Wang et al. "Arginylation of intact proteins in vivo involves a novel type of Arg linkage to amino acid side chains."

SUPPLEMENTAL ONLINE INFORMATION

Experimental Procedures.

Escherichia coli strains, Miscellaneous Reagents and Animal Care. BL21-CodonPlus® (DE3)-RIL and BL21(DE3) were purchased from Stratagene (USA). The recombinant mouse ATE1 isoforms and Escherichia coli Arginy-tRNA synthetase (RRS) were purified as previously described [13]. The care and treatment of mice were performed in accordance with the relevant National Institutes of Health (NIH) guidelines.

Peptides. Human angiotensin II (A9525) was purchased from Sigma; Acetylated and non-acetylated actin N-terminal peptide were synthesized by Dr. Henry Zebroski at the Rockefeller University Proteomics Resource Center; non-arginylated long actin Nterminal peptide, DN, DN-Me, and DN-All were synthesized by Genscript.

In vitro arginylation of peptides. In vitro arginylation reaction of peptides by ATE1 was modified from [13]. A typical reaction was performed in 100 μ l volume, containing 50 mM Hepes, pH 7.5, 25 mM KCl, 15 mM MgCl₂, 0.1 mM DTT, 3 mM ATP, 1 mM Arginine, 100 μ M tRNA-Arg from E.coli (Chemical Block), 6 μ M RRS, 3 μ M ATE1 and 100 μ M peptide substrate. Reaction was mixed and incubated at 37 °C for 1 hour, followed by heating at 95°C for 15 min, cooling down on ice for 20 min, and spinning at 13,000 rpm for 15 min in a tabletop microfuge. The supernatants were loaded

onto C18 spin columns (The Nest Group, Inc.) prewashed with 100% acetonitrile and water, then washed with 0.1% trifluoroacetic acid (TFA) and eluted with 60% acetonitrile/0.1% TFA in water. The eluted peptide was dried with speed vacuum and redissolved in 0.1% TFA in water for further analysis. L-[¹³C,¹⁵N]-Arginine (Pierce) was used for arginylation of peptides for mass spectrometry and NMR, and L-Arginine (Sigma) was used for Edman sequencing. For NMR analysis, arginylated peptides were further purified by HPLC, lyophilized, and dissolved in 20 mM K₂HPO₄/KH₂PO₄ buffer, pH 6.0 and adjusted to the concentration to 1mM. To evaluate the arginylation efficiency, 12.5 μ M L-[2,3,4-3H]-Arginine (PerkinElmer) instead of "cold" arginine was used in the reaction and the concentrations of tRNA^{Arg} and peptides were adjusted to 50 μ M. After purification with C18 spin columns, arginylated peptides were counted in a liquid scintillation counter and evaluated as counts per minute (cpm) of [3H]-Arg.

Mass spectrometry and database searches. Sample preparation and tandem MS/MS were performed as previously described [2, 14]. Protein identification was performed with Integrated Proteomics Pipeline - IP2 (Integrated Proteomics Applications, Inc., San Diego, CA. http://www.integratedproteomics.com/) using ProLuCID and DTASelect2 [14]. Spectrum raw files were extracted into ms1 and ms2 files from raw files using RawExtract 1.9.9 (http://fields.scripps.edu/downloads.php) [15], and the tandem mass spectra were searched against NCBI RefSeq protein database (http://www.ncbi.nlm.nih.gov/refseq/, downloaded on May 17, 2010). In order to accurately estimate peptide probabilities and false discovery rates, we used a decoy database containing the reversed sequences of all the proteins appended to the target database. Tandem mass spectra were matched to sequences using the ProLuCID

algorithm with 50 ppm peptide mass tolerance. ProLuCID searches were done on an Intel Xeon cluster running under the Linux operating system. The search space included all fully tryptic peptide candidates that fell within the mass tolerance window with maximum 2 miscleavages. Carbamidomethylation (+57.02146 Da) of cysteine was considered as a static modification, arginylation (+156.1011 or + 166.1093) on Aspartic acid and Glutamic acid residues were considered as variable modifications.

The validity of peptide/spectrum matches (PSMs) was assessed with DTASelect2 using two SEQUEST defined parameters, the cross-correlation score (XCorr), and normalized difference in cross-correlation scores (DeltaCN). The search results were grouped by charge state (+1, +2, +3,and greater than +3) and tryptic status (fully tryptic, half-tryptic, and non-tryptic), resulting in 12 distinct sub-groups. In each one of these sub-groups, the distribution of XCorr, DeltaCN, and DeltaMass values for (a) direct and (b) decoy database PSMs was obtained, then the direct and decoy subsets were separated by discriminant analysis. Full separation of the direct and decoy PSM subsets is not generally possible; therefore, peptide match probabilities were calculated based on a nonparametric fit of the direct and decoy score distributions. A peptide confidence of 95% was set as the minimum threshold and only peptides with delta mass less than 5 ppm were accepted. The false discovery rate was calculated as the percentage of reverse decoy PSMs among all the PSMs that passed the 95% confidence threshold. After this last filtering step, we estimate that the peptide false discovery rates were below 1%. See [14] for further details on manual data validation.

Subtractive Edman degradation. For the Edman reaction, the phenylisothiocyanate (PITC) was purchased from Pierce. All other reagents were from

Sigma Aldrich. The peptide was initially dissolved in 50% aqueous pyridine and then an equal volume of 5% PITC in pyridine was added. The coupling reaction was performed by heating for 10 minutes at 50 degrees Celsius. Extractions were performed twice with 2:1 heptane/ethyl acetate solution and then lyophilized. Cleavage was performed using anhydrous TFA and the reaction was performed for 5 minutes at 37 degrees Celsius and then lyophilized. The peptide was dissolved in water and two volumes of n-butyl acetate were added. The mixture was shaken and the acetate layer was removed after separation. The peptide was lyophilized prior to mass spectrometry analysis.

NMR experiments. All NMR spectra were recorded on a Bruker Avance II 600 MHz NMR spectrometer, equipped with a Bruker TCI triple resonance cryoprobe. All NMR samples were prepared by dissolving the lyophilized compounds in a buffer with 50 mM sodium phosphate, 50 mM NaCl, and pH 6.3. The concentrations of the samples were around 0.5 mM. The spectra were recorded with Bruker Topspin standard pulse sequences at 25 °C, and the spectra were processed with Bruker Topspin software based on the manufacture suggestions.

Analysis of the sequence context around the arginylated sites. Sequence fragments of specified lengths (+/- 5, 10 or 20 positions) around the arginylated sites were extracted from the database and the overlapping fragments (resulting from closely located sites) were merged together. Frequencies of occurrence of each amino acid in the vicinity of the arginylated sites were compared to those in the full-length proteins; the significance of the deviation for each amino acid was estimated using the binomial approximation with Bonferroni correction (binomial p-values were multiplied by a factor

of 20 to account for comparison of 20 amino acids). To avoid bias, titin, the longest protein in the mouse genome, was excluded from the analysis.

Statistical analysis. Calculation of standard deviation (SD) and standard error of mean (S.E.M.) was based on Student's t-distribution.

Supplemental Figure and Table Legends.

Figure S1 (related to Figure 2). Normalized side chain incorporation of Arg into a standard peptide in the presence and absence of tRNA. Error bar represents SEM, n=3

Figure S2 (**related to Figures 2 and 3**). MALDI TOF spectrograms showing the standard enzymatically arginylated peptide (R)-DDIAALVVDNGSGMCK before (left) and after (right) the first Edman degradation cycle. The peptide mass has reduced by one Arg, confirming its N-terminal linkage to the peptide.

Figure S3 (related to Figure 3). Colorimetric assay with Aminopeptidase B in the absence (red) and presence (green) of the inhibitor Bestatin.

Supplemental Table 1 (related to Figure 1 and Table 1). Protein sites arginylated in vivo on the side chains of Asp and Glu showing the homology of these sites to other isoforms of the identified proteins.

Supplemental Table 2 (related to Figure 1 and Table 1). Parameters of the identified peptides.

Supplemental Table 3 (related to Figure 1 and Table 1). Frequencies of amino acid occurrence within 5, 10, and 20 flanking residues to the arginylation sites.

Supplemental Dataset (related to Figure 1 and Table 1). Mass spectra of the identified side chain arginylated peptides.





Wang_et_al_Supplemental Figure 2









SUPPLEMENTAL DATASET

Mass spectra of the side chain arginylated peptides identified in vivo.



b+	b2+	#	Seq	#	у+	y2+
102.0550	51.5311	1	т	30		
203.1026	102.0550	2	т	29	3251.6630	1626.3351
260.1241	130.5657	3	G	28	3150.6153	1575.8113
373.2082	187.1077	4	Т	27	3093.5938	1547.3006
472.2766	236.6419	5	v	26	2980.5098	1490.7585
585.3606	293.1840	6	L	25	2881.4414	1441.2243
700.3876	350.6974	7	D	24	2768.3573	1384.6823
787.4196	394.2134	8	s	23	2653.3304	1327.1688
844.4411	422.7242	9	G	22	2566.2983	1283.6528
959.4680	480.2376	10	D	21	2509.2769	1255.1421
1016.4895	508.7484	11	G	20	2394.2499	1197.6286
1115.5579	558.2826	12	v	19	2337.2285	1169.1179
1216.6056	608.8064	13	т	18	2238.1600	1119.5837
1353.6645	677.3359	14	н	17	2137.1124	1069.0598
1467.7074	734.3573	15	N	16	2000.0535	1000.5304
1566.7758	783.8916	16	v	15	1886.0105	943.5089
1663.8286	832.4179	17	Ρ	14	1786.9421	893.9747
1776.9127	888.9600	18	Т	13	1689.8893	845.4483
1939.9760	970.4916	19	Y	12	1576.8053	788.9063
2225.1197	1113.0635	20	Е	11	1413.7420	707.3746
2282.1411	1141.5742	21	G	10	1128.5983	564.8028
2445.2045	1223.1059	22	Y	9	1071.5768	536.2920
2516.2416	1258.6244	23	A	8	908.5135	454.7604
2629.3256	1315.1665	24	L	7	837.4764	419.2418
2726.3784	1363.6928	25	Р	6	724.3923	362.6998
2863.4373	1432.2223	26	н	5	627.3395	314.1734
2934.4744	1467.7409	27	A	4	490.2806	245.6439
3047.5585	1524.2829	28	1	3	419.2435	210.1254
3178.5990	1589.8031	29	м	2	306.1594	153.5834
		30	R	1	175.1190	88.0631



b+	b2+	#	Seq	#	у+	y2+
100.0757	50.5415	1	v	18		
171.1128	86.0600	2	A	17	2013.0764	1007.0418
268.1656	134.5864	3	Р	16	1942.0392	971.5233
397.2082	199.1077	4	E	15	1844.9865	922.9969
682.3519	341.6796	5	Е	14	1715.9439	858.4756
819.4108	410.2090	6	н	13	1430.8002	715.9037
916.4635	458.7354	7	Р	12	1293.7413	647.3743
1017.5112	509.2592	8	т	11	1196.6885	598.8479
1130.5953	565.8013	9	L	10	1095.6408	548.3241
1243.6793	622.3433	10	L	9	982.5568	491.7820
1344.7270	672.8671	11	т	8	869.4727	435.2400
1473.7696	737.3884	12	E	7	768.4250	384.7162
1544.8067	772.9070	13	A	6	639.3824	320.1949
1641.8595	821.4334	14	Р	5	568.3453	284.6763
1754.9435	877.9754	15	L	4	471.2926	236.1499
1868.9865	934.9969	16	N	3	358.2085	179.6079
1966.0392	983.5233	17	Р	2	244.1656	122.5864
		18	к	1	147.1128	74.0600
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Static Mod C: 57.0214	lifications 16	51				













b+	b2+	#	Seq	#	у+	y2+
129.0659	65.0366	1	Q	17		
226.1186	113.5629	2	Ρ	16	1935.9784	968.4928
283,1401	142.0737	3	G	15	1838.9256	919.9664
384.1878	192.5975	4	т	14	1781.9041	891.4557
471.2198	236.1135	5	s	13	1680.8565	840.9319
584.3039	292.6556	6	L	12	1593.8244	797.4159
683.3723	342.1898	7	v	11	1480.7404	740.8738
954.5003	477.7538	8	D	10	1381.6720	691.3396
1025.5374	513.2723	9	A	9	1110.5439	555.7756
1140.5644	570.7858	10	D	8	1039.5068	520.2570
1241.6120	621.3097	11	т	7	924.4799	462.7436
1388.6805	694.8439	12	F	6	823.4322	412.2197
1525.7394	763.3733	13	н	5	676.3638	338.6855
1662.7983	831.9028	14	н	4	539.3049	270.1561
1790.8569	895.9321	15	Q	3	402.2459	201.6266
1889.9253	945.4663	16	v	2	274.1874	137.5973
		17	R	1	175.1190	88.0631

Static Modifications: Variable Modifications:



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378.2023	189.6048	3	Y	20	2478.1473	1239.5773		
515.2613	258.1343	4	н	19	2315.0840	1158.0456		
612.3140	306.6606	5	Р	18	2178.0250	1089.5162		
883.4421	442.2247	6	D	17	2080.9723	1040.9898		
940.4635	470.7354	7	G	16	1809.8442	905.4258		
1037.5163	519.2618	8	Р	15	1752.8228	876.9150		
1166.5589	583.7831	9	E	14	1655.7700	828.3886		
1223.5804	612.2938	10	G	13	1526.7274	763.8673		
1351.6389	676.3231	11	Q	12	1469.7060	735.3566		
1422.6760	711.8417	12	A	11	1341.6474	671.3273		
1585.7394	793.3733	13	Y	10	1270.6103	635.8088		
1714.7820	857.8946	14	Е	9	1107.5469	554.2771		
1813.8504	907.4288	15	v	8	978.5043	489.7558		
1928.8773	964.9423	16	D	7	879.4359	440.2216		
2075.9457	1038.4765	17	F	6	764.4090	382.7081		
2176.9934	1089.0003	18	т	5	617.3406	309.1739		
2274.0462	1137.5267	19	Р	4	516.2929	258.6501		
2371.0989	1186.0531	20	Р	3	419.2401	210.1237		
2518.1674	1259.5873	21	F	2	322.1874	161.5973		
		22	R	1	175.1190	88.0631		
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Static Modifications: C: 57 02146								

Variable Modifications:

D: 156.1011



C: 37.02146

Variable Modifications:

D: 156.1011









3595.8099 1798.4086

31 E 2

32 R

304.1615

175.1190

1

152.5844

88.0631



C: 57.02146

Variable Modifications: E: 156.1011





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Static Modifications: C: 57.02146 Variable Modifications: D: 156.1011





b+	b2+	#	Seq	#	у+	y2+				
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530.2609	265.6341	4	Е	18	2211.1193	1106.0633				
627.3137	314.1605	5	Р	17	2082.0767	1041.5420				
724.3665	362.6869	6	Р	16	1985.0239	993.0156				
1009.5101	505.2587	7	E	15	1887.9712	944.4892				
1156.5786	578.7929	8	F	14	1602.8275	801.9174				
1271.6055	636.3064	9	D	13	1455.7591	728.3832				
1328.6270	664.8171	10	G	12	1340.7321	670.8697				
1385.6484	693.3279	11	G	11	1283.7106	642.3590				
1472.6805	736.8439	12	s	10	1226.6892	613.8482				
1569.7332	785.3702	13	Р	9	1139.6572	570.3322				
1682.8173	841.9123	14	1	8	1042.6044	521.8058				
1795.9014	898.4543	15	L	7	929.5203	465.2638				
1932.9603	966.9838	16	н	6	816.4363	408.7218				
2096.0236	1048.5154	17	Y	5	679.3774	340.1923				
2195.0920	1098.0496	18	v	4	516.3140	258.6606				
2308.1761	1154.5917	19	L	3	417.2456	209.1264				
2437.2187	1219.1130	20	E	2	304.1615	152.5844				
		21	R	1	175.1190	88.0631				
[Click] to mo	ove table									
Static Modifications: C: 57.02146										
Variable M F: 156,101	odification	ns:								

FVESRDVVMT <mark>D</mark> TSITEEQA GPGEPA A PFFISKPVVQK, MH+ 4163.1118, m/z 1041.5334 File: myofibril-9, Scan: 7049, Precursor m/z: 1041.7876, Charge: 4											
115%	±										
99%	P20-										
82%											
66%	-b23++										
49%											
33%	11+ + + + + + + + + + + + + + + + + + + +										
16%											
08	400 600 800 1000 1200 1400 1600 1800										

	DZ+	b3+	÷ .	Seq	*	λ+	yZ+	y3+
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247.1441	124.0757	83.0529	2	٧	36	4016.0433	2008.5253	1339.3526
376.1867	188.5970	126.0671	3	E	35	3916.9749	1958.9911	
463.2187	232.1130	155.0778	4	s	34	3787.9323	1894.4698	
619.3198	310.1636	207.1115	s	R	33	3700.9003	1850.9538	1234.3050
734.3468	367.6770	245.4538	6	D	32	3544.7992		1182.2713
833,4152	417.2112	278.4766	7	۷	31	3429.7723	1715.3898	
932,4836	466.7454	311.4994	8	v	30	3330.7038	1665.8556	1110.9061
1063.5241	532.2657	355.1795	9	м	29	3231.6354	1616.3214	1077.8833
1164.5718	582.7895	388.8621	10	т	28	3100.5949	1550.8011	
1435.6998	718.3535	479.2381	11	D	27	2999.5473	1500.2773	1000.5206
1536.7475	768.8774	512.9207	12	т	26	2728.4192		
1623.7795	812.3934	541.9314	13	s	25	2627.3715		
1736.8636	868.9354	579.6260	14	T.	24	2540.3395		
1837.9113	919.4593	613.3086	15	т	23	2427.2555		
1966.9539	983.9806	656.3228	16	E	22	2326.2078		776.0741
2095.9964	1048.5019	699.3370	17	E	21	2197.1652	1099.0862	733.0599
2224.0550	1112,5312	742.0232	18	Q	20	2068.1226	1034.5649	
2295.0921	1148.0497	765.7022	19	A	19	1940.0640	970.5356	
2352.1136	1176.5604	784.7094	20	G	18	1869.0269	935.0171	
2449.1664	1225.0868	817.0603	21	P	17	1812.0054		
2506.1878	1253.5976	836.0675	22	G	16	1714.9527		
2635.2304	1318,1189	879.0817	23	E	15	1657.9312		
2732.2832	1366.6452	911.4326	24	P	14	1528.8886		
2803.3203	1402.1638		25	A	13	1431.8358		477.9501
2874.3574	1437.6823	958.7907	26	A	12	1360.7987	680.9030	454.2711
2971.4102	1486.2087	991,1416	27	P	11	1289.7616		430,5921
3118.4786	1559.7429	1040.1644	28	F	10	1192.7089	596.8581	398.2411
3265.5470	1633.2771	1089.1872	29	F	9	1045.6404		349.2183
3378.6311	1689.8192	1126.8819	30	Т	8	898.5720		300.1955
3465.6631	1733.3352	1155.8926	31	s	7	785.4880	393.2476	262.5008
3593.7581	1797.3827	1198.5909	32	к	6	698.4559	349.7316	233.4902
3690.8108	1845.9091	1230.9418	33	P	5	570.3610	285.6841	190.7918
3789.8792	1895.4433	1263.9646	34	v	4	473.3082	237.1577	158.4409
3888.9477	1944.9775	1296.9874	35	۷	з	374.2398	187.6235	125.4181
4017.0062	2009.0068	1339.6736	36	Q	2	275.1714	138.0893	92.3953
			37	к	1	147.1128	74.0600	49.7091





b+	b2+	#	Seq	#	у+	y2+		
129.0659	65.0366	1	Q	21				
242.1499	121.5786	2	L	20	2443.2477	1222.1275		
389.2183	195.1128	3	F	19	2330.1636	1165.5854		
526.2772	263.6423	4	н	18	2183.0952	1092.0512		
623.3300	312.1686	5	Р	17	2046.0363	1023.5218		
908.4737	454.7405	6	Е	16	1948.9835	974.9954		
1036.5323	518.7698	7	Q	15	1663.8398	832.4235		
1149.6163	575.3118	8	L	14	1535.7812	768.3943		
1262.7004	631.8538	9	1	13	1422.6972	711.8522		
1363.7481	682.3777	10	т	12	1309.6131	655.3102		
1420.7695	710.8884	11	G	11	1208.5654	604.7864		
1548.8645	774.9359	12	к	10	1151.5440	576.2756		
1677.9071	839.4572	13	E	9	1023.4490	512.2281		
1792.9340	896.9707	14	D	8	894.4064	447.7068		
1863.9712	932.4892	15	A	7	779.3795	390.1934		
1935.0083	968.0078	16	A	6	708.3424	354.6748		
2049.0512	1025.0292	17	N	5	637.3052	319,1563		
2163.0941	1082.0507	18	N	4	523.2623	262.1348		
2326.1575	1163.5824	19	Y	3	409.2194	205.1133		
2397.1946	1199.1009	20	A	2	246.1561	123.5817		
		21	R	1	175.1190	88.0631		
[Click] to mo	ove table							
Static Mod C: 57.0214	lifications: 46							
Variable Modifications: E: 156.1011								

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	300.1030	100.5551	67.3725	2	Q	29	4256.0666	2129.5490	1419.3678
	299.1714	150.0693	100.3953	3	v	28	4129.0302	2064.5167	1376.6816
	436.2303	218.6168	146.0916	A	н	27	4029.9619	2014.9945	1343.6588
	\$35.2987	268.1530	179.1044	5	۷	36	3891.9029		
	622.3307	311.6690	208,1151	6	\$	35	3792.8345	1896.9209	
AQVHVSEEGGEPEAMLQVLGPKPALPEGTEDTAKEDAANR, MH+ 4327.1259, m/z 1082.5369	751.3733	376,1903	351,1293	τ	E	24	3705.8024	1853.4049	
File: 080910WTPMA-11, Scan: 6387, Precursor m/z: 1082.7855, Charge: 4	1036.5170	\$18,7621		8		33	3576.7599	1768.6836	
	1093.5385	\$47.2729	365,1843	۰	G	32	3291.6162		
	1150.5599	\$75,7835		10	G	21	3234.5947	1617.8010	1078.8698
	1279.6025	640.3049		11	E	30	3177.5732	1589.2903	
±	1276.6553	688,8313	459.5566	12	P	29	3048.5306	1524.7690	
35	1505.6979	753.3526	502.5708	12	E	29	2951.4779		
	1576.7350	788.8711	516.2499	14	۵	27	1912.4353	1411.7213	
	1707.7755	454.3914		15		26	2751.3982	1376.2027	917.8042
	1820,8595	910,9334		16	L.	25	2620.3577		
	1948,9181	974.9627	450.3109	17	Q	24	2507.2736	1254.1404	
	2047.9866	1024.4969	683.3337	18	۷	22	2379.2150		
	2161.0706	1081.0389		19	L.	22	2290.1466		
	2218.0921	1109.5497	740.0355	20	G	21	2167.0626	1084.0349	
	2215.1448	1158.0761	772.3855	21	P	20	2110.0411		
	2662.2298	1222.1235	815.0848	22	к	19	2012.9663		
	1540.3926	1270.6499		23	P	18	1684.8934		
<u>ė</u>	2611.3297	1306.1685		24	۵	17	1787.8406	894.4229	
+ 1 +	2724.4127	1362.7105	908.8094	25	L.	16	1716.8035	858.9054	\$72,9393
	1921.4665	1411.2369		26	P	15	1603.7196		
¥ <u>⊟</u> ‡ ‡ <u>⇒</u> ‡	1950.5091	1475.7582		27	E	14	1506.6667		
	3007.5306	1504.2689		29	G	12	1277.6241		
	3108.5782	1554.7928	1036.8643	29	т	12	1320.6026	660,8049	440.8724
	3237.6208	1619-3141	1079.8785	30	E	11	1219.5549	610.3511	
	3352.6478	1676.8275	1118.2208	24	D	10	1090.5123	\$45,7598	364.1756
600 800 1000 1200 1400 1600 1800	3453.6955	1727.3514	1151.9033	32	т	9	975.4854	488.2463	325.8333
	3524.7326	1762.8699	1175.5824	33	۸	8	874.4377	437,7335	292.1508
	3652.8275	1826.9174	1218.2907	34	к	7	803.4006	402.2039	268.4717
	3751.5701	1891.4287	1261.2949	35	E	6	675.3056	338,1565	225,7724
	3896.8971	1948.9522	1299.6372	36	D	5	546.2630	273.6352	182,7592
	3967.9342	1984.4707	1323,3162	27	۵	4	431.2361	216.1217	144.4169
	4028.9713	2019,9893	1346.9953	28	۵	3	360,1990	180.6031	120.7378
	4153.0142	2077.0108	1385.0096	29	н	2	289,1619	145.0946	97.0588

118%

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		52e	b2e		Sec	e	124	1/24
	72.0444	36.5258	24.6963	1	4	40	2-1	
	200.1030	100.5551	67.3725	2	9	29 4256.06	8 2128.5480	1419.3679
	299.1714	150.0693	100.3953	2	v	39 4129.036	2 2064.5187	1376.6816
	436.2303	218.6168	146.0816	4	н	37 4029.96	8 2014.9845	1343.6568
	\$35.2987	268.1520	179,1044	5	v	36 2891.903	9 1946-4551	1297.9725
	622.3307	311.6690	208.1151	6	\$	35 2792.82	5 1896.9209	1264.9497
AOVHVSEEGGEPEAMLOVLGPKPALPEGTEDTAKEDAANR, MH+ 4327,1259, m/z 1082,5369	751.3733	376.1903	251.1292	7	•	34 3705.80	4 1952-4049	1235.9390
File: 080910WTPMA-11, Scan: 6354, Precursor m/z: 1083.0355, Charge: 4	880.4159	660.7116	294.1425	8	E	22 2576.75	9 1768.6636	1192.9248
	937.4374	469.2223	313,1506		G	22 2447.71	3 1726.3623	1149.9106
+	994.4588	497,7221	332.1578	10	G	31 2290.69	8 1695.8515	1130.9035
1	1279.6025	640.3049	427.2057	11	•	10 2222.67	a 1667.3408	1111.6963
Š.	1376.6553	688.8313	459.5566	12	P	19 2018.53	6 1524.7690	1016.8484
	1505.6979	753.3526	502.5708	12		18 2951.47	9 1476-2426	994.4975
	1576.7350	788.8711	\$26.2499	14	۵	27 2922.43	a 1411.7213	941.4833
	1707.7755	454.3914	\$69,9300	15		26 2751.39	1376.2027	917.8042
	1820,8596	910.9334	607.6247	16	L.	25 2620.35	7 1310.6625	874,1241
	1948,9181	974.9627	650.3109	17	Q	24 2507.27	6 1254.1404	\$16,4194
	2047.9666	1024.4969	682.2227	18	V.	22 2279.245	0 1190.1112	793,7432
	2161.0706	1061.0369	721.0284	19	L.	22 2260.14	4 1140.5770	760,7304
	2218.0921	1109.5697	740.0355	20	G	21 2167.063	6 1064.0349	723.0257
	2215.1448	1158.0761	772.3865	21	P	20 2110.04	1 1055,5242	704.0166
± .	2662.2398	1222.1235	815.0848	22	к	19 2012.96	1006.9978	671.6676
	1540.2926	1270.6499	\$47.4357	22	P	18 1884.89	4 942.9503	628.9693
±	2611.3297	1306.1685		24	4	17 1797.84	6 894.4239	596.6184
	2724.4127	1362.7105	908.8094	25	L	16 1716.80	5 658.9054	\$75,9393
	2921.4665	1411.2369	941.1604	26	P	15 1603.71	602.3634	\$35,3667
	2950.5091	1475.7582	984.1746	27		14 1506.66	7 753.8370	502.8937
	3007.5306	1504.2689	1003.1817	29	G	13 1377.65	1 669.3157	459.8795
★ 載1+25	3108.5782	1554.7928	1036.8643	29	т	12 1320.60	6 660.8049	440.8724
	3237.6208	1619-2141	1079.8785	30	•	11 1219.55	e10.3811	407.1696
	3352.6478	1676.8275	1118.2208	21	•	10 1090.51	3 545.7598	364.1756
0 600 800 1000 1200 1400 1600 1800	3453.4955	1727.3514	1151.9033	32	т	9 975.485	466.2462	325.4333
	3524.7326	1762.8699	1175.5824	33	Δ	8 876.637	437.7235	292.1508
	3652.8275	1826.9174	1218.3907	24	К	7 803.400	402.2039	268.4717
	3781.8701	1891.4387	1261.2949	35	•	6 675.305	228.1565	225.7724
	3896.8971	1948.9522	1299.6372	36	D	5 546.263	273.4352	182.7592
	3967.9342	1984.4707	1323.3162	27	A	4 431.234	1 216.1217	144.4169
	1 4036 9713	1 1040 0001	1246 346 3	1.26		3 1 340 400	0 1 100 6034	1 120 7276

4153.0142 2077.0106 1365.0096

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29 N 2

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145.0846

\$5,0631

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\$9.0645

110%

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