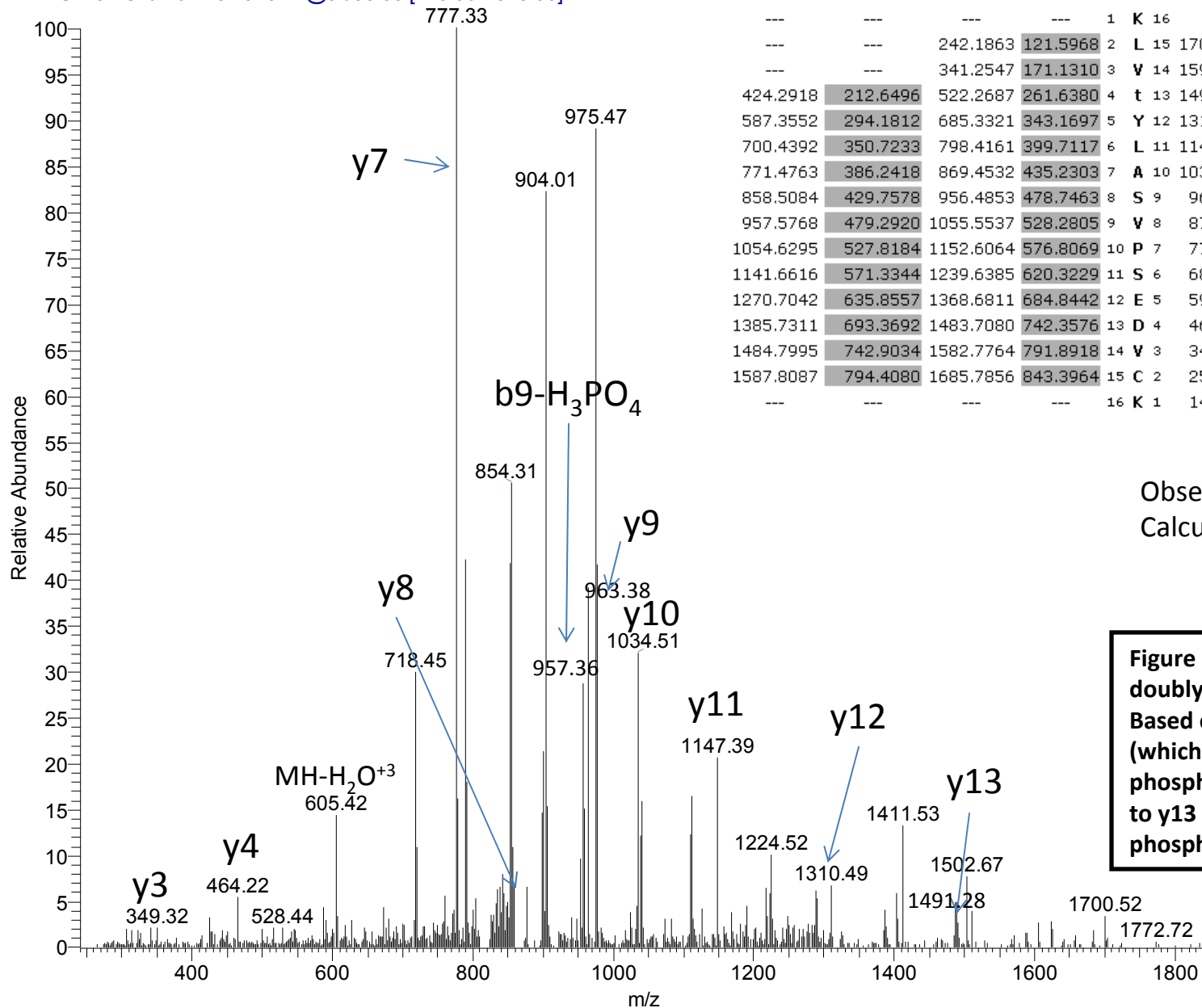


CamKII

MS/MS of peptide KLVpTYLASVPSEDVCK

OT10-3482 #3221 RT: 37.23 AV: 1 NL: 1.69E4
 T: ITMS + c NSI d Full ms2 916.44@cid35.00 [240.00-1845.00]

b-H ₃ PO ₄	b-H ₃ PO ₄ ⁺²	b	b ⁺²		y	y ⁺²	y-H ₃ PO ₄	y-H ₃ PO ₄ ⁺²	
---	---	---	---	1	K 16	---	---	---	
---	---	242.1863	121.5968	2	L 15	1703.7962	852.4017	1605.8193	803.4133
---	---	341.2547	171.1310	3	V 14	1590.7121	795.8597	1492.7352	746.8712
424.2918	212.6496	522.2687	261.6380	4	t 13	1491.6437	746.3255	1393.6668	697.3370
587.3552	294.1812	685.3321	343.1697	5	Y 12	1310.6297	655.8185	---	---
700.4392	350.7233	798.4161	399.7117	6	L 11	1147.5664	574.2868	---	---
771.4763	386.2418	869.4532	435.2303	7	A 10	1034.4823	517.7448	---	---
858.5084	429.7578	956.4853	478.7463	8	S 9	963.4452	482.2262	---	---
957.5768	479.2920	1055.5537	528.2805	9	V 8	876.4131	438.7102	---	---
1054.6295	527.8184	1152.6064	576.8069	10	P 7	777.3447	389.1760	---	---
1141.6616	571.3344	1239.6385	620.3229	11	S 6	680.2920	340.6496	---	---
1270.7042	635.8557	1368.6811	684.8442	12	E 5	593.2599	297.1336	---	---
1385.7311	693.3692	1483.7080	742.3576	13	D 4	464.2173	232.6123	---	---
1484.7995	742.9034	1582.7764	791.8918	14	V 3	349.1904	175.0988	---	---
1587.8087	794.4080	1685.7856	843.3964	15	C 2	250.1220	125.5646	---	---
---	---	---	---	16	K 1	147.1128	74.0600	---	---



Observed M=1830.8685
 Calculated M=1830.8838

Figure S1. MS/MS on the doubly charged 916.44 ion. Based on the intact mass (which indicated 1 site of phosphorylation) and the y10 to y13 ions, residue 79 is a phosphothreonine.

CamKII

MS/MS of peptide LVTYLA^pSVPS^SEDVCKR

OT10-3482 #3180 RT: 36.95 AV: 1 NL: 9.98E4
 T: ITMS + c NSI d Full ms2 931.45@cid35.00 [245.00-1875.00]

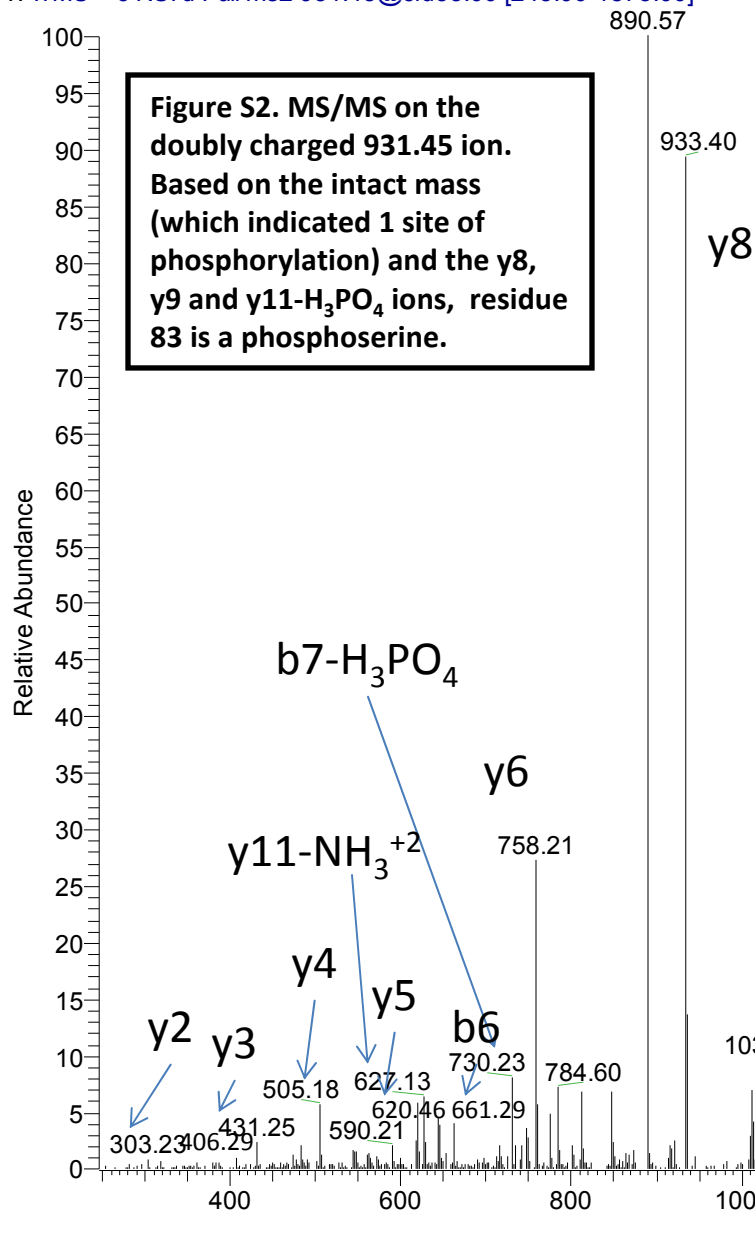
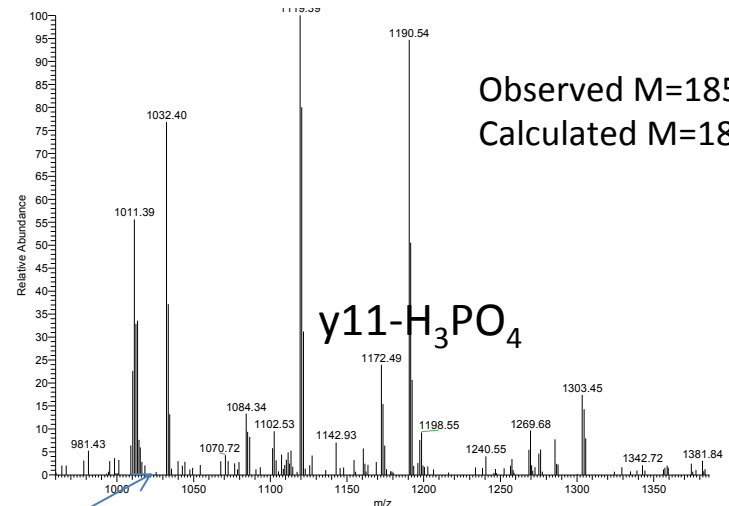


Figure S2. MS/MS on the doubly charged 931.45 ion. Based on the intact mass (which indicated 1 site of phosphorylation) and the y8, y9 and y11-H₃PO₄ ions, residue 83 is a phosphoserine.

b-H ₃ PO ₄	b-H ₃ PO ₄ ⁺²	b	b ⁺²		y	y ⁺²	y-H ₃ PO ₄	y-H ₃ PO ₄ ⁺²	
---	---	---	---	1	L 16	---	---	---	
---	---	213.1598	107.0835	2	V 15	1746.8132	873.9102	1648.8363	824.9218
---	---	314.2074	157.6074	3	T 14	1647.7448	824.3760	1549.7679	775.3876
---	---	477.2708	239.1390	4	Y 13	1546.6971	773.8522	1448.7202	724.8638
---	---	590.3548	295.6811	5	L 12	1383.6338	692.3205	1285.6569	643.3321
---	---	661.3919	331.1996	6	A 11	1270.5497	635.7785	1172.5728	586.7901
730.4134	365.7103	828.3903	414.6988	7	S 10	1199.5126	600.2599	1101.5357	551.2715
829.4818	415.2445	927.4587	464.2330	8	V 9	1032.5143	516.7608	---	---
926.5346	463.7709	1024.5115	512.7594	9	P 8	933.4458	467.2266	---	---
1013.5666	507.2869	1111.5435	556.2754	10	S 7	836.3931	418.7002	---	---
1142.6092	571.8082	1240.5861	620.7967	11	E 6	749.3610	375.1842	---	---
1257.6361	629.3217	1355.6130	678.3102	12	D 5	620.3185	310.6629	---	---
1356.7046	678.8559	1454.6815	727.8444	13	V 4	505.2915	253.1494	---	---
1459.7137	730.3605	1557.6906	779.3490	14	C 3	406.2231	203.6152	---	---
1587.8087	794.4080	1685.7856	843.3964	15	K 2	303.2139	152.1106	---	---
---	---	---	---	16	R 1	175.1190	88.0631	---	---

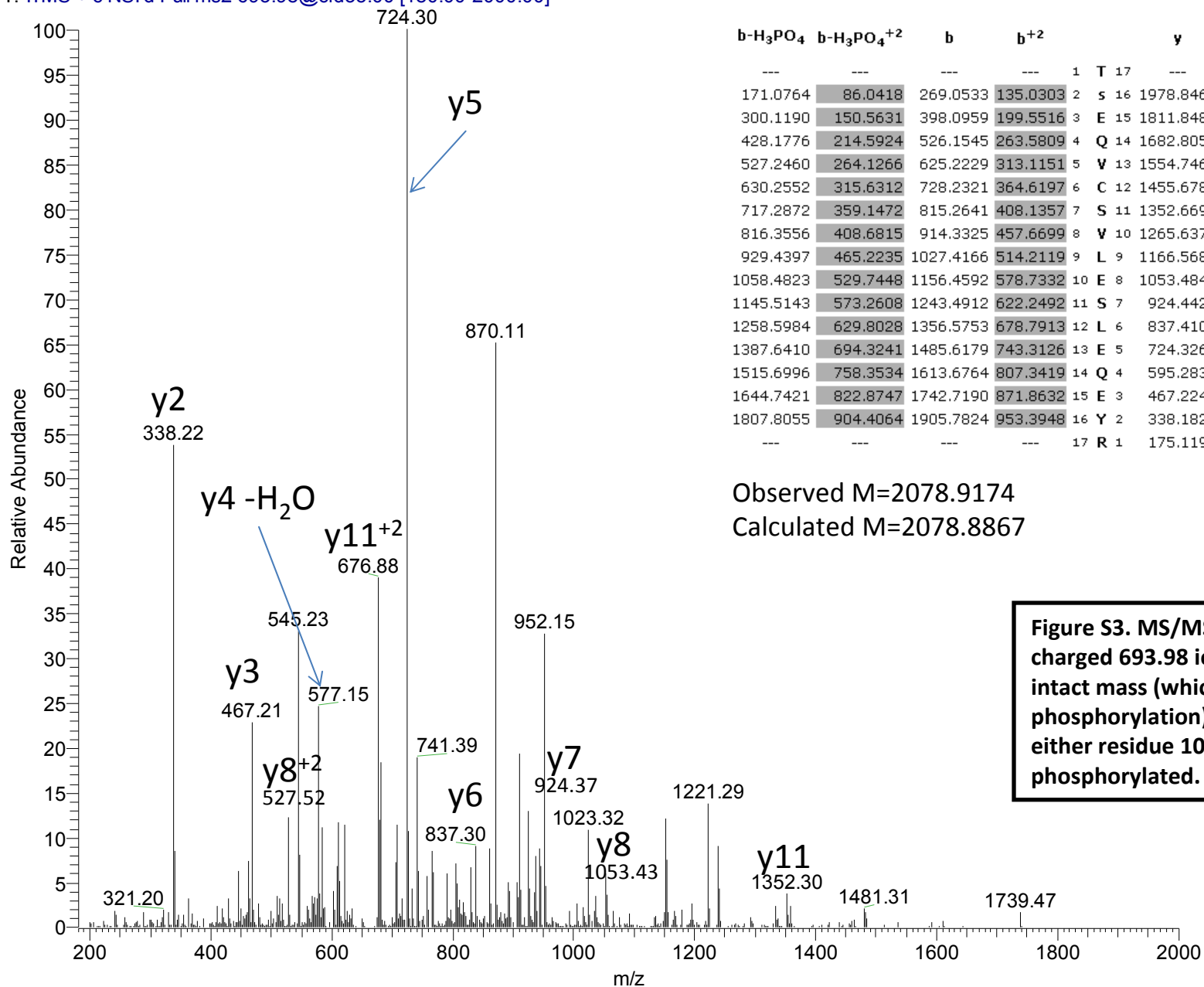


Observed M=1858.8736
 Calculated M=1858.8900

CamKII

MS/MS of peptide pT/pSEQVCSVLESLEQYR

OT10-3482 #4297 RT: 45.66 AV: 1 NL: 1.91E5
 T: ITMS + c NSI d Full ms2 693.98@cid35.00 [180.00-2000.00]



b-H ₃ PO ₄	b-H ₃ PO ₄ +2	b	b+2		y	y+2	y-H ₃ PO ₄	y-H ₃ PO ₄ +2
---	---	---	---	1	T 17	---	---	---
171.0764	86.0418	269.0533	135.0303	2	s 16	1978.8464	989.9268	1880.8695
300.1190	150.5631	398.0959	199.5516	3	E 15	1811.8480	906.4276	---
428.1776	214.5924	526.1545	263.5809	4	Q 14	1682.8054	841.9063	---
527.2460	264.1266	625.2229	313.1151	5	V 13	1554.7468	777.8771	---
630.2552	315.6312	728.2321	364.6197	6	C 12	1455.6784	728.3428	---
717.2872	359.1472	815.2641	408.1357	7	S 11	1352.6692	676.8383	---
816.3556	408.6815	914.3325	457.6699	8	V 10	1265.6372	633.3222	---
929.4397	465.2235	1027.4166	514.2119	9	L 9	1166.5688	583.7880	---
1058.4823	529.7448	1156.4592	578.7332	10	E 8	1053.4847	527.2460	---
1145.5143	573.2608	1243.4912	622.2492	11	S 7	924.4421	462.7247	---
1258.5984	629.8028	1356.5753	678.7913	12	L 6	837.4101	419.2087	---
1387.6410	694.3241	1485.6179	743.3126	13	E 5	724.3260	362.6667	---
1515.6996	758.3534	1613.6764	807.3419	14	Q 4	595.2835	298.1454	---
1644.7421	822.8747	1742.7190	871.8632	15	E 3	467.2249	234.1161	---
1807.8055	904.4064	1905.7824	953.3948	16	Y 2	338.1823	169.5948	---
---	---	---	---	17	R 1	175.1190	88.0631	---

Observed M=2078.9174
 Calculated M=2078.8867

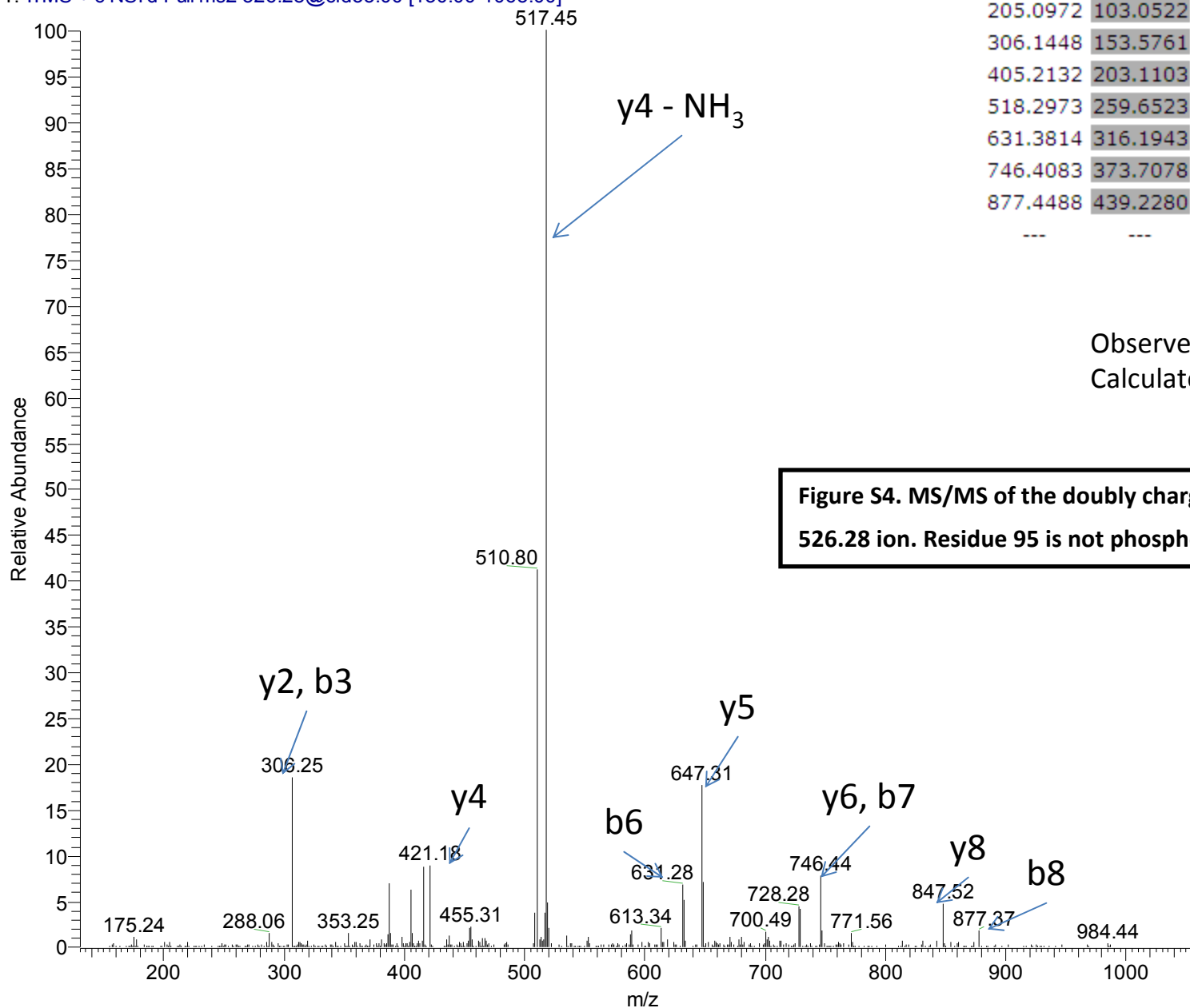
Figure S3. MS/MS on the triply charged 693.98 ion. Based on the intact mass (which indicated 1 site of phosphorylation) and the y ions, either residue 1002 or 1003 is phosphorylated.

CamKII

MS/MS of peptide GFTVIIDMR

OT10-3482 #3773 RT: 41.58 AV: 1 NL: 3.32E4

T: ITMS + c NSI d Full ms2 526.28@cid35.00 [130.00-1065.00]



b	b ⁺	y	y ⁺
---	---	1 G 9	---
205.0972	103.0522	2 F 8	994.5390
306.1448	153.5761	3 T 7	847.4706
405.2132	203.1103	4 V 6	746.4229
518.2973	259.6523	5 I 5	647.3545
631.3814	316.1943	6 I 4	534.2704
746.4083	373.7078	7 D 3	421.1864
877.4488	439.2280	8 M 2	306.1594
---	---	9 R 1	175.1190

Observed M=1050.5511
Calculated M=1050.5532

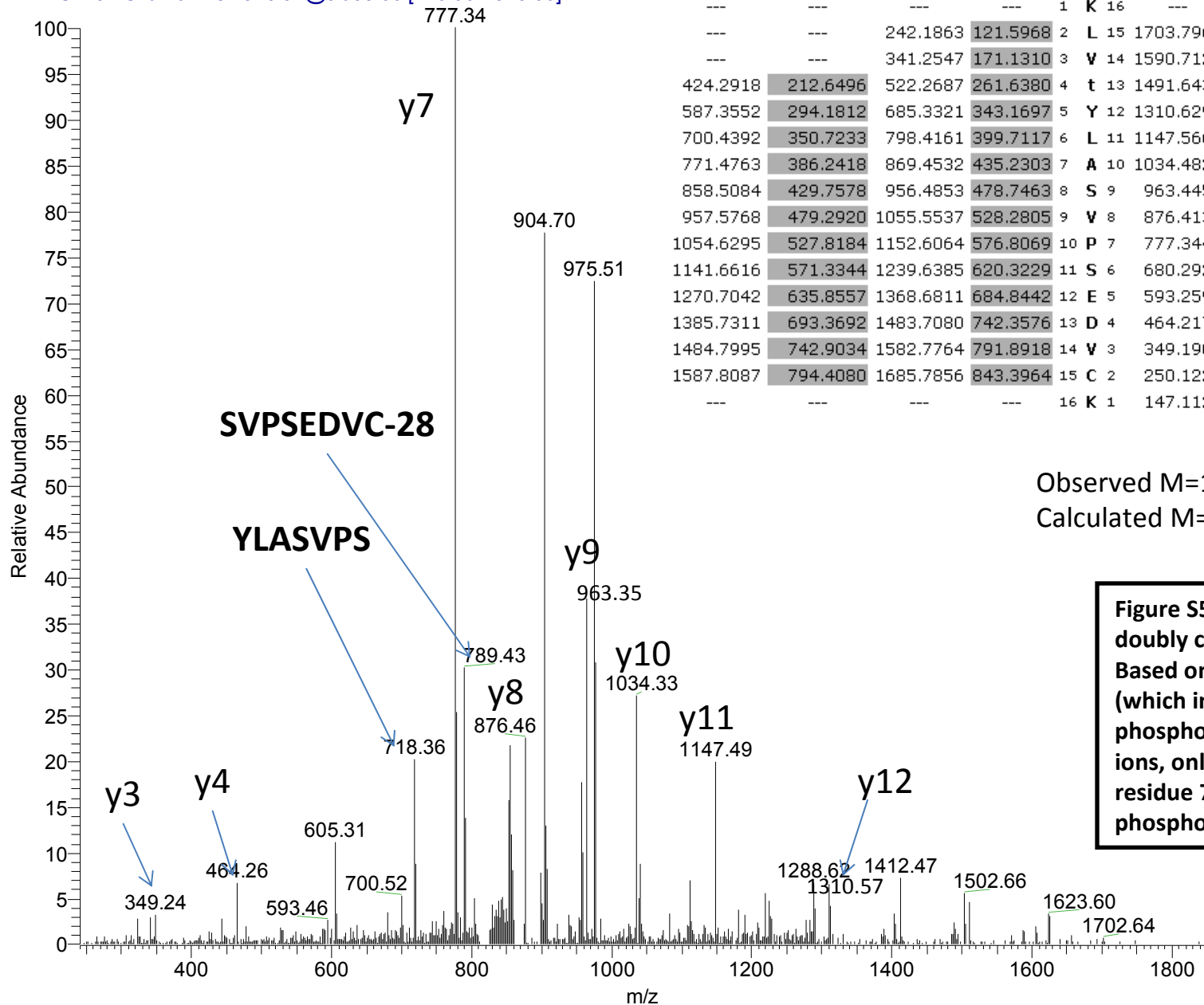
Figure S4. MS/MS of the doubly charged 526.28 ion. Residue 95 is not phosphorylated.

PKA

MS/MS of peptide KLVpTYLASVPSEDVCK

OT10-3486 #3221 RT: 37.38 AV: 1 NL: 1.13E4
 T: ITMS + c NSI d Full ms2 916.94@cid35.00 [240.00-1845.00]

b-H ₃ PO ₄	b-H ₃ PO ₄ +2	b	b+2		y	y+2	y-H ₃ PO ₄	y-H ₃ PO ₄ +2
---	---	---	---	1	K	16	---	---
---	---	242.1863	121.5968	2	L	15	1703.7962	852.4017
---	---	341.2547	171.1310	3	V	14	1590.7121	795.8597
424.2918	212.6496	522.2687	261.6380	4	t	13	1491.6437	746.3255
587.3552	294.1812	685.3321	343.1697	5	Y	12	1310.6297	655.8185
700.4392	350.7233	798.4161	399.7117	6	L	11	1147.5664	574.2868
771.4763	386.2418	869.4532	435.2303	7	A	10	1034.4823	517.7448
858.5084	429.7578	956.4853	478.7463	8	S	9	963.4452	482.2262
957.5768	479.2920	1055.5537	528.2805	9	V	8	876.4131	438.7102
1054.6295	527.8184	1152.6064	576.8069	10	P	7	777.3447	389.1760
1141.6616	571.3344	1239.6385	620.3229	11	S	6	680.2920	340.6496
1270.7042	635.8557	1368.6811	684.8442	12	E	5	593.2599	297.1336
1385.7311	693.3692	1483.7080	742.3576	13	D	4	464.2173	232.6123
1484.7995	742.9034	1582.7764	791.8918	14	V	3	349.1904	175.0988
1587.8087	794.4080	1685.7856	843.3964	15	C	2	250.1220	125.5646
---	---	---	---	16	K	1	147.1128	74.0600



Observed M=1830.8690
 Calculated M=1830.8838

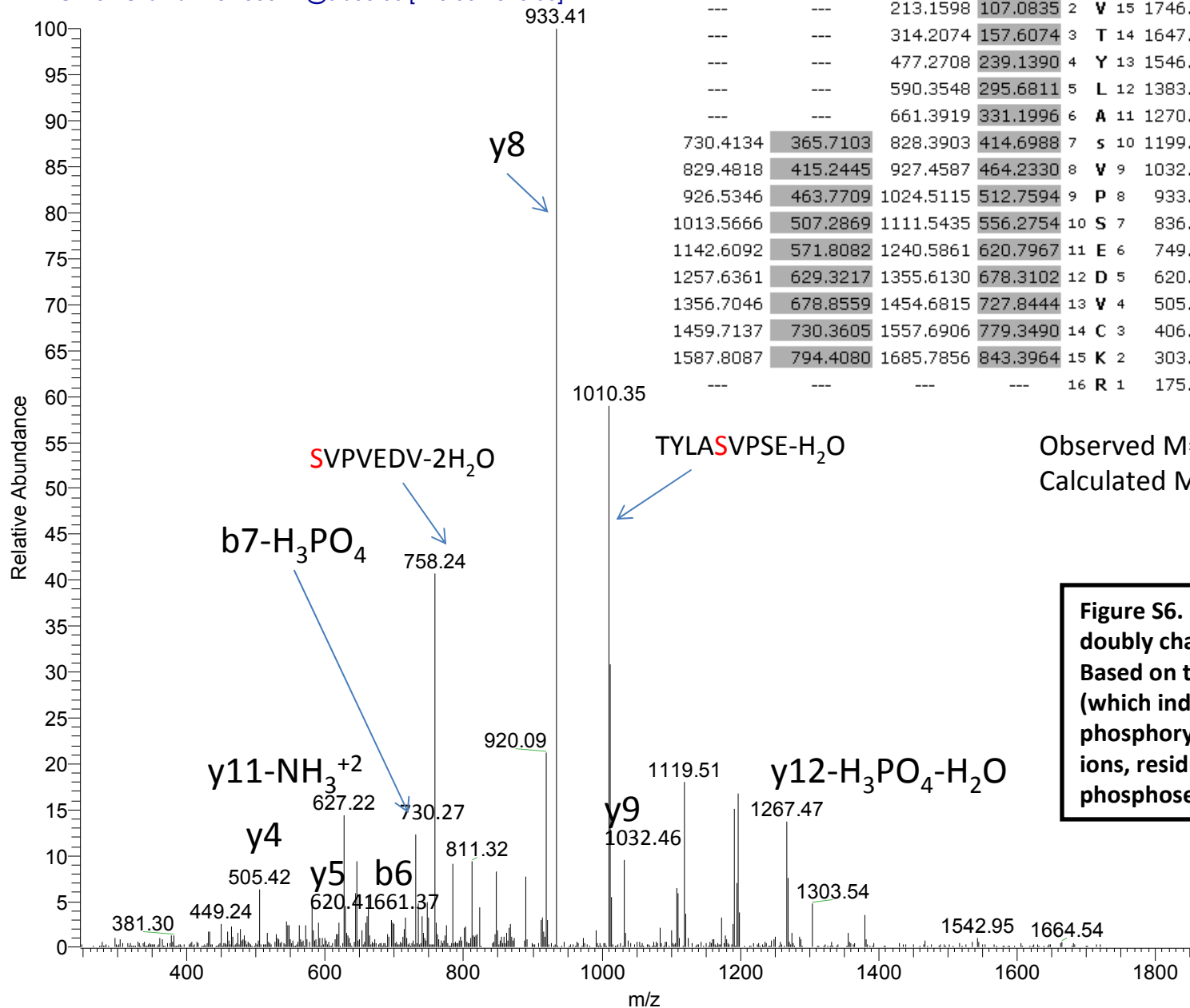
Figure S5. MS/MS on the doubly charged 916.94 ion. Based on the intact mass (which indicated 1 site of phosphorylation) and the y ions, only the threonine at residue 79 can be phosphorylated.

PKA

MS/MS of peptide LVTYLA^pSVPSE^DVCKR

OT10-3486 #3176 RT: 37.04 AV: 1 NL: 2.73E4
 T: ITMS + c NSI d Full ms2 930.44@cid35.00 [245.00-1875.00]

	b-H ₃ PO ₄	b-H ₃ PO ₄ ⁺²	b	b ⁺²		y	y ⁺²	y-H ₃ PO ₄	y-H ₃ PO ₄ ⁺²	
	---	---	---	---	1	L 16	---	---	---	
	---	---	213.1598	107.0835	2	V 15	1746.8132	873.9102	1648.8363	824.9218
	---	---	314.2074	157.6074	3	T 14	1647.7448	824.3760	1549.7679	775.3876
	---	---	477.2708	239.1390	4	Y 13	1546.6971	773.8522	1448.7202	724.8638
	---	---	590.3548	295.6811	5	L 12	1383.6338	692.3205	1285.6569	643.3321
	---	---	661.3919	331.1996	6	A 11	1270.5497	635.7785	1172.5728	586.7901
	730.4134	365.7103	828.3903	414.6988	7	s 10	1199.5126	600.2599	1101.5357	551.2715
	829.4818	415.2445	927.4587	464.2330	8	V 9	1032.5143	516.7608	---	---
	926.5346	463.7709	1024.5115	512.7594	9	P 8	933.4458	467.2266	---	---
	1013.5666	507.2869	1111.5435	556.2754	10	S 7	836.3931	418.7002	---	---
	1142.6092	571.8082	1240.5861	620.7967	11	E 6	749.3610	375.1842	---	---
	1257.6361	629.3217	1355.6130	678.3102	12	D 5	620.3185	310.6629	---	---
	1356.7046	678.8559	1454.6815	727.8444	13	V 4	505.2915	253.1494	---	---
	1459.7137	730.3605	1557.6906	779.3490	14	C 3	406.2231	203.6152	---	---
	1587.8087	794.4080	1685.7856	843.3964	15	K 2	303.2139	152.1106	---	---
	---	---	---	---	16	R 1	175.1190	88.0631	---	---



Observed M=1858.8751
 Calculated M=1858.8900

Figure S6. MS/MS on the doubly charged 930.44 ion. Based on the intact mass (which indicated 1 site of phosphorylation) and the y ions, residue 83 is a phosphoserine.

PKC

MS/MS of peptide KLVpTYLASVPSEDVCK

OT10-3470 #3256 RT: 37.31 AV: 1 NL: 1.65E4
 T: ITMS + c NSI d Full ms2 916.44@cid35.00 [240.00-1845.00]

b-H ₃ PO ₄	b-H ₃ PO ₄ +2	b	b+2		y	y+2	y-H ₃ PO ₄	y-H ₃ PO ₄ +2
---	---	---	---	1 K 16	---	---	---	---
---	---	242.1863	121.5968	2 L 15	1703.7962	852.4017	1605.8193	803.4133
---	---	341.2547	171.1310	3 V 14	1590.7121	795.8597	1492.7352	746.8712
424.2918	212.6496	522.2687	261.6380	4 t 13	1491.6437	746.3255	1393.6668	697.3370
587.3552	294.1812	685.3321	343.1697	5 Y 12	1310.6297	655.8185	---	---
700.4392	350.7233	798.4161	399.7117	6 L 11	1147.5664	574.2868	---	---
771.4763	386.2418	869.4532	435.2303	7 A 10	1034.4823	517.7448	---	---
858.5084	429.7578	956.4853	478.7463	8 S 9	963.4452	482.2262	---	---
957.5768	479.2920	1055.5537	528.2805	9 V 8	876.4131	438.7102	---	---
1054.6295	527.8184	1152.6064	576.8069	10 P 7	777.3447	389.1760	---	---
1141.6616	571.3344	1239.6385	620.3229	11 S 6	680.2920	340.6496	---	---
1270.7042	635.8557	1368.6811	684.8442	12 E 5	593.2599	297.1336	---	---
1385.7311	693.3692	1483.7080	742.3576	13 D 4	464.2173	232.6123	---	---
1484.7995	742.9034	1582.7764	791.8918	14 V 3	349.1904	175.0988	---	---
1587.8087	794.4080	1685.7856	843.3964	15 C 2	250.1220	125.5646	---	---
---	---	---	---	16 K 1	147.1128	74.0600	---	---

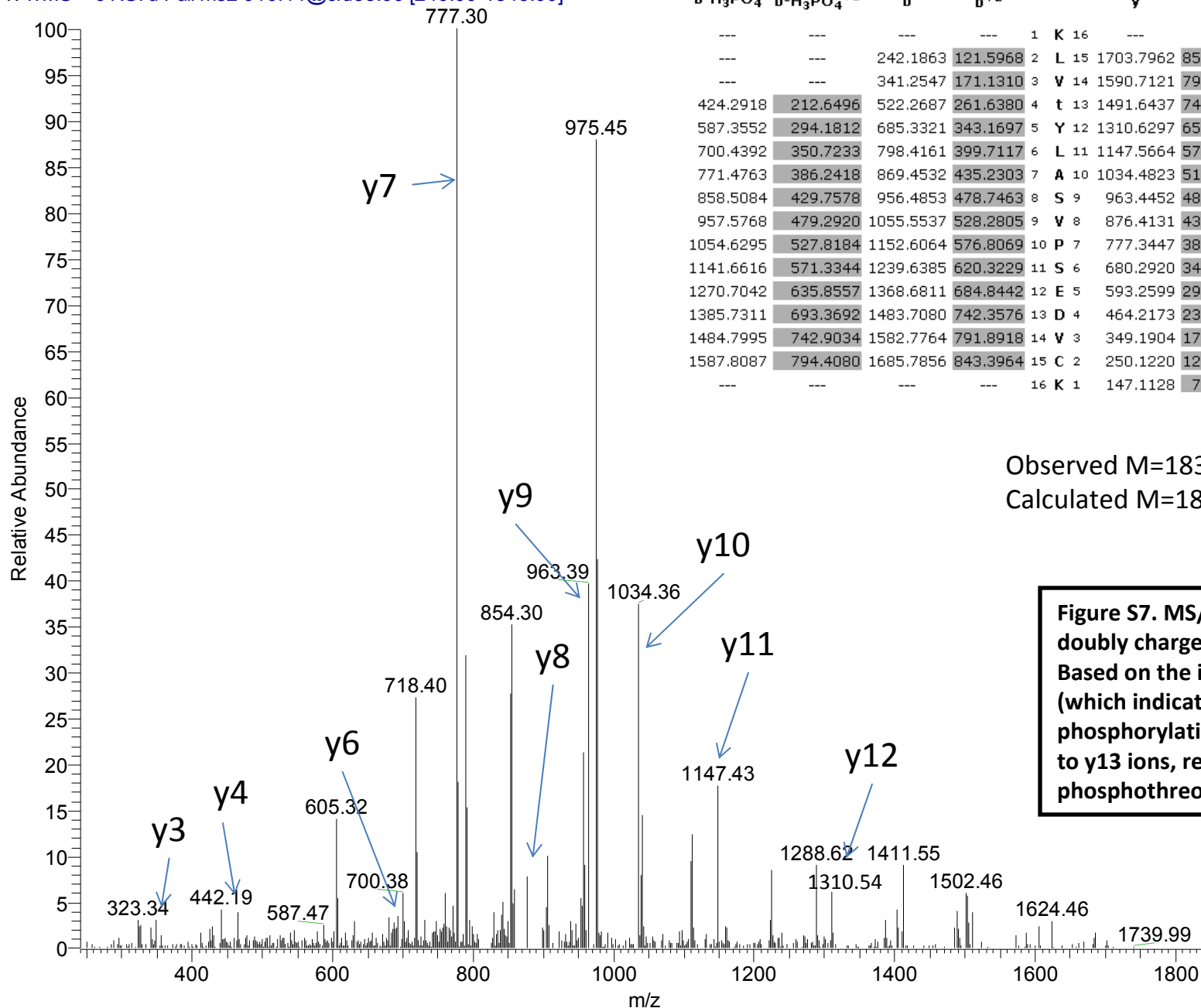
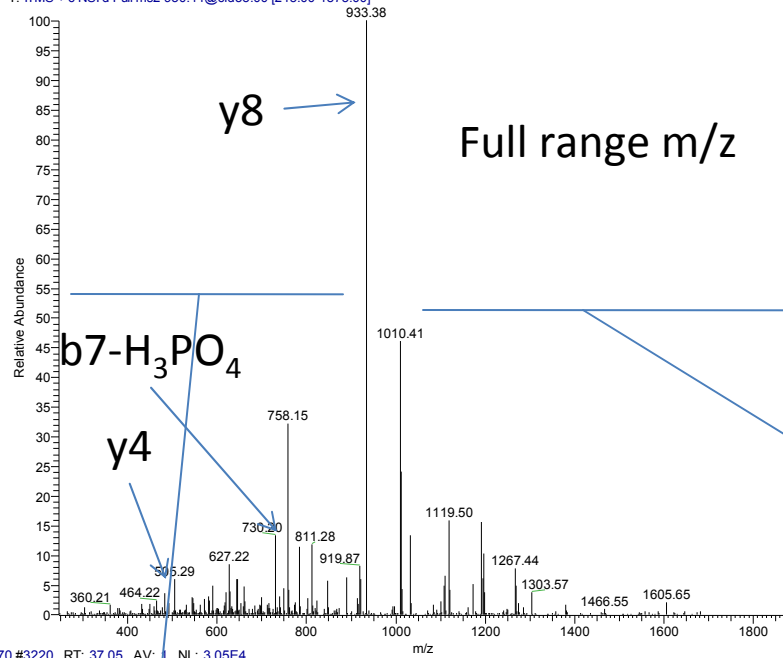


Figure S7. MS/MS on the doubly charged 916.44 ion. Based on the intact mass (which indicated 1 site of phosphorylation) and the y10 to y13 ions, residue 79 is a phosphothreonine.

PKC

MS/MS of peptide LVTYLA^pSVPS^{ED}VCKR

OT10-3470 #3220 RT: 37.05 AV: 1 NL: 9.50E4
T: ITMS + c NSI d Full ms2 930.44@cid35.00 [245.00-1875.00]



b-H ₃ PO ₄	b-H ₃ PO ₄ ⁺²	b	b ⁺²	y	y ⁺²	y-H ₃ PO ₄	y-H ₃ PO ₄ ⁺²	
---	---	---	---	1 L 16	---	---	---	
---	---	213.1598	107.0835	2 V 15	1746.8132	873.9102	1648.8363	824.9218
---	---	314.2074	157.6074	3 T 14	1647.7448	824.3760	1549.7679	775.3876
---	---	477.2708	239.1390	4 Y 13	1546.6971	773.8522	1448.7202	724.8638
---	---	590.3548	295.6811	5 L 12	1383.6338	692.3205	1285.6569	643.3321
---	---	661.3919	331.1996	6 A 11	1270.5497	635.7785	1172.5728	586.7901
730.4134	365.7103	828.3903	414.6988	7 S 10	1199.5126	600.2599	1101.5357	551.2715
829.4818	415.2445	927.4587	464.2330	8 V 9	1032.5143	516.7608	---	---
926.5346	463.7709	1024.5115	512.7594	9 P 8	933.4458	467.2266	---	---
1013.5666	507.2869	1111.5435	556.2754	10 S 7	836.3931	418.7002	---	---
1142.6092	571.8082	1240.5861	620.7967	11 E 6	749.3610	375.1842	---	---
1257.6361	629.3217	1355.6130	678.3102	12 D 5	620.3185	310.6629	---	---
1356.7046	678.8559	1454.6815	727.8444	13 V 4	505.2915	253.1494	---	---
1459.7137	730.3605	1557.6906	779.3490	14 C 3	406.2231	203.6152	---	---
1587.8087	794.4080	1685.7856	843.3964	15 K 2	303.2139	152.1106	---	---
---	---	---	---	16 R 1	175.1190	88.0631	---	---

Observed M=1858.8723
Calculated M=1858.8900

OT10-3470 #3220 RT: 37.05 AV: 1 NL: 3.05E4
T: ITMS + c NSI d Full ms2 930.44@cid35.00 [245.00-1875.00]

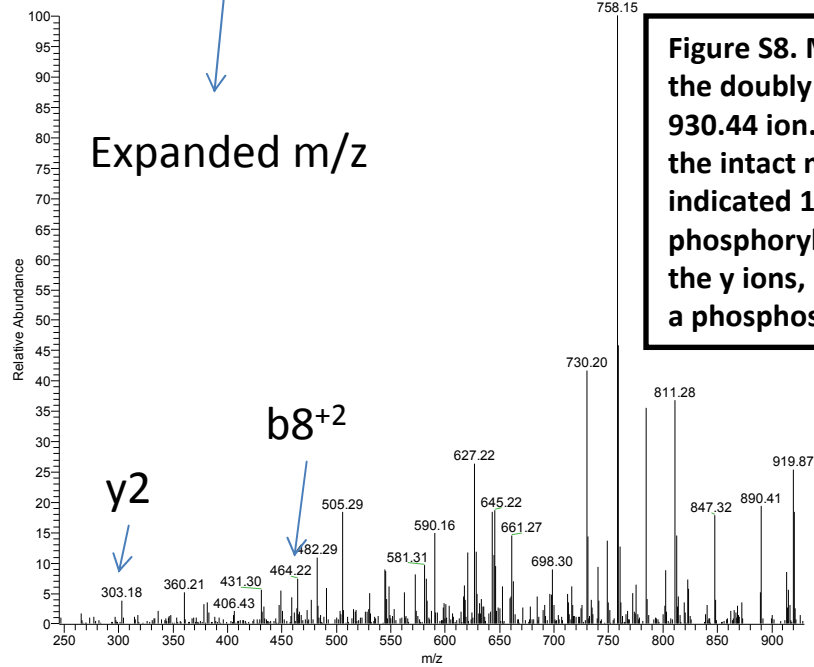
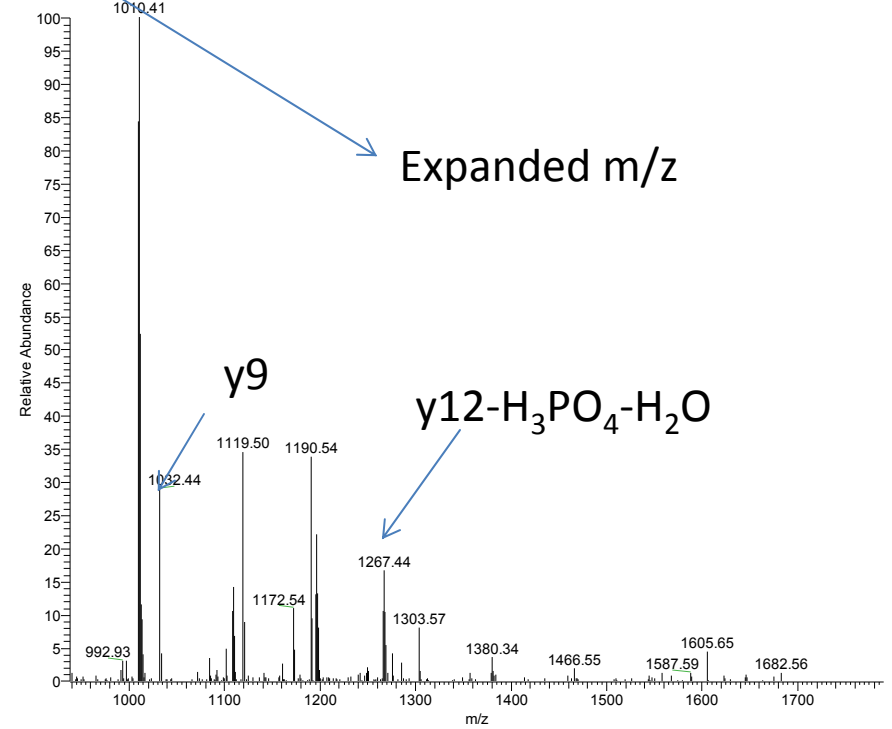


Figure S8. MS/MS on the doubly charged 930.44 ion. Based on the intact mass (which indicated 1 site of phosphorylation) and the y ions, residue 83 is a phosphoserine.

OT10-3470 #3220 RT: 37.05 AV: 1 NL: 4.38E4
T: ITMS + c NSI d Full ms2 930.44@cid35.00 [245.00-1875.00]



PKC

MS/MS of peptide LLT_pSELGVTEHVEGDPCK

OT10-3470 #3170 RT: 36.64 AV: 1 NL: 4.47E4
 T: ITMS + c NSI d Full ms2 1004.95@cid35.00 [265.00-2000.00]

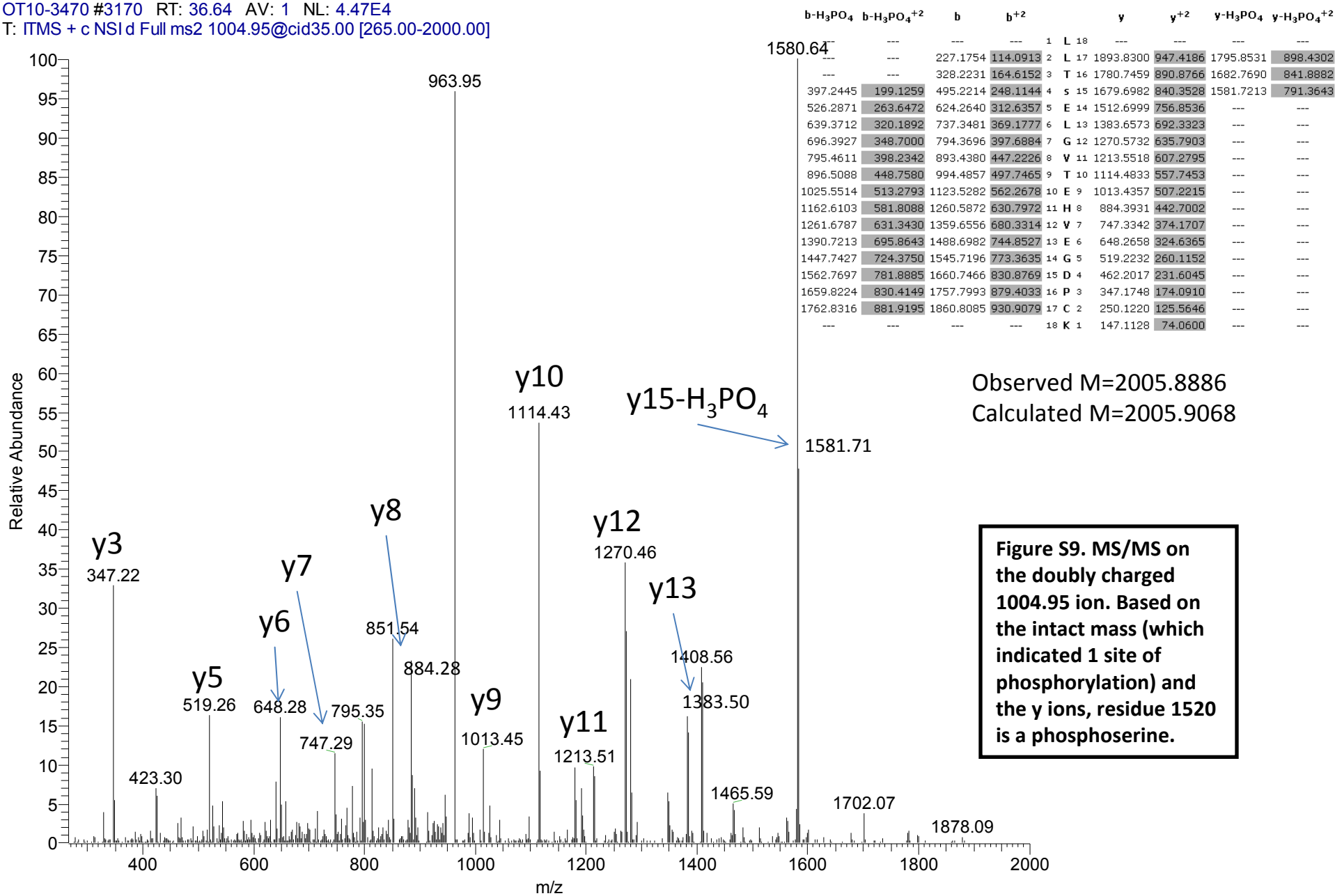


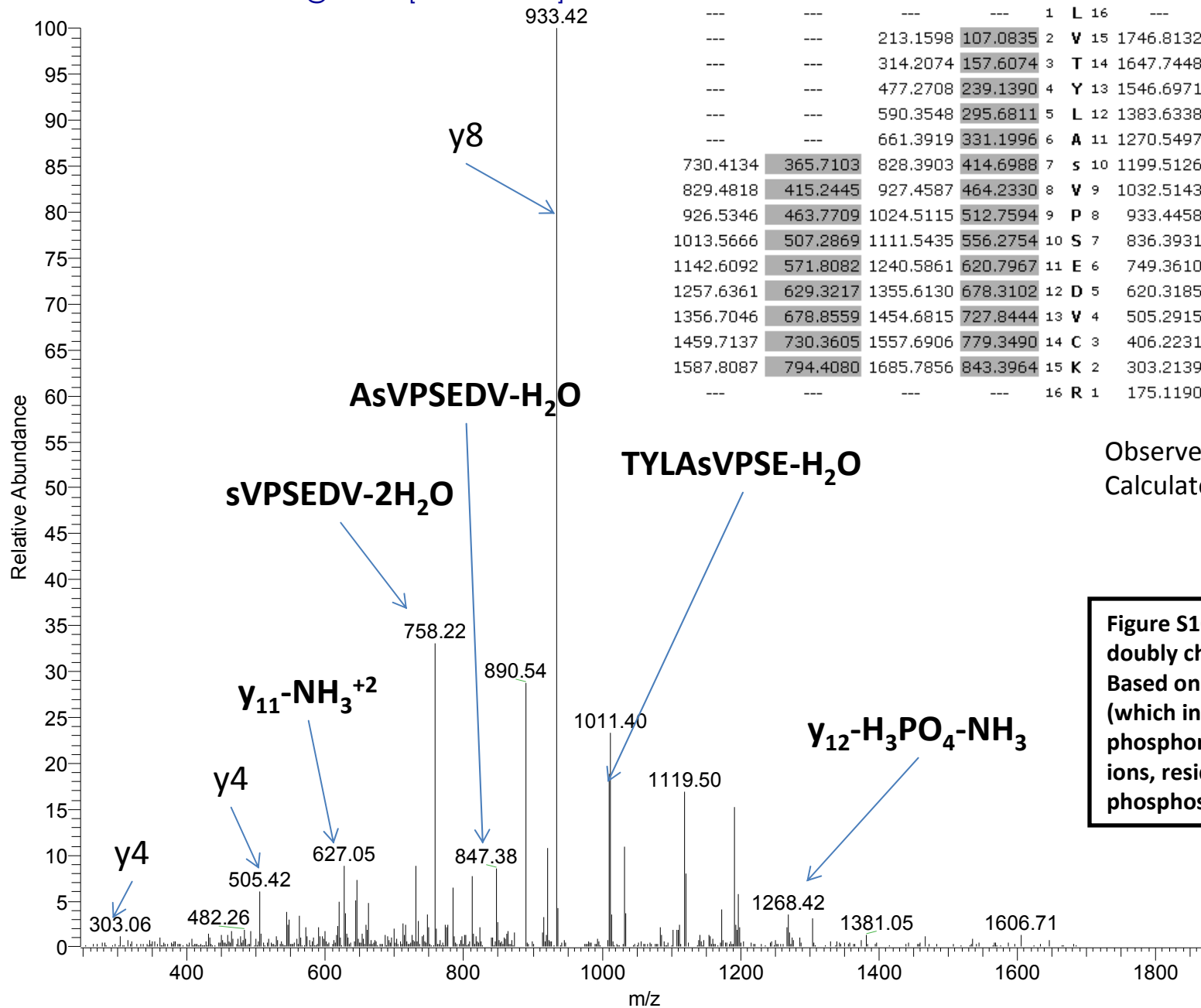
Figure S9. MS/MS on the doubly charged 1004.95 ion. Based on the intact mass (which indicated 1 site of phosphorylation) and the y ions, residue 1520 is a phosphoserine.

CKII**MS/MS of peptide LVTYLA^pSVPS^eEDVCKR**

OT10-3474 #3220 RT: 37.05 AV: 1 NL: 5.40E4

T: ITMS + c NSI d Full ms2 930.95@cid35.00 [245.00-1875.00]

	b-H ₃ PO ₄	b-H ₃ PO ₄ ⁺²	b	b ⁺²		y	y ⁺²	y-H ₃ PO ₄	y-H ₃ PO ₄ ⁺²	
	---	---	---	---	1	L 16	---	---	---	
			213.1598	107.0835	2	V 15	1746.8132	873.9102	1648.8363	824.9218
			314.2074	157.6074	3	T 14	1647.7448	824.3760	1549.7679	775.3876
			477.2708	239.1390	4	Y 13	1546.6971	773.8522	1448.7202	724.8638
			590.3548	295.6811	5	L 12	1383.6338	692.3205	1285.6569	643.3321
			661.3919	331.1996	6	A 11	1270.5497	635.7785	1172.5728	586.7901
	730.4134	365.7103	828.3903	414.6988	7	s 10	1199.5126	600.2599	1101.5357	551.2715
	829.4818	415.2445	927.4587	464.2330	8	V 9	1032.5143	516.7608	---	---
	926.5346	463.7709	1024.5115	512.7594	9	P 8	933.4458	467.2266	---	---
	1013.5666	507.2869	1111.5435	556.2754	10	S 7	836.3931	418.7002	---	---
	1142.6092	571.8082	1240.5861	620.7967	11	E 6	749.3610	375.1842	---	---
	1257.6361	629.3217	1355.6130	678.3102	12	D 5	620.3185	310.6629	---	---
	1356.7046	678.8559	1454.6815	727.8444	13	V 4	505.2915	253.1494	---	---
	1459.7137	730.3605	1557.6906	779.3490	14	C 3	406.2231	203.6152	---	---
	1587.8087	794.4080	1685.7856	843.3964	15	K 2	303.2139	152.1106	---	---
	---	---	---	---	16	R 1	175.1190	88.0631	---	---



Observed M=1858.8710
 Calculated M=1858.8900

Figure S10. MS/MS on the doubly charged 930.95 ion. Based on the intact mass (which indicated 1 site of phosphorylation) and the y ions, residue 83 is a phosphoserine.

CKII

MS/MS of peptide **pTpSEQVCSVLESLEQYR**

OT10-3474 #4333 RT: 45.74 AV: 1 NL: 1.40E5
T: ITMS + c NSI d Full ms2 1040.47@cid35.00 [275.00-2000.00]

b-H ₃ PO ₄	b-H ₃ PO ₄ +2	b	b+2		y	y+2	y-H ₃ PO ₄	y-H ₃ PO ₄ +2
---	---	---	---	1 T 17	---	---	---	---
171.0764	86.0418	269.0533	135.0303	2 s 16	1978.8464	989.9268	1880.8695	940.9384
300.1190	150.5631	398.0959	199.5516	3 E 15	1811.8480	906.4276	---	---
428.1776	214.5924	526.1545	263.5809	4 Q 14	1682.8054	841.9063	---	---
527.2460	264.1266	625.2229	313.1151	5 V 13	1554.7468	777.8771	---	---
630.2552	315.6312	728.2321	364.6197	6 C 12	1455.6784	728.3428	---	---
717.2872	359.1472	815.2641	408.1357	7 S 11	1352.6692	676.8383	---	---
816.3556	408.6815	914.3325	457.6699	8 V 10	1265.6372	633.3222	---	---
929.4397	465.2235	1027.4166	514.2119	9 L 9	1166.5688	583.7880	---	---
1058.4823	529.7448	1156.4592	578.7332	10 E 8	1053.4847	527.2460	---	---
1145.5143	573.2608	1243.4912	622.2492	11 S 7	924.4421	462.7247	---	---
1258.5984	629.8028	1356.5753	678.7913	12 L 6	837.4101	419.2087	---	---
1387.6410	694.3241	1485.6179	743.3126	13 E 5	724.3260	362.6667	---	---
1515.6996	758.3534	1613.6764	807.3419	14 Q 4	595.2835	298.1454	---	---
1644.7421	822.8747	1742.7190	871.8632	15 E 3	467.2249	234.1161	---	---
1807.8055	904.4064	1905.7824	953.3948	16 Y 2	338.1823	169.5948	---	---
---	---	---	---	17 R 1	175.1190	88.0631	---	---

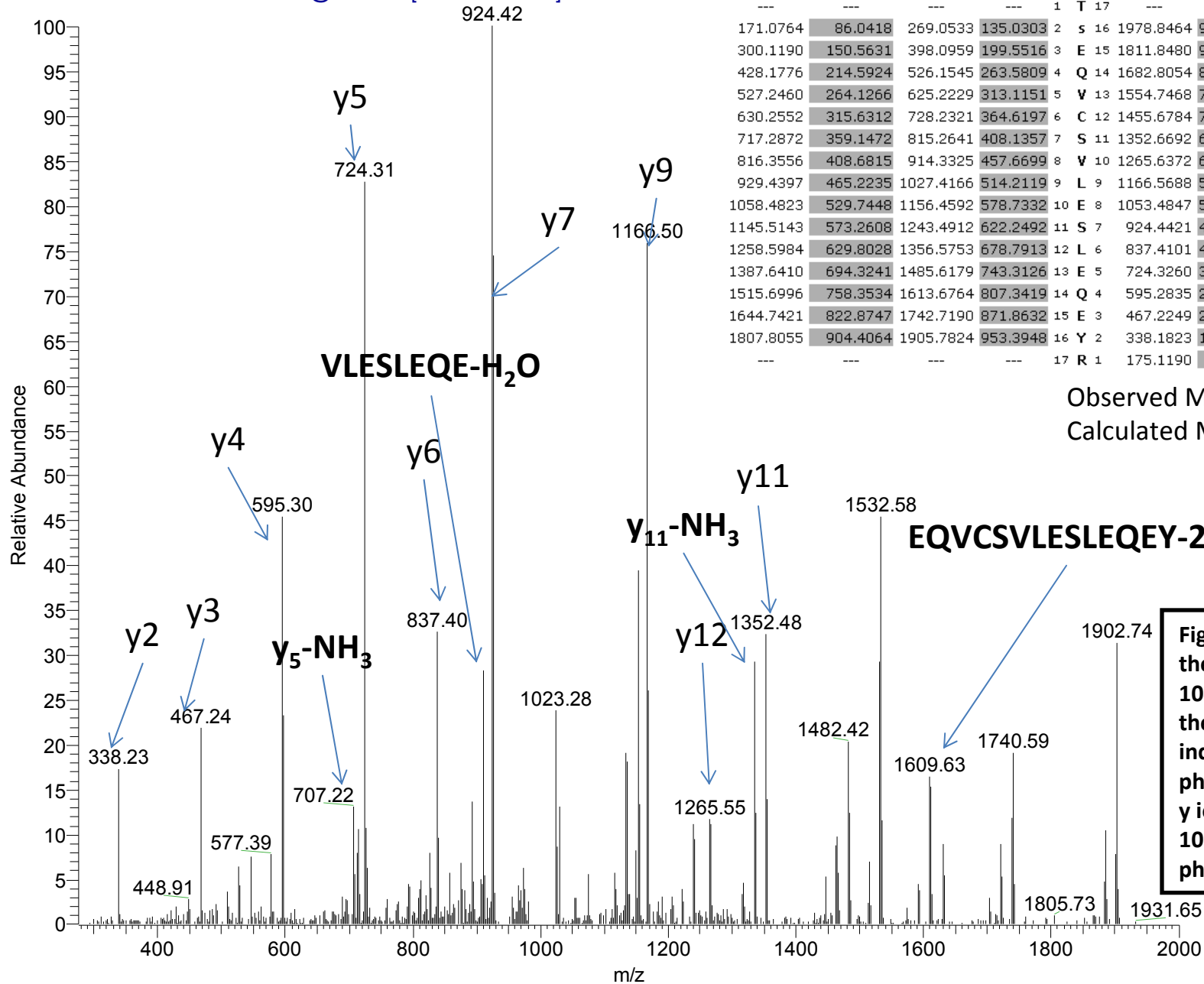


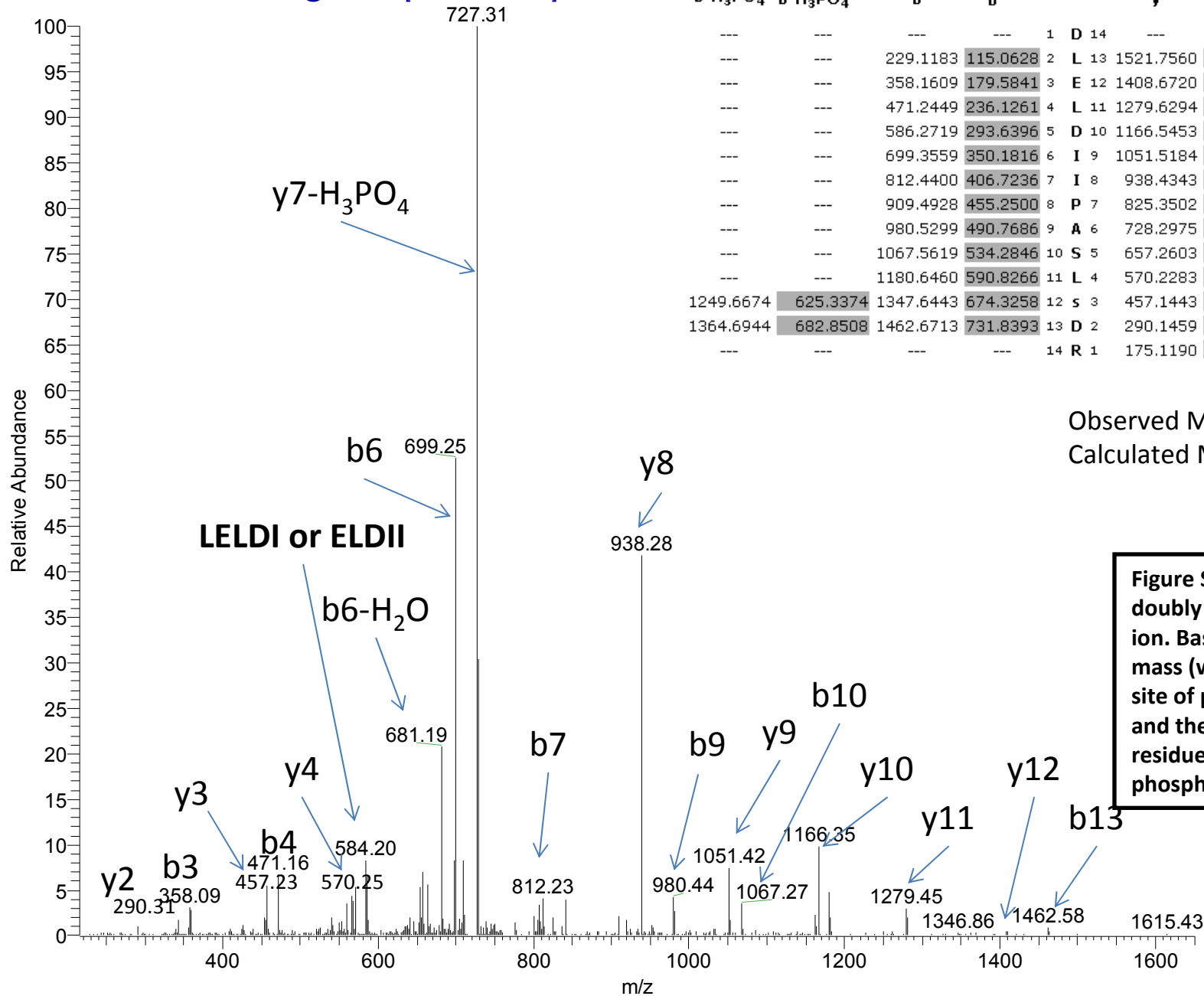
Figure S11. MS/MS on the doubly charged 1040.47 ion. Based on the intact mass (which indicated 1 site of phosphorylation) and the y ions, either residue 1002 or 1003 is phosphorylated.

CKII

MS/MS of peptide DLELDIIPASLpSDR

OT10-3473 #3543 RT: 45.24 AV: 1 NL: 1.03E4
T: ITMS + c NSI d Full ms2 818.89@cid35.00 [215.00-1650.00]

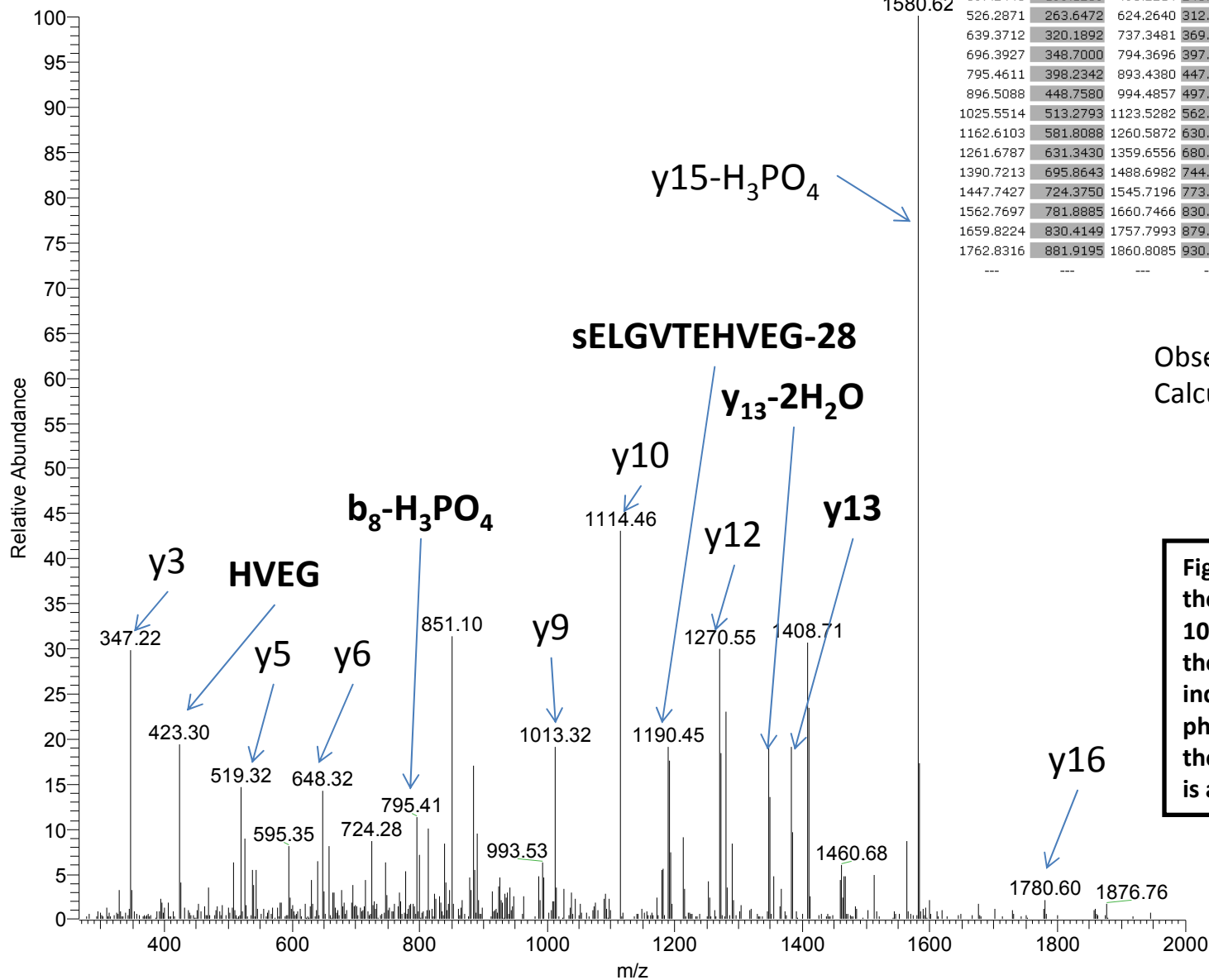
b-H ₃ PO ₄	b-H ₃ PO ₄ ⁺²	b	b ⁺²		y	y ⁺²	y-H ₃ PO ₄	y-H ₃ PO ₄ ⁺²	
---	---	---	---	1	D 14	---	---	---	
---	---	229.1183	115.0628	2	L 13	1521.7560	761.3816	1423.7791	712.3932
---	---	358.1609	179.5841	3	E 12	1408.6720	704.8396	1310.6951	655.8512
---	---	471.2449	236.1261	4	L 11	1279.6294	640.3183	1181.6525	591.3299
---	---	586.2719	293.6396	5	D 10	1166.5453	583.7763	1068.5684	534.7878
---	---	699.3559	350.1816	6	I 9	1051.5184	526.2628	953.5415	477.2744
---	---	812.4400	406.7236	7	I 8	938.4343	469.7208	840.4574	420.7323
---	---	909.4928	455.2500	8	P 7	825.3502	413.1788	727.3733	364.1903
---	---	980.5299	490.7686	9	A 6	728.2975	364.6524	630.3206	315.6639
---	---	1067.5619	534.2846	10	S 5	657.2603	329.1338	559.2835	280.1454
---	---	1180.6460	590.8266	11	L 4	570.2283	285.6178	472.2514	236.6293
1249.6674	625.3374	1347.6443	674.3258	12	s 3	457.1443	229.0758	359.1674	180.0873
1364.6944	682.8508	1462.6713	731.8393	13	D 2	290.1459	145.5766	---	---
---	---	---	---	14	R 1	175.1190	88.0631	---	---



Observed M=1635.7706
Calculated M=1635.7757

Figure S12. MS/MS on the doubly charged 818.89 ion. Based on the intact mass (which indicated 1 site of phosphorylation) and the y2, y3 and y4 ions, residue 1249 is a phosphoserine.

OT10-3474 #3173 RT: 36.68 AV: 1 NL: 4.41E4
 T: ITMS + c NSI d Full ms2 1003.95@cid35.00 [265.00-2000.00]



b-H ₃ PO ₄	b-H ₃ PO ₄ ⁺²	b	b ⁺²		y	y ⁺²	y-H ₃ PO ₄	y-H ₃ PO ₄ ⁺²
---	---	---	---	1	L	18	---	---
---	---	227.1754	114.0913	2	L	17	1893.8300	947.4186
---	---	328.2231	164.6152	3	T	16	1780.7459	890.8766
---	---	---	---	4	S	15	1679.6982	840.3528
526.2871	263.6472	624.2640	312.6357	5	E	14	1512.6999	756.8536
639.3712	320.1892	737.3481	369.1777	6	L	13	1383.6573	692.3323
696.3927	348.7000	794.3696	397.6884	7	G	12	1270.5732	635.7903
795.4611	398.2342	893.4380	447.2226	8	V	11	1213.5518	607.2795
896.5088	448.7580	994.4857	497.7465	9	T	10	1114.4833	557.7453
1025.5514	513.2793	1123.5282	562.2678	10	E	9	1013.4357	507.2215
1162.6103	581.8088	1260.5872	630.7972	11	H	8	884.3931	442.7002
1261.6787	631.3430	1359.6556	680.3314	12	V	7	747.3342	374.1707
1390.7213	695.8643	1488.6982	744.8527	13	E	6	648.2658	324.6365
1447.7427	724.3750	1545.7196	773.3635	14	G	5	519.2232	260.1152
1562.7697	781.8885	1660.7466	830.8769	15	D	4	462.2017	231.6045
1659.8224	830.4149	1757.7993	879.4033	16	P	3	347.1748	174.0910
1762.8316	881.9195	1860.8085	930.9079	17	C	2	250.1220	125.5646
---	---	---	---	18	K	1	147.1128	74.0600

Observed M=2005.8874
 Calculated M=2005.9068

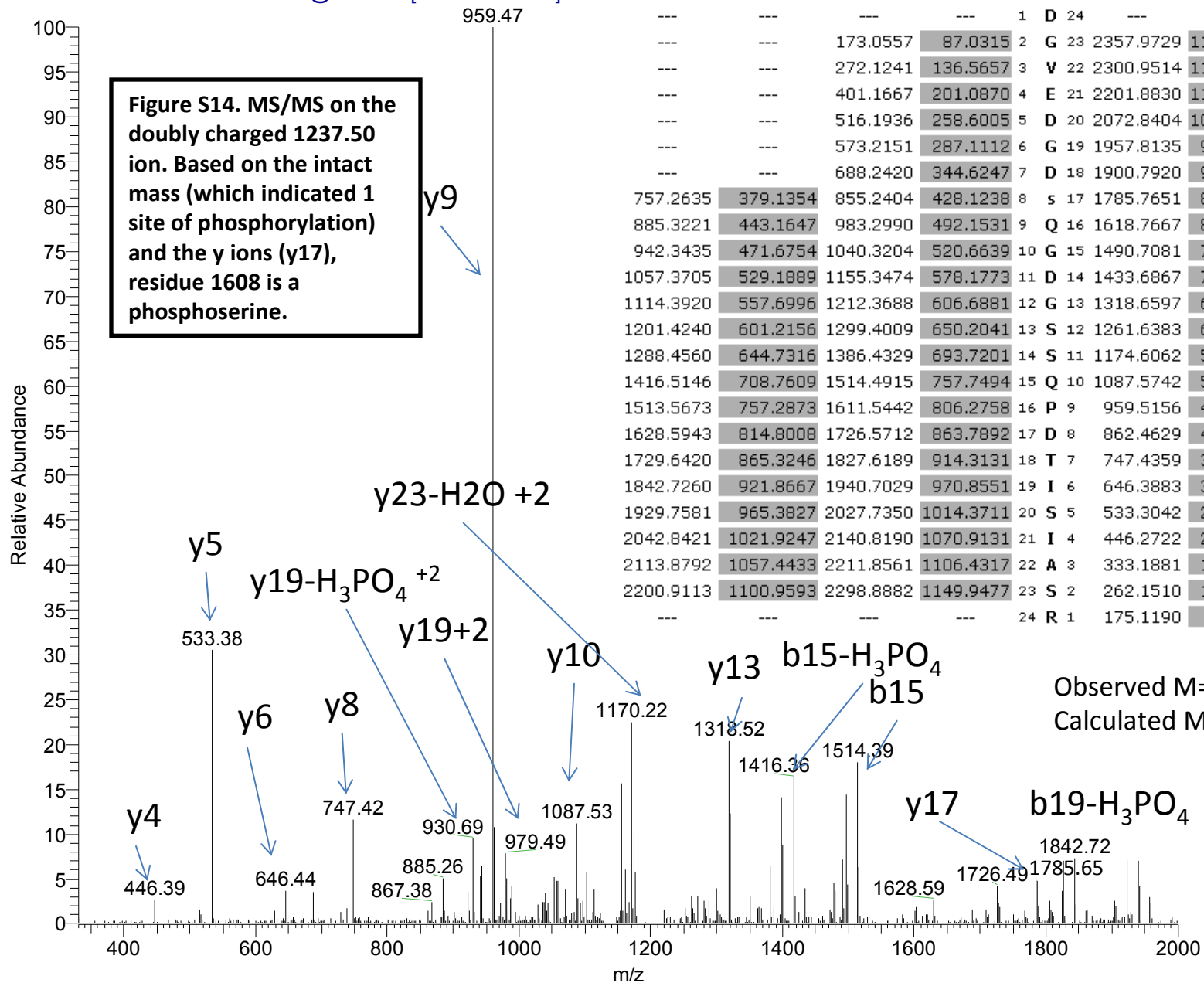
Figure S13. MS/MS on the doubly charged 1003.95 ion. Based on the intact mass (which indicated 1 site of phosphorylation) and the y ions, residue 1520 is a phosphoserine.

CKII

MS/MS of peptide DGVEDGDpSQGDGSSQPDTISIASR

OT10-3473 #2616 RT: 34.30 AV: 1 NL: 1.10E4
 T: ITMS + c NSI d Full ms2 1237.50@cid35.00 [330.00-2000.00]

	b-H ₃ PO ₄	b-H ₃ PO ₄ ⁺²	b	b ⁺²		y	y ⁺²	y-H ₃ PO ₄	y-H ₃ PO ₄ ⁺²
---	---	---	---	---	1	D 24	---	---	---
---	---	173.0557	87.0315	2	G 23	2357.9729	1179.4901	2259.9960	1130.5016
---	---	272.1241	136.5657	3	V 22	2300.9514	1150.9794	2202.9745	1101.9909
---	---	401.1667	201.0870	4	E 21	2201.8830	1101.4451	2103.9061	1052.4567
---	---	516.1936	258.6005	5	D 20	2072.8404	1036.9239	1974.8635	987.9354
---	---	573.2151	287.1112	6	G 19	1957.8135	979.4104	1859.8366	930.4219
---	---	688.2420	344.6247	7	D 18	1900.7920	950.8996	1802.8151	901.9112
757.2635	379.1354	855.2404	428.1238	8	S 17	1785.7651	893.3862	1687.7882	844.3977
885.3221	443.1647	983.2990	492.1531	9	Q 16	1618.7667	809.8870	---	---
942.3435	471.6754	1040.3204	520.6639	10	G 15	1490.7081	745.8577	---	---
1057.3705	529.1889	1155.3474	578.1773	11	D 14	1433.6867	717.3470	---	---
1114.3920	557.6996	1212.3688	606.6881	12	G 13	1318.6597	659.8335	---	---
1201.4240	601.2156	1299.4009	650.2041	13	S 12	1261.6383	631.3228	---	---
1288.4560	644.7316	1386.4329	693.7201	14	S 11	1174.6062	587.8068	---	---
1416.5146	708.7609	1514.4915	757.7494	15	Q 10	1087.5742	544.2907	---	---
1513.5673	757.2873	1611.5442	806.2758	16	P 9	959.5156	480.2615	---	---
1628.5943	814.8008	1726.5712	863.7892	17	D 8	862.4629	431.7351	---	---
1729.6420	865.3246	1827.6189	914.3131	18	T 7	747.4359	374.2216	---	---
1842.7260	921.8667	1940.7029	970.8551	19	I 6	646.3883	323.6978	---	---
1929.7581	965.3827	2027.7350	1014.3711	20	S 5	533.3042	267.1557	---	---
2042.8421	1021.9247	2140.8190	1070.9131	21	I 4	446.2722	223.6397	---	---
2113.8792	1057.4433	2211.8561	1106.4317	22	A 3	333.1881	167.0977	---	---
2200.9113	1100.9593	2298.8882	1149.9477	23	S 2	262.1510	131.5791	---	---
---	---	---	---	24	R 1	175.1190	88.0631	---	---

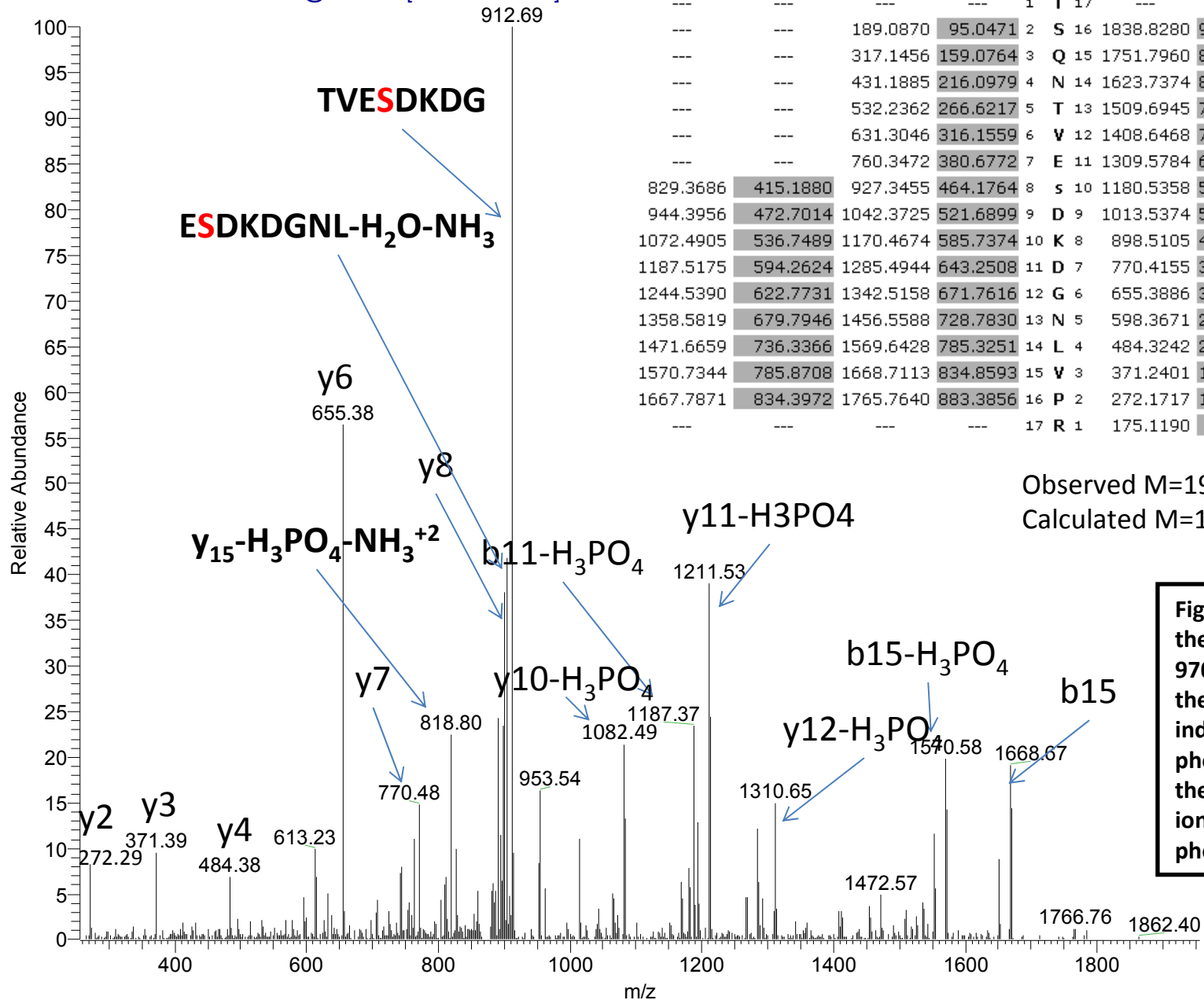


CKII

MS/MS of peptide TSQNTVEpSDKDGNLVPR

OT10-3473 #2296 RT: 30.44 AV: 1 NL: 1.44E4
T: ITMS + c NSI d Full ms2 970.94@cid35.00 [255.00-1955.00]

b-H ₃ PO ₄	b-H ₃ PO ₄ ⁺²	b	b ⁺²	y	y ⁺²	y-H ₃ PO ₄	y-H ₃ PO ₄ ⁺²	
---	---	---	---	1 T 17	---	---	---	
---	---	189.0870	95.0471	2 S 16	1838.8280	919.9176	1740.8511	870.9292
---	---	317.1456	159.0764	3 Q 15	1751.7960	876.4016	1653.8191	827.4132
---	---	431.1885	216.0979	4 N 14	1623.7374	812.3723	1525.7605	763.3839
---	---	532.2362	266.6217	5 T 13	1509.6945	755.3509	1411.7176	706.3624
---	---	631.3046	316.1559	6 V 12	1408.6468	704.8270	1310.6699	655.8386
---	---	760.3472	380.6772	7 E 11	1309.5784	655.2928	1211.6015	606.3044
829.3686	415.1880	927.3455	464.1764	8 s 10	1180.5358	590.7715	1082.5589	541.7831
944.3956	472.7014	1042.3725	521.6899	9 D 9	1013.5374	507.2724	---	---
1072.4905	536.7489	1170.4674	585.7374	10 K 8	898.5105	449.7589	---	---
1187.5175	594.2624	1285.4944	643.2508	11 D 7	770.4155	385.7114	---	---
1244.5390	622.7731	1342.5158	671.7616	12 G 6	655.3886	328.1979	---	---
1358.5819	679.7946	1456.5588	728.7830	13 N 5	598.3671	299.6872	---	---
1471.6659	736.3366	1569.6428	785.3251	14 L 4	484.3242	242.6657	---	---
1570.7344	785.8708	1668.7113	834.8593	15 V 3	371.2401	186.1237	---	---
1667.7871	834.3972	1765.7640	883.3856	16 P 2	272.1717	136.5895	---	---
---	---	---	---	17 R 1	175.1190	88.0631	---	---



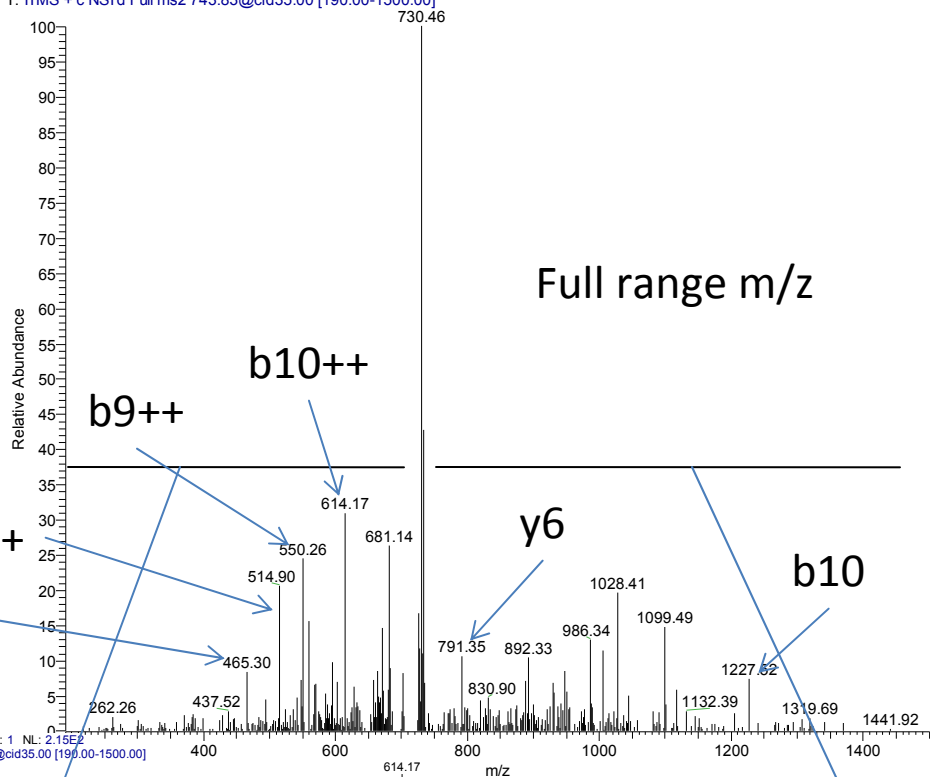
Observed M=1938.8607
Calculated M=1938.8684

Figure S15. MS/MS on the doubly charged 970.94 ion. Based on the intact mass (which indicated 1 site of phosphorylation) and the y2 to y10-H₃PO₄ ions, residue 1632 is a phosphoserine.

Fyn

MS/MS of peptide RHDDFEEVAQNTpYTNADK

OT10-3477 #2355 RT: 31.26 AV: 1 NL: 6.96E2
T: ITMS + c NSI d Full ms2 743.83@cid35.00 [190.00-1500.00]



b	b+2		y	y+2
---	---	1	R 18	---
294.1673	147.5873	2	H 17	2076.8182
409.1942	205.1008	3	D 16	1939.7593
524.2212	262.6142	4	D 15	1824.7324
671.2896	336.1484	5	F 14	1709.7054
800.3322	400.6697	6	E 13	1562.6370
929.3748	465.1910	7	E 12	1433.5944
1028.4432	514.7252	8	V 11	1304.5518
1099.4803	550.2438	9	A 10	1205.4834
1227.5389	614.2731	10	Q 9	1134.4463
1341.5818	671.2945	11	N 8	1006.3877
1442.6295	721.8184	12	T 7	892.3448
1685.6592	843.3332	13	y 6	791.2971
1786.7068	893.8571	14	T 5	548.2675
1900.7498	950.8785	15	N 4	447.2198
1971.7869	986.3971	16	A 3	333.1769
2086.8138	1043.9105	17	D 2	262.1397
---	---	18	K 1	147.1128

Observed M=2231.9017
Calculated M=2231.9120

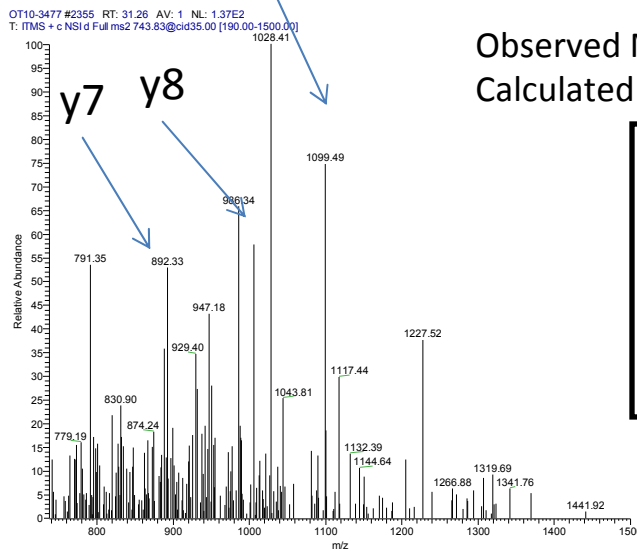
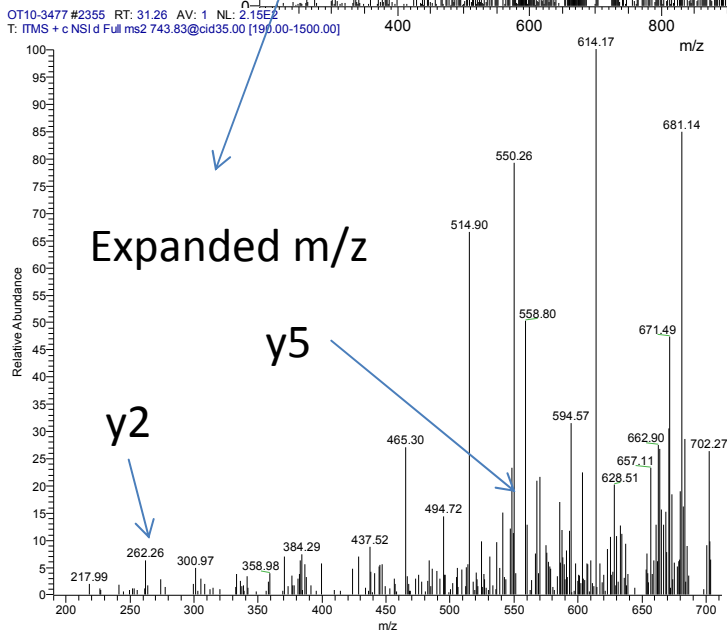
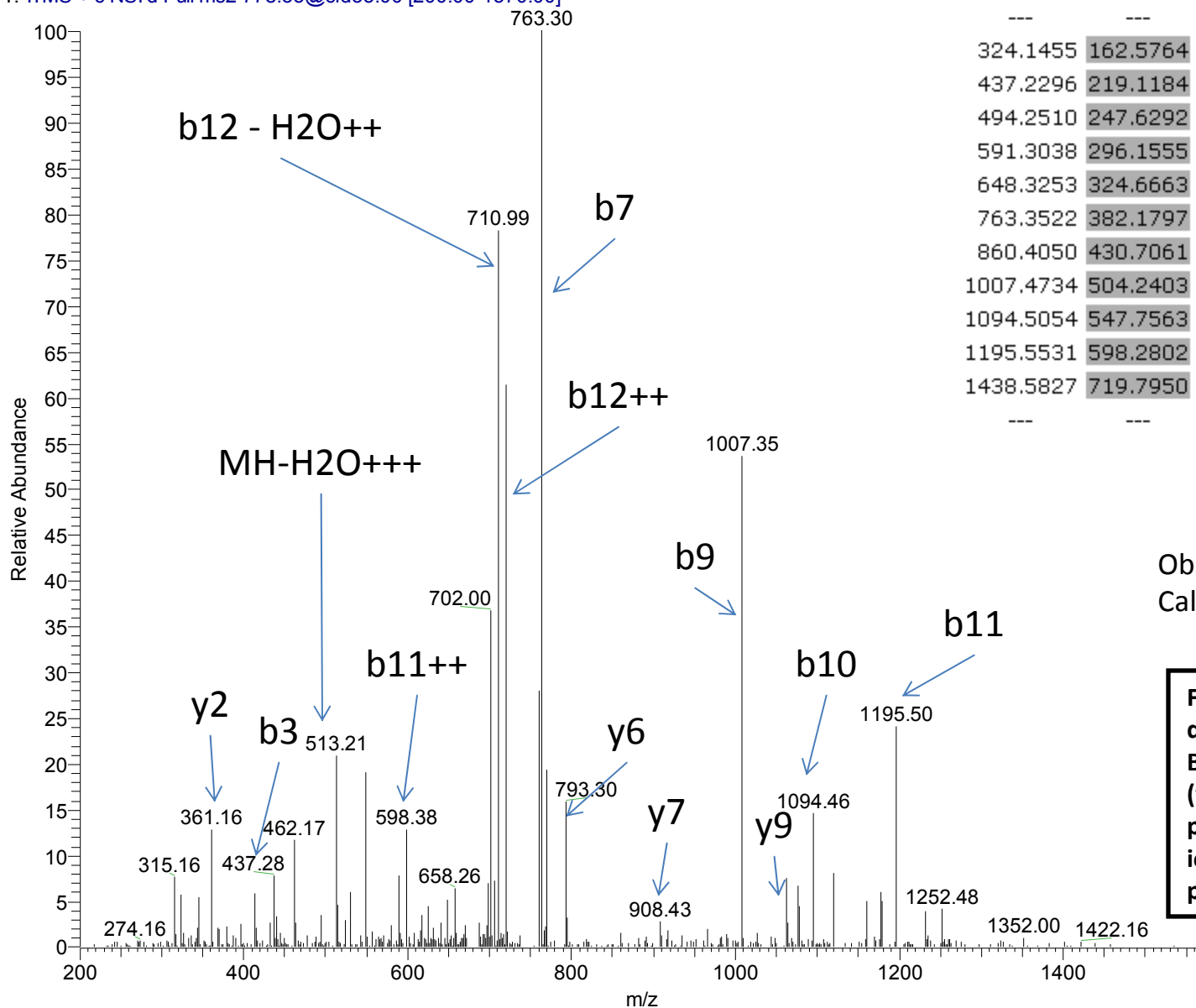


Figure S16. MS/MS on the triply charged 743.83 ion. Based on the intact mass (which indicated 1 site of phosphorylation) and the y2 to y8 ions, residue 591 is a phosphotyrosine

Fyn

MS/MS of peptide WHLGGDPFSTpYV

OT10-3477 #3451 RT: 44.62 AV: 1 NL: 2.21E3
T: ITMS + c NSI d Full ms2 778.33@cid35.00 [200.00-1570.00]



b	b ⁺²		y	y ⁺²
---	---	1	W 13	---
324.1455	162.5764	2	H 12	1369.5824
437.2296	219.1184	3	L 11	1232.5235
494.2510	247.6292	4	G 10	1119.4394
591.3038	296.1555	5	P 9	1062.4180
648.3253	324.6663	6	G 8	965.3652
763.3522	382.1797	7	D 7	908.3437
860.4050	430.7061	8	P 6	793.3168
1007.4734	504.2403	9	F 5	696.2640
1094.5054	547.7563	10	S 4	549.1956
1195.5531	598.2802	11	T 3	462.1636
1438.5827	719.7950	12	y 2	361.1159
---	---	13	V 1	118.0863

Observed M=1554.6487
Calculated M=1554.6545

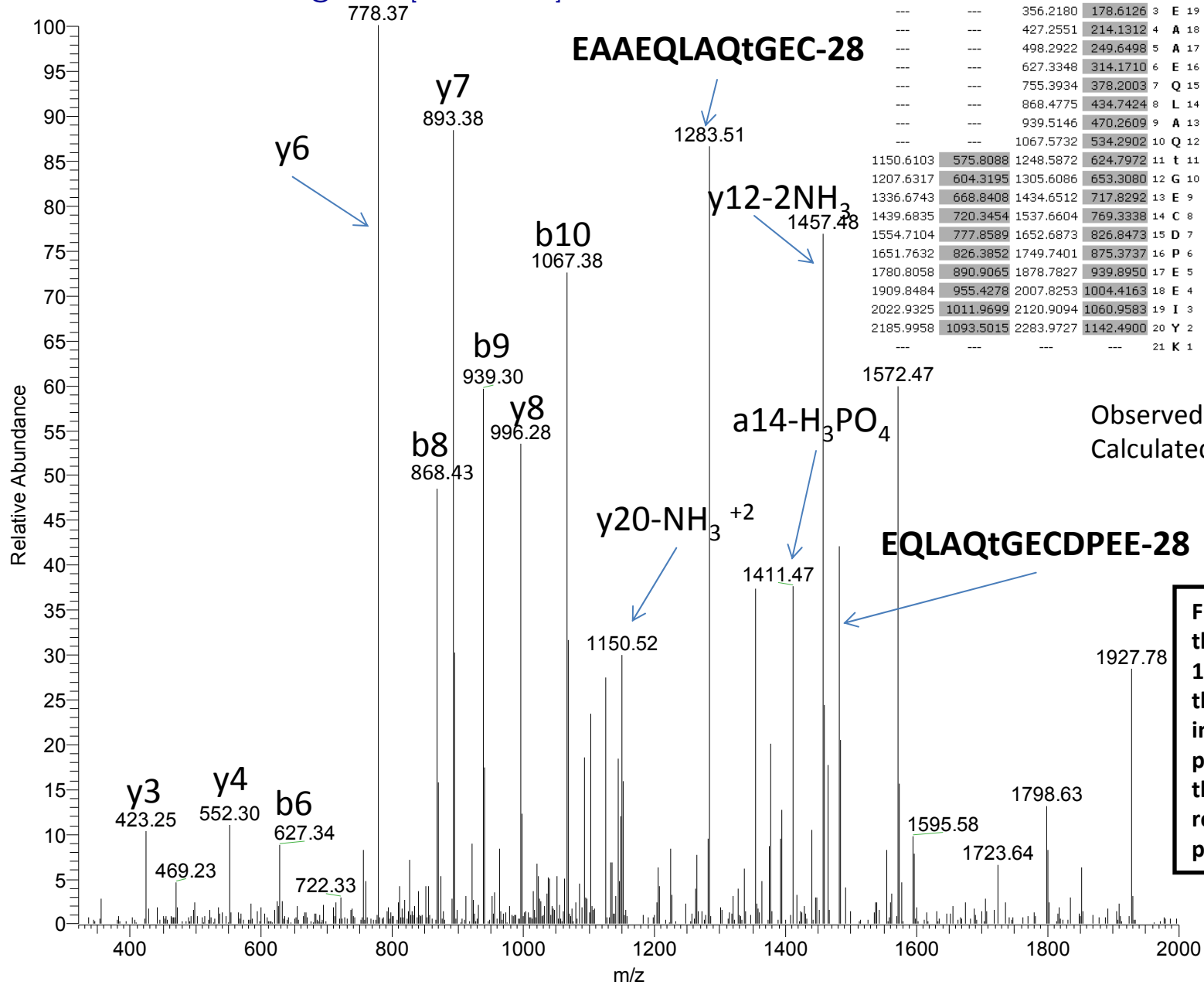
Figure S17. MS/MS on the doubly charged 778.33 ion. Based on the intact mass (which indicated 1 site of phosphorylation) and the y2 ion, residue 1653 is a phosphotyrosine.

Lambda

MS/MS of peptide LLEAAEQLAQpTGECDPEEIYK

OT10-3466 #3647 RT: 40.03 AV: 1 NL: 1.37E4
 T: ITMS + c NSI d Full ms2 1216.03@cid35.00 [320.00-2000.00]

	b-H ₃ PO ₄	b-H ₃ PO ₄ ⁺²	b	b ⁺²		y	y ⁺²	y-H ₃ PO ₄	y-H ₃ PO ₄ ⁺²		
---	---	---	---	---	1	L	21	---	---		
---	---	---	227.1754	114.0913	2	L	20	2316.9942	1159.0007	2219.0173	1110.0123
---	---	---	356.2180	178.6126	3	E	19	2203.9101	1102.4587	2105.9332	1053.4702
---	---	---	427.2551	214.1312	4	A	18	2074.8675	1037.9374	1976.8906	988.9489
---	---	---	498.2922	249.6498	5	A	17	2003.8304	1002.4188	1905.8535	953.4304
---	---	---	627.3348	314.1710	6	E	16	1932.7933	966.9003	1834.8164	917.9118
---	---	---	755.3934	378.2003	7	Q	15	1803.7507	902.3790	1705.7738	853.3905
---	---	---	868.4775	434.7424	8	L	14	1675.6921	838.3497	1577.7152	789.3612
---	---	---	939.5146	470.2609	9	A	13	1562.6080	781.8077	1464.6311	732.8192
---	---	---	1067.5732	534.2902	10	Q	12	1491.5709	746.2891	1393.5940	697.3006
1150.6103	575.8088	1248.5872	624.7972	653.3080	11	t	11	1363.5123	682.2598	1265.5354	633.2714
1207.6317	604.3195	1305.6086	717.8292	769.3338	12	G	10	1182.4983	591.7528	---	---
1336.6743	668.8408	1434.6512	826.8473	875.3737	13	E	9	1125.4769	563.2421	---	---
1439.6835	720.3454	1537.6604	939.8950	1004.4163	14	C	8	996.4343	498.7208	---	---
1554.7104	777.8589	1652.6873	1060.9583	1142.4900	15	D	7	893.4251	447.2162	---	---
1651.7632	826.3852	1749.7401	1185.8227	1248.5872	16	P	6	778.3981	389.7027	---	---
1780.8058	890.9065	1878.7827	1305.6086	1377.8292	17	E	5	681.3454	341.1763	---	---
1909.8484	955.4278	2007.8253	1434.6512	1477.8292	18	E	4	552.3028	276.6550	---	---
2022.9325	1011.9699	2120.9094	1562.6873	1577.8292	19	I	3	423.2602	212.1337	---	---
2185.9958	1093.5015	2283.9727	1682.6080	1675.6921	20	Y	2	310.1761	155.5917	---	---
---	---	---	---	---	21	K	1	147.1128	74.0600	---	---



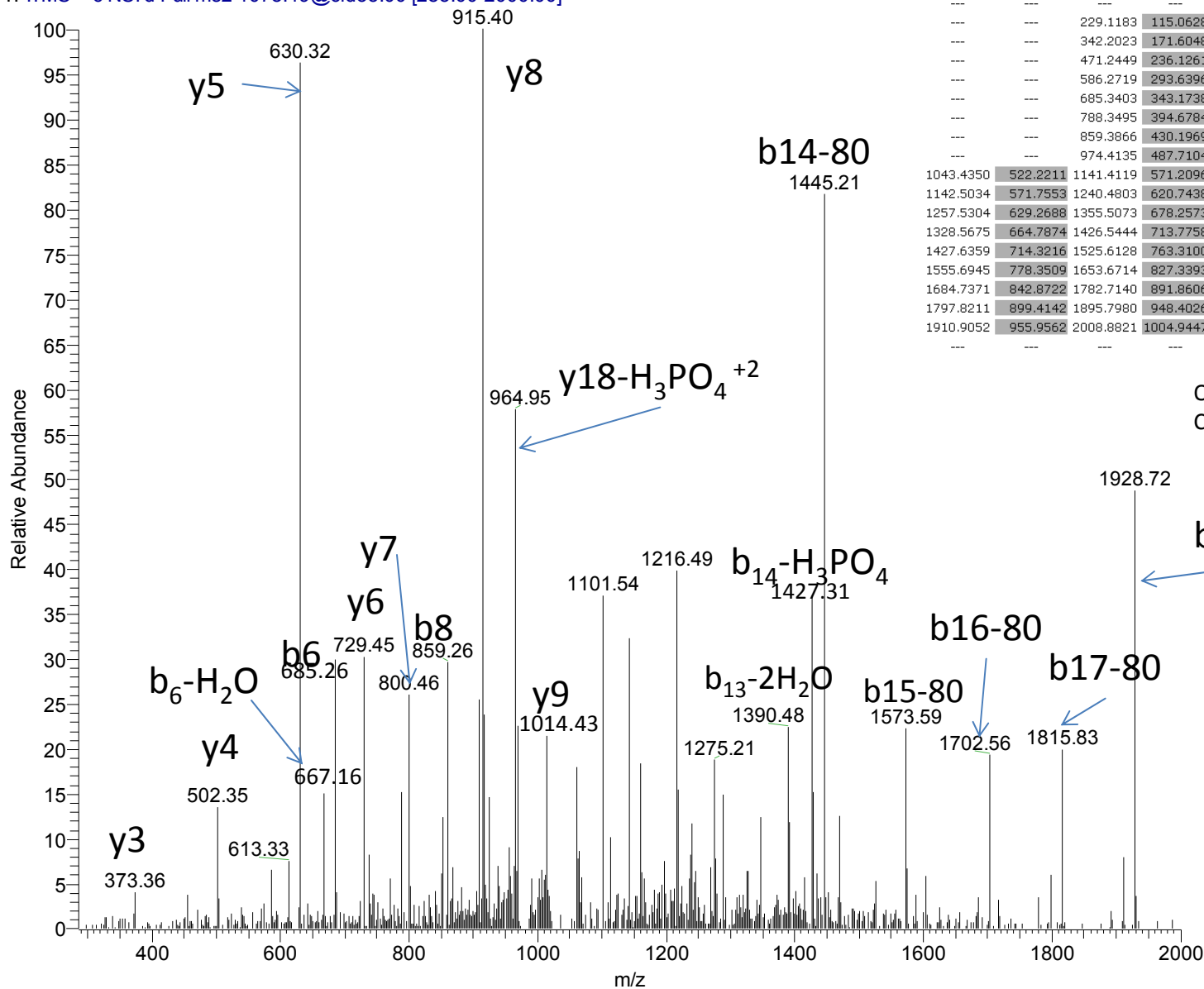
Observed M=2429.0478
 Calculated M=2429.0709

Figure S18. MS/MS on the doubly charged 1216.03 ion. Based on the intact mass (which indicated 1 site of phosphorylation) and the b10 and y ions, residue 607 is a phosphothreonine.

Lambda

MS/MS of peptide EVLEDVCADpSVDAVQELIK

OT10-3466 #4489 RT: 46.70 AV: 1 NL: 6.81E3
 T: ITMS + c NSI d Full ms2 1078.49@cid35.00 [285.00-2000.00]



b-H ₃ PO ₄	b-H ₃ PO ₄ ⁺²	b	b ⁺²	y	y ⁺²	y-H ₃ PO ₄	y-H ₃ PO ₄ ⁺²
---	---	---	---	1 E 19	---	---	---
---	---	229.1183	115.0628	2 V 18 2025.9450	1013.4761	1927.9681	964.4877
---	---	342.2023	171.6048	3 L 17 1926.8766	963.9419	1828.8997	914.9535
---	---	471.2449	236.1261	4 E 16 1813.7925	907.3999	1715.8156	858.4115
---	---	586.2719	293.6396	5 D 15 1684.7499	842.8786	1586.7730	793.8902
---	---	685.3403	343.1738	6 V 14 1569.7230	785.3651	1471.7461	736.3767
---	---	788.3495	394.6784	7 C 13 1470.6546	735.8309	1372.6777	686.8425
---	---	859.3866	430.1969	8 A 12 1367.6454	684.3263	1269.6685	635.3379
---	---	974.4135	487.7104	9 D 11 1296.6083	648.8078	1198.6314	599.8193
1043.4350	522.2211	1141.4119	571.2096	10 S 10 1181.5813	591.2943	1083.6045	542.3059
1142.5034	571.7553	1240.4803	620.7438	11 V 9 1014.5830	507.7951	---	---
1257.5304	629.2688	1355.5073	678.2573	12 D 8 915.5146	458.2609	---	---
1328.5675	664.7874	1426.5444	713.7758	13 A 7 800.4876	400.7475	---	---
1427.6359	714.3216	1525.6128	763.3100	14 V 6 729.4505	365.2289	---	---
1555.6945	778.3509	1653.6714	827.3393	15 Q 5 630.3821	315.6947	---	---
1684.7371	842.8722	1782.7140	891.8606	16 E 4 502.3235	251.6654	---	---
1797.8211	899.4142	1895.7980	948.4026	17 L 3 373.2809	187.1441	---	---
1910.9052	955.9562	2008.8821	1004.9447	18 I 2 260.1969	130.6021	---	---
---	---	---	---	19 K 1 147.1128	74.0600	---	---

Observed M=2153.9606
 Calculated M=2153.9803

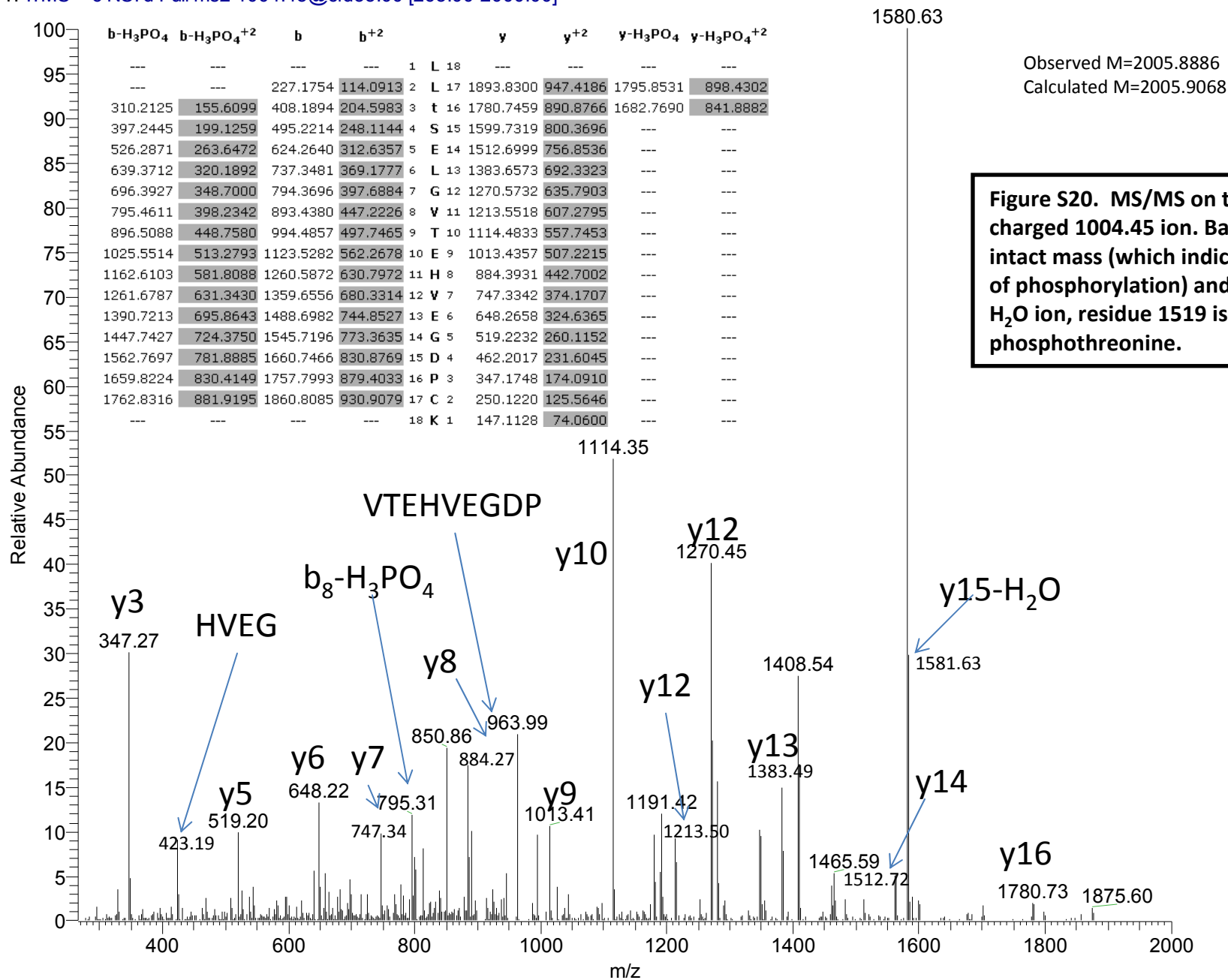
Figure S19. MS/MS on the doubly charged 1078.49 ion. Based on the intact mass (which indicated 1 site of phosphorylation) and the b14-H₃PO₄ and b-80 ions, residue 672 is a phosphoserine.

Lambda

MS/MS of peptide LLpTSELGVTEHVEGDPCCK

U110-5400 #5178 RT: 36.55 AV: 1 NL: 5.54E4

T: ITMS + c NSI d Full ms2 1004.45@cid35.00 [265.00-2000.00]



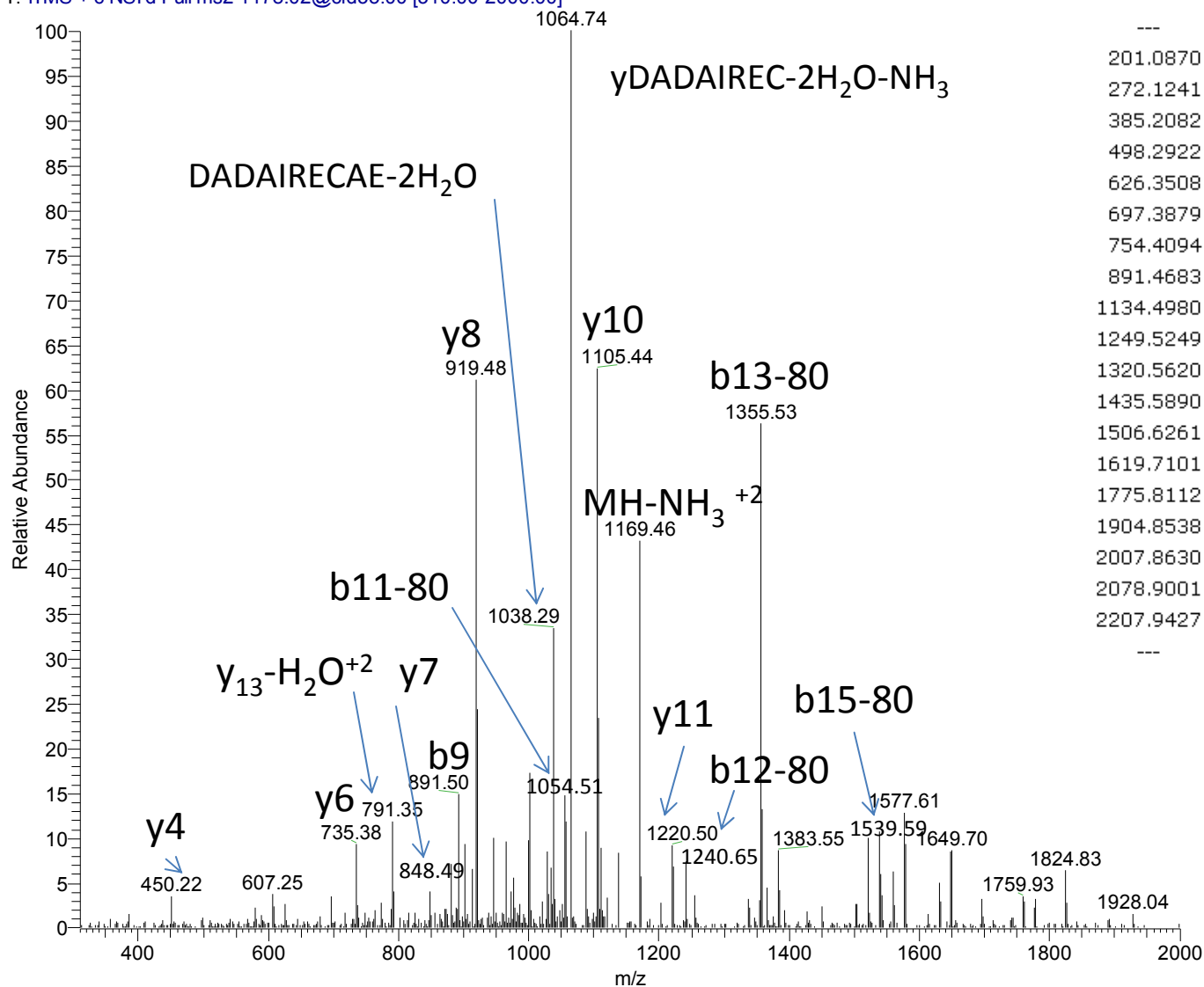
Observed M=2005.8886
Calculated M=2005.9068

Figure S20. MS/MS on the doubly charged 1004.45 ion. Based on the intact mass (which indicated 1 site of phosphorylation) and the y15-H₂O ion, residue 1519 is a phosphothreonine.

CIP

MS/MS of peptide AEALLQAGHYDADAIRECAEK

OT10-3490 #3014 RT: 35.41 AV: 1 NL: 2.01E4
 T: ITMS + c NSI d Full ms2 1178.02@cid35.00 [310.00-2000.00]



b	b ⁺²		y	y ⁺²
---	---	1	A 21	---
201.0870	101.0471	2	E 20	2283.0111 1142.0092
272.1241	136.5657	3	A 19	2153.9685 1077.4879
385.2082	193.1077	4	L 18	2082.9314 1041.9694
498.2922	249.6498	5	L 17	1969.8474 985.4273
626.3508	313.6790	6	Q 16	1856.7633 928.8853
697.3879	349.1976	7	A 15	1728.7047 864.8560
754.4094	377.7083	8	G 14	1657.6676 829.3374
891.4683	446.2378	9	H 13	1600.6461 800.8267
1134.4980	567.7526	10	y 12	1463.5872 732.2973
1249.5249	625.2661	11	D 11	1220.5576 610.7824
1320.5620	660.7846	12	A 10	1105.5306 553.2690
1435.5890	718.2981	13	D 9	1034.4935 517.7504
1506.6261	753.8167	14	A 8	919.4666 460.2369
1619.7101	810.3587	15	I 7	848.4295 424.7184
1775.8112	888.4093	16	R 6	735.3454 368.1763
1904.8538	952.9306	17	E 5	579.2443 290.1258
2007.8630	1004.4351	18	C 4	450.2017 225.6045
2078.9001	1039.9537	19	A 3	347.1925 174.0999
2207.9427	1104.4750	20	E 2	276.1554 138.5813
---	---	21	K 1	147.1128 74.0600

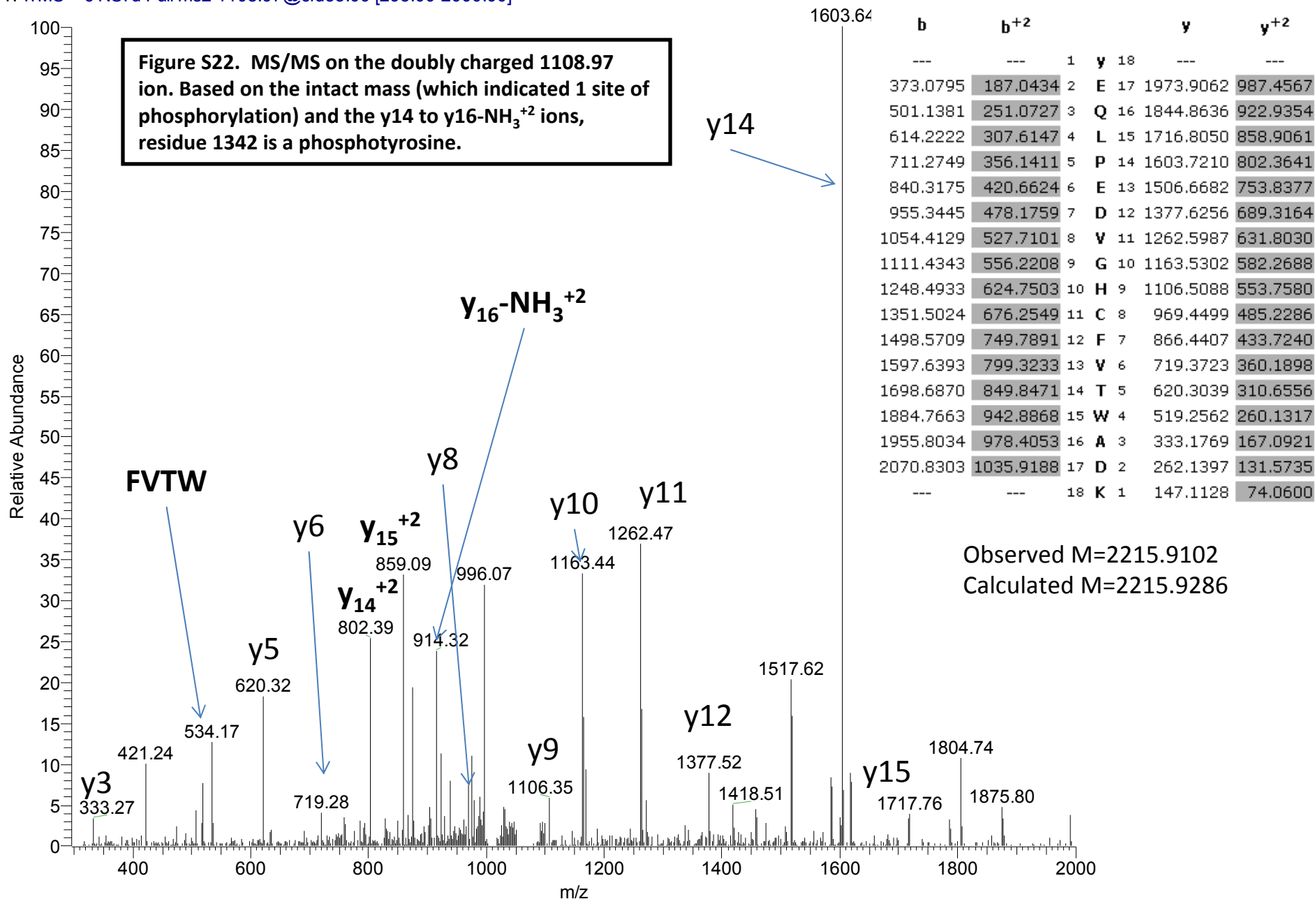
Observed M=2353.0228
 Calculated M=2353.0409

Figure S21. MS/MS on the doubly charged 1178.02 ion. Based on the intact mass (which indicated 1 site of phosphorylation) and the b ions, residue 963 is a phosphotyrosine

CIP

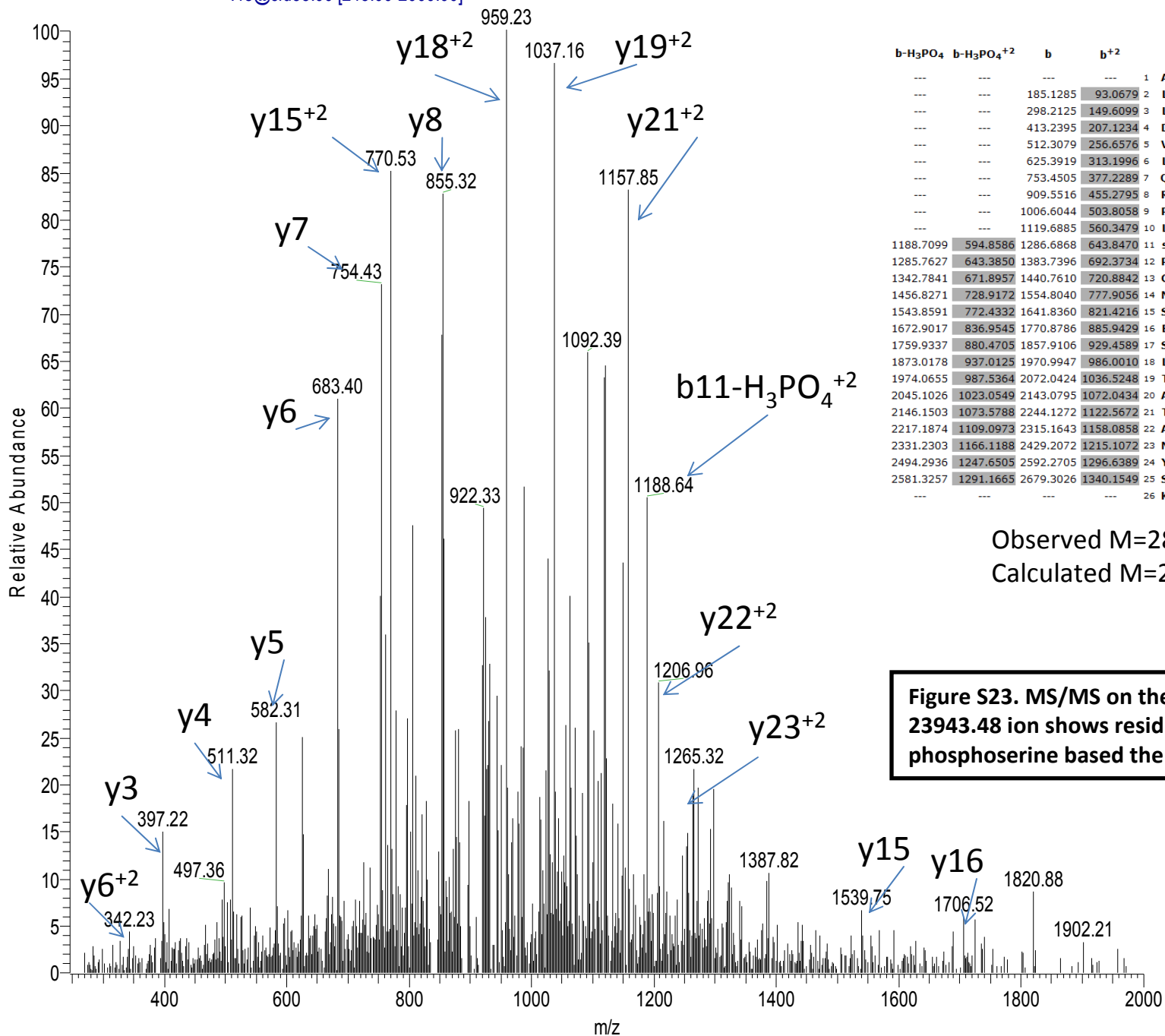
MS/MS of peptide pYEQLPEDVGHCFTWADK

OT10-3490 #3979 RT: 43.10 AV: 1 NL: 1.22E4
T: ITMS + c NSI d Full ms2 1108.97@cid35.00 [295.00-2000.00]



pEAK Rapid Cells AV: 1 NL: 9.86E3
 .48@cid35.00 [245.00-2000.00]

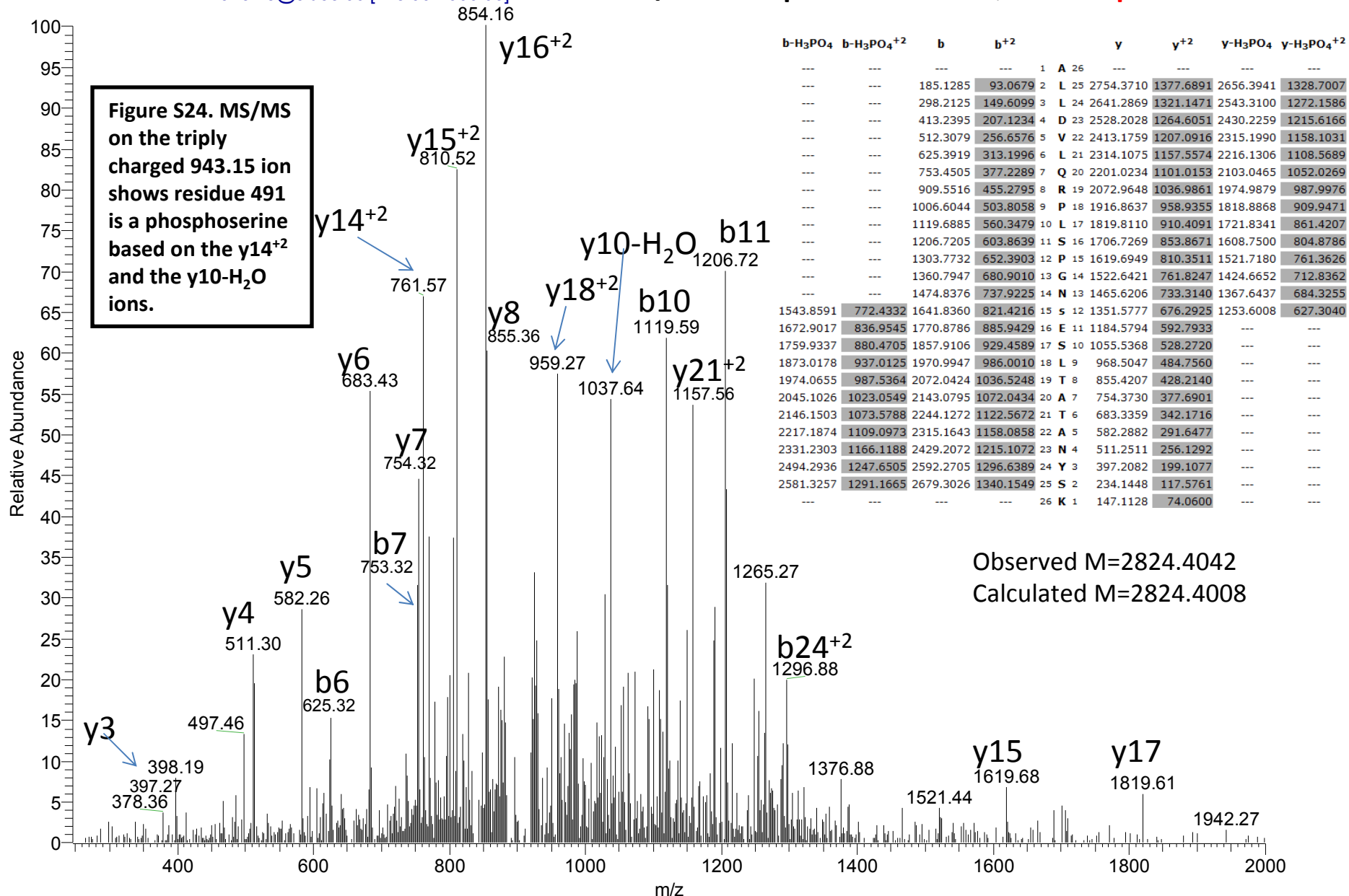
MS/MS of Peptide K.ALLDVLQRRLpSPGNSESLTATANYSK.A

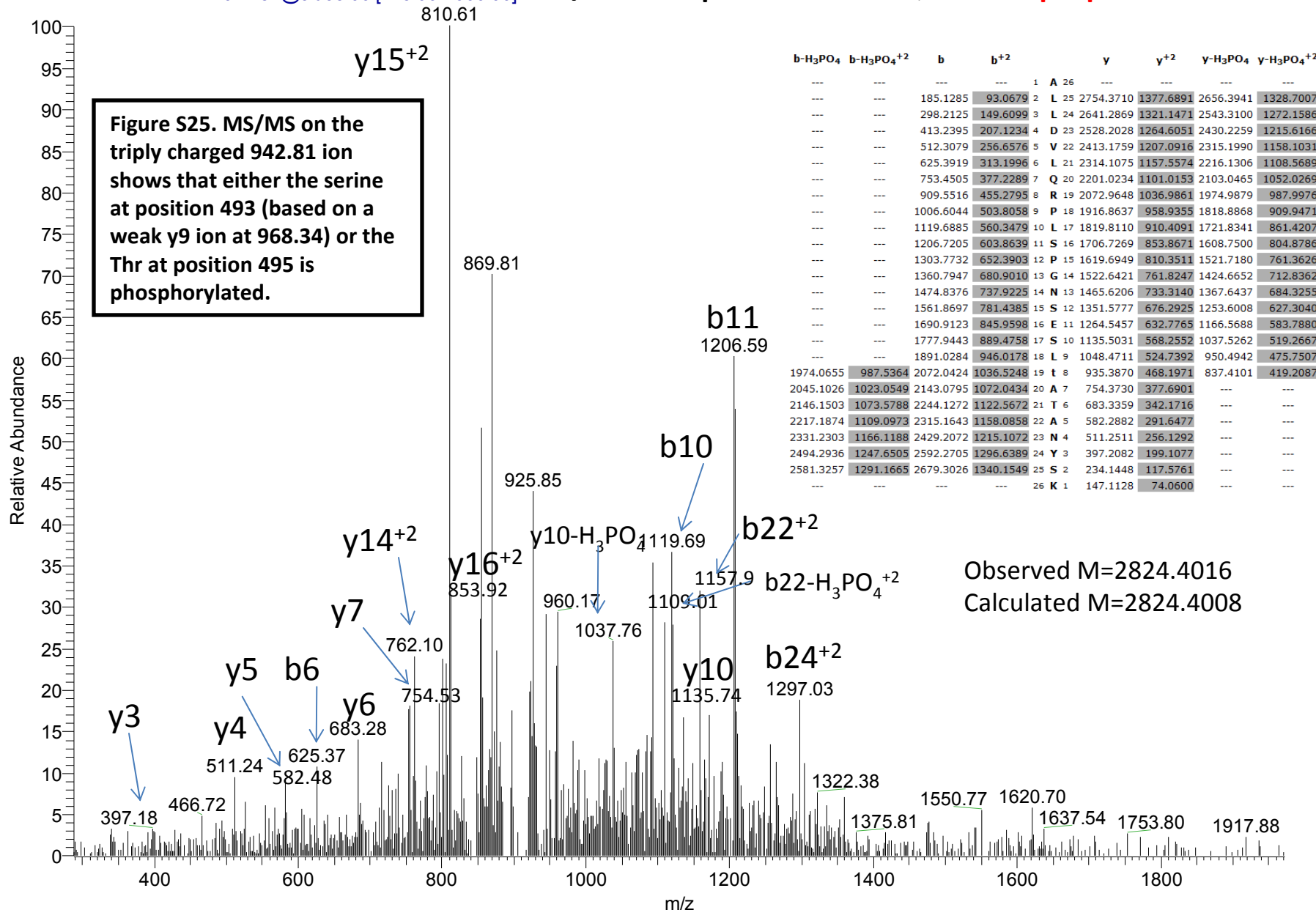


b-H ₃ PO ₄	b-H ₃ PO ₄ ⁺²	b	b ⁺²		y	y ⁺²	y-H ₃ PO ₄	y-H ₃ PO ₄ ⁺²
---	---	---	---	1	A 26	---	---	---
---	---	185.1285	93.0679	2	L 25	2754.3710	1377.6891	2656.3941
---	---	298.2125	149.6099	3	L 24	2641.2869	1321.1471	2543.3100
---	---	413.2395	207.1234	4	D 23	2528.2028	1264.6051	2430.2259
---	---	512.3079	256.6576	5	V 22	2413.1759	1207.0916	2315.1990
---	---	625.3919	313.1996	6	L 21	2314.1075	1157.5574	2216.1306
---	---	753.4505	377.2289	7	Q 20	2201.0234	1101.0153	2103.0465
---	---	909.5516	455.2795	8	R 19	2072.9648	1036.9861	1974.9879
---	---	1006.6044	503.8058	9	P 18	1916.8637	958.9355	1818.8868
---	---	1119.6885	560.3479	10	L 17	1819.8110	910.4091	1721.8341
1188.7099	594.8586	1286.6868	643.8470	11	S 16	1706.7269	853.8671	1608.7500
1285.7627	643.3850	1383.7396	692.3734	12	P 15	1539.7285	770.3679	---
1342.7841	671.8957	1440.7610	720.8842	13	G 14	1442.6758	721.8415	---
1456.8271	728.9172	1554.8040	777.9056	14	N 13	1385.6543	693.3308	---
1543.8591	772.4332	1641.8360	821.4216	15	S 12	1271.6114	636.3093	---
1672.9017	836.9545	1770.8786	885.9429	16	E 11	1184.5794	592.7933	---
1759.9337	880.4705	1857.9106	929.4589	17	S 10	1055.5368	528.2720	---
1873.0178	937.0125	1970.9947	986.0010	18	L 9	968.5047	484.7560	---
1974.0655	987.5364	2072.0424	1036.5248	19	T 8	855.4207	428.2140	---
2045.1026	1023.0549	2143.0795	1072.0434	20	A 7	754.3730	377.6901	---
2146.1503	1073.5788	2244.1272	1122.5672	21	T 6	683.3359	342.1716	---
2217.1874	1109.0973	2315.1643	1158.0858	22	A 5	582.2882	291.6477	---
2331.2303	1166.1188	2429.2072	1215.1072	23	N 4	511.2511	256.1292	---
2494.2936	1247.6505	2592.2705	1296.6389	24	Y 3	397.2082	199.1077	---
2581.3257	1291.1665	2679.3026	1340.1549	25	S 2	234.1448	117.5761	---
---	---	---	---	26	K 1	147.1128	74.0600	---

Observed M=2824.3990
 Calculated M=2824.4008

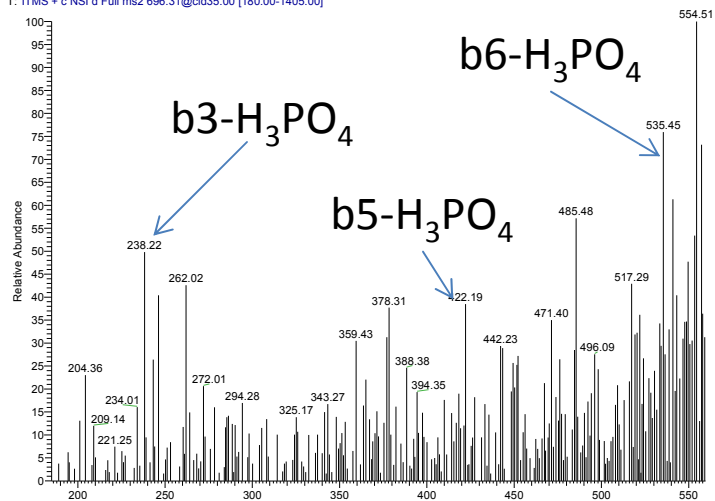
Figure S23. MS/MS on the triply charged 23943.48 ion shows residue 487 is a phosphoserine based the y15⁺² ion.





pEAK Rapid Cells

I: ITMS + c NSI d Full ms2 696.31@cid35.00 [180.00-1405.00]



OT09-0068 #1929 RT: 26.13 AV: 1 NL: 1.57E3
T: ITMS + c NSI d Full ms2 696.31@cid35.00 [180.00-1405.00]

MS/MS of Peptide R.pSAPPSLGEPTEAR.D

b-H ₃ PO ₄	b-H ₃ PO ₄ ⁺²	b	b ⁺²		y	y ⁺²
---	---	---	---	1	S 13	---
141.0659	71.0366	239.0427	120.0250	2	A 12	1224.6219
238.1186	119.5629	336.0955	168.5514	3	P 11	1153.5848
335.1714	168.0893	433.1483	217.0778	4	P 10	1056.5320
422.2034	211.6053	520.1803	260.5938	5	S 9	959.4793
535.2875	268.1474	633.2644	317.1358	6	L 8	872.4472
592.3089	296.6581	690.2858	345.6466	7	G 7	759.3632
721.3515	361.1794	819.3284	410.1679	8	E 6	702.3417
818.4043	409.7058	916.3812	458.6942	9	P 5	573.2991
919.4520	460.2296	1017.4289	509.2181	10	T 4	476.2463
1048.4946	524.7509	1146.4715	573.7394	11	E 3	375.1987
1119.5317	560.2695	1217.5086	609.2579	12	A 2	246.1561
---	---	---	---	13	R 1	175.1190
						88.0631

Observed M=1390.6141
Calculated M=1390.6129

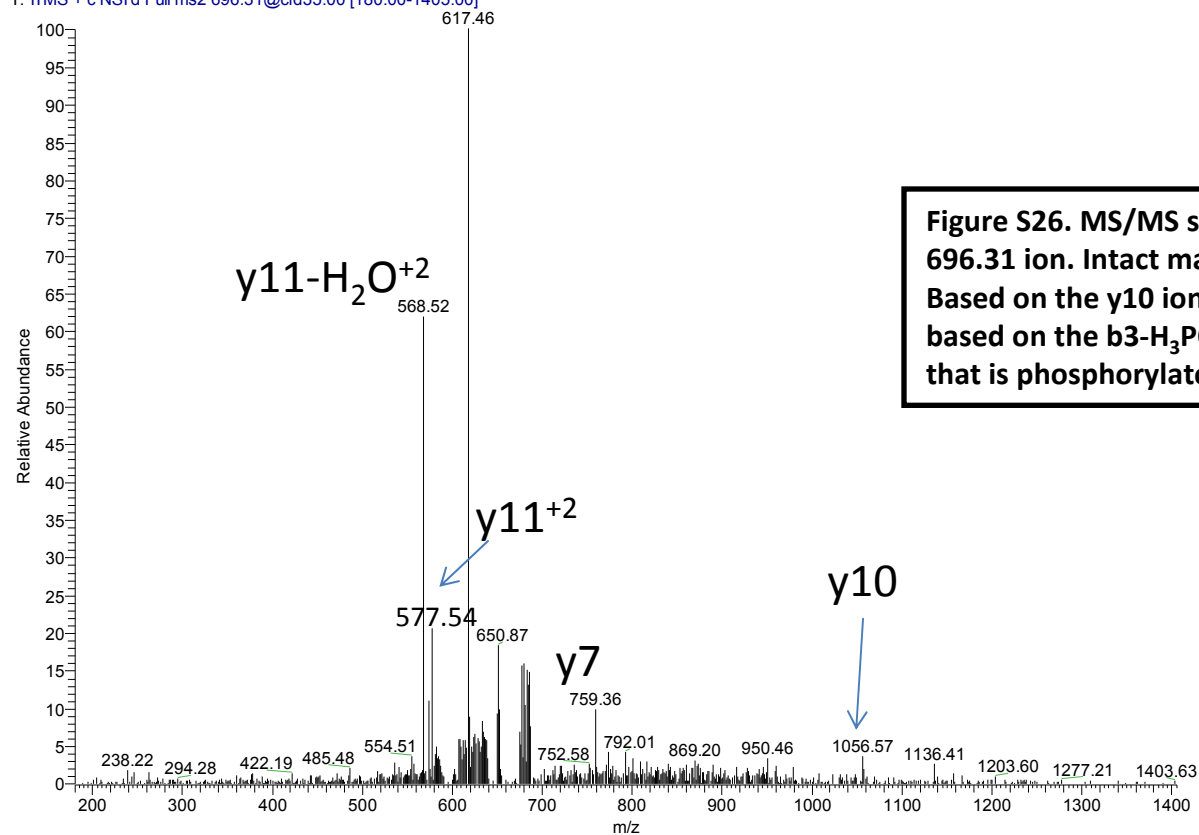
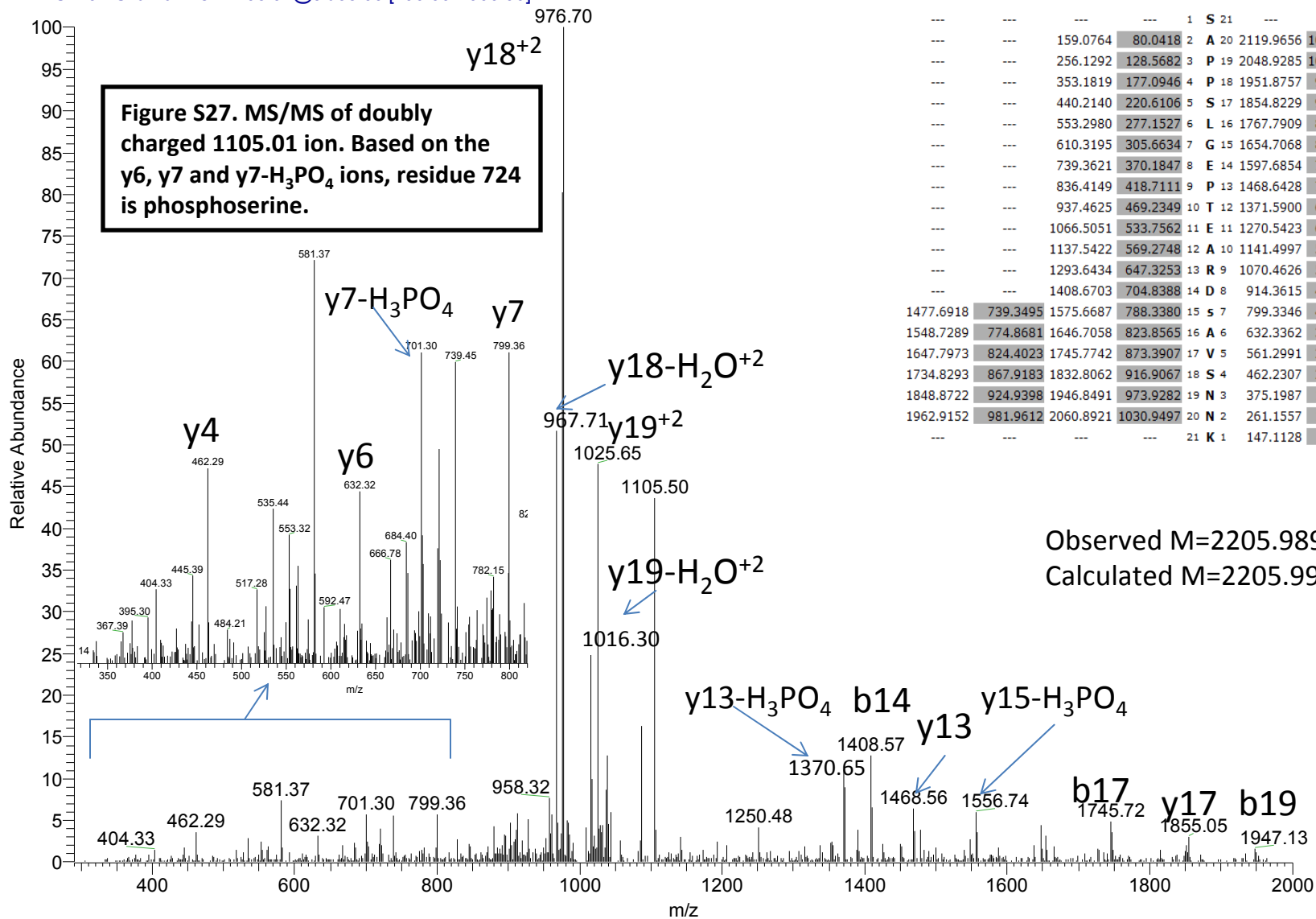


Figure S26. MS/MS spectra of doubly charged 696.31 ion. Intact mass indicates one site of phosphorylation. Based on the y10 ion, the threonine is not phosphorylated; based on the b3-H₃PO₄ ion, it is the first serine at residue 710 that is phosphorylated.

pEAK Rapid Cells

1 AV: 1 NL: 1.00E3
 T: ITMS + c NSI d Full ms2 1105.01@cid35.00 [290.00-2000.00]

MS/MS of Peptide R.SAPPSLGEPTEARpSAVSNNK.T



b-H ₃ PO ₄	b-H ₃ PO ₄ +2	b	b+2	y	y+2	y-H ₃ PO ₄	y-H ₃ PO ₄ +2
---	---	---	---	1 S 21	---	---	---
---	---	159.0764	80.0418	2 A 20	2119.9656	1060.4864	2021.9887
---	---	256.1292	128.5682	3 P 19	2048.9285	1024.9679	1950.9516
---	---	353.1819	177.0946	4 P 18	1951.8757	976.4415	1853.8988
---	---	440.2140	220.6106	5 S 17	1854.8229	927.9151	1756.8460
---	---	553.2980	277.1527	6 L 16	1767.7909	884.3991	1669.8140
---	---	610.3195	305.6634	7 G 15	1654.7068	827.8571	1556.7299
---	---	739.3621	370.1847	8 E 14	1597.6854	799.3463	1499.7085
---	---	836.4149	418.7111	9 P 13	1468.6428	734.8250	1370.6659
---	---	937.4625	469.2349	10 T 12	1371.5900	686.2986	1273.6131
---	---	1066.5051	533.7562	11 E 11	1270.5423	635.7748	1172.5654
---	---	1137.5422	569.2748	12 A 10	1141.4997	571.2535	1043.5228
---	---	1293.6434	647.3253	13 R 9	1070.4626	535.7350	972.4857
---	---	1408.6703	704.8388	14 D 8	914.3615	457.6844	816.3846
1477.6918	739.3495	1575.6687	788.3380	15 S 7	799.3346	400.1709	701.3577
1548.7289	774.8681	1646.7058	823.8565	16 A 6	632.3362	316.6717	---
1647.7973	824.4023	1745.7742	873.3907	17 V 5	561.2991	281.1532	---
1734.8293	867.9183	1832.8062	916.9067	18 S 4	462.2307	231.6190	---
1848.8722	924.9398	1946.8491	973.9282	19 N 3	375.1987	188.1030	---
1962.9152	981.9612	2060.8921	1030.9497	20 N 2	261.1557	131.0815	---
---	---	---	---	21 K 1	147.1128	74.0600	---

Observed M=2205.9892
 Calculated M=2205.9903

pEAK Rapid Cells

MS/MS of Peptide K.AEALLQAGH**p**YDADAIRECAEK.V

OT09-0081 #2883 RT: 30.49 AV: 1 NL: 2.70E5
T: ITMS + c NSI d Full ms2 1177.52@cid35.00 [310.00-2000.00]

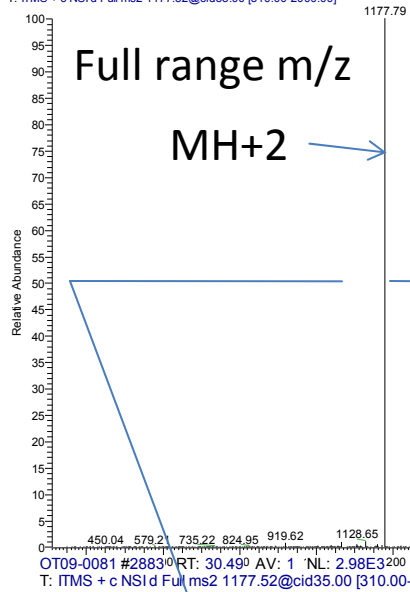


Figure S28. MS/MS of doubly charged 1177.52 ion. Intact mass is plus 80 amu from the parent mass, which suggests a single site of phosphorylation. Tyr963 is phosphorylated based on 1) the internal fragments and 2) it is the only possible phosphosite.

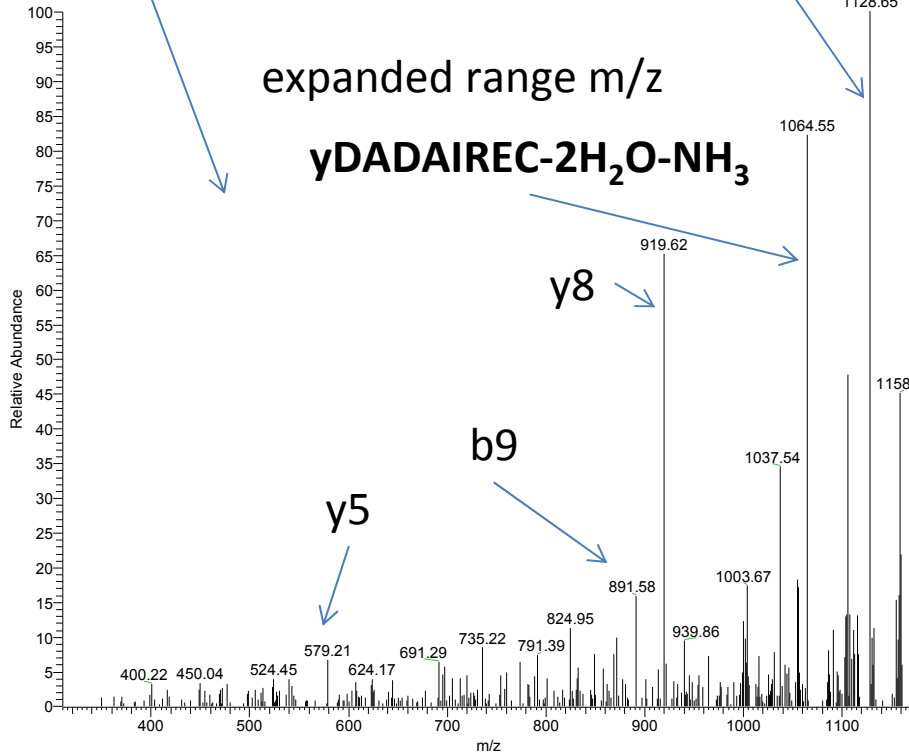
Observed M=2353.0296
Calculated M=2353.0409

b	b+2	y	y+2
---	---	1 A 21	---
201.0870	101.0471	2 E 20	2283.0111 1142.0092
272.1241	136.5657	3 A 19	2153.9685 1077.4879
385.2082	193.1077	4 L 18	2082.9314 1041.9694
498.2922	249.6498	5 L 17	1969.8474 985.4273
626.3508	313.6790	6 Q 16	1856.7633 928.8853
697.3879	349.1976	7 A 15	1728.7047 864.8560
754.4094	377.7083	8 G 14	1657.6676 829.3374
891.4683	446.2378	9 H 13	1600.6461 800.8267
1134.4980	567.7526	10 Y 12	1463.5872 732.2973
1249.5249	625.2661	11 D 11	1220.5576 610.7824
1320.5620	660.7846	12 A 10	1105.5306 553.2690
1435.5890	718.2981	13 D 9	1034.4935 517.7504
1506.6261	753.8167	14 A 8	919.4666 460.2369
1619.7101	810.3587	15 I 7	848.4295 424.7184
1775.8112	888.4093	16 R 6	735.3454 368.1763
1904.8538	952.9306	17 E 5	579.2443 290.1258
2007.8630	1004.4351	18 C 4	450.2017 225.6045
2078.9001	1039.9537	19 A 3	347.1925 174.0999
2207.9427	1104.4750	20 E 2	276.1554 138.5813
---	---	21 K 1	147.1128 74.0600

LLQAGHyDAD-2H₂O

expanded range m/z

yDADAIREC-2H₂O-NH₃

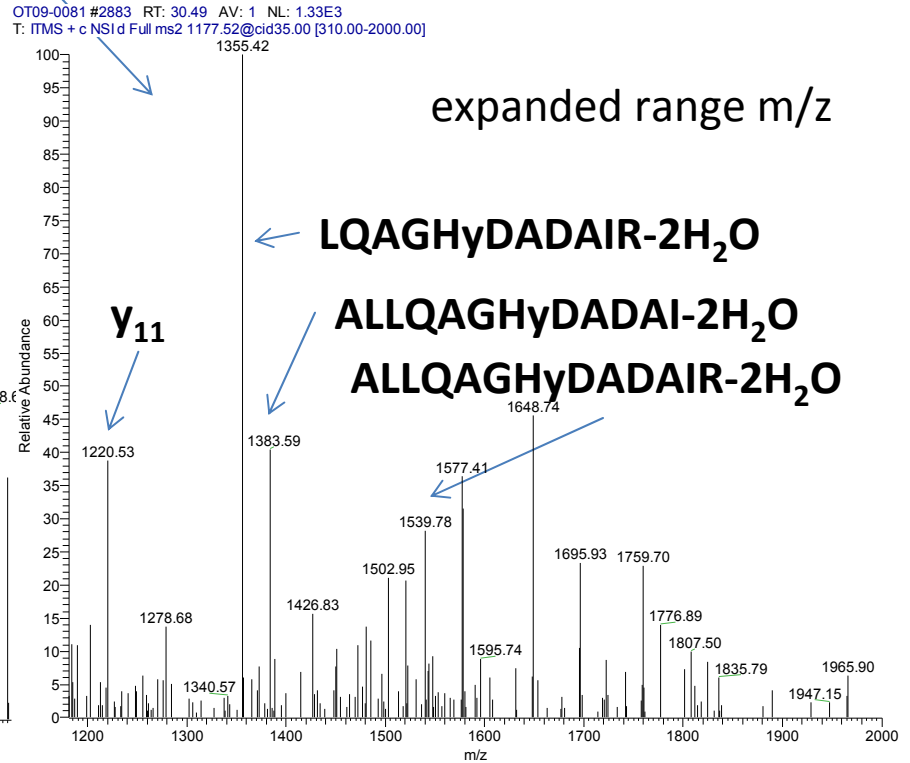


expanded range m/z

LQAGHyDADAIR-2H₂O

ALLQAGHyDADAI-2H₂O

ALLQAGHyDADAIR-2H₂O

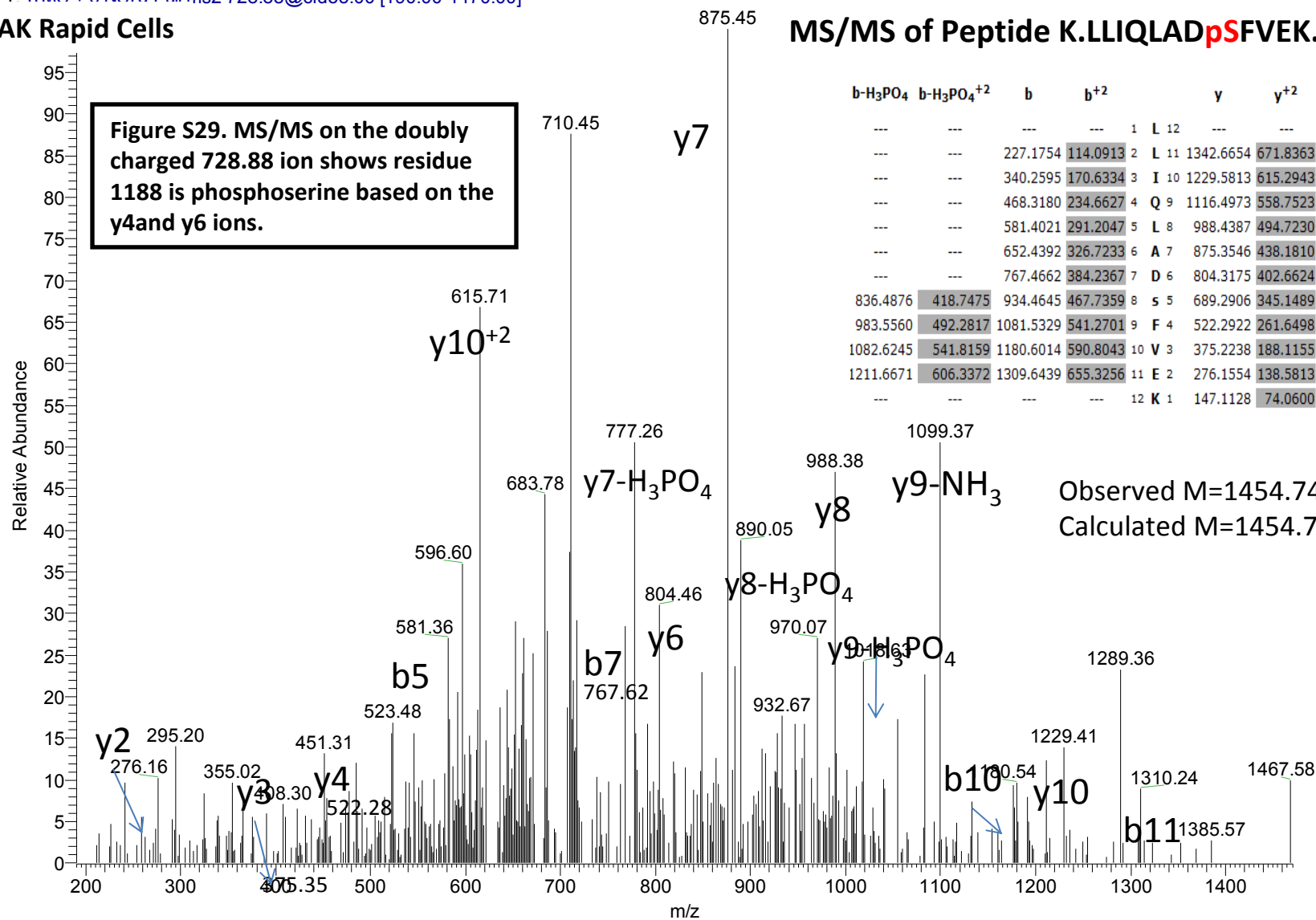


OT09-0060 #3122 RT: 41.21 AV: 1 NL: 8.40E1
 T: ITMS + c NSI d Full ms2 728.88@cid35.00 [190.00-1470.00]

pEAK Rapid Cells

MS/MS of Peptide K.LLIQLADpSFVEK.G

Figure S29. MS/MS on the doubly charged 728.88 ion shows residue 1188 is phosphoserine based on the y4 and y6 ions.



Observed M=1454.7472
 Calculated M=1454.7422

pEAK Rapid Cells 4 AV: 1 NL: 9.24E2
19.40@cid35.00 [215.00-1650.00]

MS/MS of Peptide K.DLELDIIPASLpSDR.E

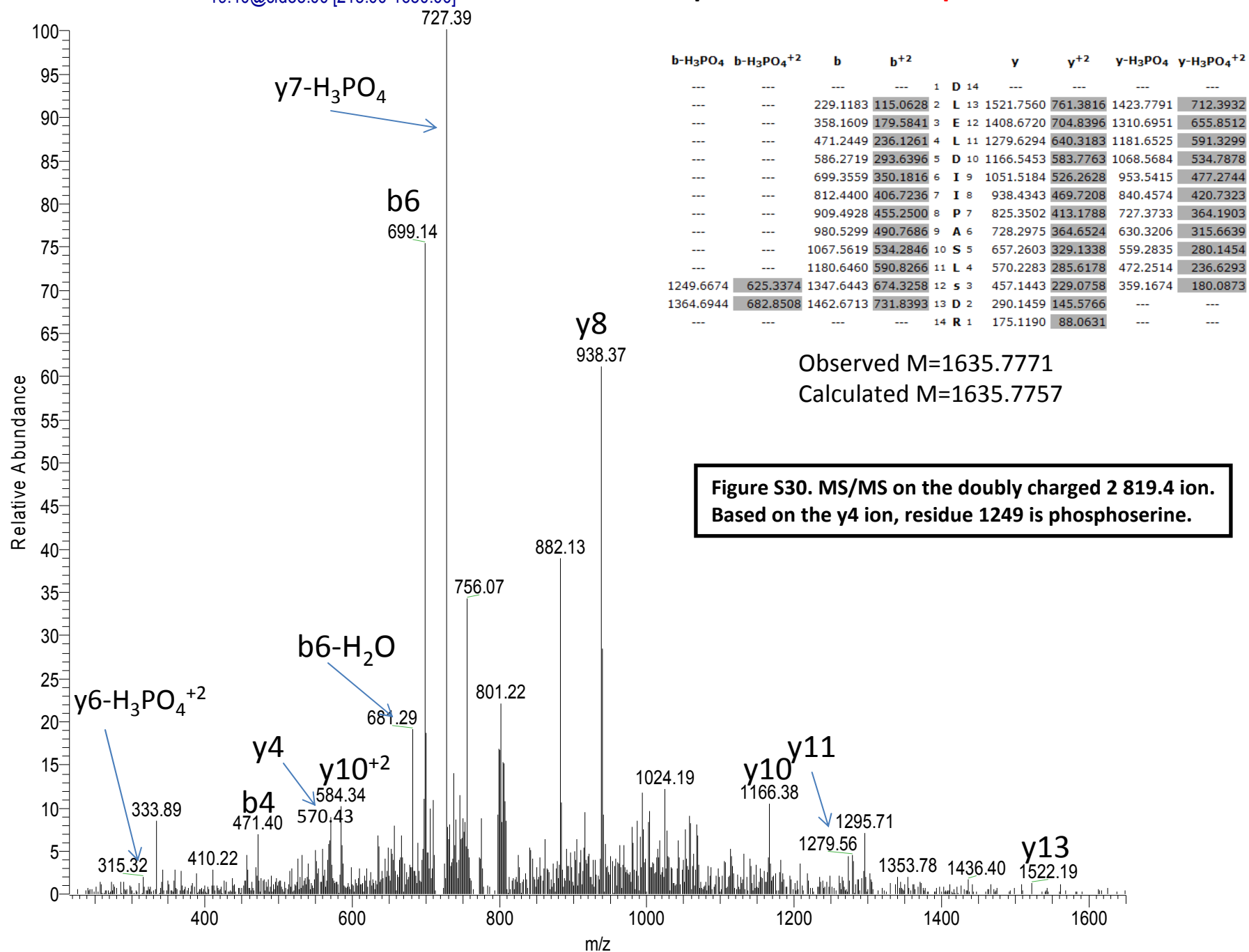
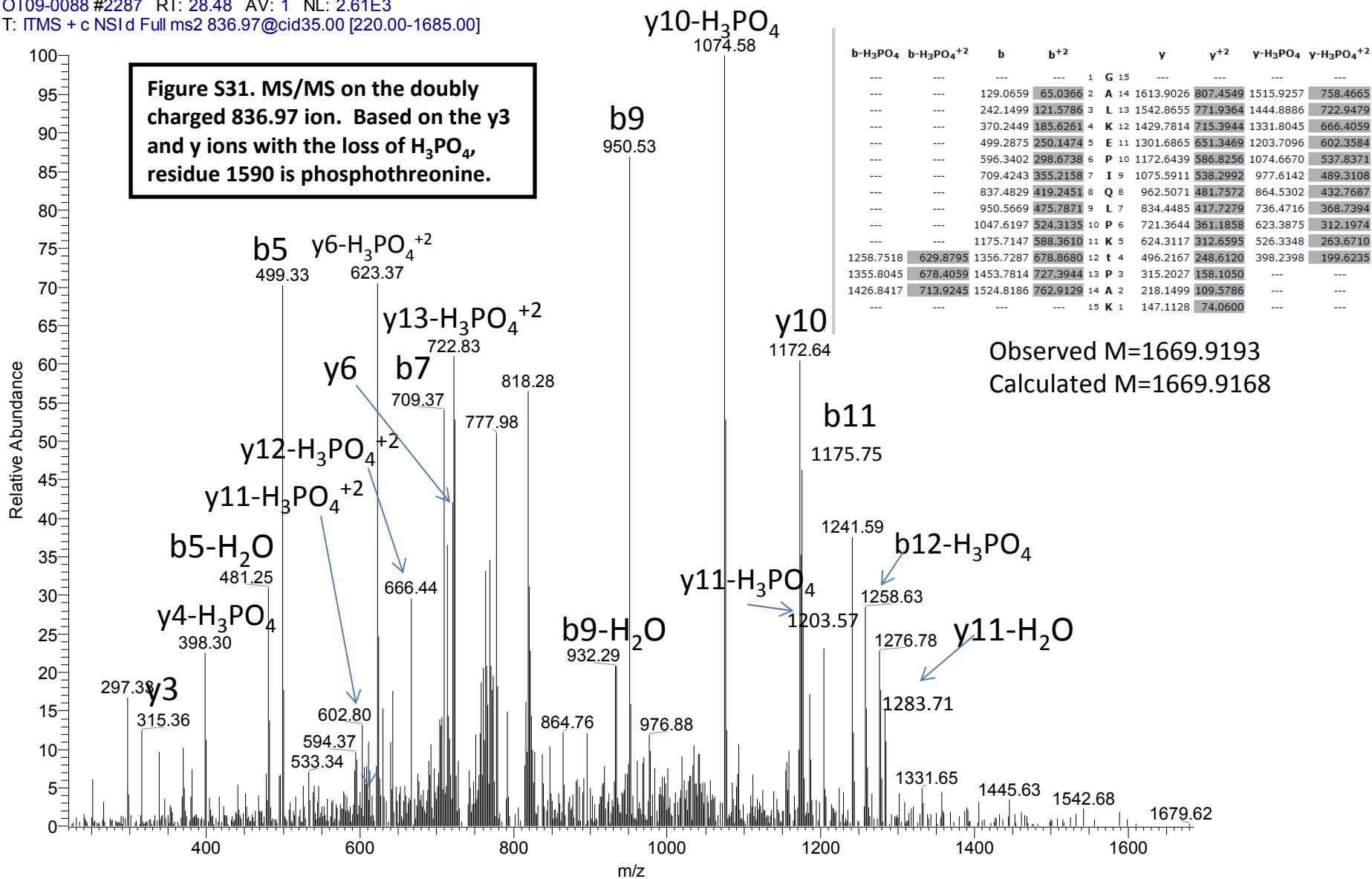


Figure S30. MS/MS on the doubly charged 2 819.4 ion. Based on the y4 ion, residue 1249 is phosphoserine.

pEAK Rapid Cells

OT09-0088 #2287 RT: 28.48 AV: 1 NL: 2.61E3
 T: ITMS + c NSI d Full ms2 836.97@cid35.00 [220.00-1685.00]

MS/MS of Peptide K.GALKEIPIQLPK^pTPAK.L



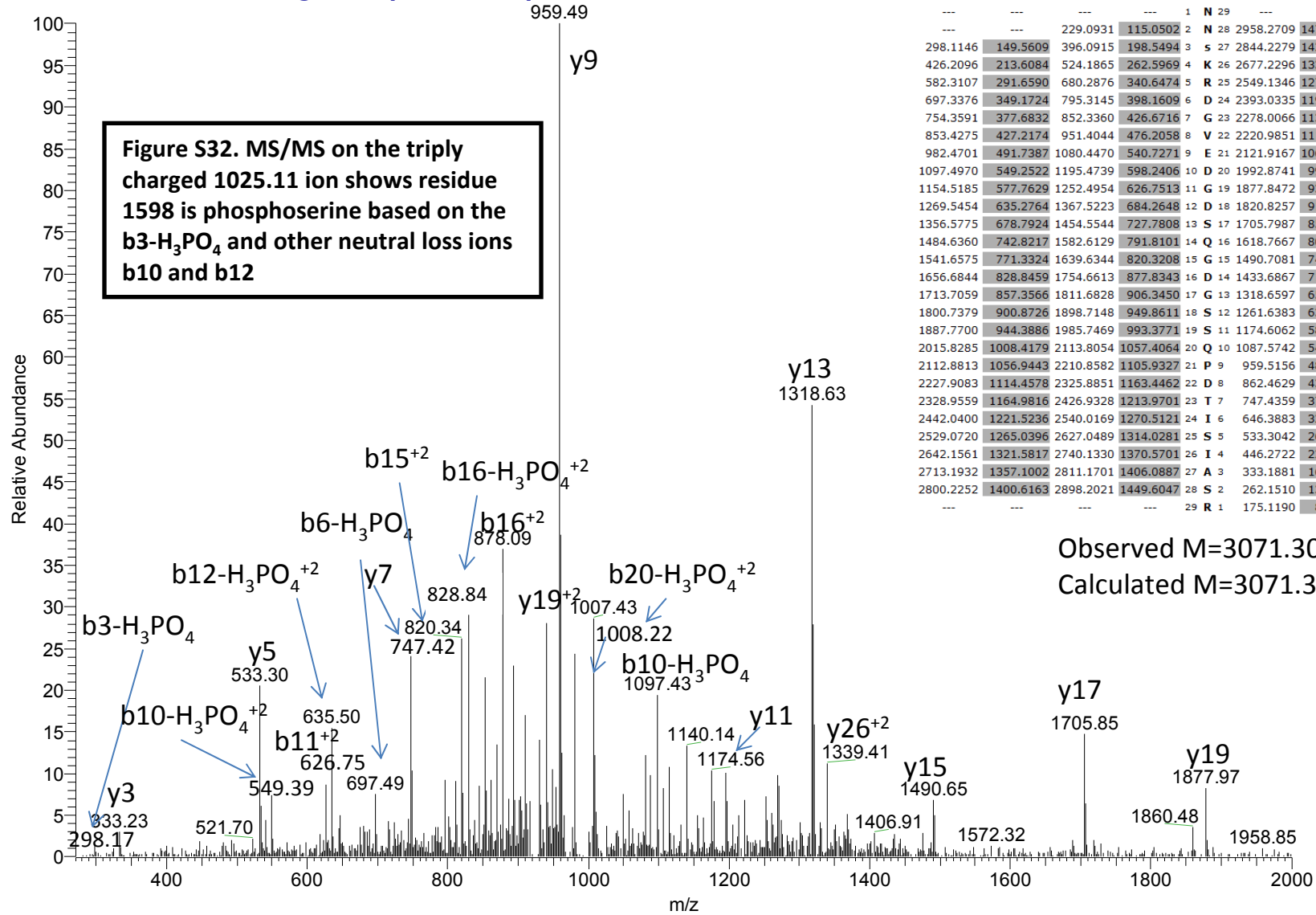
pEAK Rapid Cells

OT09-0060 #1896 RT: 25.69 AV: 1 NL: 3.66E3
 T: ITMS + c NSI d Full ms2 1025.11@cid35.00 [270.00-2000.00]

MS/MS of Peptide R.NNpSKRDGVEDGDSQGDGSSQPDTISIAsR.T

b-H ₃ PO ₄	b-H ₃ PO ₄ ⁺²	b	b ⁺²	y	y ⁺²	y-H ₃ PO ₄	y-H ₃ PO ₄ ⁺²
---	---	---	---	1	N 29	---	---
---	---	229.0931	115.0502	2	N 28	2958.2709	1479.6391
298.1146	149.5609	396.0915	198.5494	3	S 27	2844.2279	1422.6176
426.2096	213.6084	524.1865	262.5969	4	K 26	2677.2296	1339.1184
582.3107	291.6590	680.2876	340.6474	5	R 25	2549.1346	1275.0709
697.3376	349.1724	795.3145	398.1609	6	D 24	2393.0335	1197.0204
754.3591	377.6832	852.3360	426.6716	7	G 23	2278.0066	1139.5069
853.4275	427.2174	951.4044	476.2058	8	V 22	2220.9851	1110.9962
982.4701	491.7387	1080.4470	540.7271	9	E 21	2121.9167	1061.4620
1097.4970	549.2522	1195.4739	598.2406	10	D 20	1992.8741	996.9407
1154.5185	577.7629	1252.4954	626.7513	11	G 19	1877.8472	939.4272
1269.5454	635.2764	1367.5223	684.2648	12	D 18	1820.8257	910.9165
1356.5775	678.7924	1454.5544	727.7808	13	S 17	1705.7987	853.4030
1484.6360	742.8217	1582.6129	791.8101	14	Q 16	1618.7667	809.8870
1541.6575	771.3324	1639.6344	820.3208	15	G 15	1490.7081	745.8577
1656.6844	828.8459	1754.6613	877.8343	16	D 14	1433.6867	717.3470
1713.7059	857.3566	1811.6828	906.3450	17	G 13	1318.6597	659.8335
1800.7379	900.8726	1898.7148	949.8611	18	S 12	1261.6383	631.3228
1887.7700	944.3886	1985.7469	993.3771	19	S 11	1174.6062	587.8068
2015.8285	1008.4179	2113.8054	1057.4064	20	Q 10	1087.5742	544.2907
2112.8813	1056.9443	2210.8582	1105.9327	21	P 9	959.5156	480.2615
2227.9083	1114.4578	2325.8851	1163.4462	22	D 8	862.4629	431.7351
2328.9559	1164.9816	2426.9328	1213.9701	23	T 7	747.4359	374.2216
2442.0400	1221.5236	2540.0169	1270.5121	24	I 6	646.3883	323.6978
2529.0720	1265.0396	2627.0489	1314.0281	25	S 5	533.3042	267.1557
2642.1561	1321.5817	2740.1330	1370.5701	26	I 4	446.2722	223.6397
2713.1932	1357.1002	2811.1701	1406.0887	27	A 3	333.1881	167.0977
2800.2252	1400.6163	2898.2021	1449.6047	28	S 2	262.1510	131.5791
---	---	---	---	29	R 1	175.1190	88.0631

Observed M=3071.3071
 Calculated M=3071.3065

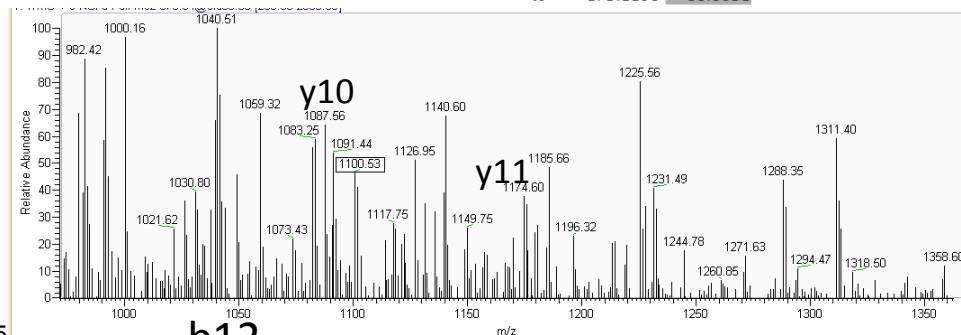
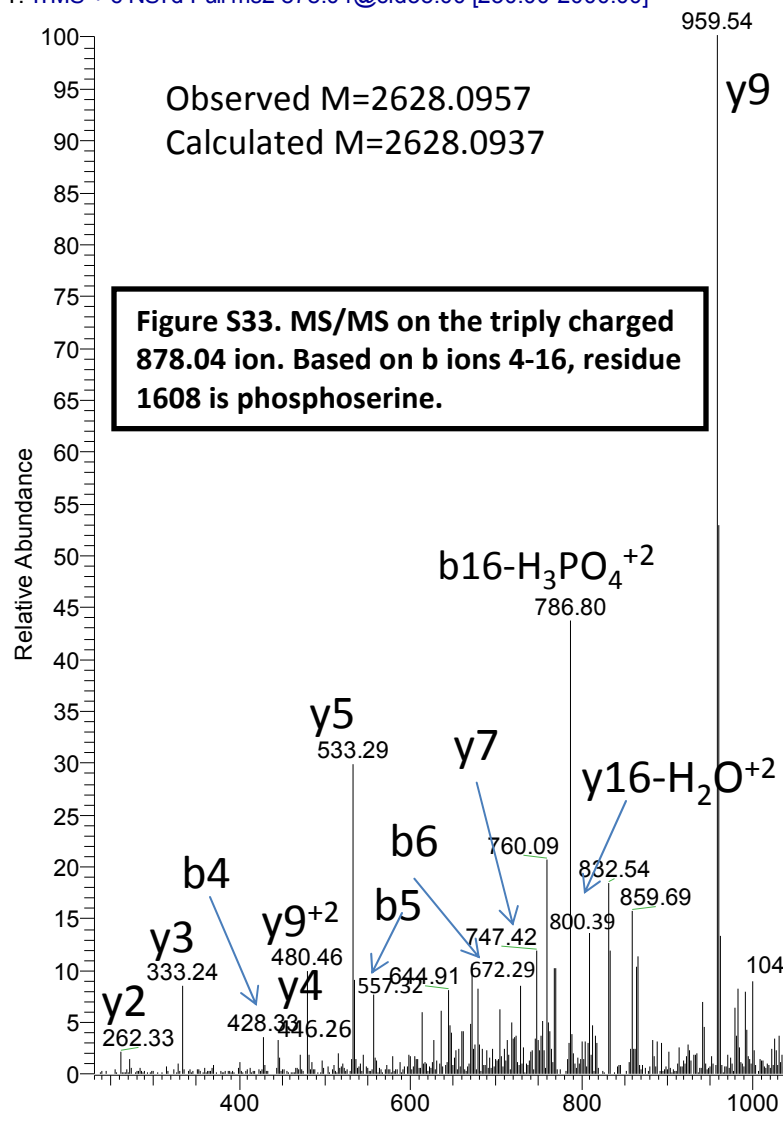


pEAK Rapid Cells

OT09-0072 #2027 RT: 27.41 AV: 1 NL: 6.16E3
 T: ITMS + c NSI d Full ms2 878.04@cid35.00 [230.00-2000.00]

MS/MS of Peptide K.RDGVEDGDpSQGDGSSQPDTISIASR.T

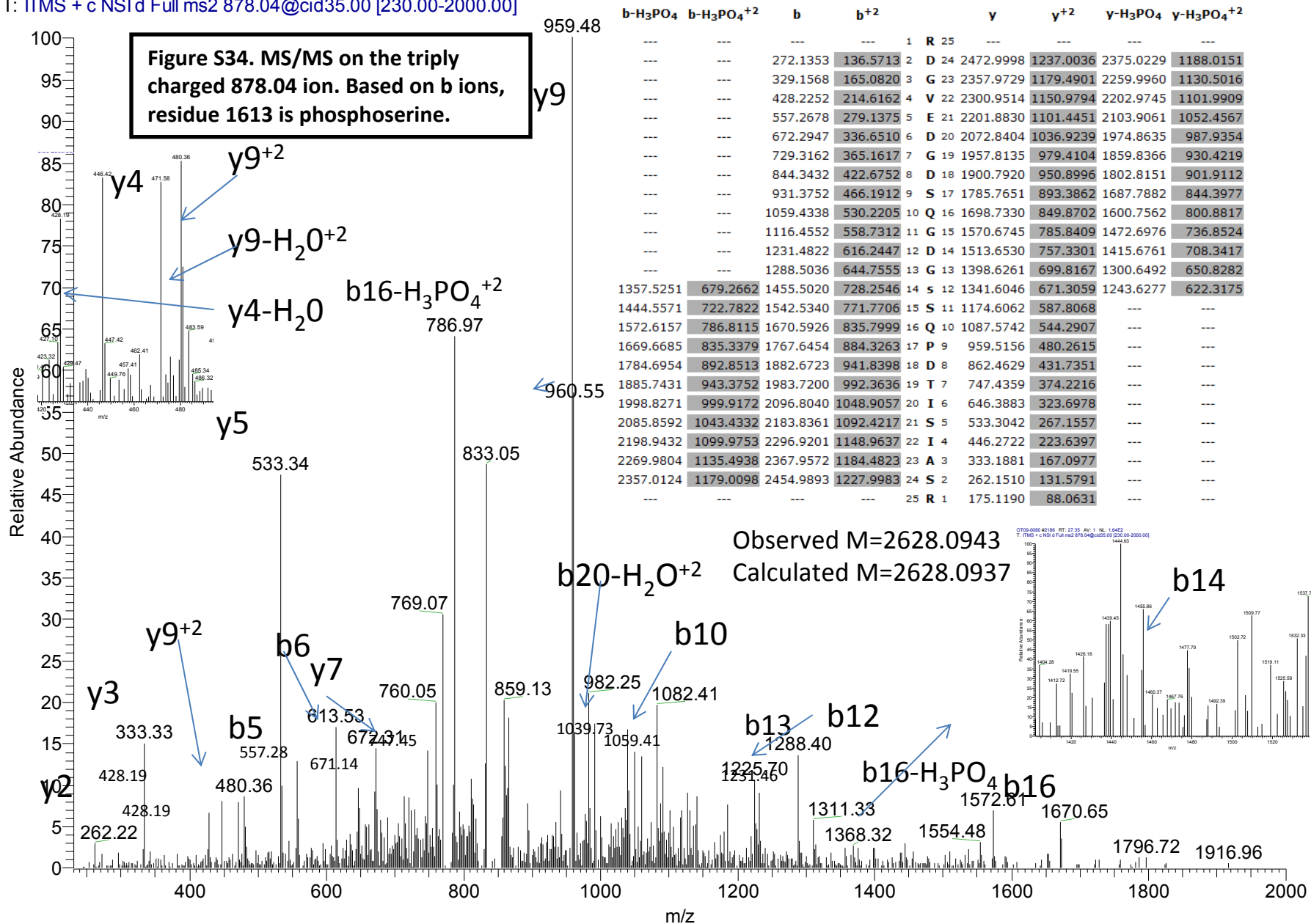
b-H ₃ PO ₄	b-H ₃ PO ₄ ⁺²	b	b ⁺²	y	y ⁺²	y-H ₃ PO ₄	y-H ₃ PO ₄ ⁺²		
---	---	---	---	1	R 25	---	---		
---	---	272.1353	136.5713	2	D 24	2472.9998	1237.0036	2375.0229	1188.0151
---	---	329.1568	165.0820	3	G 23	2357.9729	1179.4901	2259.9960	1130.5016
---	---	428.2252	214.6162	4	V 22	2300.9514	1150.9794	2202.9745	1101.9909
---	---	557.2678	279.1375	5	E 21	2201.8830	1101.4451	2103.9061	1052.4567
---	---	672.2947	336.6510	6	D 20	2072.8404	1036.9239	1974.8635	987.9354
---	---	729.3162	365.1617	7	G 19	1957.8135	979.4104	1859.8366	930.4219
---	---	844.3432	422.6752	8	D 18	1900.7920	950.8996	1802.8151	901.9112
913.3646	457.1859	1011.3415	506.1744	9	S 17	1785.7651	893.3862	1687.7882	844.3977
1041.4232	521.2152	1139.4001	570.2037	10	Q 16	1618.7667	809.8870	---	---
1098.4447	549.7260	1196.4216	598.7144	11	G 15	1490.7081	745.8577	---	---
1213.4716	607.2394	1311.4485	656.2279	12	D 14	1433.6867	717.3470	---	---
1270.4931	635.7502	1368.4700	684.7386	13	G 13	1318.6597	659.8335	---	---
1357.5251	679.2662	1455.5020	728.2546	14	S 12	1261.6383	631.3228	---	---
1444.5571	722.7822	1542.5340	771.7706	15	S 11	1174.6062	587.8068	---	---
1572.6157	786.8115	1670.5926	835.7999	16	Q 10	1087.5742	544.2907	---	---
1669.6685	835.3379	1767.6454	884.3263	17	P 9	959.5156	480.2615	---	---
1784.6954	892.8513	1882.6723	941.8398	18	D 8	862.4629	431.7351	---	---
1885.7431	943.3752	1983.7200	992.3636	19	T 7	747.4359	374.2216	---	---
1998.8271	999.9172	2096.8040	1048.9057	20	I 6	646.3883	323.6978	---	---
2085.8592	1043.4332	2183.8361	1092.4217	21	S 5	533.3042	267.1557	---	---
2198.9432	1099.9753	2296.9201	1148.9637	22	I 4	446.2722	223.6397	---	---
2269.9804	1135.4938	2367.9572	1184.4823	23	A 3	333.1881	167.0977	---	---
2357.0124	1179.0098	2454.9893	1227.9983	24	S 2	262.1510	131.5791	---	---
---	---	---	---	25	R 1	175.1190	88.0631	---	---



pPEAK Rapid Cells

OT09-0080 #2186 RT: 27.35 AV: 1 NL: 5.54E3
 T: ITMS + c NSI d Full ms2 878.04@cid35.00 [230.00-2000.00]

MS/MS of Peptide K.RDGVEDGDSQGDGpSSQPDTISIASR.T



OT09-0088 #2355 RT: 29.10 AV: 1 NL: 4.52E3
 T: ITMS + c NSI d Full ms2 826.01@cid35.00 [215.00-2000.00]

MS/MS of Peptide R.DGVEDGDSQGDGSpSQPDTISIASR.T

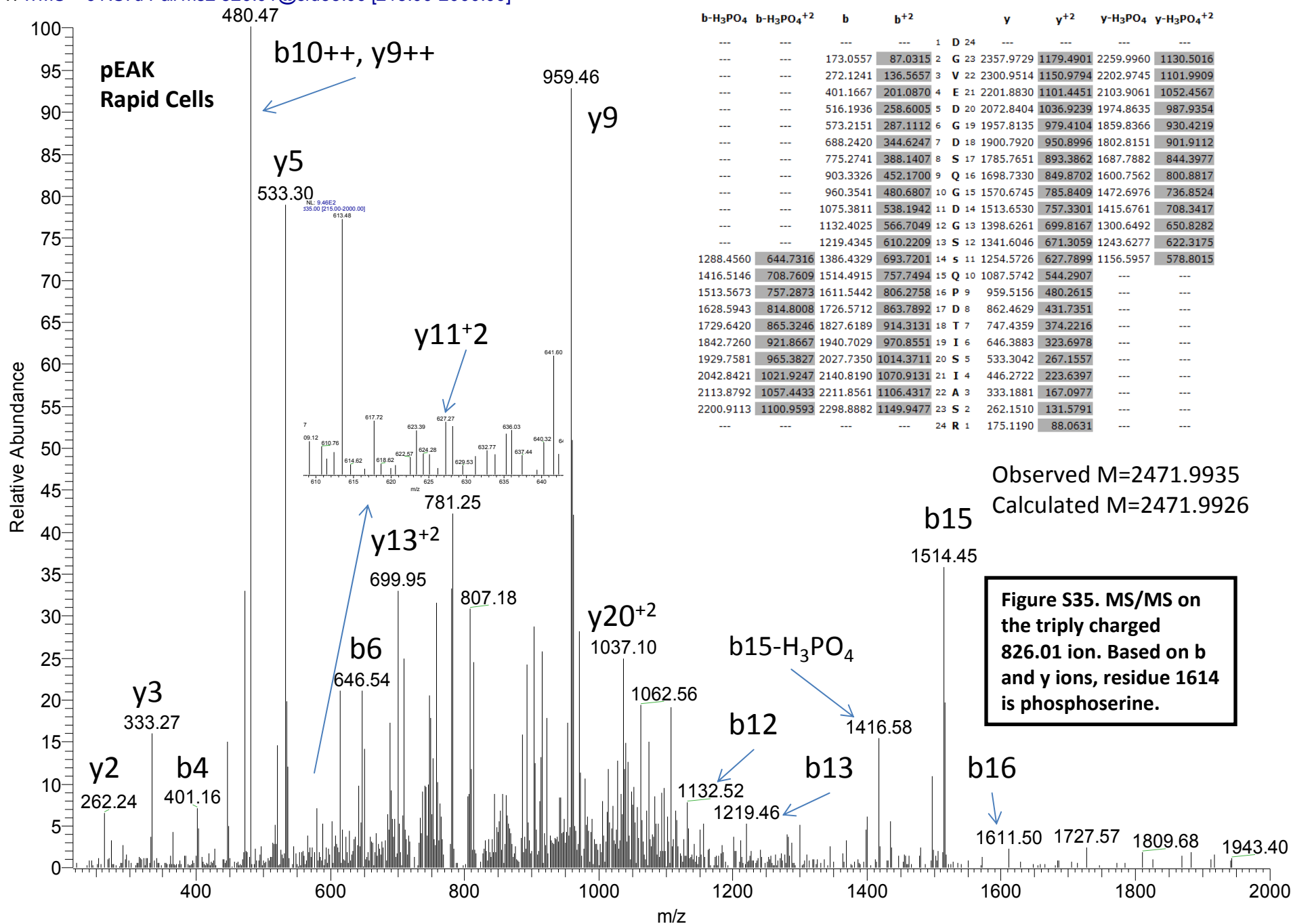
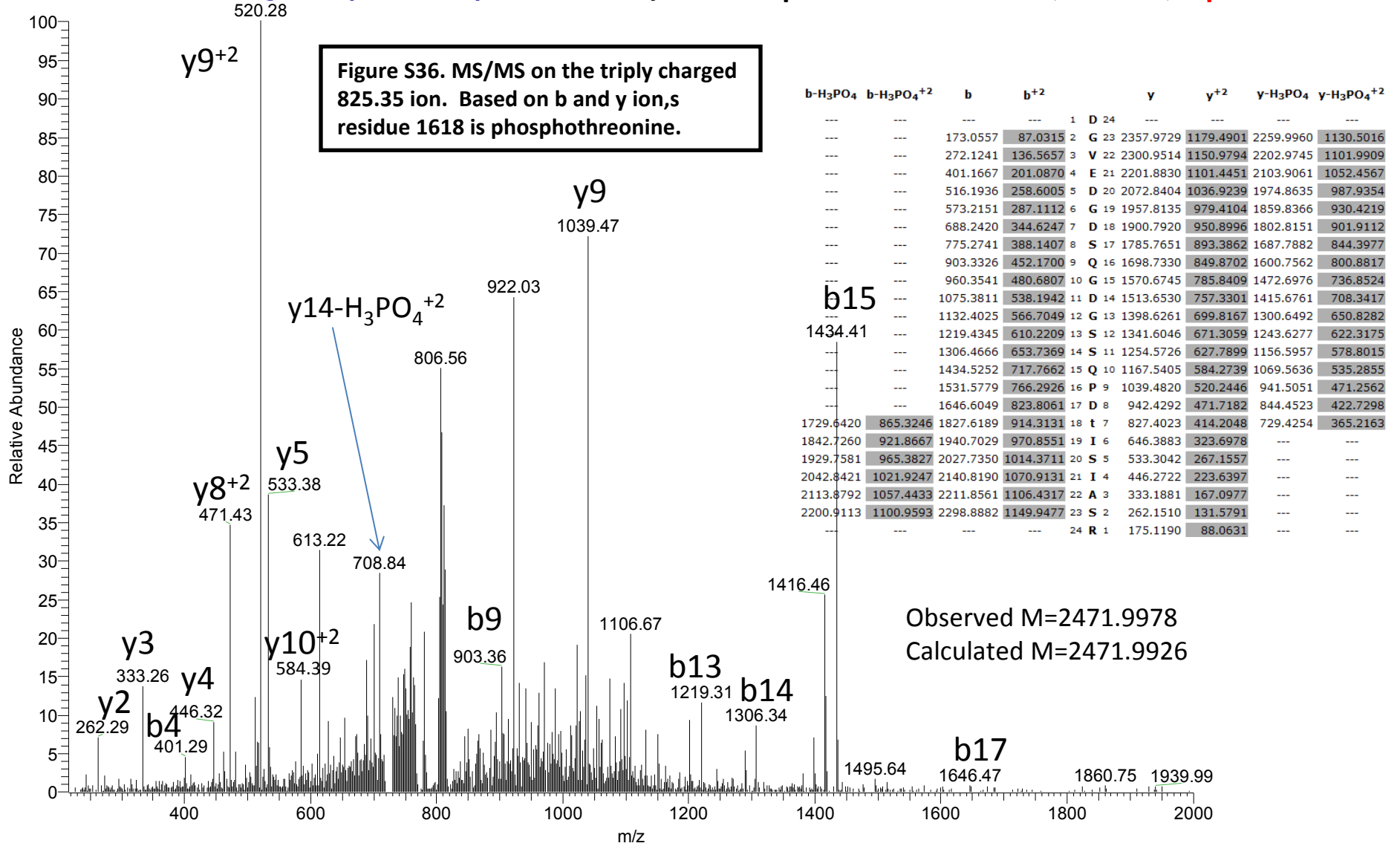


Figure S35. MS/MS on the triply charged 826.01 ion. Based on b and y ions, residue 1614 is phosphoserine.

pEAK Rapid Cells

OT09-0068 #2096 RT: 28.19 AV: 1 NL: 7.79E2
 T: ITMS + c NSI d Full ms2 825.35@cid35.00 [215.00-2000.00]

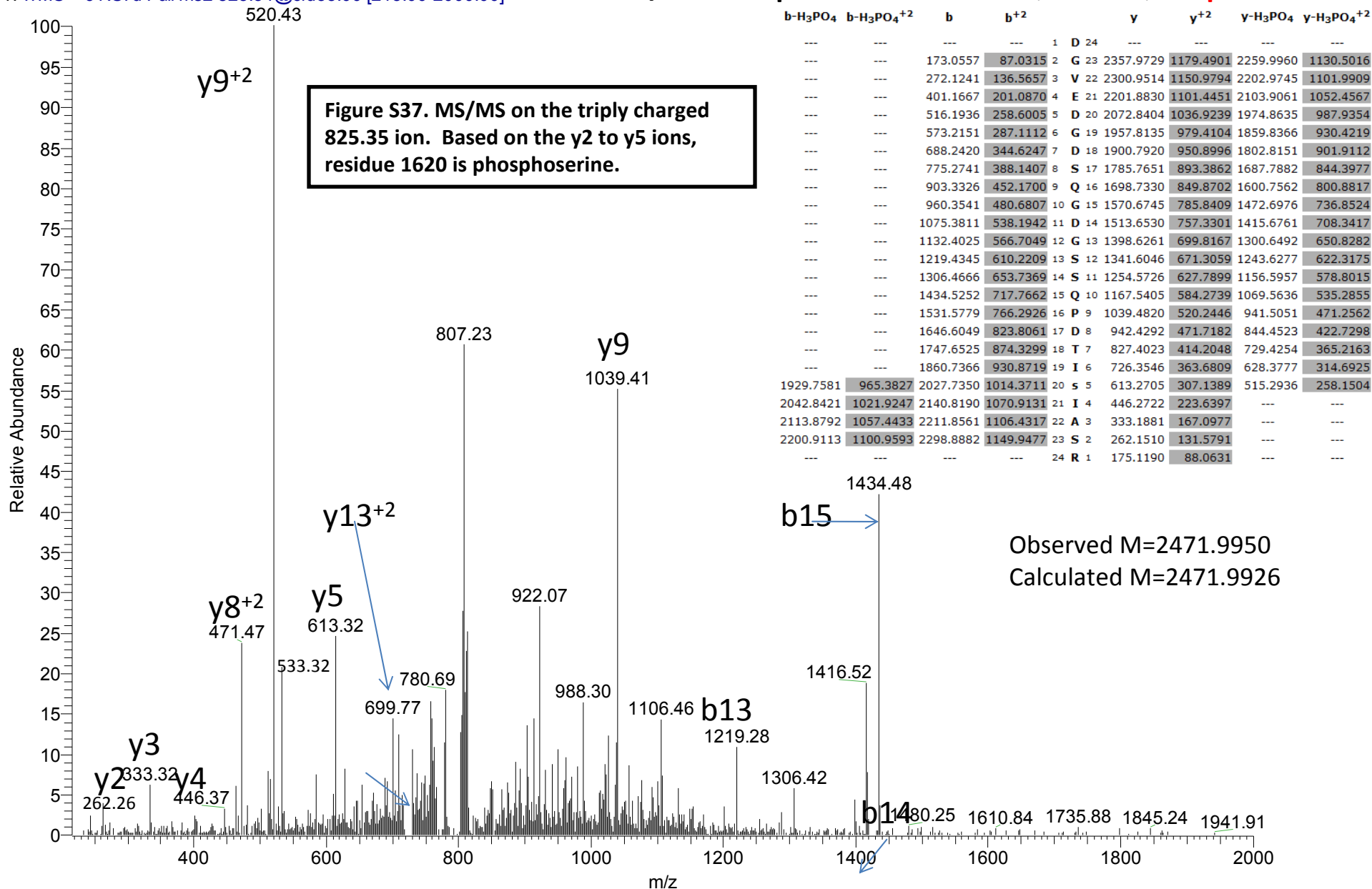
MS/MS of Peptide R.DGVEDGDSQGDSQPDP**T**ISIASR.T



pEAK Rapid Cells

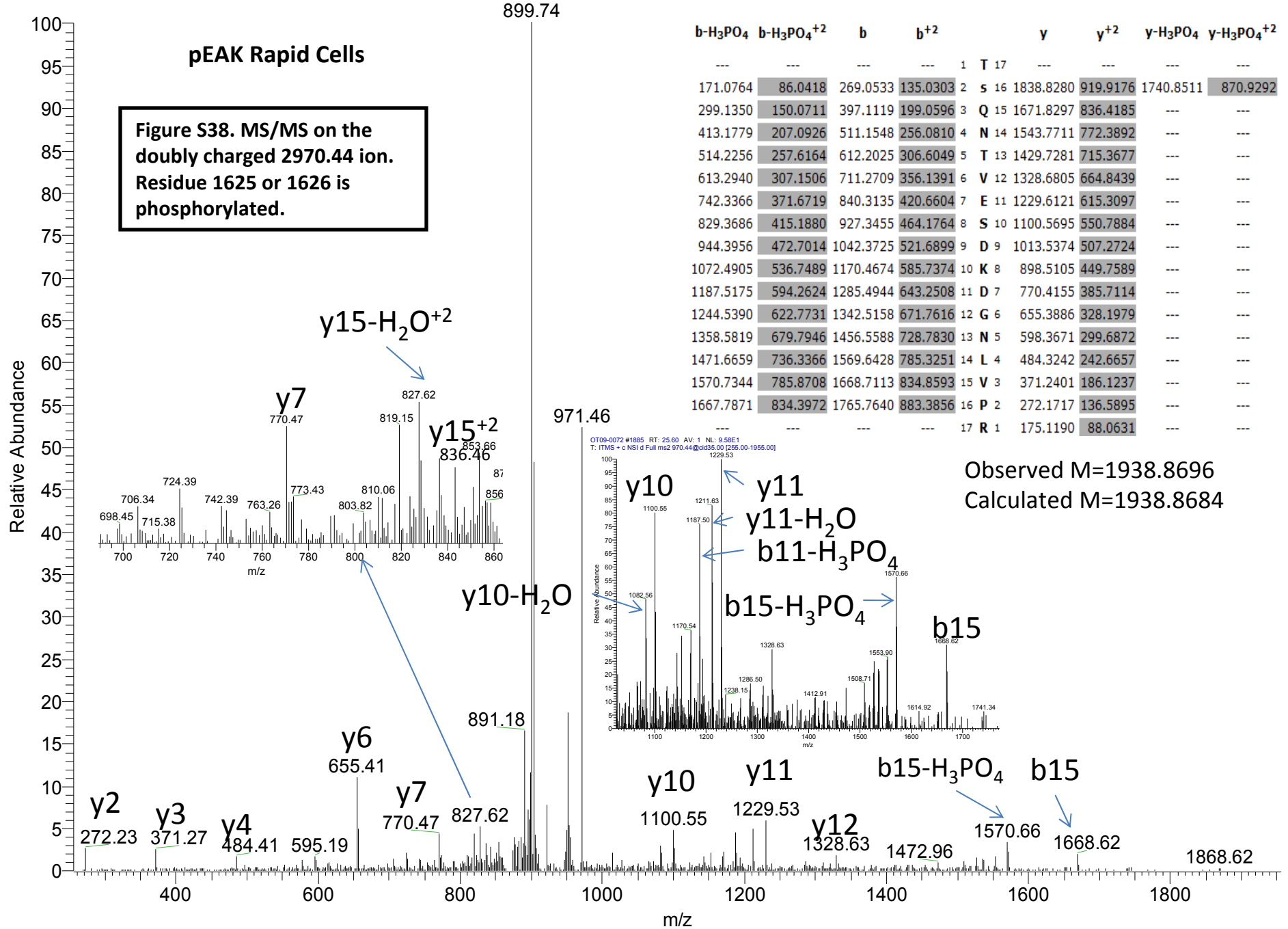
OT09-0060 #2098 RT: 28.21 AV: 1 NL: 8.61E2
 T: ITMS + c NSI d Full ms2 825.34@cid35.00 [215.00-2000.00]

MS/MS of Peptide R.DGVEDGDSQGDSQPDTI**p**SIASR.T



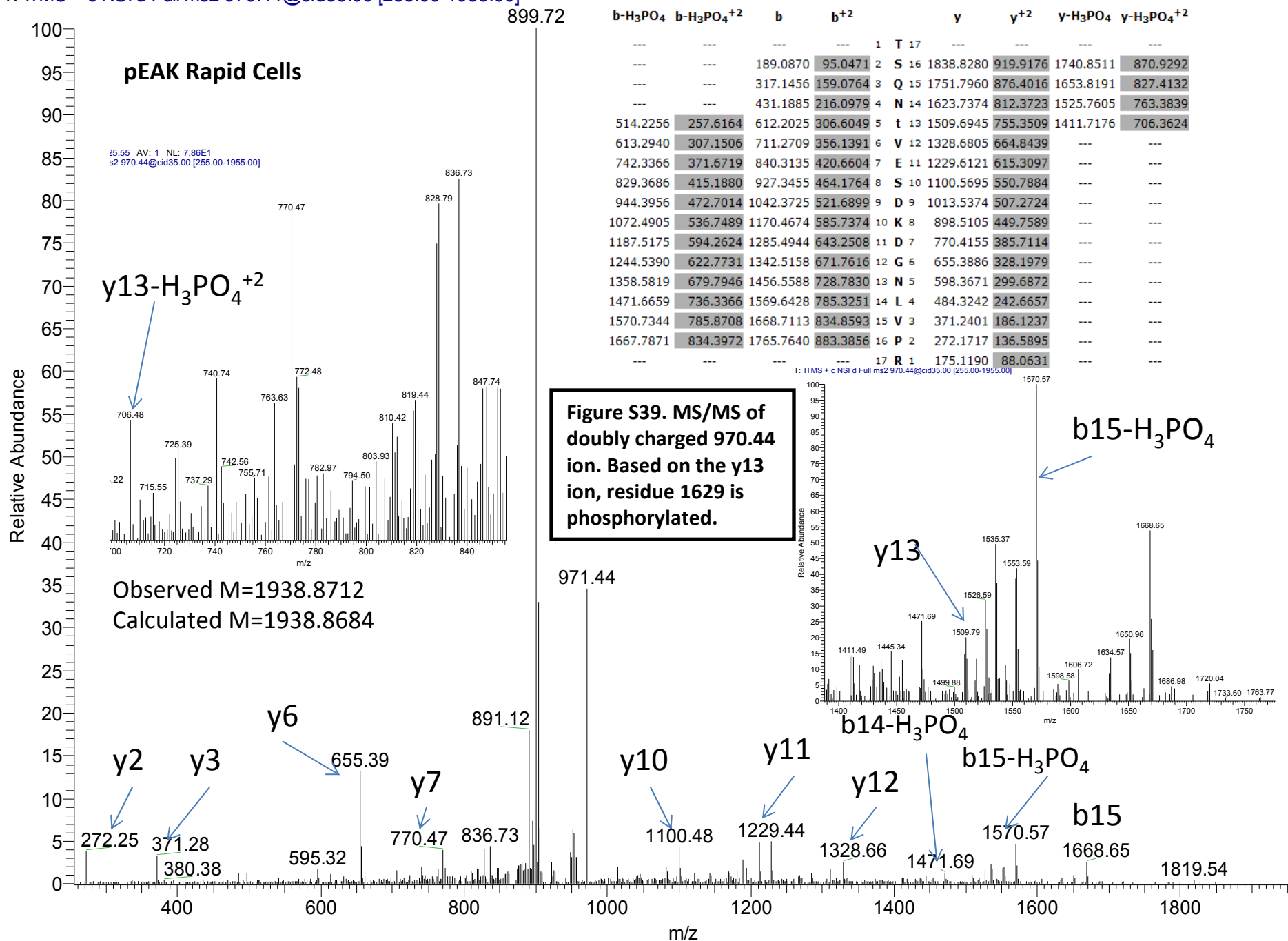
OT09-0072 #1885 RT: 25.60 AV: 1 NL: 1.62E3
 T: ITMS + c NSI d Full ms2 970.44@cid35.00 [255.00-1955.00]

MS/MS of Peptide R.pTpSQNTVESDKDGNLVPR.W



OT09-0068 #1881 RT: 25.55 AV: 1 NL: 1.81E3
 T: ITMS + c NSI d Full ms2 970.44@cid35.00 [255.00-1955.00]

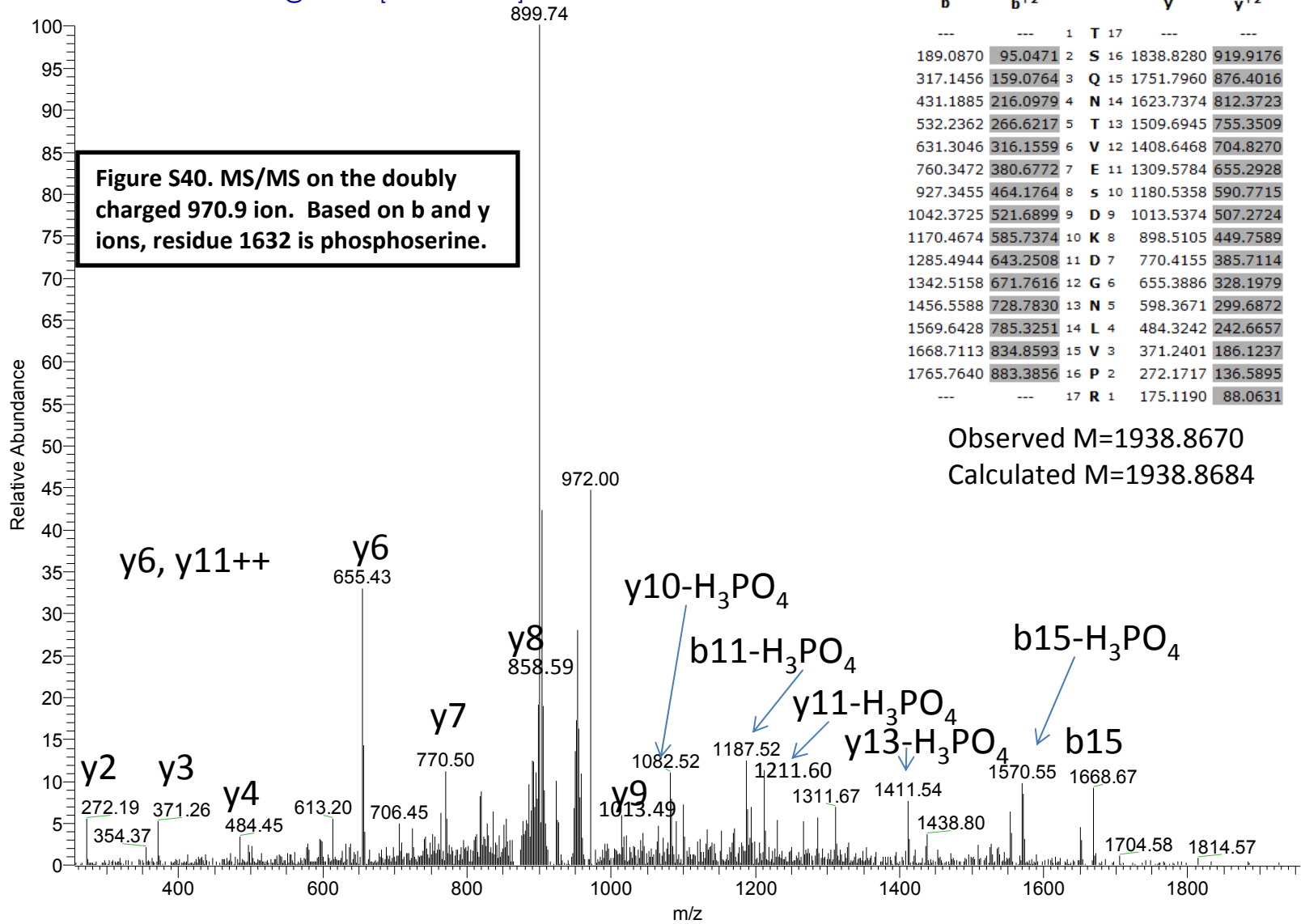
MS/MS of Peptide R.TSQNpTVESDKDGNLVPR.W



pEAK Rapid Cells

OT09-0076 #1922 RT: 25.28 AV: 1 NL: 1.05E3
 T: ITMS + c NSI d Full ms2 970.94@cid35.00 [255.00-1955.00]

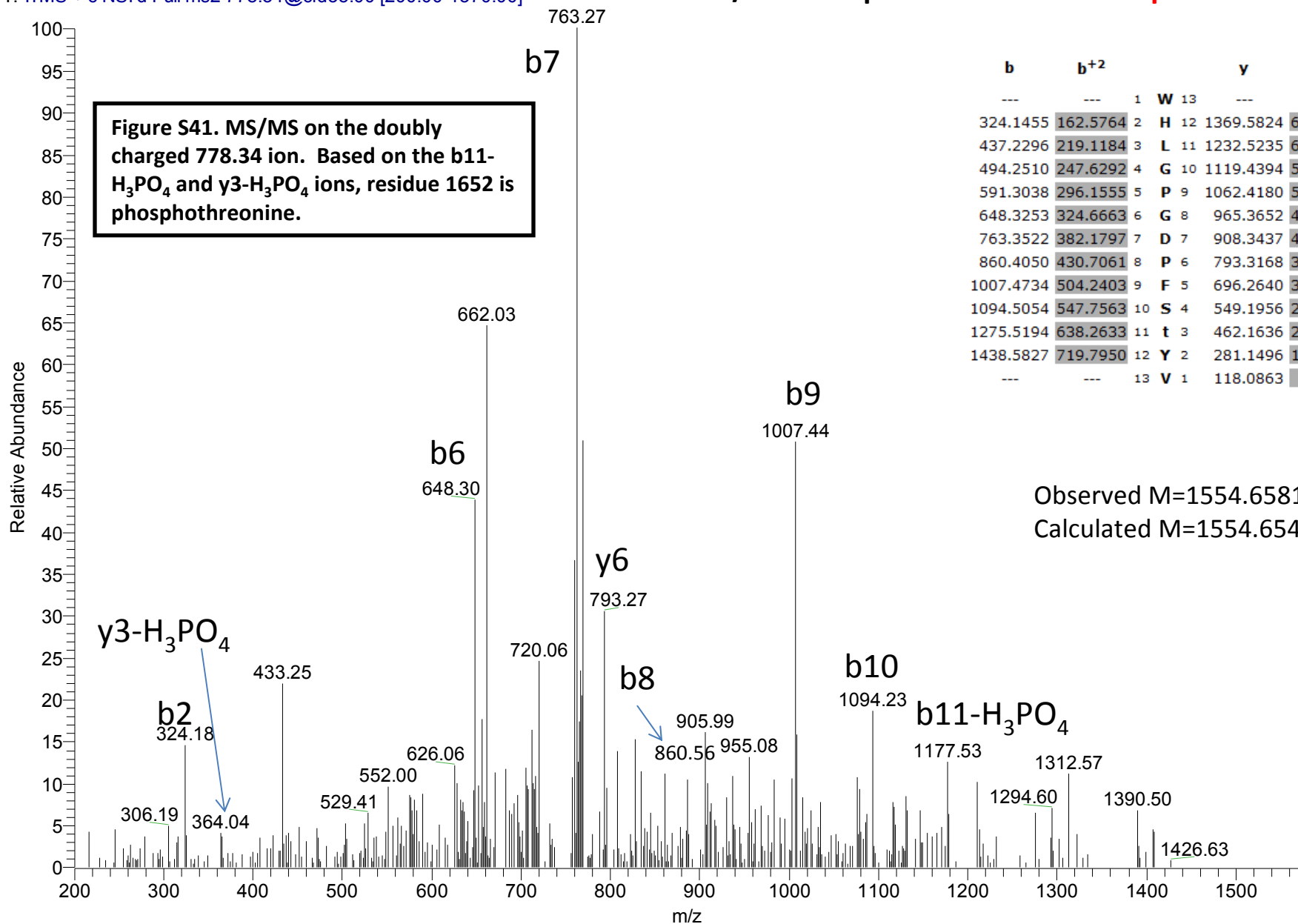
MS/MS of Peptide R.TSQNTVEpSDKDGNLVPR.W



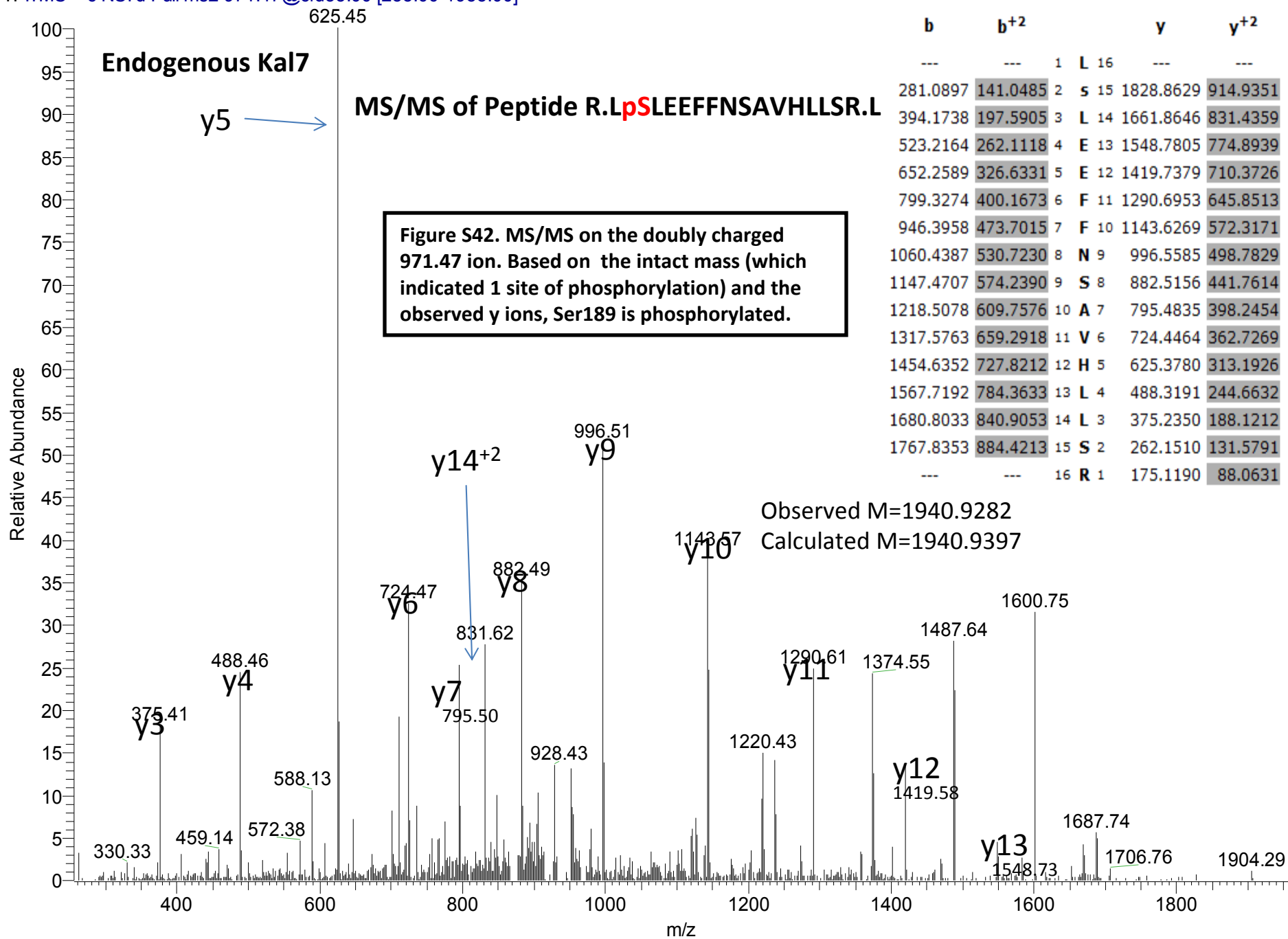
pEAK Rapid Cells

OT09-0068 #3200 RT: 42.11 AV: 1 NL: 1.02E2
 T: ITMS + c NSI d Full ms2 778.34@cid35.00 [200.00-1570.00]

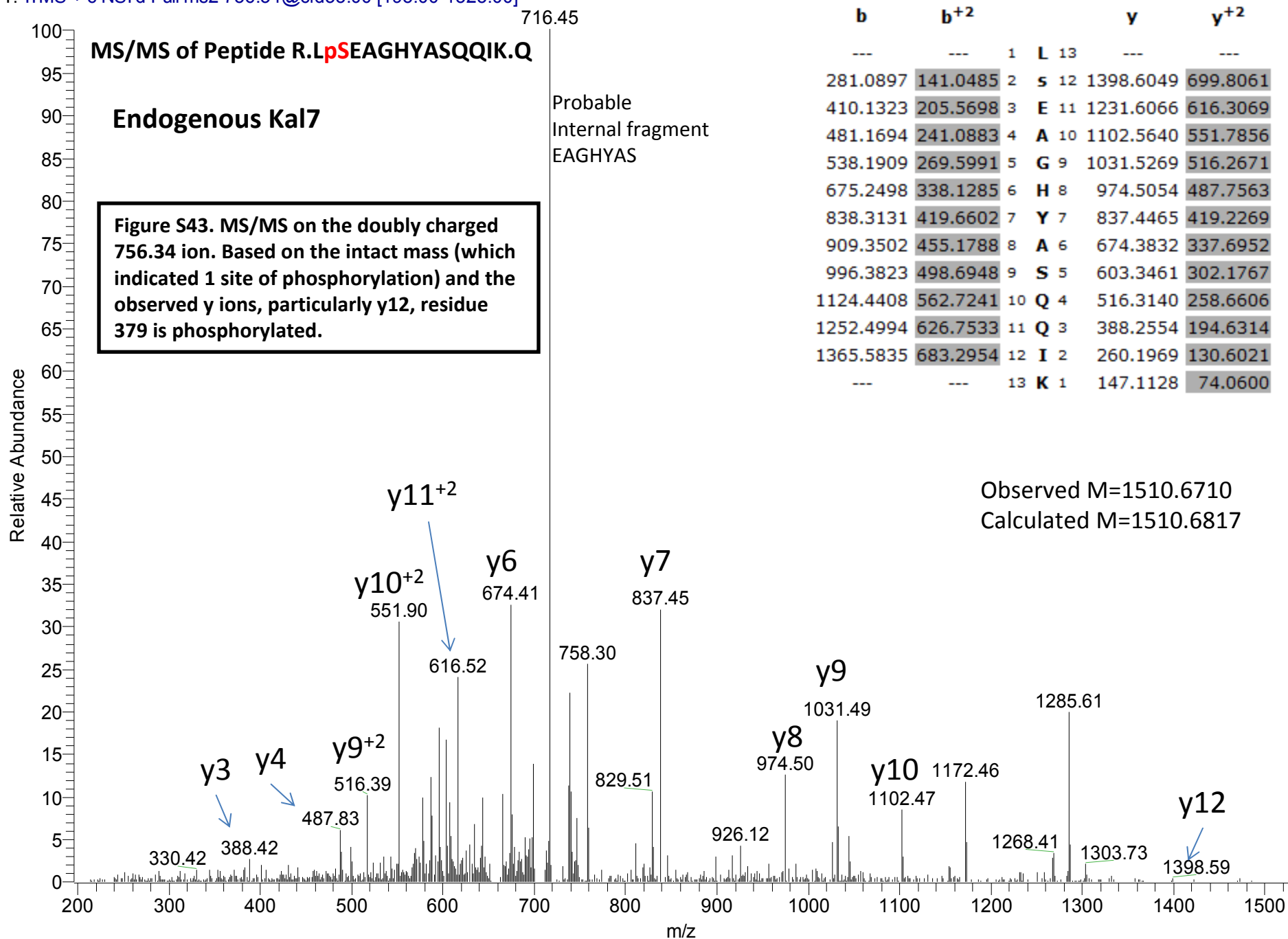
MS/MS of Peptide R.WHLGPGDPFS**pTYV**.-



OT09-4225 #5102 RT: 58.54 AV: 1 NL: 5.55E3
 T: ITMS + c NSI d Full ms2 971.47@cid35.00 [255.00-1955.00]

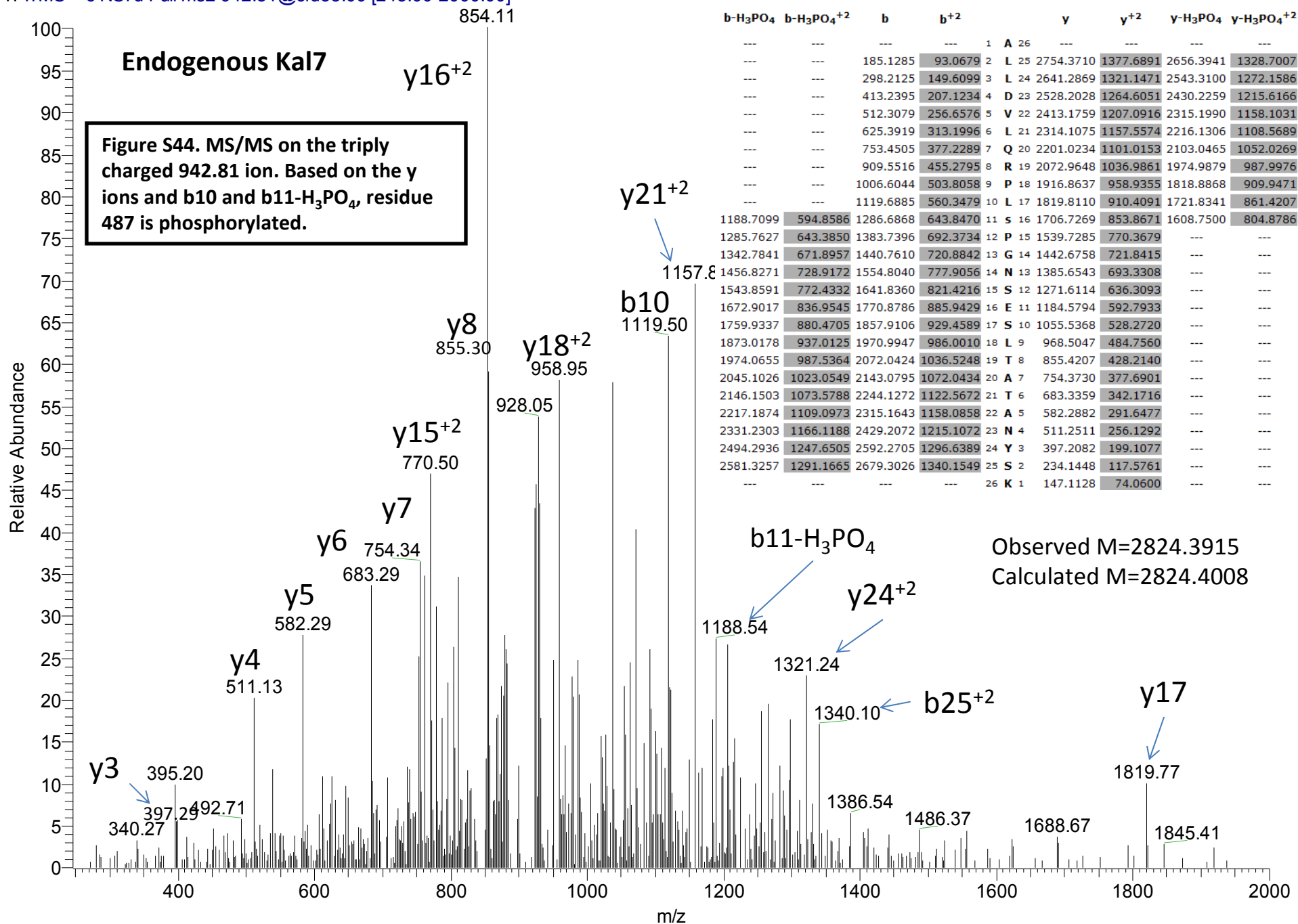


OT09-4225 #2541 RT: 33.88 AV: 1 NL: 2.11E3
 T: ITMS + c NSI d Full ms2 756.34@cid35.00 [195.00-1525.00]



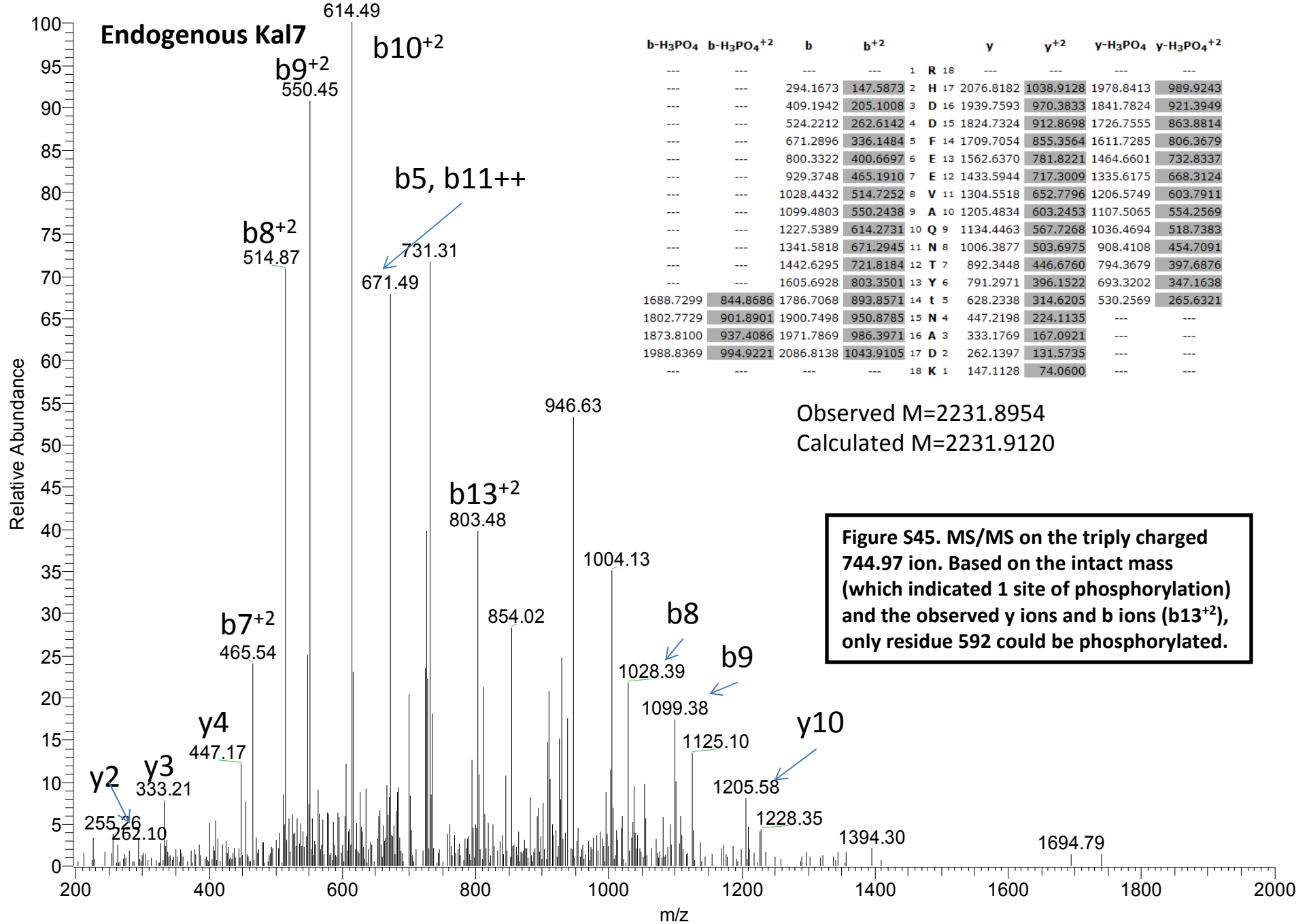
OT09-3336 #3319 RT: 47.01 AV: 1 NL: 8.07E1
 T: ITMS + c NSI d Full ms2 942.81@cid35.00 [245.00-2000.00]

MS/MS of Peptide K.ALLDVLQRPLpSPGNSESLTATANYSK.A



OT09-3336 #2575 RT: 37.05 AV: 1 NL: 1.34E2
 T: ITMS + c NSI d Full ms2 744.97@cid35.00 [195.00-2000.00]

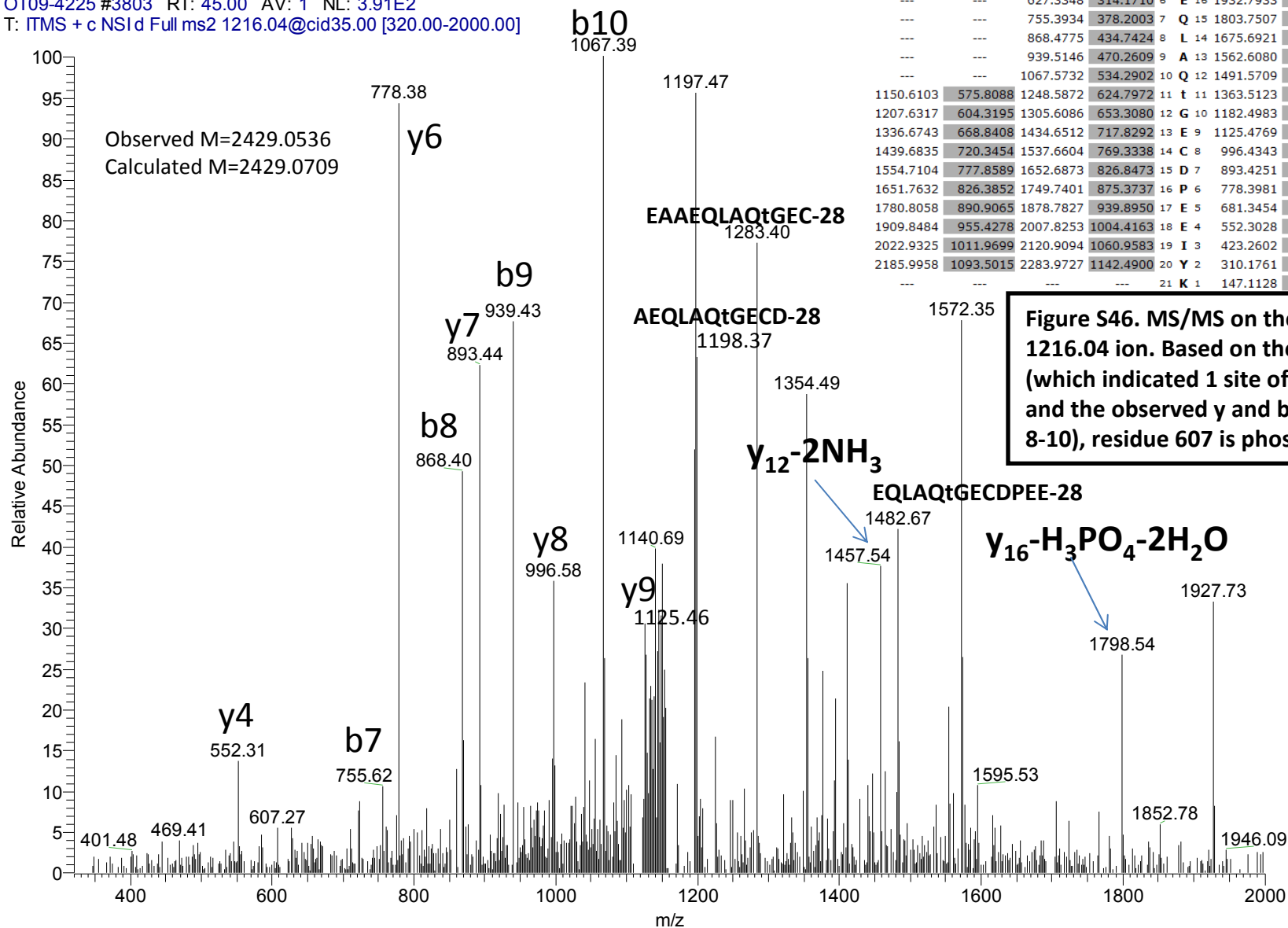
MS/MS of Peptide K.RHDDFEEVAQNTYpTNADK.L



Endogenous Kal7

MS/MS of Peptide K.LLEAAEQLAQpTGECDPEEIYK.A

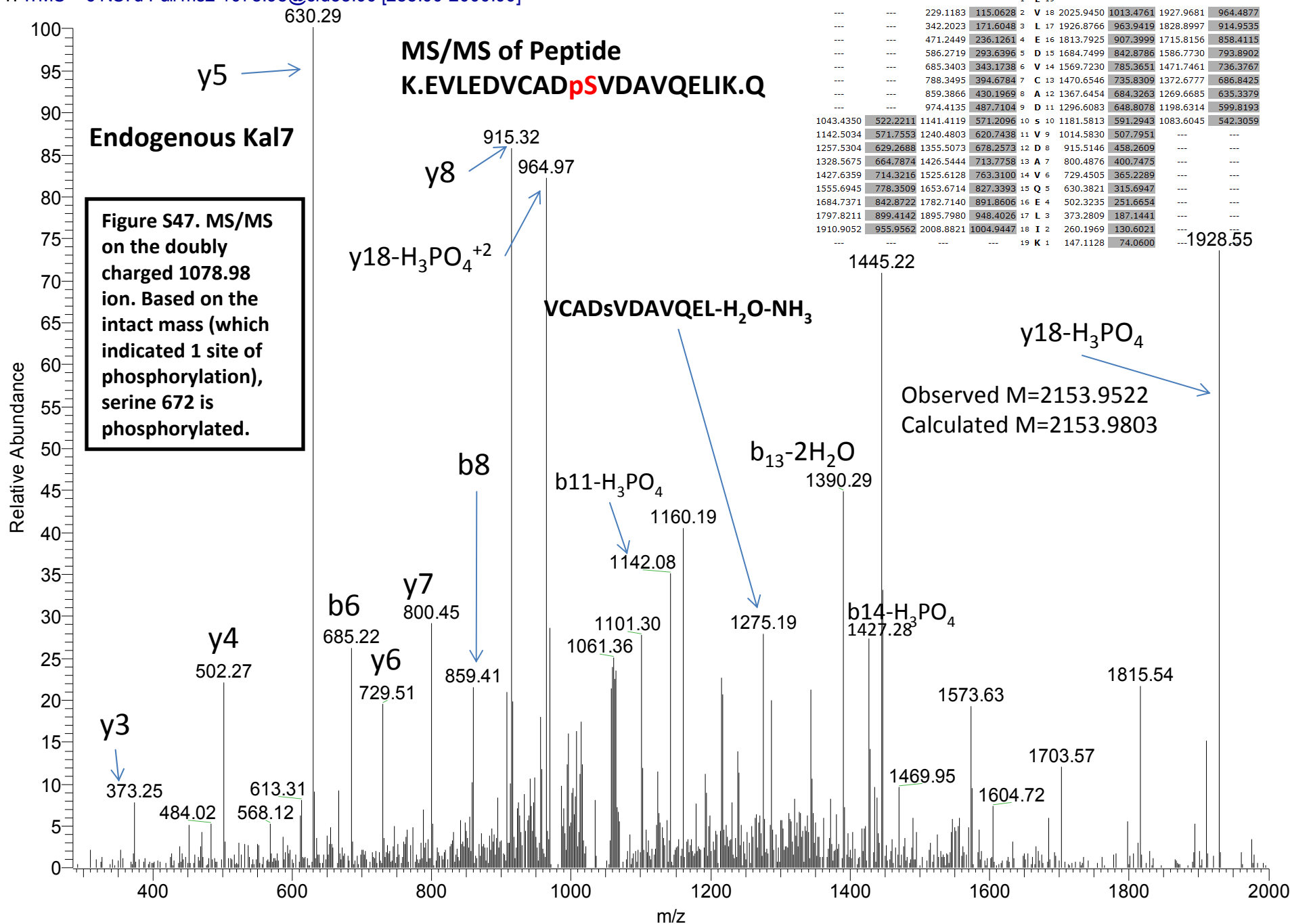
OT09-4225 #3803 RT: 45.00 AV: 1 NL: 3.91E2
 T: ITMS + c NSI d Full ms2 1216.04@cid35.00 [320.00-2000.00]



b-H ₃ PO ₄	b-H ₃ PO ₄ ⁺²	b	b ⁺²		y	y ⁺²	y-H ₃ PO ₄	y-H ₃ PO ₄ ⁺²
---	---	---	---	1	L	21	---	---
---	---	227.1754	114.0913	2	L	20	2316.9942	1159.0007
---	---	356.2180	178.6126	3	E	19	2203.9101	1102.4587
---	---	427.2551	214.1312	4	A	18	2074.8675	1037.9374
---	---	498.2922	249.6498	5	A	17	2003.8304	1002.4188
---	---	627.3348	314.1710	6	E	16	1932.7933	966.9003
---	---	755.3934	378.2003	7	Q	15	1803.7507	902.3790
---	---	868.4775	434.7424	8	L	14	1675.6921	838.3497
---	---	939.5146	470.2609	9	A	13	1562.6080	781.8077
---	---	1067.5732	534.2902	10	Q	12	1491.5709	746.2891
1150.6103	575.8088	1248.5872	624.7972	11	t	11	1363.5123	682.2598
1207.6317	604.3195	1305.6086	653.3080	12	G	10	1182.4983	591.7528
1336.6743	668.8408	1434.6512	717.8292	13	E	9	1125.4769	563.2421
1439.6835	720.3454	1537.6604	769.3338	14	C	8	996.4343	498.7208
1554.7104	777.8589	1652.6873	826.8473	15	D	7	893.4251	447.2162
1651.7632	826.3852	1749.7401	875.3737	16	P	6	778.3981	389.7027
1780.8058	890.9065	1878.7827	939.8950	17	E	5	681.3454	341.1763
1909.8484	955.4278	2007.8253	1004.4163	18	E	4	552.3028	276.6550
2022.9325	1011.9699	2120.9094	1060.9583	19	I	3	423.2602	212.1337
2185.9958	1093.5015	2283.9727	1142.4900	20	Y	2	310.1761	155.5917
---	---	---	---	21	K	1	147.1128	74.0600

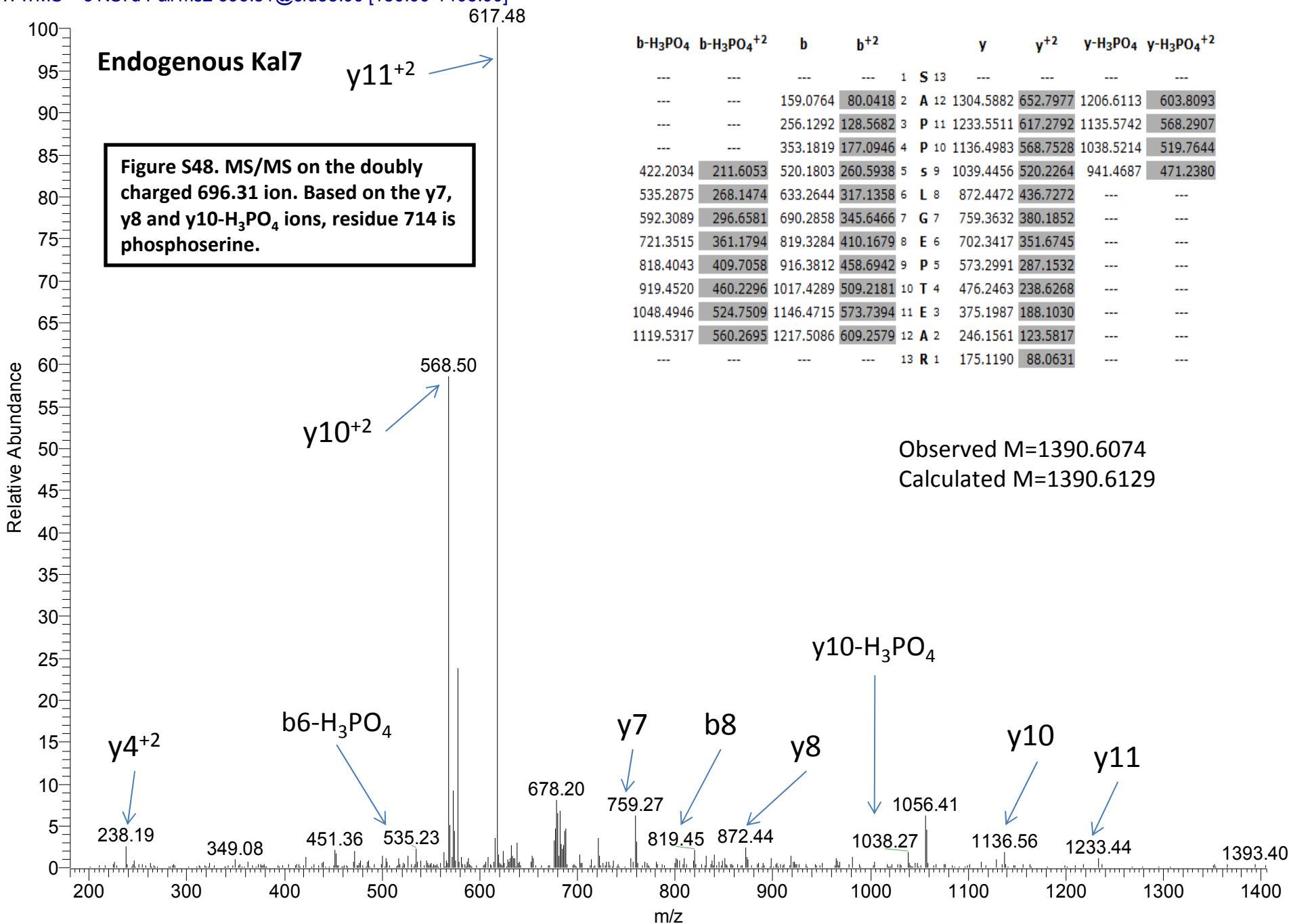
Figure S46. MS/MS on the doubly charged 1216.04 ion. Based on the intact mass (which indicated 1 site of phosphorylation) and the observed y and b ions, (y4-9 and b 8-10), residue 607 is phosphorylated.

OT09-3353 #4247 RT: 51.48 AV: 1 NL: 5.60E2
 T: ITMS + c NSI d Full ms2 1078.98@cid35.00 [285.00-2000.00]



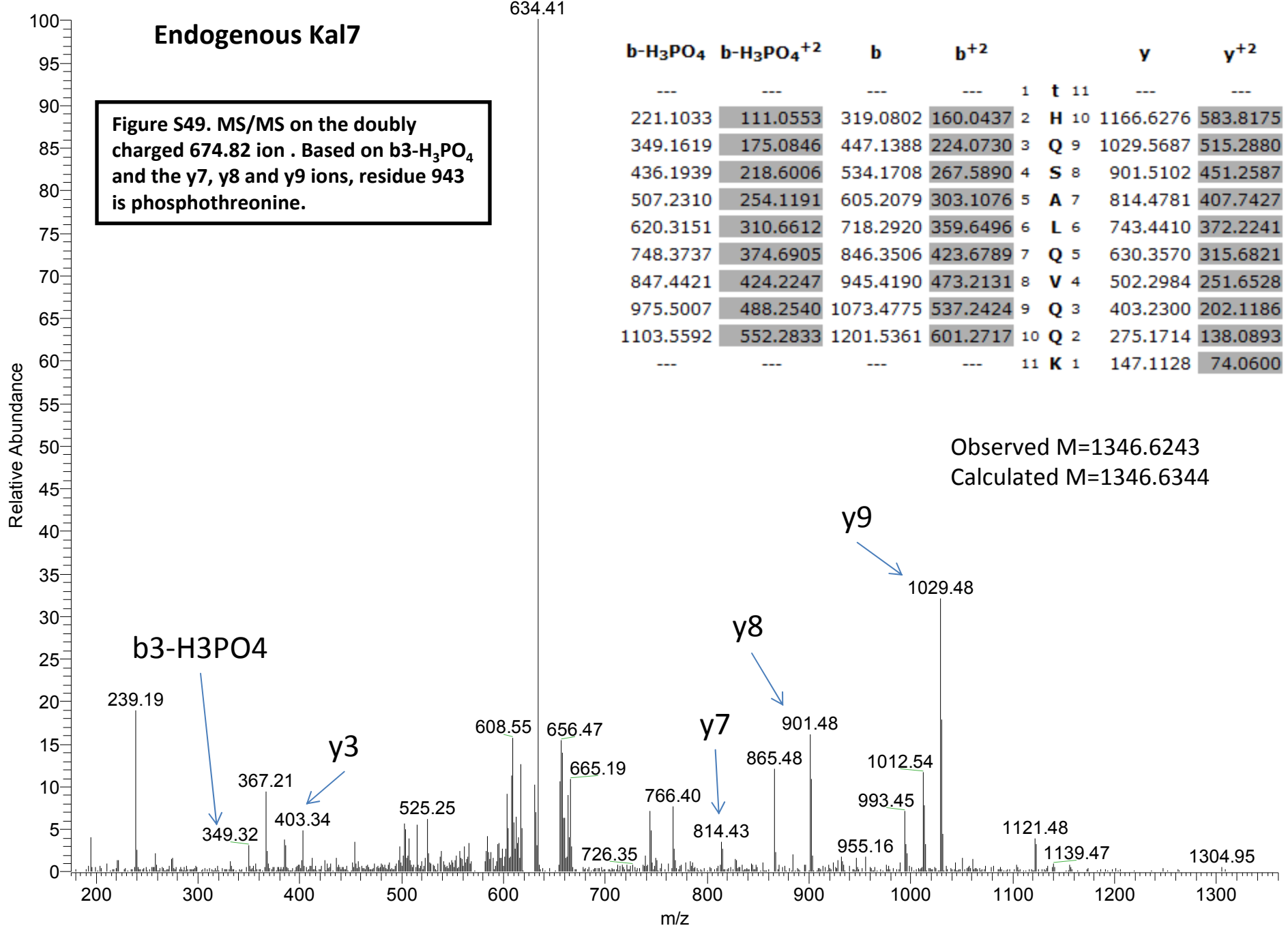
OT09-3336 #2471 RT: 35.63 AV: 1 NL: 6.60E2
 T: ITMS + c NSI d Full ms2 696.31@cid35.00 [180.00-1405.00]

MS/MS of Peptide R.SAPPpSLGEPTEAR.D



OT09-4225 #2307 RT: 31.46 AV: 1 NL: 3.28E3
 T: ITMS + c NSI d Full ms2 674.82@cid35.00 [175.00-1360.00]

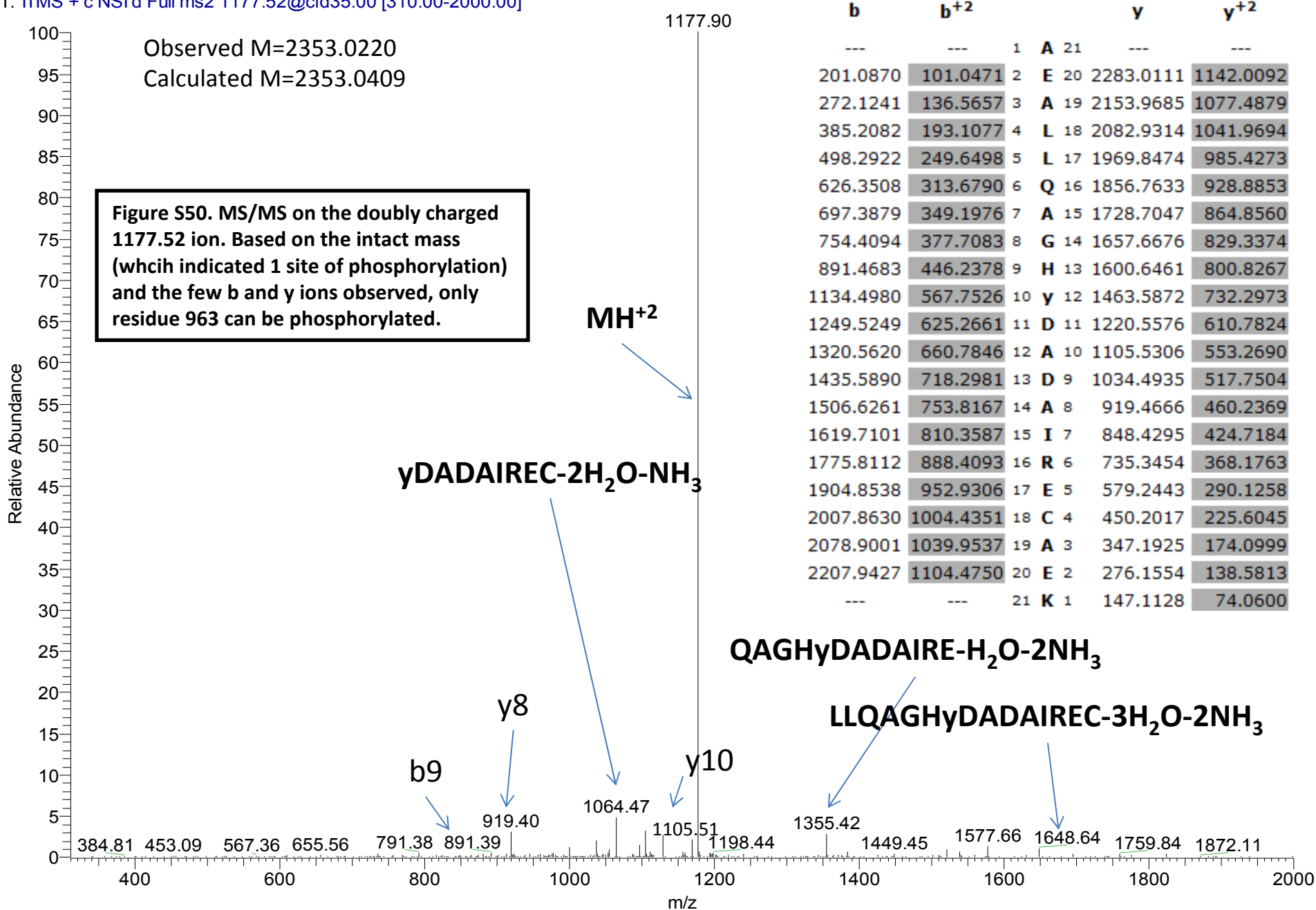
MS/MS of Peptide K.pTHQSALQVQQK.A



Endogenous Kal7

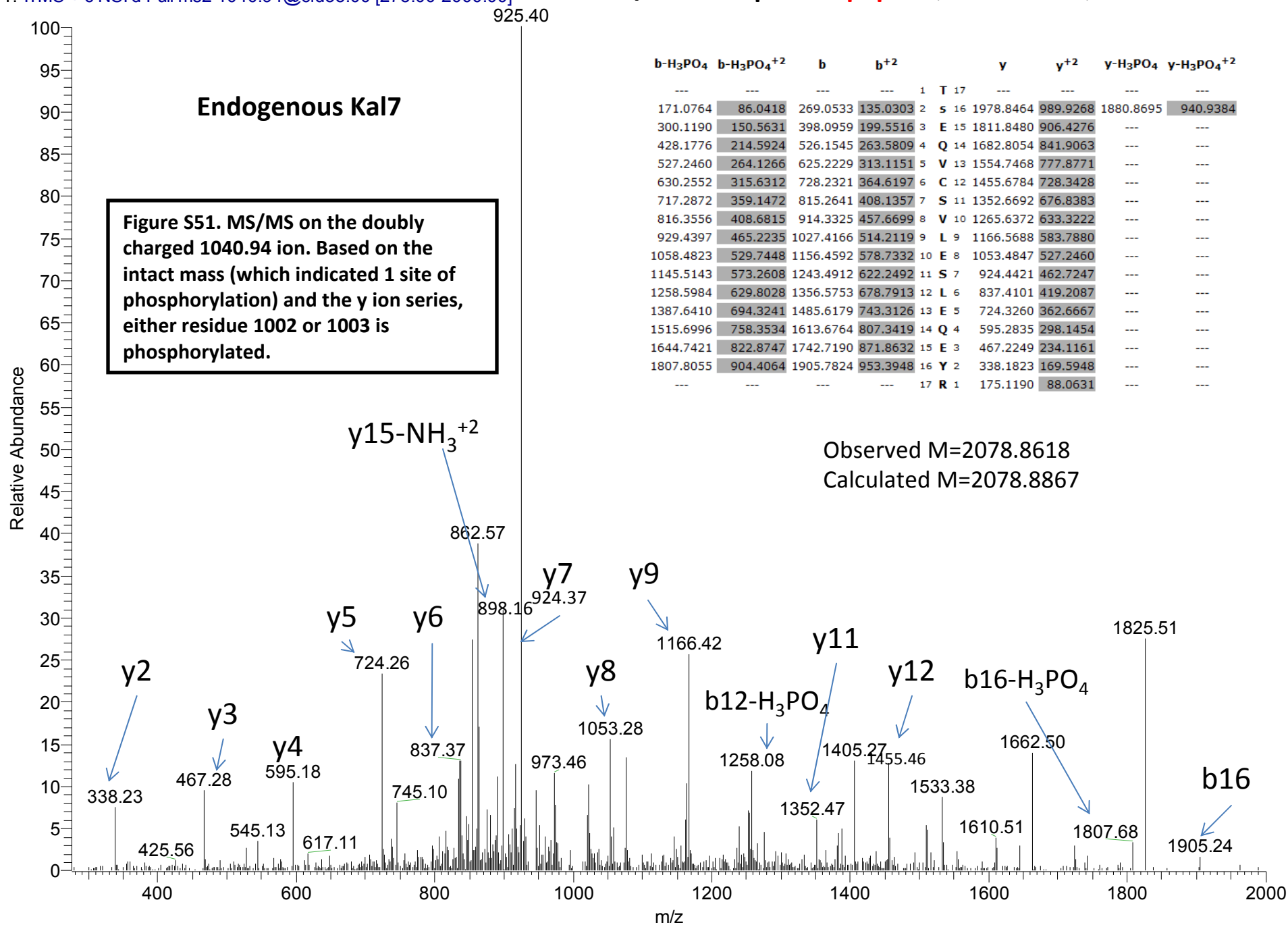
OT09-1008 #2731 RT: 34.32 AV: 1 NL: 1.97E4
 T: ITMS + c NSI d Full ms2 1177.52@cid35.00 [310.00-2000.00]

MS/MS of Peptide K.AEALLQAGH**p**YDADAIRECAEK.V

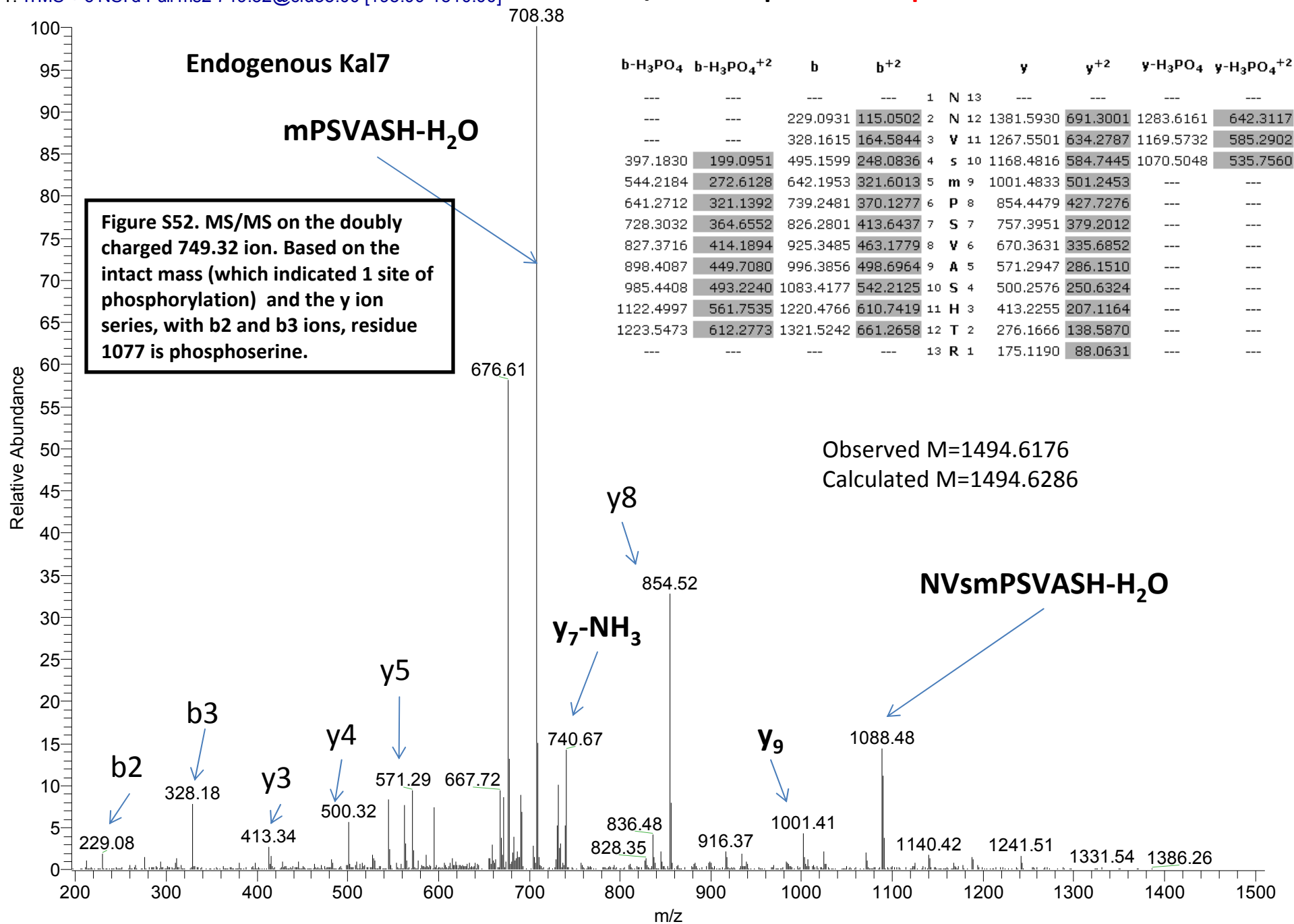


OT09-3353 #4271 RT: 51.78 AV: 1 NL: 1.53E3
 T: ITMS + c NSI d Full ms2 1040.94@cid35.00 [275.00-2000.00]

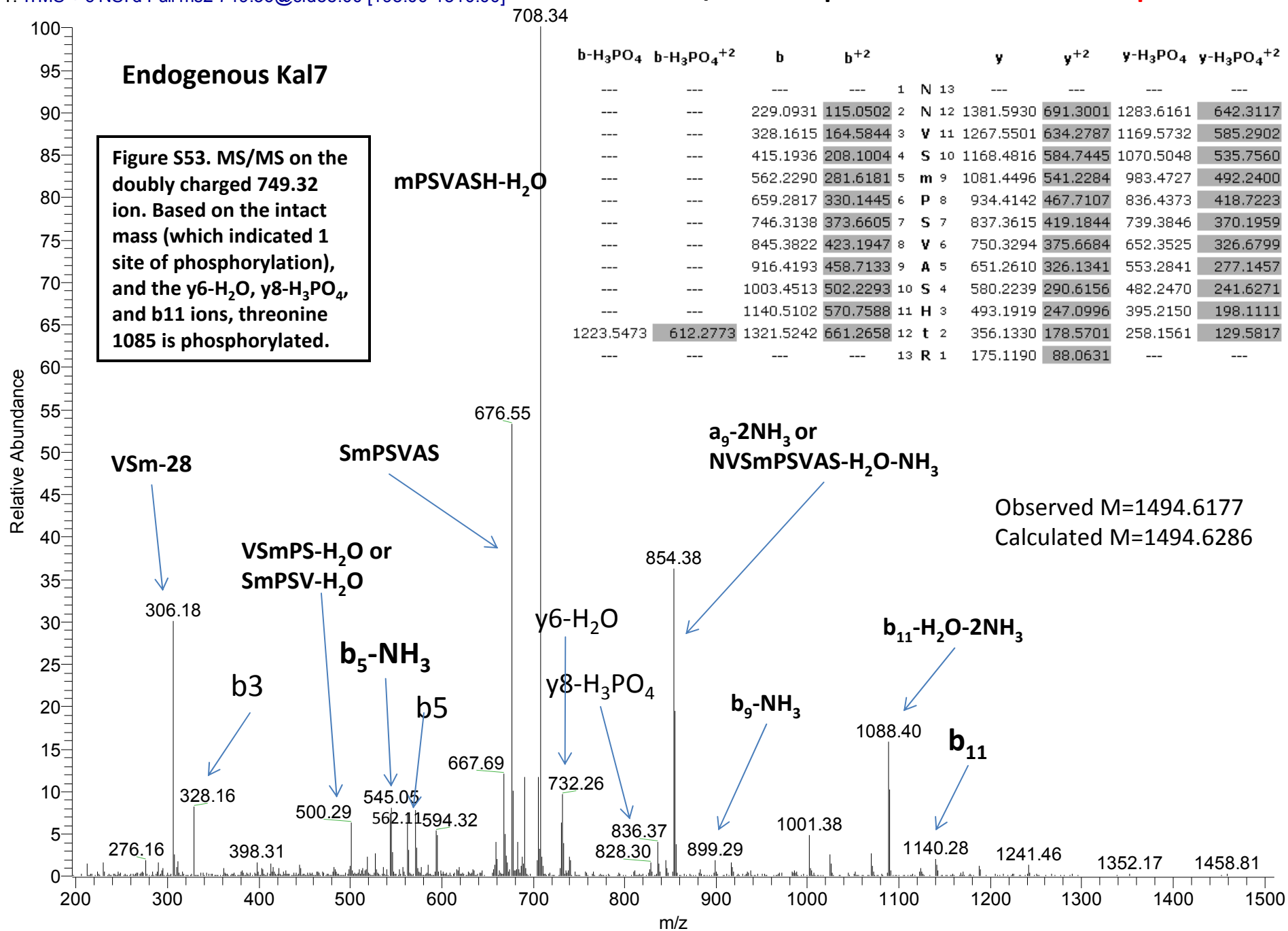
MS/MS of Peptide K.pTpSEQVCSVLESLEQEYR.R



MS/MS of Peptide R.NNVpSoxMPSVASHTR.G

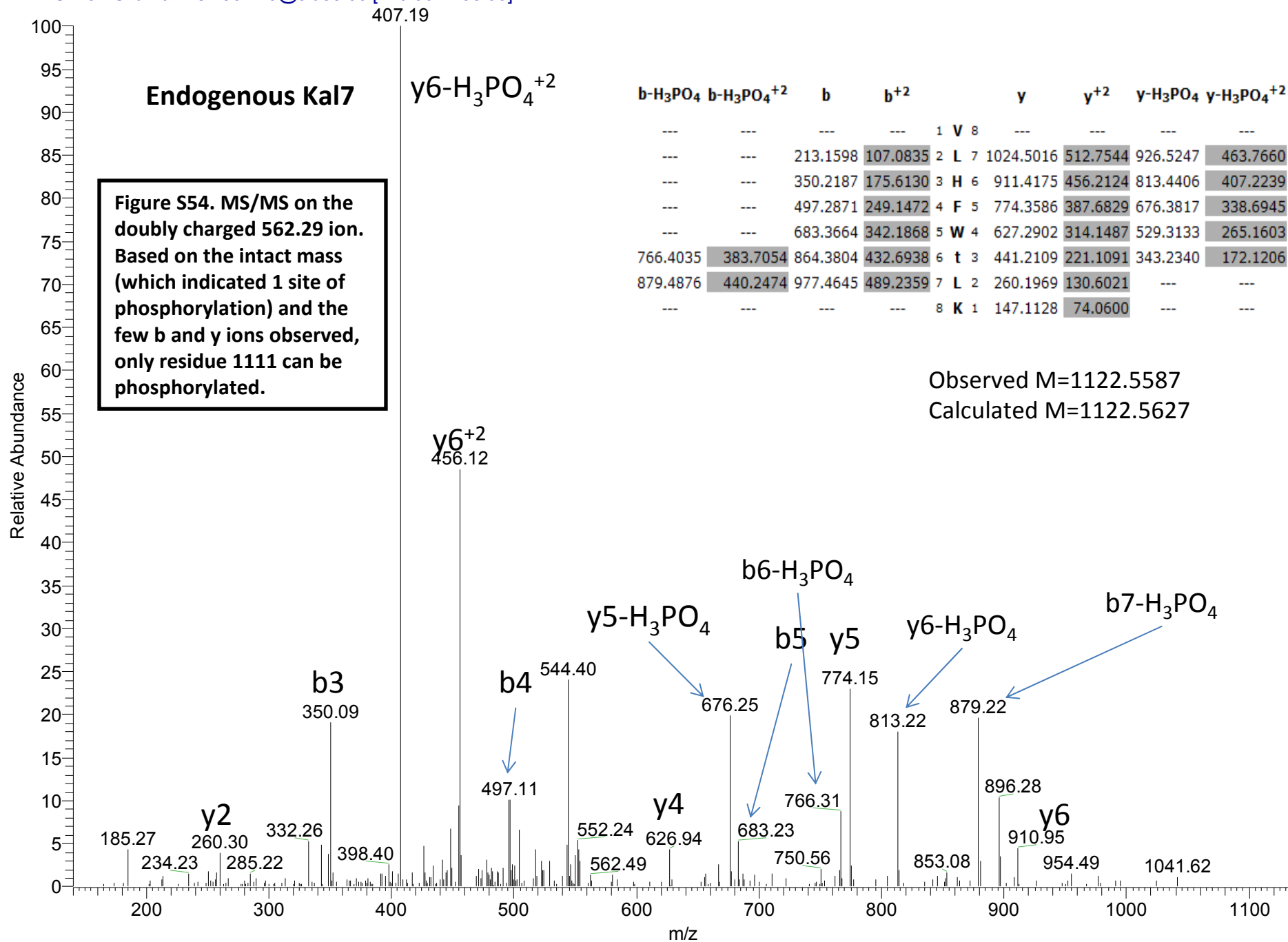


MS/MS of Peptide R.NNVSoxMPSVASHpTR.G



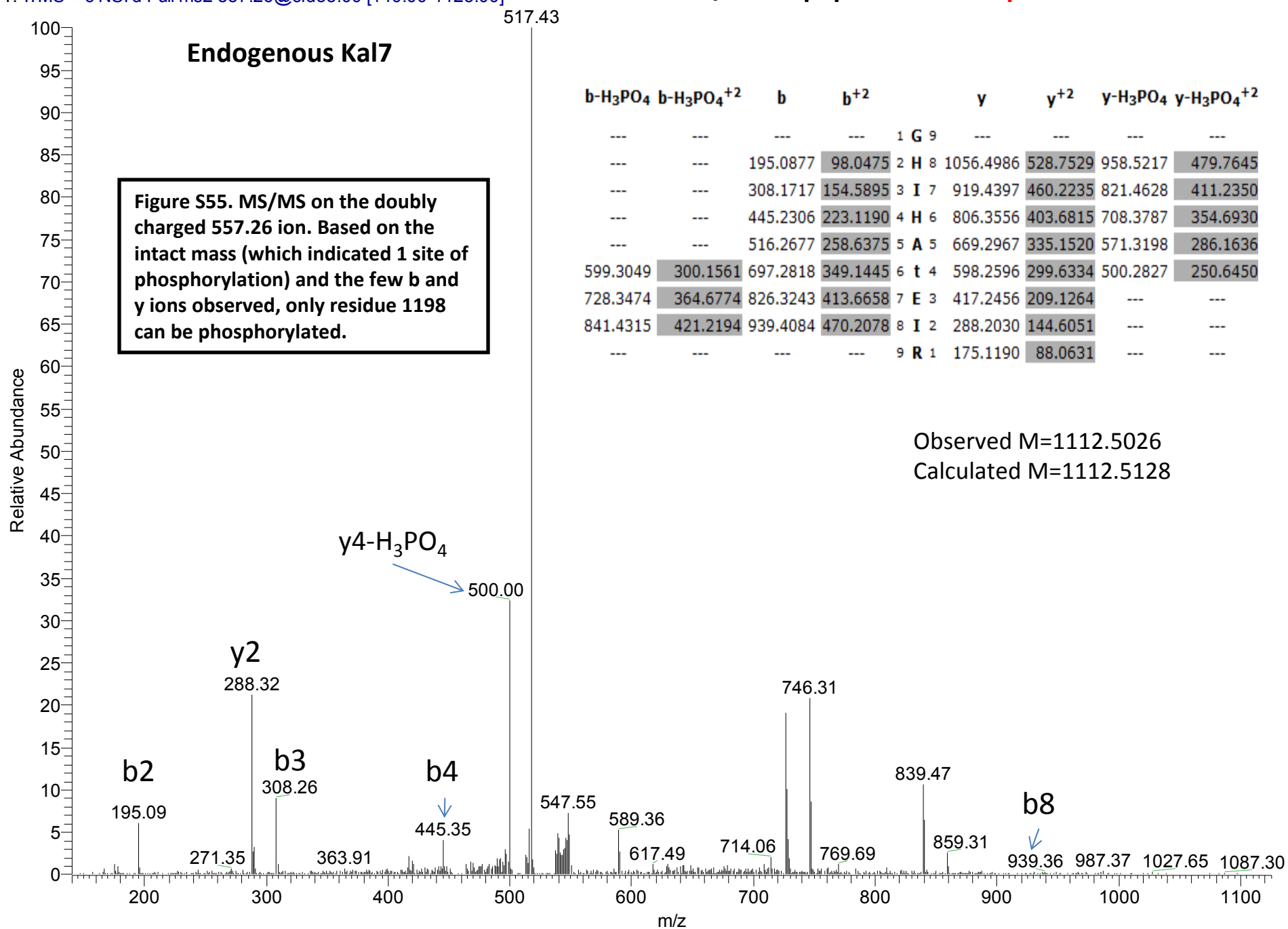
OT09-3336 #3359 RT: 47.56 AV: 1 NL: 2.45E2
 T: ITMS + c NSI d Full ms2 562.29@cid35.00 [140.00-1135.00]

MS/MS of peptide R.VLHFWpTLK.K



OT09-4228 #2284 RT: 31.38 AV: 1 NL: 2.76E3
 T: ITMS + c NSI d Full ms2 557.26@cid35.00 [140.00-1125.00]

MS/MS of peptide K.GHIHA_pTEIR.K



MS/MS of peptide R.DFpSLR.M

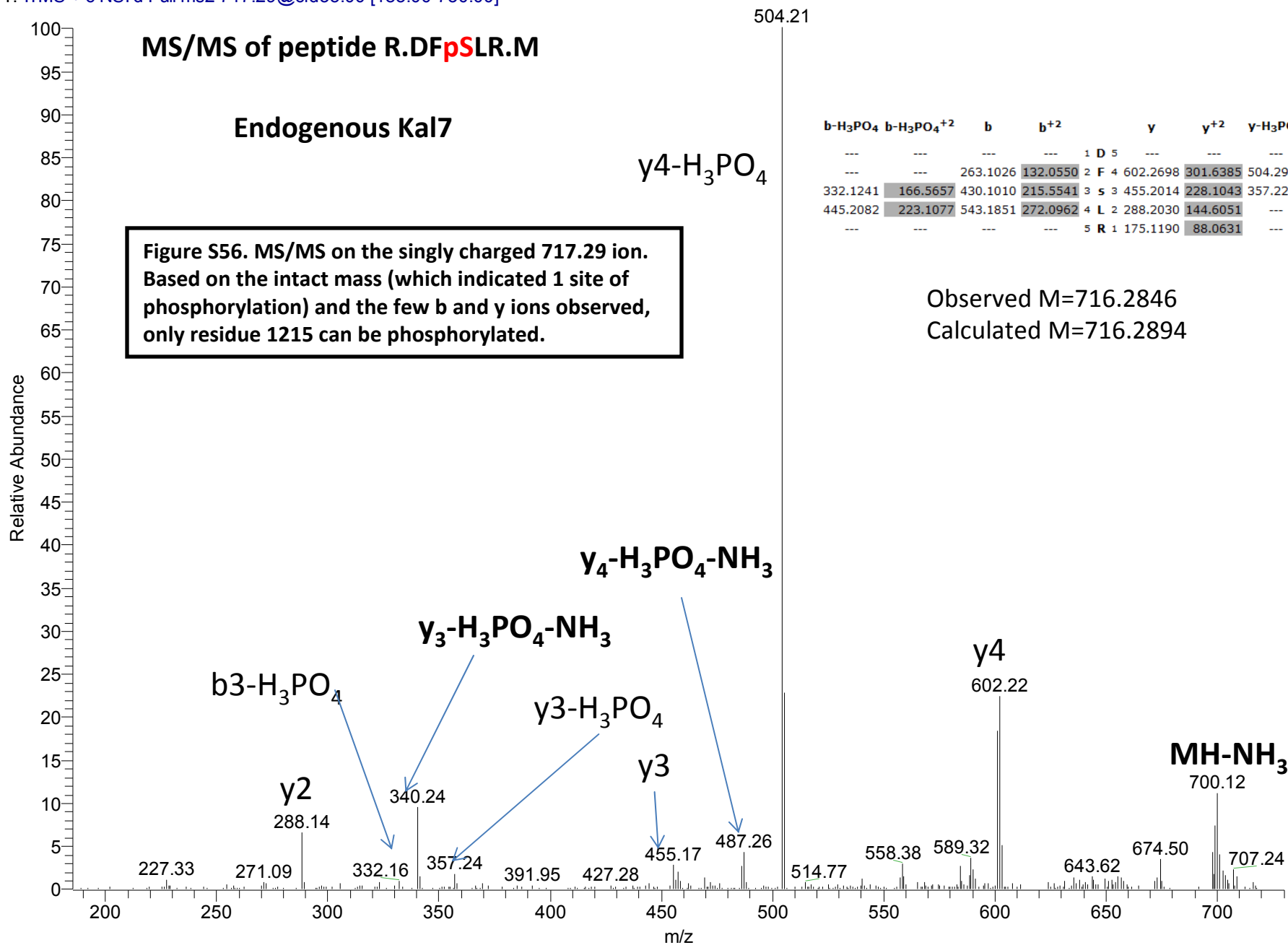
Endogenous Kal7

$\gamma_4\text{-H}_3\text{PO}_4$

Figure S56. MS/MS on the singly charged 717.29 ion. Based on the intact mass (which indicated 1 site of phosphorylation) and the few b and y ions observed, only residue 1215 can be phosphorylated.

b-H ₃ PO ₄	b-H ₃ PO ₄ ⁺²	b	b ⁺²	y	y ⁺²	$\gamma\text{-H}_3\text{PO}_4$	$\gamma\text{-H}_3\text{PO}_4^{+2}$
---	---	---	---	1 D 5	---	---	---
---	---	263.1026	132.0550	2 F 4	602.2698	301.6385	504.2929
332.1241	166.5657	430.1010	215.5541	3 S 3	455.2014	228.1043	357.2245
445.2082	223.1077	543.1851	272.0962	4 L 2	288.2030	144.6051	---
---	---	---	---	5 R 1	175.1190	88.0631	---

Observed M=716.2846
 Calculated M=716.2894

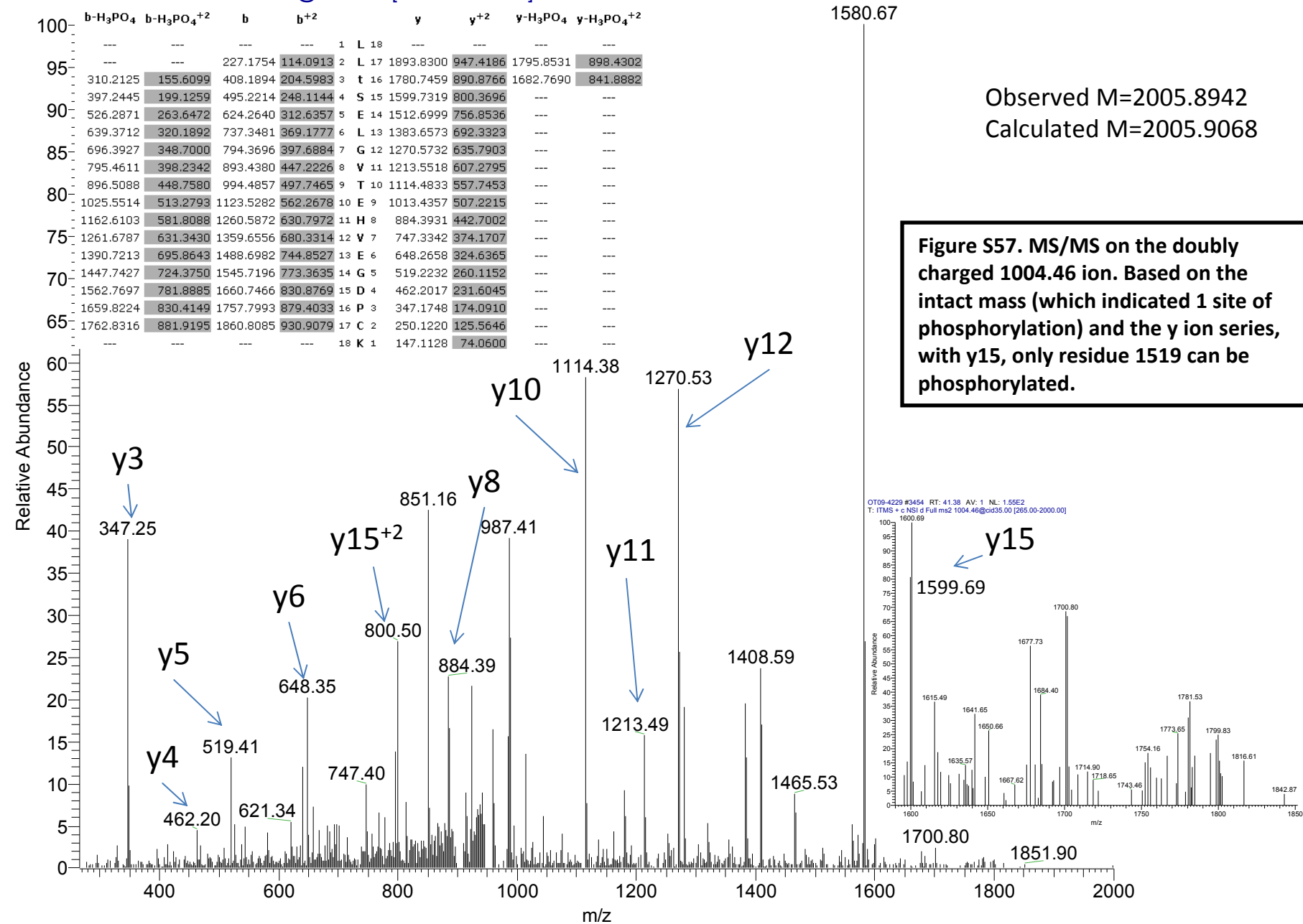


Endogenous Kal7

MS/MS of peptide LLpTSELGVTEHVEGDPCK

OT09-4229 #3454 RT: 41.38 AV: 1 NL: 4.61E3

T: ITMS + c NSI d Full ms2 1004.46@cid35.00 [265.00-2000.00]



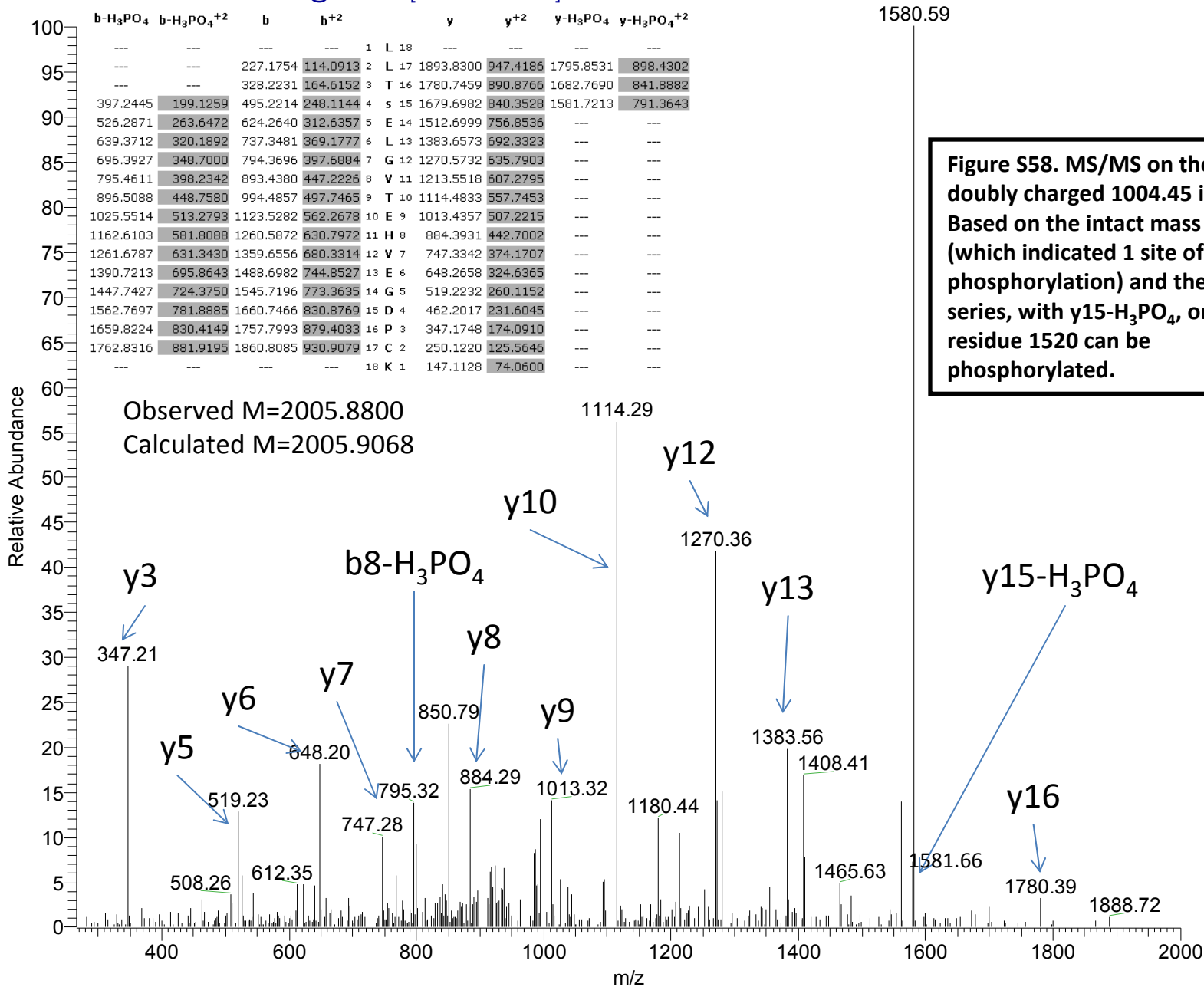
Observed M=2005.8942
Calculated M=2005.9068

Figure S57. MS/MS on the doubly charged 1004.46 ion. Based on the intact mass (which indicated 1 site of phosphorylation) and the y ion series, with y15, only residue 1519 can be phosphorylated.

Endogenous Kal7

MS/MS of peptide LLT^pSELGVTEHVEGD^pPCK

OT09-3352 #2895 RT: 41.33 AV: 1 NL: 2.11E2
 T: ITMS + c NSI d Full ms2 1004.45@cid35.00 [265.00-2000.00]

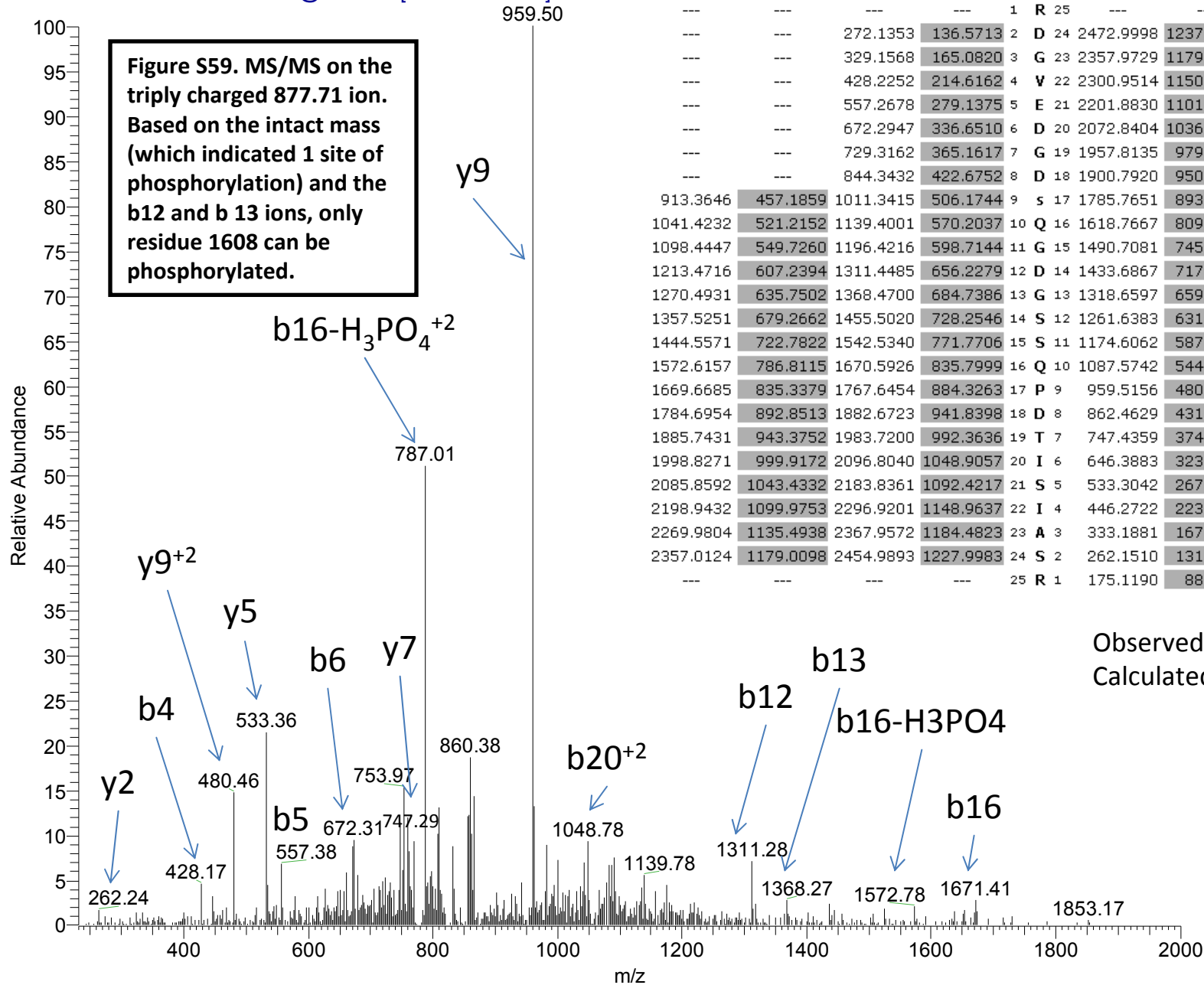


Endogenous Kal7

MS/MS of peptide RDGVEDGDpSQGDGSSQPDTISIASR

OT09-0956 #2262 RT: 30.95 AV: 1 NL: 1.11E3
 T: ITMS + c NSI d Full ms2 877.71@cid35.00 [230.00-2000.00]

	b-H ₃ PO ₄	b-H ₃ PO ₄ +2	b	b+2		y	y+2	y-H ₃ PO ₄	y-H ₃ PO ₄ +2	
	---	---	---	---	1	R 25	---	---	---	
	---	---	272.1353	136.5713	2	D 24	2472.9998	1237.0036	2375.0229	1188.0151
	---	---	329.1568	165.0820	3	G 23	2357.9729	1179.4901	2259.9960	1130.5016
	---	---	428.2252	214.6162	4	V 22	2300.9514	1150.9794	2202.9745	1101.9909
	---	---	557.2678	279.1375	5	E 21	2201.8830	1101.4451	2103.9061	1052.4567
	---	---	672.2947	336.6510	6	D 20	2072.8404	1036.9239	1974.8635	987.9354
	---	---	729.3162	365.1617	7	G 19	1957.8135	979.4104	1859.8366	930.4219
	---	---	844.3432	422.6752	8	D 18	1900.7920	950.8996	1802.8151	901.9112
	913.3646	457.1859	1011.3415	506.1744	9	s 17	1785.7651	893.3862	1687.7882	844.3977
	1041.4232	521.2152	1139.4001	570.2037	10	Q 16	1618.7667	809.8870	---	---
	1098.4447	549.7260	1196.4216	598.7144	11	G 15	1490.7081	745.8577	---	---
	1213.4716	607.2394	1311.4485	656.2279	12	D 14	1433.6867	717.3470	---	---
	1270.4931	635.7502	1368.4700	684.7386	13	G 13	1318.6597	659.8335	---	---
	1357.5251	679.2662	1455.5020	728.2546	14	S 12	1261.6383	631.3228	---	---
	1444.5571	722.7822	1542.5340	771.7706	15	S 11	1174.6062	587.8068	---	---
	1572.6157	786.8115	1670.5926	835.7999	16	Q 10	1087.5742	544.2907	---	---
	1669.6685	835.3379	1767.6454	884.3263	17	P 9	959.5156	480.2615	---	---
	1784.6954	892.8513	1882.6723	941.8398	18	D 8	862.4629	431.7351	---	---
	1885.7431	943.3752	1983.7200	992.3636	19	T 7	747.4359	374.2216	---	---
	1998.8271	999.9172	2096.8040	1048.9057	20	I 6	646.3883	323.6978	---	---
	2085.8592	1043.4332	2183.8361	1092.4217	21	S 5	533.3042	267.1557	---	---
	2198.9432	1099.9753	2296.9201	1148.9637	22	I 4	446.2722	223.6397	---	---
	2269.9804	1135.4938	2367.9572	1184.4823	23	A 3	333.1881	167.0977	---	---
	2357.0124	1179.0098	2454.9893	1227.9983	24	S 2	262.1510	131.5791	---	---
	---	---	---	---	25	R 1	175.1190	88.0631	---	---



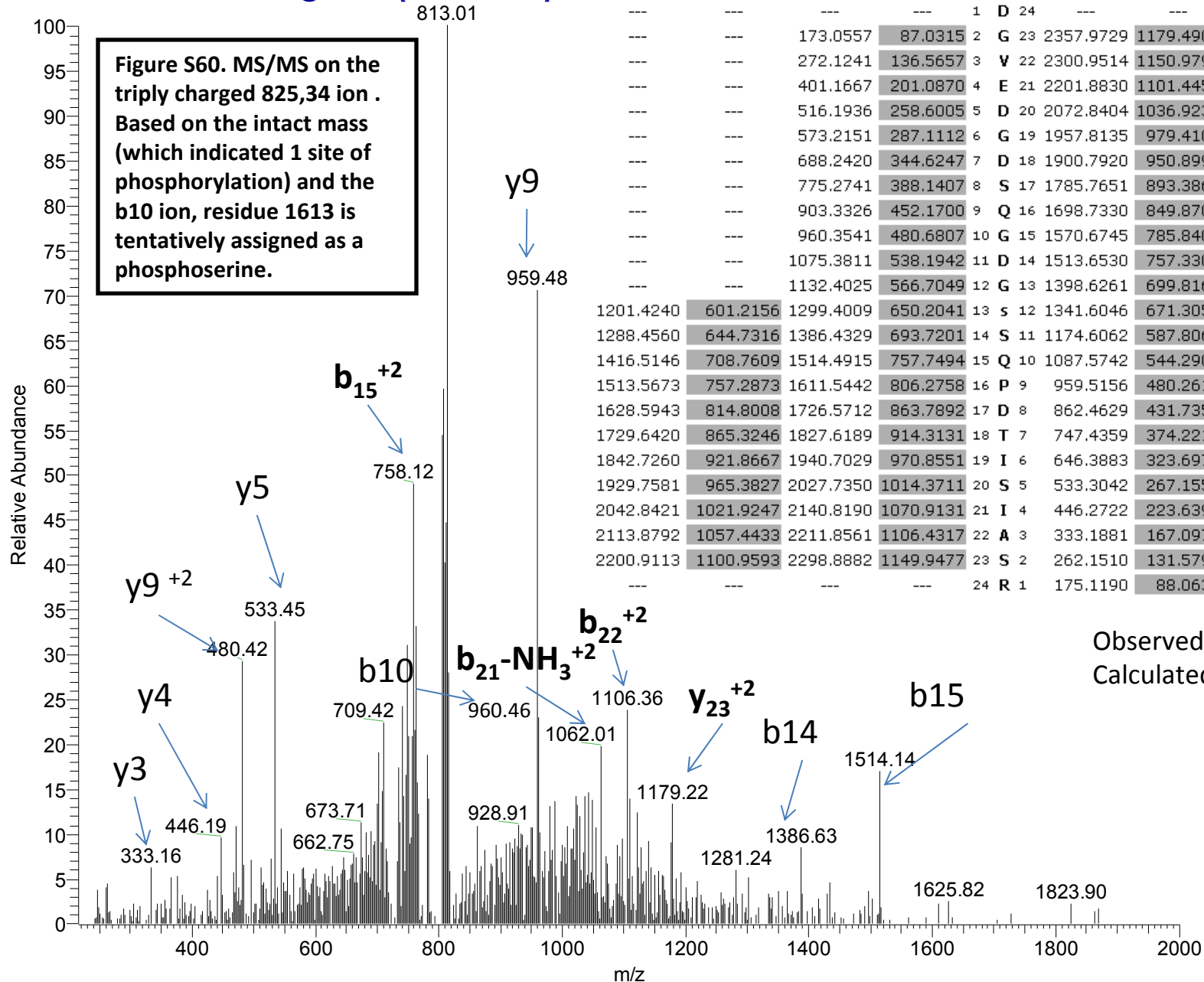
Observed M=2628.0927
 Calculated M=2628.0937

Endogenous Kal7

MS/MS of peptide DGVEDGDSQGDGpSSQPDTISIASR

OT09-4224 #2878 RT: 39.33 AV: 1 NL: 1.32E2
 T: ITMS + c NSI d Full ms2 825.34@cid35.00 [215.00-2000.00]

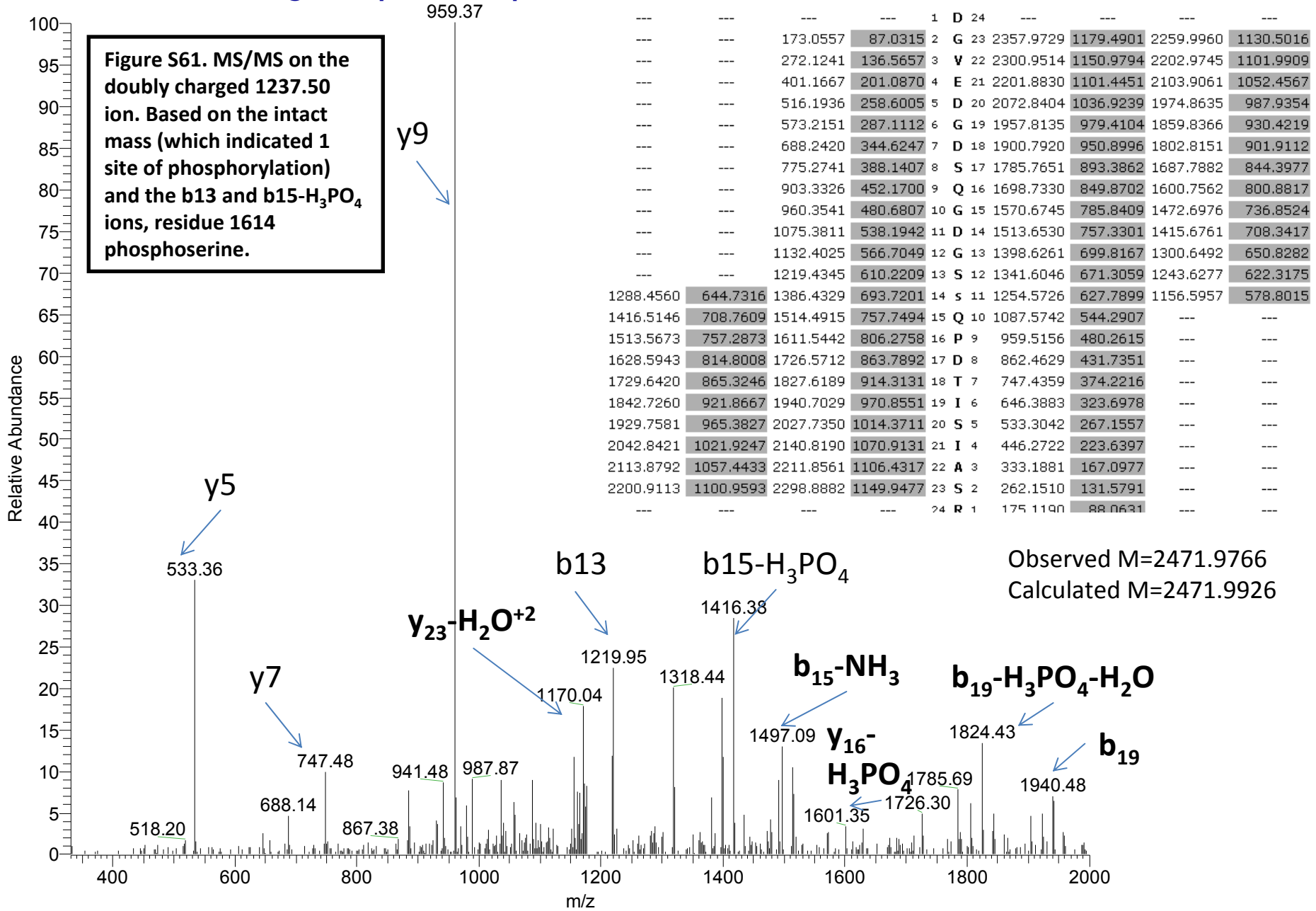
	b-H ₃ PO ₄	b-H ₃ PO ₄ ⁺²	b	b ⁺²		y	y ⁺²	y-H ₃ PO ₄	y-H ₃ PO ₄ ⁺²	
	---	---	---	---	1	D 24	---	---	---	
	---	---	173.0557	87.0315	2	G 23	2357.9729	1179.4901	2259.9960	1130.5016
	---	---	272.1241	136.5657	3	V 22	2300.9514	1150.9794	2202.9745	1101.9909
	---	---	401.1667	201.0870	4	E 21	2201.8830	1101.4451	2103.9061	1052.4567
	---	---	516.1936	258.6005	5	D 20	2072.8404	1036.9239	1974.8635	987.9354
	---	---	573.2151	287.1112	6	G 19	1957.8135	979.4104	1859.8366	930.4219
	---	---	688.2420	344.6247	7	D 18	1900.7920	950.8996	1802.8151	901.9112
	---	---	775.2741	388.1407	8	S 17	1785.7651	893.3862	1687.7882	844.3977
	---	---	903.3326	452.1700	9	Q 16	1698.7330	849.8702	1600.7562	800.8817
	---	---	960.3541	480.6807	10	G 15	1570.6745	785.8409	1472.6976	736.8524
	---	---	1075.3811	538.1942	11	D 14	1513.6530	757.3301	1415.6761	708.3417
	---	---	1132.4025	566.7049	12	G 13	1398.6261	699.8167	1300.6492	650.8282
	1201.4240	601.2156	1299.4009	650.2041	13	S 12	1341.6046	671.3059	1243.6277	622.3175
	1288.4560	644.7316	1386.4329	693.7201	14	S 11	1174.6062	587.8068	---	---
	1416.5146	708.7609	1514.4915	757.7494	15	Q 10	1087.5742	544.2907	---	---
	1513.5673	757.2873	1611.5442	806.2758	16	P 9	959.5156	480.2615	---	---
	1628.5943	814.8008	1726.5712	863.7892	17	D 8	862.4629	431.7351	---	---
	1729.6420	865.3246	1827.6189	914.3131	18	T 7	747.4359	374.2216	---	---
	1842.7260	921.8667	1940.7029	970.8551	19	I 6	646.3883	323.6978	---	---
	1929.7581	965.3827	2027.7350	1014.3711	20	S 5	533.3042	267.1557	---	---
	2042.8421	1021.9247	2140.8190	1070.9131	21	I 4	446.2722	223.6397	---	---
	2113.8792	1057.4433	2211.8561	1106.4317	22	A 3	333.1881	167.0977	---	---
	2200.9113	1100.9593	2298.8882	1149.9477	23	S 2	262.1510	131.5791	---	---
	---	---	---	---	24	R 1	175.1190	88.0631	---	---



Endogenous Kai7

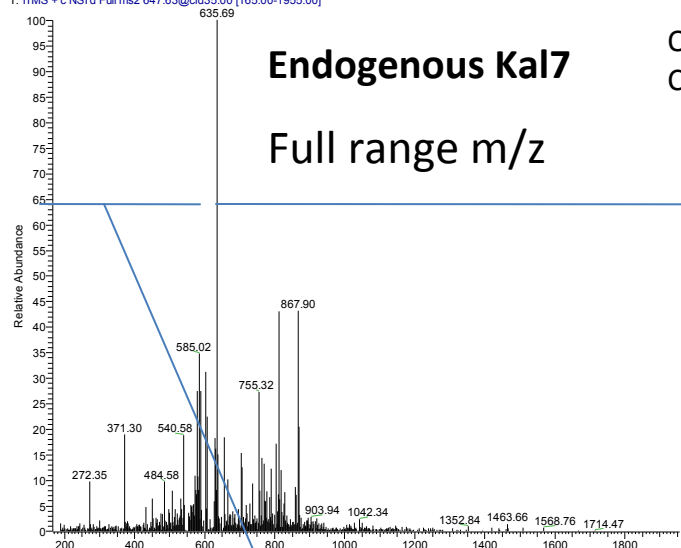
MS/MS of peptide DGVEDGDSQGDGSpSQPDTISIASR

OT09-3336 #2719 RT: 38.98 AV: 1 NL: 2.86E2
 T: ITMS + c NSI d Full ms2 1237.50@cid35.00 [330.00-2000.00]



MS/MS of peptide TSQNTVEpSDKGNLVPR

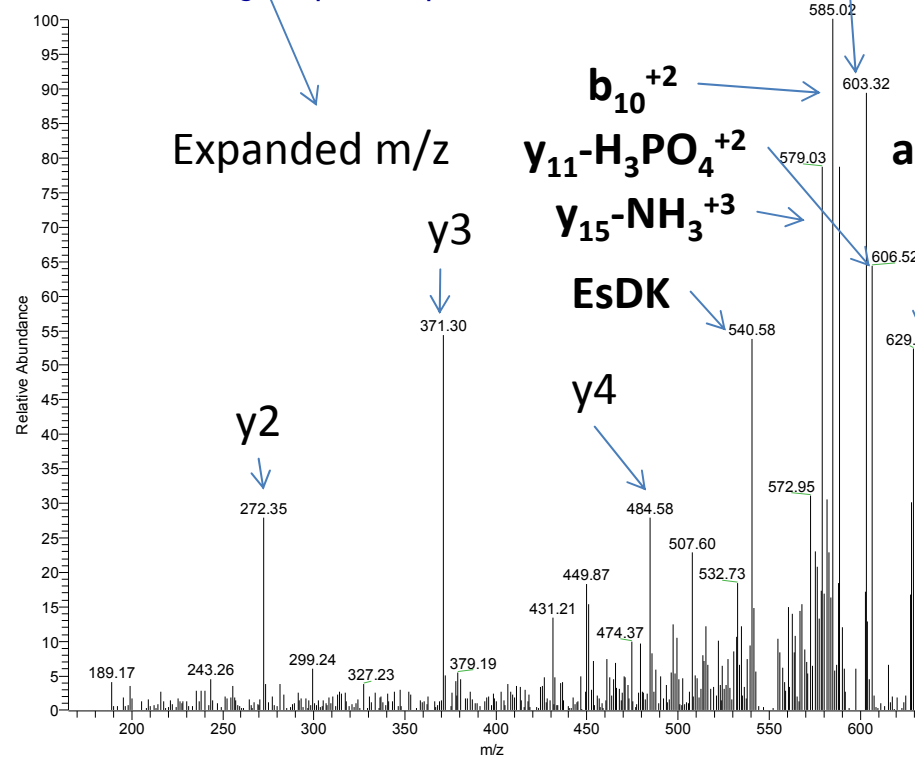
OT09-4224 #2579 RT: 35.43 AV: 1 NL: 8.08E2
T: ITMS + c NSI d Full ms2 647.63@cid35.00 [165.00-1955.00]



Observed M=1938.8669
Calculated M=1938.8684

	b-H ₃ PO ₄	b-H ₃ PO ₄ ⁺²	b	b ⁺²	y	y ⁺²	y-H ₃ PO ₄	y-H ₃ PO ₄ ⁺²	
---	---	---	---	---	1 T 17	---	---	---	
---	---	---	189.0870	95.0471	2 S 16	1838.8280	919.9176	1740.8511	870.9292
---	---	---	317.1456	159.0764	3 Q 15	1751.7960	876.4016	1653.8191	827.4132
---	---	---	431.1885	216.0979	4 N 14	1623.7374	812.3723	1525.7605	763.3839
---	---	---	532.2362	266.6217	5 T 13	1509.6945	755.3509	1411.7176	706.3624
---	---	---	631.3046	316.1559	6 V 12	1408.6468	704.8270	1310.6699	655.8386
---	---	---	760.3472	380.6772	7 E 11	1309.5784	655.2928	1211.6015	606.3044
---	---	---	829.3686	415.1880	8 s 10	1180.5358	590.7715	1082.5589	541.7831
---	---	---	944.3956	472.7014	9 D 9	1013.5374	507.2724	---	---
---	---	---	1072.4905	536.7489	10 K 8	898.5105	449.7589	---	---
---	---	---	1187.5175	594.2624	11 D 7	770.4155	385.7114	---	---
---	---	---	1244.5390	622.7731	12 G 6	655.3886	328.1979	---	---
---	---	---	1358.5819	679.7946	13 N 5	598.3671	299.6872	---	---
---	---	---	1471.6659	736.3366	14 L 4	484.3242	242.6657	---	---
---	---	---	1570.7344	785.8708	15 V 3	371.2401	186.1237	---	---
---	---	---	1667.7871	834.3972	16 P 2	272.1717	136.5895	---	---
---	---	---	---	---	17 R 1	175.1190	88.0631	---	---

OT09-4224 #2579 RT: 35.43 AV: 1 NL: 2.81E2
T: ITMS + c NSI d Full ms2 647.63@cid35.00 [165.00-1955.00]



OT09-4224 #2579 RT: 35.43 AV: 1 NL: 3.48E2
T: ITMS + c NSI d Full ms2 647.63@cid35.00 [165.00-1955.00]

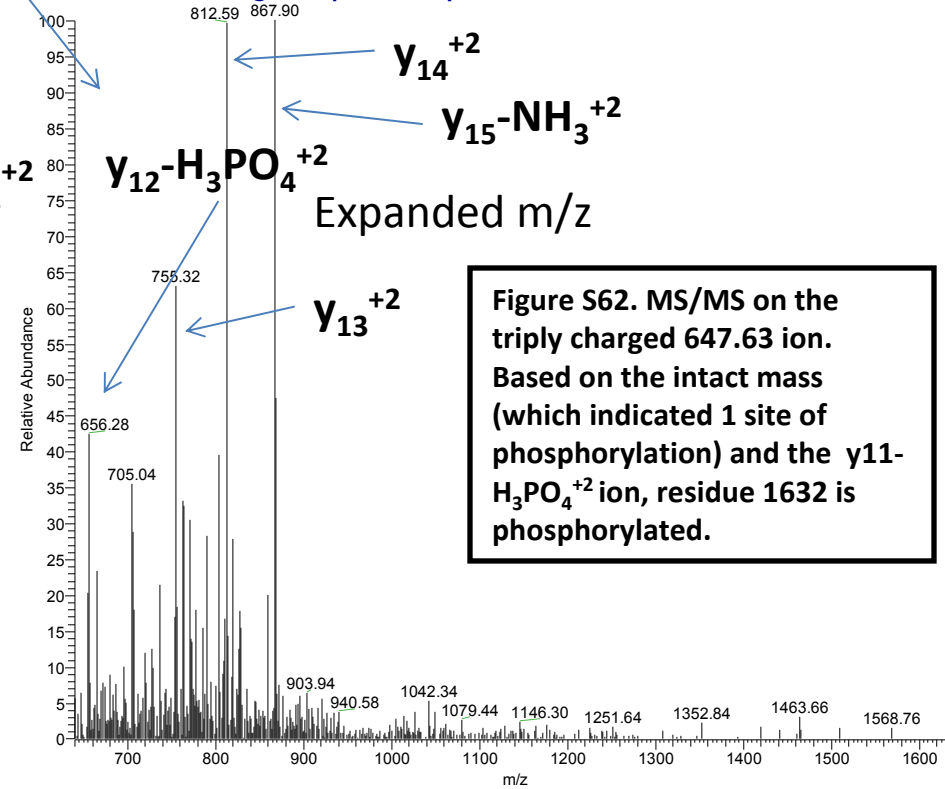


Figure S62. MS/MS on the triply charged 647.63 ion. Based on the intact mass (which indicated 1 site of phosphorylation) and the y11-H₃PO₄⁺² ion, residue 1632 is phosphorylated.