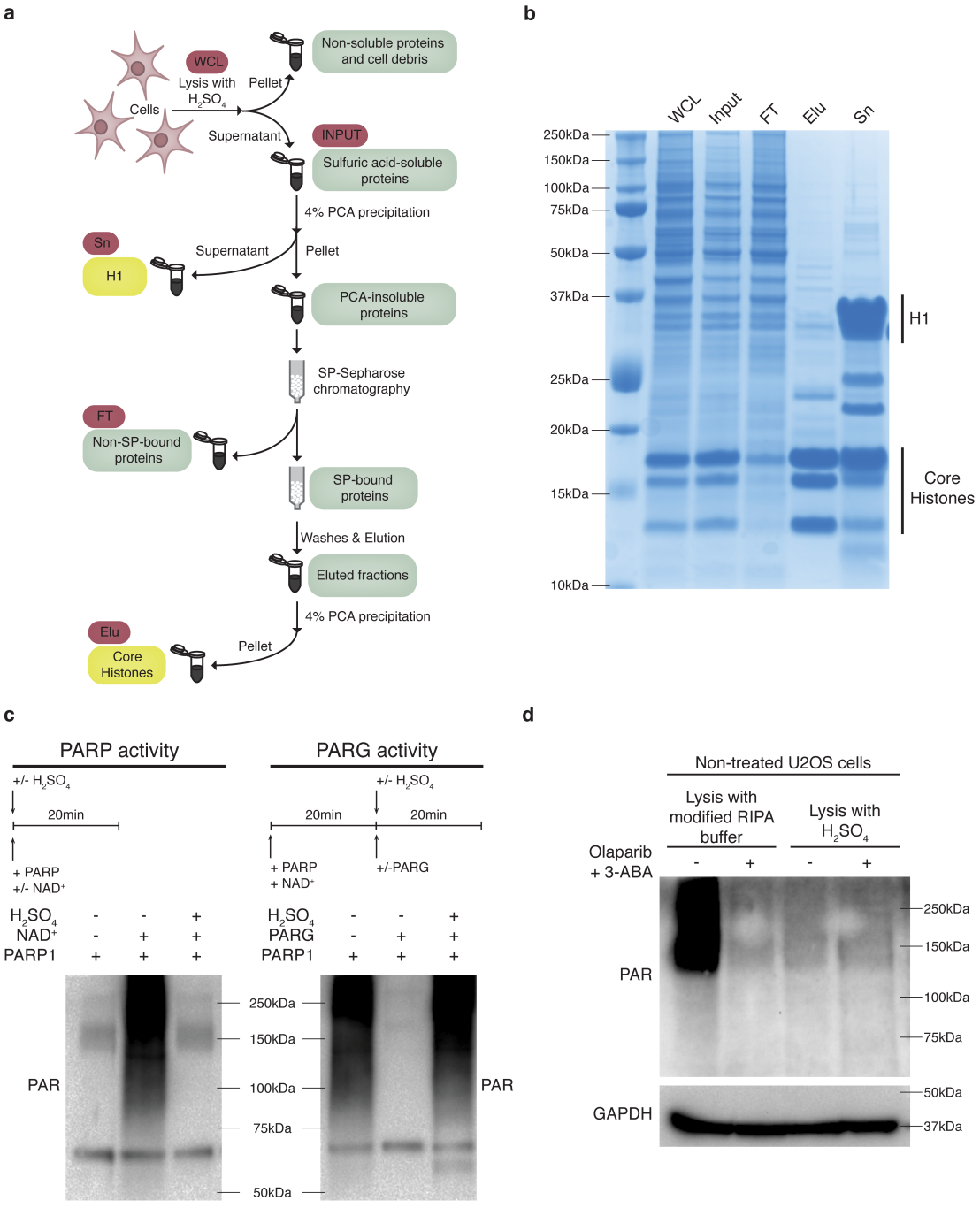


Supplementary Figure 2. Characterization of the Partial FASP method. (a) Sequence coverage of the most abundant isoforms of H1 and the core histones prepared with the Partial FASP method. The experiment was performed in triplicates. All samples represent technical replicates on the digestion level. Note: H1 digestion was done separately. **(b)** Charge distributions of fragmented precursors for histones digested with traditional FASP versus step iii of Figure 1a of Partial FASP. **(c and d)** Distributions of peptide molecular weight **(c)** and missed cleavages **(d)** from LC-MS/MS analyses of samples generated by FASP (black) or Partial FASP (red) digestion of 50 µg of purified histones.

Supplementary Figure 3



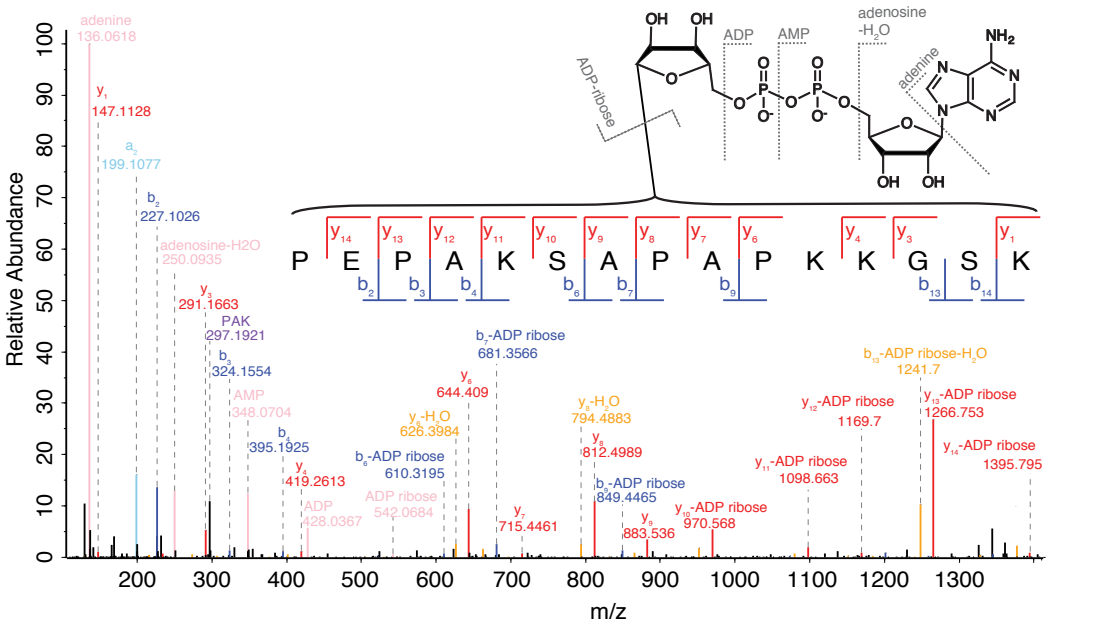
Supplementary Figure 3. Further characterization of the Partial FASP method. Schematic representation of H2A **(a)**, H2B **(b)**, H3 **(c)** and H4 **(d)** sequence coverage. Left panels show the sequence coverage of proteins digested with the FASP method, right ones with Partial FASP. Each bar represents an identified peptide, and shows the peptide's position within a protein (x axis) and number of MS/MS spectral counts (y axis). Bars were color coded according the number of experimental replicates (out of three) in which the peptide was identified. In general, the Partial FASP method results in higher number of peptides per protein that can be identified. Those peptides are longer and contain more missed cleavages (see arginine and lysine residues), while still being abundant and reaching high MS/MS counts.



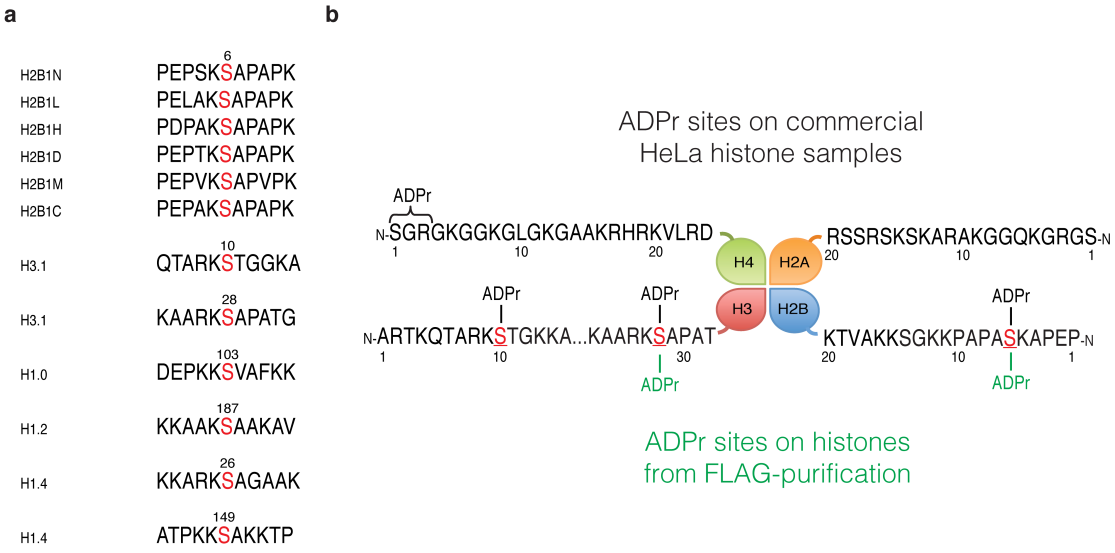
Supplementary Figure 4. Extraction and purification of histones from cells. (a) Schematic overview of the workflow for histone extraction and purification combining sulfuric acid lysis and SP-chromatography. See **Online Methods** for further experimental details. **(b)** Coomassie-staining of SDS-PAGE with the different fractions collected during the purification method outlined in (a). See **Supplementary**

Fig. 11 for original image. **(c)** Analysis of the effect of sulfuric acid on PARP or PARG activity. *In vitro* PARP activity assays (left panel) were performed in the absence or presence of 200 μM NAD^+ and/or 0.1 M sulfuric acid. *In vitro* PARG activity assays (right panel) were performed in the absence or presence of 1 μM human PARG and/or 0.1 M sulfuric acid. PARP1 poly-ADP-ribosylation was analyzed by Western blotting using anti-PAR antibodies. The blot is representative of three independent experiments. See **Supplementary Fig. 11** for original image. **(d)** Acid extraction prevents artifactual post-lysis increase in the level of PARylation and therefore preserves the endogenous level of PAR. Untreated U2OS cells were lysed with modified RIPA buffer or with sulfuric acid in the absence or presence of the PARP inhibitors olaparib (2 μM) and 3-ABA (10 mM). Total protein poly-ADP-ribosylation was analyzed by Western blotting using anti-PAR antibodies. Anti-GAPDH was used as a loading control. See Online Methods for further experimental details. The Western blot is representative of three independent experiments. See **Supplementary Fig. 11** for original image.

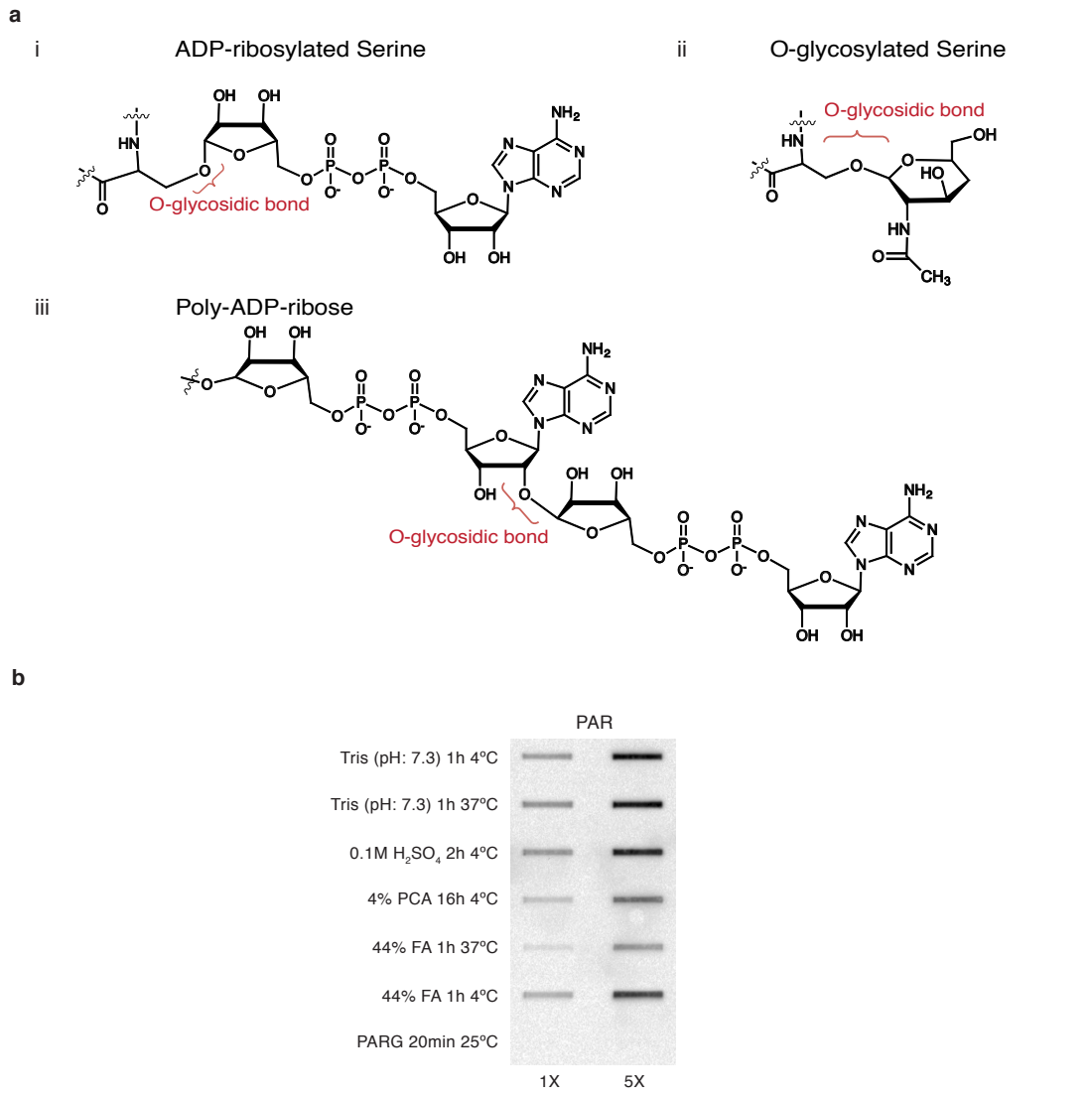
Supplementary Figure 5



Supplementary Figure 5. High-resolution HCD fragmentation spectra of an H2B peptide modified by ADP-ribose. While low-mass ions resulting from fragmentation of ADP-ribose unmistakably confirm the presence of ADP-ribose, this fragmentation mode is not suitable for confidently localizing modification sites, as the bond between ADP-ribose and the peptides is very labile during HCD, a behavior also observed for O-glycopeptides[13]. We exploited this lability of ADP-ribose for triggering acquisition of high-resolution ETD spectra based on the presence of adenine ions in rapidly-acquired HCD spectra. See **Online Methods** for further details.

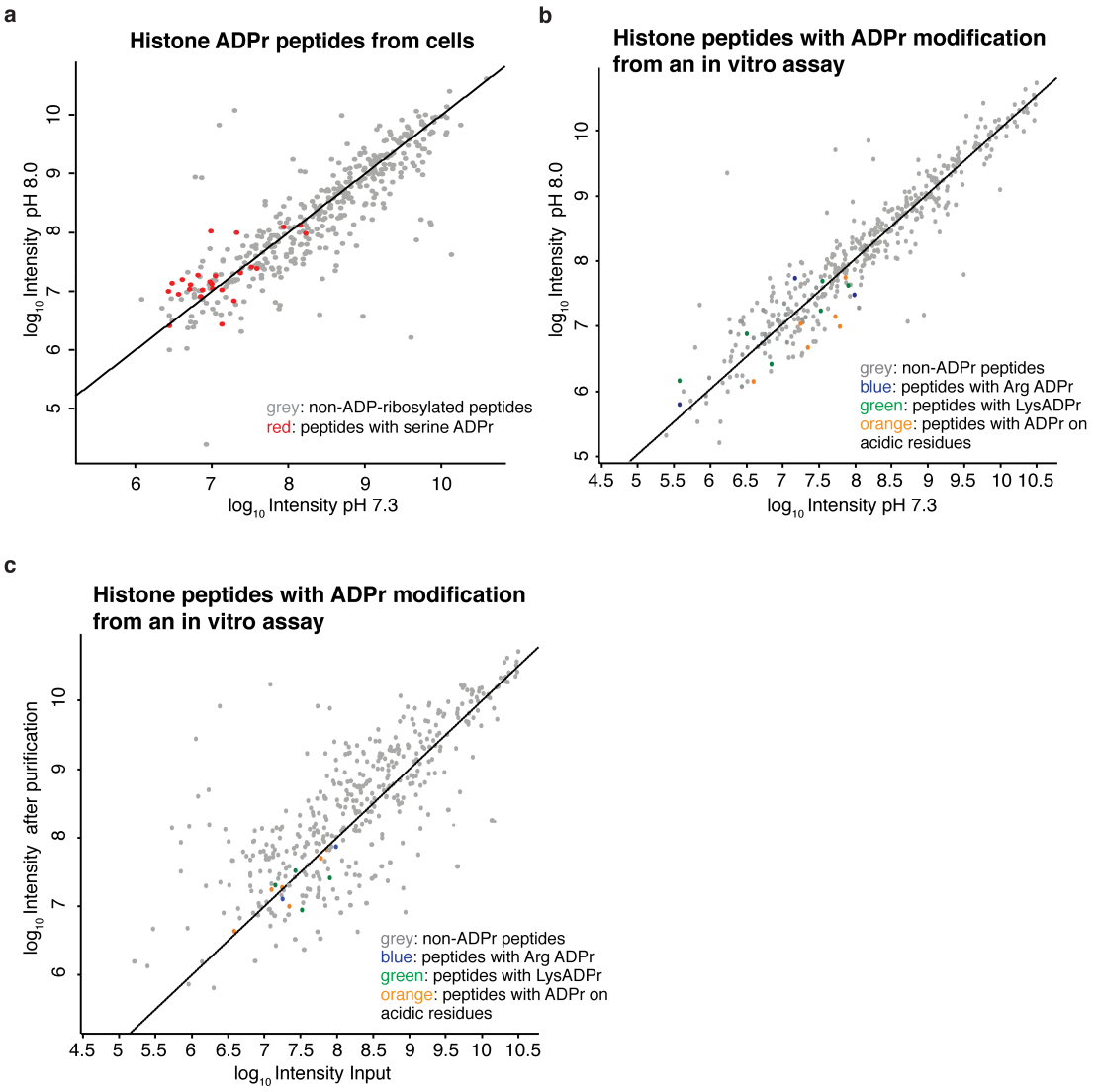


Supplementary Figure 6. Serine ADPr sites on different histone variants and on commercial core histone samples. (a) Sequences show the similarity between the regions of the proteins where serine ADPr was found. One site was detected on six different H2B variants. **(b)** Schematic representation of core histones. Four out of six previously identified histone serine ADP-ribosylation sites are also found on commercial HeLa core histones and two of them in an immunoprecipitation experiment, digested with the Partial FASP method.



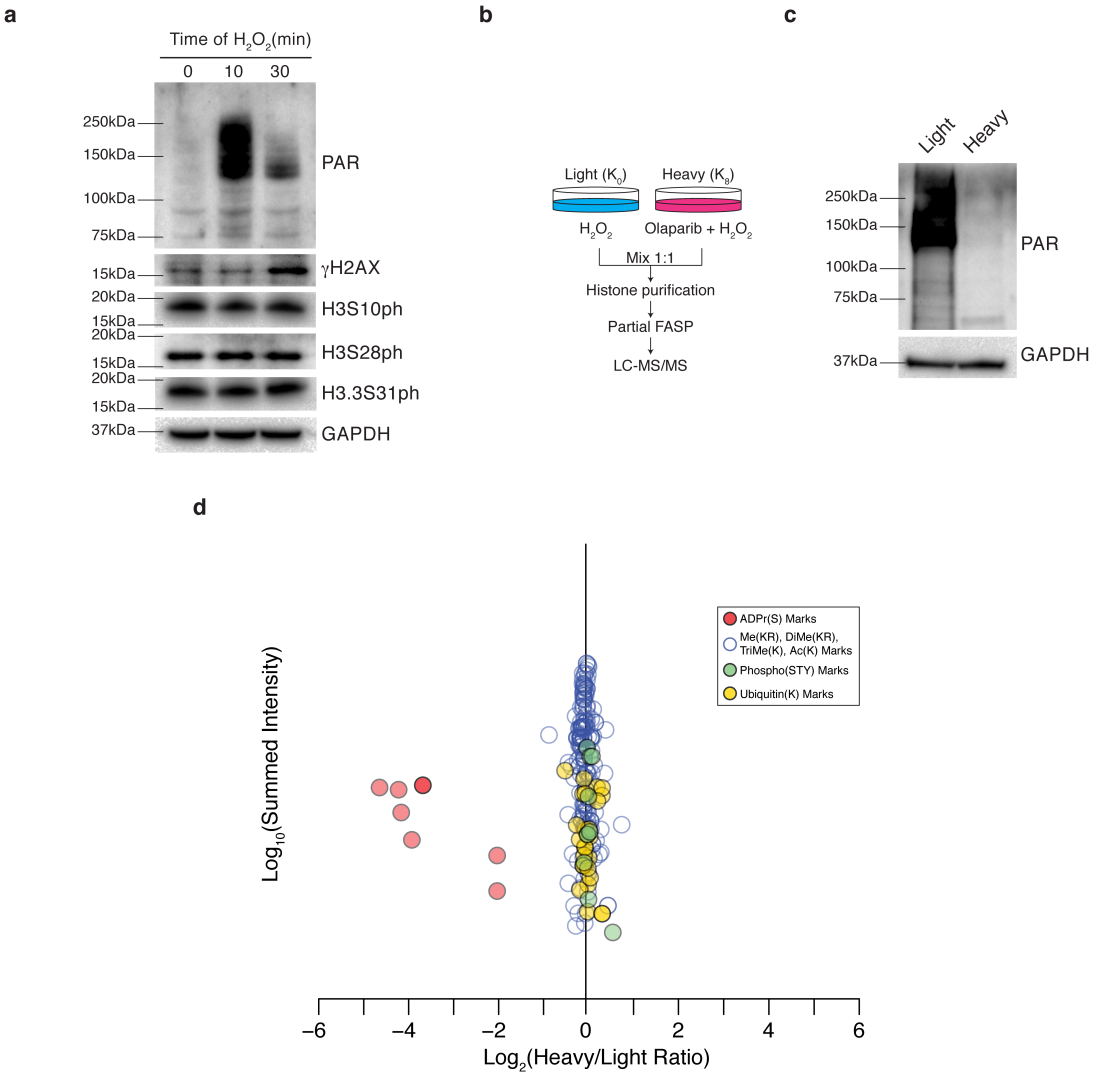
Supplementary Figure 7. PAR stability under acidic conditions (a) Chemical structures of ADP-ribosylated Serine (i), O-glycosylated Serine (ii) and a poly-ADP-ribose chain represented by two monomers (iii). ADP-ribosylation is attached to Serine (i) through the same linkage that exists between sugars, such as N-acetylglucosamine, and O-glycosylated proteins (ii), and that links together poly-ADP-ribose (iii). **(b)** Analysis of PAR degradation under acidic conditions. PAR chains were subjected to the indicated treatments. PAR stability was analyzed by immune-slot blot using anti-PAR antibodies. 1X and 5X indicate the relative amount

of sample loaded into the slots after treatments. See **Online Methods** for further experimental details. The slot-blot is representative of four independent experiments. See **Supplementary Fig. 11** for original image. The degradation of PAR observed in 44% formic acid at 37°C is consistent with the degradation of low molecular weight acetal model conjugates observed by Cervantes-Lauren et al. JBC 1995. Incubation temperature is an important factor for the cleavage of the acetal bond: much less PAR is degraded by formic acid at 4°C compared to 37°C. Importantly, acid extraction (H2SO4 for 2h at 4°C), which is the essential first step of our histone purification protocol, does not appear to degrade any significant amount of PAR. Even after overnight incubation (16h) in 4% percloric acid at 4°C, approximately half of the PAR signal remains.



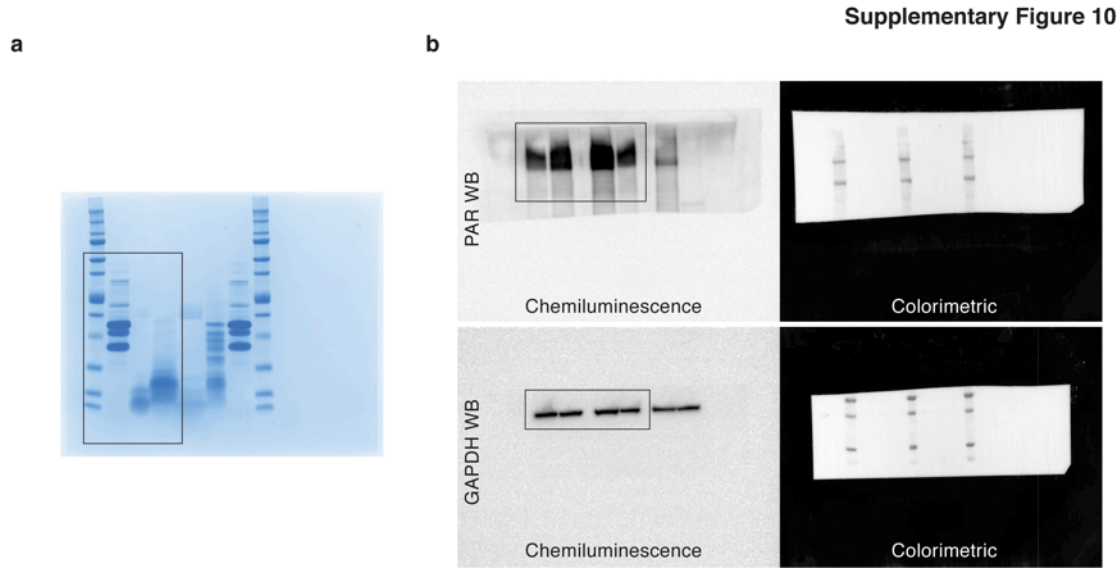
Supplementary Figure 8. Validation of the histone digestion and purification method. (a) Histones were digested with the Partial FASP method, at pH 7.3 or pH 8.0, as indicated. Log_{10} intensities of the peptides obtained from the different digestion conditions were compared on the scatterplot. **(b)** ADP-ribosylation of histones was performed with PARP10 in an *in vitro* assay. The proteins were then digested with the Partial FASP method, at pH 7.3 or pH 8.0, as indicated. Log_{10} intensities of the peptides obtained from the different digestion conditions were compared on the scatterplot. **(c)** Histones were incubated with PARP10 in the

presence of NAD+, and then subjected to another round of histone purification and digestion. ADPr was detected on arginine, lysine and acidic residues both before and after purification. Log_{10} intensities of the repurified histone peptides were compared to the input (taken after the *in vitro* assay)**.**



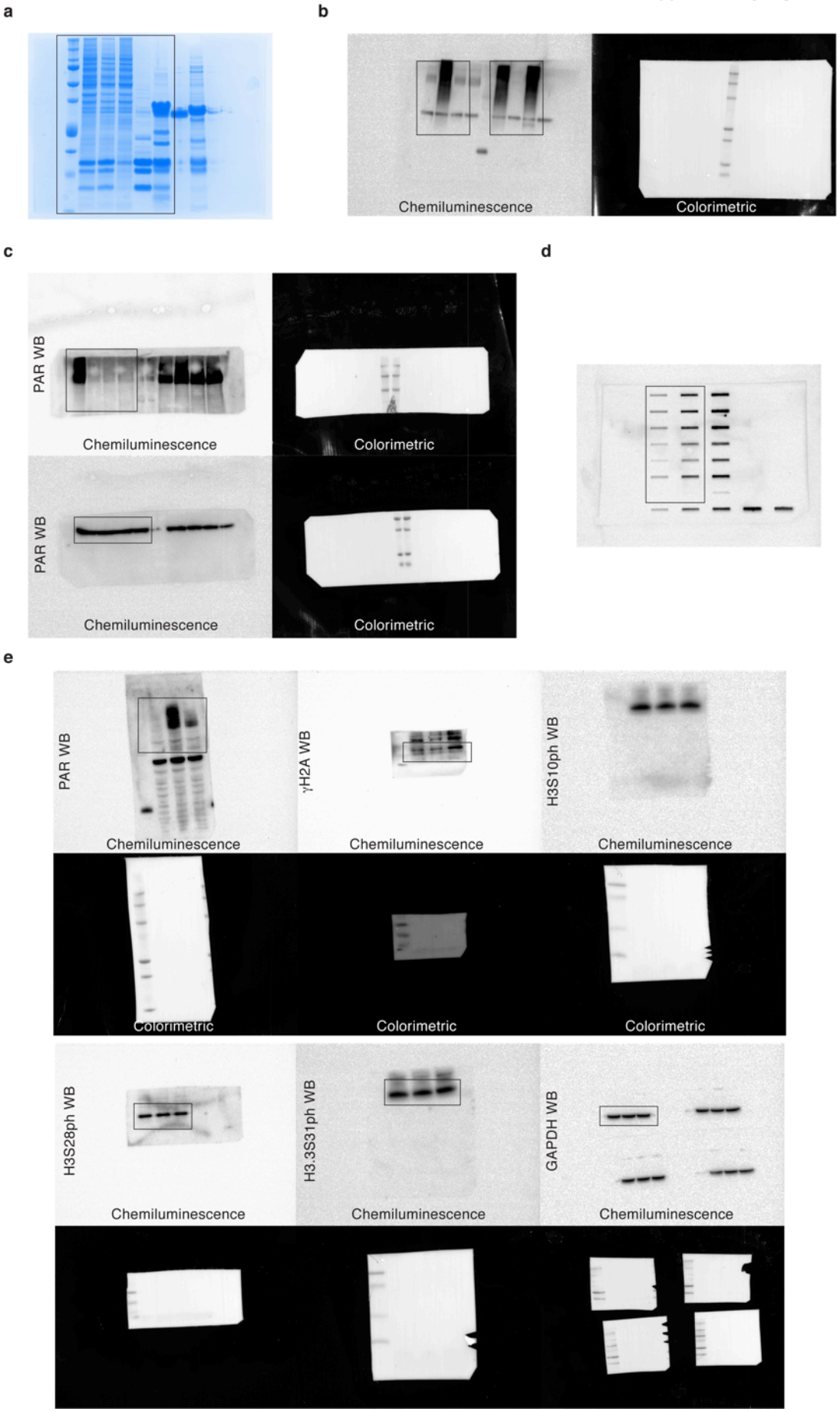
Supplementary Figure 9. Histone Serine ADPr modification inhibition by Olaparib. (a) Analysis of histone phosphorylation marks after H_2O_2-induced DNA damage. U2OS cells were stimulated with 2 mM H_2O_2 for the indicated time points and lysed with Laemmli buffer. Total protein poly-ADP-ribosylation, phosphorylation of histone H2A.X (Ser139), phosphorylation of histone H3 (Ser10 and Ser28) and phosphorylation of histone H3.3 (Ser31) were analyzed by Western blotting. Anti-GAPDH was used as a loading control. See **Online Methods** for further experimental details. The Western blot is representative of three independent experiments, which are biological replicates. See **Supplementary Fig. 11** for original images. **(b)** Schematic representation of the SILAC-based strategy to quantify core histone marks

upon 10 minutes of 2 mM H_2O_2-induced DNA damage in the absence or presence of the PARP inhibitor olaparib. **(c)** Western blot analysis of total protein poly-ADP-ribosylation levels prior to mixing light and heavy lysates from the SILAC experiment. Anti-GAPDH was used as a loading control. See **Supplementary Fig. 11** for original images. **(d)** Log_{10} of summed peptide intensities were plotted against log_2 Heavy/Light SILAC ratios. Each point represents a unique histone mark. ADPr marks are of medium to low abundance compared to the rest of the peptide population. As expected, other marks did not respond to treatment with olaparib.



Supplementary Figure 10. **Original images of gels and Western blots of main figures. (a)** Full Coomassie gel of Fig. 1b. The box indicates the part displayed in Fig. 1b **(b)** Full Western blots of Fig. 3b. The boxes indicate the parts displayed in Fig. 3b. Right panels show the colorimetric images for molecular mass (kDa) estimation.

Supplementary Figure 11



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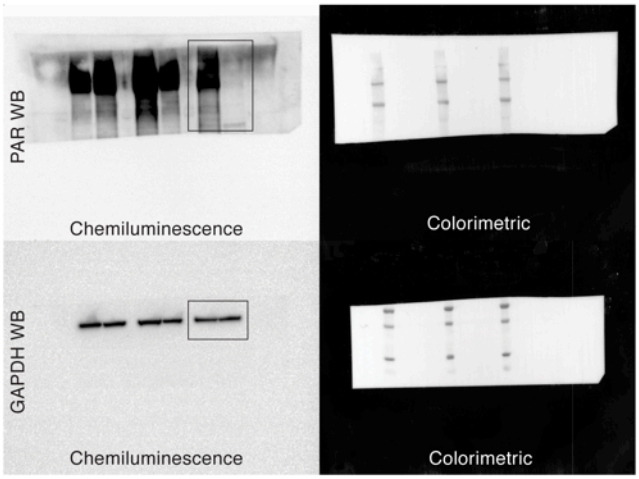


Supplementary Figure 11. **Original images of gels and Western blots of supplementary figures. (a)** Full Coomassie gel of Supplementary Fig. 4b. The box indicates the part displayed in Supplementary Fig. 4b. **(b)** Full Western blot of Supplementary Fig. 4c. The boxes indicate the parts displayed in Supplementary Fig. 4c. Right panel show the colorimetric images for molecular mass (kDa) estimation. **(c)** Full Western blot of Supplementary Fig. 4d. The boxes indicate the parts displayed in Supplementary Fig. 4d. Right panels show the colorimetric images for molecular mass (kDa) estimation. **(d)** Full immune-slot blot of Supplementary Fig. 7b. The box indicate the part displayed in Supplementary Fig. 7b. **(e)** Full Western blots of Supplementary Fig. 9a. The boxes indicate the parts displayed in Supplementary Fig. 9a. Bottom panels show the colorimetric images for molecular mass (kDa) estimation. **(f)** Full Western blot of Supplementary Fig. 9c. The boxes indicate the parts displayed in Supplementary Fig. 9c. Right panels show the colorimetric images for molecular mass (kDa) estimation.

Supplementary Tables

Histone	ADPr mark	Score	Localization probabilities (DEKRSTYCMNQHM)
H1.0	S103	280.19	LAKSDEPKK(0.001)S(0.999)VAFKK
H1.0	S103	286.59	S(1)VAFKK
H1.0	S103	246.41	SDEPKKS(1)VAFKK
H1.2	S187	174.4	KAAK(0.002)S(0.998)AAKAVKPK
H1.2	S187	313.75	S(1)AAKAVKPK
H1.4	S26	366.95	SETAPAAPAAPAPAEKTPVKKKARK(0.004)S(0.996)AGAAKR
H1.4	S54	142.04	S(1)GVSLAALKK
H1.4	S112	155.07	KAAS(1)GEAKPK
H1.4	S149	183.65	KATGAATPKK(0.034)S(0.966)AK
H2A	S1 or R3	336.31	S(0.5)GR(0.5)GKQGGKARAKAKTRSSR
H2A	S1 or R3	179.51	S(0.5)GR(0.5)GKQGGKAR
H2B1C	S6	492.3	PEPAKS(1)APAPKKGSK
H2B1C	S6	472.56	PEPAKS(1)APAPKKGSKKAVTK
H2B1D	S6	381.89	PEPTKS(1)APAPKKGSK
H2B1D	S6	357.75	PEPTKS(1)APAPKKGSK
H2B1H	S6	195.79	PDPAKS(1)APAPKKGSK
H2B1H	S6	237.68	PDPAKS(1)APAPKKGSK
H2B1L	S6	394.72	PELAKS(1)APAPKKGSK
H2B1L	S6	323.54	PELAKS(1)APAPKKGSK
H2B1M	S6	311.7	PEPVKS(1)APVPKKGSK
H2B1M	S6	280.19	PEPVKS(1)APVPKKGSK
H2B1N	S6	254.19	PEPSK(0.001)S(0.999)APAPKKGSK
H2B1N	S6	265.61	PEPSKS(1)APAPKKGSK
H2B1C	S14	290.04	PEPAKSAPAPKKGS(0.999)K(0.001)K
H3.1	S10	571.27	ARTKQTARKS(1)TGGKAPRKQLATK
H3.1	S10	272.94	QTARKS(0.998)T(0.002)GGKAPR
H3.1	S28	446.3	S(1)APATGGVKKPHR
H3.1	S28	286.73	S(1)APATGGVKKPHR
H4	S1 or R3	382.17	S(0.5)GR(0.5)GKGGKGLGKGGAKR
H4	S1 (or R3)	440.72	S(0.986)GR(0.014)GKGGKGLGKGGAKR

Supplementary Table 1. *In vivo* histone ADP-ribosylation modification sites identified in this study

Histone	ADPr mark	Score	Localization probabilities (DEKRSTYCMNQHM)
H2A	R3	186,46	S(0.07)GR(0.93)GKQGGKAR
H2A	R29	123,67	AGLQFPVGR(1)VHR
H2A	E41	301,48	LLRKGNYAE(1)R
H2A	E121	291,46	VTIAQGGVLPNIQAVLLPKK(0.003)TE(0.945)S(0.052)HHKAKGK
H2B	E2	213,63	PE(1)PAKSAPAPKKGSKK
H2B	D51	106,2	VLK(0.002)QVHPD(0.985)TGISSK
H2B	K125	119,18	HAVSEGTKAVTKYTS(0.002)AK(0.998)
H3	R2	114,54	AR(1)TKQTAR
H3	K4	146,21	ARTK(1)QTAR
H3	K9	327,6	K(0.934)S(0.063)TGGK(0.002)APR(0.001)KQLATKAAR
H3	K14	267,24	KSTGGK(1)APRKQLATKAAR
H3	K18	223,78	K(1)QLATKAAR
H3	R49	194,2	YRPGTVALR(0.972)E(0.028)IR
H3	E50	281,12	YRPGTVALRE(1)IRR
H3	R52	171,85	YRPGTVALRE(0.047)IR(0.95)R(0.003)YQK
H3	K56	155,51	YQK(0.999)S(0.001)TELLIR
H3	E59	193,41	YQKSTE(1)LLIR
H3	R116	227,94	R(1)VTIMPKDIQLARR
H3	K122	222,3	RVTIMPK(0.986)D(0.006)IQLAR(0.006)R(0.001)
H3	E133	105,82	IRGE(1)RA
H4	R3	351,63	S(0.045)GR(0.954)GKGGK(0.001)GLGKGGAKRHR
H4	K8	234,05	SGRGKGGK(1)GLGKGGAKR

Supplementary Table 2. A list of identified ADPr modification sites on histones after *in vitro* ADPr reaction with PARP10.

Supplementary Data Set 1. SILAC-based quantification of histone marks: DNA damage and olaparib experiments.

Supplementary Note. Representative high-resolution ETD fragmentation MS/MS spectra of all identified ADP-ribosylation sites.

Supplementary Note

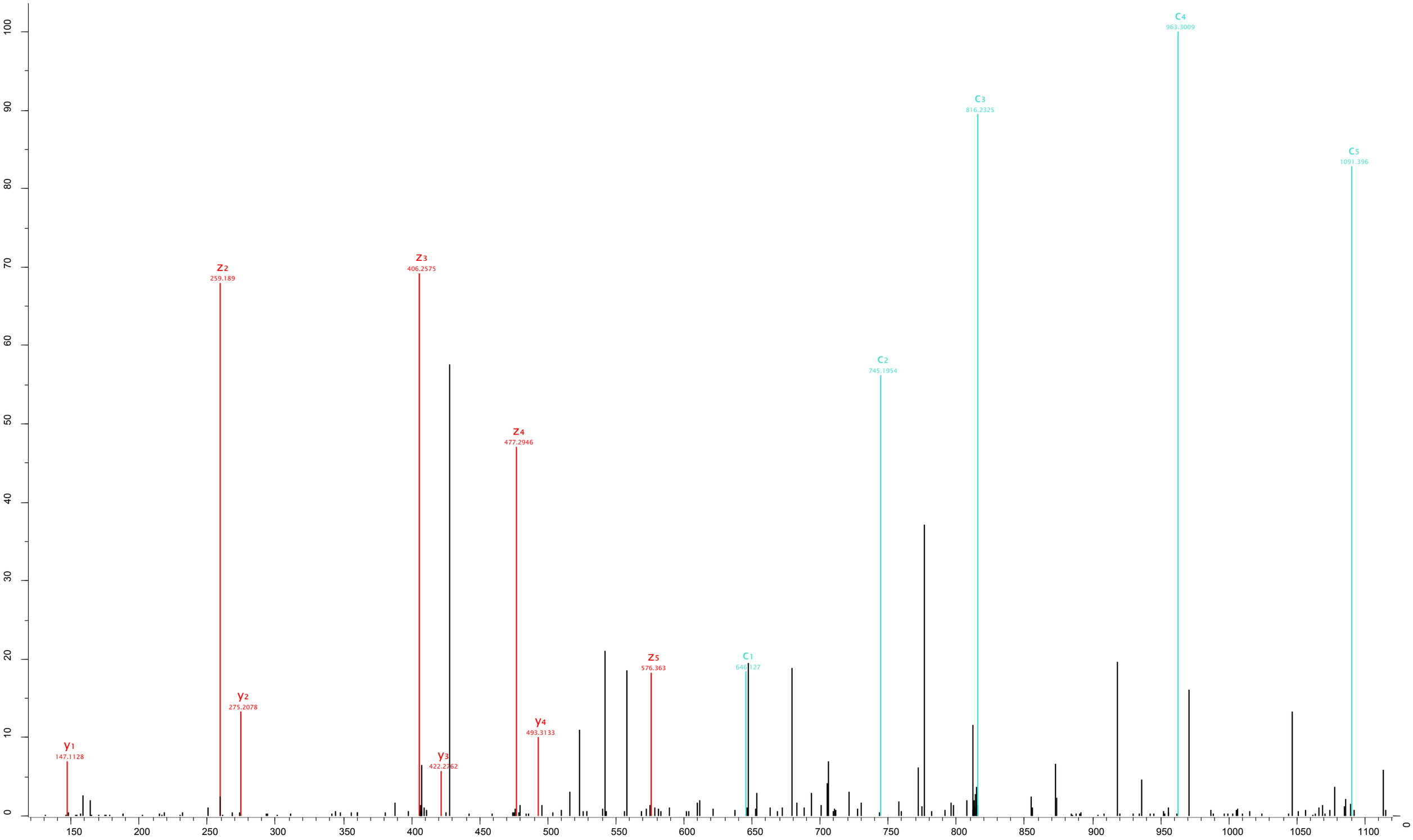
Representative high-resolution ETD fragmentation MS/MS spectra of confidently identified ADP-ribosylation sites on histones purified from cells.

ADPr = ADP-ribosylation

pRib = ribose phosphate

H1.0 ADP-ribosylation on Ser103

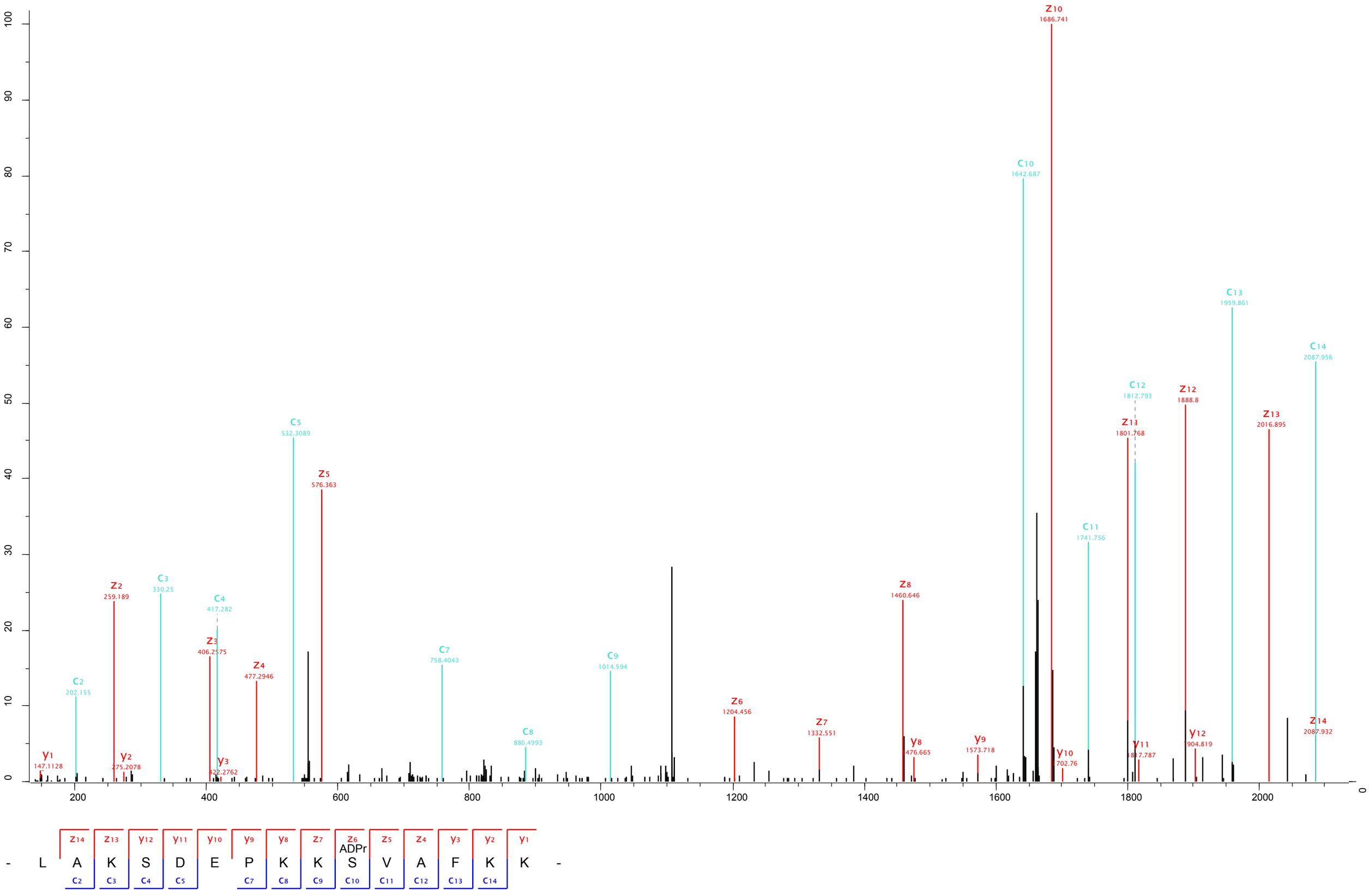
m/z 407.49647 charge 3+ mass error [ppm] 0.6



		z5	y4	y3	y2	y1	
-	ADPr	V	A	F	K	K	-
	S						
	c1	c2	c3	c4	c5		

H1.0 ADP-ribosylation on Ser103

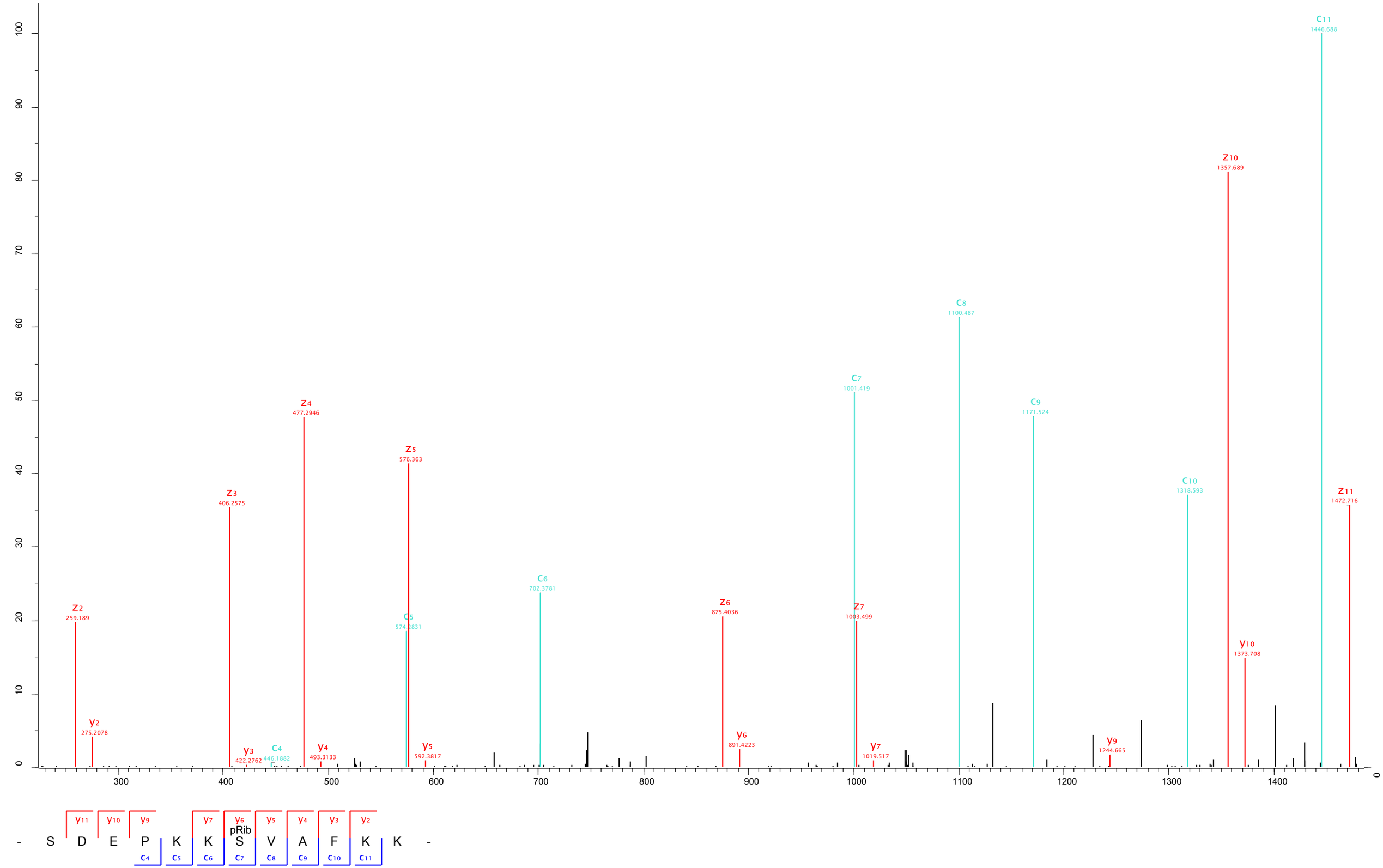
m/z 555.01427 charge 4+ mass error [ppm] -0.6



	z14	z13	y12	y11	y10	y9	y8	z7	z6 ADPr	z5	z4	y3	y2	y1	
-	L	A	K	S	D	E	P	K	K	S	V	A	F	K	K	-
		C2	C3	C4	C5		C7	C8	C9	C10	C11	C12	C13	C14		

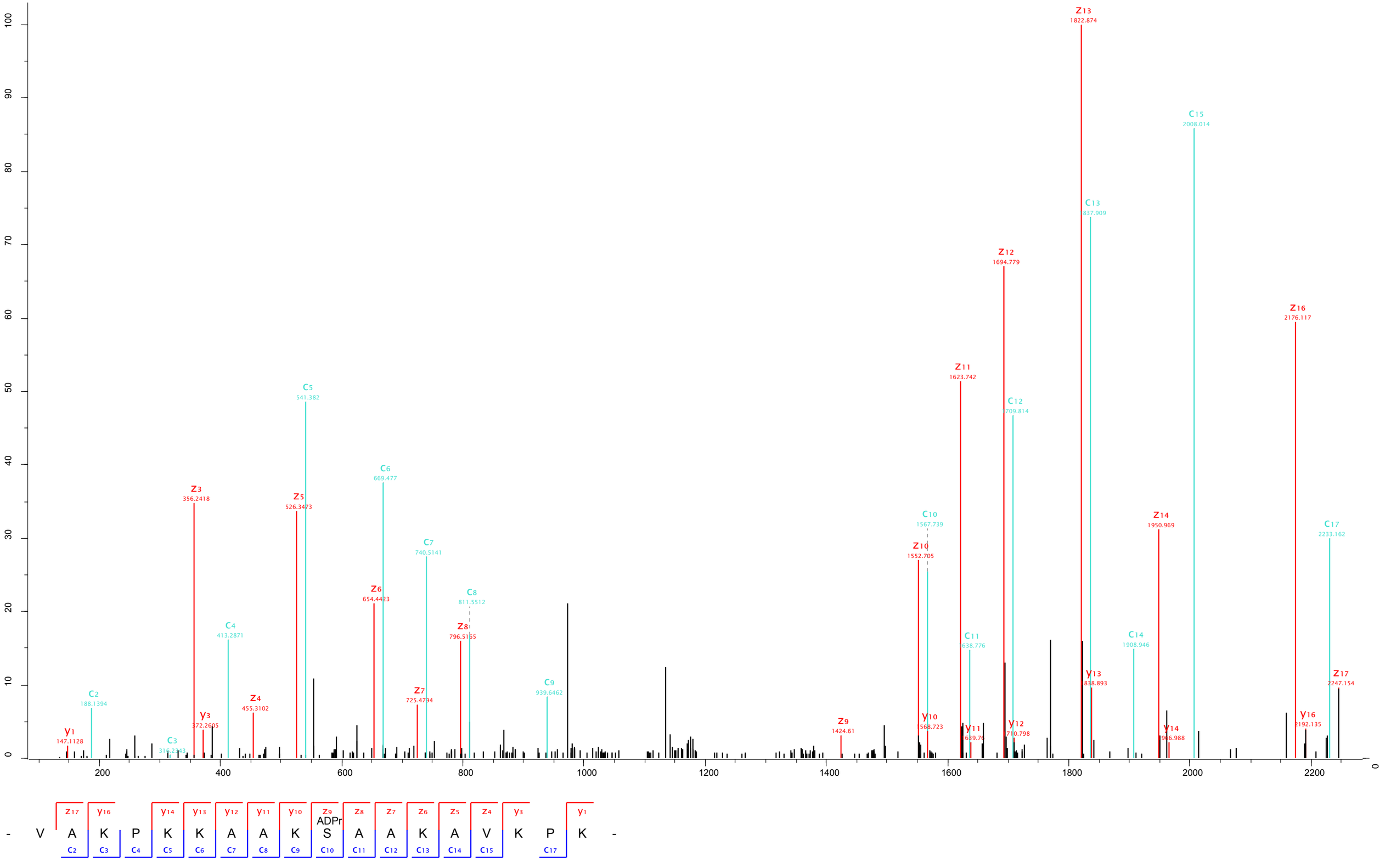
H1.0 phospho-ribose on Ser103

m/z 525.92704 charge 3+ mass error [ppm] 1.0



H1.2 ADP-ribosylation on Ser187

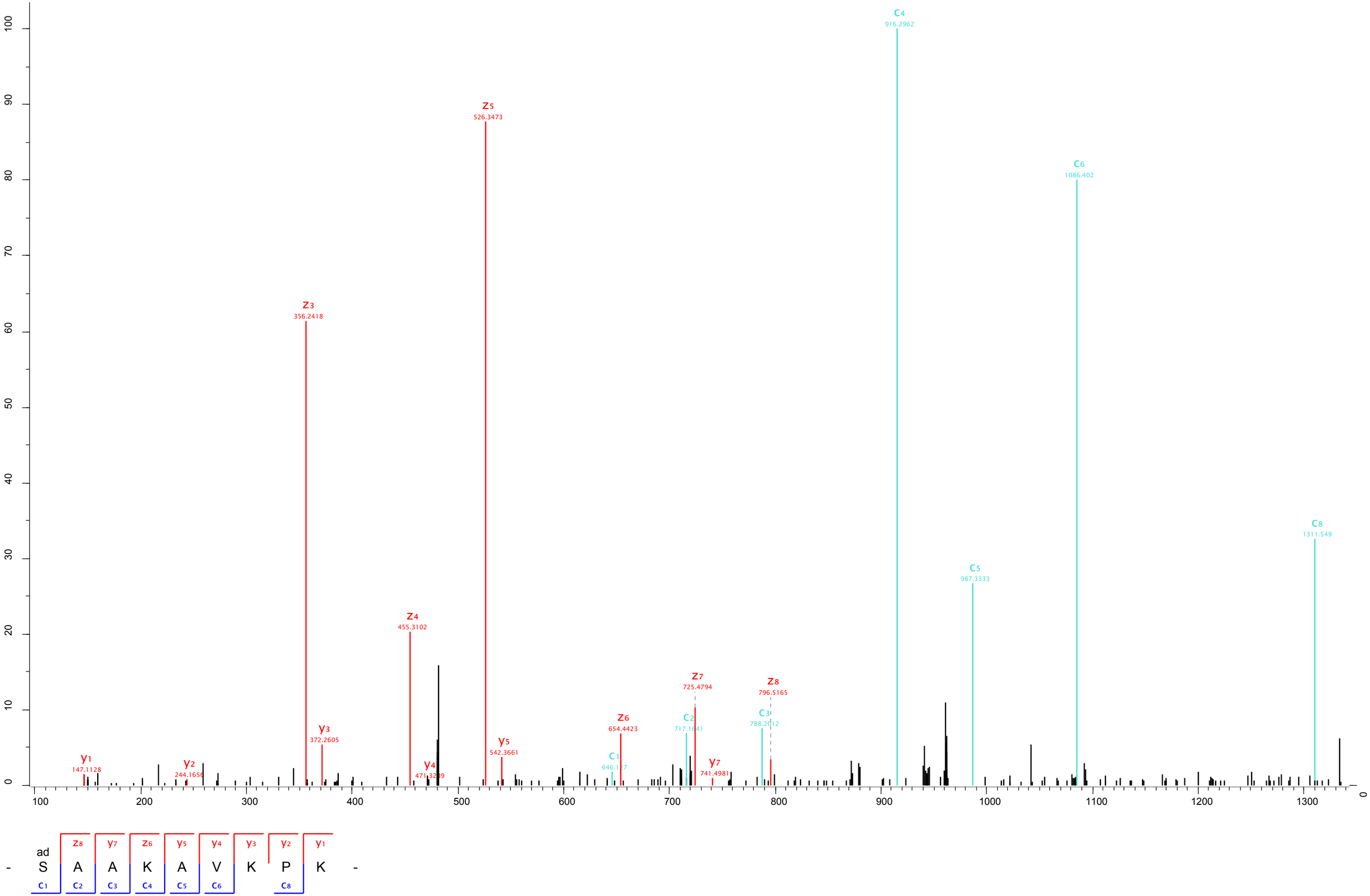
m/z 591.31565 charge 4+ mass error [ppm] -0.6



- V A K P K K A A K S(ADPr) A A K A V K P K -

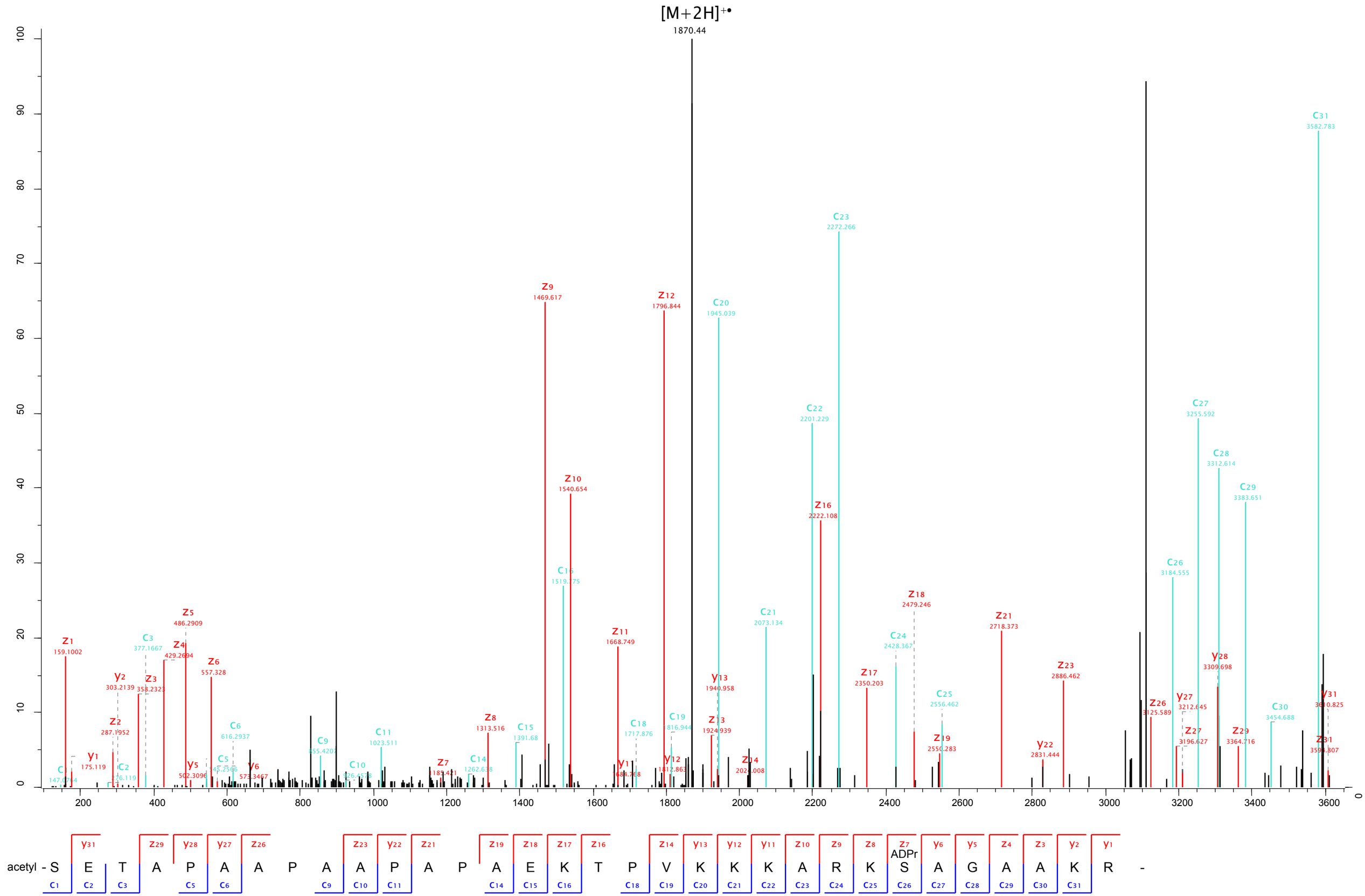
H1.2 ADP-ribosylation on Ser187

m/z 480.88098 charge 3+ mass error [ppm] 1.2



H1.4 ADP-ribosylation on Ser26

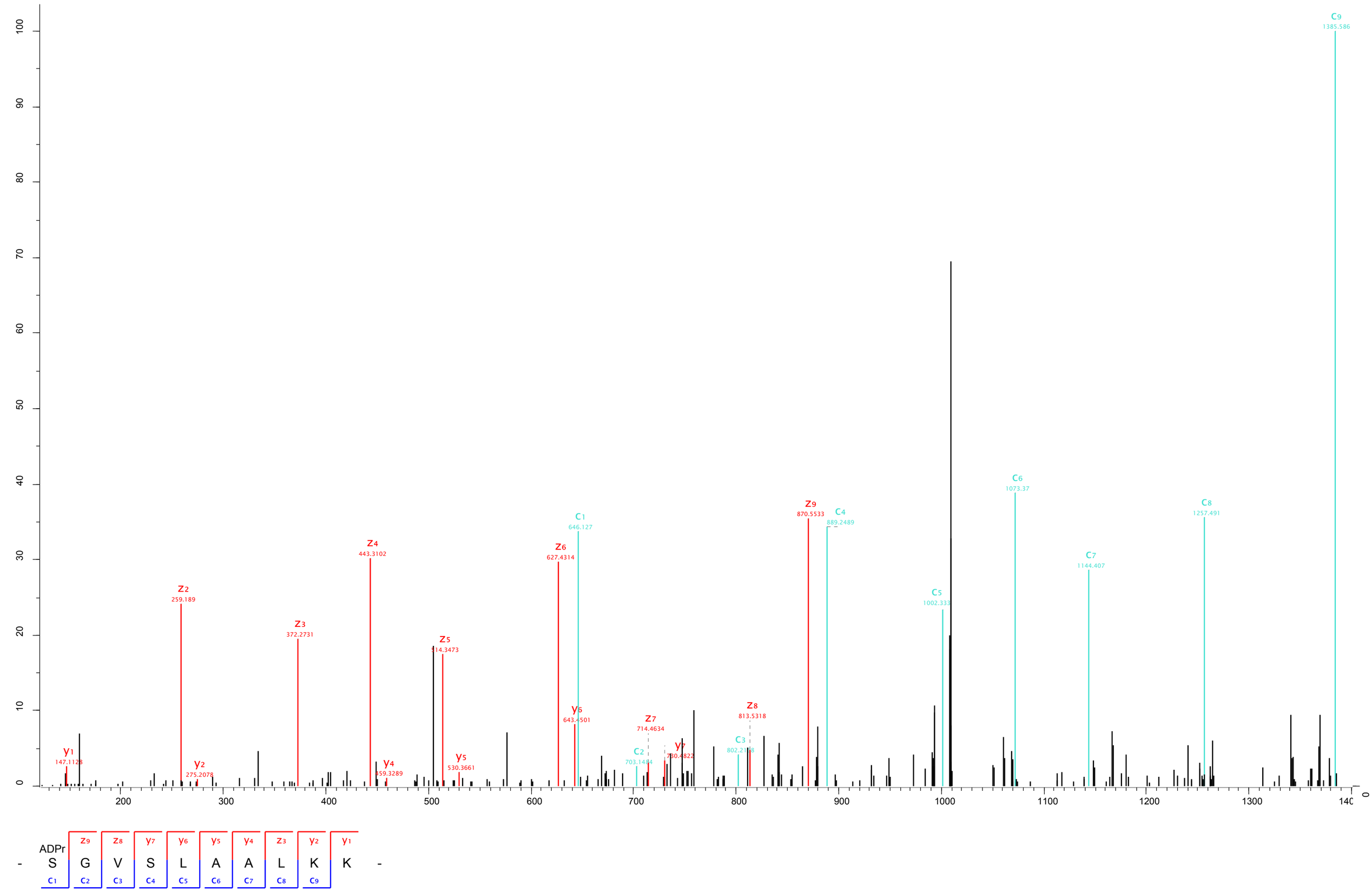
m/z 624.15074 charge 3+ mass error [ppm] 0.2



acetyl - S E T A P A A P A A P A P A E K T P V K K K A R K S(ADPr) A G A A K R -

H1.4 ADP-ribosylation on Ser54

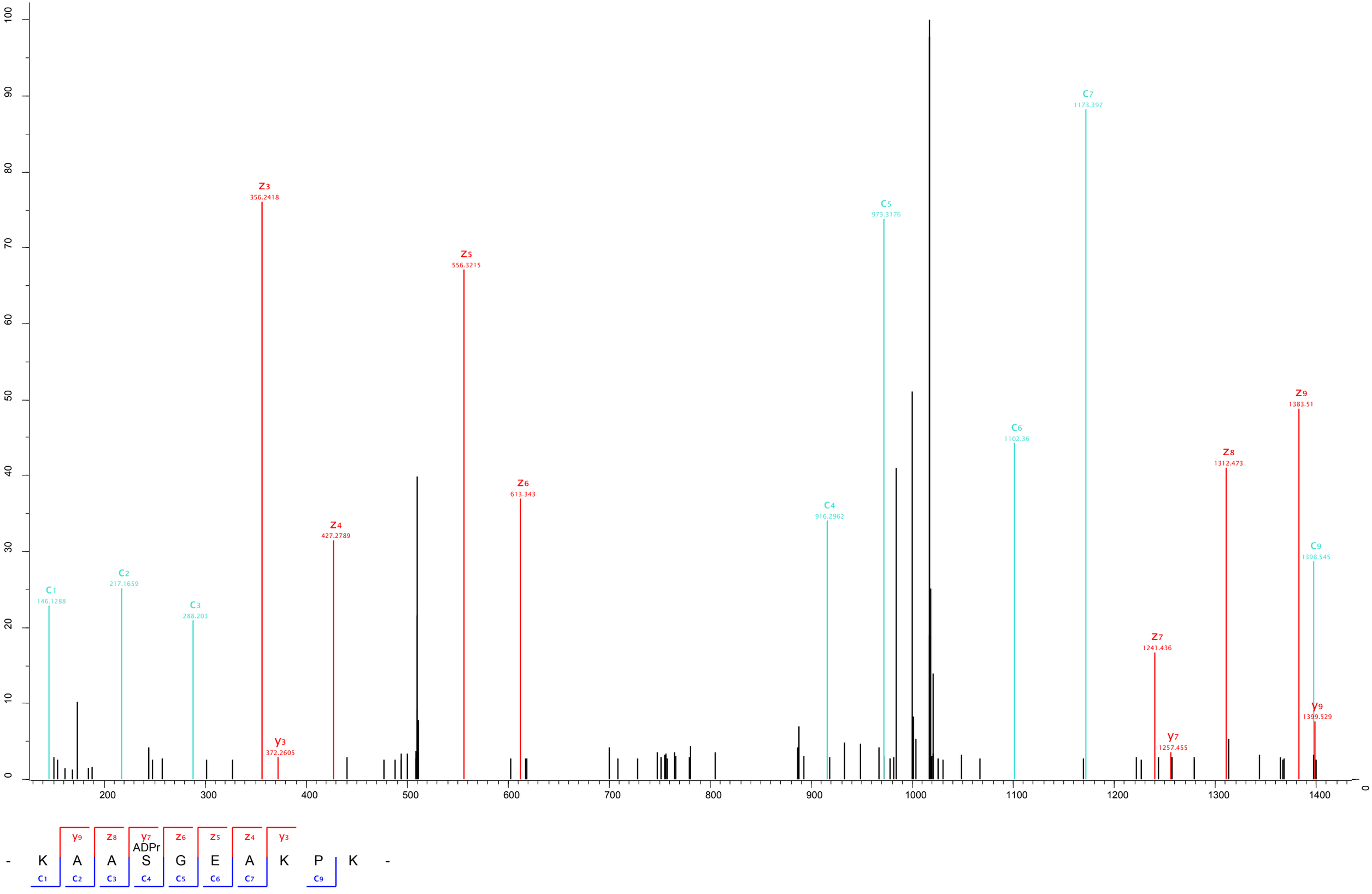
m/z 505.55991 charge 3+ mass error [ppm] -2.3



		z9	z8	y7	y6	y5	y4	z3	y2	y1	
	ADPr										
-	S	G	V	S	L	A	A	L	K	K	-
	C1	C2	C3	C4	C5	C6	C7	C8	C9		

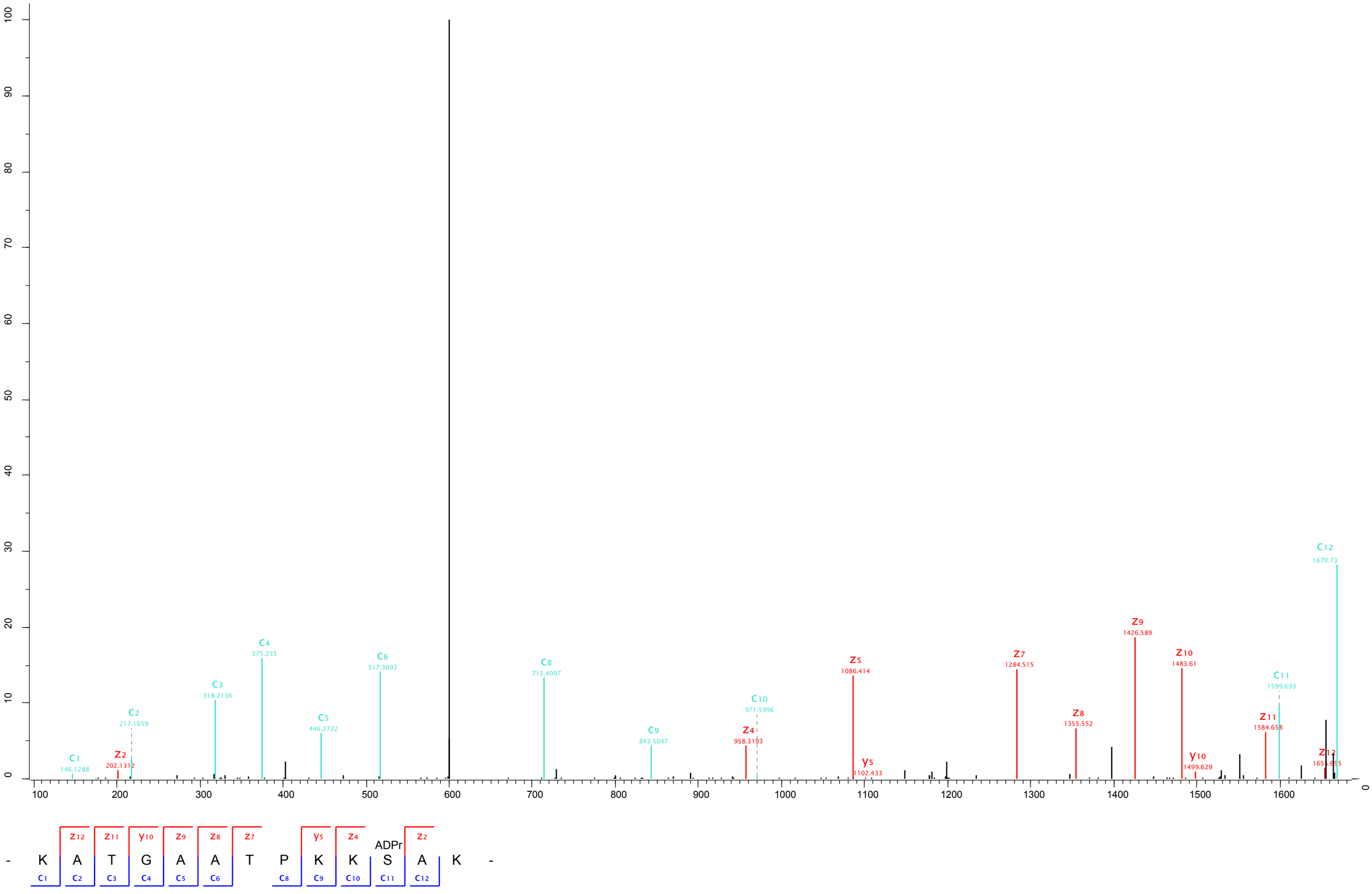
H1.4 ADP-ribosylation on Ser112

m/z 509.87953 charge 3+ mass error [ppm] 0.7



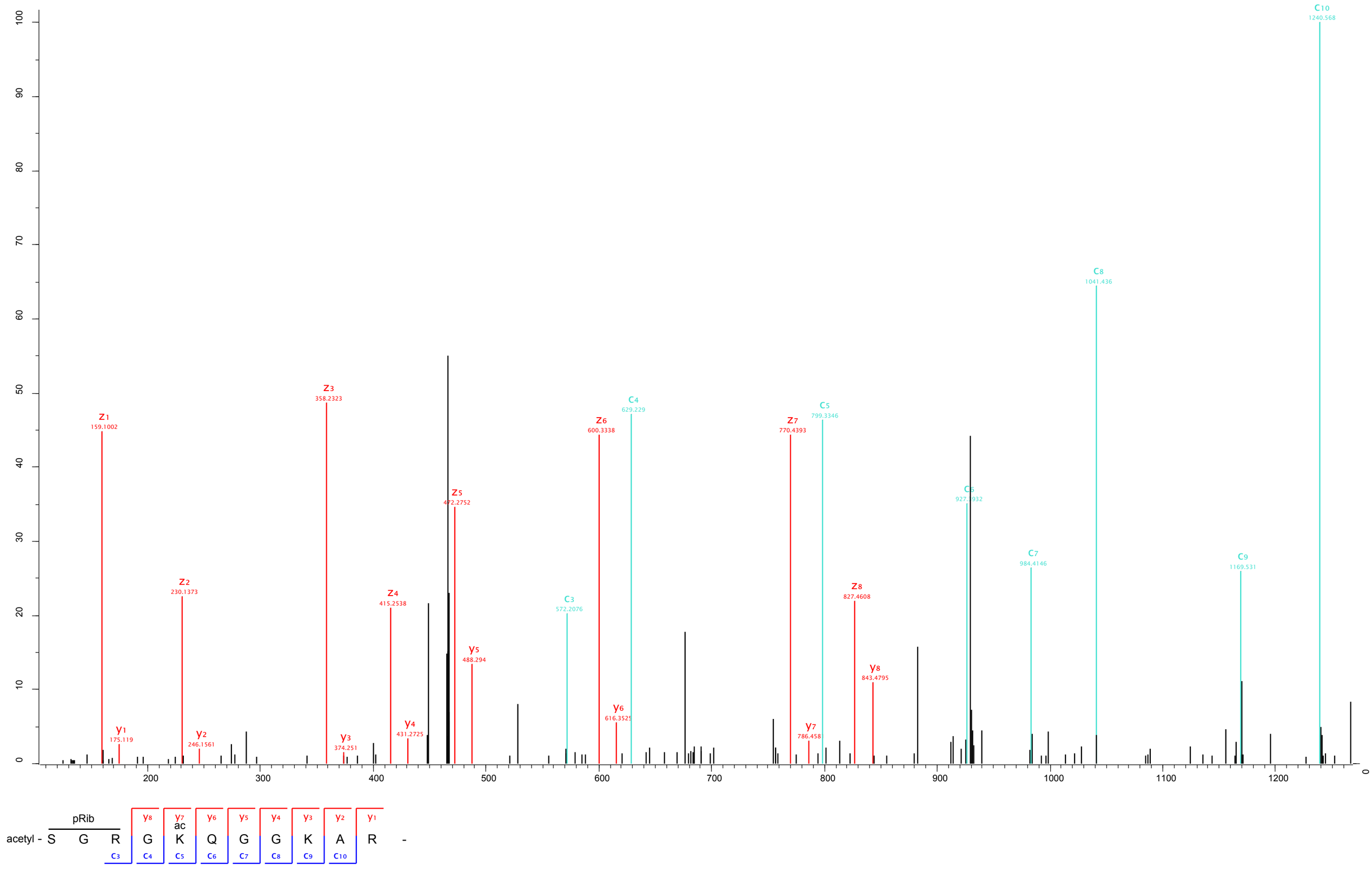
H1.4 ADP-ribosylation on Ser149

m/z 600.6078 charge 3+ mass error [ppm] 0.5



H2A phospho ribose on Ser1 or Arg3

m/z 466.55595 charge 3+ mass error [ppm] 0.2



H2A ADP-ribosylation on Ser1 or Arg3

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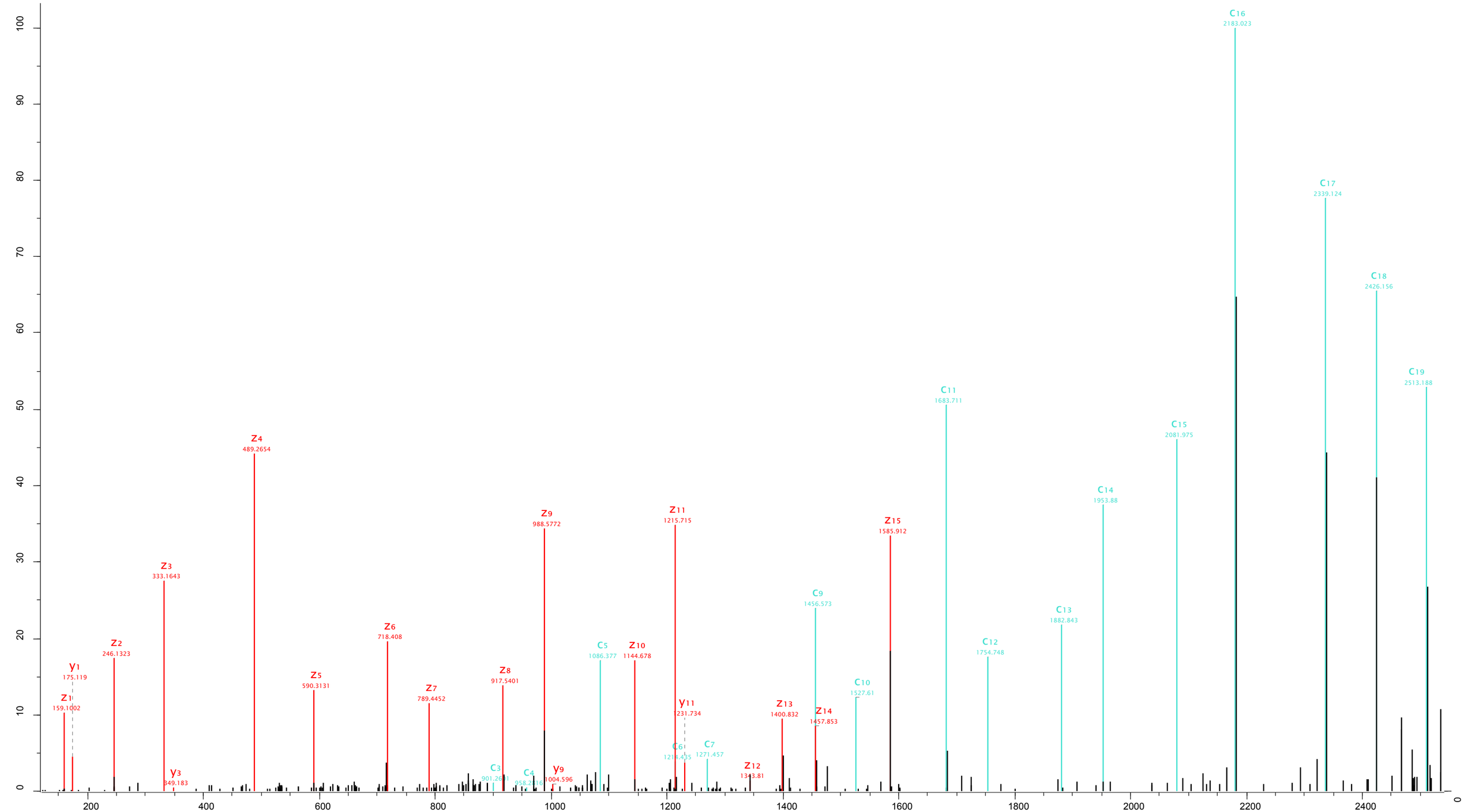
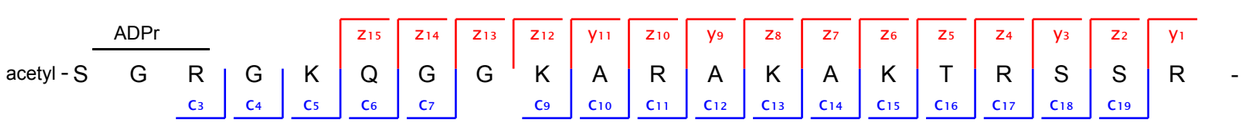
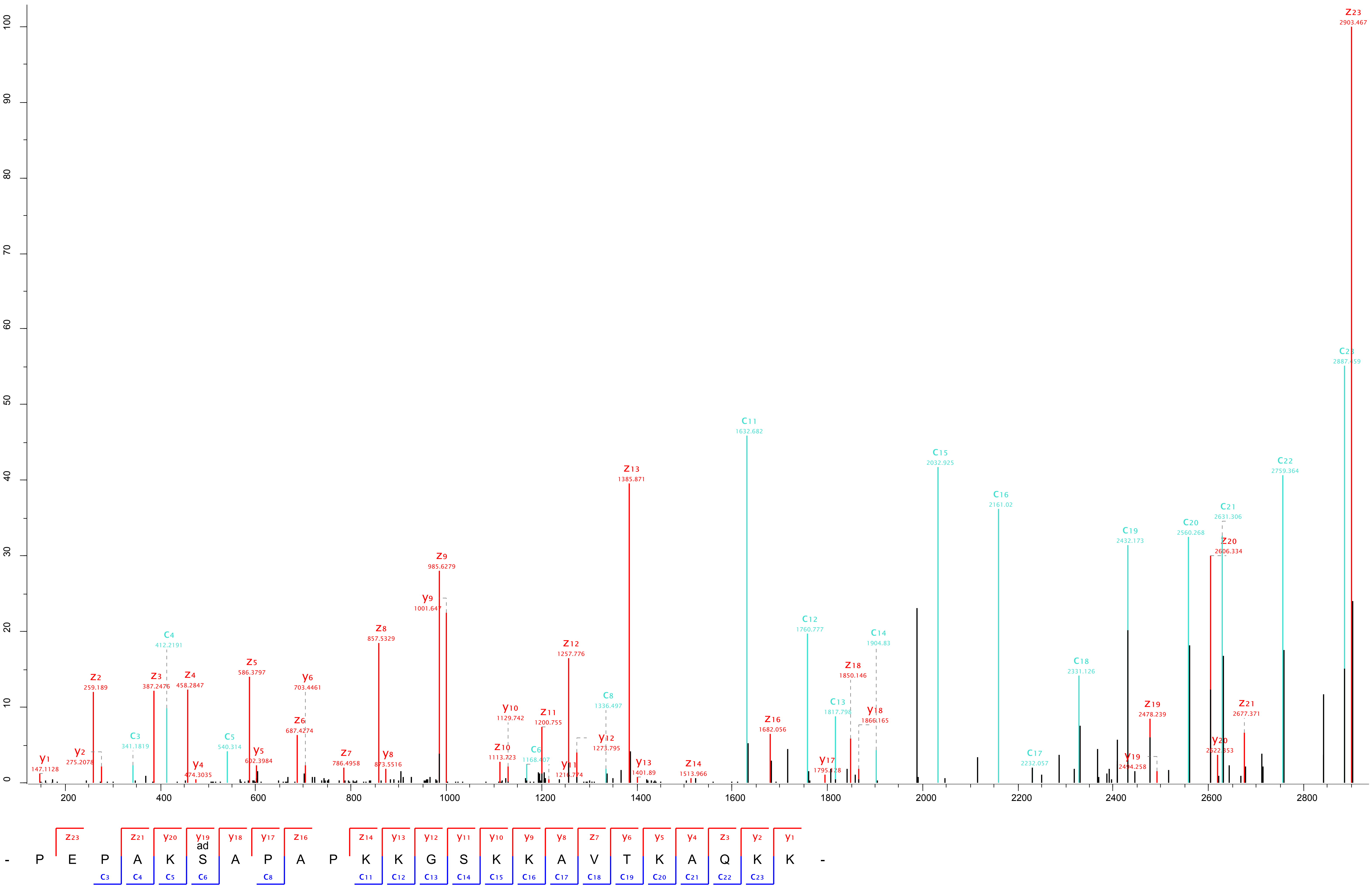




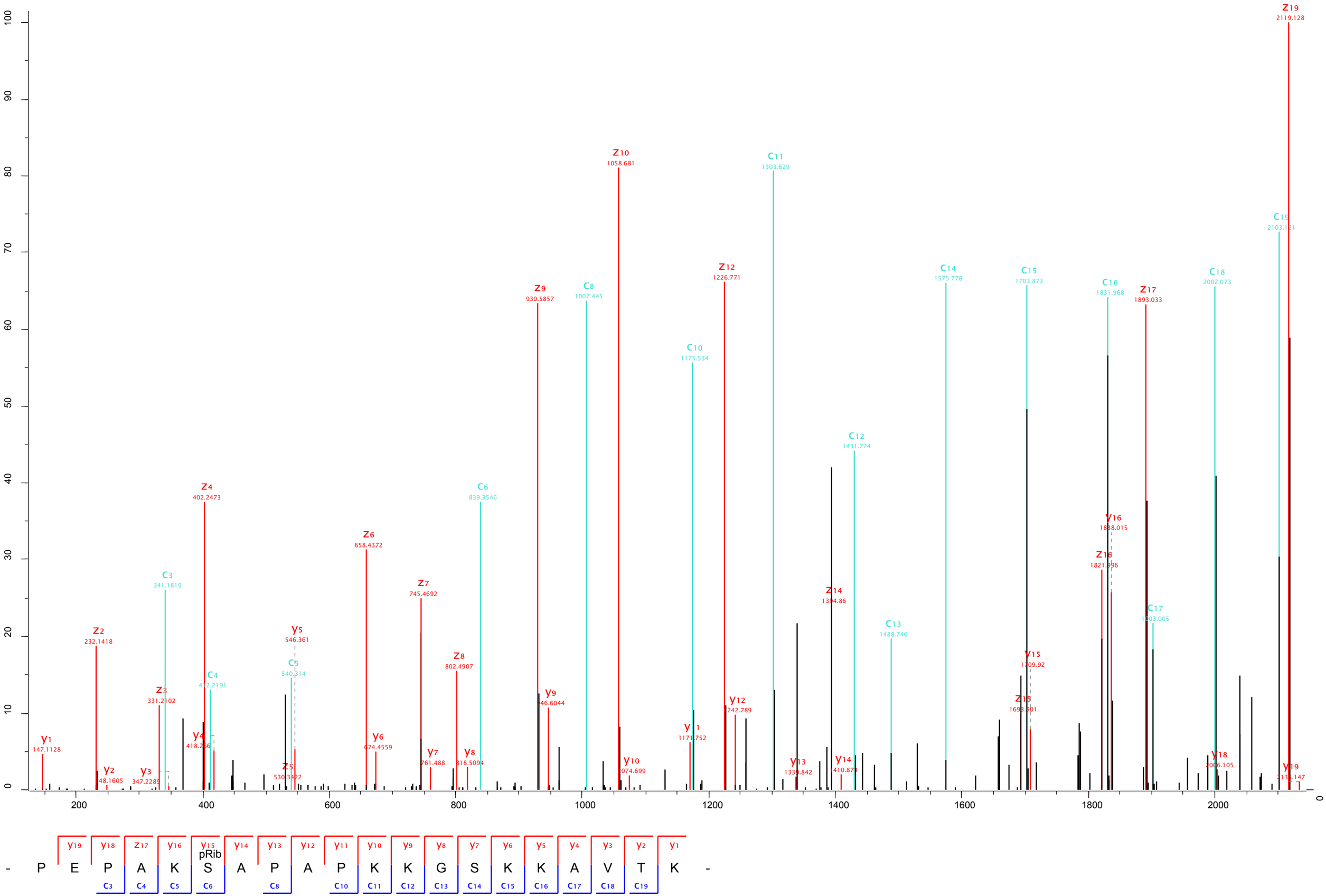
H2B1C ADP-ribosylation on Ser6

m/z 604.11343 charge 5+ mass error [ppm] -0.5



H2B1C phospho ribose on Ser6

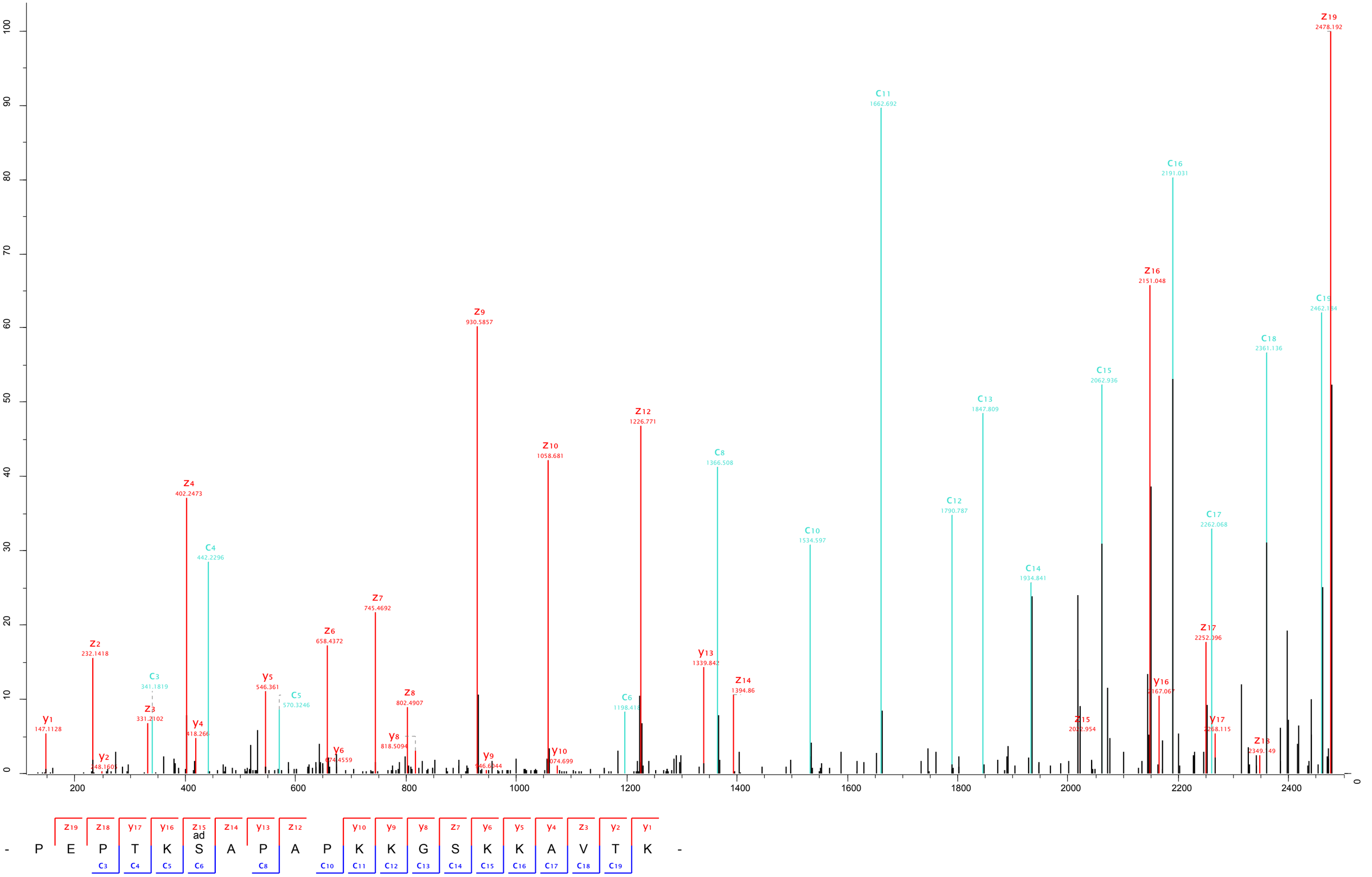
m/z 447.2481 charge 5+ mass error [ppm] -0.4



	y19	y18	z17	y16	y15	y14	y13	y12	y11	y10	y9	y8	y7	y6	y5	y4	y3	y2	y1	
-	P	E	P	A	K	S(pRib)	A	P	A	P	K	K	G	S	K	K	A	V	T	K	-
			C3	C4	C5	C6		C8		C10	C11	C12	C13	C14	C15	C16	C17	C18	C19		

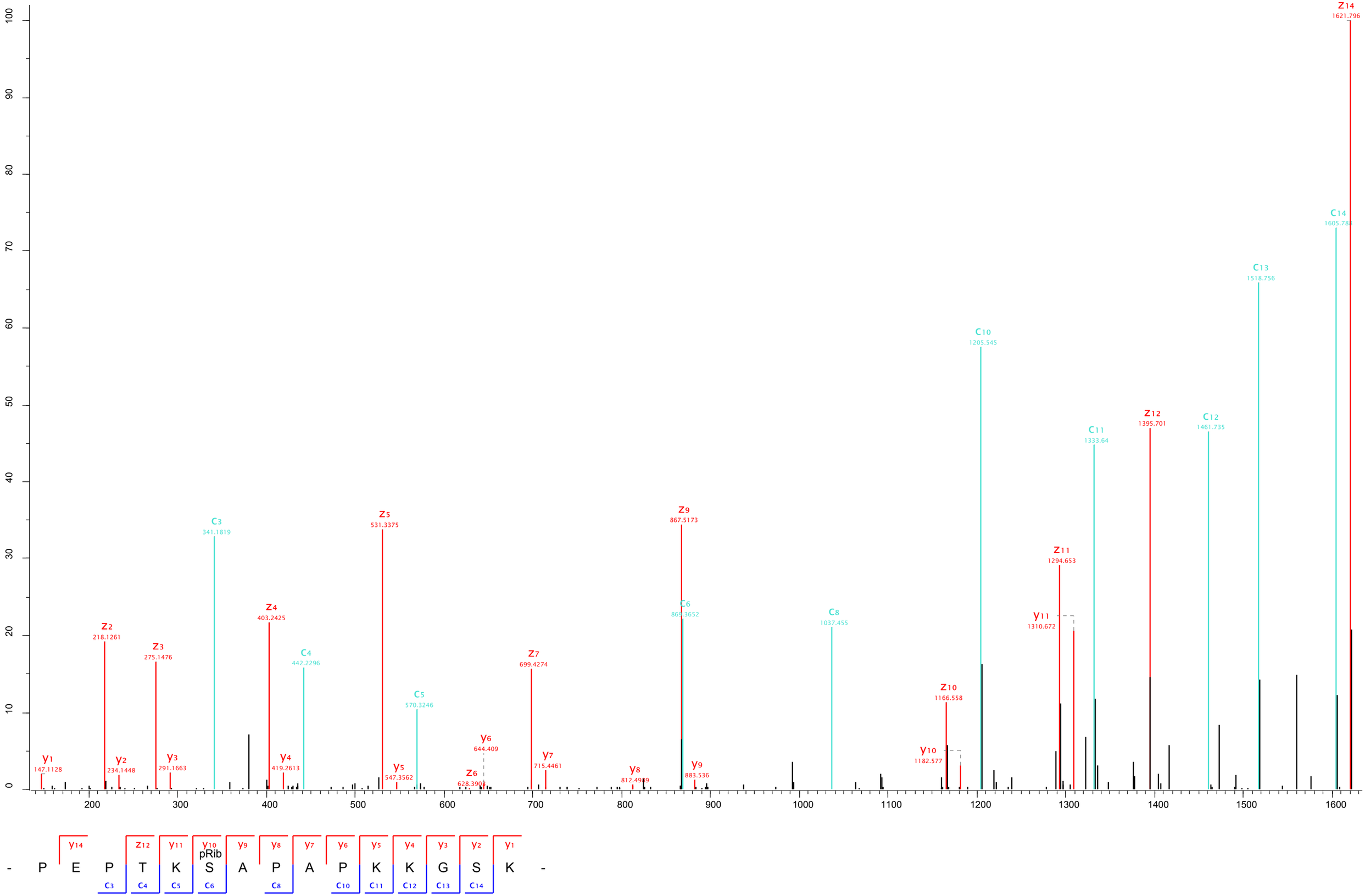
H2B1D ADP-ribosylation on Ser6

m/z 519.05842 charge 5+ mass error [ppm] 0.5



H2B1D phospho ribose on Ser6

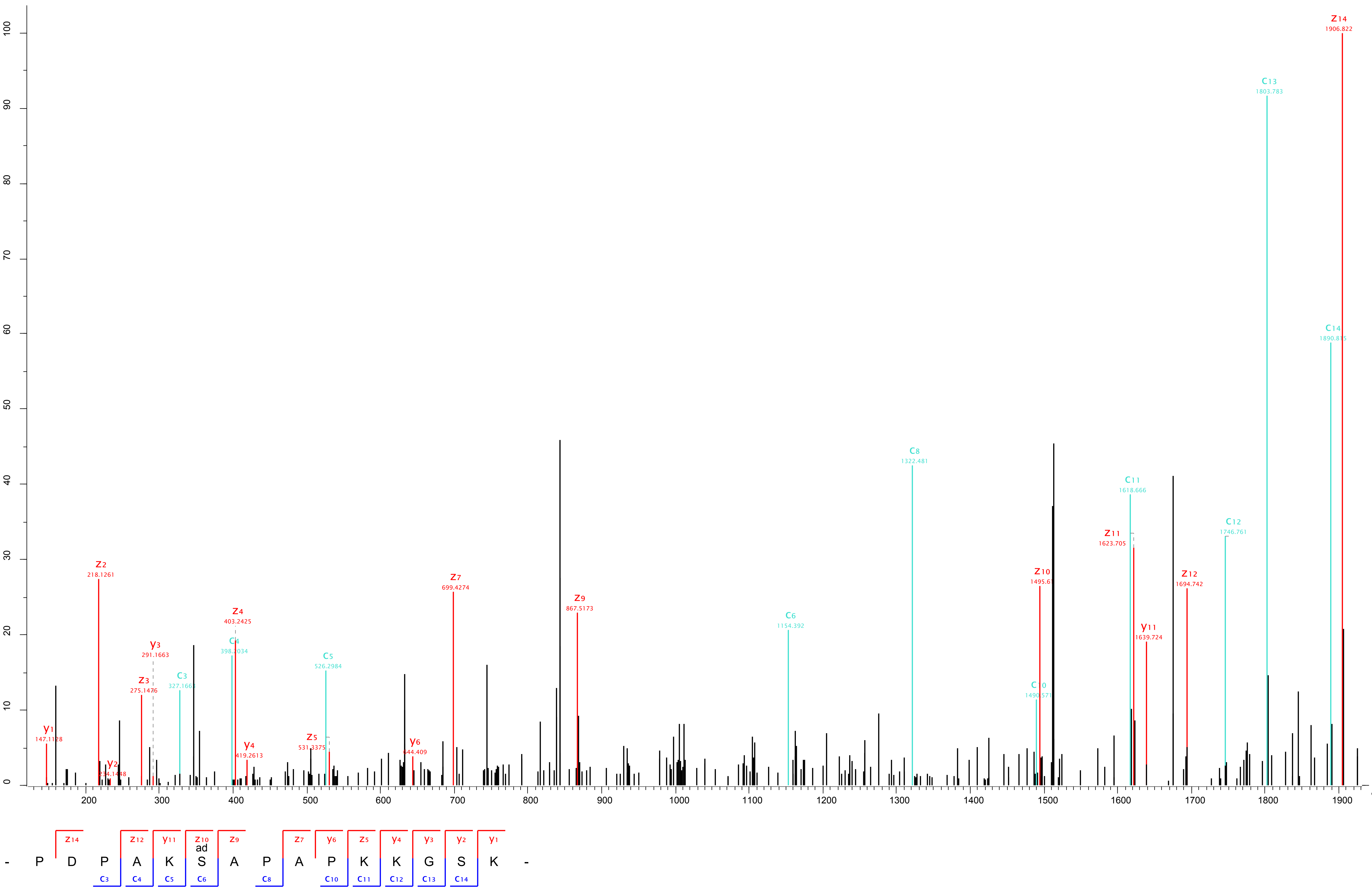
m/z 434.4723 charge 4+ mass error [ppm] -0.1



	y14		z12	y11	y10	y9	y8	y7	y6	y5	y4	y3	y2	y1	
-	P	E	P	T	K	S (pRib)	A	P	A	P	K	K	G	S	K	-
			C3	C4	C5	C6		C8		C10	C11	C12	C13	C14		

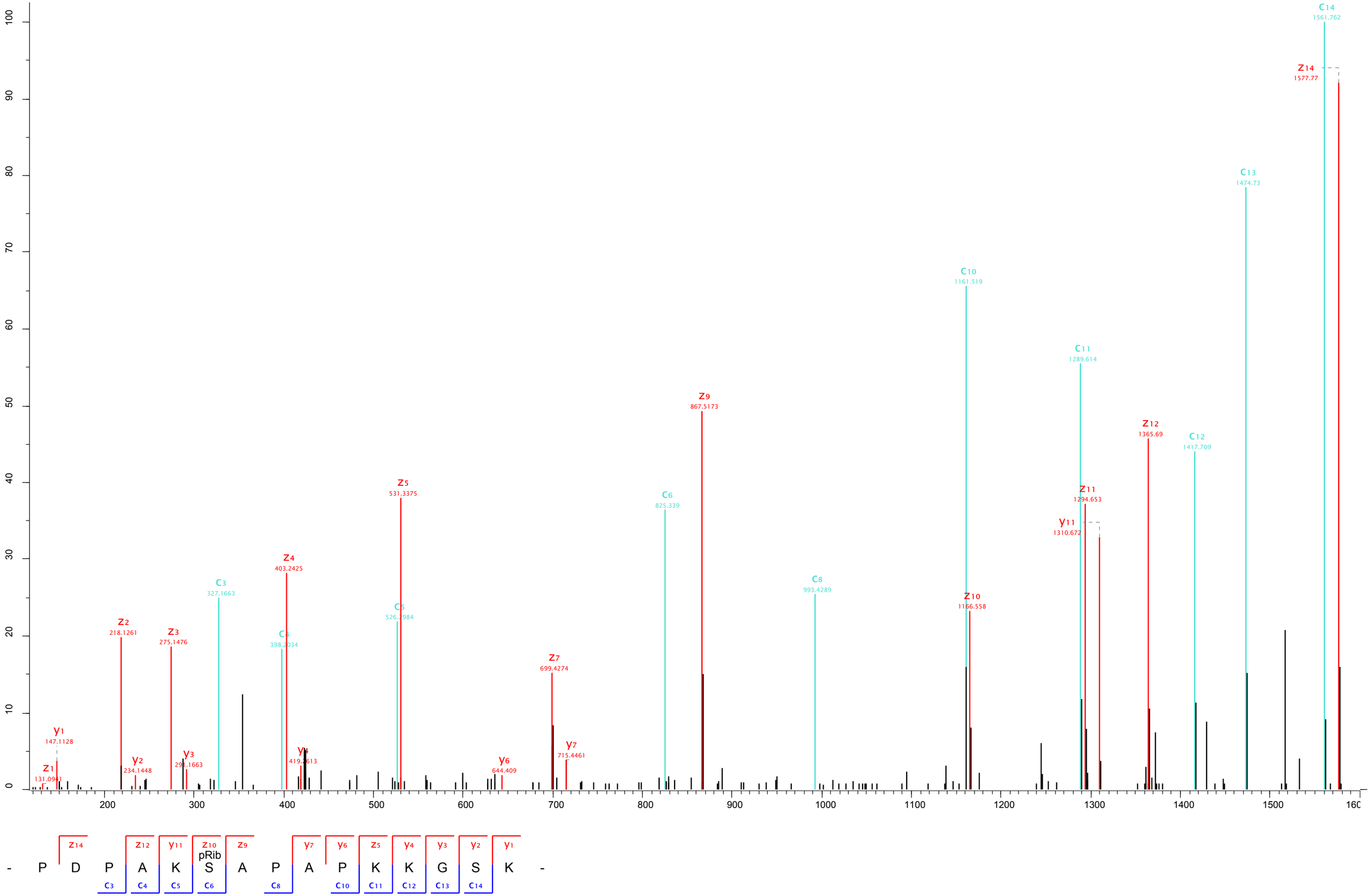
H2B1H ADP-ribosylation on Ser6

m/z 505.72887 charge 4+ mass error [ppm] 0.6



H2B1H phospho ribose on Ser6

m/z 423.46574 charge 4+ mass error [ppm] 0.2

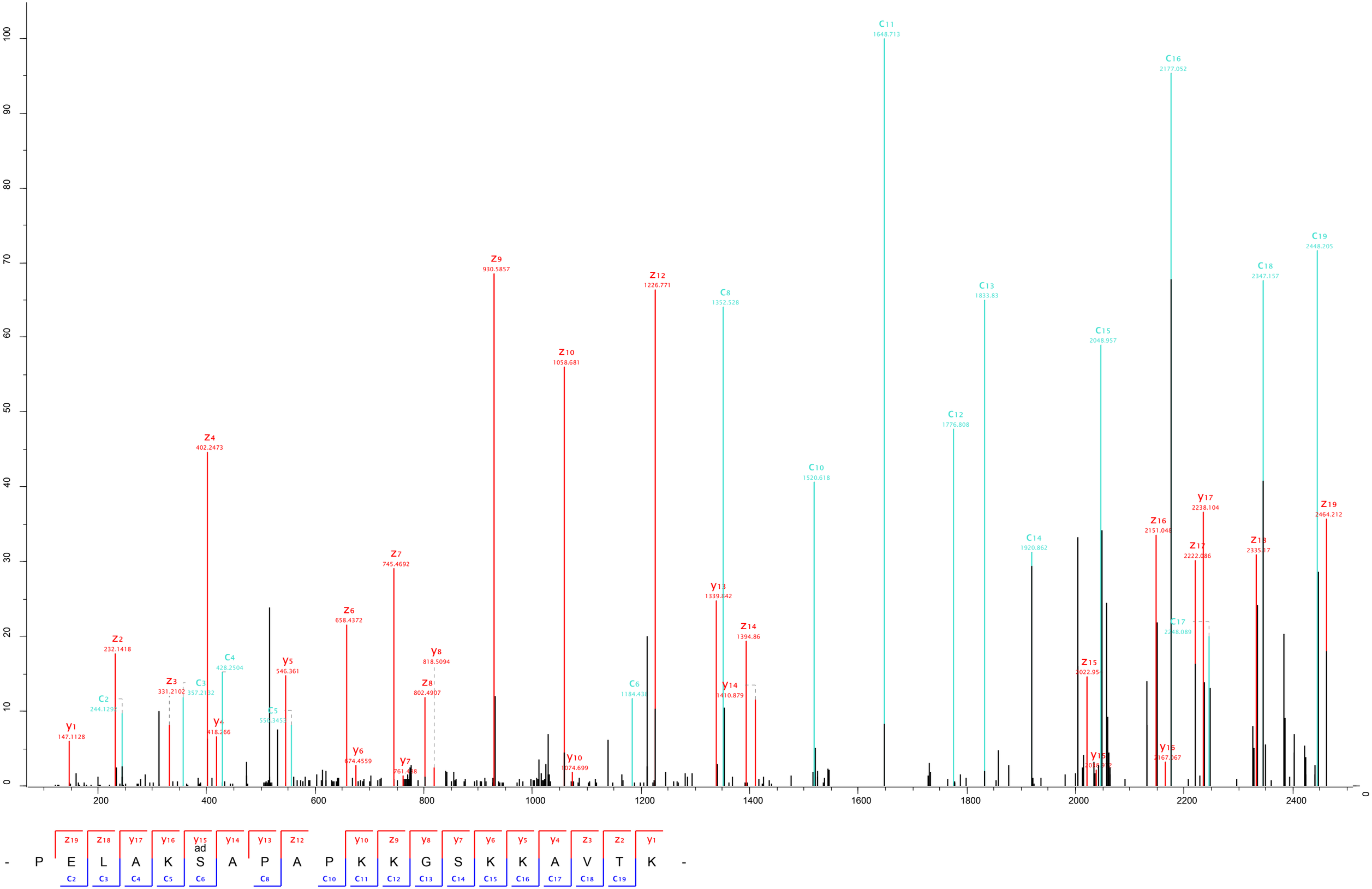


	z14		z12	y11	z10	z9		y7	y6	z5	y4	y3	y2	y1		
-	P	D	P	A	K	S	A	P	A	P	K	K	G	S	K	-
			C3	C4	C5	C6		C8	C10	C11	C12	C13	C14			

pRib

H2B1L ADP-ribosylation on Ser6

m/z 516.26257 charge 5+ mass error [ppm] -1.5

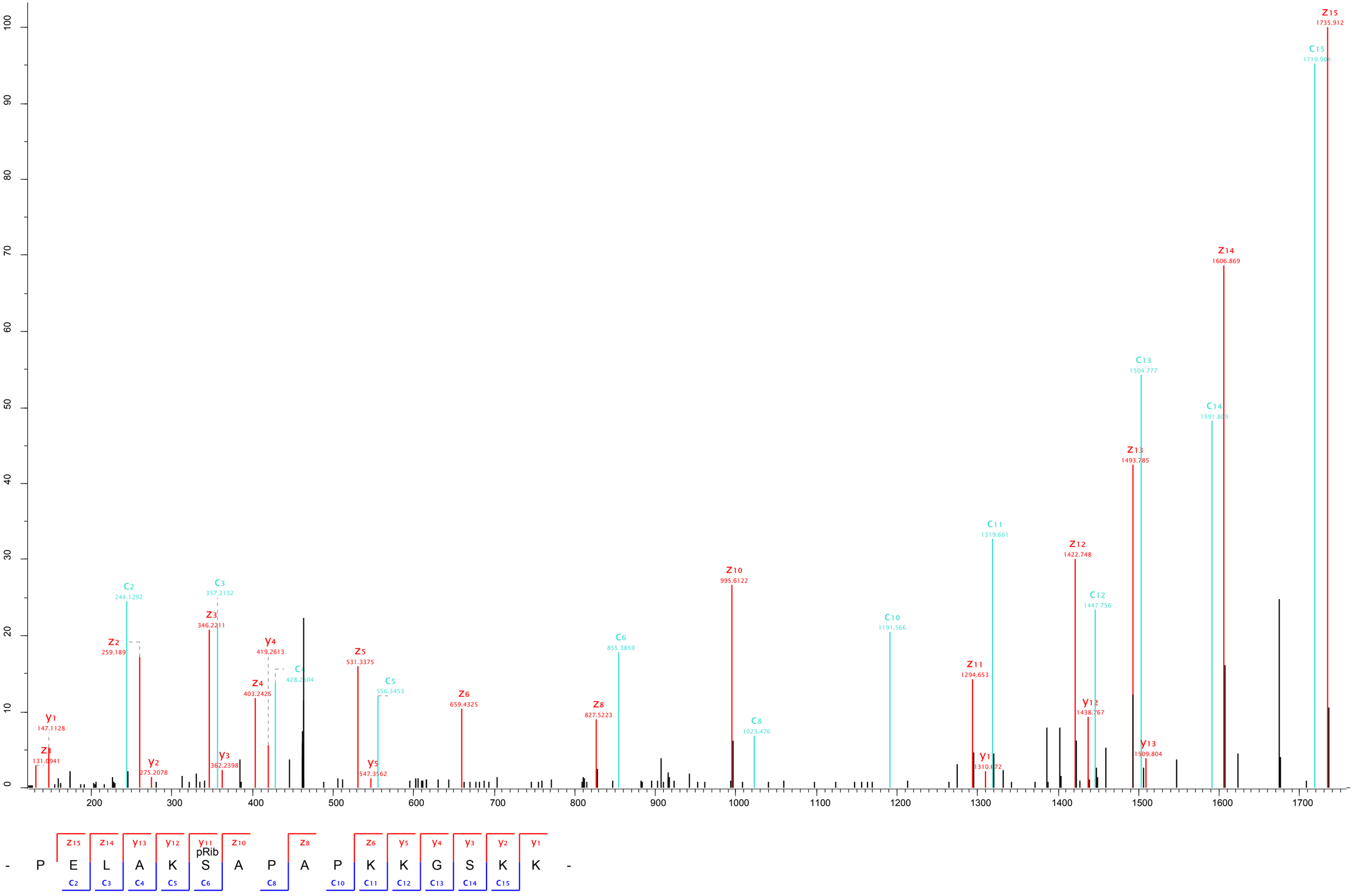


	z19	z18	y17	y16	y15	y14	y13	z12		y10	z9	y8	y7	y6	y5	y4	z3	z2	y1	
-	P	E	L	A	K	S	A	P	A	P	K	K	G	S	K	K	A	V	T	K	-
		c2	c3	c4	c5	c6		c8		c10	c11	c12	c13	c14	c15	c16	c17	c18	c19		

ad

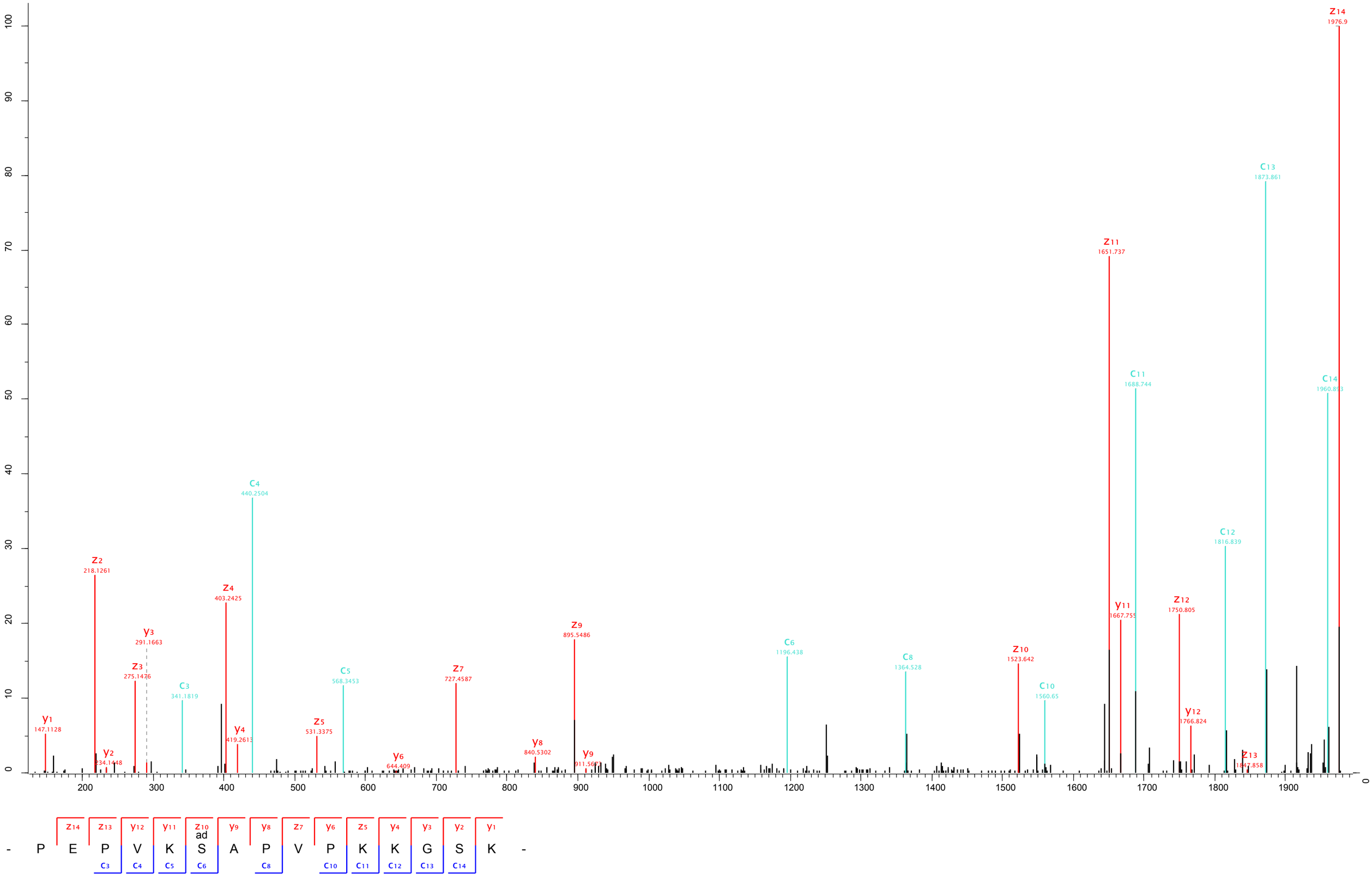
H2B1L phospho ribose on Ser6

m/z 463.00122 charge 4+ mass error [ppm] -0.5



H2B1M ADP-ribosylation on Ser6

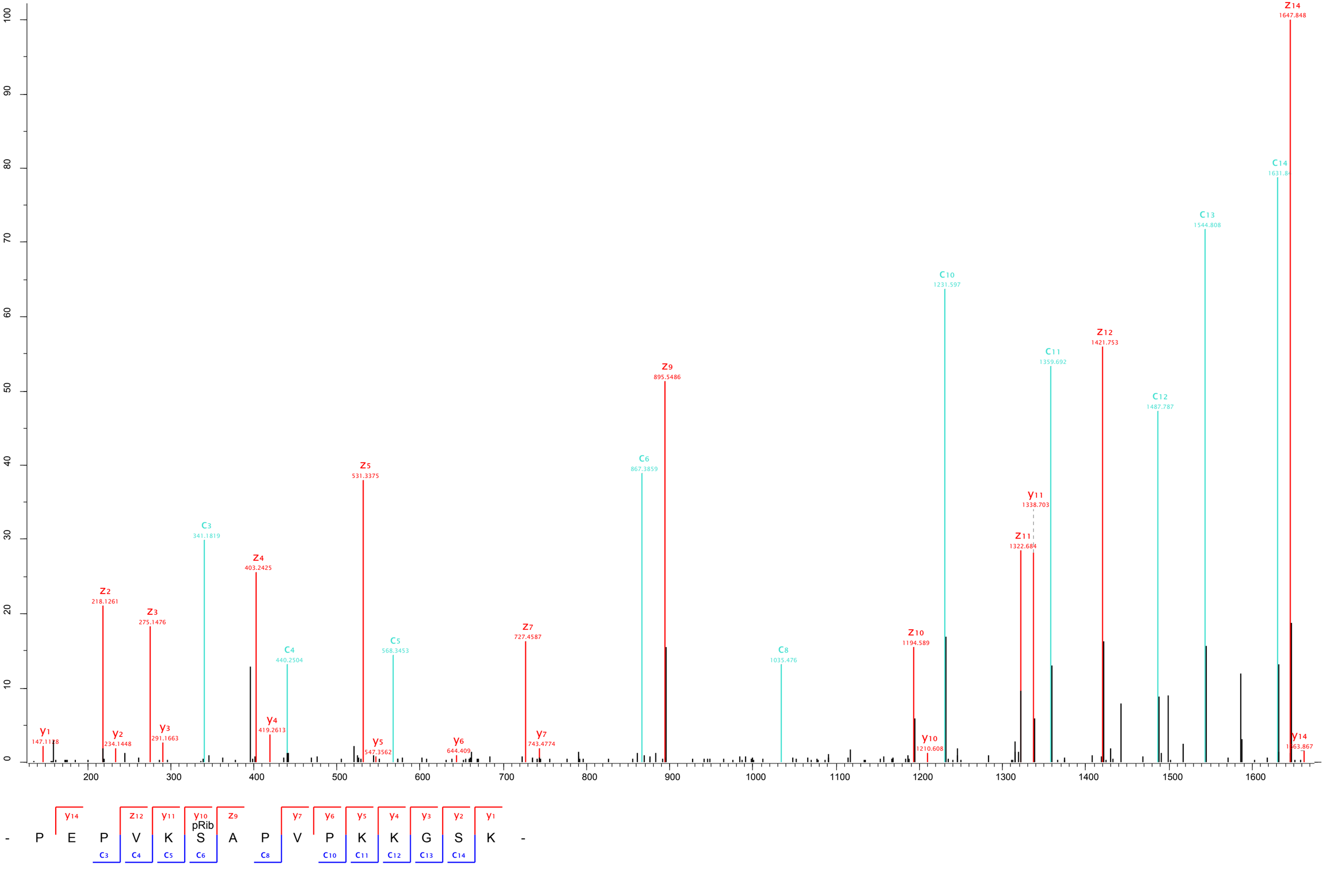
m/z 523.24844 charge 4+ mass error [ppm] 1.4



	z14	z13	y12	y11	z10 ad	y9	y8	z7	y6	z5	y4	y3	y2	y1	
-	P	E	P	V	K	S	A	P	V	P	K	K	G	S	K	-
		C3	C4	C5	C6		C8		C10	C11	C12	C13	C14		

H2B1M phospho ribose on Ser6

m/z 440.98531 charge 4+ mass error [ppm] -1.5

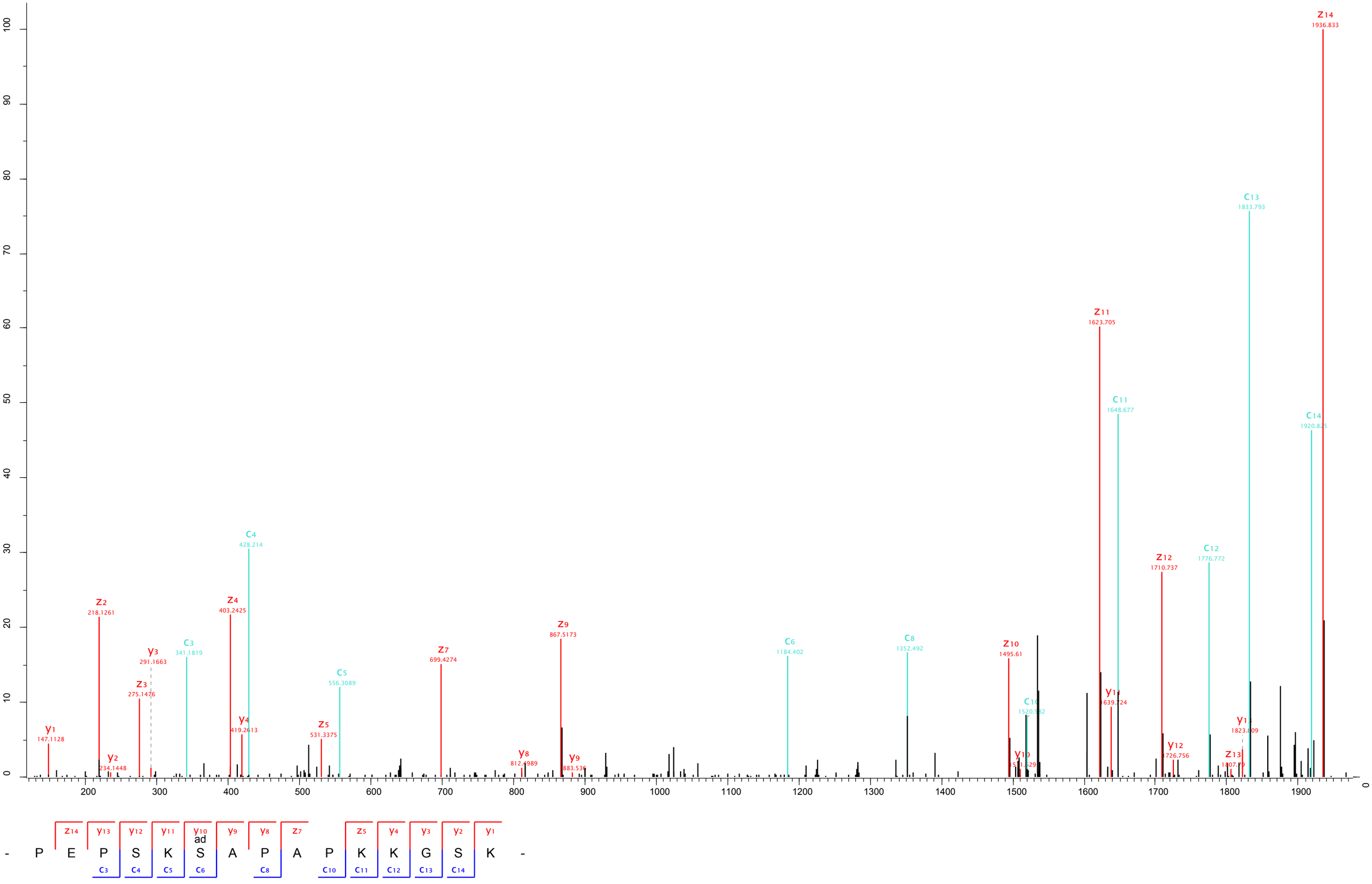


	y14		z12	y11	y10	z9		y7	y6	y5	y4	y3	y2	y1		
-	P	E	P	V	K	S	A	P	V	P	K	K	G	S	K	-
			C3	C4	C5	C6		C8		C10	C11	C12	C13	C14		

pRib

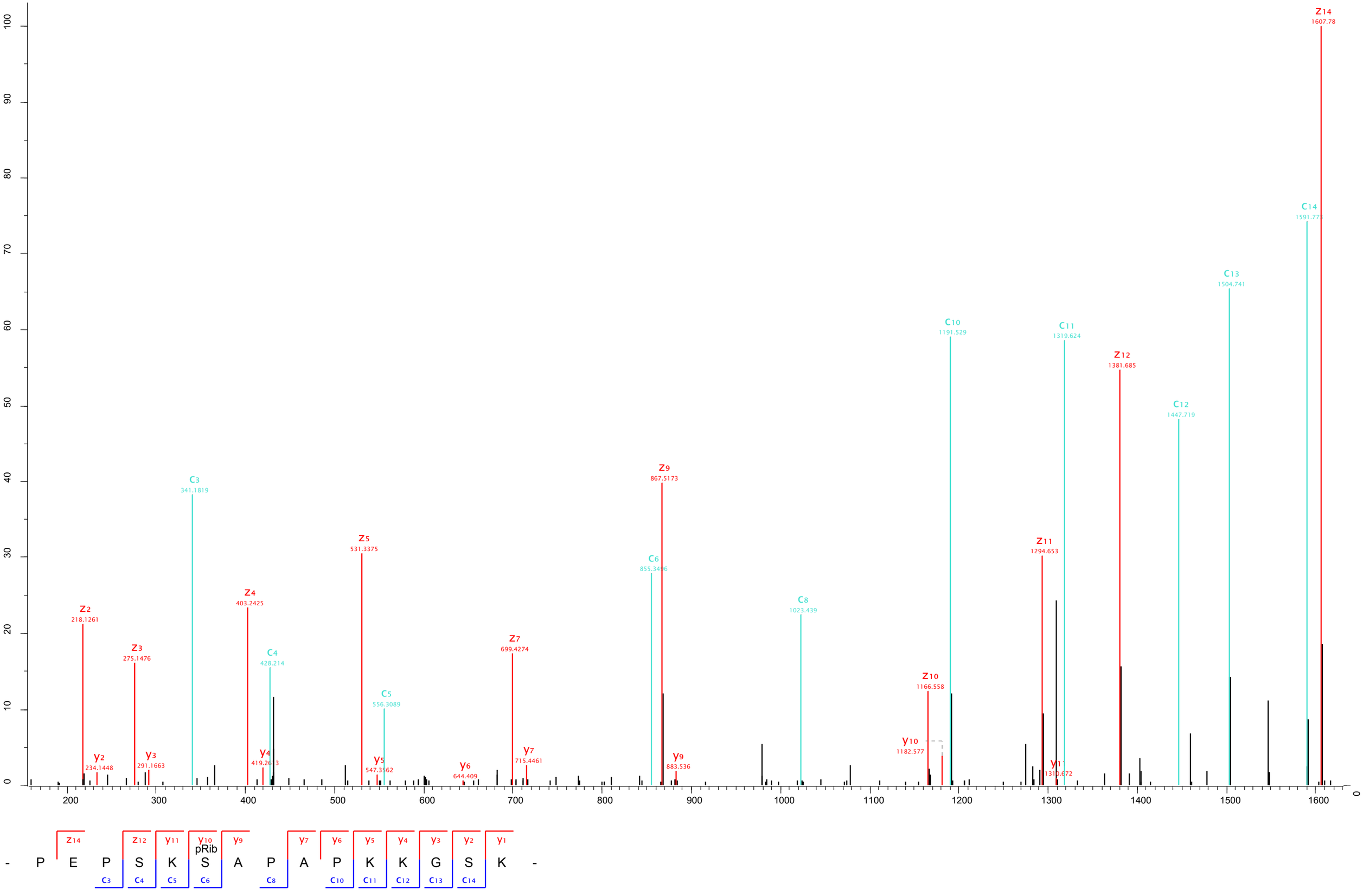
H2B1N ADP-ribosylation on Ser6

m/z 513.23151 charge 4+ mass error [ppm] 0.5



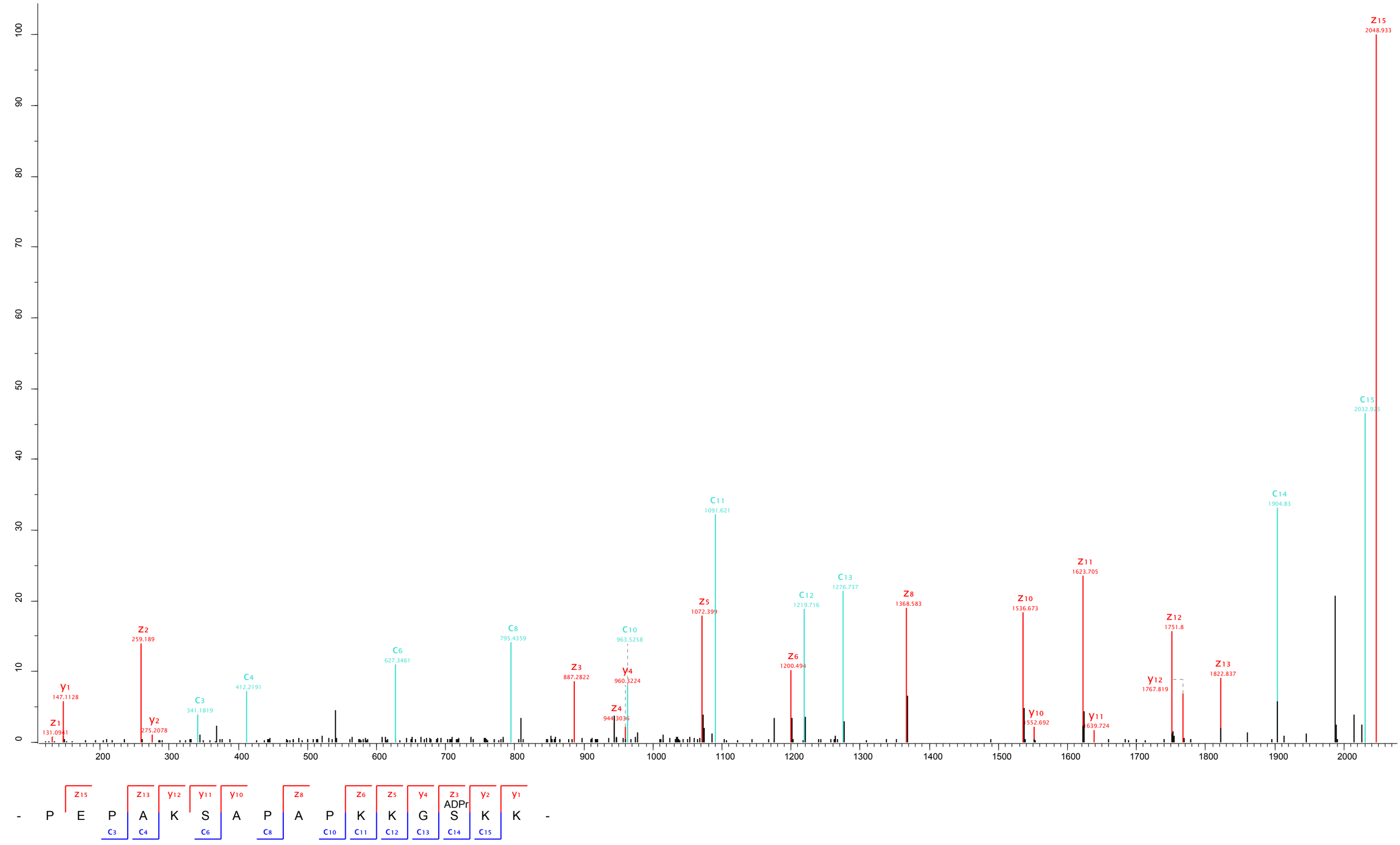
H2B1N phospho ribose on Ser6

m/z 430.96838 charge 4+ mass error [ppm] -0.3



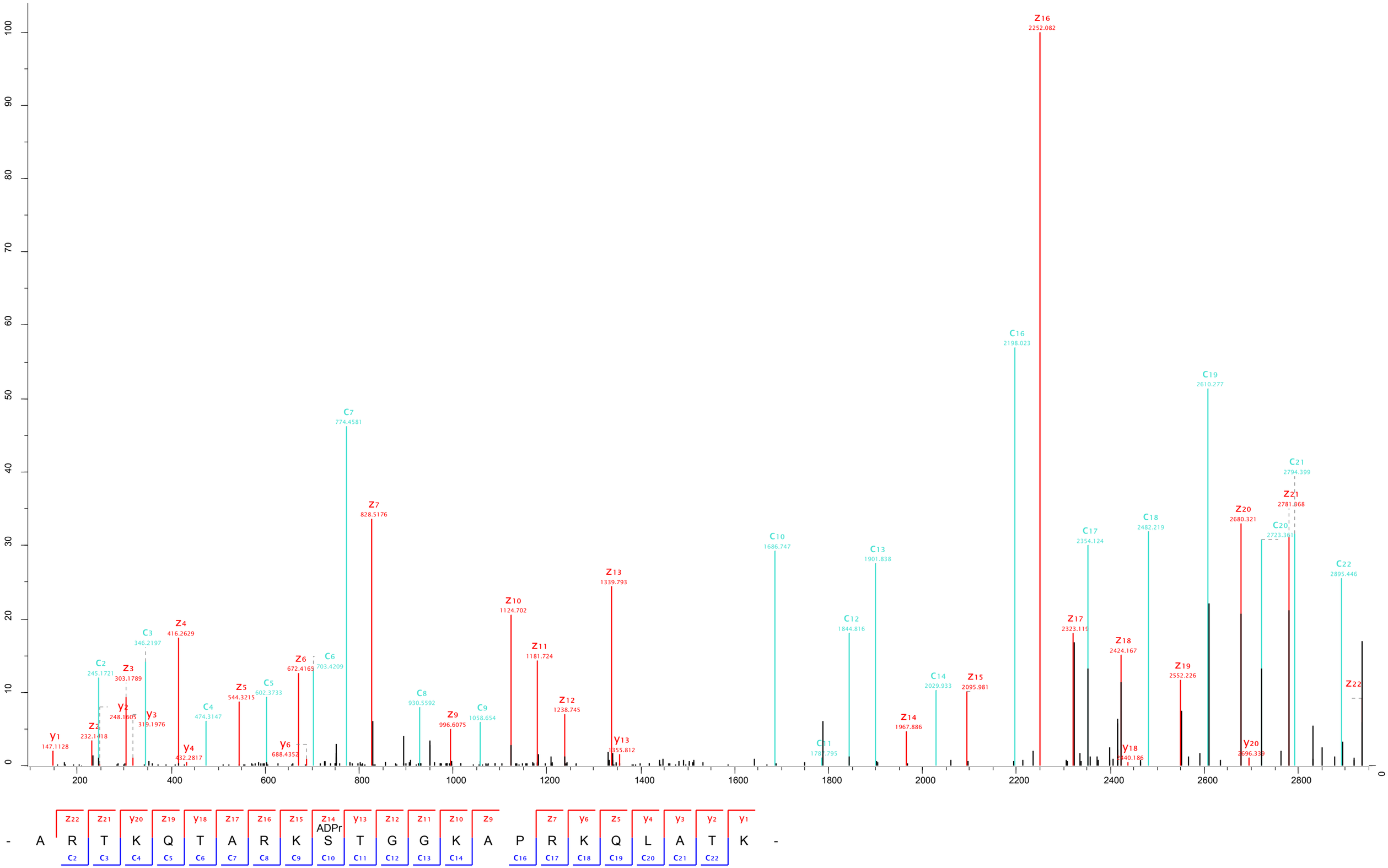
H2B1C ADP-ribosylation on Ser14

m/z 541.25653 charge 4+ mass error [ppm] -0.7



H3.1 ADP-ribosylation on Ser10

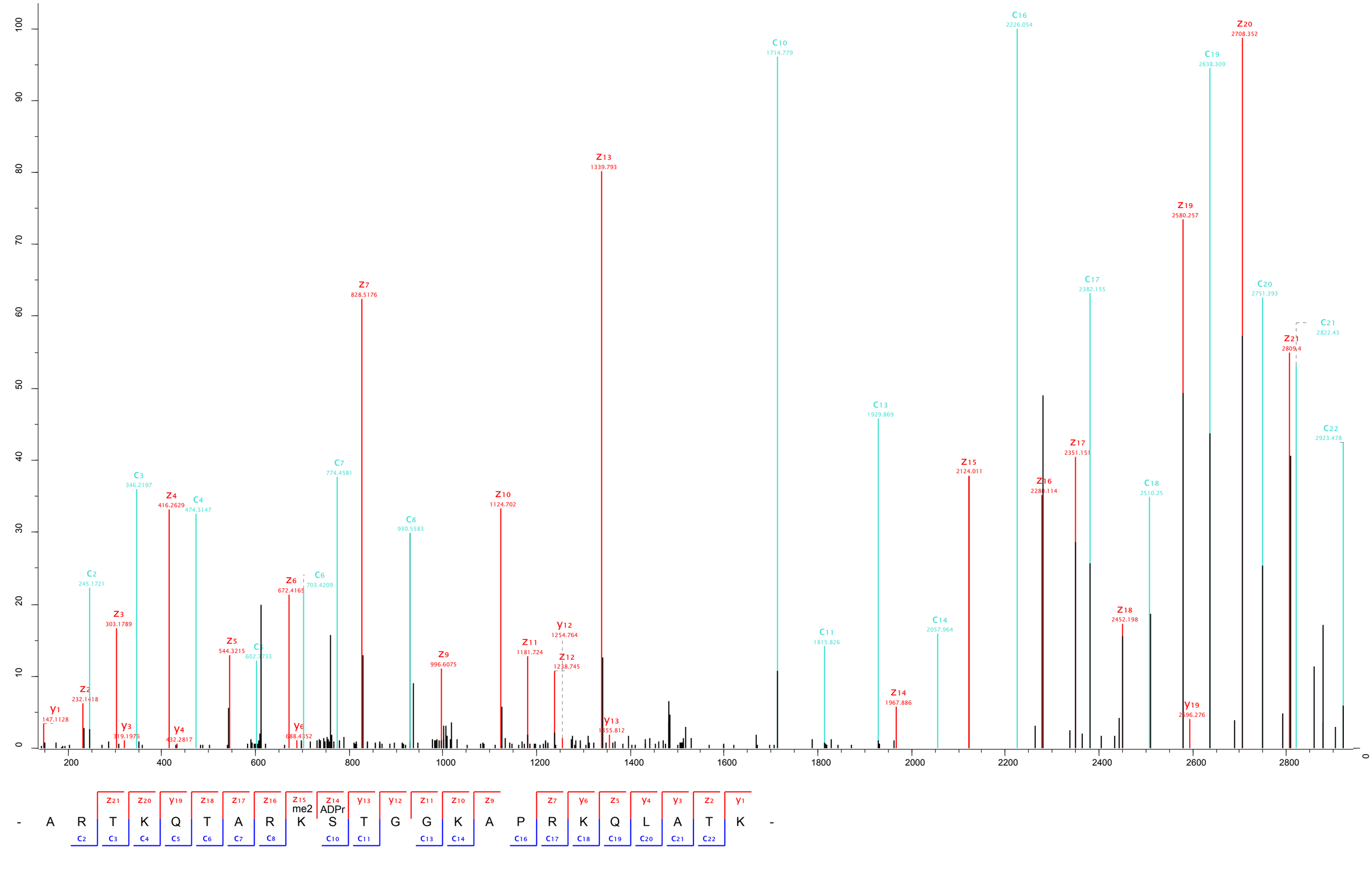
m/z 605.71086 charge 5+ mass error [ppm] -0.4



	z_{22}	z_{21}	y_{20}	z_{19}	y_{18}	z_{17}	z_{16}	z_{15}	z_{14} ADPr	y_{13}	z_{12}	z_{11}	z_{10}	z_9		z_7	y_6	z_5	y_4	y_3	y_2	y_1	
-	A	R	T	K	Q	T	A	R	K	S	T	G	G	K	A	P	R	K	Q	L	A	T	K	-
		c_2	c_3	c_4	c_5	c_6	c_7	c_8	c_9	c_{10}	c_{11}	c_{12}	c_{13}	c_{14}		c_{16}	c_{17}	c_{18}	c_{19}	c_{20}	c_{21}	c_{22}		

H3.1 ADP-ribosylation on Ser10

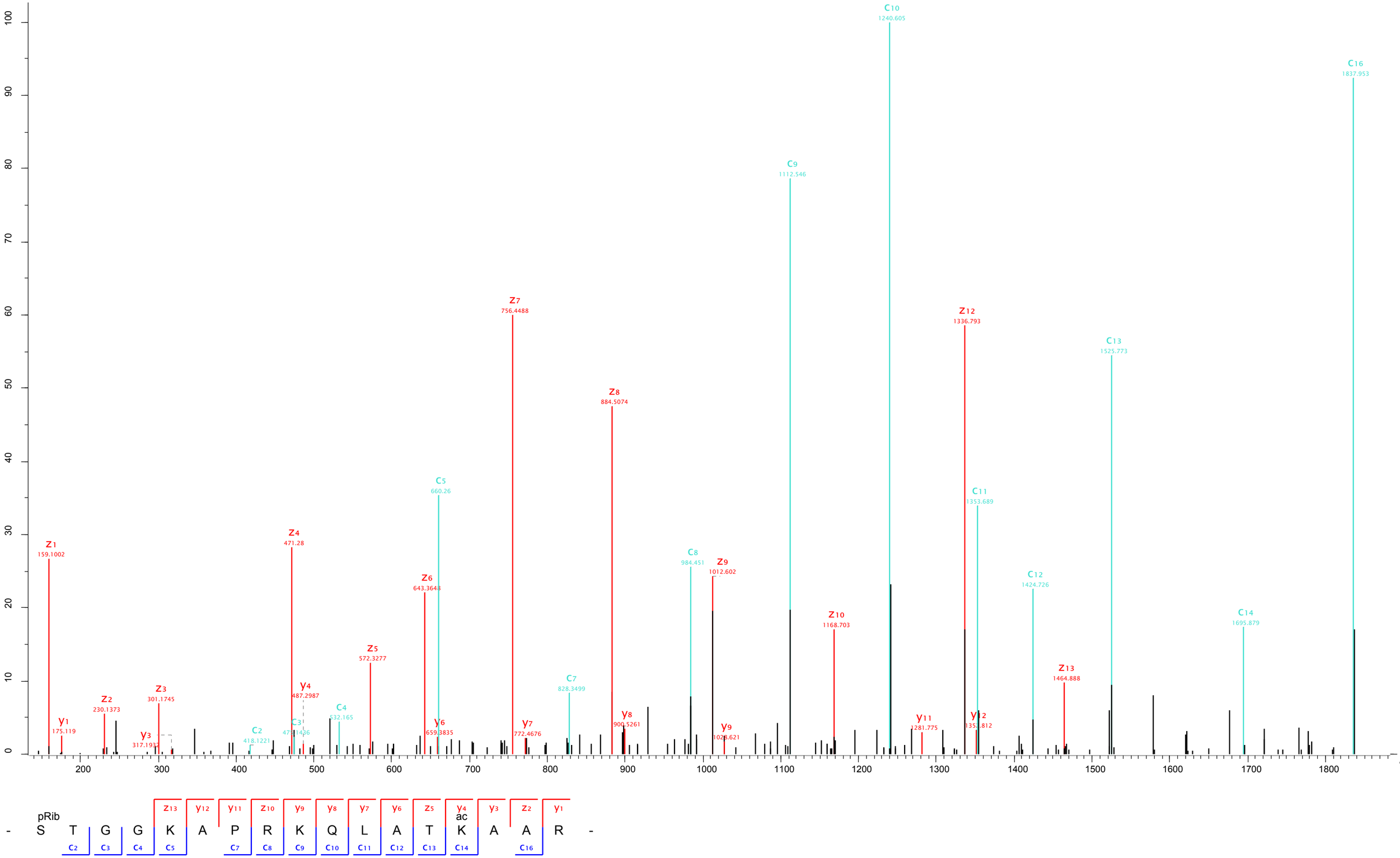
m/z 611.31712 charge 5+ mass error [ppm] 0.5



		z21	z20	y19	z18	z17	z16	z15	z14	y13	y12	z11	z10	z9		z7	y6	z5	y4	y3	z2	y1				
-	A	R	T	K	Q	T	A	R	K	me2 S	ADPr	T	G	G	K	A	P	R	K	Q	L	A	T	K	-	
		C2	C3	C4	C5	C6	C7	C8		C10	C11		C13	C14		C16	C17	C18	C19	C20	C21	C22				

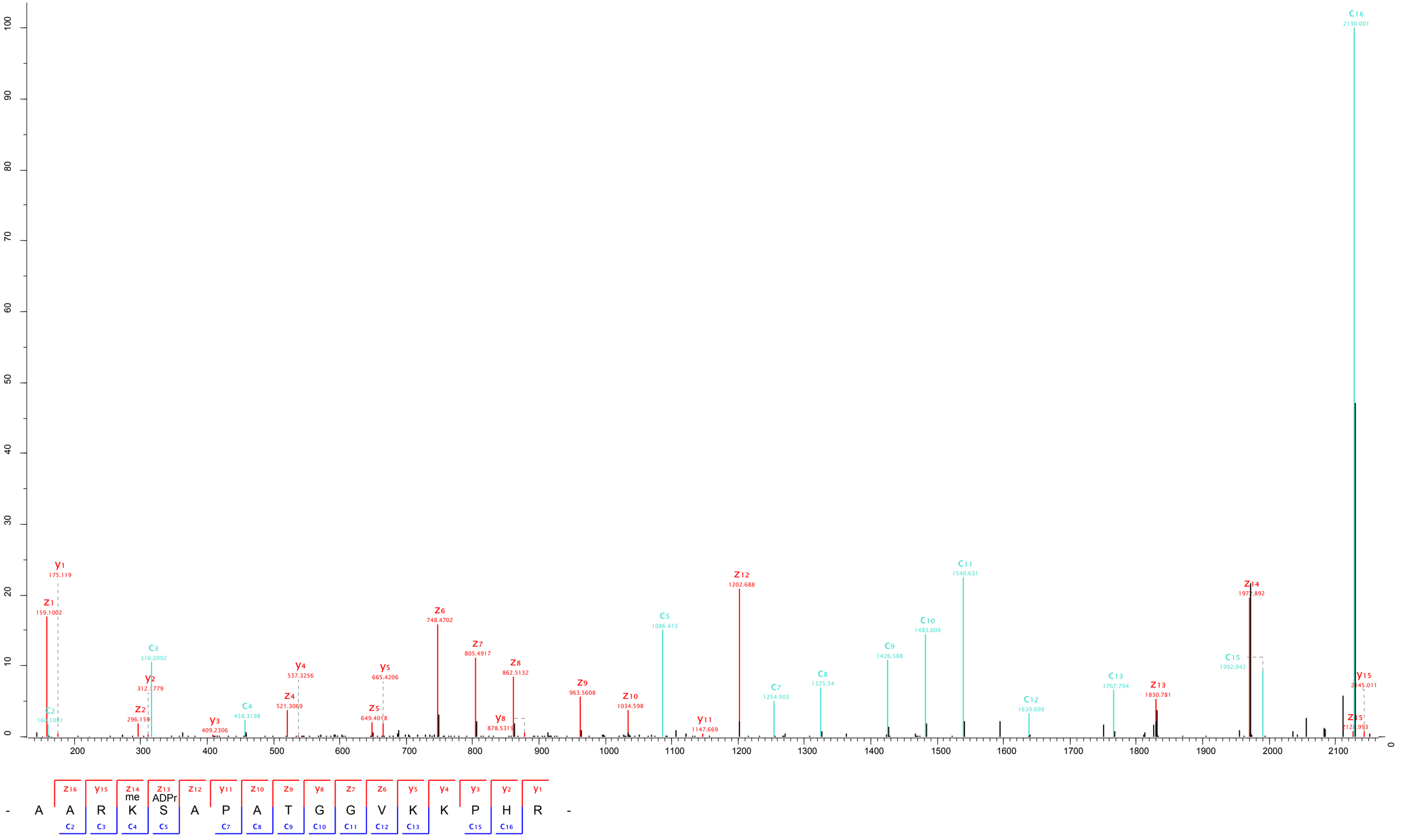
H3.1 phospho-ribose on Ser10

m/z 499.51503 charge 4+ mass error [ppm] -2.3



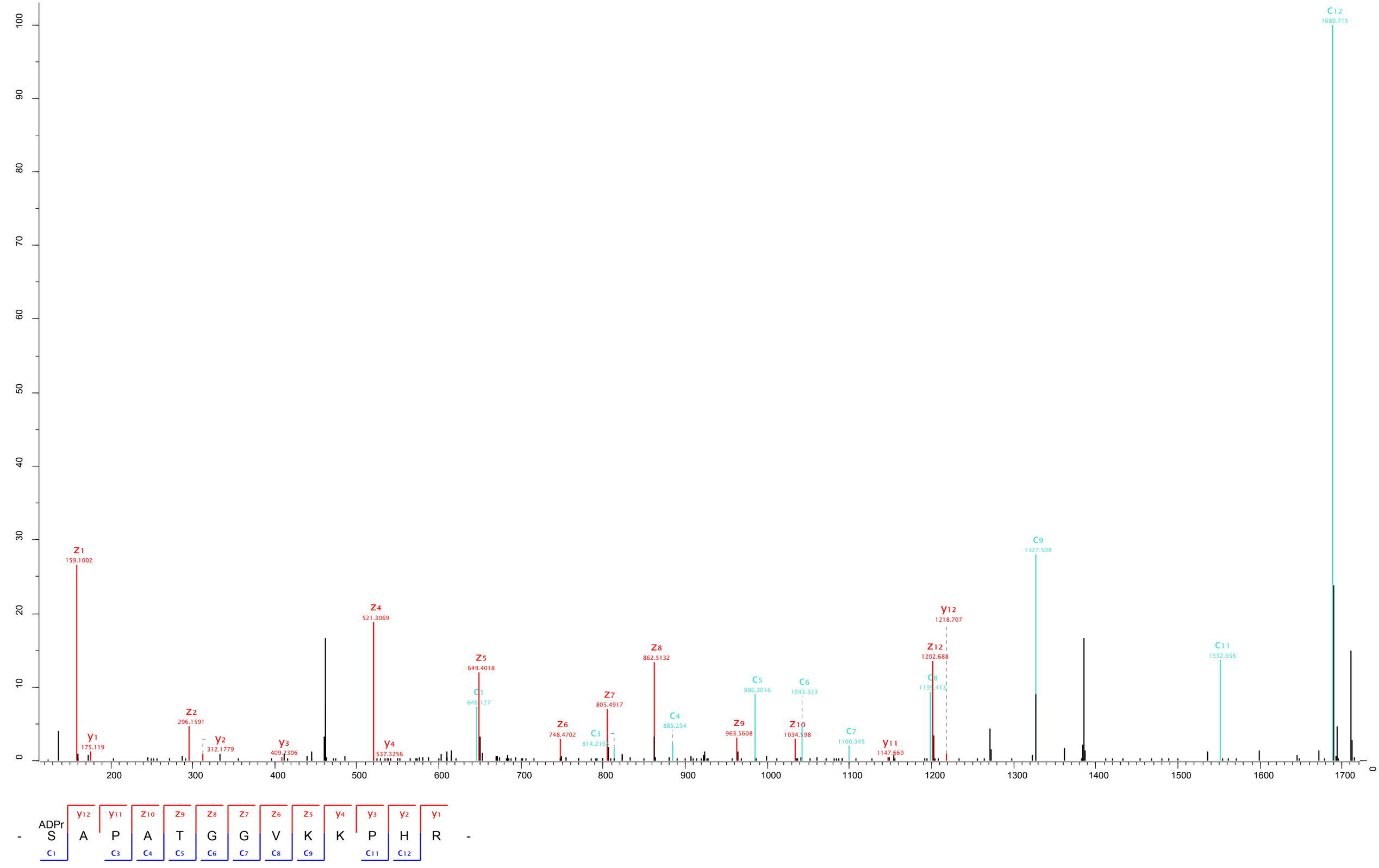
H3.1 ADP-ribosylation on Ser28

m/z 458.22295 charge 5+ mass error [ppm] -0.1



H3.1 ADP-ribosylation on Ser28

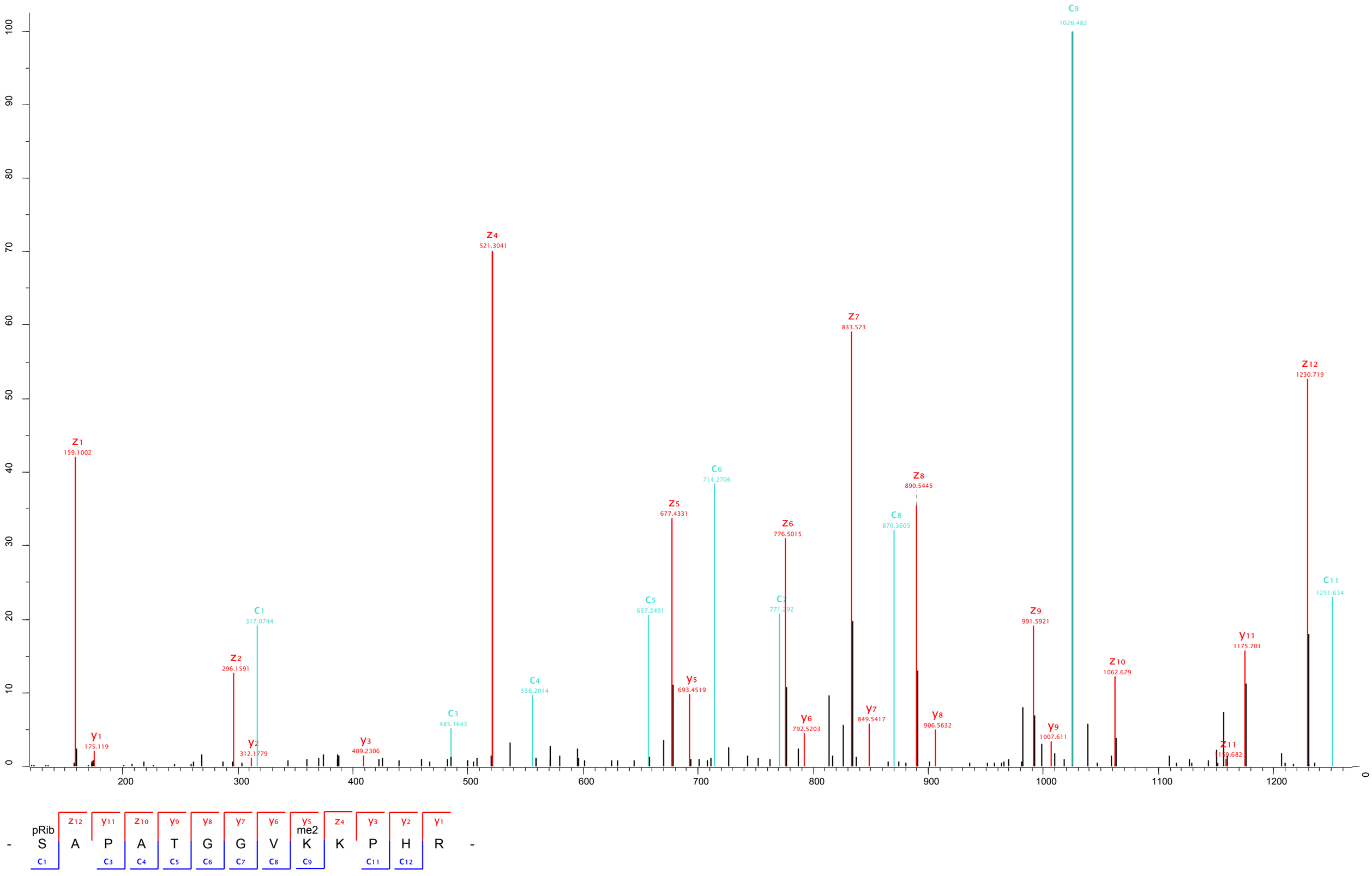
m/z 462.45538 charge 4+ mass error [ppm] 1.2



	ADPr													
-	S	A	P	A	T	G	G	V	K	K	P	H	R	-
	c1	y12	y11	c3	c4	c5	c6	c7	c8	c9	y4	c11	c12	
		z10	z9								y3	y2	y1	
			z8											
			z7											
			z6											
			z5											

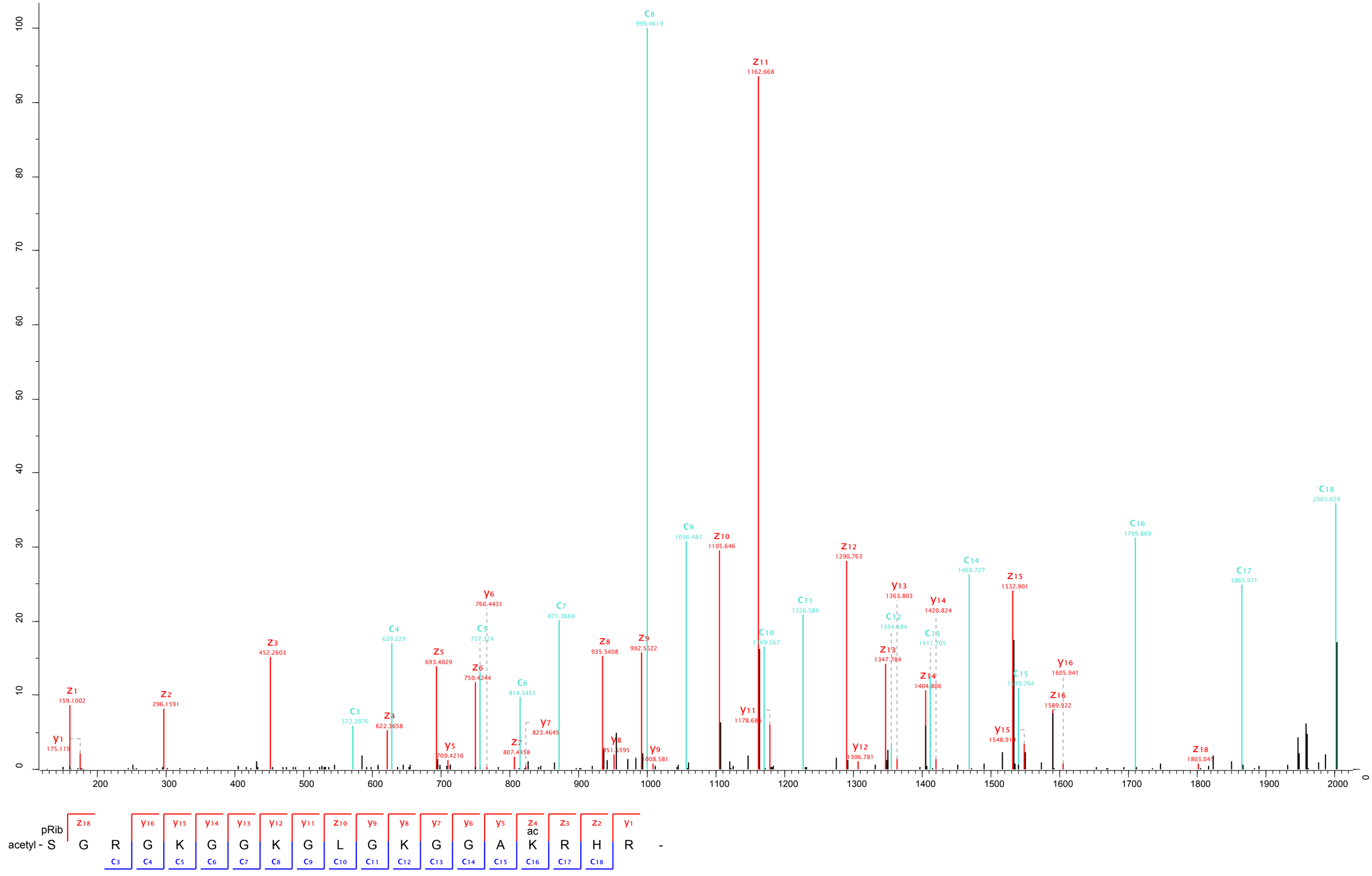
H3.1 phospho-ribose on Ser28

m/z 387.20008 charge 4+ mass error [ppm] -0.3



H4 phospho riboseon Ser1 or Arg3

m/z 432.82874 charge 5+ mass error [ppm] -0.8



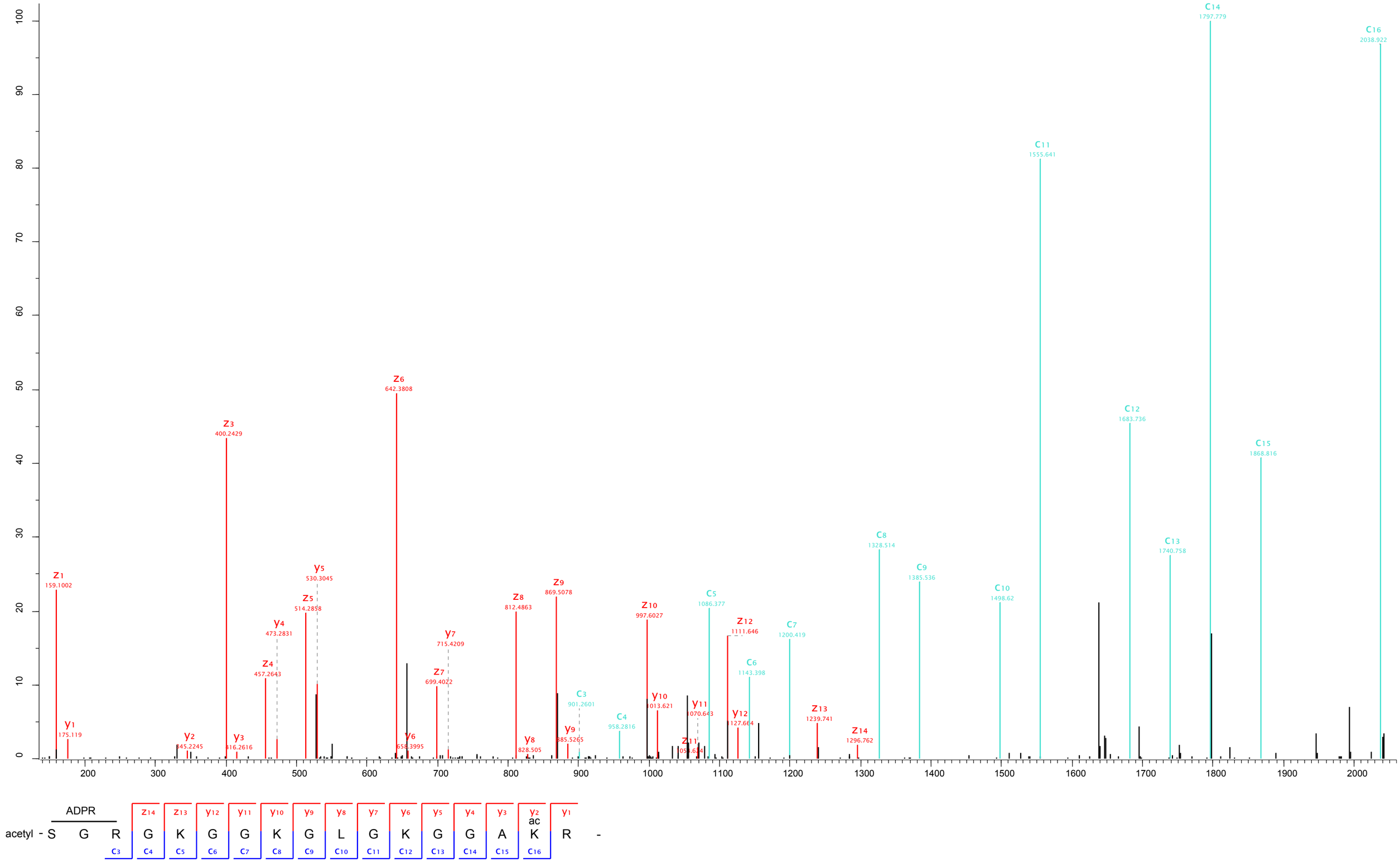
pRib

acetyl - S G R G K G G K G L G K G G A K R H R -

z18	y16	y15	y14	y13	y12	y11	z10	y9	y8	y7	y6	y5	z4 ac	z3	z2	y1	
	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	

H4 ADP-ribosylation on Ser1 or Arg3

m/z 549.75722 charge 4+ mass error [ppm] 1.9



ADPR

acetyl -	S	G	R	G	K	G	G	K	G	L	G	K	G	G	A	K	R	-
			C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16		
				z14	z13	y12	y11	y10	y9	y8	y7	y6	y5	y4	y3	y2 ac	y1	