From PKA to HCN: The cAMP-Capture Compound Mass Spectrometry as a novel tool for targeting cAMP binding proteins

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Supporting Material

Content

1. NMR and MS data of synthesized cAMP-CCs (5a-b, 6a-b, 7a-d)

6-AE-cAMP-CC, **5a**

^{1}H NMR (400 M Hz, MeOD): δ = 8.3 (s, 1H, purine-H), 8.2 (s, 1H, purine-H), 7.9 (d, 2H, aromatic-H), 6.8 (d, 2H, aromatic-H), 6.2 (s, 1H, sugar-H), 4.9 (m, 1H, sugar-H), 4.8 (m, 1H, sugar-H), 4.65 (m, 2H, biotin-H), 4.5 (m, 1H, Asp-H), 4.3 (m, 3H, sugar-H), 3.7 (m, 4H), 3.4-3.6 (m, 10H), 3.35 (m, 1H), 3.25 (m, 4H), 3.2 (m, 1H), 2.8 (t, 2H, Asp-H), 2.7 (m, 4H), 2.2 (t, 2H, biotin-H), 1.5 – 1.8 (m, 10 H, PEG-H, biotin-H).

MS: m/z = 1127.5 $[M+H]^+$, 564.8 $[M+2H]^{2+}$

6-AH-cAMP-CC, **5b**

^{1}H NMR (400 M Hz, MeOD): δ =8.3 (s, 1H, purine-H), 8.2 (s, 1H, purine-H), 7.9 (d, 2H, aromatic-H), 6.8 (d, 2H, aromatic-H),), 6.2 (s, 1H, sugar-H), 4.9 (m, 1H, sugar-H), 4.8 (m, 1H, sugar-H), 4.65 (m, 2H, biotin-H), 4.5 (m, 1H, Asp-H), 4.3 (m, 3H, sugar-H), 2.2 (t, 2H, biotin-H), 1.5 – 1.8 (m, 18 H, PEG-H, biotin-H).

MS: m/z =1183.6 $[M+H]^+$, 592.8 $[M+2H]^{2+}$

2-AEA-cAMP-CC, **6a**

^{1}H NMR (400 M Hz, MeOD): δ =7.95 (s, 1H, purine-H), 7.9 (d, 2H, aromatic-H), 7.2 (d, 2H, aromatic-H), 5.9 (s, 1H, sugar-H), 5.3 (m, 1H, sugar-H), 5.2 (m, 1H, sugar-H),5.1 (m, 1H, Asp-H), 4.1-4.2 (m, 4H, sugar-H, biotin-H),3.6-3.8 (m, 15H), 3.0-3.2 (m, 6H), 2.85 (m, 1H, biotin-H), 2.7 (m, 1H, biotin-H), 2.6-2.8 (m, 2H, Asp-H), 2.1 (t, 2H, biotin-H), 1.5-1.8 (m, 10H, PEG-H, biotin-H).

MS: m/z =1143.2 $[M+H]^+$, 572.3 $[M+2H]^{2+}$

2-AHA-cAMP-CC, **6b**

^{1}H NMR (400 M Hz, MeOD): δ =7.9 (d, 2H, aromatic-H), 7.85 (s, 1H, purine-H), 7.3 (d, 2H, aromatic-H), 5.9 (s, 1H, sugar-H), 5.1 (m, 1H, sugar-H), 4.7 (m, 1H, sugar-H), 4.5 (m, 1H, Asp-H), 4.1-4.2 (m, 4H, sugar-H, biotin-H),3.6-3.8 (m, 15H), 3.0-3.2 (m, 6H), 2.85 (m, 1H, biotin-H), 2.7 (m, 1H, biotin-H), 2.6-2.8 (m, 2H, Asp-H), 2.3 (t, 2H, biotin-H), 1.5-1.8 (m, 18H, PEG-H, biotin-H).

MS: m/z = 1198.6 $[M+H]^+$, 600.3 $[M+2H]^{2+}$

8-AEA-cAMP-CC, **7a**

^{1}H NMR (400 M Hz, MeOD): δ =8.0 (s, 1H, purine-H), 7.9 (d, 2H, aromatic-H), 7.3 (d, 2H. aromatic-H), 6.3 (s, 1H, sugar-H), 5.2 (m,1H, sugar-H), 5.05 (m, 1H, sugar-H), 4.85 (m, 1H, Asp-H), 4.5 (m,1H, biotin-H), 4.1-4.3 (m, 4H, sugar-H, biotin-H), 3.5-3.6 (m, 12H), 3.4 (m, 2H), 3.05-3.15 (m, 7H), 2.9(m, 2H), 2.7 (m, 2H), 2.2 (m, 2H, biotin-H), 1.5-1.8 (m, 10H, PEG-H, biotin-H).

MS: m/z = 1142.6 $[M+H]^{+}$, 572.3 $[M+2H]^{2+}$

8-ABA-cAMP-CC, **7b**

^{1}H NMR (400 MHz, MeOD): δ =7.8 (s, 1H, purine-H), 7.7 (d, 2H, aromatic-H), 7.1 (d, 2H. aromatic-H), 5.6 (s, 1H, sugar-H), 5.0 (m,1H, sugar-H), 4.8 (m, 1H, sugar-H), 4.3 (m, 1H, Asp-H), 3.9-4.1 (m, 4H, sugar-H, biotin-H),), 3.0-3.5 (m, 20H), 2.5-2.7(m, 4H), 2.0 (m, 2H, biotin-H), 1.5-1.8 (m, 14H, PEG-H, biotin-H).

MS: m/z = 1171.5 $[M+H]^{+}$, 586.3 $[M+2H]^{2+}$

8-AHA-cAMP-CC, **7c**

^{1}H NMR (400 M Hz, MeOD): δ =8.1 (s, 1H, purine-H), 7.9 (d, 2H, aromatic-H), 7.4 (d, 2H. aromatic-H),. 5.8 (s, 1H, sugar-H), 5.2 (m,1H, sugar-H), 5.05 (m, 1H, sugar-H), 4.85 (m, 1H, Asp-H), 4.5 (m,1H, biotin-H), 4.1-4.3 (m, 4H, sugar-H, biotin-H), 3.5-3.6 (m, 12H), 3.4 (m, 2H), 3.05-3.15 (m, 7H), 2.7-2.9 (m, 4H), 2.05 (m, 2H, biotin-H), 1.5-1.8 (m, 18H, PEG-H, biotin-H).

MS: m/z = 1199.0 $[M+H]^{+}$, 600.5 $[M+2H]^{2+}$

8-ADOA-cAMP-CC, **7d**

^{1}H NMR (400 M Hz, MeOD): δ =8.04 (s, 1H, purine-H), 7.98 (d, 2H, aromatic-H), 7.37 (d, 2H. aromatic-H), 5.86 (s, 1H, sugar-H), 5.20 (m,1H, sugar-H), 5.06 (m, 1H, sugar-H), 4.9 (m, 1H, Asp-H), 4.51 (m, 1H, biotin-H), 4.36 (m, 1H, sugar-H), 4,32 (m, 1H, biotin-H), 4.1-4.3 (m, 2H, sugar-H), 3.73-3.80 (m, 4H, ADOA-H), 3.50-3.70 (m, 20H, PEG-H, ADOA-H), 3.2-3.3 (m, 5H, PEG-H, biotin-H), 2.94 (dd, 1H, biotin-H), 2.85 (dd, 1H, Asp-H), 2.77 (dd, 1H, Asp-H), 2.73 (d, 1H, biotin-H), 2.21 (t, 2H, biotin-H), 1.5-1.8 (m, 10H, PEG-H, biotin-H).

MS: m/z = 1231.5 $[M+H]^{+}$, 616.3 $[M+2H]^{2+}$

2. Technical details of the caproBox™ and the caproMag™

The caproBox™ (Figure F1-A) uses three Philips PL-S/12 lamps for the photo-crosslinking of cooled protein-samples with Capture Compounds. The UV-spectrum of the lamps, which was kindly provided by Philips B.V., is shown in Figure F1-B (the spectrum refers to the Philips TL/12 which has exactly the same coating (and spectrum) as the PL-S/12). The caproMag™ used to collect the magnetic beads and the geometry of the PCR-tube used for photo-crosslinking is shown in Figure F1-C.

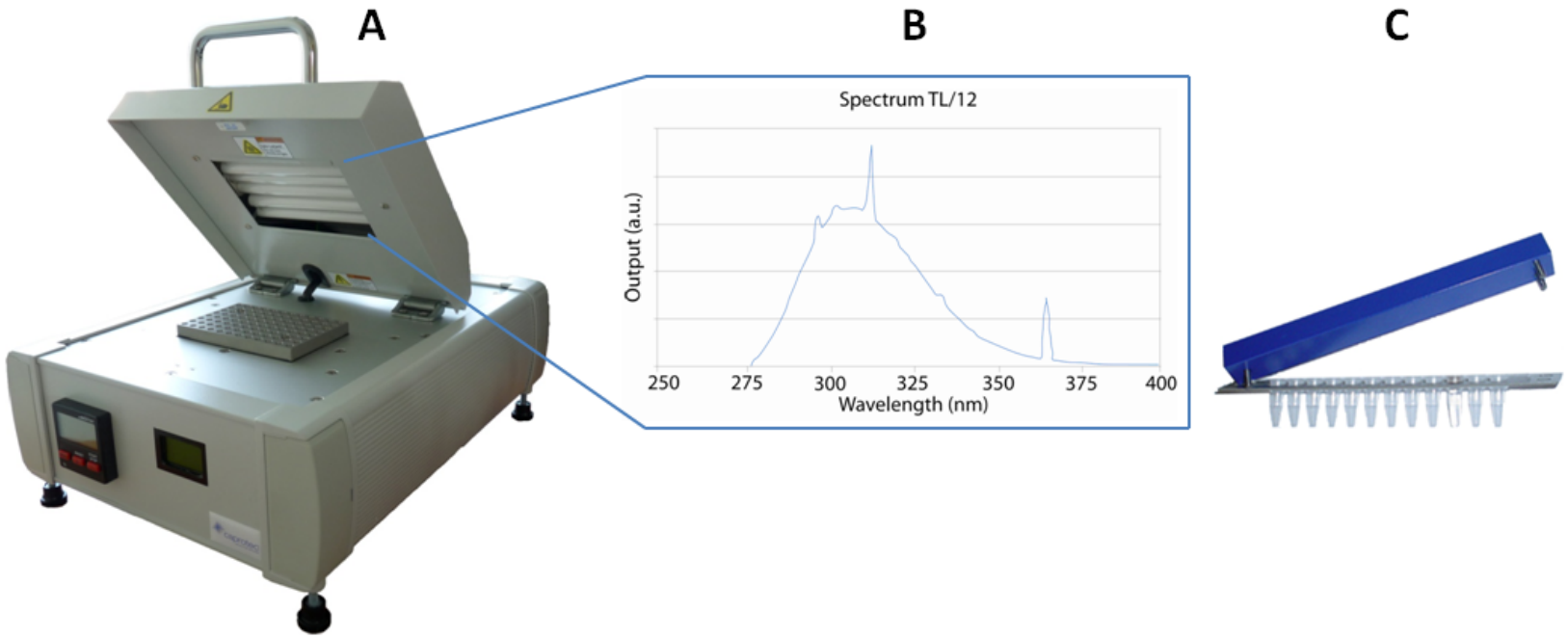


Figure F1. (A) caproBox™, (B) spectrum of the lamps, (C) caproMag™

The irradiation energy (*Q*) applied to a given sample having an irradiation area (*A*) is calculated as follows:

$$\boldsymbol{Q = I \times A \times t} \qquad \textbf{(1)}$$

The irradiance (*I*) of the caproBox used for the experiments described in this report was determined to be 11 mW x cm^{-2}.

The irradiation energy (*Q*) for a sample in an **off-bead experiment** was calculated as follows: the sample was irradiated in an open PCR-tube having a 5.4 mm diameter (area (*A*) of the open PCR-tube is 0.229 cm^{-2}) for *t* = 600 s. Following the equation (1)

Q = 0.011 J x s^{-1} x cm^{-2} **x** 0.229 cm^{-2} **x** 600 s = **1.51 J**

The irradiation energy (*Q*) for a sample in an **on-bead experiment** was calculated as follows: the sample was irradiated in capped PCR-tubes having a 4.4 mm diameter (area (*A*) of the capped PCR-tube is 0.152 cm^{-2}) for *t* = 1200 s. The transmission of the PCR-tube cap is only about 70% of the transmission in air (between 300–400 nm). This value was estimated from UV-spectroscopic studies of the PCR-tube material (R-5). Following the equation (1)

Q = 0.011 J x s^{-1} x cm^{-2} **x** 0.7 **x** 0.152 cm^{-2} **x** 1200 s = **1.40 J**

3. Biological stability of Capture compound 7c, precursor/competitor 4c and Capture Compound 5b

The biological stability of Capture Compound **7c** and the precursor **4c**, used as competitor for the present study, as well as one example of a different Capture Compound, derivative **5b**, has been tested against *E.coli* at 37 °C (all compounds), HepG2 at 4 and 30 °C (**7c** and **4c**), and PDE at 30 °C (**4c** and **5b**). The general Scheme for this study is shown in Figure F2. The total ion count and extracted masses for each starting material and the corresponding hydrolysis product as obtained from the MS experiments, typically at 0 min (column A) and after 120 – 300 min (column B) are displayed in Figures F3 – F11.

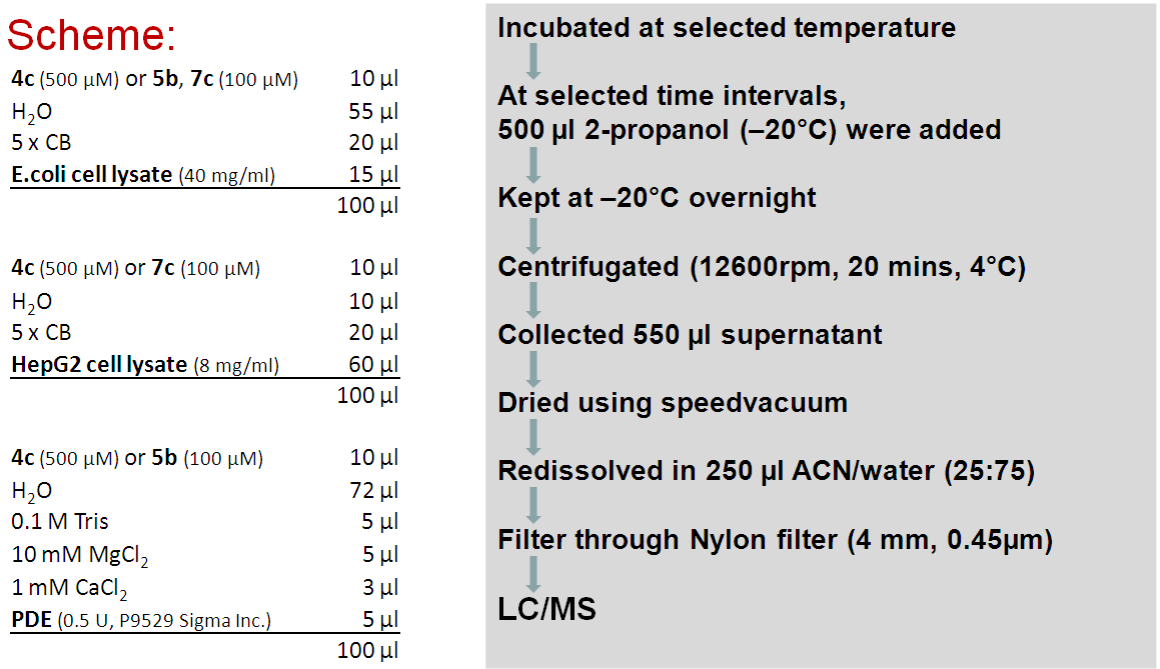


Figure F2. Overview of the reaction scheme for testing the biostability of cAMP derivatives in *E. coli*, HepG2, and against PDE (Sigma Inc.).

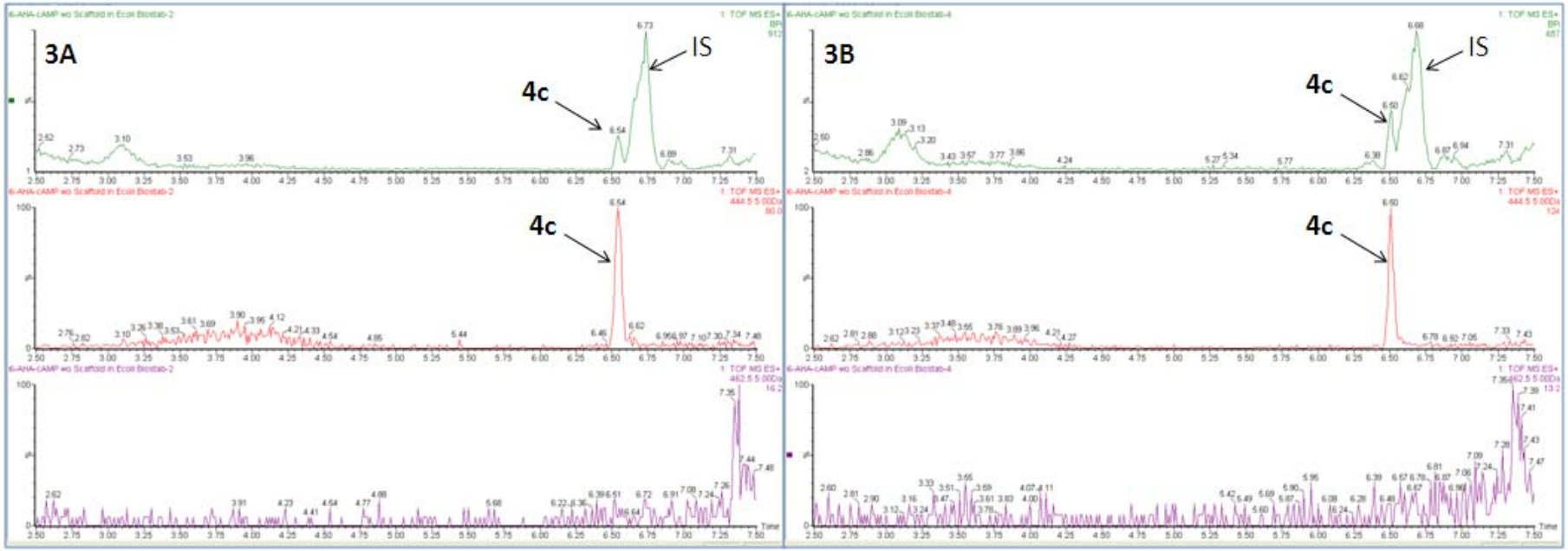


Figure F3. Stability of 8-AHA-cAMP (**4c**) in *E. coli* cell lysate at 37 °C, (F3-A) at 0 min, (F3-B) after 180 min. (green lane: BPI (IS = internal standard), red lane: extracted mass for **4c** (444.5), pink lane: extracted mass for the hydrolysis product 8-AHA-AMP (462.5))

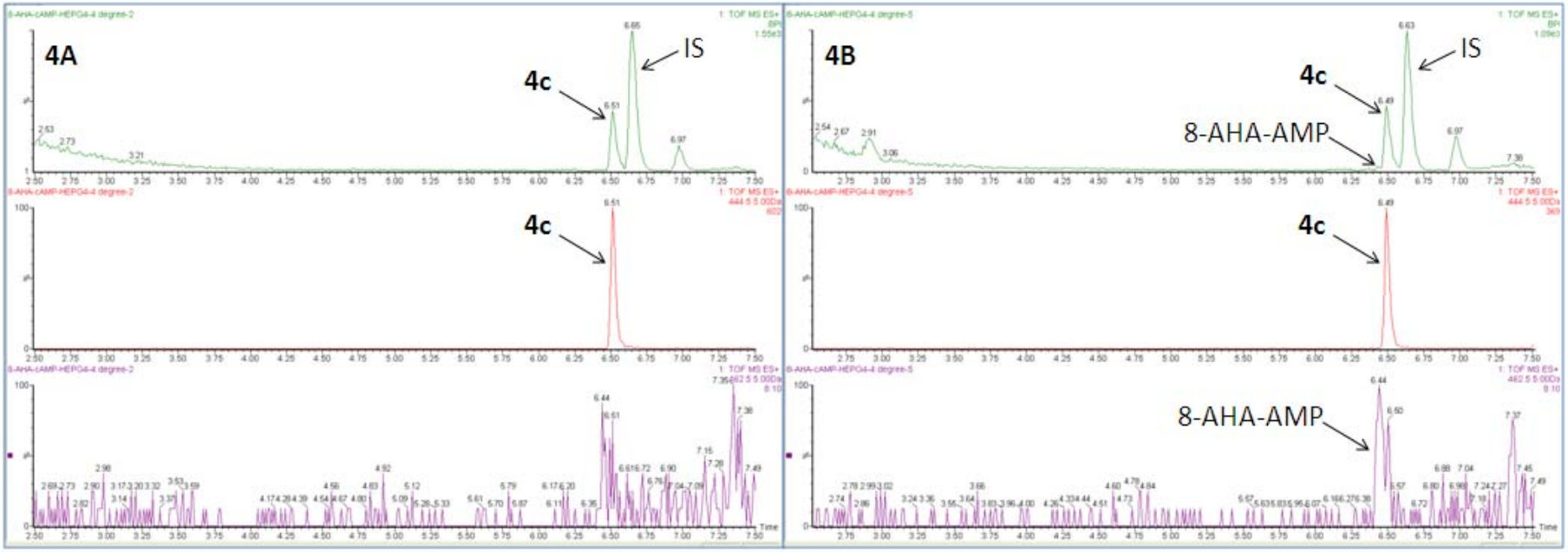


Figure F4. Stability of 8-AHA-cAMP (**4c**) in HepG2 cell lysate at 4 °C, (F4-A) after 30 min, (F4-B) after 180 min. (green lane: BPI (IS = internal standard), red lane: extracted mass for **4c** (444.5), pink lane: extracted mass for the hydrolysis product 8-AHA-AMP (462.5))

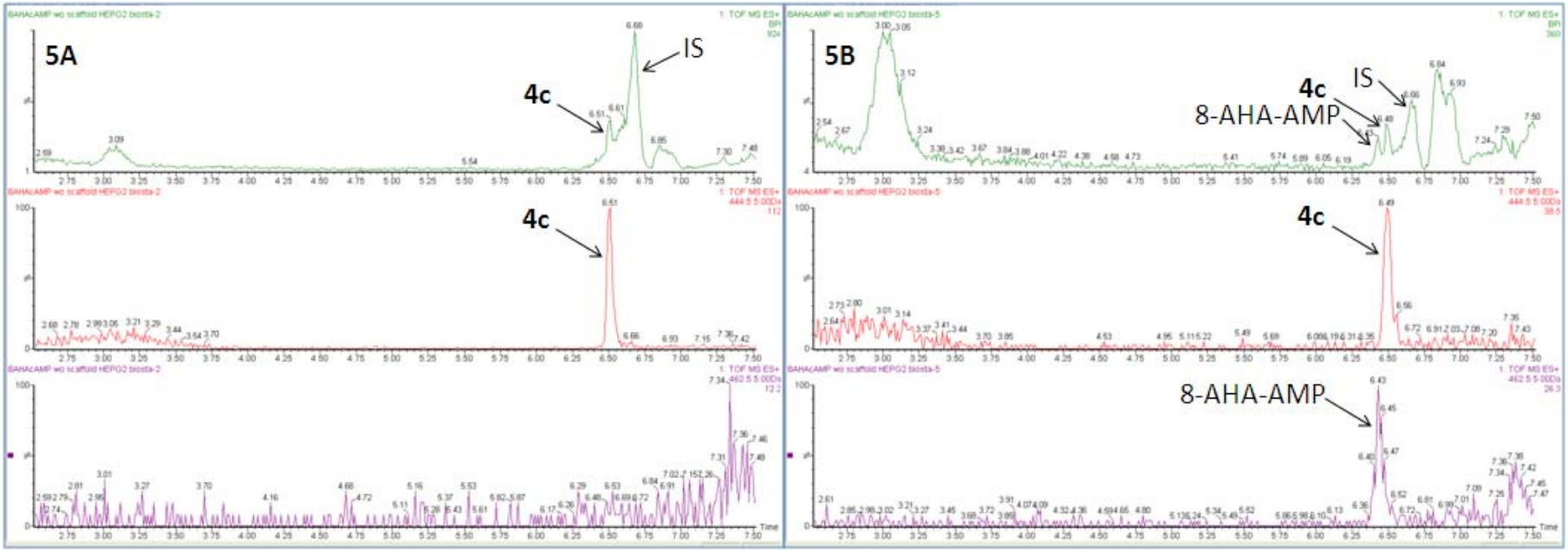


Figure F5. Stability of 8-AHA-cAMP (**4c**) in HepG2 cell lysate at 30 °C, (F5-A) after 30 min, (F5-B) after 180 min. (green lane: BPI (IS = internal standard), red lane: extracted mass for **4c** (444.5), pink lane: extracted mass for the hydrolysis product 8-AHA-AMP (462.5))

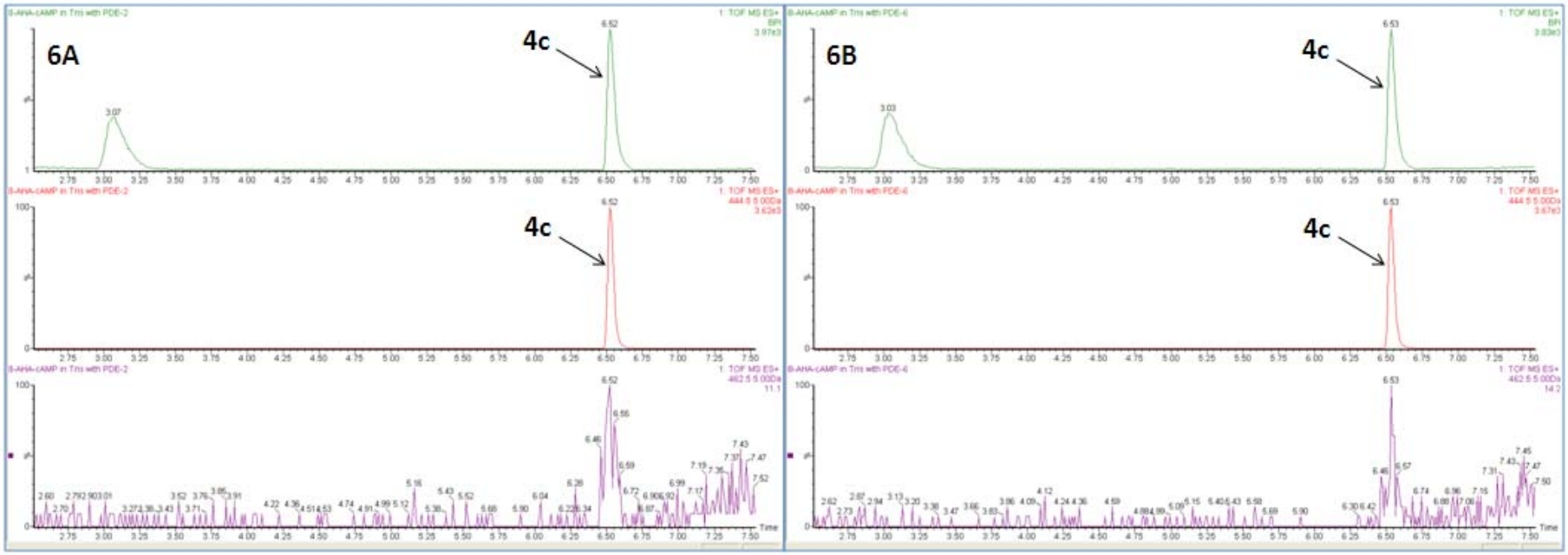


Figure F6. Stability of 8-AHA-cAMP (**4c**) in PDE (Tris-buffer) at 30 °C, (F6-A) after 30 min, (F6-B) after 180 min. (green lane: BPI, red lane: extracted mass for **4c** (444.5), pink lane: extracted mass for the hydrolysis product 8-AHA-AMP (462.5))

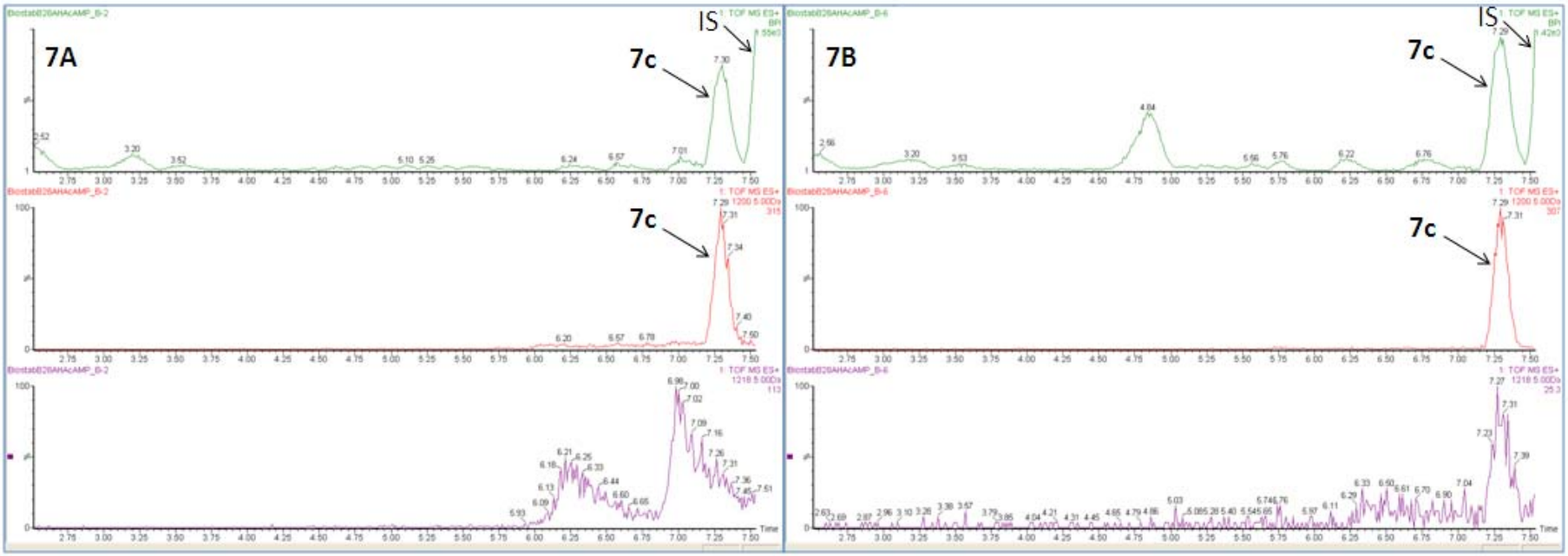


Figure F7. Stability of 8-AHA-cAMP-CC (**7c**) in *E. coli* cell lysate at 37 °C, (F7-A) at 0 min, (F7-B) after 240 min. (green lane: BPI (IS = internal standard), red lane: extracted mass for **7c** (1200.0), pink lane: extracted mass for the hydrolysis product 8-AHA-AMP-CC (1218.0))

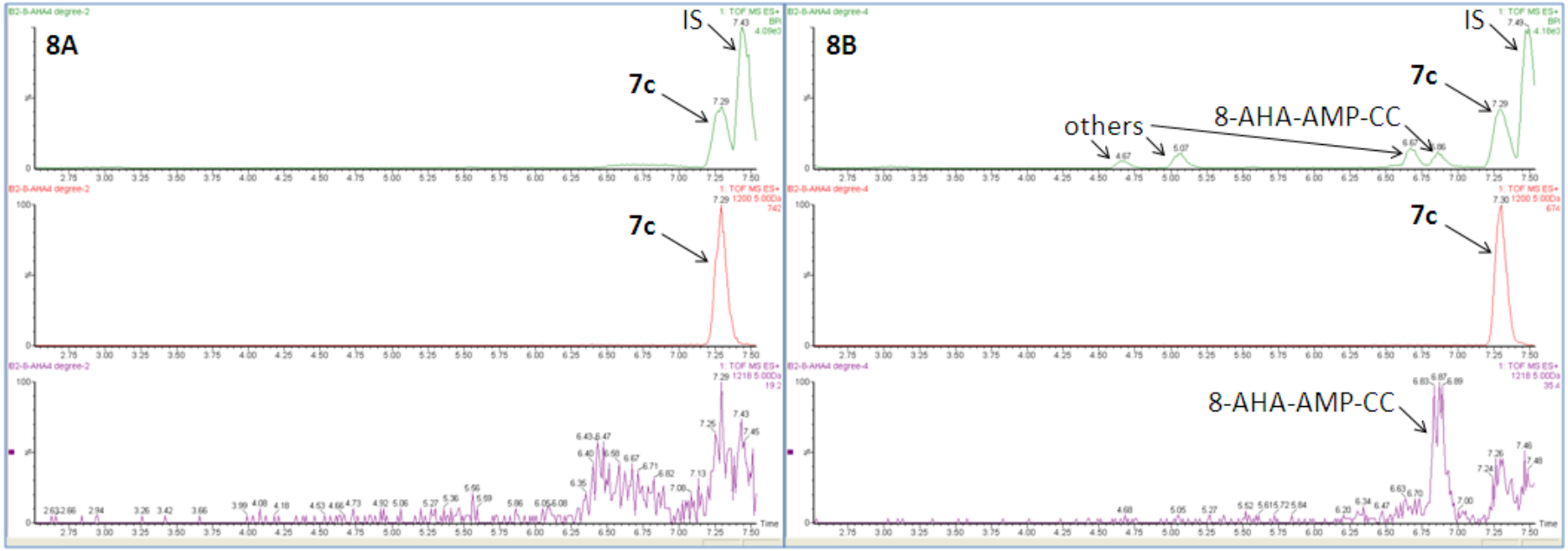


Figure F8. Stability of 8-AHA-cAMP-CC (**7c**) in HepG2 cell lysate at 4 °C, (F8-A) after 30 min, (F8-B) after 300 min. (green lane: BPI (IS = internal standard), red lane: extracted mass for **7c** (1200.0), pink lane: extracted mass for the hydrolysis product 8-AHA-AMP-CC (1218.0))

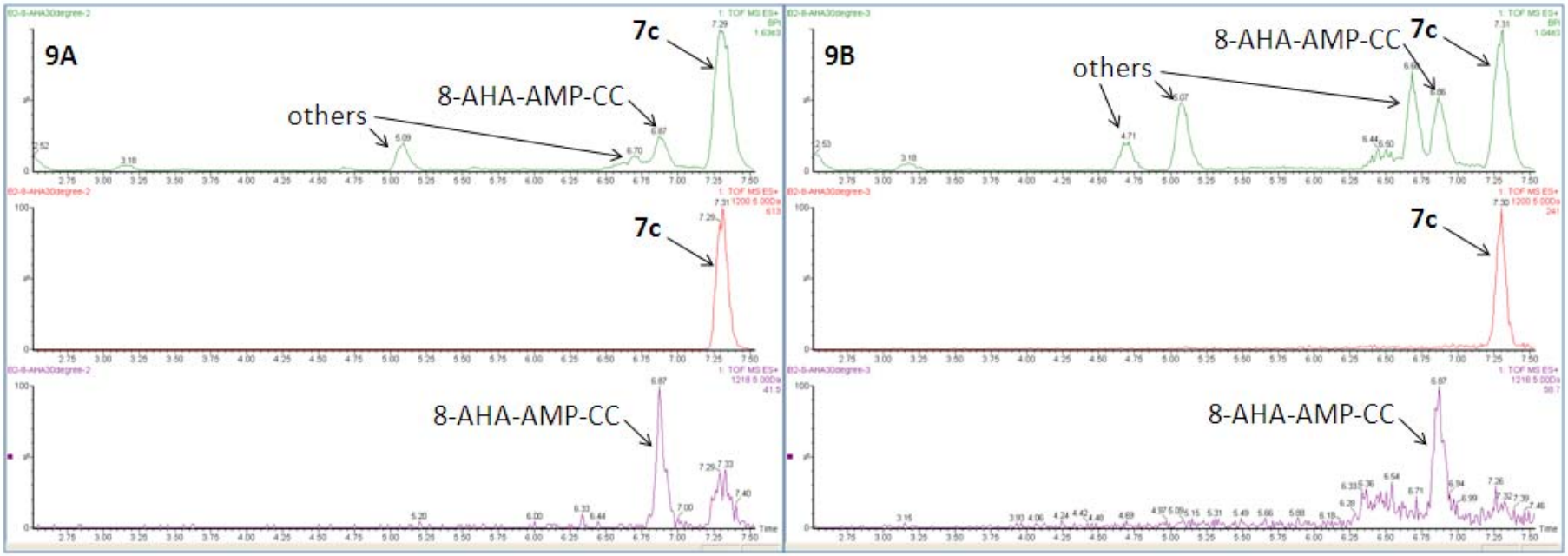


Figure F9. Stability of 8-AHA-cAMP-CC (**7c**) in HepG2 cell lysate at 30 °C, (F9-A) after 60 min, (F9-B) after 180 min. (green lane: BPI, red lane: extracted mass for **7c** (1200.0), pink lane: extracted mass for the hydrolysis product 8-AHA-AMP-CC (1218.0))

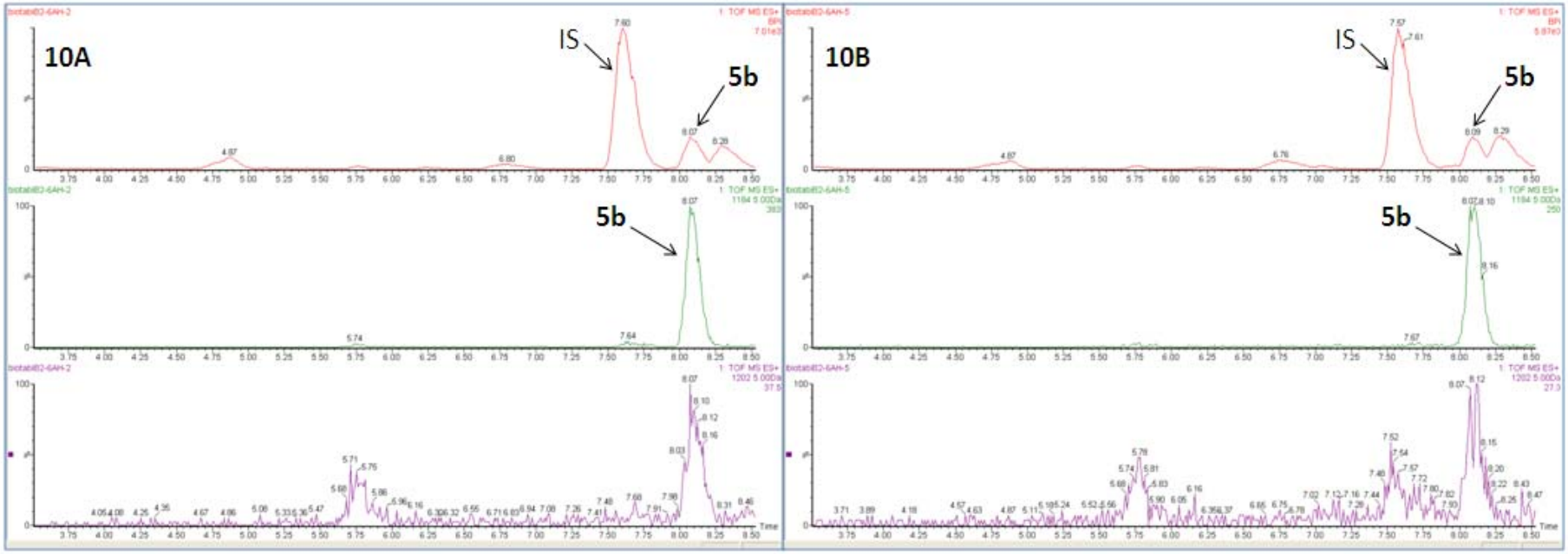


Figure F10. Stability of 6-AH-cAMP-CC (**5b**) in *E. coli* cell lysate at 37 °C, (F10-A) at 0 min, (F10-B) after 120 min. (green lane: BPI (IS = internal standard), red lane: extracted mass for **5b** (1184.0), pink lane: extracted mass for the hydrolysis product 6-AH-AMP-CC (1202.0))

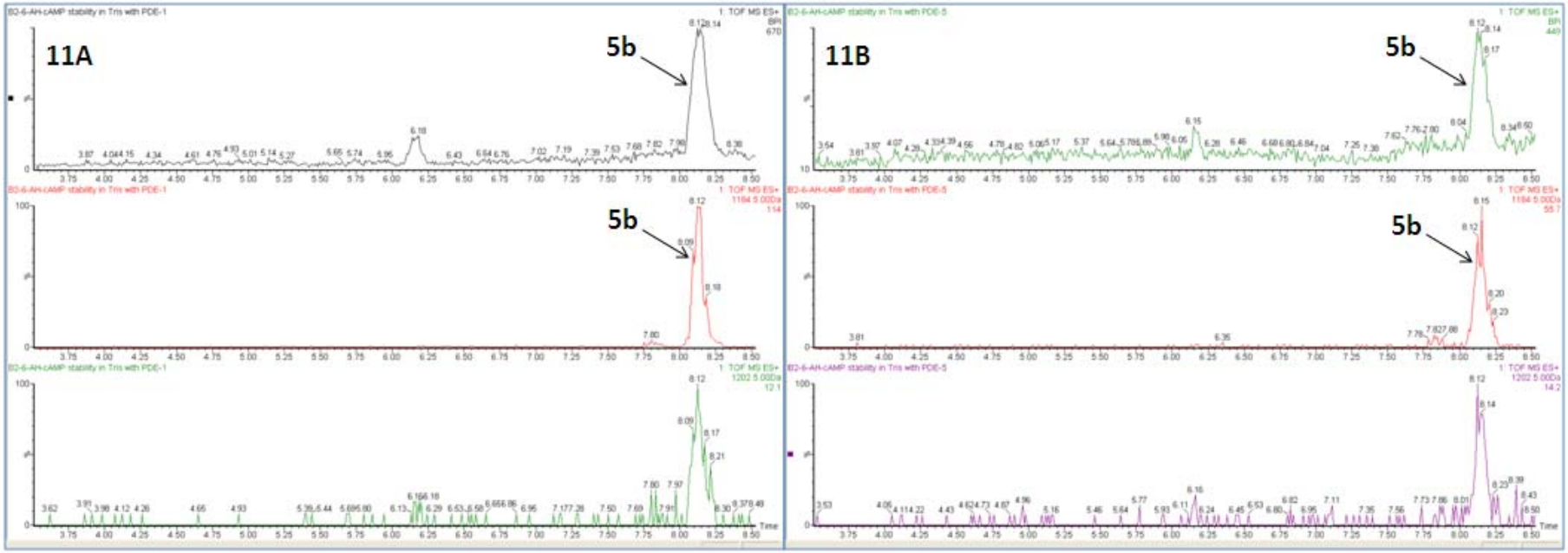


Figure F11. Stability of 6-AH-cAMP-CC (**5b**) in PDE (Tris-buffer) at 30 °C, (F11-A) at 0 min, (F11-B) after 120 min. (green lane: BPI, red lane: extracted mass for **5b** (1184.0), pink lane: extracted mass for the hydrolysis product 6-AH-AMP-CC (1202.0))

4. Fluorescence polarization (FP) competitive binding experiment

Material. Adenosine-3',5'-cyclic monophosphate (cAMP), 8-(2- [fluoresceinyl]aminoethylthio)adenosine-3',5'-cyclic monophosphate (8-Fluo-cAMP), and 8-(2- aminoethylamino)adenosine-3',5'-cyclic monophosphorothioate, Sp-isomer, immobilized on agarose (Sp-8-AEA-cAMPS-agarose) were purchased from Biolog Life Science Institute (Bremen, Germany). Fine chemicals (research grade) were purchased from Sigma (Deisenhofen, Germany). Recombinant human regulatory subunit Iα (hRIα) of the cAMP dependent protein kinase was overexpressed in Escherichia coli BL21 (DE3) RIL (Novagen) and purified following the procedure from Bertinetti *et al.* (R-1) using Sp-8-AEA-cAMPS-agarose. The purity of the hRIα was confirmed by SDS-PAGE and the biological activity of the protein was checked as described before (R-2).

Method. The fluorescence polarization (FP) competitive binding experiment was performed following in principle the procedure from Moll *et al*. (R-3). FP measurements were performed in 150 mM NaCl, 20 mM MOPS, 0.005% (v/v) CHAPS, pH 7 using the Fusion™ α-FP microtiterplate reader. Increasing concentrations of cyclic nucleotide (cAMP or Capture Compound, ranging from 3 pM to 5 µM) were mixed with 1 nM 8-Fluo-cAMP and 2.5 nM of the hRIα in a 384 well microtiterplate (Perkin Elmer, Optiplate, black). The fluorescence polarization signal was detected at 20°C for 2 seconds at Ex 485 nm / Em FP Filter 535 nm with a PMT Voltage of 1,100. Data were analyzed with GraphPad Prism 5.01 (GraphPad Software, San Diego, CA) by plotting the normalized polarization signal against the logarithm of the cyclic nucleotide concentration.

5. Determination of capture yields of bovine PKARII and human PKARIα with Capture Compound 7c

The capture yields of bovine PKARII and human PKARIα with Capture Compound **7c** have been determined by triplicate capture experiments which were compared on two SDS-PAGE gels with concentration series of the corresponding PKA (Figure F12). The gel bands were analyzed using the Image J software (http://rsbweb.nih.gov/ij/). The capture yields for bovine PKARII and human PKARIα were estimated as follows:

1. PKARII (Figure F12, left column): The mean plotted area of PKARII is 13194 (15227, 11204, 13153), the zero offset is 748. The adjusted mean is 12463. This value corresponds to 0.113 µg of PKARII, thus **23%** of the initial 0.5 µg.
2. PKARIα (Figure F12, right column): The mean plotted area of PKARIα is 8100 (8981, 6314, 9004), the zero offset is 1249. The adjusted mean is 6851. This value corresponds to 0.184 µg of PKARIα, thus **37%** of the initial 0.5 µg.

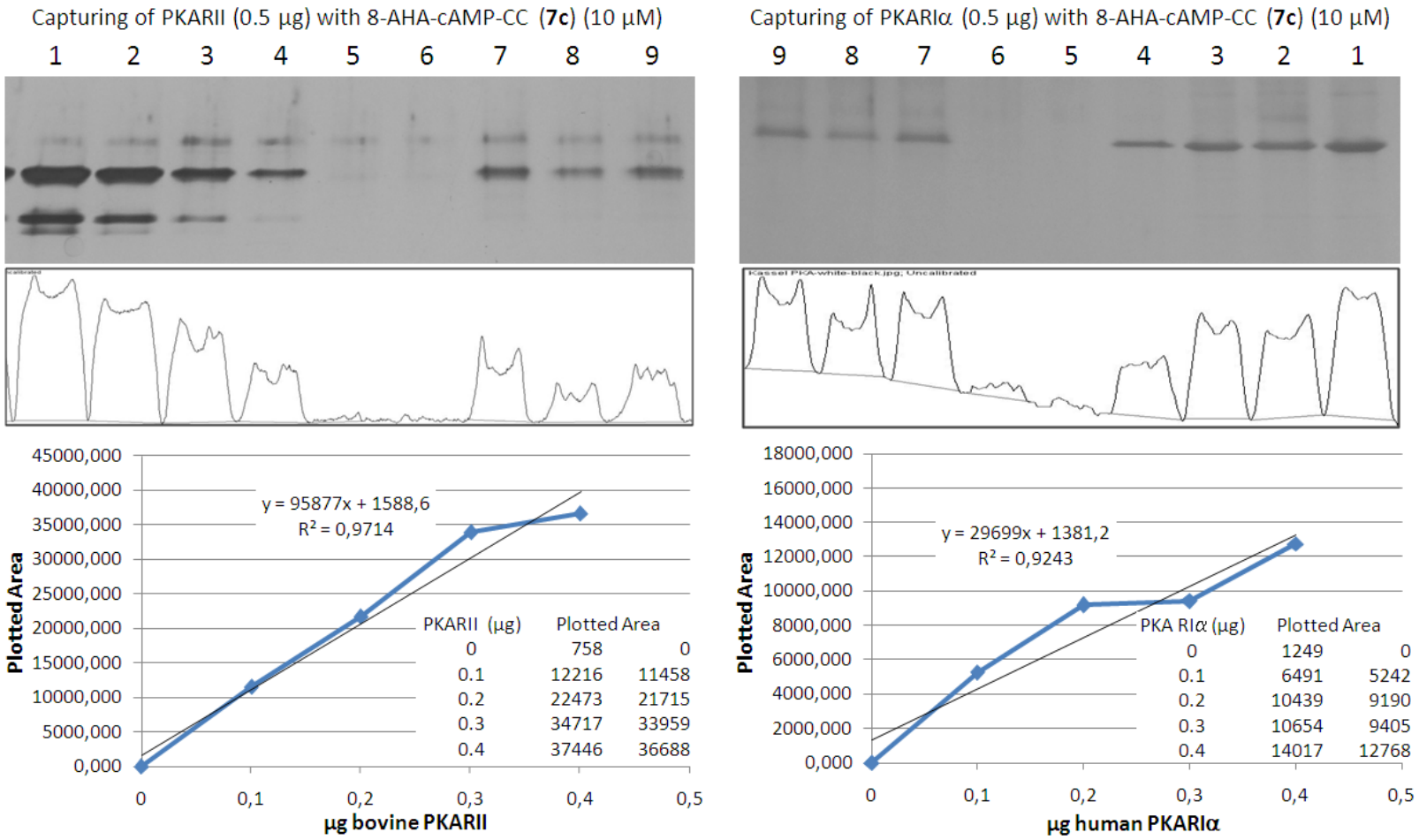


Figure F12. Determination of the capture yield of bovine PKARII (left column) and human PKARIα (right column) with Capture Compound (**7c**). **Top**: SDS-PAGE: Lane 1: 0.4 µg PKAR, lane 2: 0.3 µg PKAR, lane 3: 0.2 µg PKAR, lane 4: 0 .1 µg PKAR, lane 5: 0 µg PKAR, lane 6: repetition 0 µg PKAR, lane 7: 10 µM 8-AHA-cAMP-CC (**7c**) capturing PKAR (0.5 µg, run 1), lane 8: 10 µM 8-AHA-cAMP-CC (**7c**) capturing PKAR (0.5 µg, run 2), lane 9: 10 µM 8-AHA-cAMP-CC (**7c**) capturing PKAR (0.5 µg, run 3). **Middle**: Quantitative analyses of the gel bands using Image J software. **Bottom**: Plotted areas and normalized graph for the two regulatory subunits of the cAMP-dependent protein kinase.

6. List of identified peptides and their corresponding precursor $[MH]^+$, charge state and modification originating from the five cAMP-dependent proteins identified by capturing in HepG2 lysate (corresponding to Table 2)[a]

No.	Protein	Run	Identified Peptide(s)[b]	$[MH]^+$	Charge	Modification	Other Protein(s)[c]
1	KAP0	1	(R)EDEISPPPPNPVVK(G)	1516,78	2		
			(R)GAISAEVYTEEDAASYVR(K)	1929,89	2		
			(K)HNIQALLK(D)	935,55	2		
			(R)ILmGSTLR(K)	905,50	2	Oxidation (+16)	KAP1
			(R)LTVADALEPVQFEDGQK(I)	1858,93	2		
			(K)MYEEFLSK(V)	1045,48	1		KAP1
			(K)NVLFSHLDDNER(S)	1457,69	2		
			(K)QIQNLQK(A)	870,49	1		
			(R)RSENEEFVEVGR(L)	1449,68	2		
			(K)TMAALAK(A)	704,39	1		CHM1B
			(K)VSILESLDK(W)	1002,56	1		
			(K)VSILESLDKWER(L)	1473,78	2		
		2	(R)EDEISPPPPNPVVK(G)	1516,78	2		
			(R)GAISAEVYTEEDAASYVR(K)	1929,90	2		
			(K)HNIQALLK(D)	935,55	2		
			(R)KMYEEFLSK(V)	1173,57	2		
			(R)LGPSDYFGEIALLMNR(P)	1794,90	2		RS15A
			(R)LGPSDYFGEIALLMNRPR(A)	2048,05	3		
			(R)LTVADALEPVQFEDGQK(I)	1859,94	2		
			(K)LWGIDR(D)	758,41	1		
			(K)MYEEFLSK(V)	1045,48	2		KAP1
			(K)NVLFSHLDDNER(S)	1457,69	2		
			(R)RILMGSTLR(K)	1045,61	2		KAP1
			(R)RSENEEFVEVGR(L)	1449,68	2		
			(R)SENEEFVEVGR(L)	1293,58	2		

			(R)TDSREDEISPPPPNPVVK(G)	1975,98	2		
			(K)TMAALAK(A)	704,39	1		CHM1B
			(K)VSILESLDKWER(L)	1473,78	2		
		3	(R)GAISAEVYTEEDAASYVR(K)	1929,89	2		
			(K)IVVQGEPGDEFFIILEGSAAVLQR(R)	2586,37	3		
			(R)LTVADALEPVQFEDGQK(I)	1858,93	2		
			(-)MEsGSTAASEEAR(S)	1366,57	2	Acetyl (+42)	
			(K)MYEEFLSK(V)	1045,48	2		KAP1
			(K)NVLFSHLDDNER(S)	1457,69	2		
			(R)SENEEFVEVGR(L)	1293,58	2		
			(R)TDSREDEISPPPPNPVVK(G)	1975,99	2		
2	KAP1	1	(K)VSILESLEK(W)	1016,57	2		
		2	(R)SPNEEYVEVGR(L)	1277,59	2		
			(K)VSILESLEK(W)	1016,57	2		
3	KAP2	1	(R)AASAYAVGDVK(C)	1050,53	2		
			(R)AATIVATSEGSLWGLDR(V)	1745,90	2		
			(K)ADEHVIDQGDDGDNFYVIER(G)	2306,01	2		
			(R)APASVLPAATPR(Q)	1149,65	2		
			(K)DGGNQEVEIAR(C)	1186,56	2		
			(K)GDSEsEEDEDLEVPVPSR(F)	2067,81	2	Phospho (+80)	
			(R)GSFGELALMYNTPR(A)	1554,75	2		
			(R)GTYDILVTK(D)	1008,55	2		
			(K)IVDVIGEK(I)	871,50	2		
			(R)KMFESFIESVPLLK(S)	1666,90	2		
			(K)MFESFIESVPLLK(S)	1538,81	2		
			(R)NISHYEEQLVK(M)	1358,68	2		PGAM1, MACF1
			(K)NLDQEQLSQVLDAMFER(I)	2034,97	2		

			(K)SNKDGGNQEVEIAR(C)	1515,73	3		
			(R)SVGQYDNR(G)	937,42	2		
		2	(R)AASAYAVGDVK(C)	1050,53	2		
			(R)AATIVATSEGSLWGLDR(V)	1745,90	2		
			(K)ADEHVIDQGDDGDNFYVIER(G)	2306,01	2		
			(R)APASVLPAATPR(Q)	1149,65	2		
			(K)DGGNQEVEIAR(C)	1186,56	2		
			(K)DILLFK(N)	747,45	1		
			(K)GDSESEEDEDLEVPVPSR(F)	1987,85	2		
			(K)GQYFGELALVTNKPR(A)	1691,90	3		
			(R)GSFGELALMYNTPR(A)	1554,75	3		
			(R)GTYDILVTK(D)	1008,55	2		
			(K)IVDVIGEK(I)	871,50	2		
			(K)MFESFIESVPLLK(S)	1538,81	2		
			(R)NISHYEEQLVK(M)	1358,68	2		PGAM1, MACF1
			(K)NLDQEQLSQVLDAMFER(I)	2034,97	2		
			(R)QQPPDLVEFAVEYFTR(L)	1937,95	2		
			(R)qSLGHPPPEPGPDR(V)	1465,69	2	Pyro-cmC (-17)	RL4
			(R)SVGQYDNR(G)	937,42	2		
			(R)VADAKGDSESEEDEDLEVPVPSR(F)	2472,11	3		
		3	(K)ADEHVIDQGDDGDNFYVIER(G)	2306,01	3		
			(K)GQYFGELALVTNKPR(A)	1691,90	3		
			(R)IVKADEHVIDQGDDGDNFYVIER(G)	2646,25	3		
			(K)MFESFIESVPLLK(S)	1538,80	3		
			(R)NISHYEEQLVK(M)	1358,68	2		PGAM1, MACF1
			(K)NLDQEQLSQVLDAMFER(I)	2034,97	2		
			(R)QQPPDLVEFAVEYFTR(L)	1937,95	2		
			(K)SNKDGGNQEVEIAR(C)	1515,73	3		

4	AKAP1	1	(K)DDAAPAPPVADAK(A)	1236,60	2		
			(R)ETGGAEGTGDAVLGEK(V)	1489,69	2		
			(R)GVEGELGNEESLDR(N)	1502,69	2		
			(K)VLEEALLSR(E)	1028,59	2		
			(K)VVSTPPSVTEPPEK(E)	1465,76	2		
		2	(K)DDAAPAPPVADAK(A)	1236,60	2		
			(R)EHVLELENSK(G)	1196,60	2		
			(R)ETGGAEGTGDAVLGEK(V)	1489,69	2		
			(R)FIESAHTELAK(D)	1244,64	2		
			(R)KVQPGYPVVPAEK(R)	1410,79	2		
			(K)VLEEALLSR(E)	1028,59	2		
5	AKAP9	1	(K)LqVLPqK(A)	826,48	1	Deamidation (+1), Deamidation (+1)	
		2	(R)LLQLESTVSAK(D)	1187,67	2		
			(R)VTDESIPSYSGSDMPR(N)	1739,77	2		
			(K)VTEEGTELSQR(L)	1247,60	2		
			(K)LqVLPqK(A)	826,48	1	Deamidation (+1), Deamidation (+1)	

[a] Scaffold (version Scaffold_02_04_00, proteome Software, Portland, OR, USA) was used to compile and assign probability scores. All peptides were identified with a ≥95% probability as specified by the Peptide Prophet algorithm (R-4).

[b] Modified amino acids are displayed in small letters. The modification is listed in a subsequent column.

[c] The column 'other proteins' listed alternative proteins for an identified peptide sequence. In general, proteins that comprise similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony.

7. List of identified peptides and their corresponding precursor $[MH]^+$, charge state and modification originating from the 13 nucleotide-binding proteins identified by capturing in HepG2 lysate (corresponding to Table 3)[a]

No.	Protein	Run	Identified Peptide(s)[b]	$[MH]^+$	Charge	Modification	Other Protein(s)[c]
1	RL6	1	(R)ASITPGTILIILTGR(H)	1.524,92	3		
			(K)AVDSQILPK(I)	969,55	2		
			(R)HQEGEIFDTEK(E)	1.331,60	2		
			(K)KPFSQHVR(K)	997,54	2		
			(K)VLATVTKPVGGDK(N)	1.283,74	2		
		2	(R)ASITPGTILIILTGR(H)	1.524,92	2		
			(K)HLTDAYFK(K)	993,49	2		
			(K)QLASGLLLVTGPLVLNR(V)	1.763,07	2		
			(K)VLATVTKPVGGDK(N)	1.283,74	2		
		3	(K)HLTDAYFK(K)	993,49	2		
			(K)QLASGLLLVTGPLVLNR(V)	1.763,07	2		
			(K)VLATVTKPVGGDK(N)	1.283,74	2		
2	RL18A	1	(R)AHSIQIMK(V)	926,50	2		
			(R)FWYFVSQLKK(M)	1.344,72	3		
			(R)IFAPNHVVAK(S)	1.094,62	2		
			(K)NFGIWLR(Y)	904,49	2		
			(K)RPNTFF(-)	780,39	1		
			(K)SRFWYFVSQLKK(M)	1.587,85	3		
			(K)VEEIAASK(C)	845,45	1		
		2	(R)AHSIQIMK(V)	926,50	2		

			(R)FWYFVSQLK(K)	1.216,63	2		
			(R)IFAPNHVVAK(S)	1.094,62	2		
			(K)NFGIWLR(Y)	904,49	2		
			(K)RPNTFF(-)	780,39	1		
			(R)SGTHNMYR(E)	964,42	2		
			(K)VEEIAASK(C)	845,45	1		
		3	(R)AHSIQIMKVEEIAASK(C)	1.753,94	3		
			(K)VEEIAASK(C)	845,45	2		
3	NCPR	1	(K)ATTPVImVGPGTGVAPFIGFIQER(A)	2.473,30	3	Oxidation (+16)	
			(K)GVATNWLR(A)	915,49	2		
			(K)NPFLAAVTTNR(K)	1.202,64	2		
		2	(K)ATTPVIMVGPGTGVAPFIGFIQER(A)	2.457,31	2		
			(K)VYVQHLLK(Q)	998,59	2		
			(R)YESGDHVAVYPANDSALVNQLGK(I)	2.446,17	3		
		3	(K)ATTPVIMVGPGTGVAPFIGFIQER(A)	2.457,31	3		
			(R)TNVLYELAQYASEPSEQELLRK(M)	2.580,31	3		
4	GNL3	1	(K)ENLESWLNYLK(K)	1.407,70	2		
			(R)NSLEFFTMLAQR(R)	1.455,72	3		
			(K)SGFNLEELEK(N)	1.164,57	2		
			(R)SPASIEVVKPMEAASAILSQADAR(Q)	2.440,26	3		
			(R)VGVIGFPNVGK(S)	1.085,62	2		
			(K)VIEASDVVLEVLDAR(D)	1.626,88	3		
		2	(R)NSLEFFTMLAQR(R)	1.455,72	2		
			(R)SPASIEVVKPMEAASAILSQADAR(Q)	2.440,26	3		
			(R)VGVIGFPNVGK(S)	1.085,62	2		

			(K)VIEASDVVLEVLDAR(D)	1.626,88	2		
		3	(R)SPASIEVVKPMEAASAILSQADAR(Q)	2.440,26	3		
5	LU	1	(R)LEVPVEMNPEGYMTSR(T)	1.850,85	2		
			(K)LSWSQLGGSPAEPIPGR(Q)	1.750,90	2		
			(R)SPPYQLDSQGR(L)	1.246,59	2		
			(R)VAYLDPLELSEGK(V)	1.432,75	2		
			(R)VEDYDAADDVQLSK(T)	1.566,70	2		
		2	(R)AGAAGTAEATAR(L)	1.045,51	2		
			(R)LVLAEAQVGDER(D)	1.298,68	2		
			(R)VAYLDPLELSEGK(V)	1.432,74	2		
			(R)VEDYDAADDVQLSK(T)	1.566,70	2		
		3	(R)SPPYQLDSQGR(L)	1.246,60	2		
6	M6PBP	1	(K)DTVATQLSEAVDATR(G)	1.575,77	2		
			(K)SVVTGGVQSVMGSR(L)	1.362,69	2		
			(R)TLTAAAVSGAQPILSK(L)	1.526,87	2		
			(K)VSGAQEMVSSAK(D)	1.192,57	2		
		2	(K)SVVTGGVQSVMGSR(L)	1.362,69	2		
			(R)TLTAAAVSGAQPILSK(L)	1.526,87	2		
			(K)VSGAQEMVSSAK(D)	1.192,57	2		
		3	(R)TLTAAAVSGAQPILSK(L)	1.526,87	2		
7	VDAC2	1	(K)LTFDTTFSPNTGK(K)	1.427,69	2		
			(K)LTLSALVDGK(S)	1.015,59	2		

		2	(K)LTFDTTFSPNTGK(K)	1.427,69	2		
			(K)LTLSALVDGK(S)	1.015,59	2		
			(K)VTGTLETK(Y)	847,46	1		
			(K)YQLDPTASISAK(V)	1.292,66	2		
		3	(R)NNFAVGYR(T)	939,46	2		
8	IMMT	1	(R)ELDSITPEVLPGWK(G)	1.582,82	2		
			(K)EVAGAKPHITAAEGK(L)	1.477,79	3		OTU7B, 2AAA, SAS10, SPS2L
			(K)LSEQELQFR(R)	1.148,58	2		
			(K)SEFEQNLSEK(L)	1.209,55	2		
		2	(K)SEFEQNLSEK(L)	1.209,55	2		
			(K)VVSQYHELVVQAR(D)	1.526,82	3		
		3	(R)QTASVTLQAIAAQNAAVQAVNAHSNILK(A)	2.831,53	3		
			(K)TIPYSDKLFEMVLGPAAYNVPLPK(K)	2.662,41	3		
9	TXTP	1	(R)FGMFEFLSNHMR(D)	1.514,68	3		
			(K)FIHDQTSPNPK(Y)	1.282,63	2		
			(K)GTYQGLTATVLK(Q)	1.250,69	2		
		2	(R)FGMFEFLSNHMR(D)	1.514,68	3		
			(K)FIHDQTSPNPK(Y)	1.282,63	2		
			(R)GLSSLLYGSIPK(A)	1.233,70	2		
			(K)GTYQGLTATVLK(Q)	1.250,69	2		
			(R)SHGVLGLYR(G)	1.000,55	2		
10	NDUS3	1	(K)QLSAFGEYVAEILPK(Y)	1.663,88	2		
			(R)VVAEPVELAQEFR(K)	1.485,78	2		

		2	(K)LEAGDKKPDAK(-)	1.170,62	2		
			(K)QLSAFGEYVAEILPK(Y)	1.663,88	2		
			(R)VVAEPVELAQEFR(K)	1.485,78	2		
		3	(R)ESAGADTRPTVRPR(N)	1.511,78	3		
			(K)TYTDELTPIESAVSVFK(A)	1.898,96	2		
11	SSRD	1	(R)FFDEESYSLLR(K)	1.404,66	2		
			(K)SAHAGTYEVR(F)	1.089,52	2		
			(R)VQNMALYADVGGK(Q)	1.364,67	2		
		2	(R)FFDEESYSLLR(K)	1.404,66	2		
			(R)NNEDISIIPPLFTVSVDHR(G)	2.165,11	3		
			(K)SAHAGTYEVR(F)	1.089,52	2		
			(R)VQNMALYADVGGK(Q)	1.364,68	2		
		3	(R)NNEDISIIPPLFTVSVDHR(G)	2.165,11	3		
12	STML2	1	(K)AEQINQAAGEASAVLAK(A)	1.669,86	2		
			(K)APVPGTPDSLSSGSSR(D)	1.513,74	2		
			(R)ATVLESEGTR(E)	1.061,53	2		
			(R)DVQGTDASLDEELDR(V)	1.661,74	2		
		2	(K)AEQINQAAGEASAVLAK(A)	1.669,86	2		
			(K)APVPGTPDSLSSGSSR(D)	1.513,74	2		
			(R)ATVLESEGTR(E)	1.061,53	2		
			(R)ILEPGLNILIPVLDR(I)	1.674,01	2		
			(R)NTVVLFVPQQEAWVVER(M)	2.013,07	2		
		3	(R)ILEPGLNILIPVLDR(I)	1.674,01	2		

13	NDK8[d]	1	(R)VMLGETNPADSK(P)	1.260,60	2		
		2	(R)NIIHGSDSVK(S)	1.068,56	2		
			(R)VMLGETNPADSK(P)	1.260,60	2		
			(K)YMNSGPVVAMVWEGLNVVK(T)	2.092,05	2		
		3	(K)DRPFFPGLVK(Y)	1.174,65	2		

[a]Scaffold (version Scaffold_02_04_00, proteome Software, Portland, OR, USA) was used to compile and assign probability scores. All peptides were identified with a ≥95% probability as specified by the Peptide Prophet algorithm (R-4).

[b] Modified amino acids are displayed in small letters. The modification is listed in a subsequent column.

[c] The column 'other proteins' listed alternative proteins for an identified peptide sequence. In general, proteins that comprise similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony.

[b] Some peptide sequences suitable for NDK8 and NDKA were associated with NDKA by the Peptide Prophet algorithm and are not displayed in this table.

8. List of identified peptides and their corresponding precursor $[MH]^+$, charge state and modification originating from the nine cAMP-dependent proteins identified by capturing in rat synaptosome preparations (corresponding to Table 4)[a]

No.	Protein	Run	Identified Peptide[b]	$[MH]^+$	Charge	Modification	Other Protein(s)[c]
1	KAP0	1	(R)GAISAEVYTEEDAASYVR(K)	1929,90	2		
			(R)KMYEEFLSK(V)	1173,57	2		
			(R)LGPSDYFGEIALLMNRPR(A)	2048,05	3		
			(R)LTVADALEPVQFEDGQK(I)	1858,93	2		
			(K)LWGIDRDSYR(R)	1279,63	2		
			(K)MYEEFLSK(V)	1045,48	2		
			(K)NVLFSHLDDNER(S)	1457,69	2		
			(R)QIQSLQK(S)	843,48	1		
			(R)RSENEEFVEVGR(L)	1449,68	2		
			(K)VSILESLDKWER(L)	1473,78	2		
		2	(R)GAISAEVYTEEDAASYVR(K)	1929,90	2		
			(R)KMYEEFLSK(V)	1173,57	2		
			(K)MYEEFLSK(V)	1045,48	2		
			(R)RSENEEFVEVGR(L)	1449,68	2		
			(K)VSILESLDKWER(L)	1473,78	2		
		3	(K)MYEEFLSK(V)	1045,48	2		
		4	(R)KMYEEFLSK(V)	1173,57	2		
			(R)LTVADALEPVQFEDGQK(I)	1858,93	2		
			(K)MYEEFLSK(V)	1045,48	2		
			(R)RSENEEFVEVGR(L)	1449,69	2		
			(K)VSILESLDKWER(L)	1473,78	3		

2	KAP2	1	(R)AASAYAVGDVK(C)	1050,53	2		
			(R)AATIVATSDGSLWGLDR(V)	1731,88	2		
			(K)DILLFK(N)	747,45	1		
			(K)GQYFGELALVTNKPR(A)	1691,90	3		KAP3
			(R)GSFGELALMYNTPR(A)	1554,75	2		KAP3
			(R)GTYDILVTK(D)	1008,55	1		
			(R)GTYDILVTKDNQTR(S)	1622,83	2		
			(K)IVDVIGEK(I)	871,50	1		
			(K)IVKTDEHVIDQGDDGDNFYVIER(G)	2676,27	3		
			(R)KMFESFIESVPLFK(S)	1700,88	3		
			(K)MFESFIESVPLFK(S)	1572,79	2		
			(R)mKIVDVIGEK(I)	1146,63	2	Oxidation (+16)	
			(R)NISHYEEQLVK(M)	1358,68	2		
			(K)NLDQEQLSQVLDAMFEK(I)	2006,96	2		
			(R)QQPPDLVDFAVEYFTR(L)	1923,94	2		
			(R)SVGQYDNR(G)	937,42	2		EF2
			(K)TDEHVIDQGDDGDNFYVIER(G)	2336,02	3		
		2	(R)AASAYAVGDVK(C)	1050,53	2		
			(R)AATIVATSDGSLWGLDR(V)	1731,88	2		
			(K)GQYFGELALVTNKPR(A)	1691,90	3		
			(R)GSFGELALMYNTPR(A)	1554,75	3		KAP3
			(R)GTYDILVTK(D)	1008,55	2		KAP3
			(R)GTYDILVTKDNQTR(S)	1622,82	2		
			(K)IVDVIGEK(I)	871,50	1		
			(K)IVDVIGEKIYK(D)	1275,74	2		
			(R)KMFESFIESVPLFK(S)	1700,88	2		
			(K)MFESFIESVPLFK(S)	1572,79	2		
			(R)mKIVDVIGEK(I)	1146,63	2	Oxidation (+16)	
			(R)NISHYEEQLVK(M)	1358,68	2		
			(K)NLDQEQLSQVLDAMFEK(I)	2006,96	3		

			(R)QQPPDLVDFAVEYFTR(L)	1923,94	2		
			(K)RNISHYEEQLVK(M)	1514,78	3		
			(R)SVGQYDNR(G)	937,42	1		EF2
		3	(R)AASAYAVGDVK(C)	1050,53	2		
			(R)AATIVATSDGSLWGLDR(V)	1731,88	2		
			(K)DILLFK(N)	747,45	2		
			(R)GTYDILVTK(D)	1008,55	2		
			(R)KmFESFIESVPLFK(S)	1716,88	3	Oxidation (+16)	
			(K)MFESFIESVPLFK(S)	1572,79	2		
			(K)mFGSNLDLLDPGQ(-)	1421,65	2	Oxidation (+16)	
			(R)NISHYEEQLVK(M)	1358,68	2		
			(K)NLDQEQLSQVLDAMFEK(I)	2006,96	2		
			(R)SVGQYDNR(G)	937,42	2		EF2
		4	(R)AASAYAVGDVK(C)	1050,54	2		
			(R)AATIVATSDGSLWGLDR(V)	1731,88	2		
			(R)GTYDILVTK(D)	1008,55	2		
			(R)GTYDILVTKDNQTR(S)	1622,83	3		
			(K)IVDVIGEK(I)	871,50	2		
			(K)IVKTDEHVIDQGDDGDNFYVIER(G)	2676,27	3		
			(K)mFESFIESVPLFK(S)	1588,79	2	Oxidation (+16)	
			(R)NISHYEEQLVK(M)	1358,68	2		
			(K)NLDQEQLSQVLDAMFEK(I)	2006,97	2		
			(R)SVGQYDNR(G)	937,42	2		EF2
			(K)TDEHVIDQGDDGDNFYVIER(G)	2336,02	3		
3	KAP3	1	(R)AASAHAIGTVK(C)	1024,57	1		
			(R)AATITATSPGALWGLDR(V)	1699,89	2		
			(K)EGEHVIDQGDDGDNFYVIDR(G)	2291,99	3		
			(R)GTFDIYVK(C)	941,48	2		

		(R)HQPADLLEFALQHFTR(L)	1921,98	3	
		(R)KMYESFIESLPFLK(S)	1730,89	2	
		(R)LKVVDVIGTK(V)	1070,67	2	
		(K)MYESFIESLPFLK(S)	1602,80	2	
		(K)NLDPEQMSQVLDAMFEK(L)	1993,91	2	
		(K)SDIEEnGAVEIAR(C)	1402,66	2	Deamidation (+1)
		(R)TWGDAGAAAGGGTPSK(G)	1402,65	2	
		(K)VVDVIGTK(V)	829,49	1	
	2	(R)AASAHAIGTVK(C)	1024,57	1	
		(R)AATITATSPGALWGLDR(V)	1699,89	2	
		(K)GKSDIEEnGAVEIAR(C)	1587,77	2	Deamidation (+1)
		(R)GTFDIYVK(C)	941,49	2	
		(R)HQPADLLEFALQHFTR(L)	1921,98	3	
		(R)KGKSDIEEnGAVEIAR(C)	1715,87	3	Deamidation (+1)
		(R)KMYESFIESLPFLK(S)	1730,90	3	
		(R)LKVVDVIGTK(V)	1070,67	2	
		(K)MYESFIESLPFLK(S)	1602,80	2	
		(K)NLDPEQMSQVLDAMFEK(L)	1993,91	3	
		(K)SDIEEnGAVEIAR(C)	1402,66	2	Deamidation (+1)
		(R)TWGDAGAAAGGGTPSK(G)	1402,64	2	
		(K)VVDVIGTK(V)	829,49	1	
		(K)VYNDGEQIIAQGDSADSFFIVESGEVR(I)	2944,38	3	
	3	(R)AASAHAIGTVK(C)	1024,57	2	
		(R)AATITATSPGALWGLDR(V)	1699,89	2	
		(K)MYESFIESLPFLK(S)	1602,80	2	
		(K)NLDPEQMSQVLDAMFEK(L)	1993,91	2	
		(K)SDIEEnGAVEIAR(C)	1402,66	2	Deamidation (+1)
		(R)TWGDAGAAAGGGTPSK(G)	1402,65	2	

		4	(R)AASAHAIGTVK(C)	1024,57	2		
			(R)AATITATSPGALWGLDR(V)	1699,89	2		
			(K)EGEHVIDQGDDGDNFYVIDR(G)	2291,99	3		
			(K)GVNFAEEPMRSDSEnGEEEEAAEAGAFNAPVINR(F)	3636,59	3	Deamidation (+1)	
			(R)LKVVDVIGTK(V)	1070,67	2		
			(R)LQQENER(K)	915,44	2		
			(K)NLDPEQmSQVLDAMFEK(L)	2009,91	2	Oxidation (+16)	
			(K)SDIEEnGAVEIAR(C)	1402,66	2	Deamidation (+1)	
			(R)TWGDAGAAAGGGTPSK(G)	1402,65	2		
4	AKAP5	1	(K)AQIQPDEQATQAK(S)	1426,70	2		
			(K)ATMGQAEEATVGHIEK(T)	1670,79	2		
			(K)ETVLSQAEEVK(L)	1231,63	2		
			(K)ETVLSQAEEVKLSQIEEPAISQAK(K)	2626,36	3		
			(K)LSQAEEATVAQAK(E)	1344,69	2		
			(K)LSQIEEPAISQAK(K)	1412,75	2		
			(-)METSVSEIQIETK(D)	1493,73	2		
			(K)QKPSEAEMQPEDGALPK(K)	1853,88	3		
			(R)TSEQYETLLIETASSLVK(N)	2011,04	2		
			(R)VQGEADDLEIK(A)	1215,60	2		
		2	(K)AQIQPDEQATQAK(S)	1426,70	2		
			(K)DEKRPEAASPQK(E)	1354,68	3		
			(K)DEKRPEAASPQKER(Q)	1639,83	3		
			(K)ETVLSQAEEVKLSQIEEPAISQAK(K)	2626,37	3		
			(R)HASDLKVnGVDAEKPR(S)	1735,88	4	Deamidation (+1)	
			(R)HASDLKVnGVDAEKPRSEESK(R)	2296,13	3	Deamidation (+1)	
			(K)LSQAEEATVAQAK(E)	1344,69	2		
			(K)LSQIEEPAISQAK(K)	1412,75	2		
			(K)LTEDSGYVR(V)	1038,50	2		
			(R)LVTHRKPSESAEK(Q)	1480,80	3		

			(-)METSVSEIQIETK(D)	1493,73	2			
			(K)qKPSEAEMQPEDGALPK(K)	1836,86	2	Pyro-cmC (-17)		
			(K)RMEPIAIIITDTEISEFDVK(K)	2319,20	3			
			(K)RMEPIAIIITDTEISEFDVKK(S)	2447,30	3			
			(K)RPEAAsPQKER(Q)	1347,63	3	Phospho (+80)		
			(R)TSEQYETLLIETASSLVK(N)	2011,04	2			
			(R)VQGEADDLEIK(A)	1215,60	2			
		3	(K)AQIQPDEQATQAK(S)	1426,70	2			
			(K)LTEDSGYVR(V)	1038,50	2			
			(R)TSEQYETLLIETASSLVK(N)	2011,04	3			
			(R)VQGEADDLEIK(A)	1215,60	2			
		4	(K)AQIQPDEQATQAK(S)	1426,71	2			
			(K)ATVGQAEEPIVGQAEETVLR(H)	2096,08	2			
5	AKAP18	1	(K)AVQQYLEETQNK(K)	1449,71	2			
			(K)DRKEPEDAELVR(L)	1455,73	3			
			(R)LVENAVLK(A)	884,53	2			
		2	(K)DRKEPEDAELVR(L)	1455,73	3			
			(R)LVENAVLK(A)	884,53	2			
			(K)RLVENAVLK(A)	1040,63	2			
		3	(K)DRKEPEDAELVR(L)	1455,73	3			
		4	(K)AVQQYLEETQNK(K)	1449,71	2			
6	KGP2	1	(K)AGVSAEPTSR(T)	973,48	2			
			(R)DLKPENLILDADGYLK(L)	1815,96	2			
			(K)HSKHPDGQSGNLSNEALR(S)	1945,93	3			

			(K)TLQKGEYFGEK(A)	1298,65	3			
			(R)WLnGFNWEGLK(A)	1363,66	2	Deamidation (+1)		
		2	(R)DLKPENLILDADGYLK(L)	1815,96	2			
			(K)HSKHPDGQSGNLSNEALR(S)	1945,93	3			
			(K)LITDALNKNQFLK(R)	1516,86	2			
			(K)RLDPQQIK(D)	996,57	2			
			(R)SKVAELER(E)	930,51	2			
			(R)TYDLNKPPEFSFEK(A)	1713,82	3			
			(K)VKNENIAFAMK(C)	1263,66	2			
			(K)VKVTQSTEGHDQPQLIK(T)	1907,01	3			
			(K)VTQSTEGHDQPQLIK(T)	1679,85	2			
			(R)WLnGFNWEGLK(A)	1363,66	2	Deamidation (+1)		
		3	(K)ALISDDVR(S)	887,47	2			
			(R)DLKPENLILDADGYLK(L)	1815,96	3			
			(R)TYDLNKPPEFSFEK(A)	1713,82	3			
		4	(K)ALSLEMIQLK(E)	1144,65	2			
			(R)EGEEGSTFFILAK(G)	1426,70	2			
7	HCN1	1	(K)APATGPAAADKR(L)	1124,59	2			
			(K)DLNTGVFNNQENEILK(Q)	1846,91	2			
			(R)ESPSVLNKDPDAEKPR(F)	1780,89	3			
			(R)KLVATMPLFANADPNFVTAMLSK(L)	2478,30	3			
			(K)LVATMPLFANADPNFVTAMLSK(L)	2350,21	3			
			(R)QFTSMLQPGVNK(F)	1348,68	2			
		2	(K)APATGPAAADKR(L)	1124,59	2			
			(K)DLNTGVFNNQENEILK(Q)	1846,91	2			
			(R)ESPSVLNKDPDAEKPR(F)	1780,89	3			

			(K)FQKDLNTGVFNNQENEILK(Q)	2250,12	3		
			(K)LVATMPLFANADPNFVTAMLSK(L)	2350,21	3		
			(K)mYFIQHGVAGVITK(S)	1578,82	2	Oxidation (+16)	
			(R)QFTSMLQPGVNK(F)	1348,68	2		
			(R)TGTVNEDSSEIILDPK(V)	1716,84	2		
		3	(K)APATGPAAADKR(L)	1124,59	3		
			(K)DLNTGVFNNQENEILK(Q)	1846,90	2		
			(R)ESPSVLNKDPDAEKPR(F)	1780,90	3		LRRC7
			(K)mYFIQHGVAGVITK(S)	1578,82	3	Oxidation (+16)	
			(R)TGTVNEDSSEIILDPK(V)	1716,84	2		
		4	(K)DLNTGVFNNQENEILK(Q)	1846,91	2		
			(R)TGTVNEDSSEIILDPK(V)	1716,84	2		
8	HCN2	1	(R)DSASPGAASGLDPLDSAR(S)	1685,78	2		
			(R)EMVQQAELGQR(V)	1287,62	2		
			(K)IHDYYEHR(Y)	1131,51	2		HCN1
			(K)LKFEVFQPGDYIIR(E)	1723,93	3		
			(K)LVASMPLFANADPNFVTAMLTK(L)	2350,20	2		
			(R)LYSLSVDNFNEVLEEYPMMR(R)	2448,13	2		HCN1
			(R)RAPPGPLPPAASPGPPAASPPAAPSSPR(A)	2567,36	3		
			(R)TSPYGVPGSPATR(V)	1288,64	2		
		2	(R)DSASPGAASGLDPLDSAR(S)	1685,79	2		
			(R)EMVQQAELGQR(V)	1287,62	2		
			(R)GAASGPAAAEEAGSEEAGPAGEPR(G)	2137,95	2		
			(R)GSQASFLQR(Q)	992,50	2		
			(K)IHDYYEHR(Y)	1131,51	2		HCN1
			(R)KLVASMPLFANADPNFVTAMLTK(L)	2478,30	3		
			(K)LKFEVFQPGDYIIR(E)	1723,93	3		

			(K)LVASMPLFANADPNFVTAMLTK(L)	2350,21	2		
			(R)LYSLSVDNFNEVLEEYPMMR(R)	2448,14	2		HCN1
			(R)QFGALLQPGVNK(F)	1270,70	2		
			(R)RAPPGPLPPAASPGPPAASPPAAPSSPR(A)	2567,36	3		
		3	(R)AFETVAIDRLDR(I)	1404,74	2		HCN1
			(R)DSASPGAASGLDPLDSAR(S)	1685,79	2		
			(K)FEVFQPGDYIIR(E)	1482,75	2		HCN1
			(K)IHDYYEHR(Y)	1131,51	2		HCN1
			(R)TAAPSPDRR(D)	969,50	3		DUOX1
		4	(R)DSASPGAASGLDPLDSAR(S)	1685,79	2		
			(R)EMVQQAELGQR(V)	1287,62	2		
			(K)FEVFQPGDYIIR(E)	1482,75	2		HCN1
9	RPGF4	1	(K)TFIDNLVNFEK(M)	1338,68	2		
		2	(K)TFIDNLVNFEK(M)	1338,68	2		
			(K)VLLQQFNTGDER(A)	1418,71	2		
		4	(K)VLLQQFNTGDER(A)	1418,72	2		

[a]Scaffold (version Scaffold_02_04_00, proteome Software, Portland, OR, USA) was used to compile and assign probability scores. All peptides were identified with a ≥95% probability as specified by the Peptide Prophet algorithm (R-4).

[b] Modified amino acids are displayed in small letters. The modification is listed in a subsequent column.

[c] The column 'other proteins' listed alternative proteins for an identified peptide sequence. In general, proteins that comprise similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony.

9. List of identified peptides and their corresponding precursor $[MH]^+$, charge state and modification originating from the 13 nucleotide-binding proteins identified by capturing in rat synaptosome preparations (corresponding to Table 5)[a]

No.	Protein	Run	Identified Peptide(s)[b]	$[MH]^+$	Charge	Modification	Other Protein(s)[c]
1	DHE3	1	(K)ALASLmTYK(C)	1012,52	2	Oxidation (+16)	
			(R)DDGSWEVIEGYR(A)	1424,62	2		
			(K)DIVHSGLAYTMER(S)	1490,72	2		
			(K)GFIGPGIDVPAPDMSTGER(E)	1914,91	2		
			(K)HGGTIPVVPTAEFQDR(I)	1722,87	2		H10, NMDE2, B5DFL7, HNRPQ
			(K)IIAEGANGPTTPEADKIFLER(N)	2241,16	3		
			(R)ISGASEKDIVHSGLAYTMER(S)	2163,06	4		
			(K)MVEGFFDR(G)	999,45	2		
			(K)NLNHVSYGR(L)	1058,52	2		
			(K)NYTDNELEKITR(R)	1494,73	2		
			(R)RDDGSWEVIEGYR(A)	1580,72	2		
			(R)TAAYVNAIEK(V)	1078,56	2		
			(K)YNLGLDLR(T)	962,52	2		
			(R)YSTDVSVDEVK(A)	1240,58	2		AT2B2, SYGP1
		2	(K)AKIIAEGAnGPTTPEADKIFLER(N)	2441,28	3	Deamidation (+1)	
			(K)ALASLMTYK(C)	996,53	2		
			(R)DDGSWEVIEGYR(A)	1424,62	2		
			(K)DIVHSGLAYTMER(S)	1490,72	3		
			(R)DSNYHLLMSVQESLER(K)	1919,90	2		
			(R)DSNYHLLMSVQESLERK(F)	2048,00	3		
			(K)ELEDFKLQHGSILGFPK(A)	1957,03	3		
			(R)FTMELAK(K)	838,43	1		

			(R)FTMELAKK(G)	966,52	2		
			(K)GFIGPGIDVPAPDMSTGER(E)	1914,91	2		
			(K)HGGTIPVVPTAEFQDR(I)	1722,87	2		H10, NMDE2, B5DFL7, HNRPQ
			(K)HGGTIPVVPTAEFQDRISGASEKDIVHSGLAYTMER(S)	3867,92	4		
			(K)IIAEGANGPTTPEADKIFLER(N)	2241,16	2		
			(R)ISGASEKDIVHSGLAYTMER(S)	2163,06	2		
			(K)KGFIGPGIDVPAPDMSTGER(E)	2043,01	2		
			(K)MVEGFFDR(G)	999,45	2		
			(K)MVEGFFDRGASIVEDKLVEDLK(T)	2496,26	3		
			(K)NLNHVSYGR(L)	1058,52	2		
			(K)NYTDNELEK(I)	1124,50	2		
			(K)NYTDNELEKITR(R)	1494,73	2		
			(R)RDDGSWEVIEGYR(A)	1580,72	2		
			(R)TAAYVNAIEK(V)	1078,57	1		
			(R)TAmKYNLGLDLR(T)	1409,73	3	Oxidation (+16)	
			(K)TFVVQGFGNVGLHSmR(Y)	1763,88	3	Oxidation (+16)	
			(K)VYNEAGVTFT(-)	1099,52	2		
			(K)YNLGLDLR(T)	962,52	2		
			(R)YSTDVSVDEVK(A)	1240,58	2		AT2B2, SYGP1
		3	(K)AKIIAEGAnGPTTPEADKIFLER(N)	2441,28	3	Deamidation (+1)	
			(K)ALASLmTYK(C)	1012,53	2	Oxidation (+16)	
			(R)DDGSWEVIEGYR(A)	1424,62	2		
			(K)DIVHSGLAYTmER(S)	1506,71	2	Oxidation (+16)	
			(K)ELEDFKLQHGSILGFPK(A)	1957,03	3		
			(R)FTmELAK(K)	854,42	2	Oxidation (+16)	
			(R)GASIVEDKLVEDLK(T)	1514,82	2		
			(K)GFIGPGIDVPAPDmSTGER(E)	1930,91	2	Oxidation (+16)	
			(K)HGGTIPVVPTAEFQDR(I)	1722,87	2		H10, NMDE2, B5DFL7, HNRPQ

			(K)IIAEGANGPTTPEADKIFLER(N)	2241,16	3		
			(R)ISGASEKDIVHSGLAYTMER(S)	2163,06	3		
			(K)LQHGSILGFPK(A)	1195,67	3		
			(K)MVEGFFDR(G)	999,45	2		
			(K)NLNHVSYGR(L)	1058,52	2		
			(K)NYTDNELEK(I)	1124,50	2		
			(K)NYTDNELEKITR(R)	1494,73	2		
			(R)RDDGSWEVIEGYR(A)	1580,72	3		
			(R)RFTmELAK(K)	1010,52	2	Oxidation (+16)	
			(R)TAAYVnAIEK(V)	1079,55	2	Deamidation (+1)	
			(K)YNLGLDLR(T)	962,52	2		
			(R)YSTDVSVDEVK(A)	1240,58	2		AT2B2, SYGP1
			(R)YSTDVSVDEVKALASLMTYK(C)	2219,11	3		
		4	(R)YSTDVSVDEVK(A)	1240,58	2		
			(K)ALASLmTYK(C)	1012,53	2	Oxidation (+16)	
			(R)DDGSWEVIEGYR(A)	1424,62	2		
			(K)DIVHSGLAYTMER(S)	1490,72	3		
			(K)ELEDFKLQHGSILGFPK(A)	1957,03	3		
			(R)GASIVEDKLVEDLK(T)	1514,82	2		
			(K)GFIGPGIDVPAPDMSTGER(E)	1914,91	2		
			(K)HGGTIPVVPTAEFQDR(I)	1722,87	2		H10, NMDE2, B5DFL7, HNRPQ
			(K)IIAEGANGPTTPEADKIFLER(N)	2241,17	3		
			(R)ISGASEKDIVHSGLAYTMER(S)	2163,06	3		
			(K)MVEGFFDR(G)	999,45	2		
			(K)NYTDNELEKITR(R)	1494,73	2		
			(R)TAAYVNAIEK(V)	1078,56	2		
			(K)YNLGLDLR(T)	962,52	2		
			(R)YSTDVSVDEVK(A)	1240,58	2		AT2B2, SYGP1

2	IMMT	1	(R)DDFRKELDSITPDITPGWK(G)	2232,11	3
			(R)EIAGATPYITAAEEK(L)	1562,78	2
			(K)ELDSITPDITPGWK(G)	1570,79	2
			(K)FEFEQDLSEK(L)	1270,57	2
			(R)GVYSEETLR(A)	1052,51	2
			(R)KAVDEAADALLK(A)	1242,68	2
			(K)KPIQSGPLK(I)	966,58	2
			(R)LRGIEQAVQSHAVAEEEAR(K)	2092,07	3
			(R)QTITAQNAAVQAVK(A)	1441,79	2
			(K)SLEDALNQTATVTR(Q)	1517,77	2
			(K)TSSAEMPTIPLGSAVEAIR(V)	1928,99	2
			(K)VVSQYHELVVQAR(D)	1526,82	3
			(R)YSTSSSSGVTAGK(I)	1230,57	2
		2	(K)ALEHHRSEIQAEQDR(K)	1817,87	3
			(R)DDFRKELDSITPDITPGWK(G)	2232,11	3
			(R)EIAGATPYITAAEEK(L)	1562,78	2
			(R)GIEQAVQSHAVAEEEAR(K)	1822,88	2
			(R)GVYSEETLR(A)	1052,51	2
			(R)KAVDEAADALLK(A)	1242,68	2
			(K)KPIQSGPLK(I)	966,59	2
			(R)LRGIEQAVQSHAVAEEEAR(K)	2092,06	3
			(R)QTITAQNAAVQAVK(A)	1441,79	2
			(K)RAFDSAVAK(A)	963,51	2
			(K)SLEDALNQTATVTR(Q)	1517,77	2
			(K)TSSAEMPTIPLGSAVEAIR(V)	1928,99	2
			(K)VVSQYHELVVQAR(D)	1526,82	2
			(R)YSTSSSSGVTAGK(I)	1230,57	2
		3	(R)GIEQAVQSHAVAEEEAR(K)	1822,88	3
			(R)GVYSEETLR(A)	1052,51	2

			(R)RQAAAHTDHLR(D)	1274,66	3		
			(K)SLEDALNQTATVTR(Q)	1517,77	2		
		4	(K)AVDEAADALLK(A)	1114,59	2		
			(R)EIAGATPYITAAEEK(L)	1562,78	2		
			(K)ELDSITPDITPGWK(G)	1570,79	2		
			(R)GIEQAVQSHAVAEEEAR(K)	1822,88	3		
			(R)GVYSEETLR(A)	1052,51	2		
			(R)QTITAQNAAVQAVK(A)	1441,79	2		
			(K)SLEDALNQTATVTR(Q)	1517,77	2		
			(K)TSSAEMPTIPLGSAVEAIR(V)	1928,99	2		
3	MPCP	1	(K)EKGSTASQVLQR(L)	1302,69	2		
			(R)LPRPPPPEMPESLK(K)	1586,85	3		
			(R)LPRPPPPEMPESLKK(K)	1714,94	4		
		2	(K)ALYSNILGEENTYLWR(T)	1940,96	2		
			(K)EKGSTASQVLQR(L)	1302,69	2		
			(K)GSTASQVLQR(L)	1045,55	1		
			(R)IQTQPGYANTLR(E)	1360,71	2		
			(K)MYKEEGLNAFYK(G)	1491,71	2		
		3	(K)EKGSTASQVLQR(L)	1302,69	2		
			(K)GSTASQVLQR(L)	1045,55	2		
			(R)IQTQPGYANTLR(E)	1360,71	2		
			(R)IQTQPGYAnTLR(E)	1361,69	2	Deamidation (+1)	
			(R)LPRPPPPEmPESLKK(K)	1730,94	3	Oxidation (+16)	
			(R)MQVDPQK(Y)	844,41	2		
			(R)MQVDPQKYK(G)	1135,57	2		
			(K)MYKEEGLNAFYK(G)	1491,71	2		

			(R)QIPYTmmK(F)	1042,48	2	Oxidation (+16), Oxidation (+16)	ARL8B, SPKAP
		4	(K)ALYSNILGEENTYLWR(T)	1940,97	2		
			(K)EKGSTASQVLQR(L)	1302,69	2		
			(R)IQTQPGYANTLR(E)	1360,71	2		
			(R)LPRPPPPEmPESLK(K)	1602,84	3	Oxidation (+16)	
			(R)mQVDPQKYK(G)	1151,56	2	Oxidation (+16)	
			(K)MYKEEGLNAFYK(G)	1491,71	2		
4	ATPB	1	(R)AIAELGIYPAVDPLDSTSR(I)	1987,03	2		
			(R)LVLEVAQHLGESTVR(T)	1649,91	3		
			(R)TIAMDGTEGLVR(G)	1261,63	2		
			(K)TVLIMELINNVAK(A)	1456,83	2		
			(K)VALVYGQMNEPPGAR(A)	1600,80	2		
			(K)VLDSGAPIKIPVGPETLGR(I)	1918,09	3		
		2	(K)AHGGYSVFAGVGER(T)	1405,67	2		
			(R)AIAELGIYPAVDPLDSTSR(I)	1987,02	2		
			(R)FLSQPFQVAEVFTGHMGK(L)	2022,00	2		
			(K)IGLFGGAGVGK(T)	974,55	2		
			(R)IMDPNIVGSEHYDVAR(G)	1814,86	2		
			(R)IMNVIGEPIDER(G)	1384,70	2		
			(R)IMNVIGEPIDERGPIKTK(Q)	2009,10	3		
			(R)IPSAVGYQPTLATDMGTMQER(I)	2265,08	2		
			(K)KGSITSVQAIYVPADDLTDPAPATTFAHLDATTVLSR(A)	3841,99	4		
			(R)LVLEVAQHLGESTVR(T)	1649,91	3		
			(R)TIAMDGTEGLVR(G)	1261,63	2		
			(K)TVLImELINNVAK(A)	1472,83	2	Oxidation (+16)	
			(K)VALVYGQMNEPPGAR(A)	1600,80	2		
			(K)VLDSGAPIKIPVGPETLGR(I)	1918,09	3		

			(K)VVDLLAPYAK(G)	1087,63	2			
		3	(R)AIAELGIYPAVDPLDSTSR(I)	1987,03	2			
			(R)ImDPNIVGSEHYDVAR(G)	1830,86	3	Oxidation (+16)		
			(R)ImNVIGEPIDER(G)	1400,70	2	Oxidation (+16)		
			(R)LVLEVAQHLGESTVR(T)	1649,91	3			
			(R)TIAMDGTEGLVR(G)	1261,63	2			
			(K)VALVYGQMNEPPGAR(A)	1600,81	2			
			(K)VALVYGQmNEPPGAR(A)	1616,80	2	Oxidation (+16)		
			(K)VLDSGAPIKIPVGPETLGR(I)	1918,09	3			
			(K)VVDLLAPYAK(G)	1087,63	2			
		4	(R)AIAELGIYPAVDPLDSTSR(I)	1987,03	2			
			(R)IMDPNIVGSEHYDVAR(G)	1814,87	3			
			(R)IMNVIGEPIDER(G)	1384,70	2			
			(R)TIAMDGTEGLVR(G)	1261,63	2			
			(K)VALVYGQMNEPPGAR(A)	1600,81	2			
5	EFTU	1	(R)AEAGDNLGALVR(G)	1184,61	2			
			(R)DKPHVNVGTIGHVDHGK(T)	1808,93	3			
			(R)GTVVTGTLER(G)	1031,56	2			
			(K)LLDAVDTYIPVPTR(D)	1571,86	2			
		2	(R)AEAGDNLGALVR(G)	1184,61	2			
			(R)DKPHVNVGTIGHVDHGK(T)	1808,93	4			
			(K)FKKYEEIDNAPEER(A)	1766,84	3			
			(R)GITINAAHVEYSTAAR(H)	1672,85	3			
			(K)LLDAVDTYIPVPTR(D)	1571,86	2			
			(K)SLERAEAGDNLGALVR(G)	1669,87	2			
		3	(R)AEAGDNLGALVR(G)	1184,62	2			

			(R)DKPHVNVGTIGHVDHGK(T)	1808,93	3			
			(R)DLEKPFLLPVESVYSIPGR(G)	2158,17	3			
			(R)GTVVTGTLER(G)	1031,56	2			
			(K)LLDAVDTYIPVPTR(D)	1571,86	2			
			(K)SLERAEAGDNLGALVR(G)	1669,88	3			
			(K)TTLTAAITK(I)	918,54	2			
			(R)TVVTGIEmFHK(S)	1276,65	2	Oxidation (+16)		
		4	(R)AEAGDNLGALVR(G)	1184,62	2			
			(R)DLEKPFLLPVESVYSIPGR(G)	2158,17	3			
			(K)LLDAVDTYIPVPTR(D)	1571,85	2			
6	ATAD3	1	(R)AKADRENADIIR(E)	1370,72	3			
			(R)ISVLEALR(H)	899,54	2			
			(R)KQEESVQKQEAIR(R)	1571,82	3			
			(K)LKEYEAAVEQLKSEQIR(V)	2033,08	3			
			(R)NVLMYGPPGTGK(T)	1232,62	2			
			(R)VQDAVQQHQQK(M)	1307,66	2			
		2	(R)KQEESVQKQEAIR(R)	1571,83	3			
			(K)LKEYEAAVEQLKSEQIR(V)	2033,08	3			
			(R)MYFDKYVLKPATEGK(Q)	1788,91	3			
			(R)VQDAVQQHQQK(M)	1307,66	3			
			(R)VRDIAIATR(N)	1013,60	2			
		3	(R)KQEESVQKQEAIR(R)	1571,83	3			
			(R)VQDAVQQHQQK(M)	1307,66	3			
		4	(R)KQEESVQKQEAIR(R)	1571,83	3			
			(K)LKEYEAAVEQLKSEQIR(V)	2033,08	3			

7	ITPR1	1	(R)AIAIPVDLDSQVNNLFLK(S)	1969,09	2			
			(R)FAQTMEFVEEYLR(D)	1661,77	2			
		2	(K)AAKPGANSTTDAVLLNK(L)	1669,90	2			
			(K)ELVEVIKK(A)	956,59	2			
			(K)LVTNLSGQLSELKDQMTEQR(K)	2289,16	3			
			(R)NLQEKLESTMK(L)	1319,68	2			
			(R)SGGGVGDVLR(K)	915,48	2			
			(K)VLGSIAGKLEK(G)	1113,67	2			
			(R)VLSDVAK(S)	730,42	1			
		3	(R)AIAIPVDLDSQVNNLFLK(S)	1969,09	2			
			(K)LHHAADLEK(K)	1032,54	3			
			(K)LHHAADLEKK(Q)	1160,63	3			
			(R)LPALLEK(N)	782,49	2			
			(R)NLDWFPR(M)	946,47	2			
			(R)RDSVLAASR(D)	973,53	2			
		4	(K)AAKPGANSTTDAVLLNK(L)	1669,90	3			
			(R)AMSLVSSDSEGEQNELR(N)	1850,83	2			
			(K)AVLNPTNADILIETK(L)	1610,89	2			
			(R)EADPDDHYQSGEGTQATTDK(A)	2163,88	3			
			(R)ESLTSFGnGPLSPGGPSKPGGGGGGPGSGSTSR(G)	2900,35	3	Deamidation (+1)		
			(R)GGGFLPMTPMAAAPEGNVK(Q)	1843,90	2			
			(K)INDFFLR(S)	923,49	2			
			(R)LQDIVSALEDR(L)	1257,66	2			
			(R)RDSVLAASR(D)	973,53	2			
			(R)SGGGVGDVLR(K)	915,48	2			
8	SEPT7	1	(K)LKDSEAELQR(R)	1187,61	2			
			(K)SPLAQMEEERR(E)	1344,64	3			

			(K)STLINSLFLTDLYSPEYPGPSHR(I)	2606,30	3		
		2	(K)DVTNNVHYENYR(S)	1522,68	2		
			(R)ILEQQnSSR(T)	1074,53	2	Deamidation (+1)	
			(K)KNLEAQHKELEEK(R)	1594,83	3		
			(K)LKDSEAELQR(R)	1187,61	2		
			(K)NLEGYVGFANLPNQVYR(K)	1952,98	2		
			(K)NLEGYVGFANLPNQVYRK(S)	2081,07	3		
			(R)QFEEEKANWEAQQR(I)	1791,82	2		
			(K)SPLAQMEEERR(E)	1344,64	2		
			(K)STLINSLFLTDLYSPEYPGPSHR(I)	2606,30	3		
			(R)THMQDLKDVTNNVHYENYR(S)	2376,09	4		
		3	(K)LKDSEAELQR(R)	1187,61	2		
			(R)QFEEEKANWEAQQR(I)	1791,82	3		
			(K)SPLAQmEEERR(E)	1360,64	3	Oxidation (+16)	
		4	(R)ILEQQNSSR(T)	1073,55	2		
			(K)LKDSEAELQR(R)	1187,62	2		
			(R)QFEEEKANWEAQQR(I)	1791,82	3		
			(K)SPLAQMEEERR(E)	1344,65	3		
			(K)VNIIPLIAK(A)	979,64	2		SEP10, B0BNF1, B3DMA8
9	GNAO	1	(R)IGAADYQPTEQDILR(T)	1688,84	2		
			(R)MEDTEPFSAELLSAMMR(L)	1956,86	2		
			(K)NLKEDGISAAK(D)	1144,61	2		
			(K)TTGIVETHFTFK(N)	1379,71	2		
			(K)YYLDSLDR(I)	1043,49	2		
		2	(R)AMDTLGVEYGDKER(K)	1582,73	2		GIT1

			(R)IGAADYQPTEQDILR(T)	1688,83	2		
			(K)IIHEDGFSGEDVK(Q)	1444,68	2		Q9QX19, B1H279
			(K)LLLLGAGESGK(S)	1056,62	2		
			(R)MEDTEPFSAELLSAMMR(L)	1956,86	2		
			(K)NLKEDGISAAK(D)	1144,61	2		
			(K)YYLDSLDRIGAADYQPTEQDILR(T)	2714,32	3		
		3	(R)AmDTLGVEYGDKER(K)	1598,73	3	Oxidation (+16)	GIT1
			(R)IGAADYQPTEQDILR(T)	1688,84	2		
			(K)LLLLGAGESGK(S)	1056,62	2		
		4	(R)AMDTLGVEYGDKER(K)	1582,73	3		GIT1
			(R)IGAADYQPTEQDILR(T)	1688,84	2		
			(K)IIHEDGFSGEDVK(Q)	1444,68	3		Q9QX19, B1H279
			(K)LLLLGAGESGK(S)	1056,62	2		
			(R)MEDTEPFSAELLSAMmR(L)	1972,86	2	Oxidation (+16)	
			(K)NLKEDGISAAK(D)	1144,61	2		
			(K)YYLDSLDR(I)	1043,49	2		
10	STXB1	1	(R)HKHIAEVSQEVTR(S)	1532,80	3		
			(K)LIQHAQIPPEDSEIITNMAHLGVPIVTDSTLR(R)	3507,84	4		
			(R)SSASFSTTAVSAR(Y)	1270,61	2		
		2	(K)HIAEVSQEVTR(S)	1267,65	2		
			(R)HKHIAEVSQEVTR(S)	1532,80	3		
			(R)ISEQTYQLSR(W)	1223,61	2		
			(K)LIQHAQIPPEDSEIITNMAHLGVPIVTDSTLR(R)	3507,84	4		
			(R)SSASFSTTAVSAR(Y)	1270,62	2		
			(K)SVHSLISDFKDPPTAK(Y)	1740,90	3		

			(K)VLVVDQLSMR(M)	1158,64	2		
		3	(R)ISEQTYQLSR(W)	1223,62	2		
			(K)SVHSLISDFKDPPTAK(Y)	1740,91	4		
		4	(R)SQLLILDR(G)	956,57	2		
			(R)SSASFSTTAVSAR(Y)	1270,61	2		
			(K)VLVVDQLSMR(M)	1158,64	2		
11	AP3D1	1	(K)GVPVAEEVSALFAGELNPVAPK(A)	2193,17	2		
			(R)ILIEDSDQNLK(Y)	1286,67	2		
			(K)VTTLPGHIQAVYVQNVVK(L)	1965,10	3		
		2	(K)AIFHEEEPR(H)	1126,54	2		
			(K)GVPVAEEVSALFAGELNPVAPK(A)	2193,17	2		
			(R)ILIEDSDQNLK(Y)	1286,67	2		
			(R)RRQPEEDEEELAR(R)	1655,79	3		
		3	(K)EQAADTEGAQEVTqLLVER(L)	2086,99	2	Deamidation (+1)	
			(K)GVPVAEEVSALFAGELNPVAPK(A)	2193,17	3		
			(R)ILIEDSDQNLK(Y)	1286,67	2		
			(R)RRQPEEDEEELAR(R)	1655,79	3		
		4	(R)ALDIDLDKPLADSEKLPVQK(H)	2207,21	3		
			(K)EQAADTEGAQEVTQLLVER(L)	2086,02	2		
			(R)ILIEDSDQNLK(Y)	1286,67	2		
			(R)MFDKNLQDLVR(G)	1377,71	2		
			(K)NMELNVLDSLNTK(M)	1489,75	2		
			(R)RRQPEEDEEELAR(R)	1655,79	3		
12	AT2B1	1	(R)IRNEKGEIEQER(I)	1499,77	3		AT2B2, AT2B3

			(R)QVVAVTGDGTNDGPALK(K)	1640,84	2		AT2B2, AT2B3, AT2B4
			(R)SSPTDKHTLVK(G)	1211,65	2		AT2B2, AT2B4
		2	(R)IRNEKGEIEQER(I)	1499,77	3		AT2B2, AT2B3
			(R)QVVAVTGDGTNDGPALK(K)	1640,84	2		AT2B2, AT2B3, AT2B4
			(R)SSPTDKHTLVK(G)	1211,65	2		AT2B2, AT2B4
			(K)TVIEPMASEGLR(T)	1301,66	2		
		3	(R)MVTGDNINTAR(A)	1190,57	2		AT2B2, AT2B3
			(R)QVVAVTGDGTNDGPALK(K)	1640,84	2		AT2B2, AT2B3, AT2B4
		4	(K)EASDIILTDDNFTSIVK(A)	1879,94	2		AT2B4
			(R)IRNEKGEIEQER(I)	1499,77	3		AT2B2, AT2B3
			(R)QVVAVTGDGTNDGPALK(K)	1640,84	2		AT2B2, AT2B3, AT2B4
			(R)qVVAVTGDGTNDGPALKK(A)	1751,90	2	Pyro-cmC (-17)	AT2B2
			(R)SSPTDKHTLVK(G)	1211,65	2		AT2B2, AT2B4
			(K)TVIEPMASEGLR(T)	1301,67	2		
			(K)YGDLLPADGILIQGNDLK(I)	1914,01	2		AT2B4
13	AT1A1	1	(K)GVGIISEGNETVEDIAAR(L)	1828,92	2		AT1A2, AT1A3
			(R)LNIPVNQVNPR(D)	1262,71	2		
			(K)VDNSSLTGESEPQTR(S)	1618,74	2		AT1A2, AT1A3
			(K)VIMVTGDHPITAK(A)	1380,74	2		AT1A2, AT1A3
		2	(R)GIVVYTGDR(T)	978,51	2		
			(K)GVGIISEGNETVEDIAAR(L)	1828,92	2		AT1A2, AT1A3
			(R)LNIPVNQVNPR(D)	1262,71	2		

			(K)VIMVTGDHPITAK(A)	1380,74	2		AT1A2, AT1A3
		3	(K)GVGIISEGNETVEDIAAR(L)	1828,92	2		AT1A2, AT1A3
		4	(R)AVAGDASESALLK(C)	1230,64	2		
			(R)GIVVYTGDR(T)	978,51	2		
			(K)GVGIISEGNETVEDIAAR(L)	1828,92	2		AT1A2, AT1A3
			(R)LNIPVNQVNPR(D)	1262,71	2		
			(K)NMVPQQALVIR(N)	1267,71	2		AT1A2, AT1A3
			(R)QGAIVAVTGDGVNDSPALK(K)	1810,94	2		AT1A3
			(R)SPDFTNENPLETR(N)	1518,69	2		
			(K)TDKLVNER(L)	973,52	3		AT1A2, AT1A3
			(K)VIMVTGDHPITAK(A)	1380,74	2		AT1A2, AT1A3
14	RAB3A	1	(K)ILIIGNSSVGK(T)	1.099,66	2		RAB3C, RAB3B
			(K)MSESLDTADPAVTGAK(Q)	1.591,74	2		
			(R)qLADHLGFEFFEASAK(D)	1.791,85	2	Pyro-cmC (-17)	
			(R)YADDSFTPAFVSTVGIDFK(V)	2.078,98	2		
		2	(K)ILIIGNSSVGK(T)	1.099,66	2		RAB3C, RAB3B
		4	(K)ESSDQNFDYMFK(I)	1.509,61	2		
			(K)MSESLDTADPAVTGAK(Q)	*1.591,74*	*2*		
15	SEPT8	1	(R)ISNAEPEPR(S)	1011,50	2		
			(R)LRPQTYDLQESNVHLK(L)	1940,01	3		
		2	(R)ISNAEPEPR(S)	1011,50	2		
			(R)LRPQTYDLQESNVHLK(L)	1940,01	3		
		3	(R)ISNAEPEPR(S)	1011,50	2		

		4	(R)ISNAEPEPR(S)	1011,50	2		
16	GBRA1	1	(R)GDPGLATIAK(S)	941,52	2		
			(R)ITEDGTLLYTMR(L)	1411,70	2		
		2	(K)EVKPETKPPEPK(K)	1377,75	2		
			(R)ITEDGTLLYTMR(L)	1411,70	2		
			(R)LLDGYDNR(L)	964,46	2		
			(K)NNTYAPTATSYTPNLAR(G)	1853,89	2		
			(R)SVVVAEDGSR(L)	1017,51	2		
		3	(R)SVVVAEDGSR(L)	1017,51	2		
17	CN37	1	(K)ATGAEEYAQQDVVR(R)	1535,72	2		
			(K)GGSQGEEVGELPR(G)	1313,62	2		
			(R)HFISGDEPK(E)	1028,49	2		
		2	(K)ATGAEEYAQQDVVR(R)	1535,72	2		
			(K)GGSQGEEVGELPR(G)	1313,62	2		
			(R)HFISGDEPK(E)	1028,49	2		
		3	(K)AGQVFLEELGNHK(A)	1440,73	3		
		4	(R)ADFSEEYKR(L)	1143,52	2		
			(K)ATGAEEYAQQDVVR(R)	1535,72	2		
			(K)GGSQGEEVGELPR(G)	1313,62	2		
			(R)HFISGDEPK(E)	1028,49	2		
			(R)KAGQVFLEELGNHK(A)	1568,83	3		
18	SV2A	1	(R)HLQAVDYAAR(T)	1142,58	2		

			(R)MADGAPLAGVR(G)	1056,54	2			
		2	(R)MADGAPLAGVR(G)	1056,54	2			
		3	(R)HLQAVDYAAR(T)	1142,58	2			
			(R)MADGAPLAGVR(G)	1056,54	2			
		4	(K)DREELAQQYETILR(E)	1762,89	2			
			(R)HLQAVDYAAR(T)	1142,58	2			
			(R)MADGAPLAGVR(G)	1056,54	2			

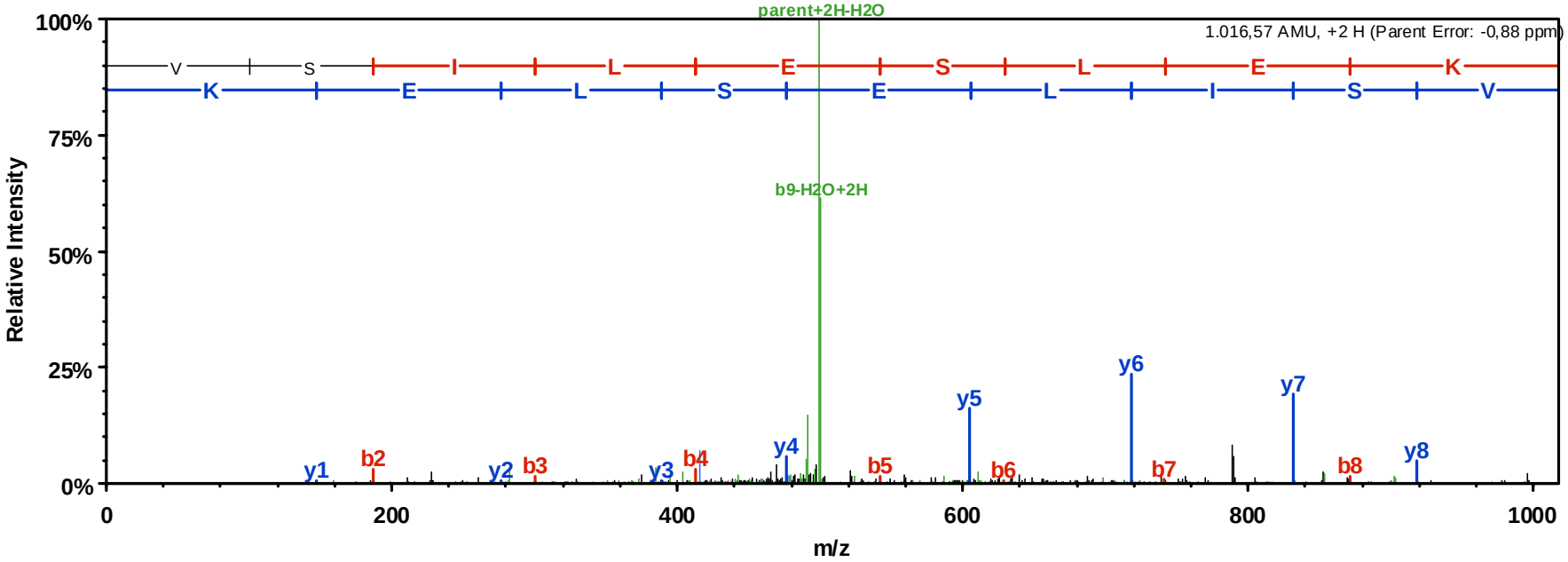
[a]Scaffold (version Scaffold_02_04_00, proteome Software, Portland, OR, USA) was used to compile and assign probability scores. All peptides were identified with a ≥95% probability as specified by the Peptide Prophet algorithm (R-4).

[b] Modified amino acids are displayed in small letters. The modification is listed in a subsequent column.

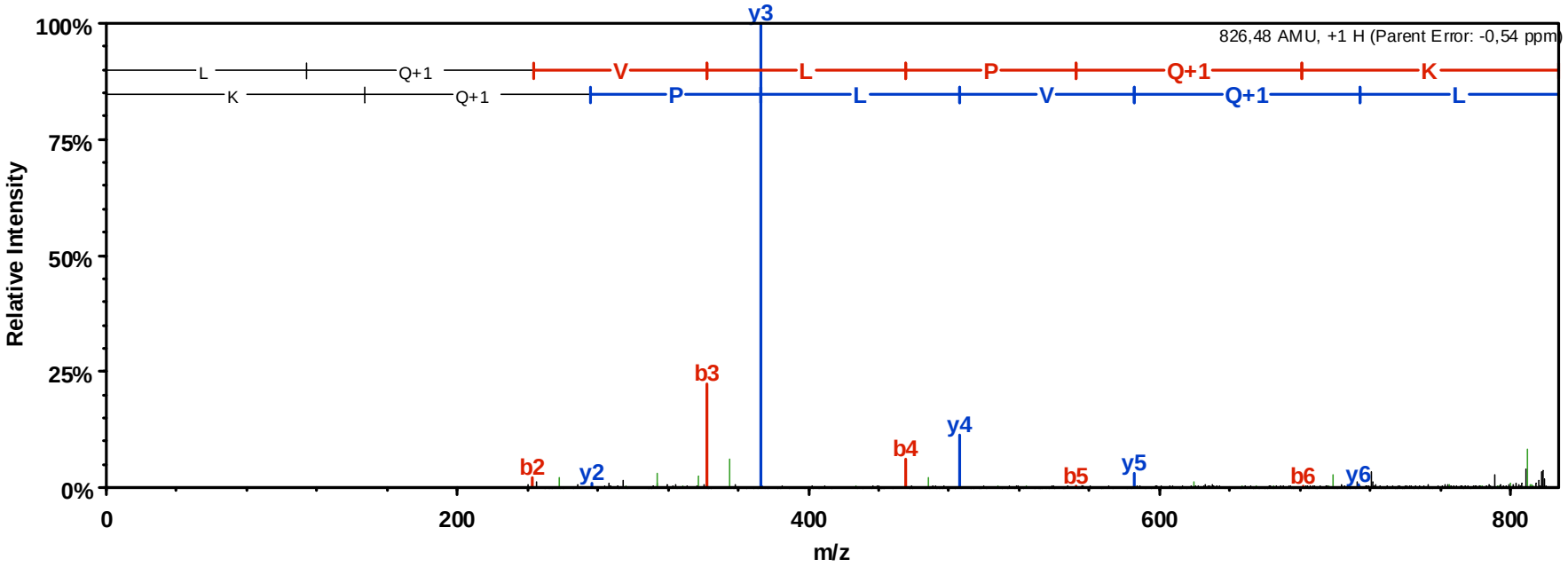
[c] The column 'other proteins' listed alternative proteins for an identified peptide sequence. In general, proteins that comprise similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony.

10. MS/MS spectra of all one peptide based proteins (as obtained from the Scaffold 2_04_00 software)

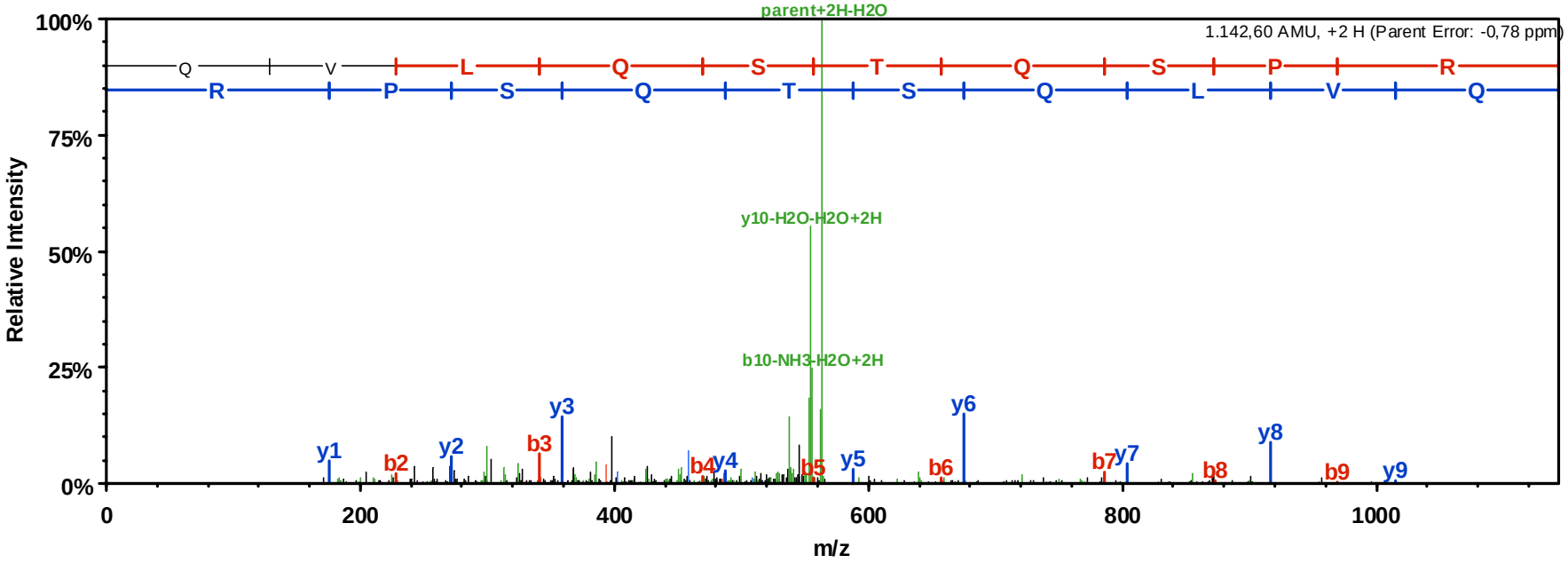
MS/MS spectrum of KAP1, cAMP-dependent protein kinase tryptic peptide (K)VSILESLEK(W) **(corresponding to Table 2, run 1)**.



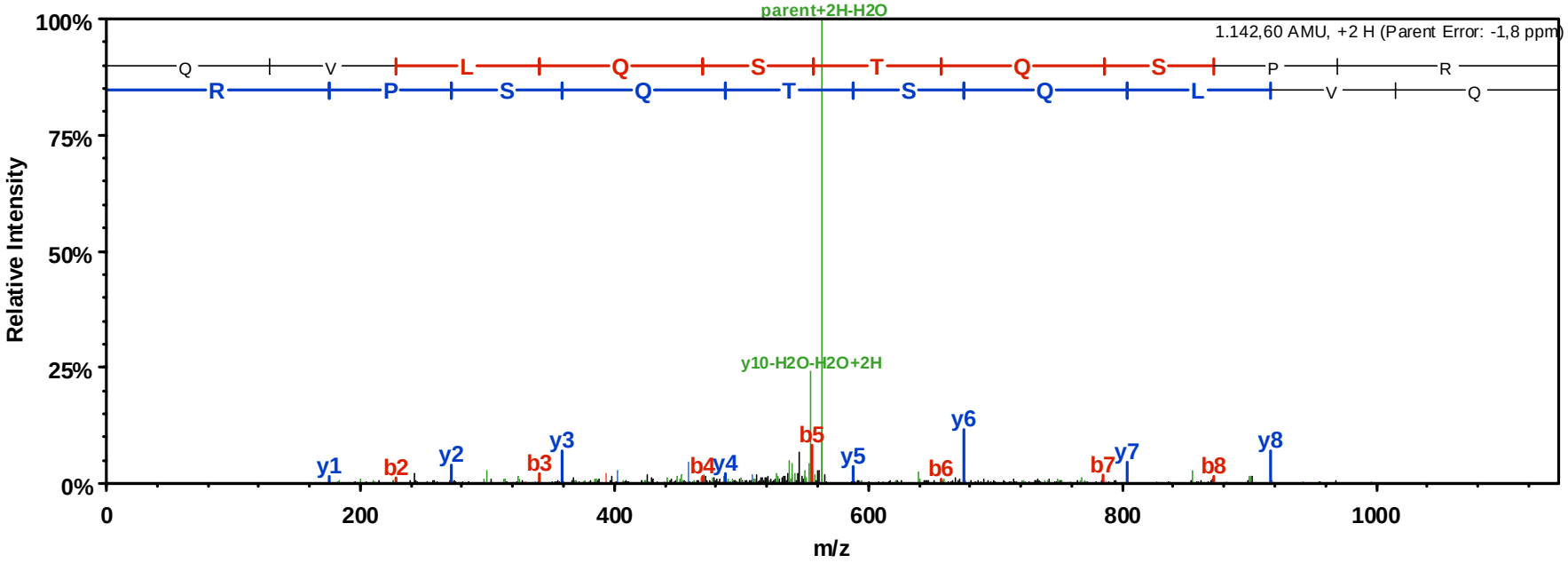
MS/MS spectrum of AKAP9, A-kinase anchor protein 9 tryptic peptide (K)LqVLPqK(A) **(corresponding to Table 2, run 1)**.



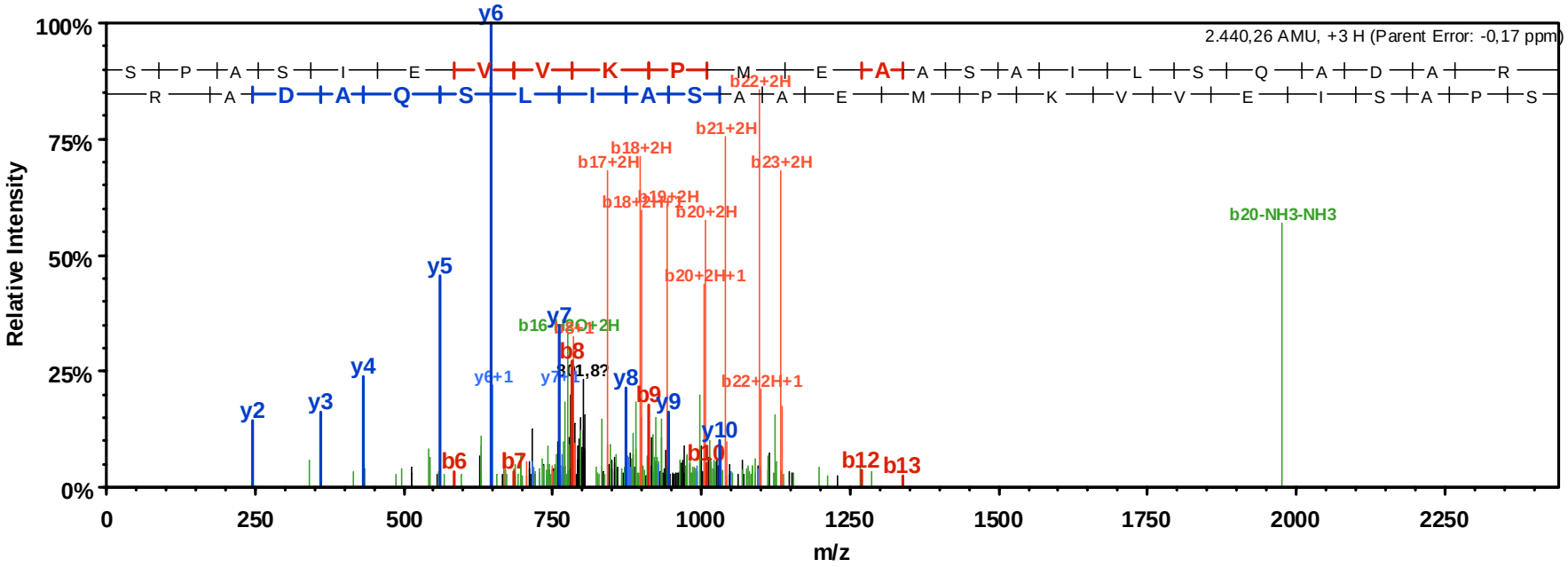
MS/MS spectrum of AKAP2, A-kinase anchor protein 2 tryptic peptide (R)QVLQSTQSPR(T) (**Footnote to Table 2, run 1**).



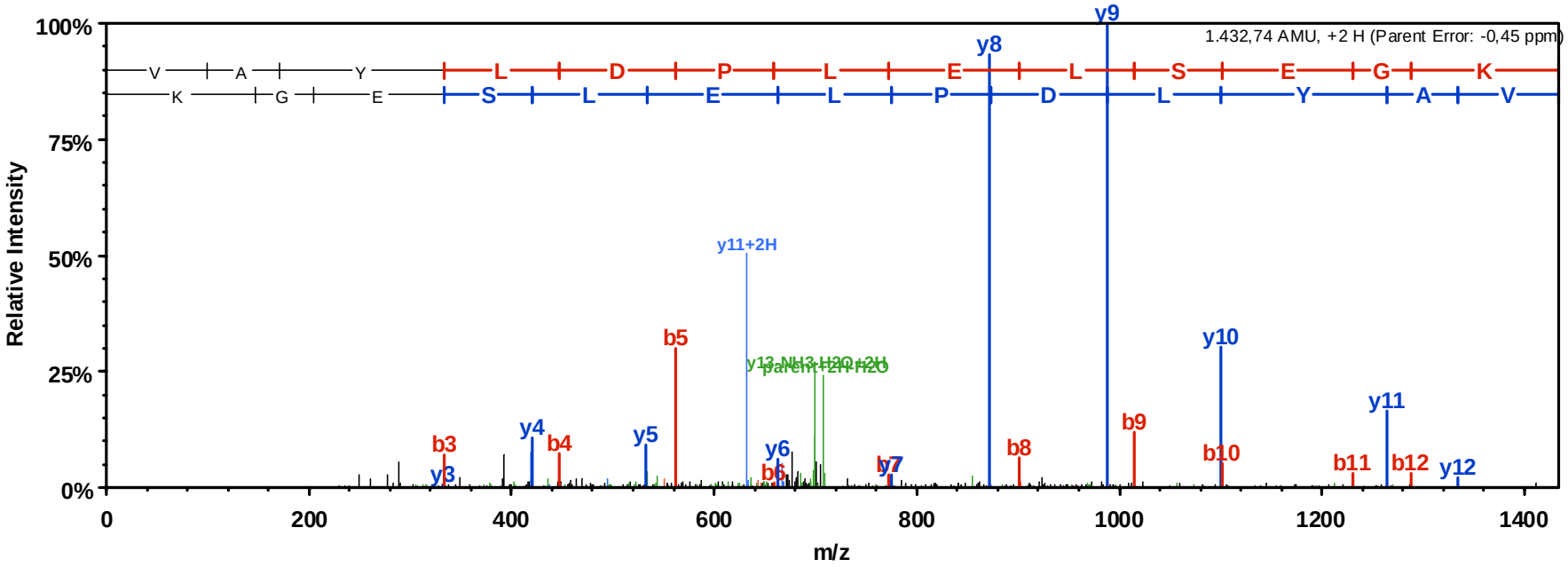
MS/MS spectrum of AKAP2, A-kinase anchor protein 2 tryptic peptide (R)QVLQSTQSPR(T) (**Footnote to Table 2, run 2**).



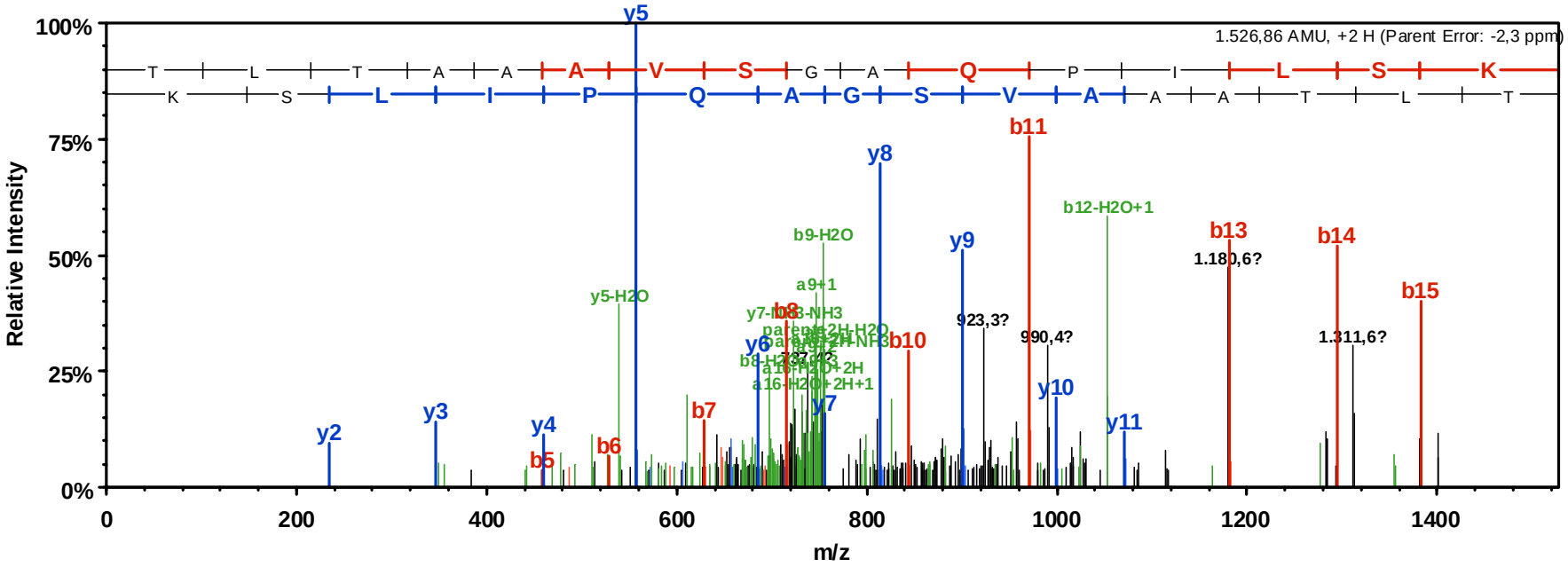
MS/MS spectrum of GNL3, Guanine nucleotide-binding protein tryptic peptide (R)SPASIEVVKPMEAASAILSQADAR(Q) (**corresponding to Table 3, run 3**).



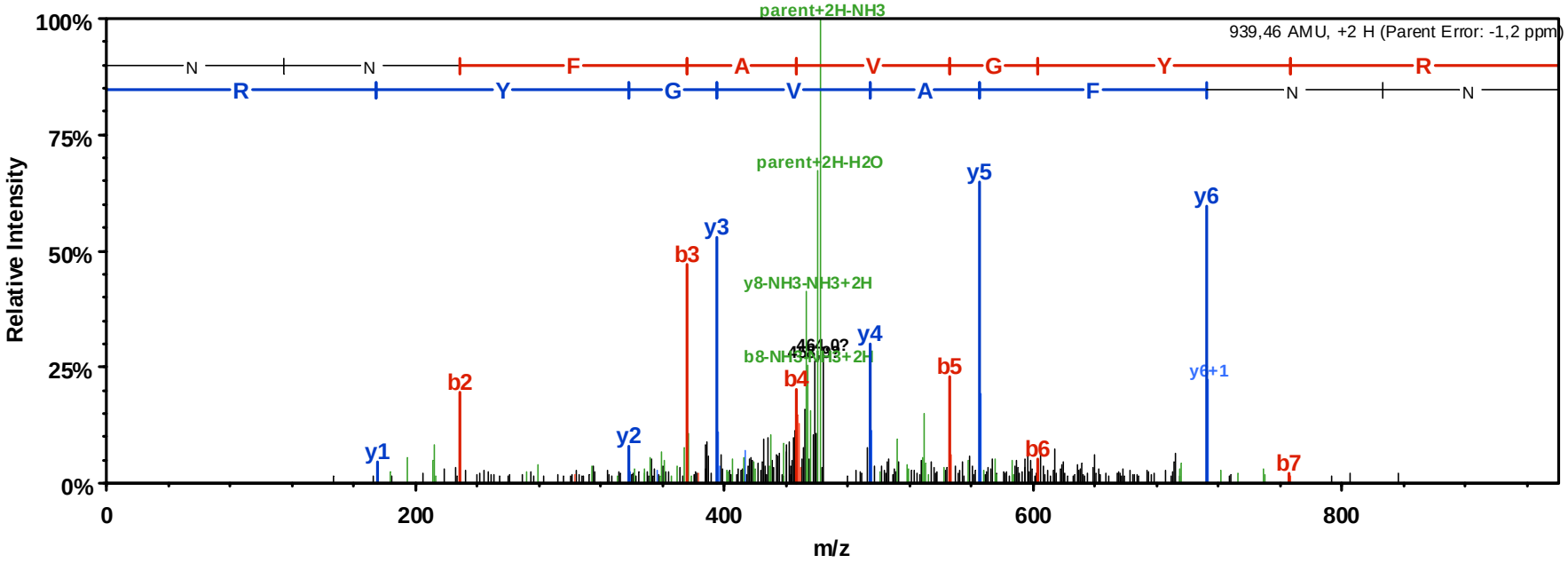
MS/MS spectrum of LU, Lutheran blood group glycoprotein tryptic peptide (R)SPPYQLDSQGR(L) (**corresponding to Table 3, run 3**).



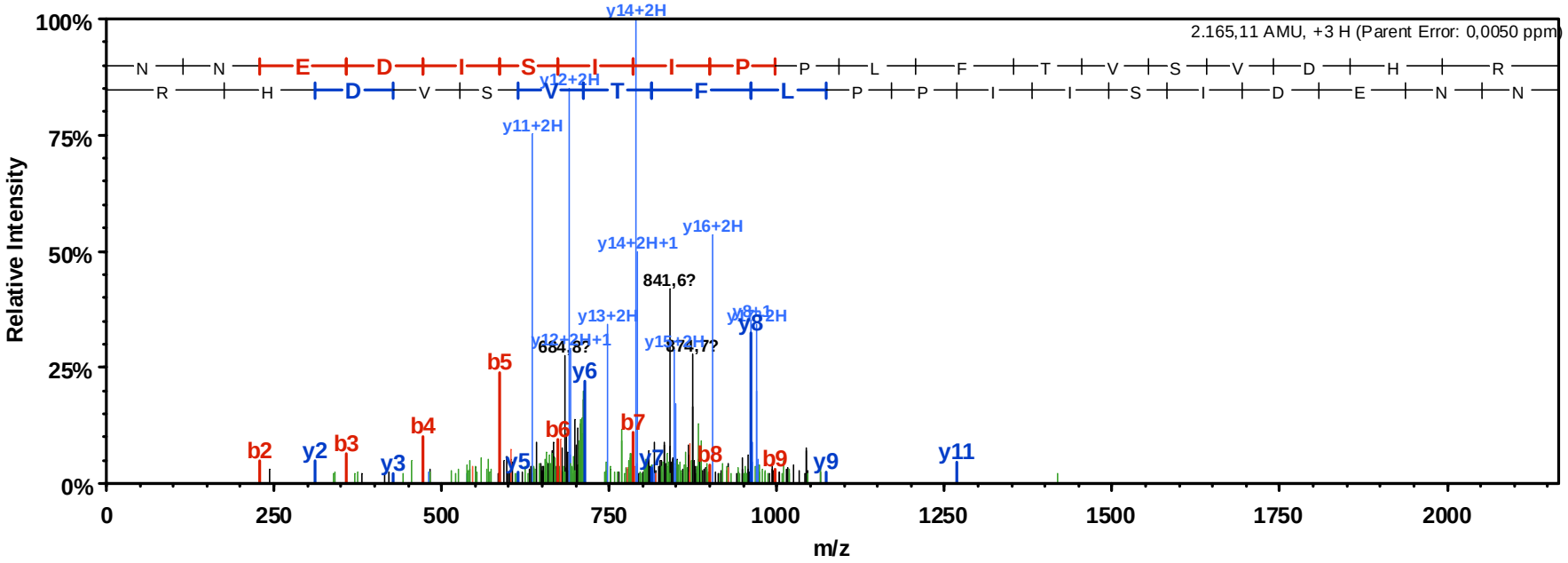
MS/MS spectrum of M6PBP, Mannose-6-phosphate receptor-binding protein 1 tryptic peptide (R)TLTAAAVSGAQPILSK(L) (**corresponding to Table 3, run 3**).



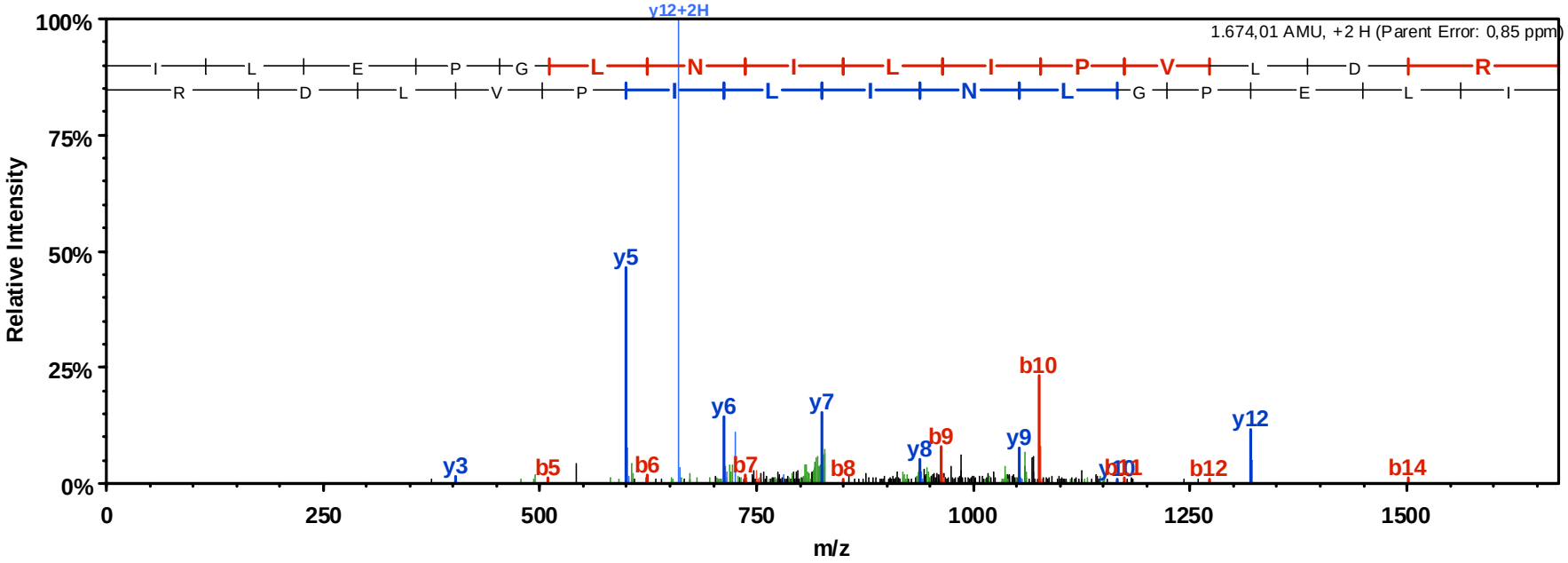
MS/MS spectrum of VDAC2, Voltage-dependent anion-selective channel protein 2 tryptic peptide (R)NNFAVGYR(T) (**corresponding to Table 3, run 3**).



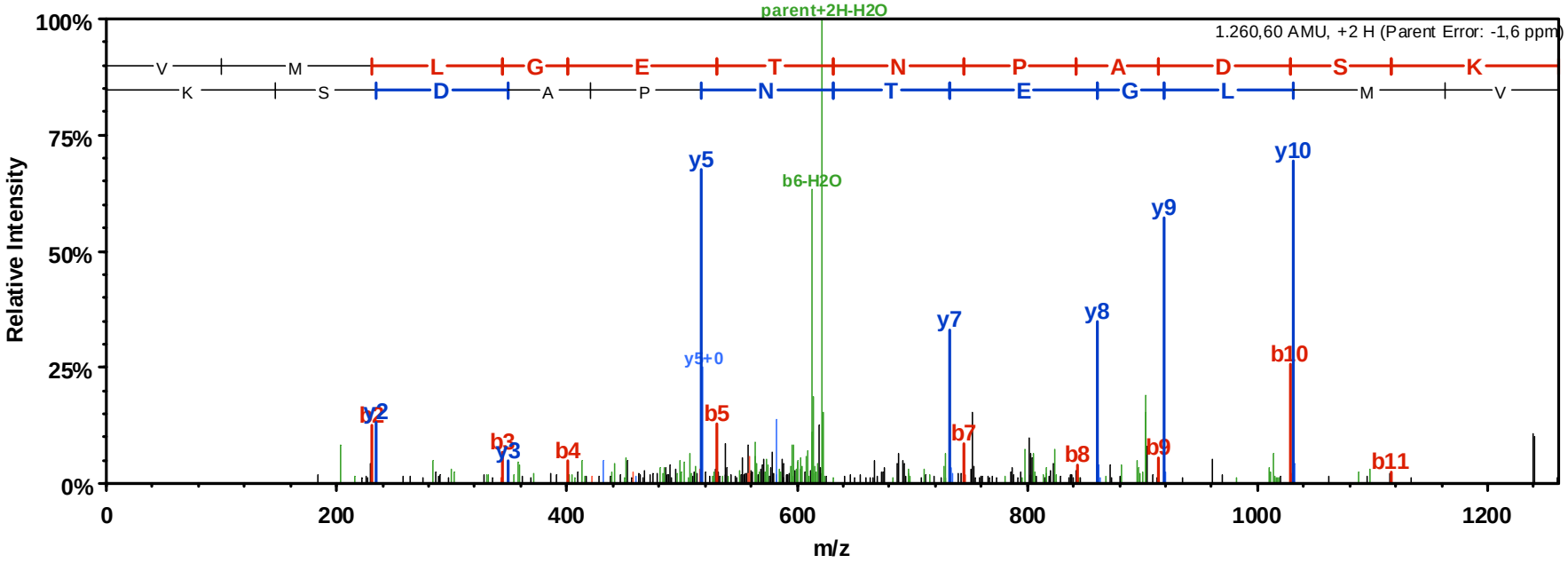
MS/MS spectrum of SSRD, Translocon-associated protein tryptic peptide (R)NNEDISIIPPLFTVSVDHR(G) (**corresponding to Table 3, run 3**).



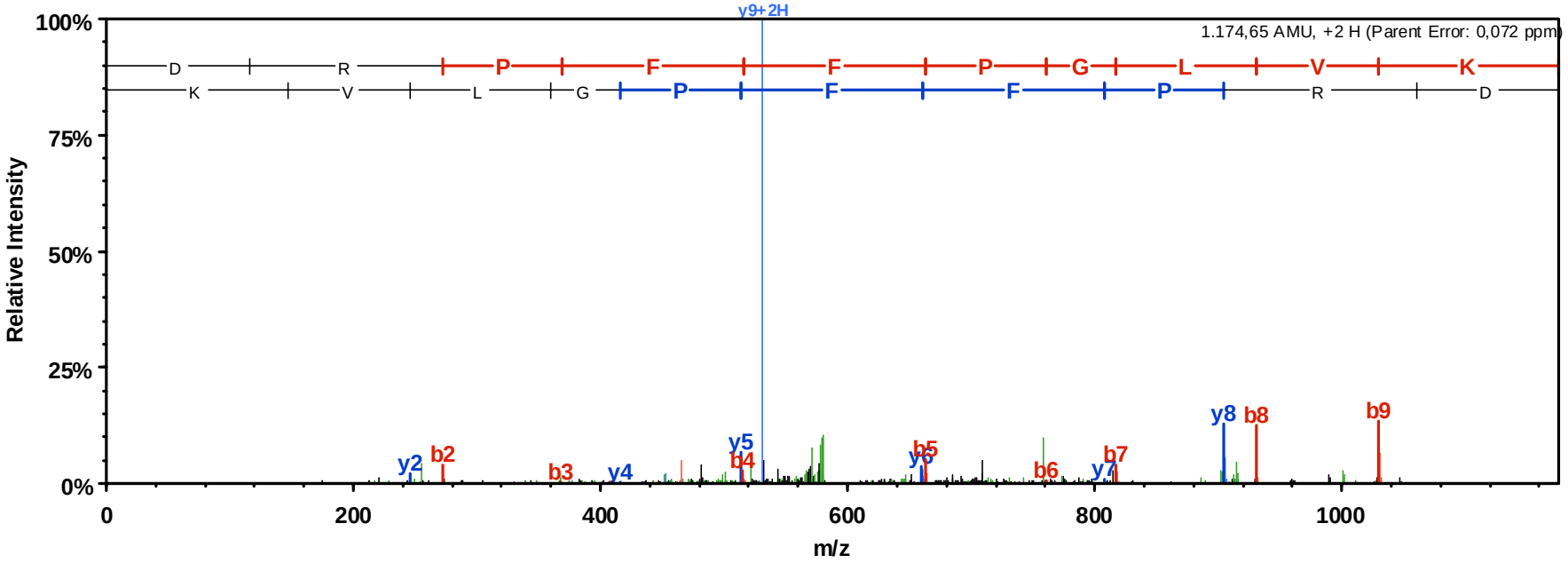
MS/MS spectrum of STML2, Stomatin-like protein 2 tryptic peptide (R)ILEPGLNILIPVLDR(I) (**corresponding to Table 3, run 3**).



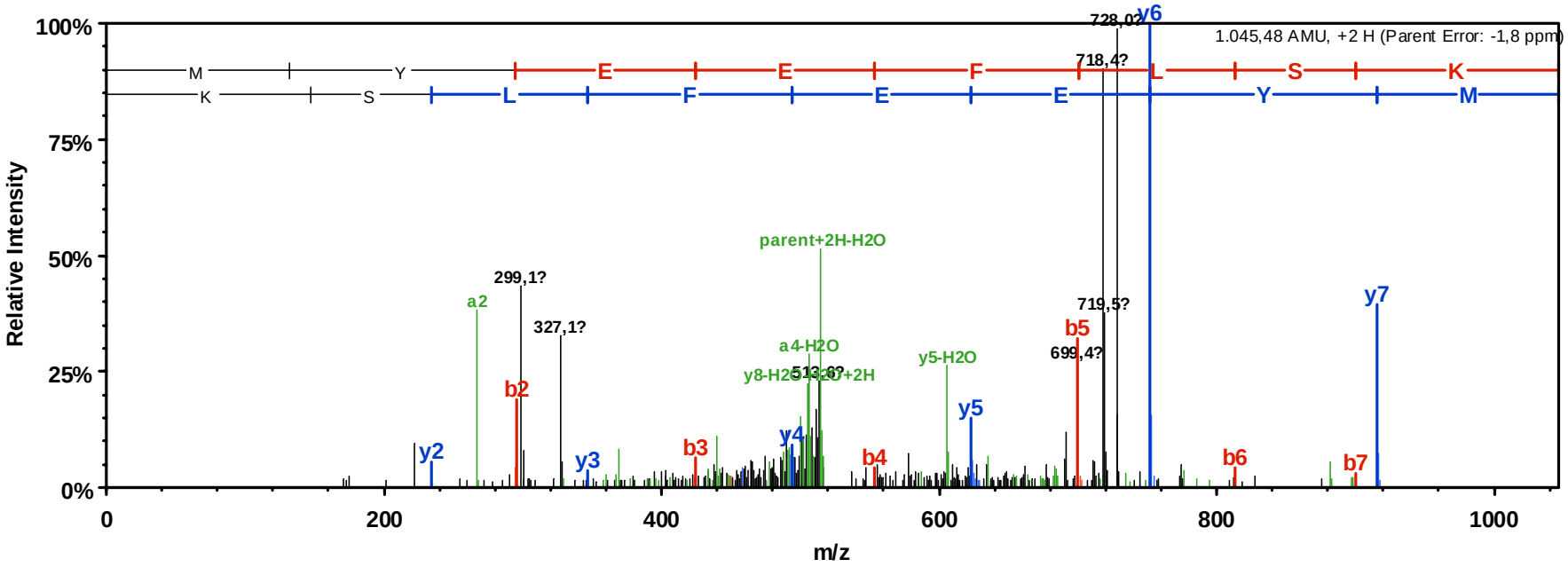
MS/MS spectrum of NDK8, Putative nucleoside diphosphate kinase tryptic peptide (R)VMLGETNPADSK(P) (**corresponding to Table 3, run 1**).



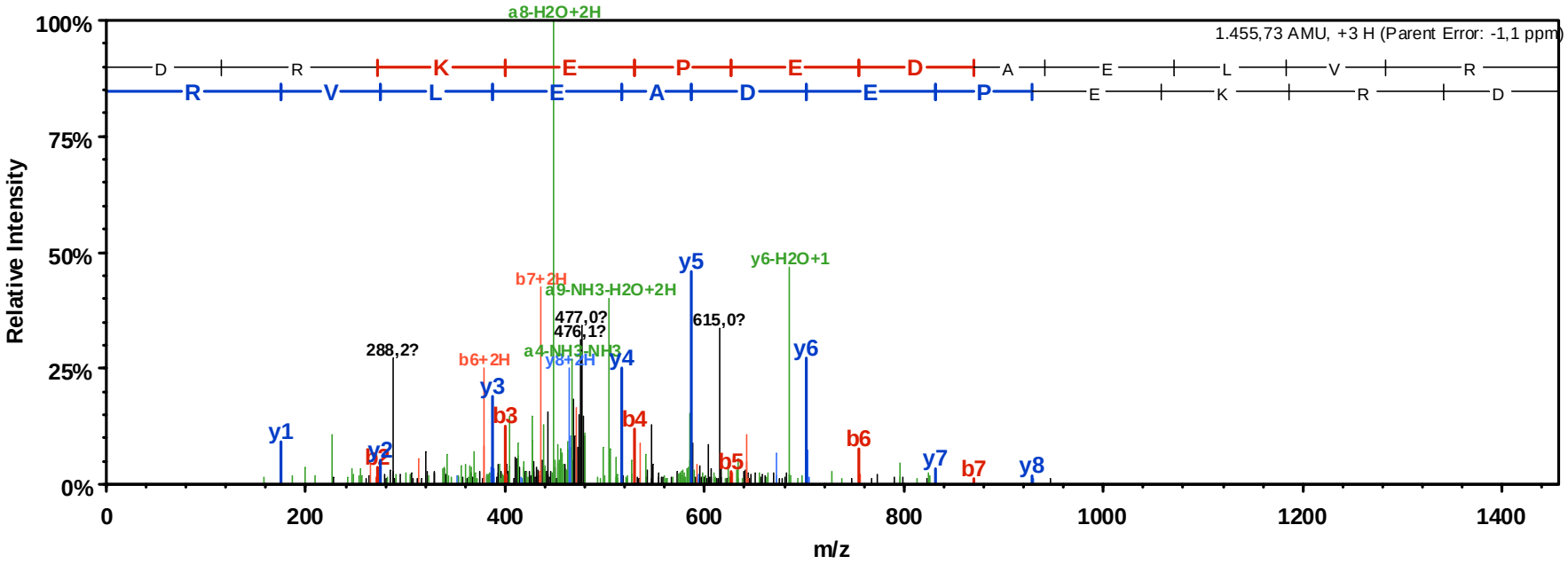
MS/MS spectrum of NDK8, Putative nucleoside diphosphate kinase tryptic peptide (K)DRPFFPGLVK(Y) (**corresponding to Table 3, run 3**).



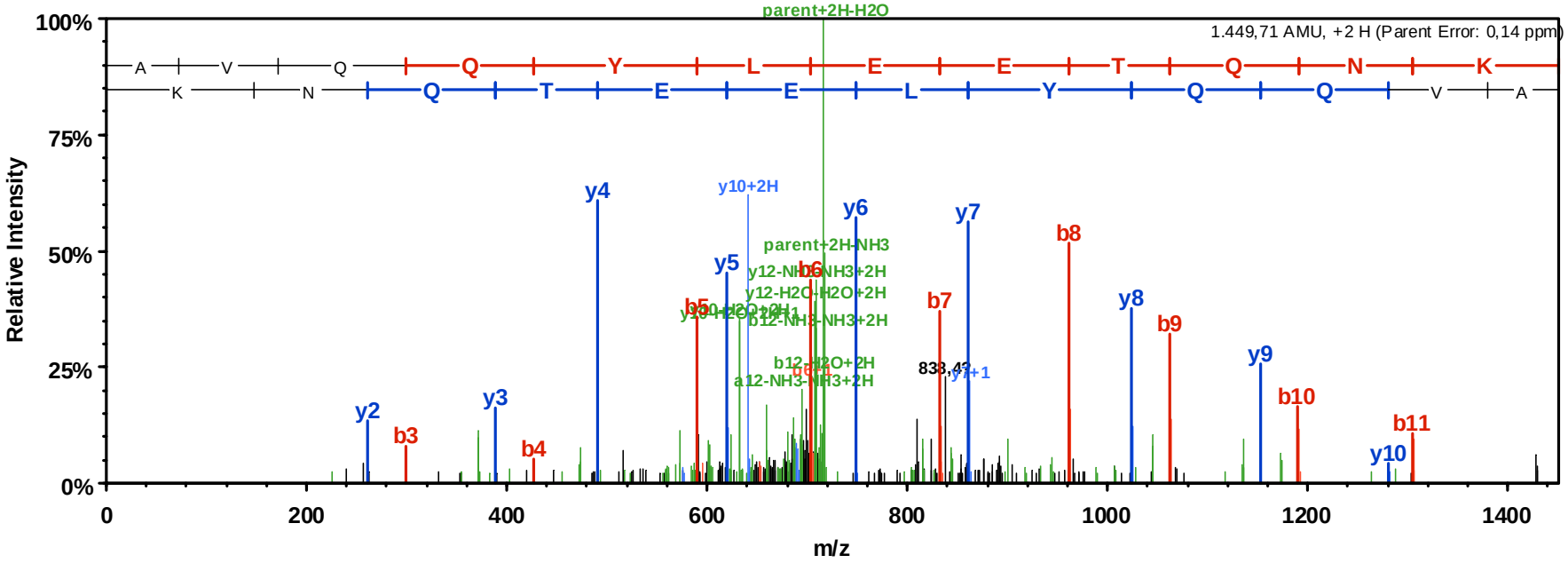
MS/MS spectrum of KAP 0, cAMP-dependent protein kinase tryptic peptide (K)MYEEFLSK(V) (**corresponding to Table 4, run 3**).



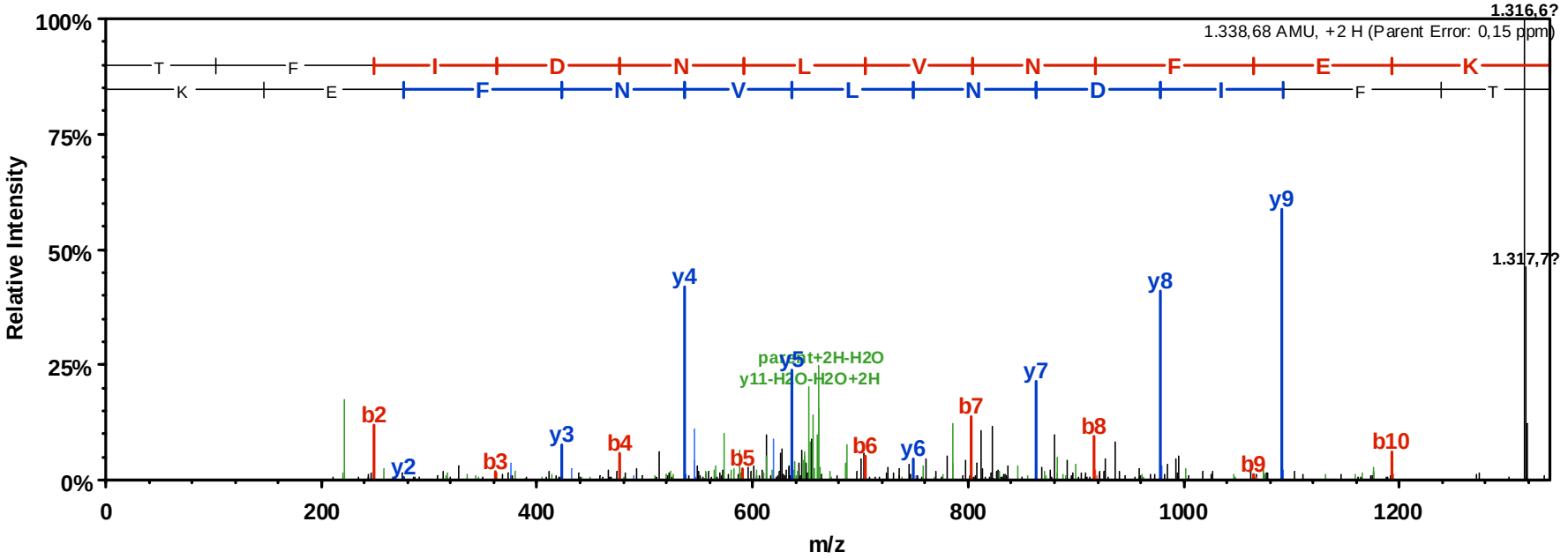
MS/MS spectrum of AKAP18 A-kinase anchor protein, delta isoform tryptic peptide (K)DRKEPEDAELVR(L) (**corresponding to Table 4, run 3**).



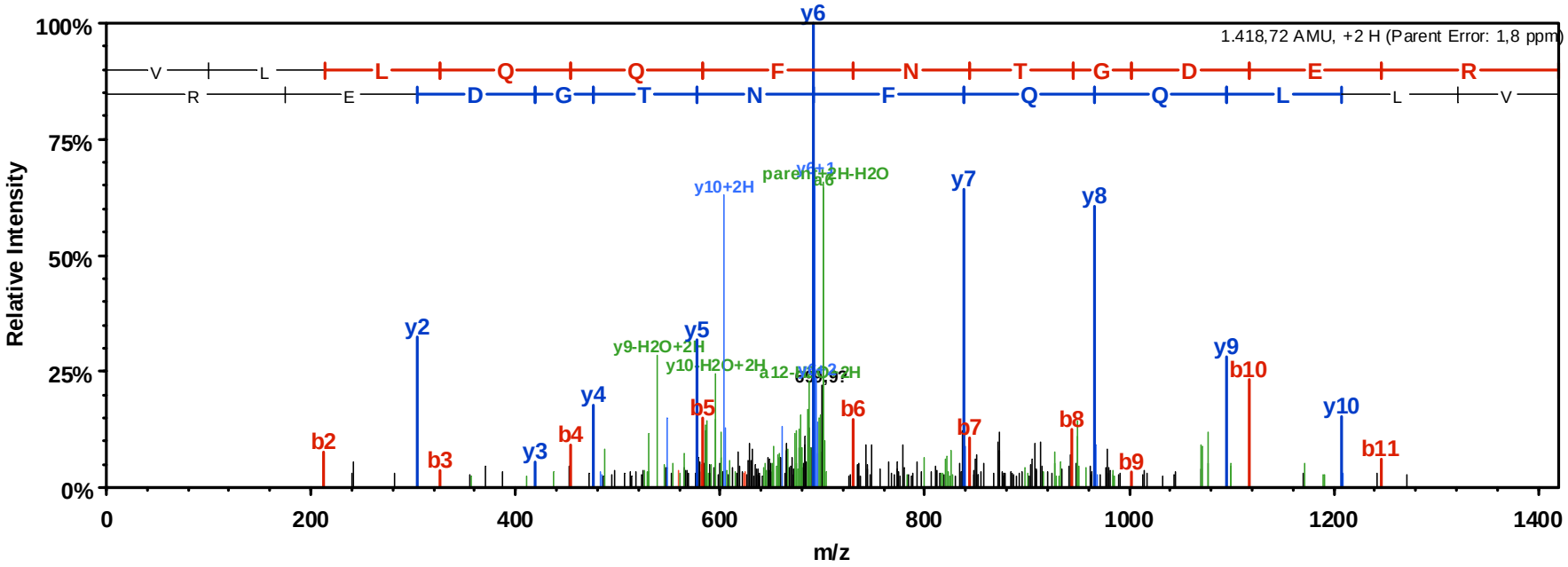
MS/MS spectrum of AKAP18 A-kinase anchor protein, delta isoform tryptic peptide (K)AVQQYLEETQNK(K) (**corresponding to Table 4, run 4**).



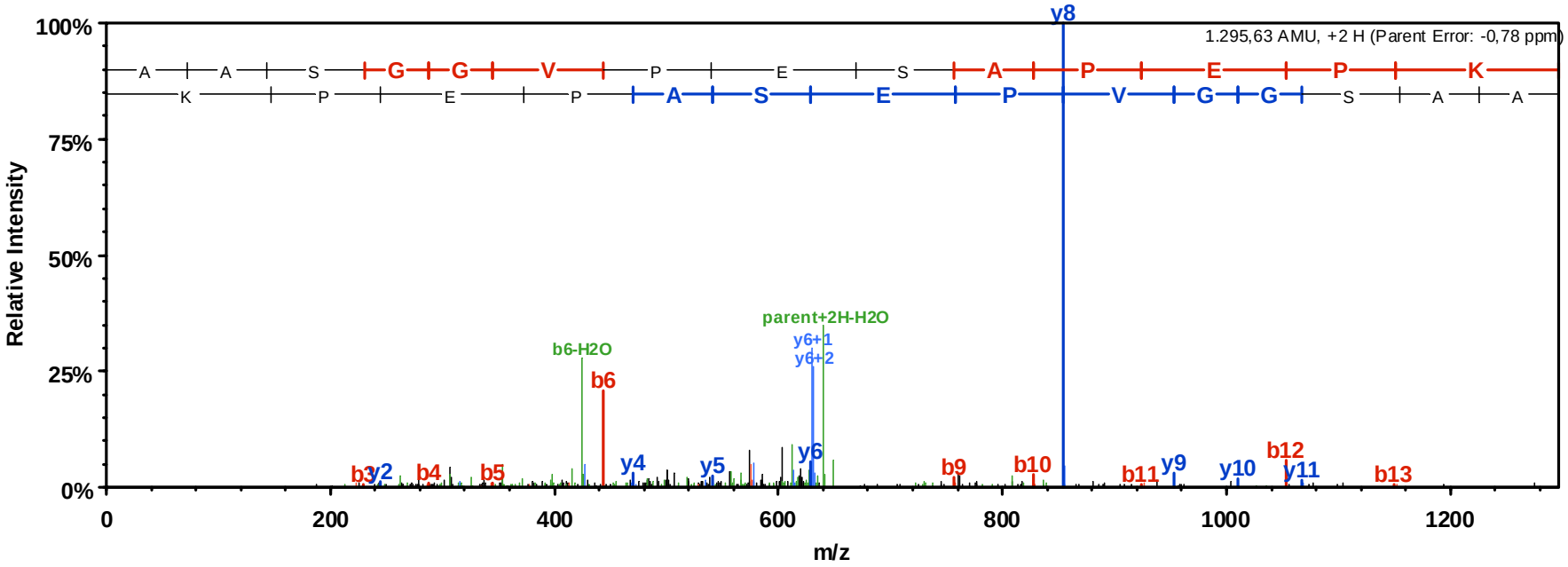
MS/MS spectrum of RPGF4, Rap guanine nucleotide exchange factor 4 tryptic peptide (K)TFIDNLVNFEK(M) (**corresponding to Table 4, run 1**).



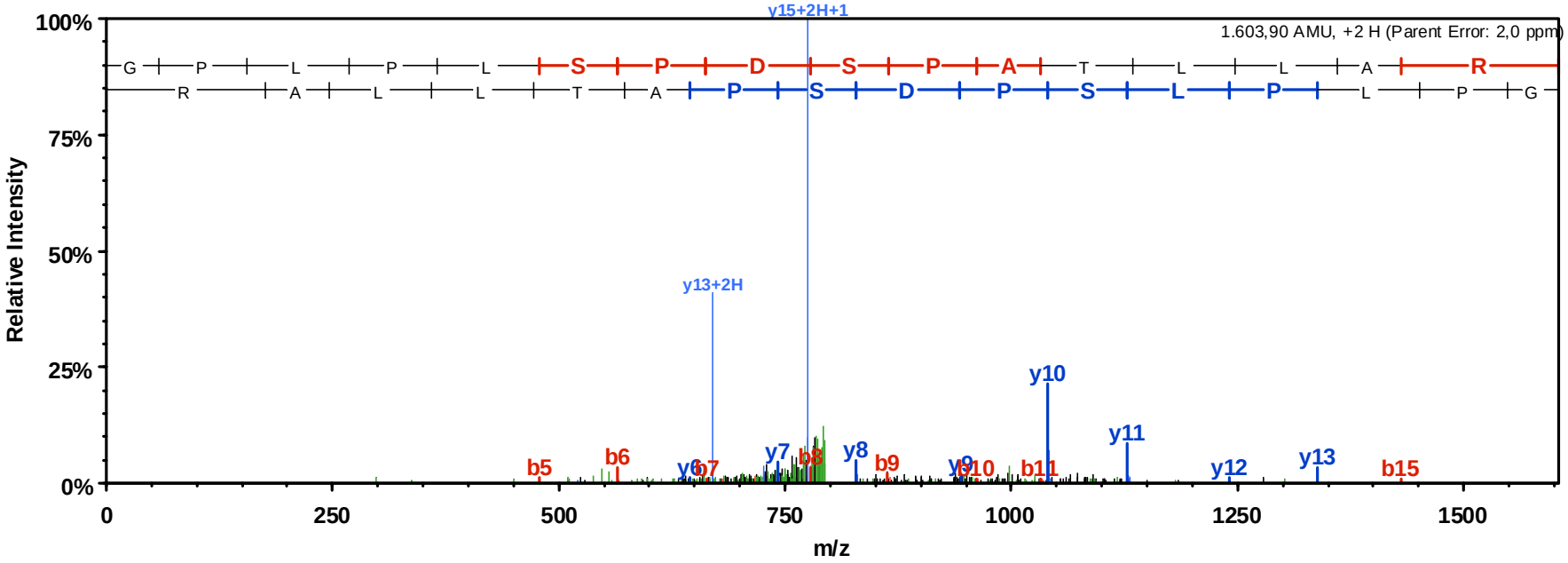
MS/MS spectrum of RPGF4, Rap guanine nucleotide exchange factor 4 tryptic peptide (K)VLLQQFNTGDER(A) (**corresponding to Table 4, run 1**).



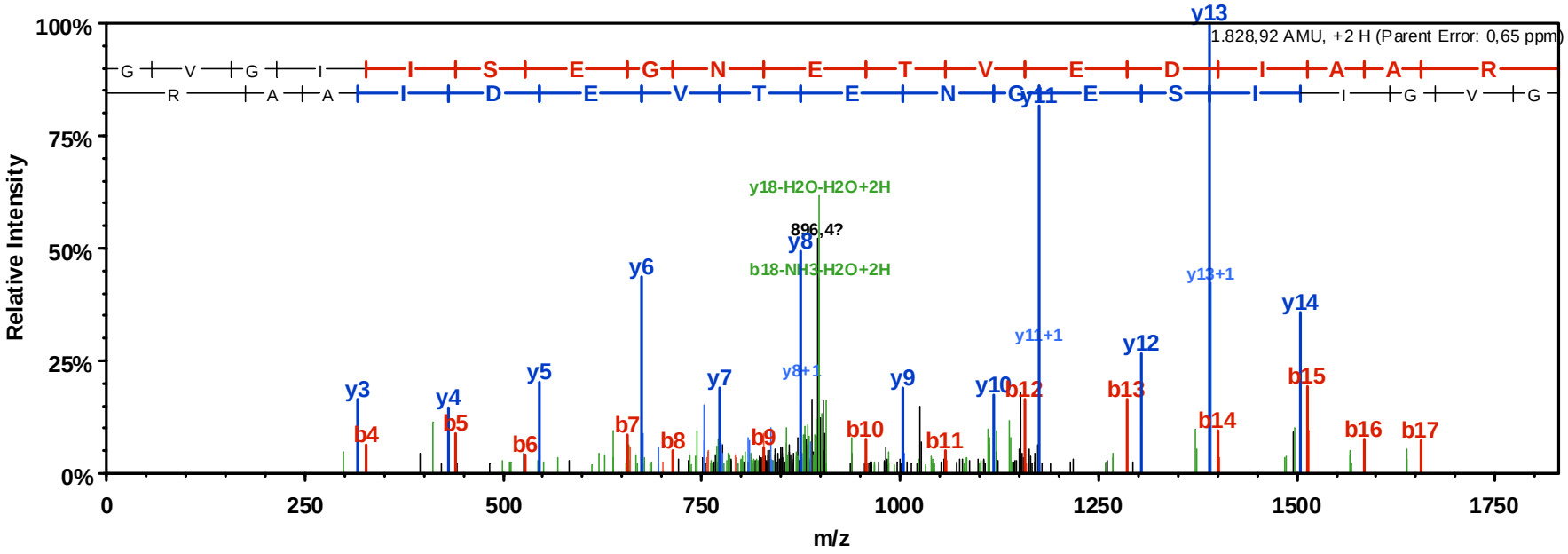
MS/MS spectrum of HCN3, Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 3 tryptic peptide (R)AASGGVPESAPEPK(R) (**Footnote to Table 4, run 2**).



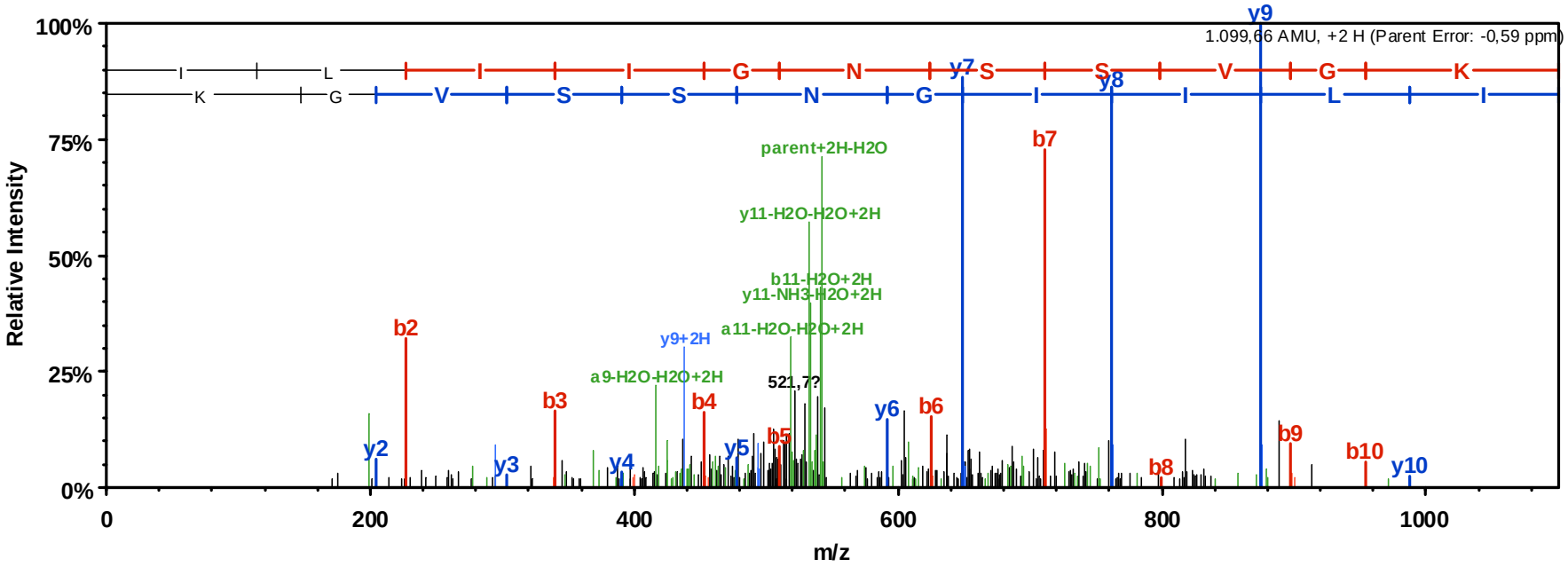
MS/MS spectrum of HCN3, Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 3 tryptic peptide (R)GPLPLSPDSPATLLAR(S) (**Footnote to Table 4, run 3**).



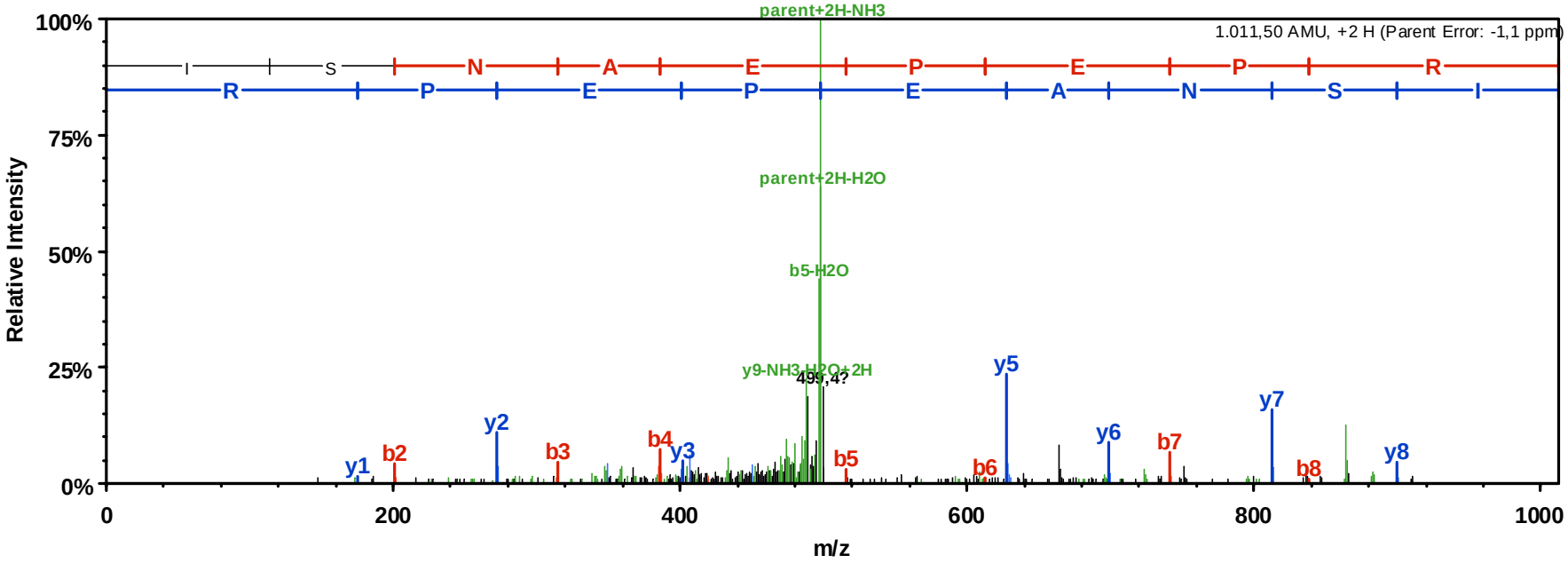
MS/MS spectrum of AT1A1, Sodium/potassium-transporting ATPase tryptic peptide (K)GVGIISEGNETVEDIAAR(L) (**corresponding to Table 5, run 3**).



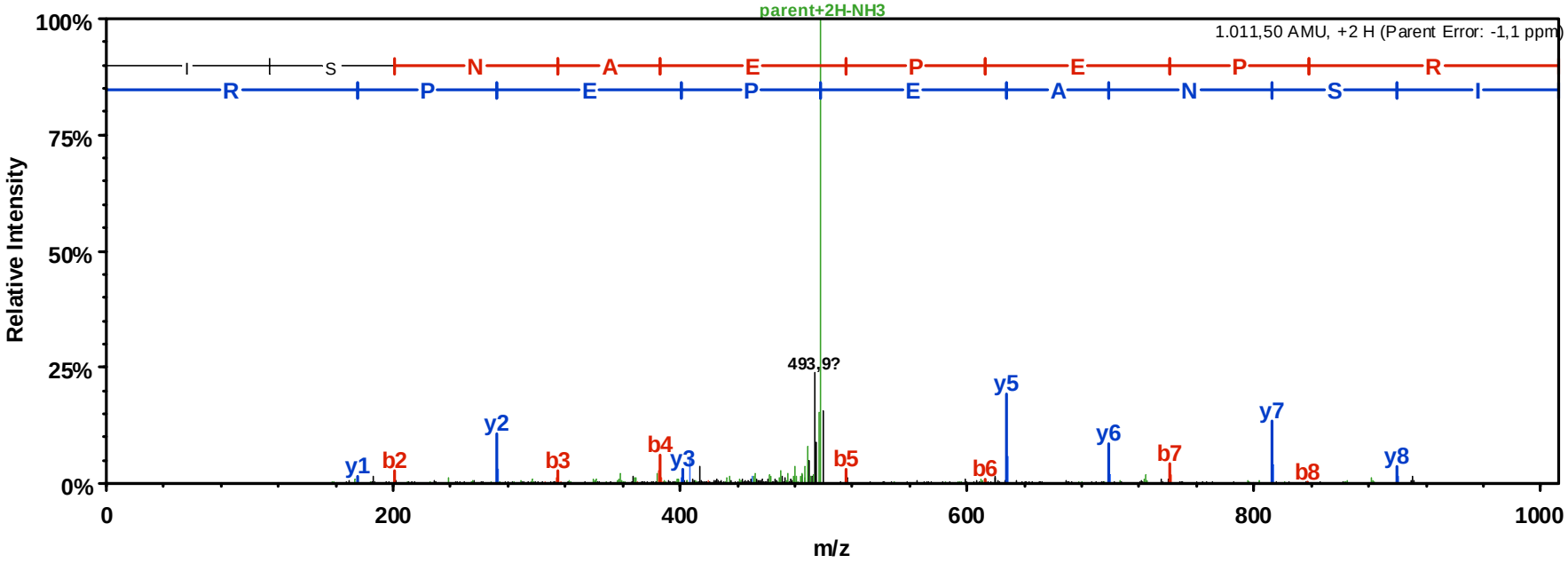
MS/MS spectrum of RAB3A, Ras-related protein Rab-3A tryptic peptide (K)ILIIGNSSVGK(T) (**corresponding to Table 5, run 2**).



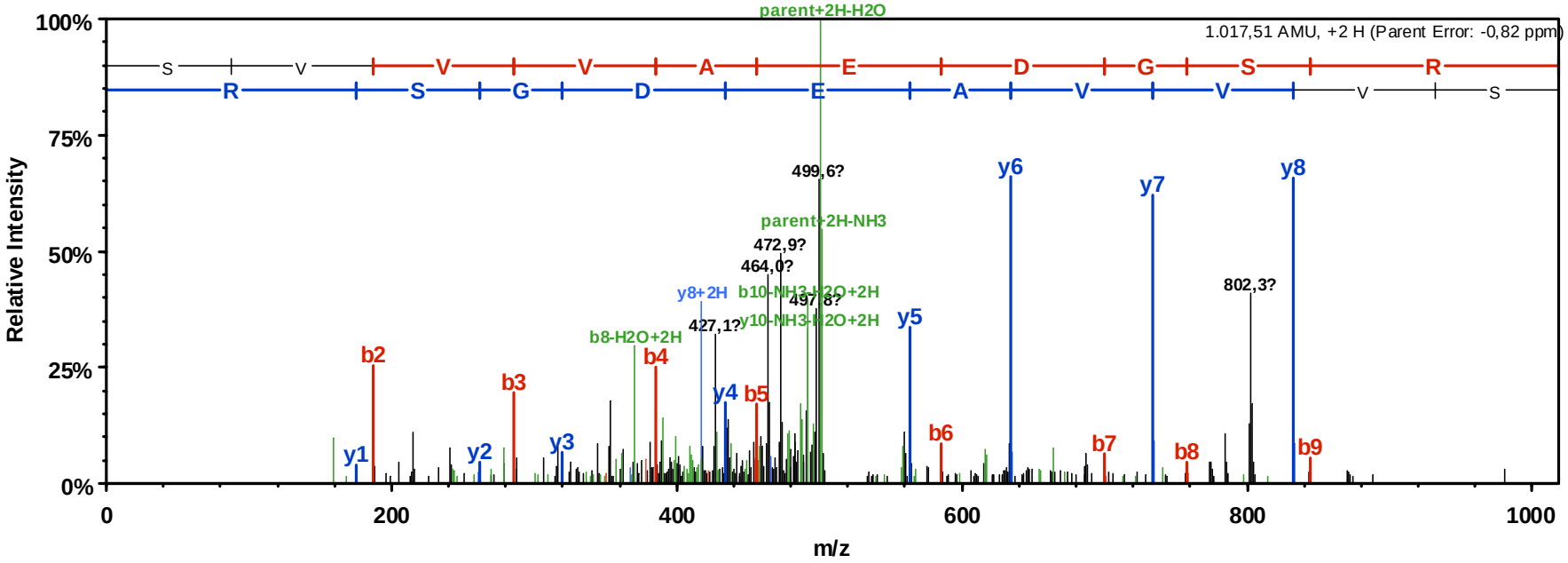
MS/MS spectrum of SEPT8, Sept8 protein tryptic peptide (R)ISNAEPEPR(S) (**corresponding to Table 5, run 3**).



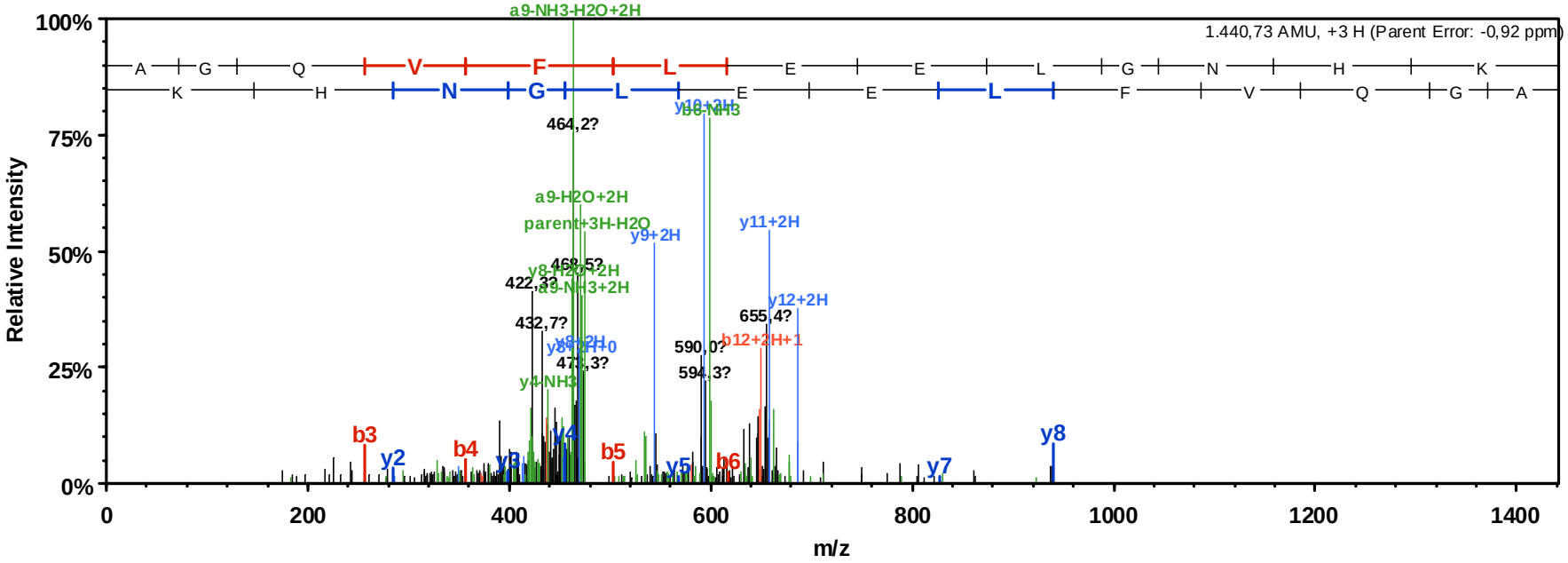
MS/MS spectrum of SEPT8, Sept8 protein tryptic peptide (R)ISNAEPEPR(S) (**corresponding to Table 5, run 4**).



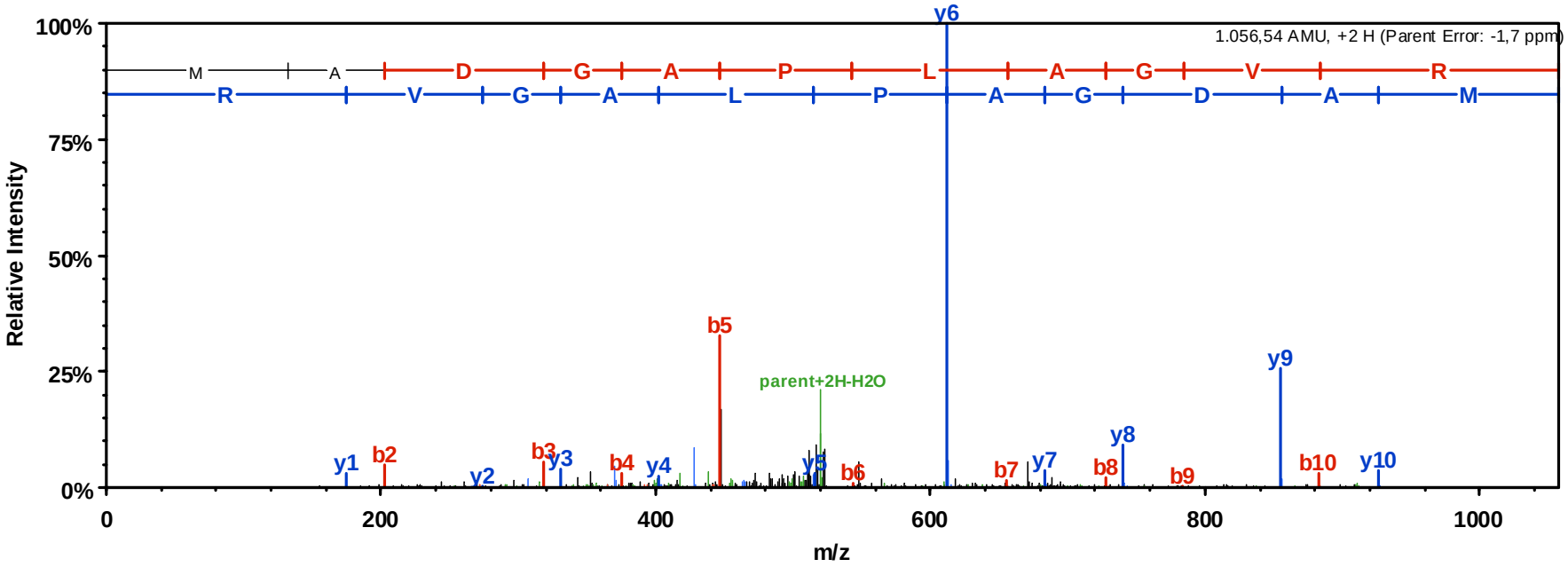
MS/MS spectrum of GBRA1, Gamma-aminobutyric acid receptor tryptic peptide (R)SVVVAEDGSR(L) (**corresponding to Table 5, run 3**).



MS/MS spectrum of CN37, 2',3'-cyclic-nucleotide 3'-phosphodiesterase tryptic peptide (K)AGQVFLEELGNHK(A) (**corresponding to Table 5, run 3**).



MS/MS spectrum of SV2A, Synaptic vesicle glycoprotein 2A tryptic peptide (R)MADGAPLAGVR(G) (**corresponding to Table 5, run 2**).



11. References

1. Bertinetti, D., Schweinsberg, S., Hanke, S. E., Schwede, F., Bertinetti, O., Drewianka, S., Genieser, H. G., and Herberg, F.W. (2009) Chemical tools selectively target components of the PKA system. *BMC Chem Biol* **9**, 3.

2. Cook, P. F., Neville, M. E., Jr., Vrana, K. E., Hartl, F. T., and Roskoski, R., Jr. (1982) Adenosine cyclic 3',5'-monophosphate dependent protein kinase: kinetic mechanism for the bovine skeletal muscle catalytic subunit. *Biochemistry* **21**, 5794-5799.

3. Moll, D., Prinz, A., Gesellchen, F., Drewianka, S., Zimmermann, B., and Herberg, F.W. (2006) Biomolecular interaction analysis in functional proteomics. *J Neural Transm* **113**, 1015-1032.

4. Keller, A., Nesvizhskii, A. I., Kolker, E., and Aebersold, R. (2002) Empirical statistical model to estimate the accuracy of peptide identifications made by MS/MS and database search. *Anal Chem* **74**, 5383–5392.

5. Lenz, T. caprotec bioanalytics GmbH, unpublished results.