

## **Supplementary Data**

## SUPPLEMENTARY TABLES

**Supplementary Table 1: Targets and cleavage sites identified in a proteome-wide COFRADIC analysis with caspase-1.** The complete list of internally located, tri-deuteroacetylated peptides cleaved after residues other than Asp in P<sub>1</sub> that were uniquely identified in the digested proteome of recombinant caspase-1 treated mouse Mf/f4 lysates, is shown. Protein names are identified by using the SIB BLAST network service, and the accession numbers are according to the UniProtKB/Swiss-Prot database. The cleavage sites represent the amino acids preceding the identified peptide (i.e. P4 – P1) as well as the P1'. Start/end, sequence, modified sequence, number of identified spectra (spectra #), Mascot ion score of highest scoring spectra, m/z, z, error (Da), e-value, threshold and  $\Delta$  threshold of the identified spectra are given.

Accession Nr.	Protein name	Sequence	Modified sequence	Cleavage site
O08756	HADH2	TIENPFLNGEIVR	AcD3-TIENPFLN<Dam>GEVIR-COOH	HLVQ <sub>237</sub> -T <sub>238</sub>
Q01WR3	ASCC2	QVGANDADSDDELISR	AcD3-Q<Dam>VGANDADSDDELISR-COOH	YDGN <sub>16</sub> -Q <sub>617</sub>
Q9JKX6	Nudt5	FPAGFIEDGESPEAAALR	AcD3-FPAGFIEDGESPEAAALR-COOH	YCLE <sub>92</sub> -F <sub>93</sub>
P17427	Ap2a2	VAPLAPGSEDNFAR	AcD3-VAPLAPGSEDNFAR-COOH	SASA <sub>693</sub> -V <sub>694</sub>
Q7TQH0	Atxn2l	SVPGQQGKYR	AcD3-SVPGQQGKYR-COOH	PVSN <sub>735</sub> -S <sub>739</sub>
P01887	Beta-2-microglobulin	KTIPIQIVYSR	AcD3-KTIPIQIVYSR-COOH	YAIQ <sub>22</sub> -K <sub>23</sub>
Q88TV2	Cpsf7	ATPSENLVPSAR	AcD3-ATPSENLVPSAR-COOH	ADGR <sub>201</sub> -A <sub>202</sub>
P97310	MCM2	GLALDDEDVEELTASQR	AcD3-GLALDDEDVEELTASQR-COOH	YEAE <sub>93</sub> -G <sub>94</sub>
P60898	RNA polymerase II subunit B9	YEPGFVIGR	AcD3-YEPGFVIGR-COOH	PDGT <sub>6</sub> -Y <sub>7</sub>
Q9WUJ2	eIF-4H	KEALTYDGALLGDR	AcD3-KEALTYDGALLGDR-COOH	VDSL <sub>94</sub> -K <sub>95</sub>
P97323	Fbpase 3	EFIMVER	AcD3-EFIMVER-COOH	PSIG <sub>39</sub> -E <sub>40</sub>
P49710	LckBP1	GFGGKYGVER	AcD3-GFGGKYGVER-COOH	DYSH <sub>118</sub> -G <sub>119</sub>
P51859	HDFG	GPPAEEEEEEEEAAKEEAQAQGV	AcD3-GPPAEEEEEEEEAAKEEAQAQGV-COOH	DSGQ <sub>208</sub> -G <sub>209</sub>
Q02780	NF1-A	GEEPFYTGQGR	AcD3-GEEPFYTGQGR-COOH	MDSP <sub>311</sub> -G <sub>312</sub>
Q9D903	EBP2	LASDQELQDAFSR	AcD3-LASDQELQDAFSR-COOH	SDAC <sub>16</sub> -L <sub>17</sub>
Q8K3X4	Protein C14orf4 homolog	TKPAVLAAPSGLER	AcD3-TKPAVLAAPSGLER-COOH	VDGS <sub>115</sub> -T <sub>116</sub>
P26350	Prothymosin alpha	EGDGEEDGDEDEEAAPTGKR	AcD3-EGDGEEDGDEDEEAAPTGKR-COOH	EEEE <sub>67</sub> -E <sub>68</sub>
Q9QZS2	RING finger protein 4	VVSSDDEELSR	AcD3-VVSSDDEELSR-COOH	ADSC <sub>95</sub> -V <sub>96</sub>
Q60710	MG11	STDPQLSEAQSILR	AcD3-STDPQLSEAQSILR-COOH	EVLH <sub>444</sub> -S <sub>445</sub>
Q8BT18	Srrm2	MAPALSGANLTSPR	AcD3-M<Mox>APALSGANLTSPR-COOH	SSSR <sub>2323</sub> -M <sub>2324</sub>
P27048	snRNP-B	GENLVSMITVEGPPPKDTGIAR	AcD3-GENLVSM<Mox>TVEGPPPKDTGIAR-COOH	VLLR <sub>73</sub> -G <sub>74</sub>
P26369	U2AF2	TPTPVPVWGSQMTR	AcD3-TPTPVPVWGSQM<Mox>TR-COOH	GLAV <sub>132</sub> -T <sub>133</sub>
O54988	STE20-related kinase	GEAAATEVDLER	AcD3-GEAAATEVDLER-COOH	LGSE <sub>667</sub> -G <sub>668</sub>
O35988	Syndecan-4	EVIDPQDLLEGR	AcD3-EVIDPQDLLEGR-COOH	IRET <sub>29</sub> -E <sub>30</sub>
P26039	Talin-1	SPKSTVLQQQYNR	AcD3-SPKSTVLQQQYNR-COOH	EDSV <sub>424</sub> -S <sub>425</sub>
Q62318	TIF1-beta	QPGGTLDLTLIR	AcD3-QPGGTLDLTLIR-COOH	FSME <sub>731</sub> -Q <sub>732</sub>
Q08784	Treacle protein	GGTLDLTLIR	AcD3-GGTLDLTLIR-COOH	MEQP <sub>733</sub> -G <sub>734</sub>
Q9JHJ0	Tropomodulin-3	SSPALAKGTQR	AcD3-SSPALAKGTQR-COOH	SASL <sub>591</sub> -S <sub>592</sub>
Q8R5H6	WAVE-1	DVLGSSNGVNQER	AcD3-DVLGSSN<Dam>GVNQR-COOH	TPFC <sub>150</sub> -D <sub>151</sub>
		ALPFSQMSSELLTR	AcD3-ALPFSQM<Mox>SELLTR-COOH	YLSL <sub>253</sub> -A <sub>254</sub>

Accession Nr.	Protein name	Spectra #	Start	End	m/z	z	error [Da]	Threshold	Score of highest scoring spectra	$\Delta$ Threshold	e-value
O08756	HADH2	1	238	251	830.92	2	-0.0656	47	57	10	0.005012942
O91WR3	ASCC2	1	617	632	875.898	2	0.0082	42	102	60	5.07816E-08
O9JKX6	Nuclt5	1	93	110	961.461	2	-0.0222	43	50	7	0.009994381
P17427	Ap2a2	1	694	707	744.929	2	0.0987	42	65	23	0.000252088
O7TQH0	Atxn2l	1	739	748	605.437	2	0.2174	41	48	7	0.009994381
P01887	Beta-2-microglobulin	3	23	32	655.371	2	-0.0034	42	62	20	0.000502592
O8BTV2	Cpsf7	1	202	214	687.405	2	0.0929	42	62	20	0.000502592
P97310	MCM2	1	94	110	953.444	2	-0.0308	42	50	8	0.007940871
P60898	RNA polymerase II subunit B9	1	7	15	541.831	2	0.0839	45	50	5	0.015831838
O9WUK2	eIF-4H	1	95	108	806.484	2	0.1112	42	97	55	1.60378E-07
P97323	Fbpase 3	1	40	46	484.85	2	0.1978	33	36	3	0.025078803
P49710	LckBP1	1	119	128	580.317	2	0.0255	41	71	30	5.03893E-05
P51859	HDFG	1	209	232	877.714	3	-0.0535	43	51	8	0.007940871
O02780	NF1-A	1	312	322	643.346	2	0.0961	41	52	11	0.003982951
O9D903	EBP2	2	17	29	762.861	2	-0.0221	42	65	23	0.000252088
O8K3X4	Protein C14orf4 homolog	1	116	129	750.431	2	-0.0151	42	75	33	2.52741E-05
P26350	Prothymosin alpha	1	68	89	818.719	3	0.1608	35	41	6	0.012578928
O9QZS2	RING finger protein 4	1	96	106	640.898	2	0.1843	42	51	9	0.006309289
O60710	MG11	1	445	458	795.417	2	0.0058	42	76	34	2.00811E-05
O8BT18	Srrm2	1	2324	2337	723.95	2	0.1479	30	45	15	0.001587282
P27048	snRNP-B	1	74	94	758.728	3	0.0143	46	62	16	0.001261149
P26369	U2AF2	4	133	146	765.91	2	0.0103	42	68	26	0.000126441
O54988	STE20-related kinase	1	668	679	653.168	2	-0.3073	45	52	7	0.009994381
O35988	Syndecan-4	1	30	41	714.856	2	-0.0361	42	62	20	0.000502592
P26039	Talin-1	1	425	438	906.519	2	0.0347	42	53	11	0.003982951
O62318	TIF1-beta	1	732	743	664.949	2	0.1296	42	42	0	0.05
O08784	Treacle protein	1	592	602	603.424	2	0.1652	42	44	2	0.031564182
O9JHJ0	Tropomodulin-3	2	151	163	710.876	2	0.0706	42	74	32	3.18099E-05
O8R5H6	WAVE-1	1	254	266	777.416	2	0.0176	47	58	11	0.003982951

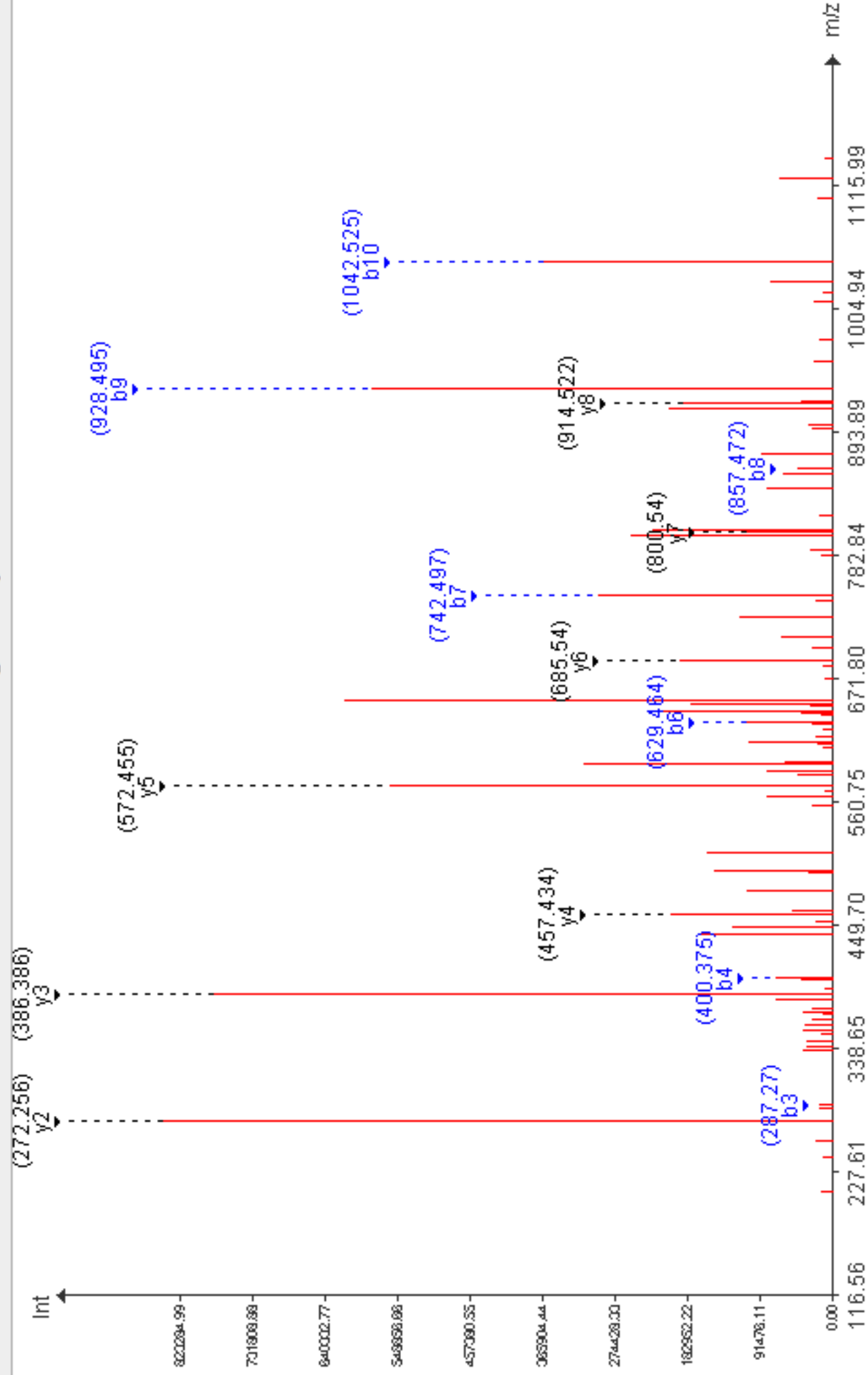
## **MS/MS-spectra of proteins in Table 1**

AcD3-SGPINDIDANPR-COOH

precursor mass: 657.3940 Da (charge: +2) protein: P97864 (199-210)

score (threshold): 39.0 (31.0)

instrument: Agilent Esquire HCT

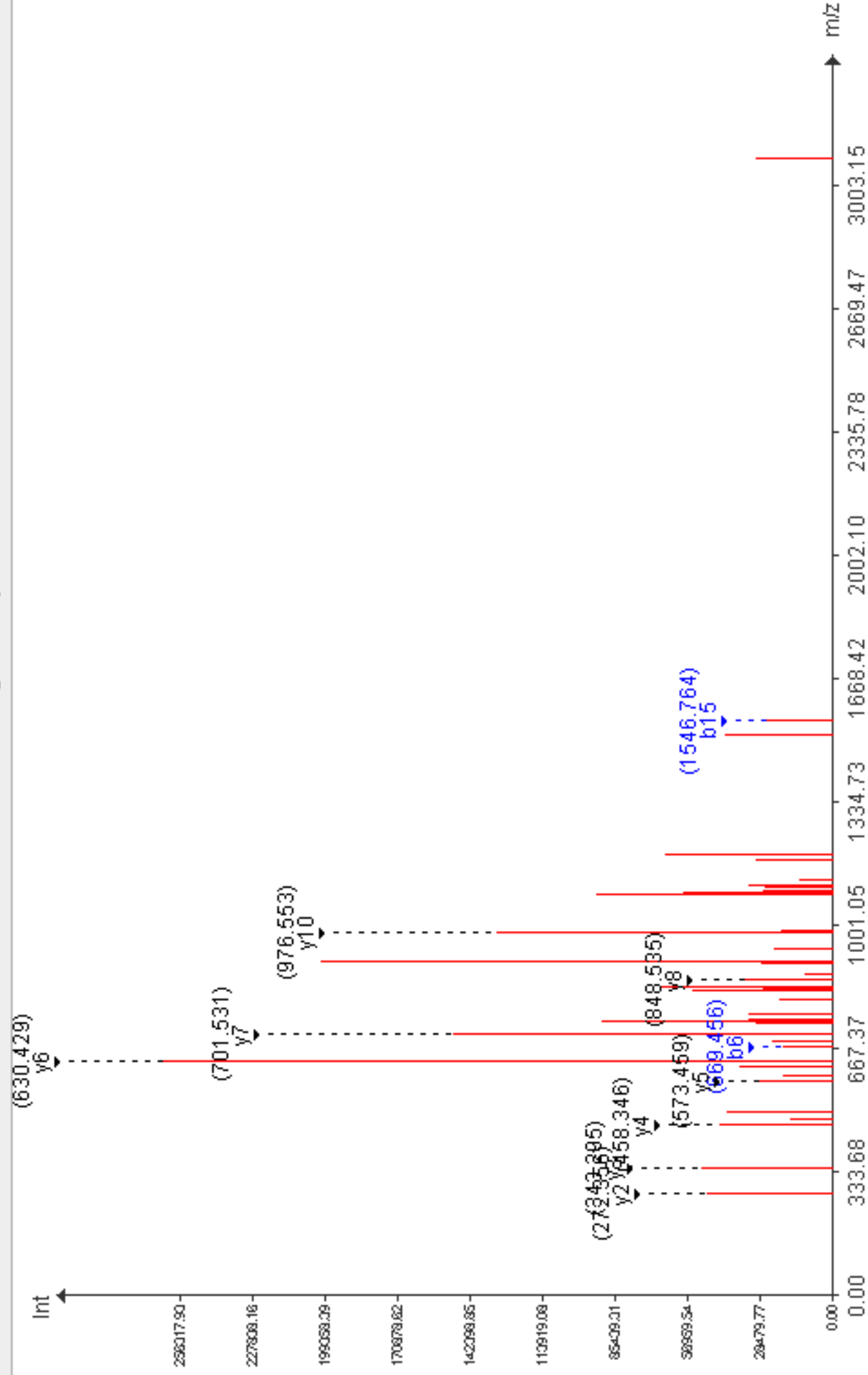


**AcD3-N(Dam)GSGM(Mox)C(Cmm\*)K(AcD3K\*)AGFAGDDAPR-COOH**

precursor mass: 909.3720 Da (charge: +2) protein: P60710 (12-28)

score (threshold): 46.0 (42.0)

instrument: Agilent Esquire HCT

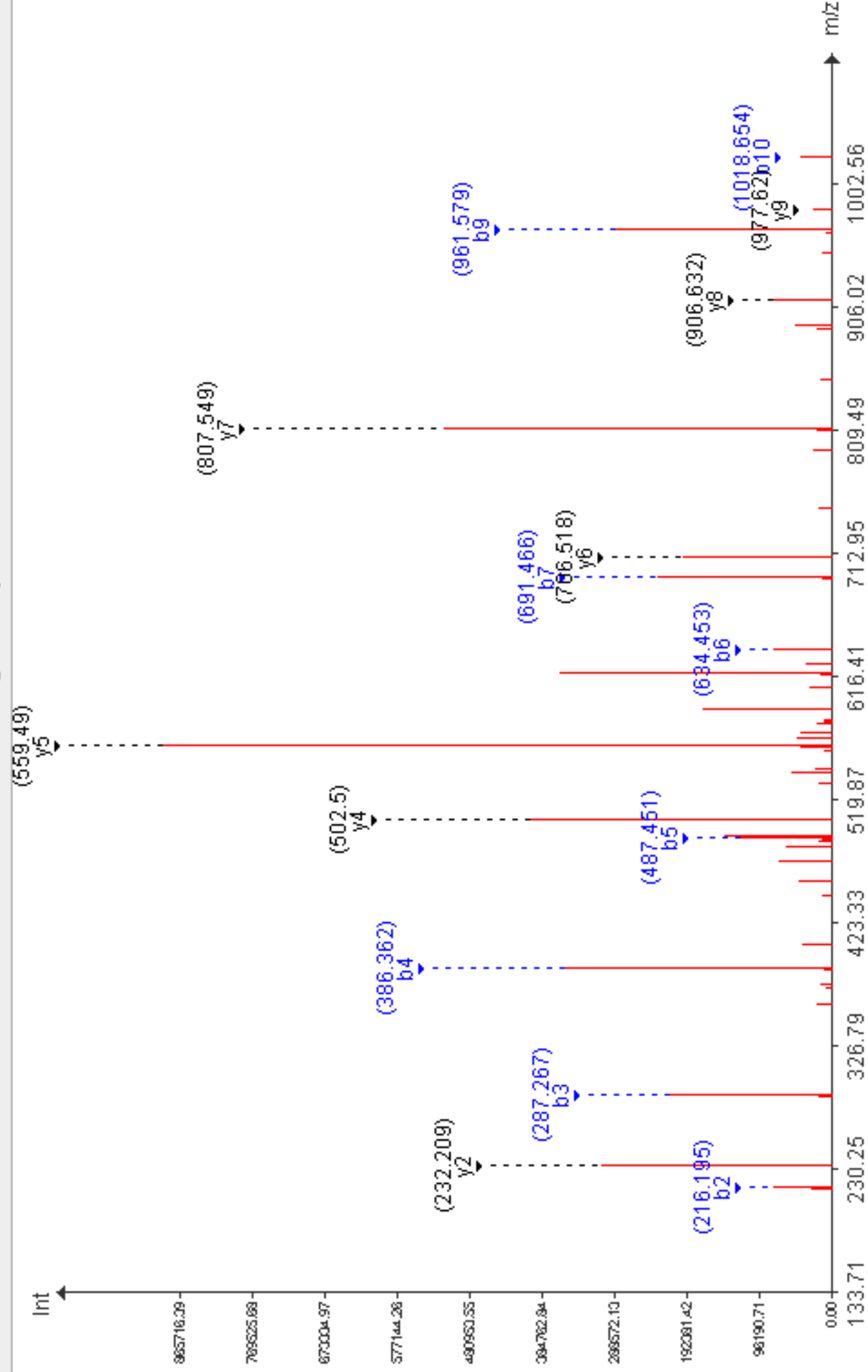


**AcD3-AVAVTM(Mox)GPK(AcD3K\*)GR-COOH**

precursor mass: 596.8780 Da (charge: +2) protein: P63038 (50-60)

score (threshold): 66.0 (42.0)

instrument: Agilent Esquire HCT



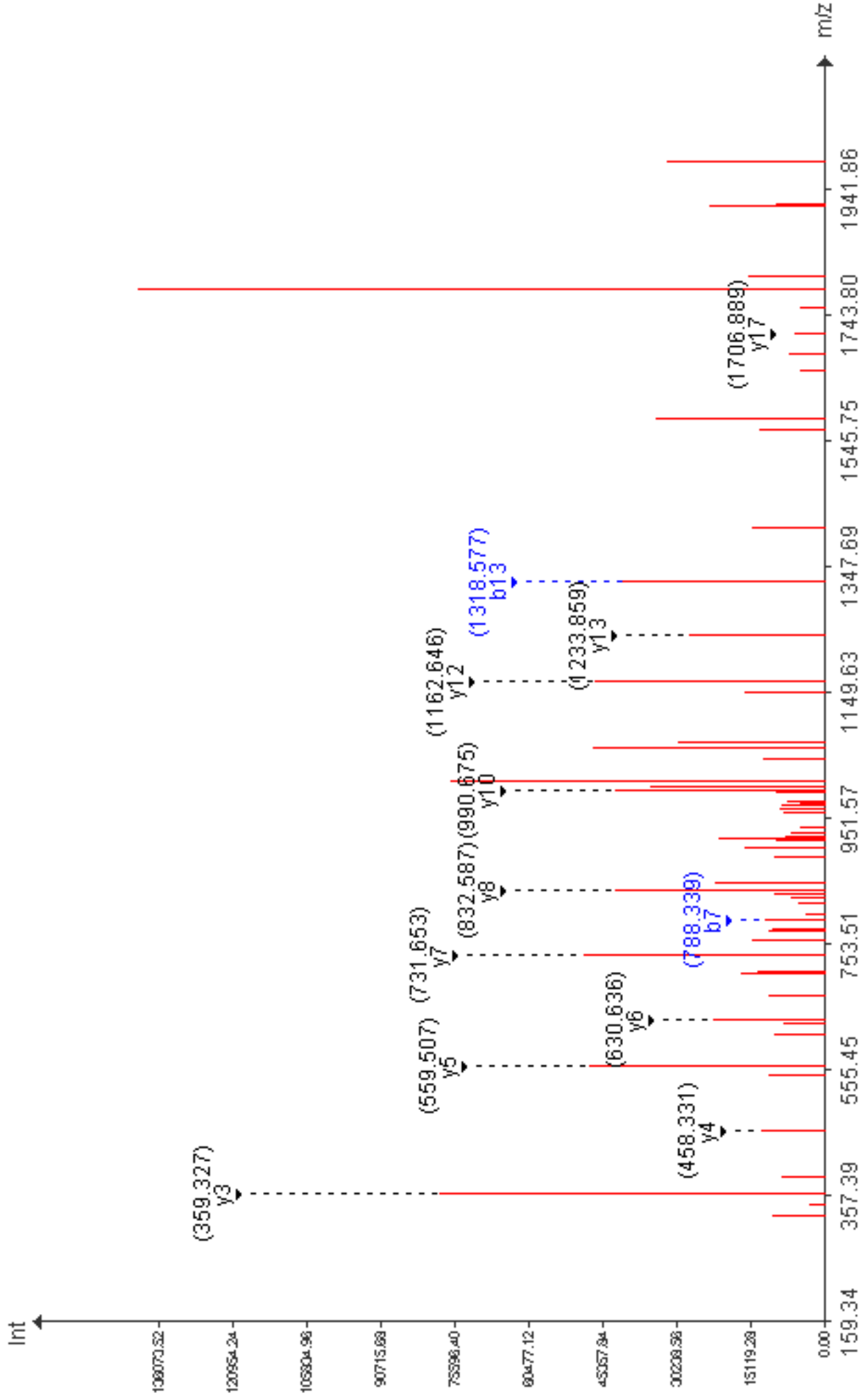


**AcD3-VANNTNEEAGDGTITATVLAR-COOH**

precursor mass: 1075.4600 Da (charge: +2) protein: P63038 (101-121)

score (threshold): 60.0 (43.0)

instrument: Agilent Esquire HCT

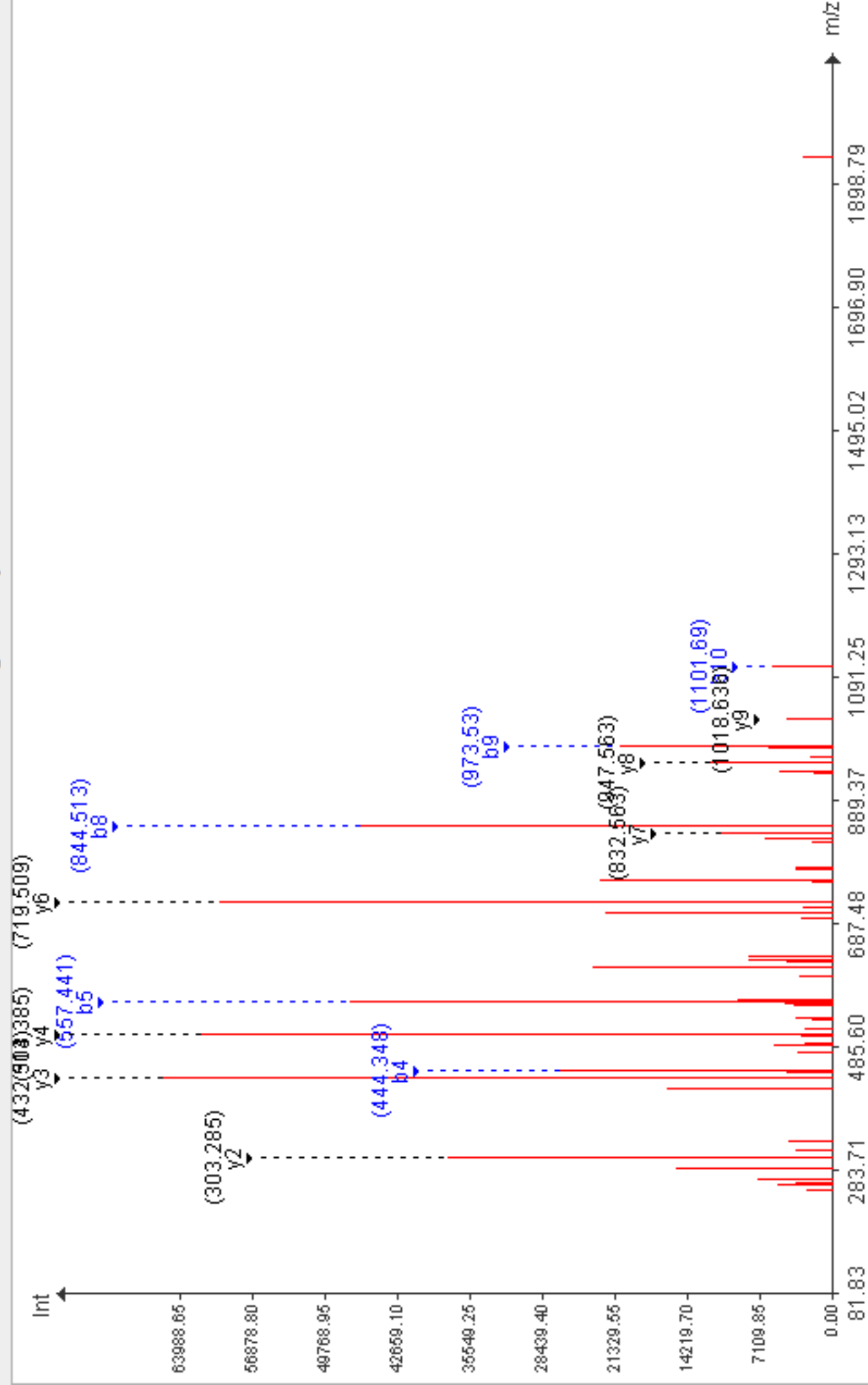


AcD3-LVADISEAEQR-COOH

precursor mass: 638.4350 Da (charge: +2) protein: Q8R5A3 (71-81)

score (threshold): 56.0 (42.0)

instrument: Agilent Esquire HCT

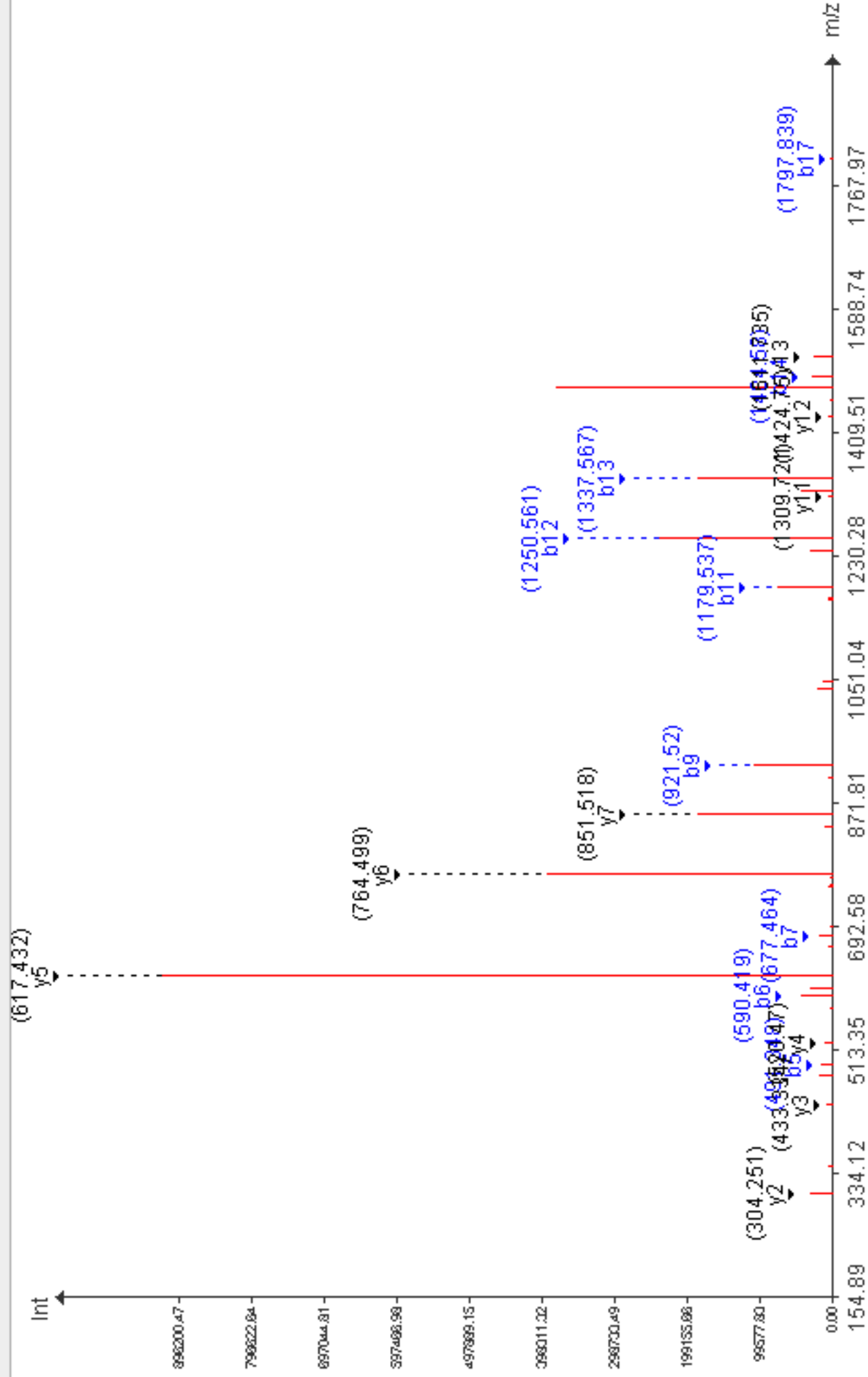


**AcD3-GDGSEVSDEEEASFPSEER-COOH**

precursor mass: 1050.9230 Da (charge: +2) protein: Q99KY4 (821-839)

score (threshold): 98.0 (43.0)

instrument: Agilent Esquire HCT

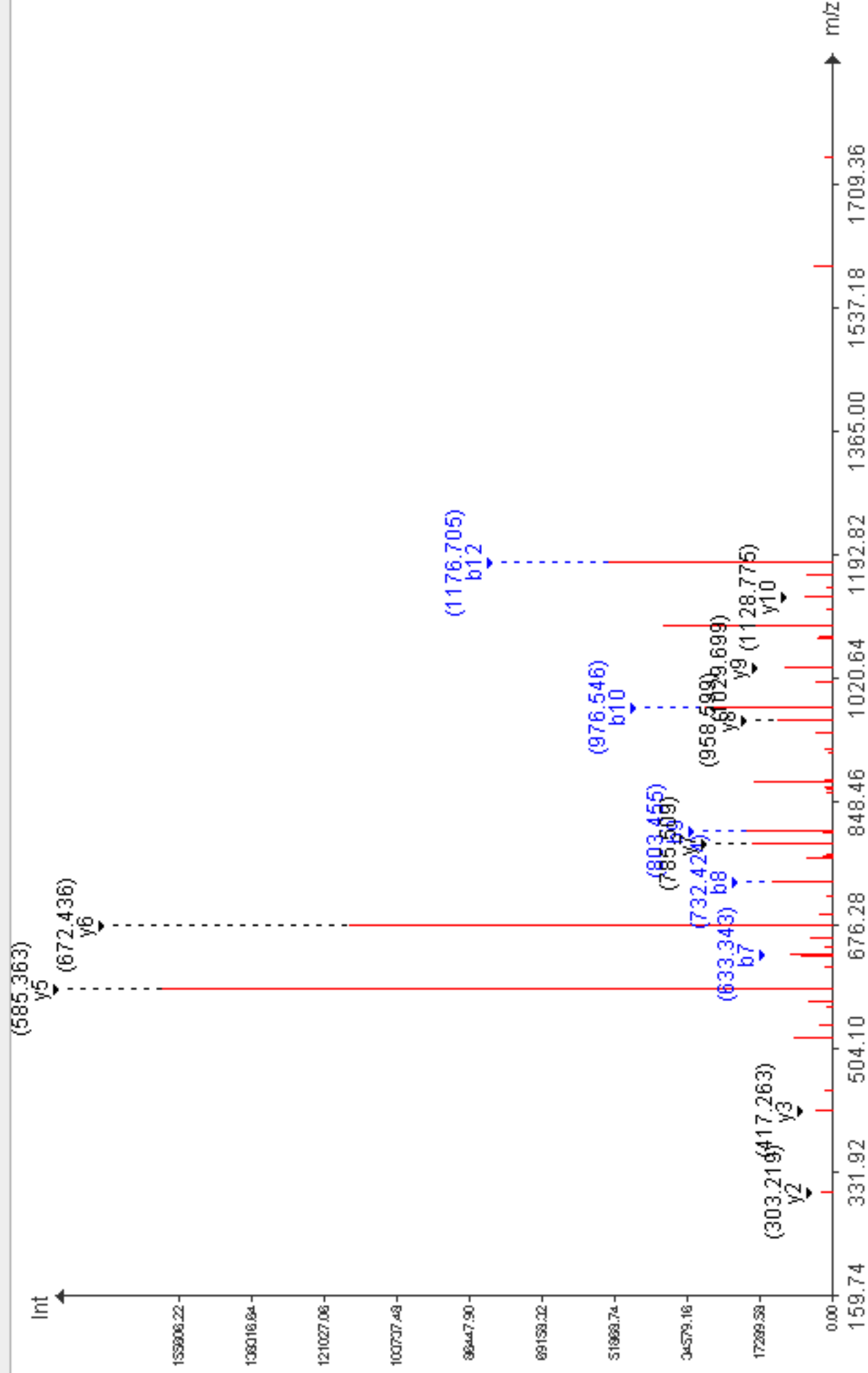


**ACD3-GADSTGVVAK(AcD3K\*)LSPANQR-COOH**

**precursor mass: 880.9750 Da (charge: +2) protein: Q62318 (685-701)**

**score (threshold): 62.0 (42.0)**

**instrument: Agilent Esquire HCT**

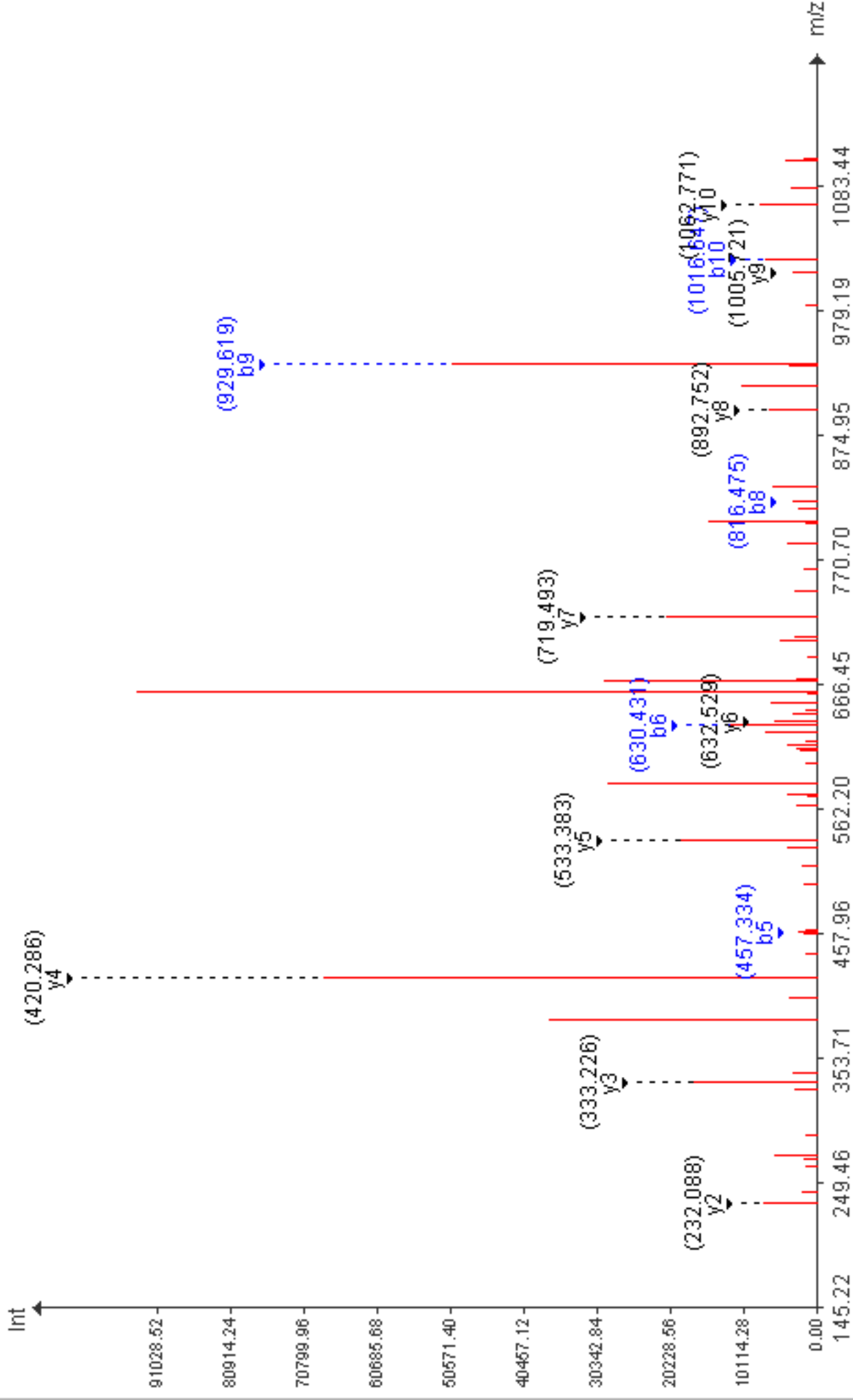


**AcD3-GSPGLK(AcD3K\*)SVLSTGR-COOH**

precursor mass: 674.9280 Da (charge: +2) protein: Q5R.JH6 (518-530)

score (threshold): 64.0 (42.0)

instrument: Agilent Esquire HCT

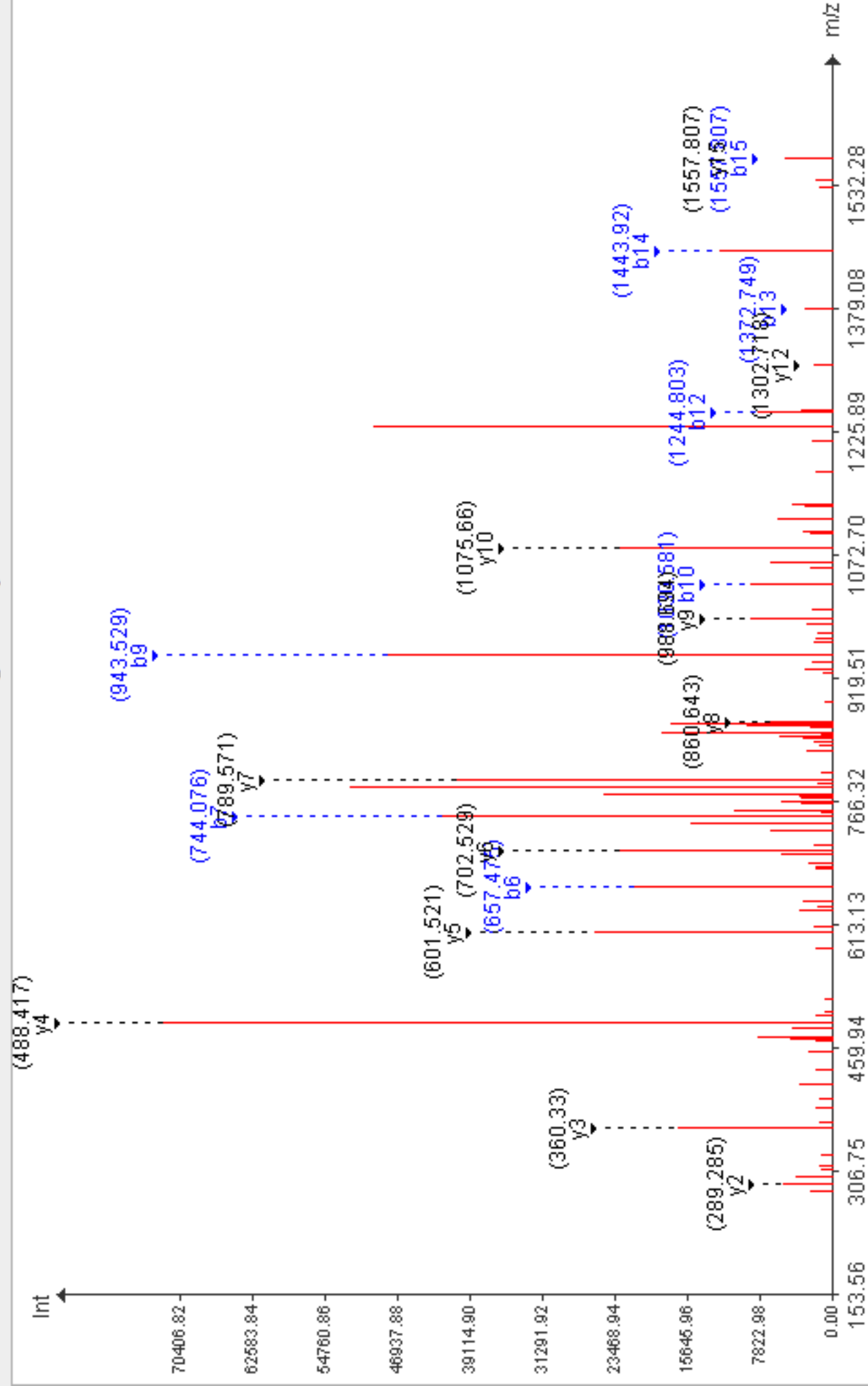


**ACD3-EAPSNLSQASTLQANR-COOH**

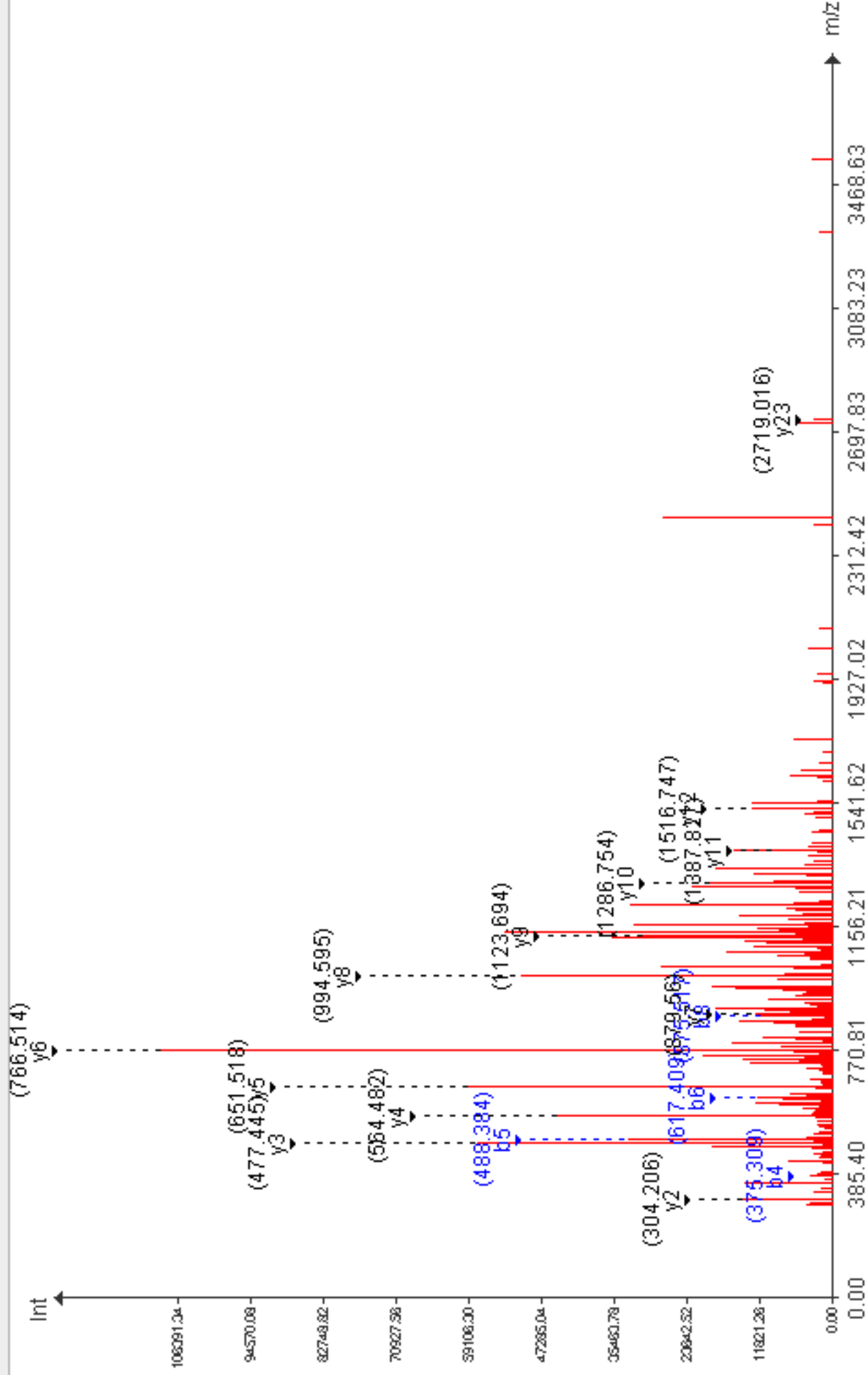
precursor mass: 866.4930 Da (charge: +2) protein: Q7TMY8 (2385-2400)

score (threshold): 63.0 (42.0)

instrument: Agilent Esquire HCT



**AcD3-GTGNLEEEQ(Dam)ESEGETYEDIDSSK(AcD3K\*)ER-COOH**  
precursor mass: 974.7710 Da (charge: +3) protein: O35601 (435-459)  
score (threshold): 54.0 (43.0)  
instrument: Agilent Esquire HCT

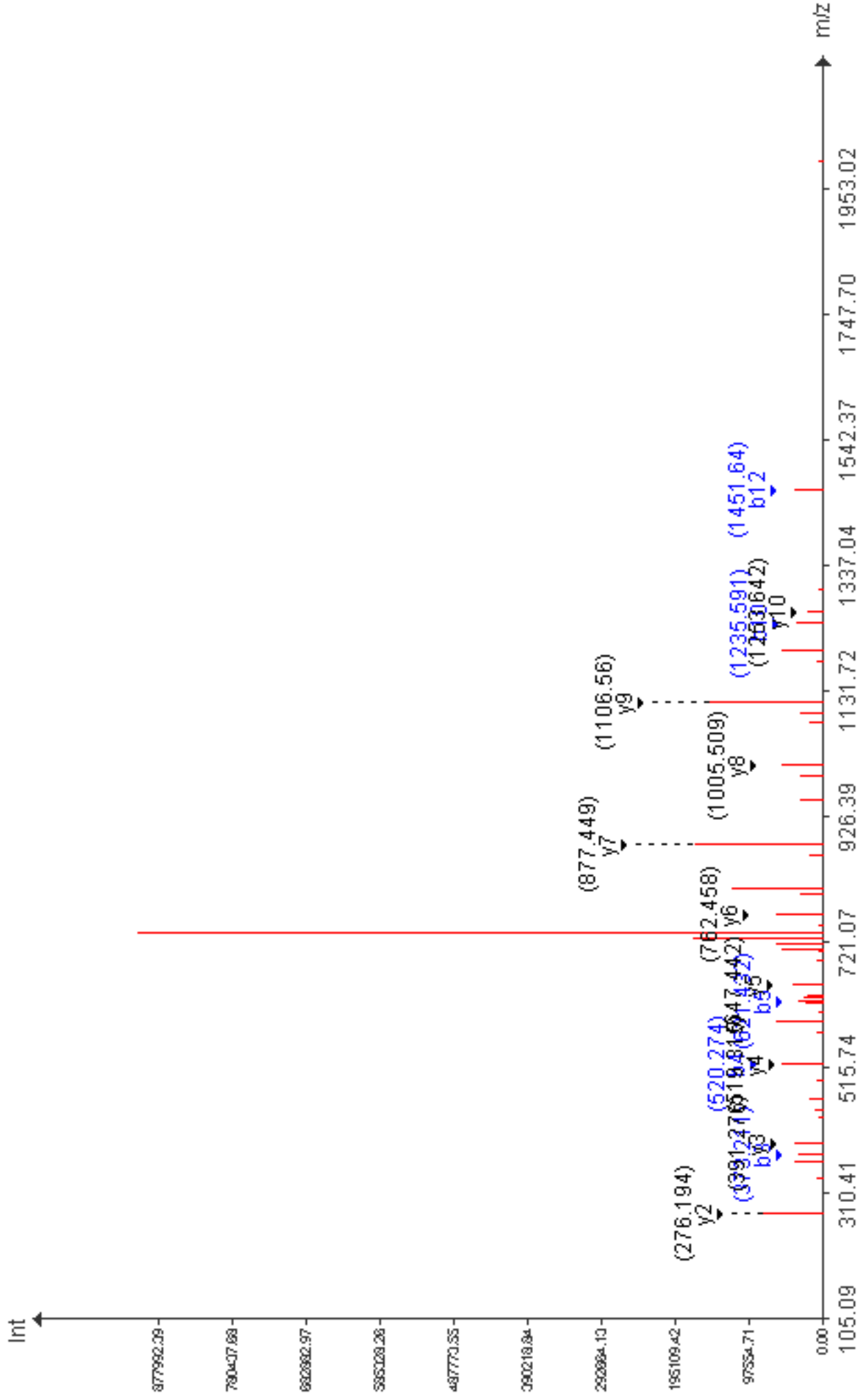


**AcD3-DPDPFTQDDQDTR-COOH**

precursor mass: 813.3640 Da (charge: +2) protein: P25206 (531-543)

score (threshold): 63.0 (42.0)

instrument: Agilent Esquire HCT



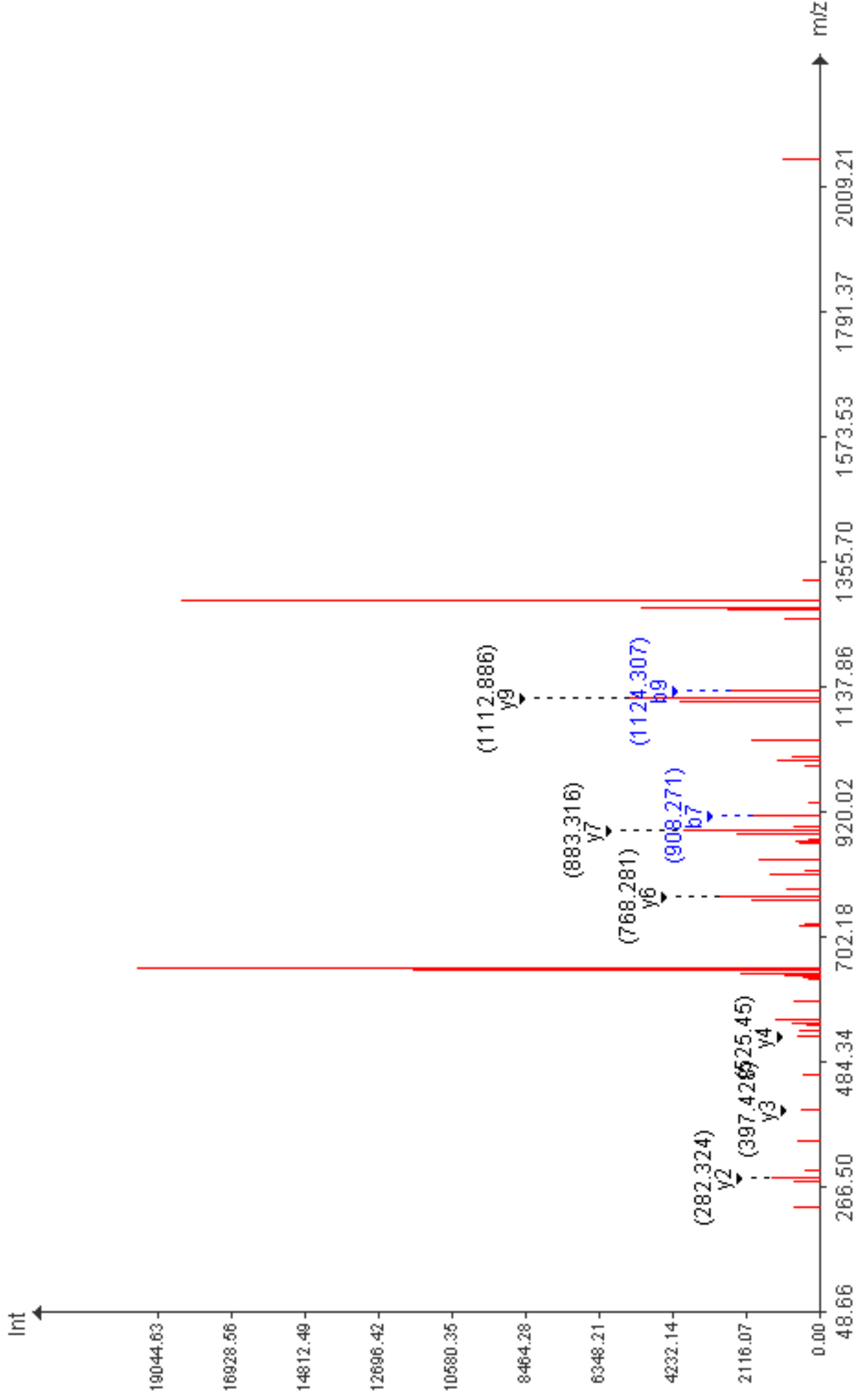


**AcD3-FTQDDQDTR(C13\*)-COOH**

precursor mass: 652.8030 Da (charge: +2) protein: P25206 (534-543)

score (threshold): 35.0 (31.0)

instrument: Bruker Esquire HCT

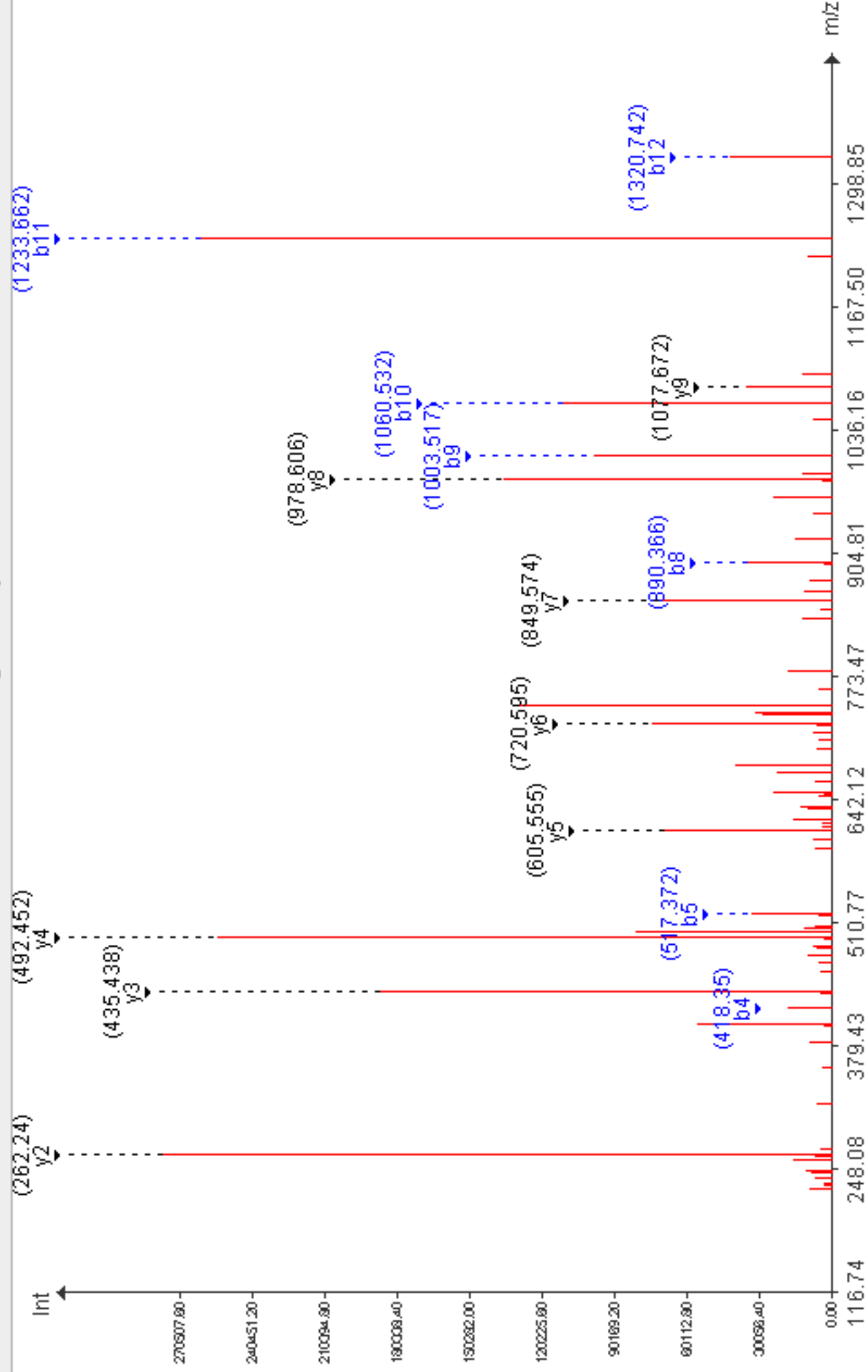


**AcD3-VDGTVVEEDLGK(AcD3K\*)SR-COOH**

precursor mass: 747.9110 Da (charge: +2) protein: P08113 (27-39)

score (threshold): 76.0 (42.0)

instrument: Agilent Esquire HCT

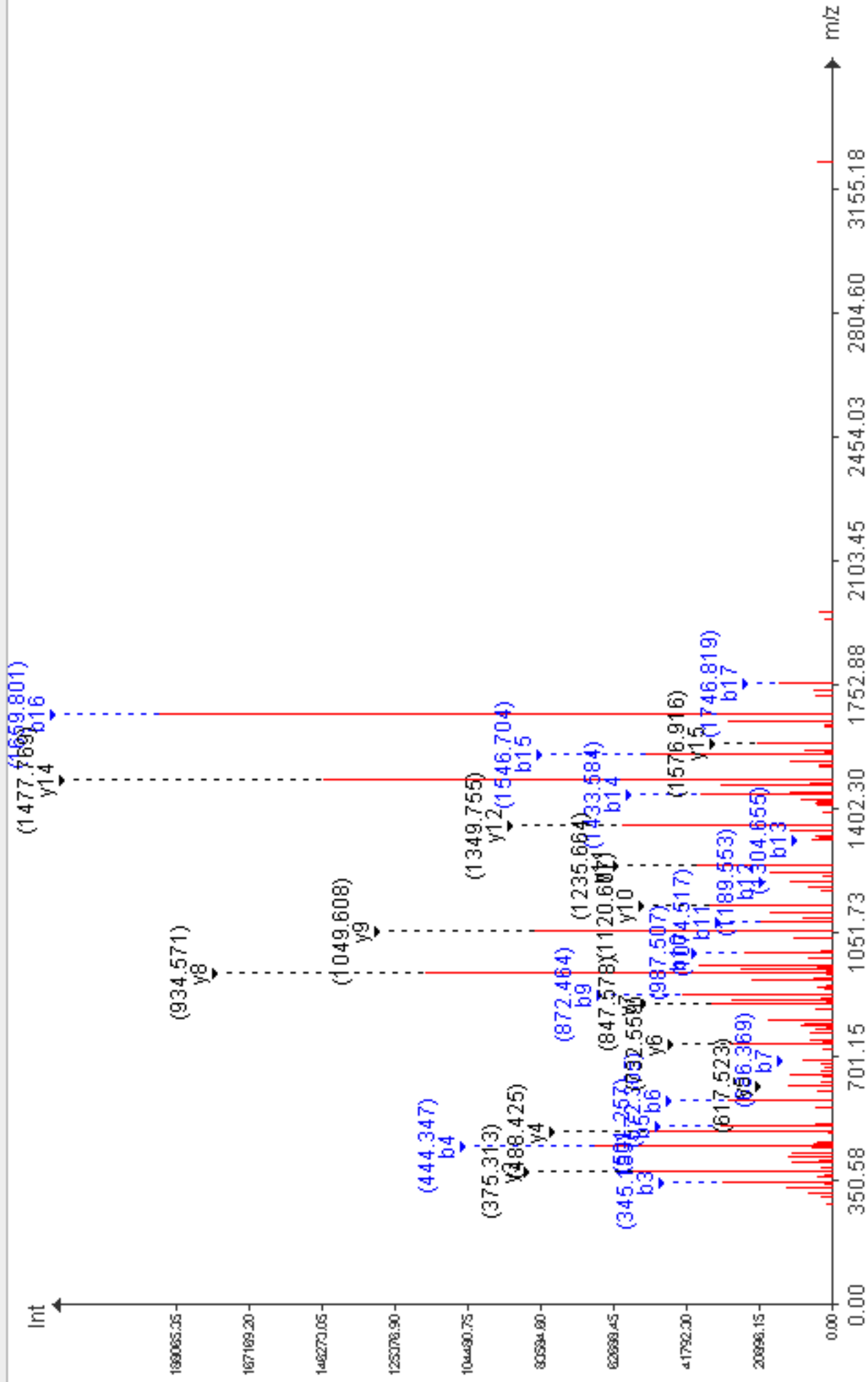


AcD3-GNQVGANDSDDELISR-COOH

precursor mass: 960.9580 Da (charge: +2) protein: Q91WR3 (615-632)

score (threshold): 131.0 (42.0)

instrument: Agilent Esquire HCT

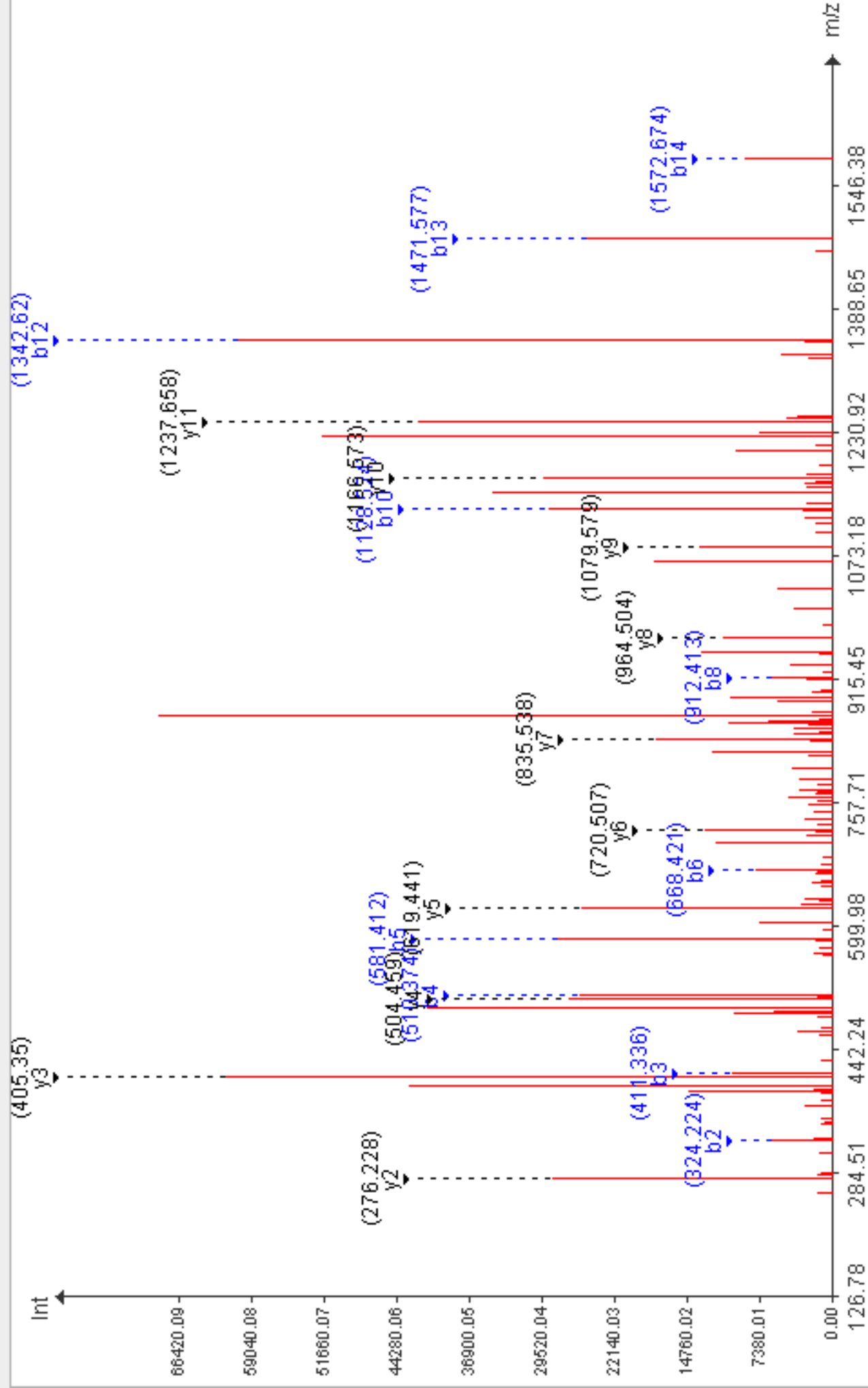


**AcD3-YDSVASEDEDIVETR-COOH**

precursor mass: 873.8850 Da (charge: +2) protein: Q9JLQ2 (391-405)

score (threshold): 90.0 (42.0)

instrument: Agilent Esquire HCT

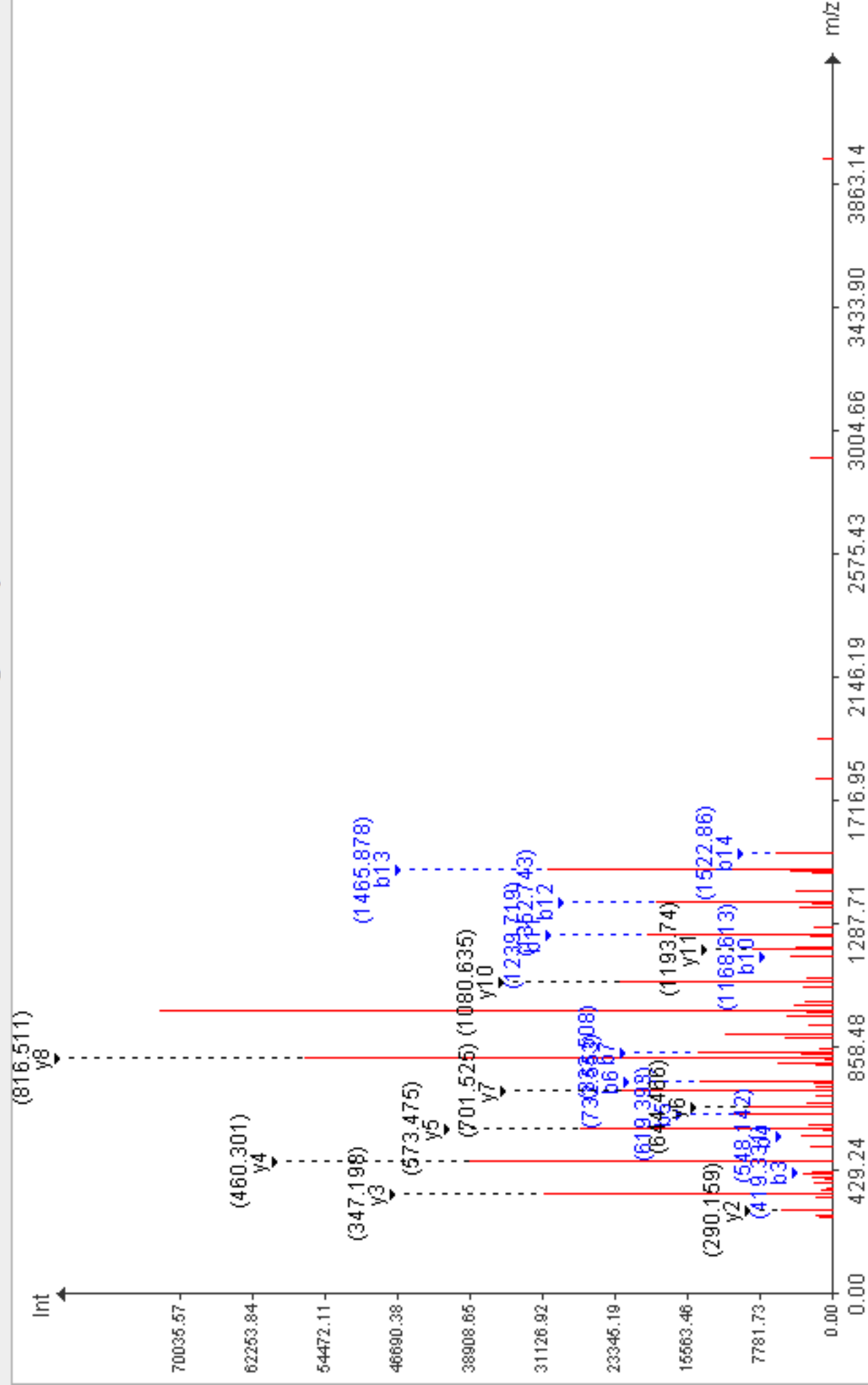


**AcD3-SLK(AcD3K\*)EALTYDGALLGDR-COOH**

precursor mass: 906.3990 Da (charge: +2) protein: Q9WUJ2 (93-108)

score (threshold): 72.0 (47.0)

instrument: Agilent Esquire HCT

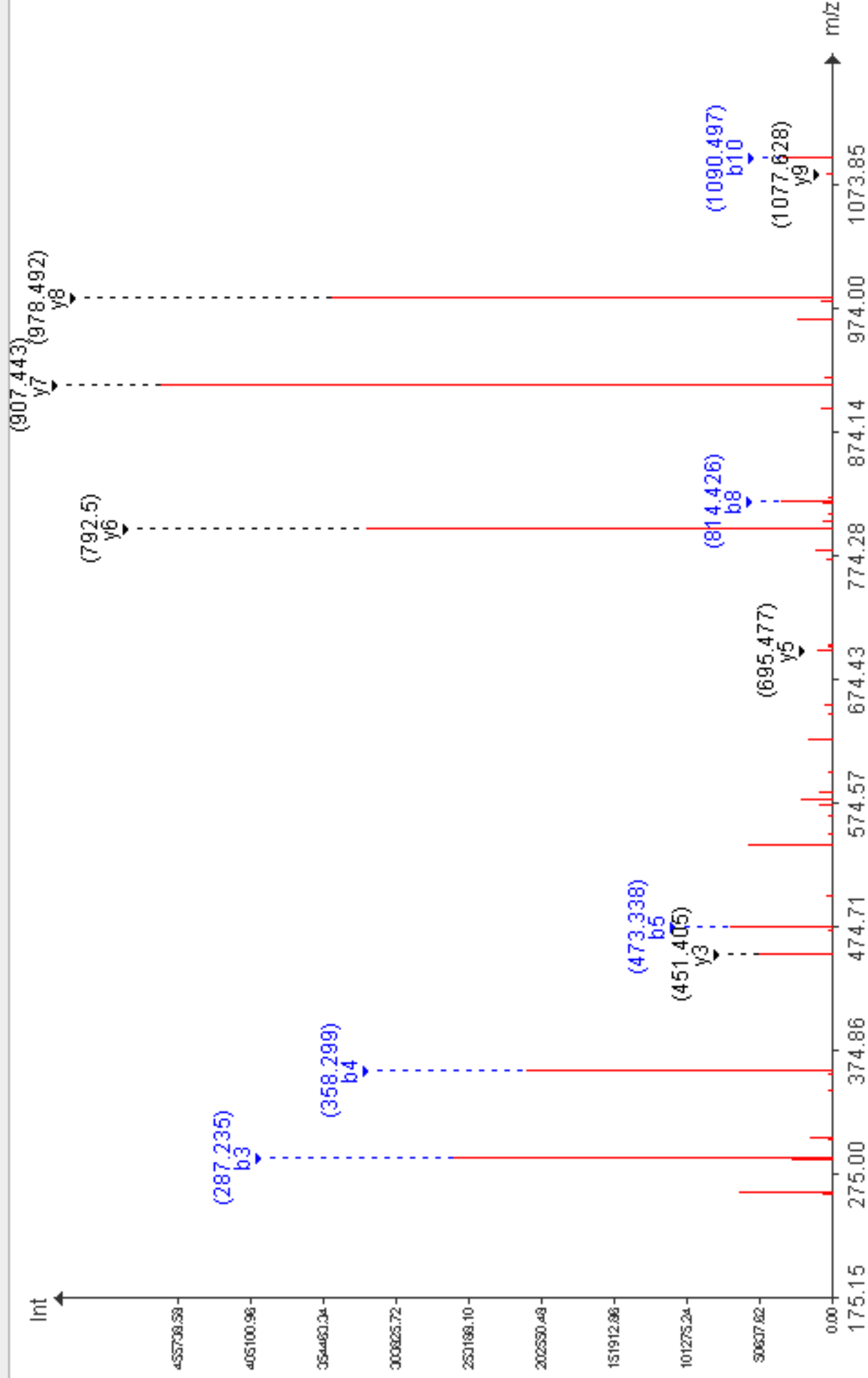


**AcD3-AAVADPDEFER-COOH**

precursor mass: 632.8480 Da (charge: +2) protein: P47713 (522-532)

score (threshold): 58.0 (42.0)

instrument: Agilent Esquire HCT

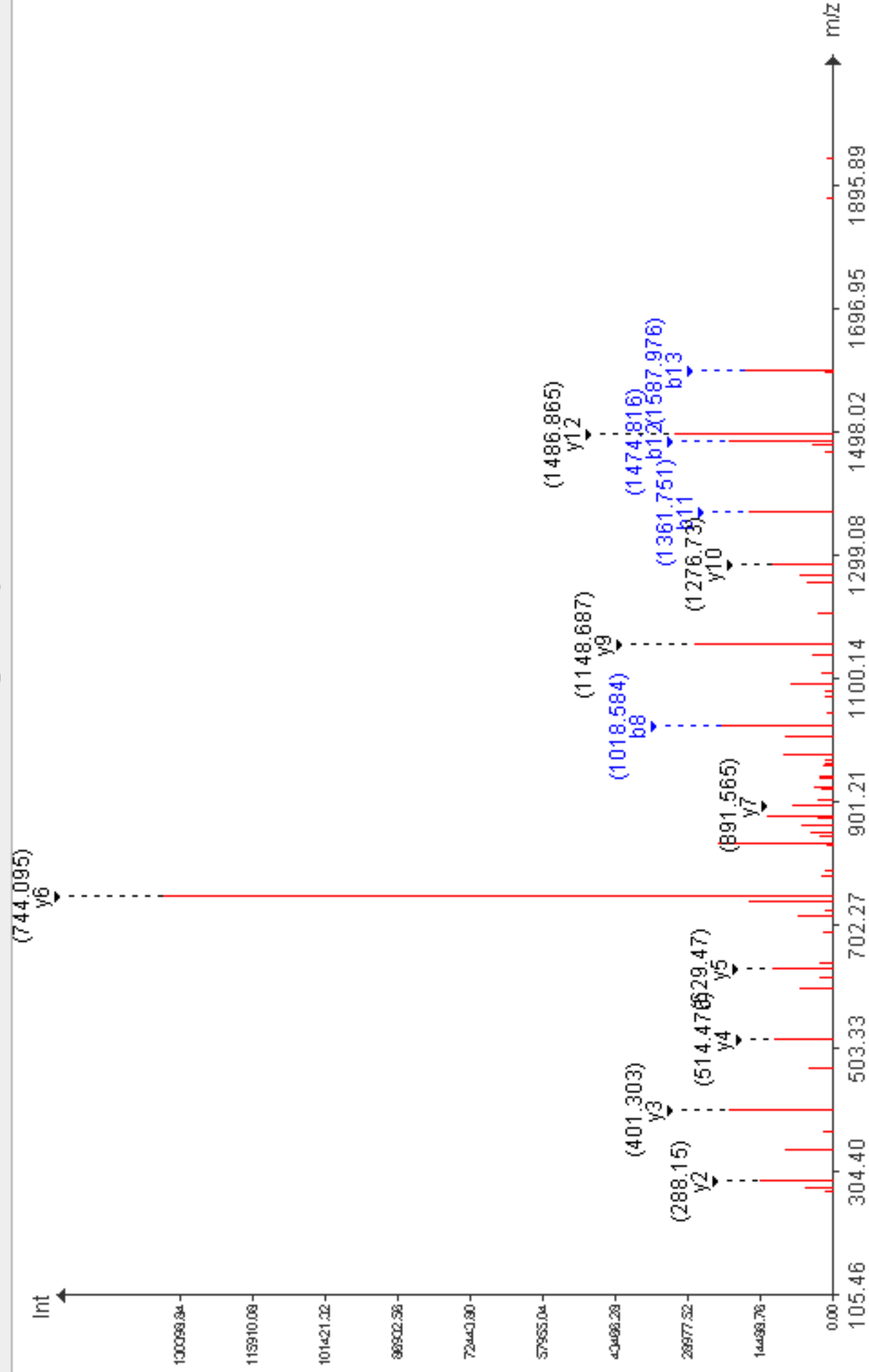


**AcD3-GK(AcD3K\*)PLQEQM(Mox)DLLLLR-COOH**

precursor mass: 881.5200 Da (charge: +2) protein: Q61211 (244-257)

score (threshold): 66.0 (42.0)

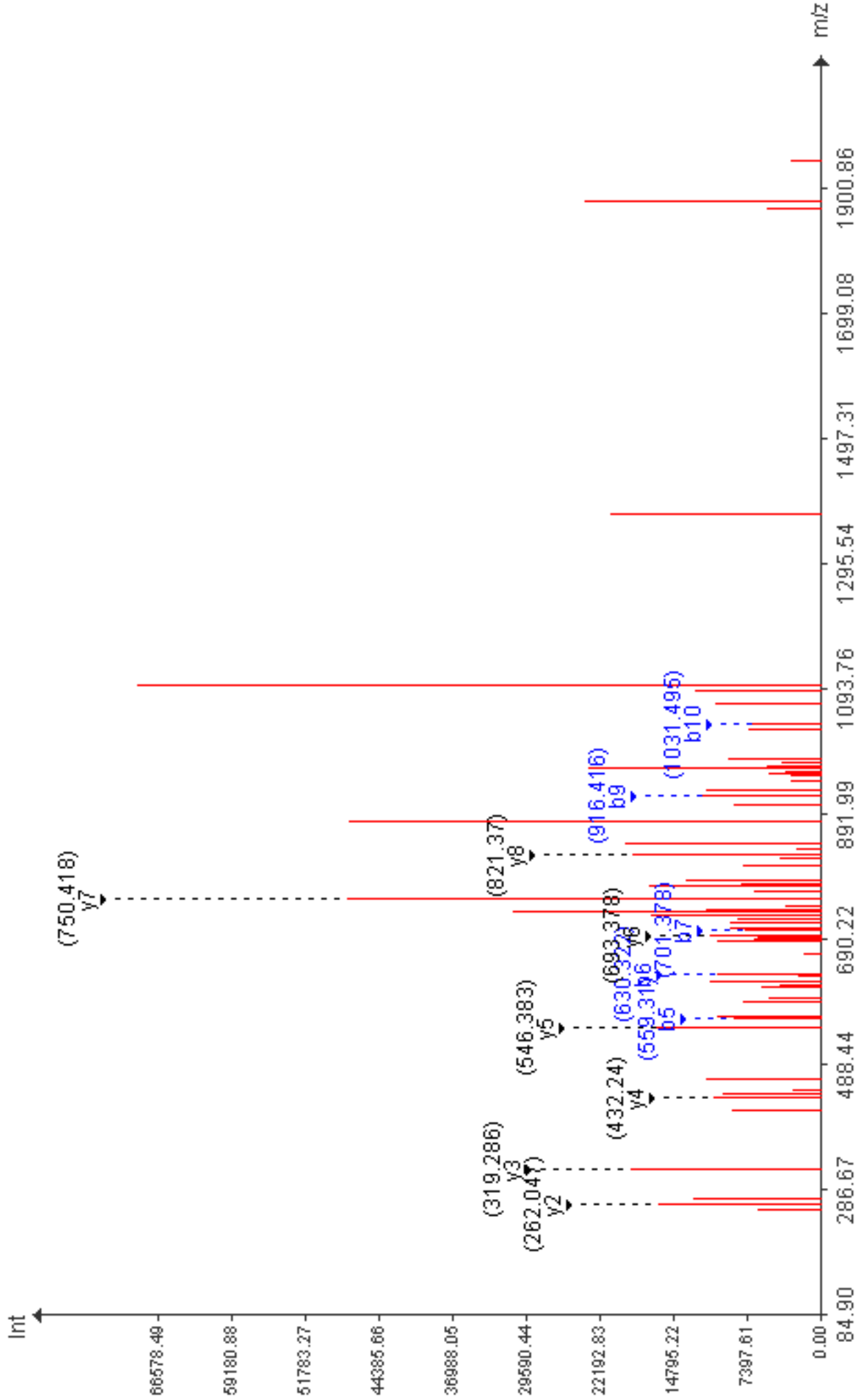
instrument: Agilent Esquire HCT



**AcD3-M(Mox)GPDPAATNN(Dam)LSQLAGM(Mox)NLGSR-COOH**  
precursor mass: 794.0430 Da (charge: +3) protein: O88746 (325-347)

score (threshold): 45.0 (43.0)

instrument: Agilent Esquire HCT



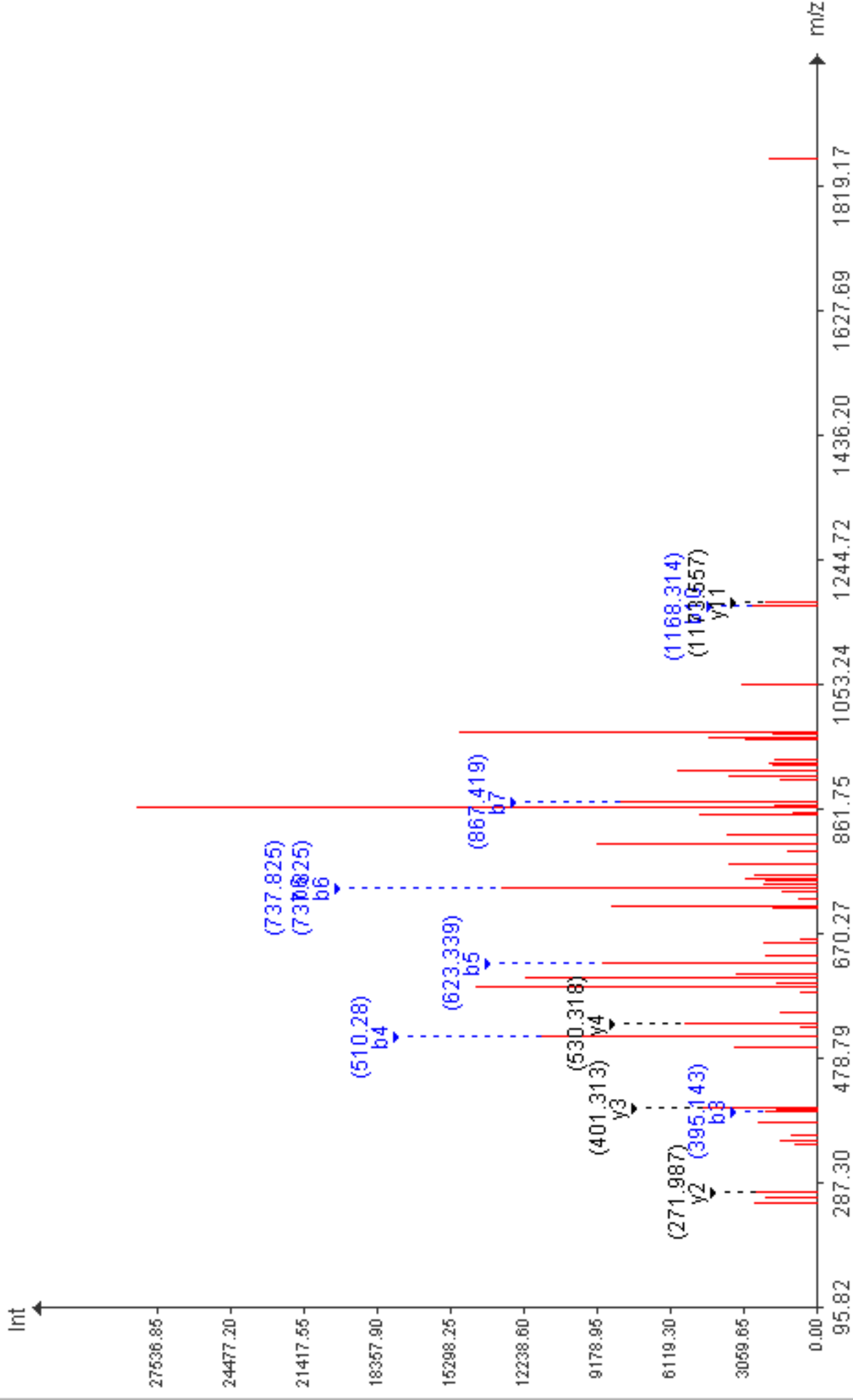


**AcD3-SDFIDEGDEPSSDGEAEPR-COOH**

precursor mass: 780.9510 Da (charge: +3) protein: Q62481 (59-79)

score (threshold): 40.0 (38.0)

instrument: Agilent Esquire HCT

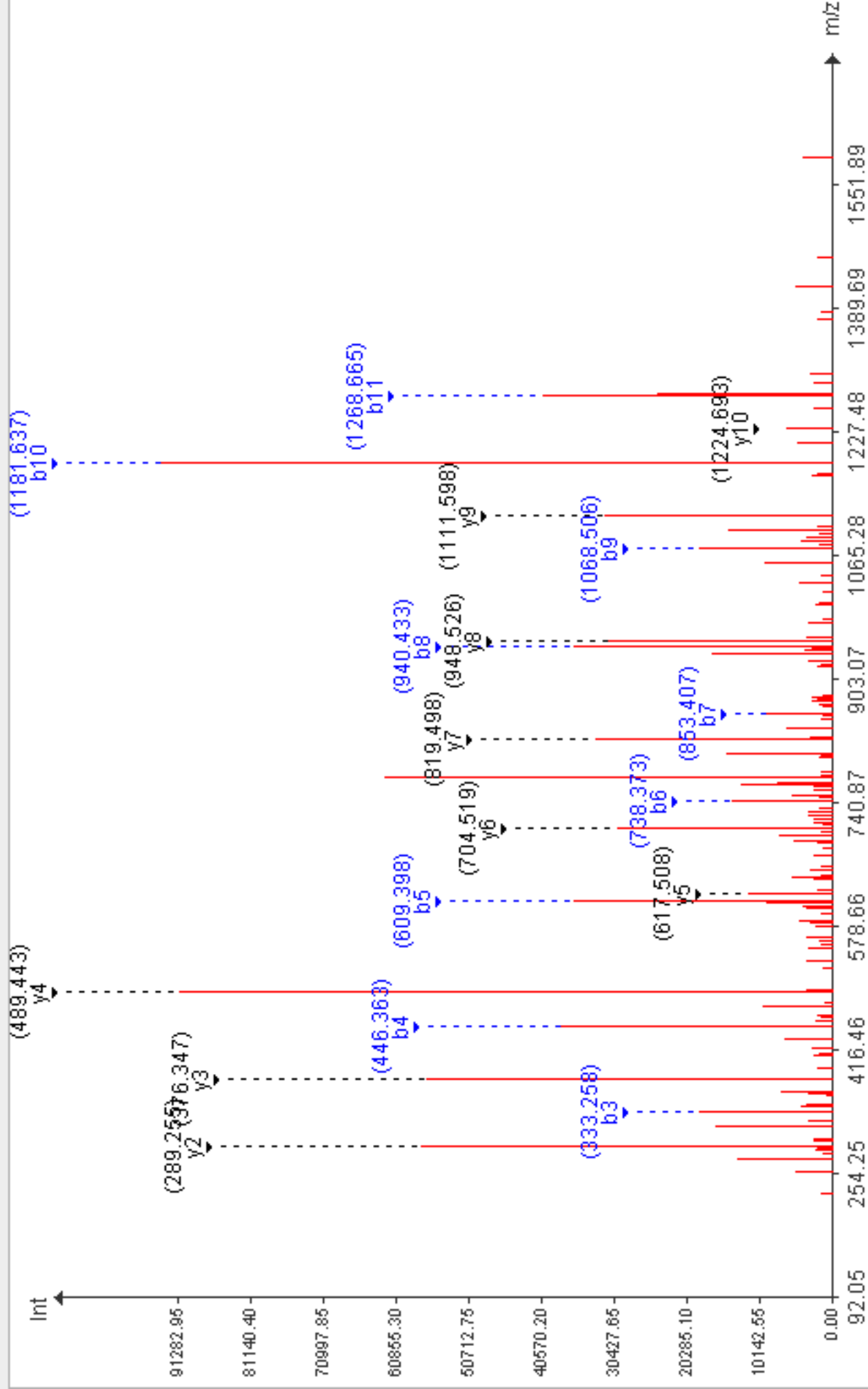


**AcD3-GTELYEDSQLSNR-COOH**

precursor mass: 778.8580 Da (charge: +2) protein: Q8K2H1 (292-304)

score (threshold): 71.0 (47.0)

instrument: Agilent Esquire HCT

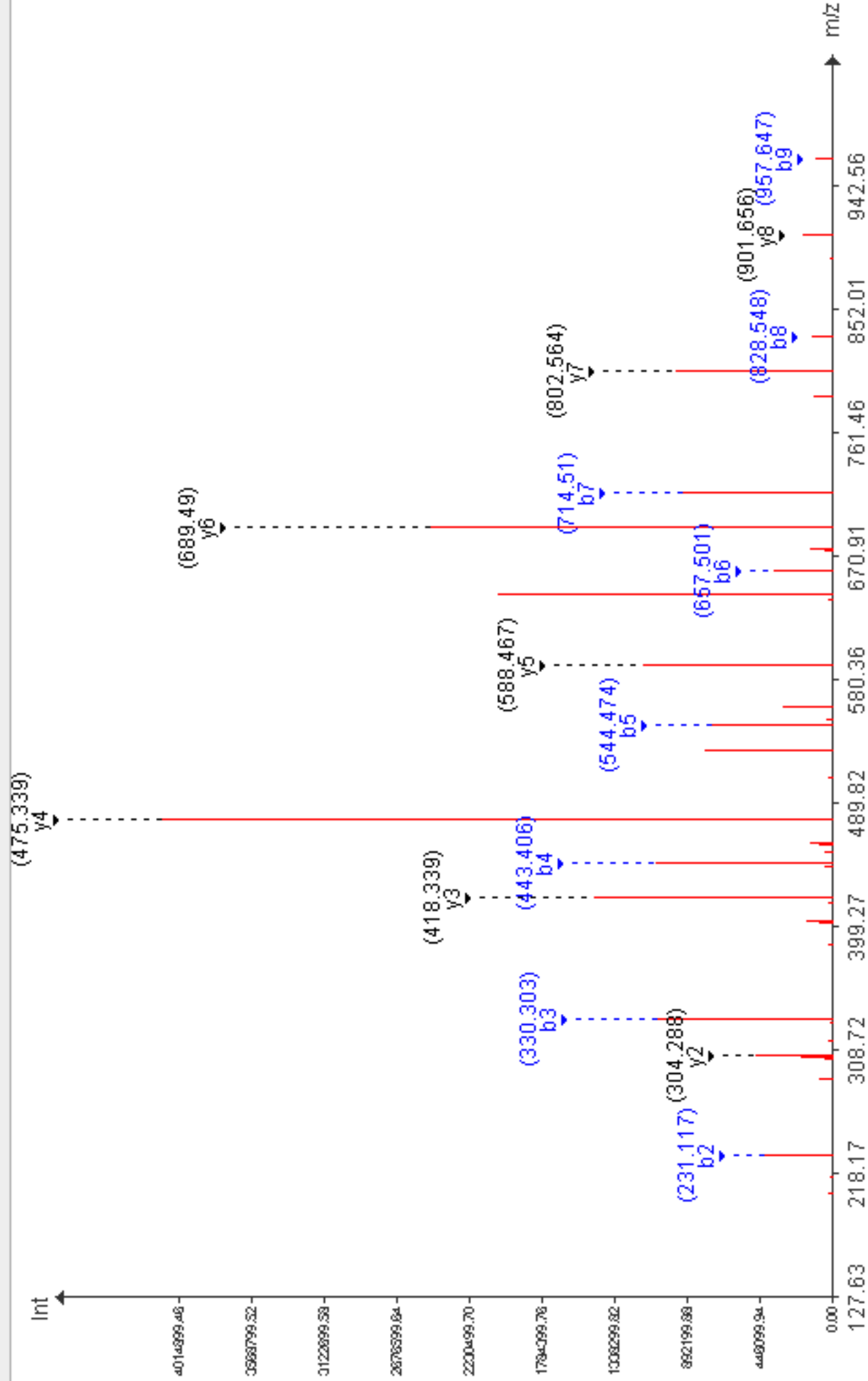


**AcD3-GQVITIGNER-COOH**

precursor mass: 566.3810 Da (charge: +2) protein: P62737 (247-256)

score (threshold): 87.0 (41.0)

instrument: Agilent Esquire HCT

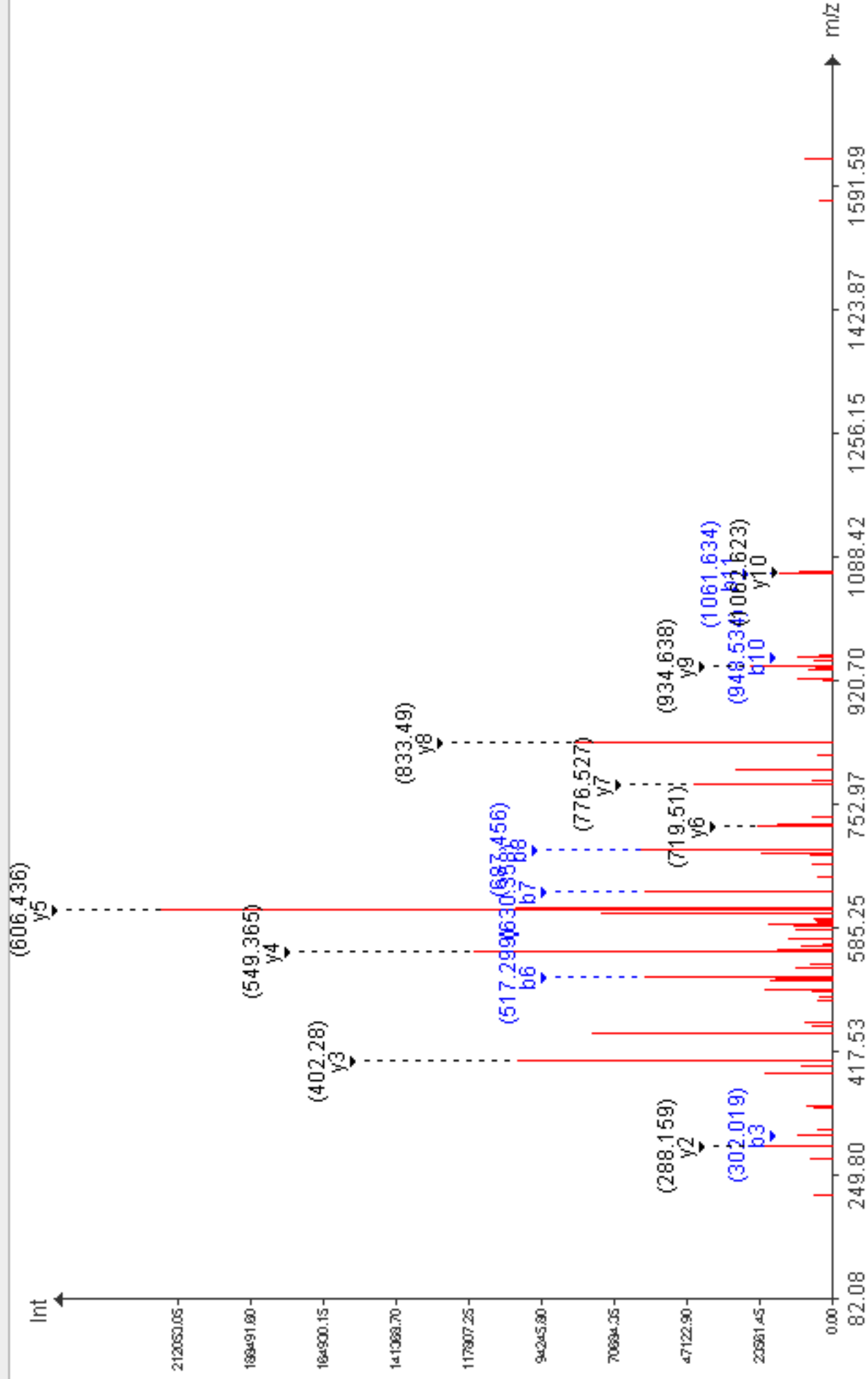


AcD3-GAQTGGLGFNLR-COOH

precursor mass: 618.3240 Da (charge: +2) protein: Q61152 (425-436)

score (threshold): 81.0 (42.0)

instrument: Agilent Esquire HCT



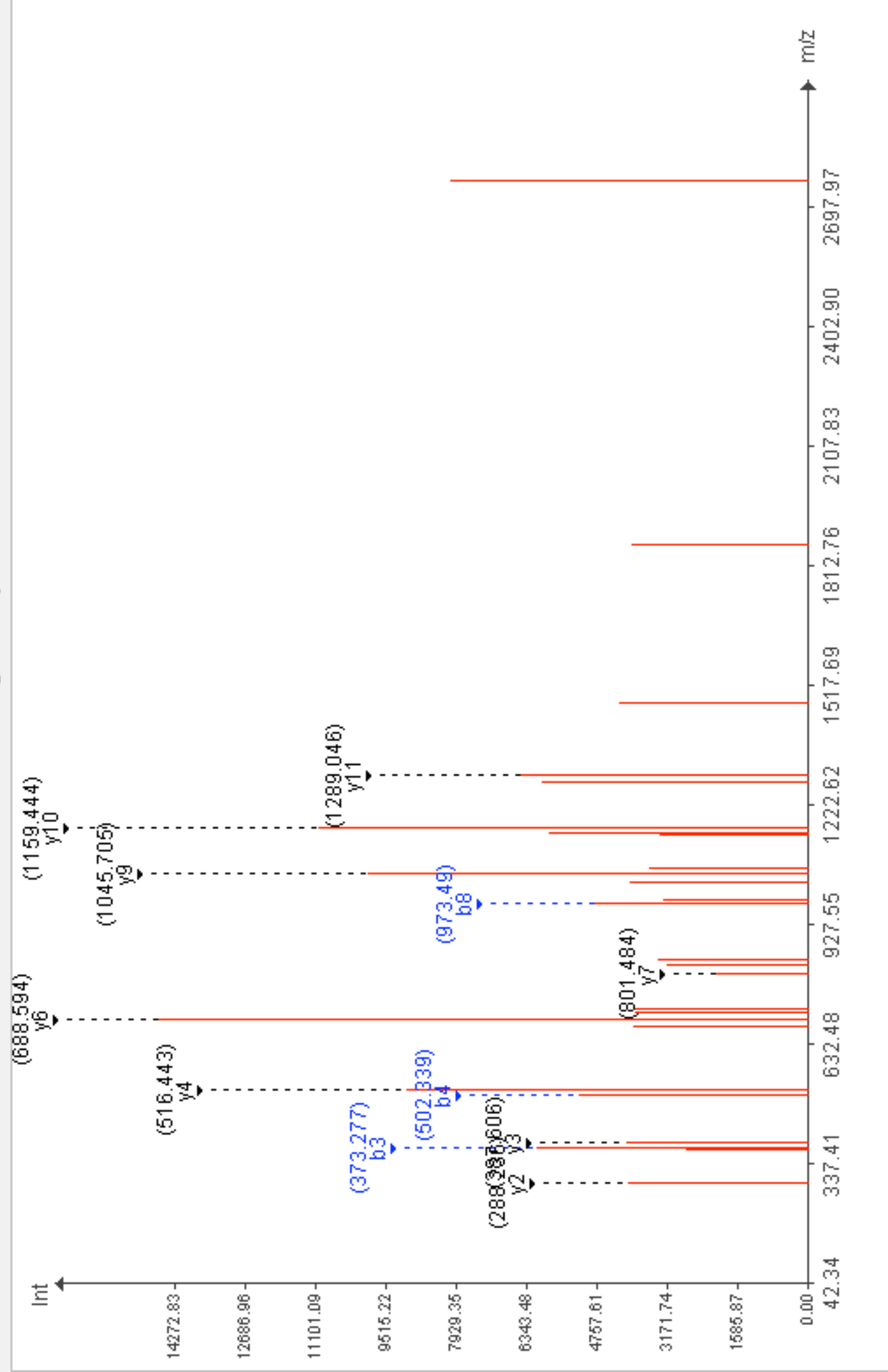
## **MS/MS-spectra of proteins in Supplementary Table 1**

**ACD3-TIIENPFLN(Dam)GEVIR-COOH**

precursor mass: 830.9200 Da (charge: +2) protein: O08756 (238-251)

score (threshold): 57.0 (47.0)

instrument: Agilent Esquire HCT

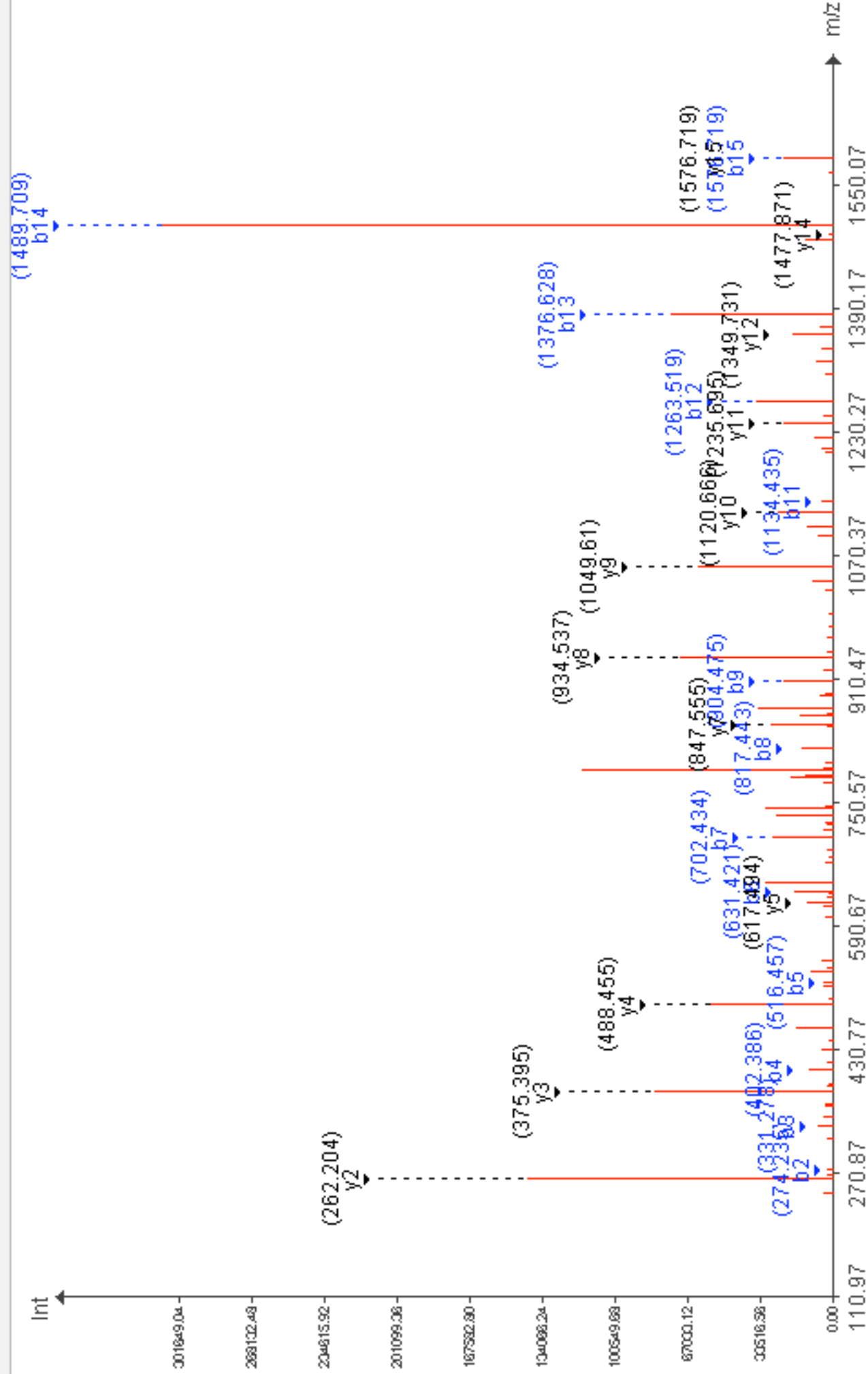


AcD3-Q(Dam)VGANDADSDDELISR-COOH

precursor mass: 875.8980 Da (charge: +2) protein: Q91WR3 (617-632)

score (threshold): 102.0 (42.0)

instrument: Agilent Esquire HCT

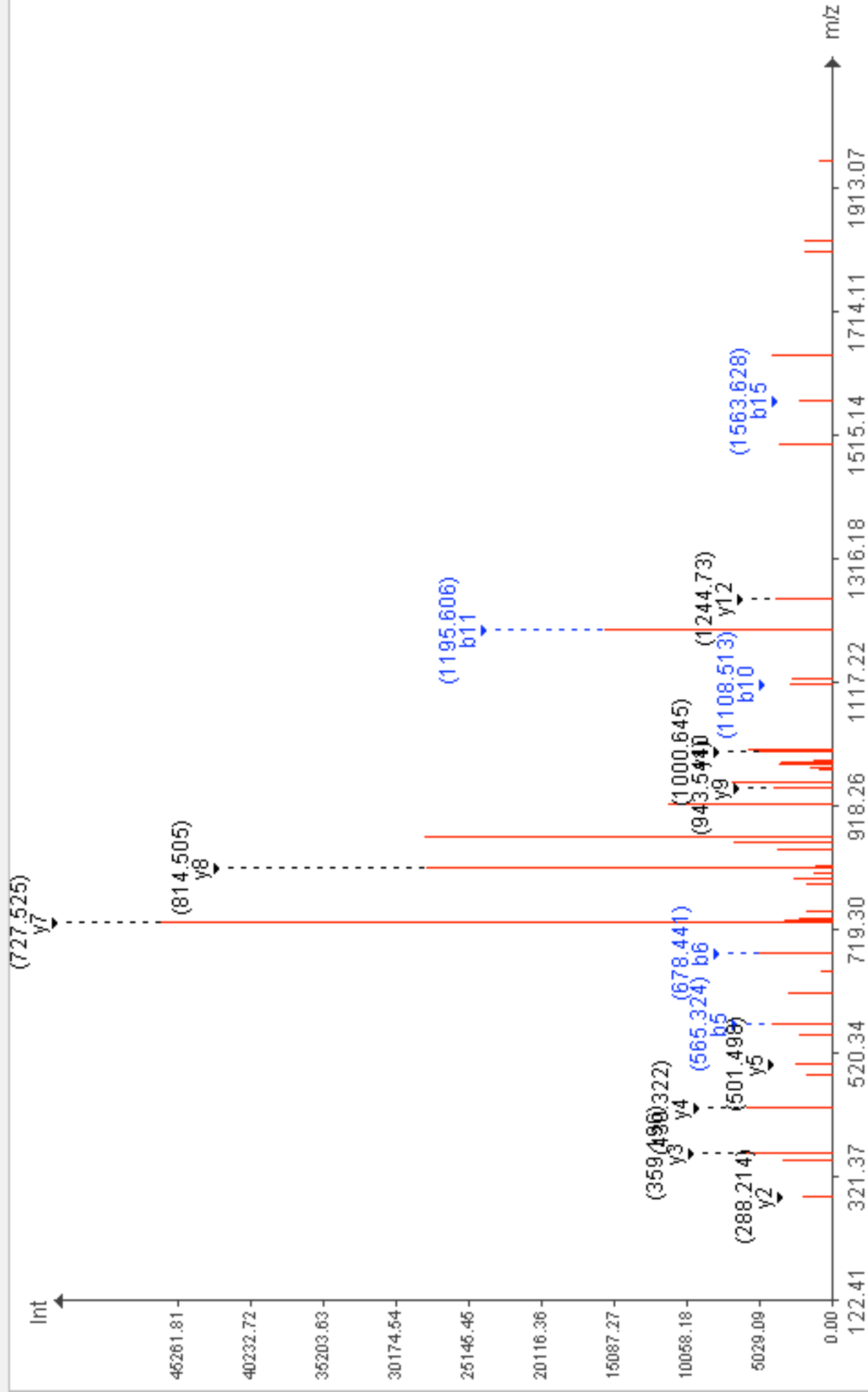


AcD3-FPAGFIEDGESPEEAALR-COOH

precursor mass: 961.4610 Da (charge: +2) protein: Q9JKX6 (93-110)

score (threshold): 50.0 (43.0)

instrument: Agilent Esquire HCT



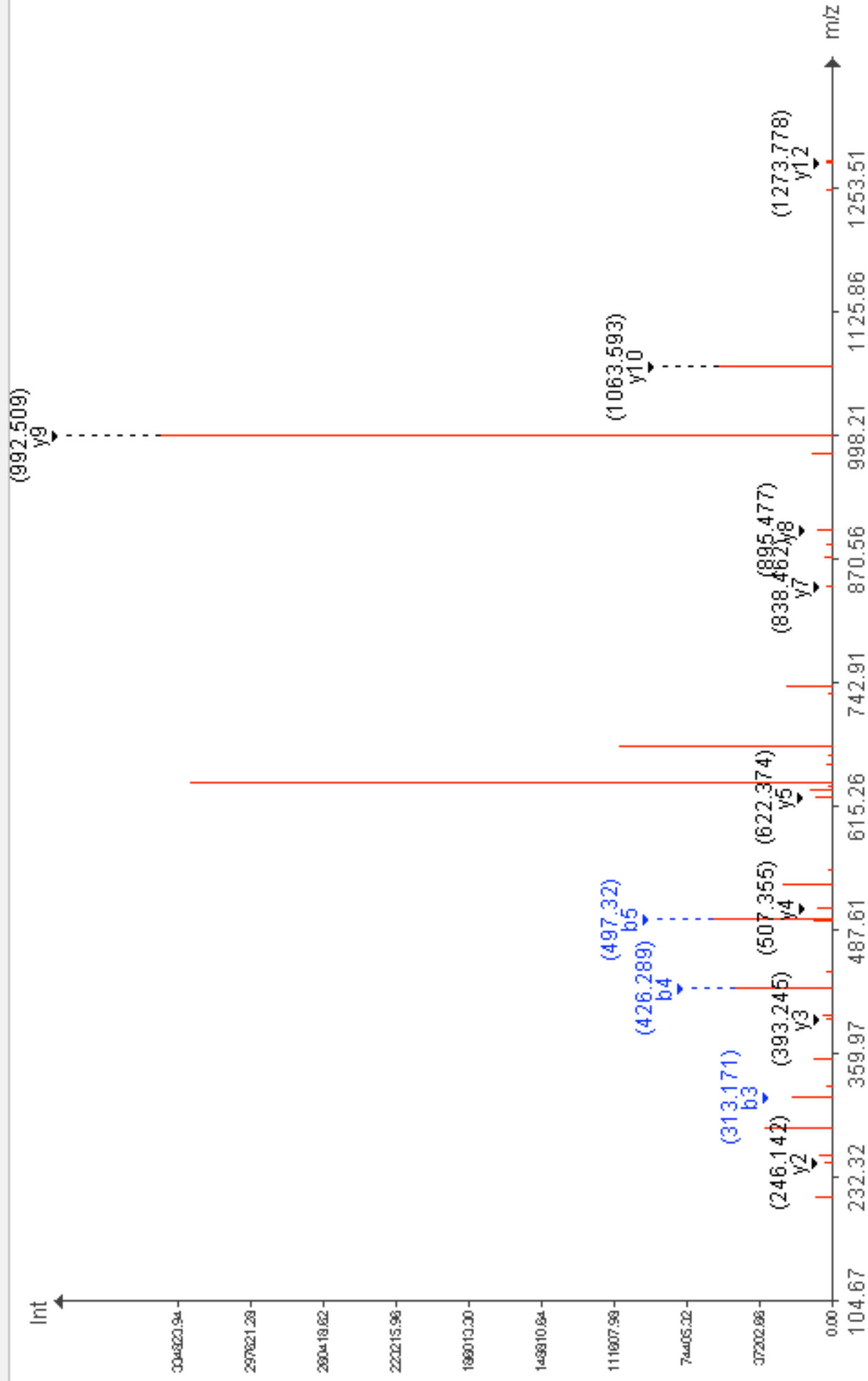


AcD3-VAPLAPGSEDNFAR-COOH

precursor mass: 744.9290 Da (charge: +2) protein: P17427 (694-707)

score (threshold): 65.0 (42.0)

instrument: Agilent Esquire HCT

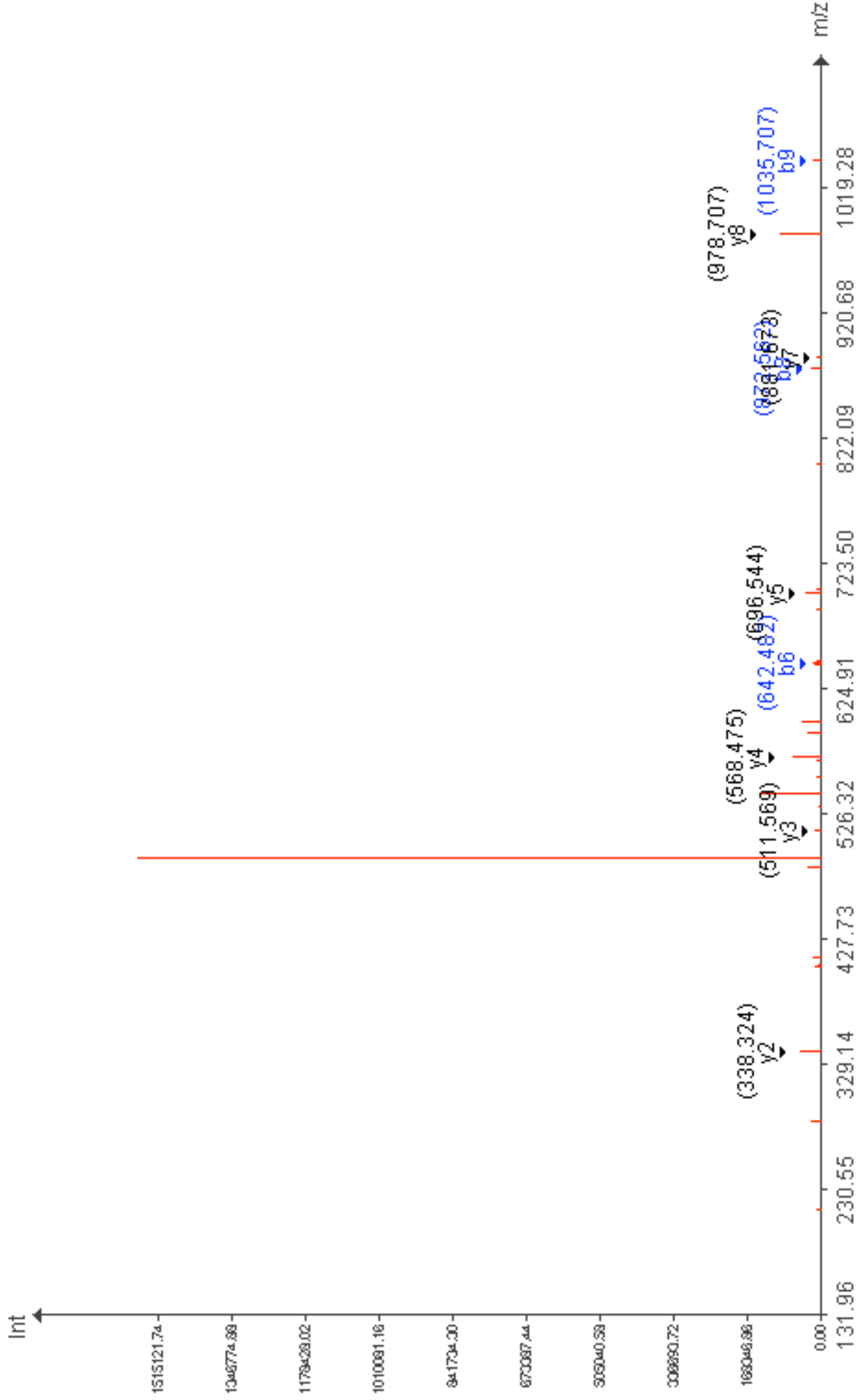


**AcD3-SVPGQQGK(AcD3K\*)YR-COOH**

**precursor mass: 605.4370 Da (charge: +2) protein: Q7TQH0 (739-748)**

**score (threshold): 48.0 (41.0)**

**instrument: Agilent Esquire HCT**

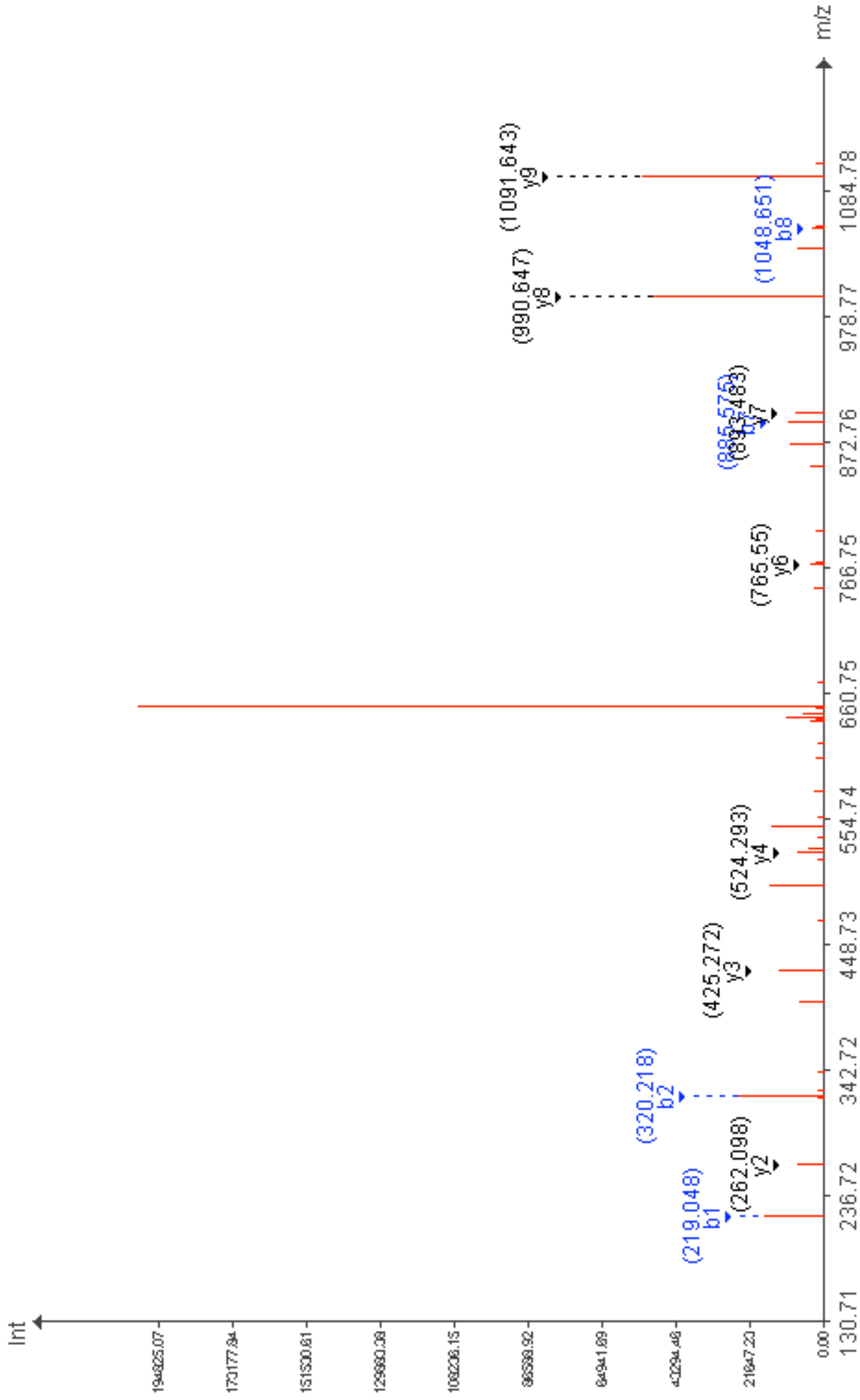


**AcD3-K(AcD3K\*)TPQIQVYSR-COOH**

precursor mass: 655.3710 Da (charge: +2) protein: P01887 (23-32)

score (threshold): 62.0 (42.0)

instrument: Agilent Esquire HCT

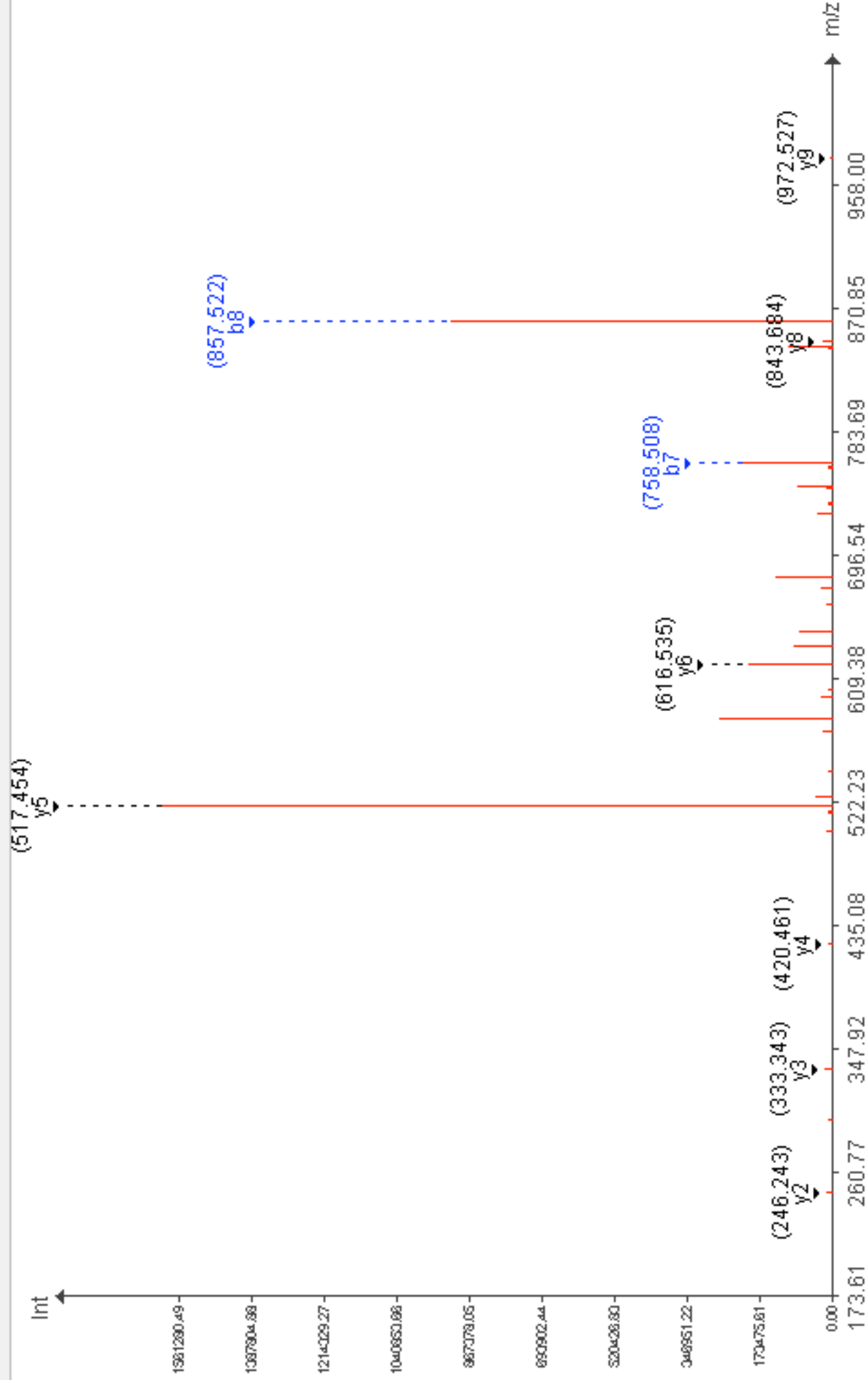


**AcD3-ATPSENLPSSAR-COOH**

precursor mass: 687.4050 Da (charge: +2) protein: Q8BTV2 (202-214)

score (threshold): 62.0 (42.0)

instrument: Agilent Esquire HCT

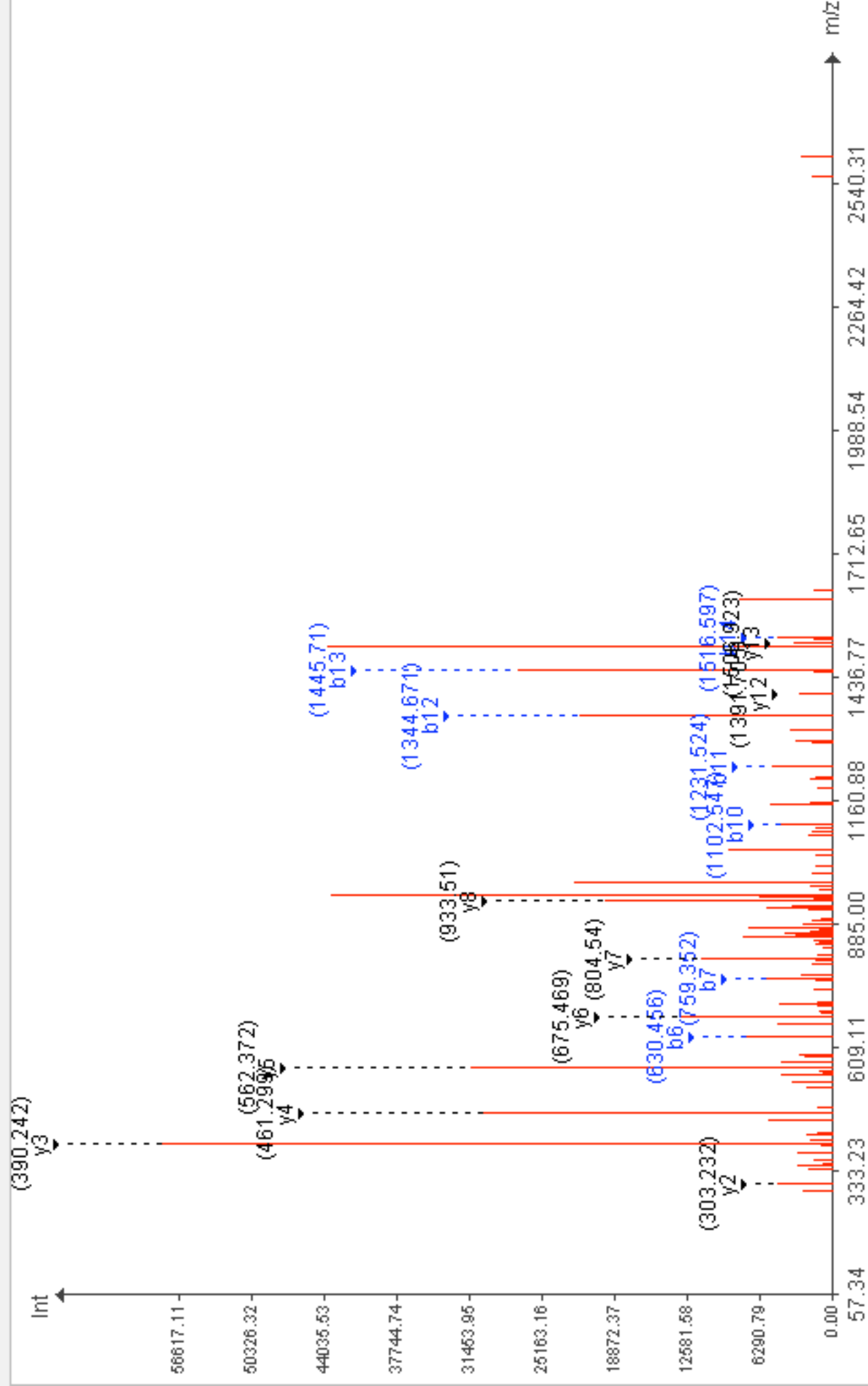


**AcD3-GLALDDEDVEELTASQR-COOH**

precursor mass: 953.4440 Da (charge: +2) protein: P97310 (94-110)

score (threshold): 50.0 (42.0)

instrument: Agilent Esquire HCT

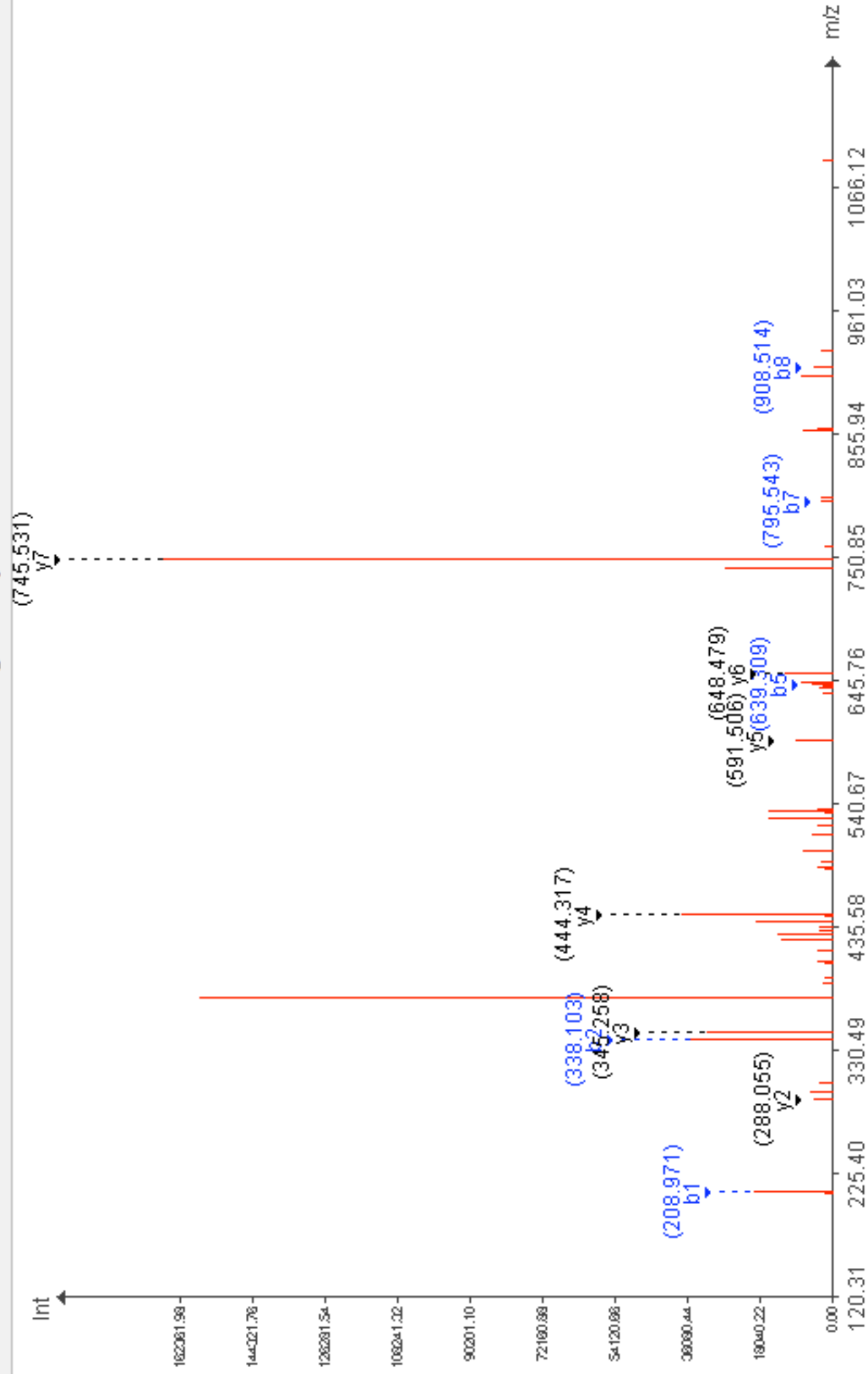


**AcD3-YEPGFV GIR-COOH**

precursor mass: 541.8310 Da (charge: +2) protein: P60898 (7-15)

score (threshold): 50.0 (45.0)

instrument: Agilent Esquire HCT

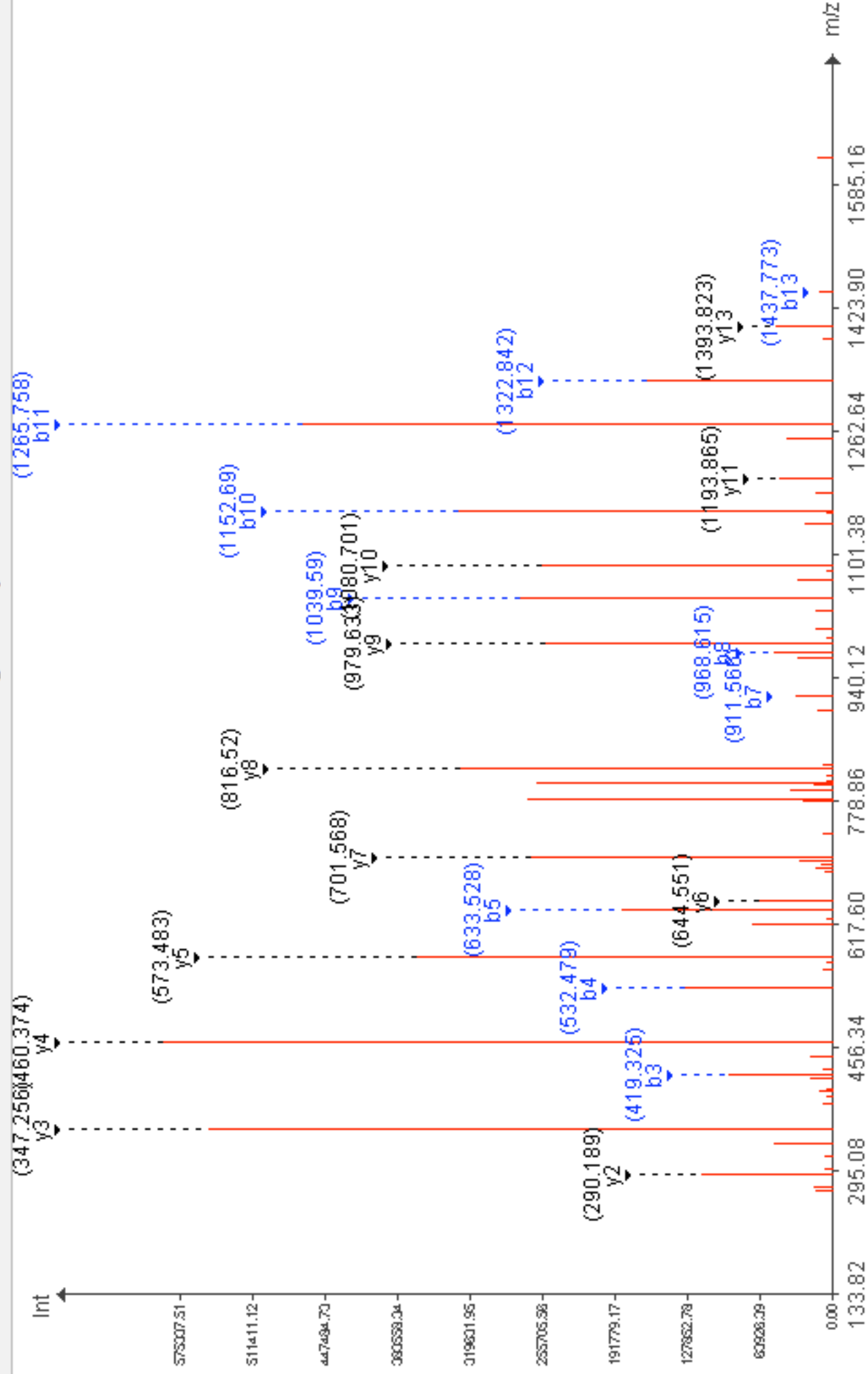


**ACD3-K(AcD3K\*)EALTYDGALLGDR-COOH**

precursor mass: 806.4840 Da (charge: +2) protein: Q9WUK2 (95-108)

score (threshold): 108.0 (42.0)

instrument: Agilent Esquire HCT

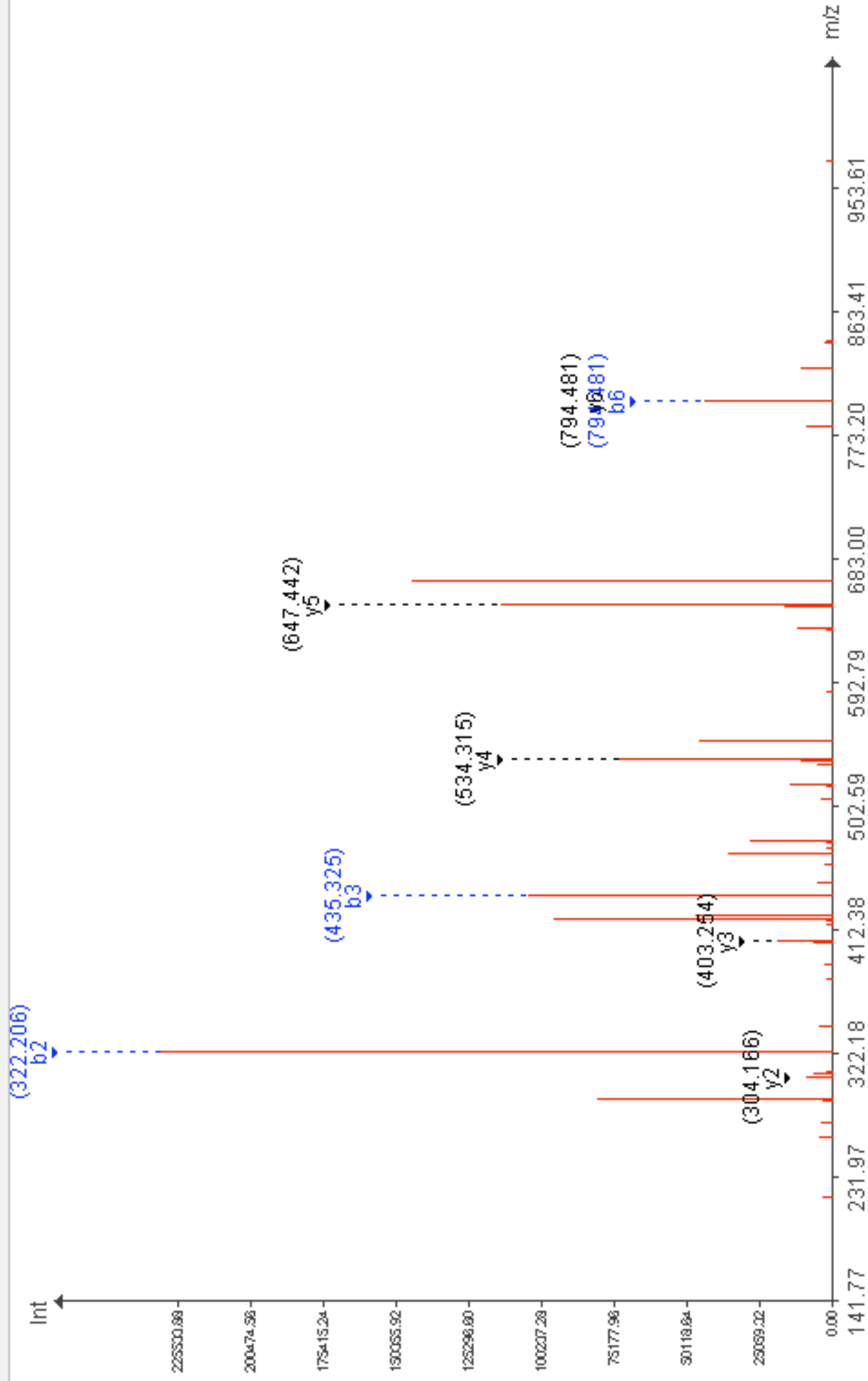


**AcD3-EFIMVER-COOH**

precursor mass: 484.8500 Da (charge: +2) protein: P97323 (40-46)

score (threshold): 36.0 (33.0)

instrument: Agilent Esquire HCT



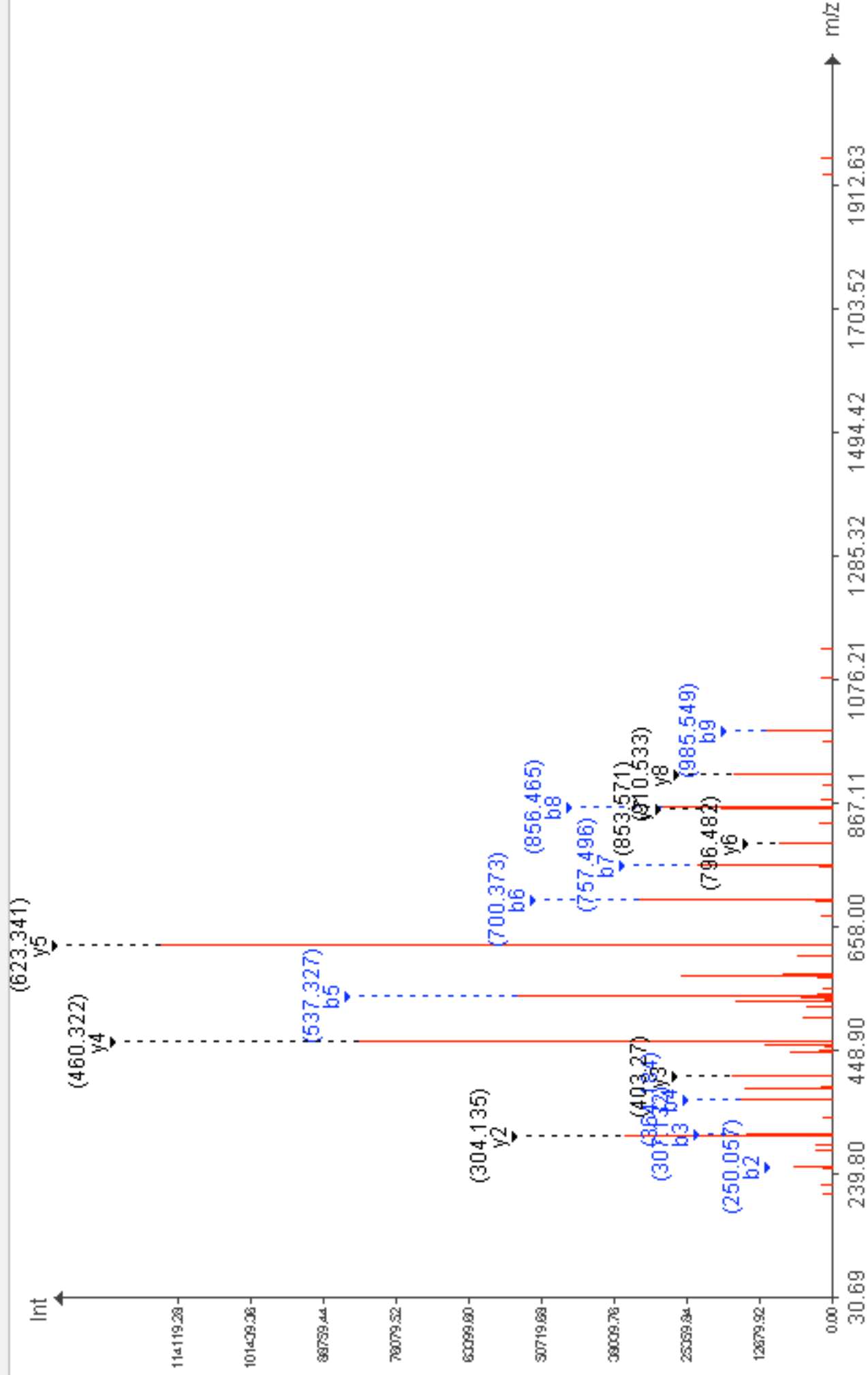


**AcD3-GFGGK(AcD3K\*)YGVVER-COOH**

precursor mass: 580.3170 Da (charge: +2) protein: P49710 (119-128)

score (threshold): 71.0 (41.0)

instrument: Agilent Esquire HCT

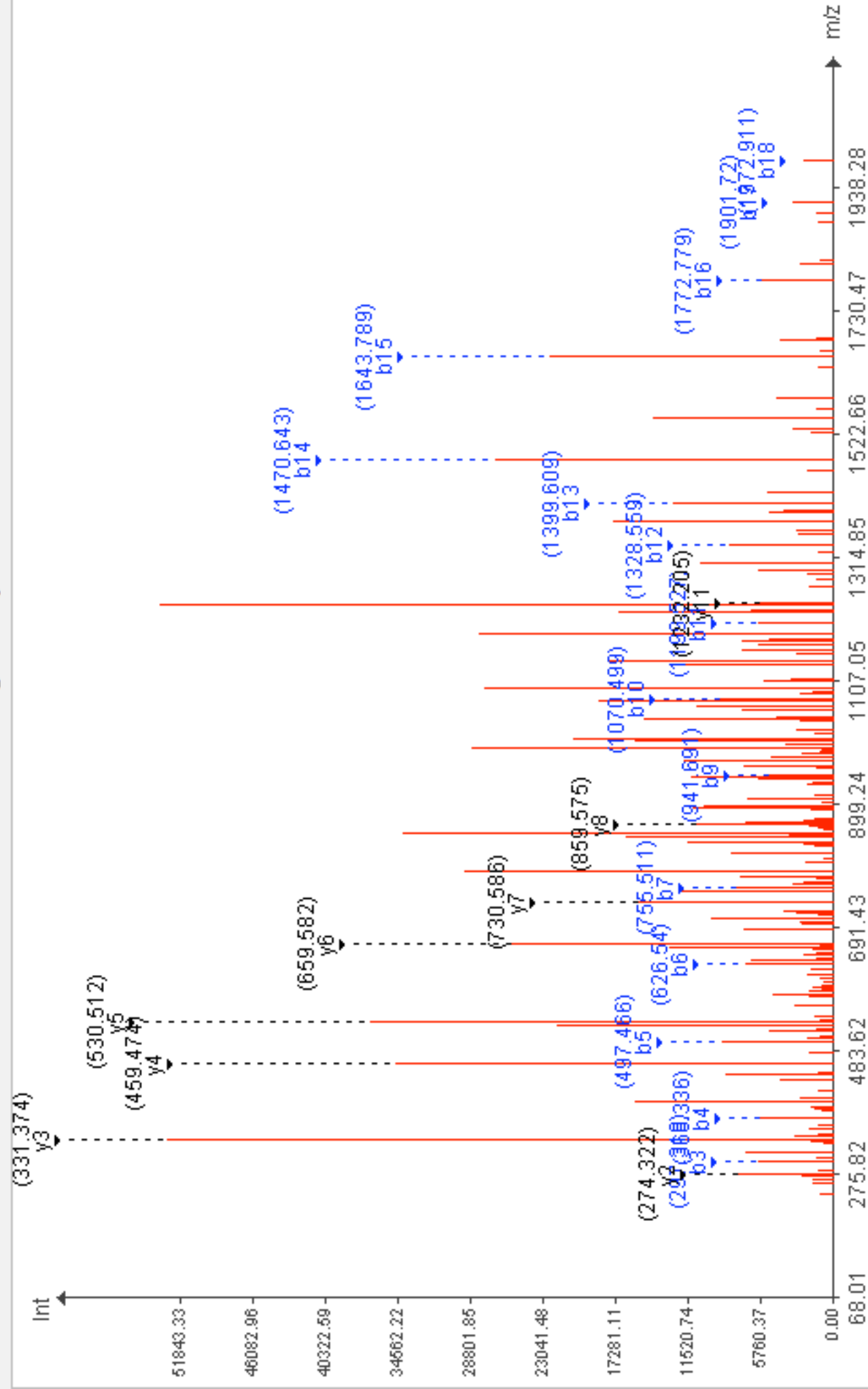


ACD3-GPPAEEEEEEAAK(AcD3K\*)EEEAQGVK-COOH

precursor mass: 877.7140 Da (charge: +3) protein: P51859 (209-232)

score (threshold): 51.0 (43.0)

instrument: Agilent Esquire HCT

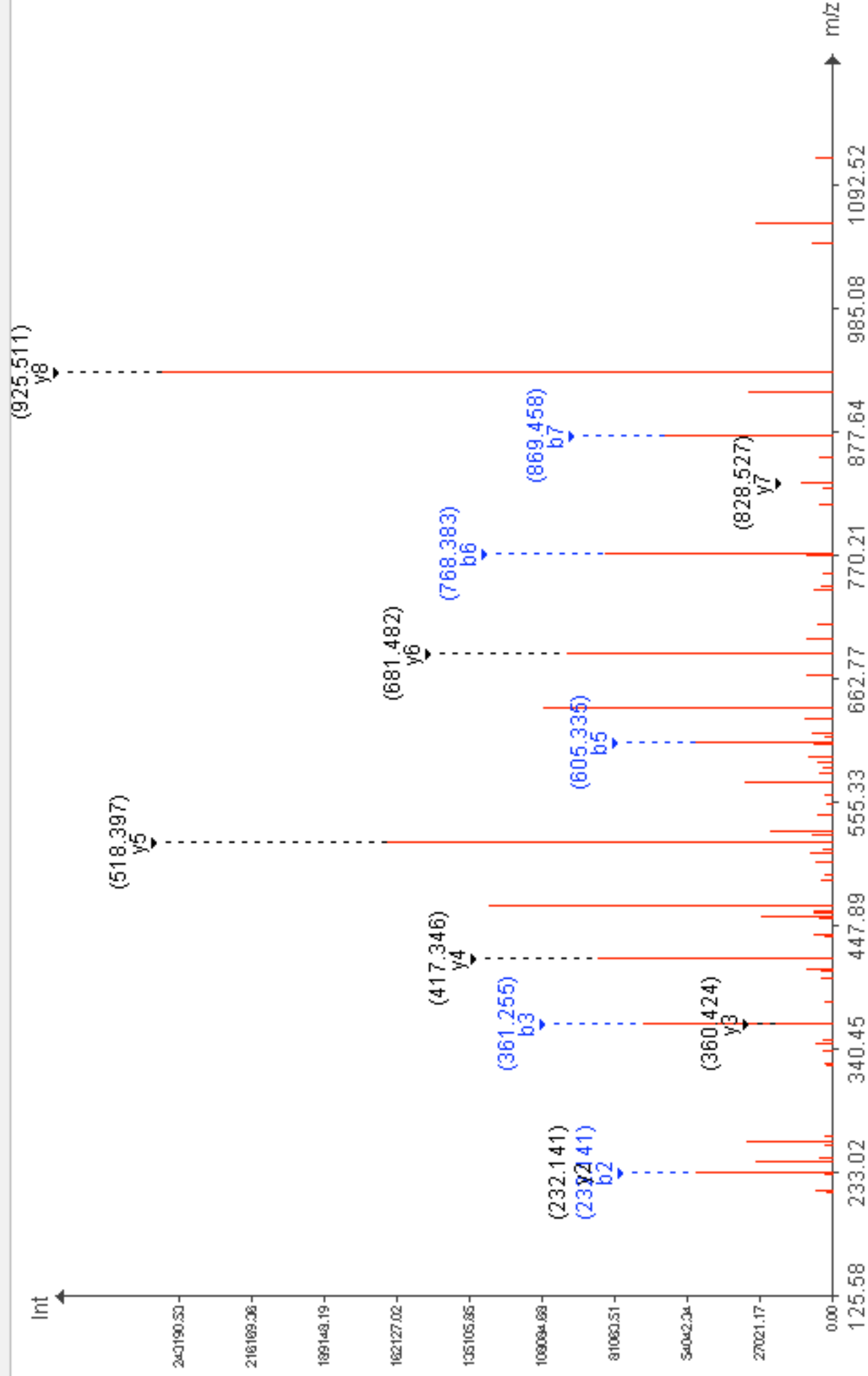


AcD3-GEEPFYTGQR-COOH

precursor mass: 643.3460 Da (charge: +2) protein: Q02780 (312-322)

score (threshold): 52.0 (41.0)

instrument: Agilent Esquire HCT

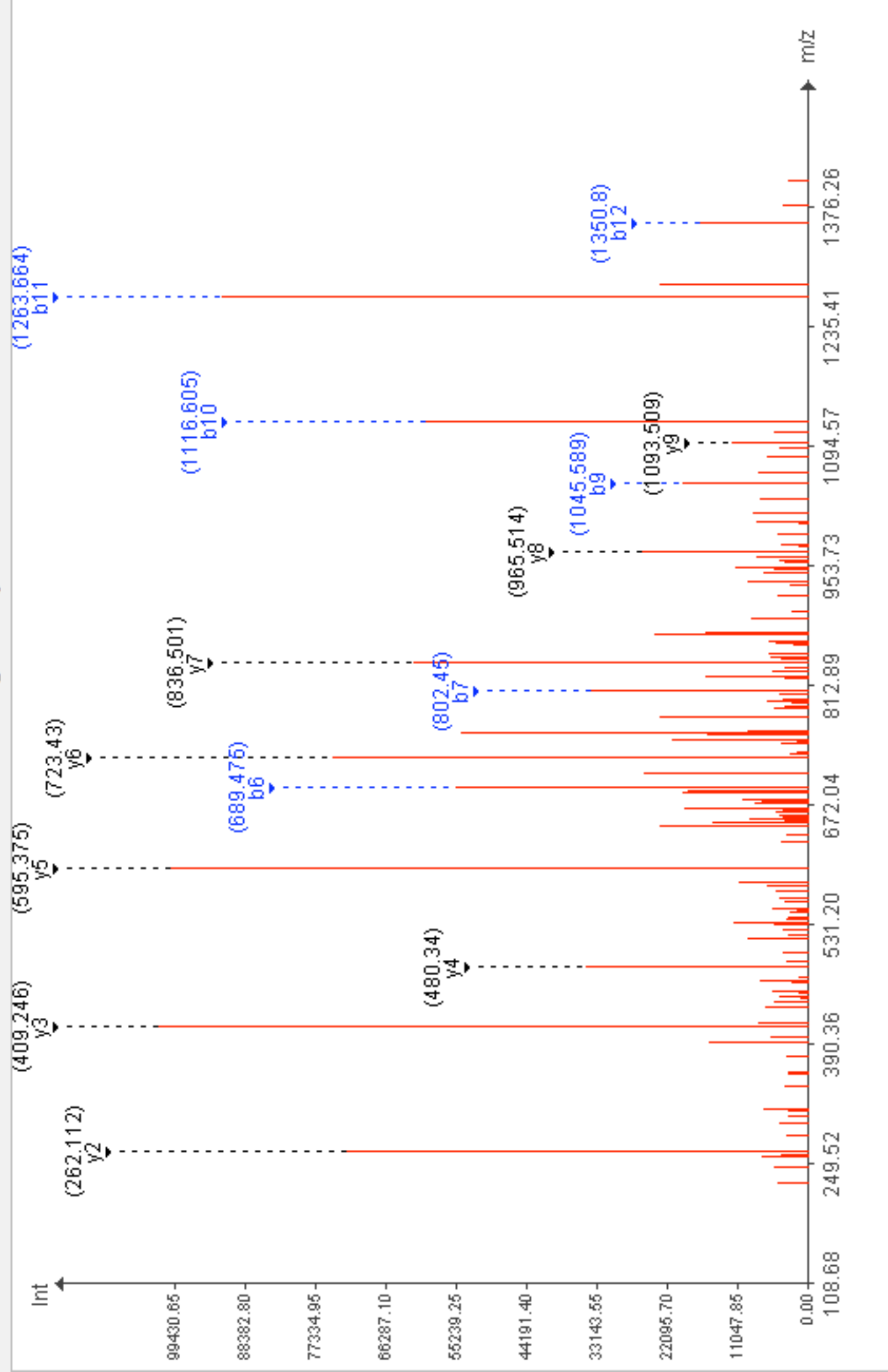


**AcD3-LASDQELQDAFSR-COOH**

precursor mass: 762.8610 Da (charge: +2) protein: Q9D903 (17-29)

score (threshold): 65.0 (42.0)

instrument: Agilent Esquire HCT

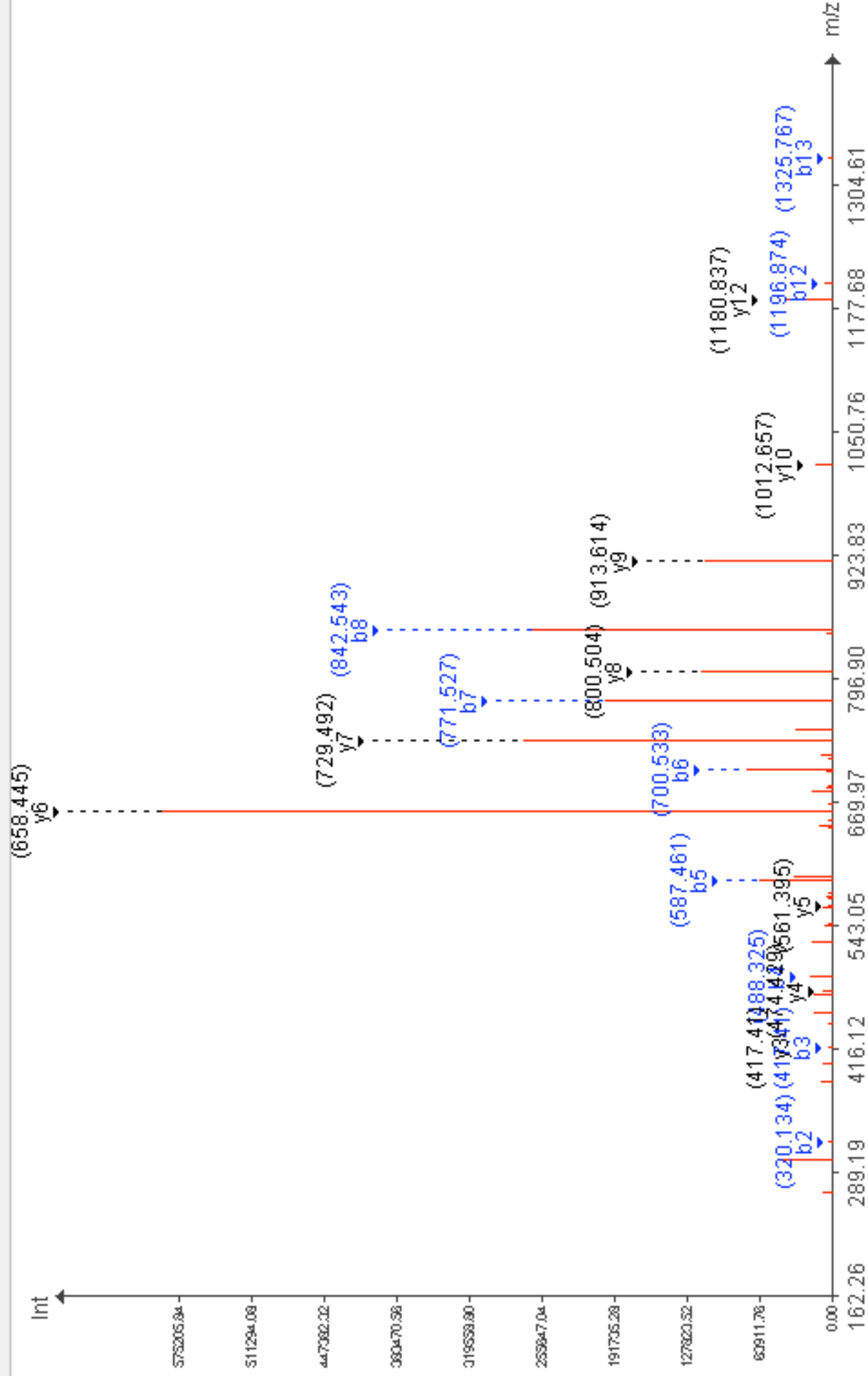


**AcD3-TK(AcD3K\*)PAVLAAPSGLER-COOH**

precursor mass: 750.4310 Da (charge: +2) protein: Q8K3X4 (116-129)

score (threshold): 75.0 (42.0)

instrument: Agilent Esquire HCT

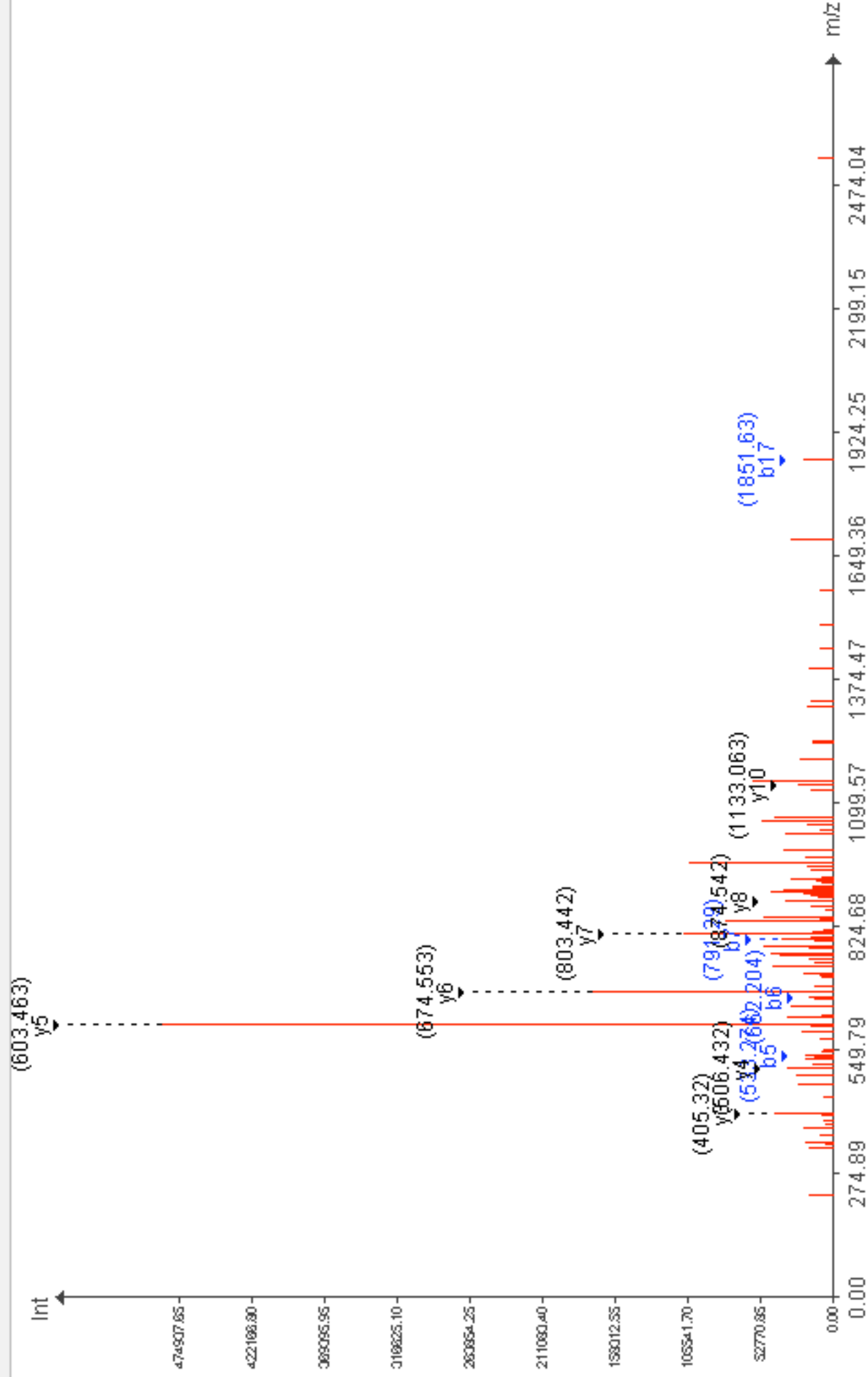


AcD3-EGDGEEDGDEDEEEAEAPTGK(AcD3K\*)R-COOH

precursor mass: 818.7190 Da (charge: +3) protein: P26350 (68-89)

score (threshold): 41.0 (35.0)

instrument: Agilent Esquire HCT

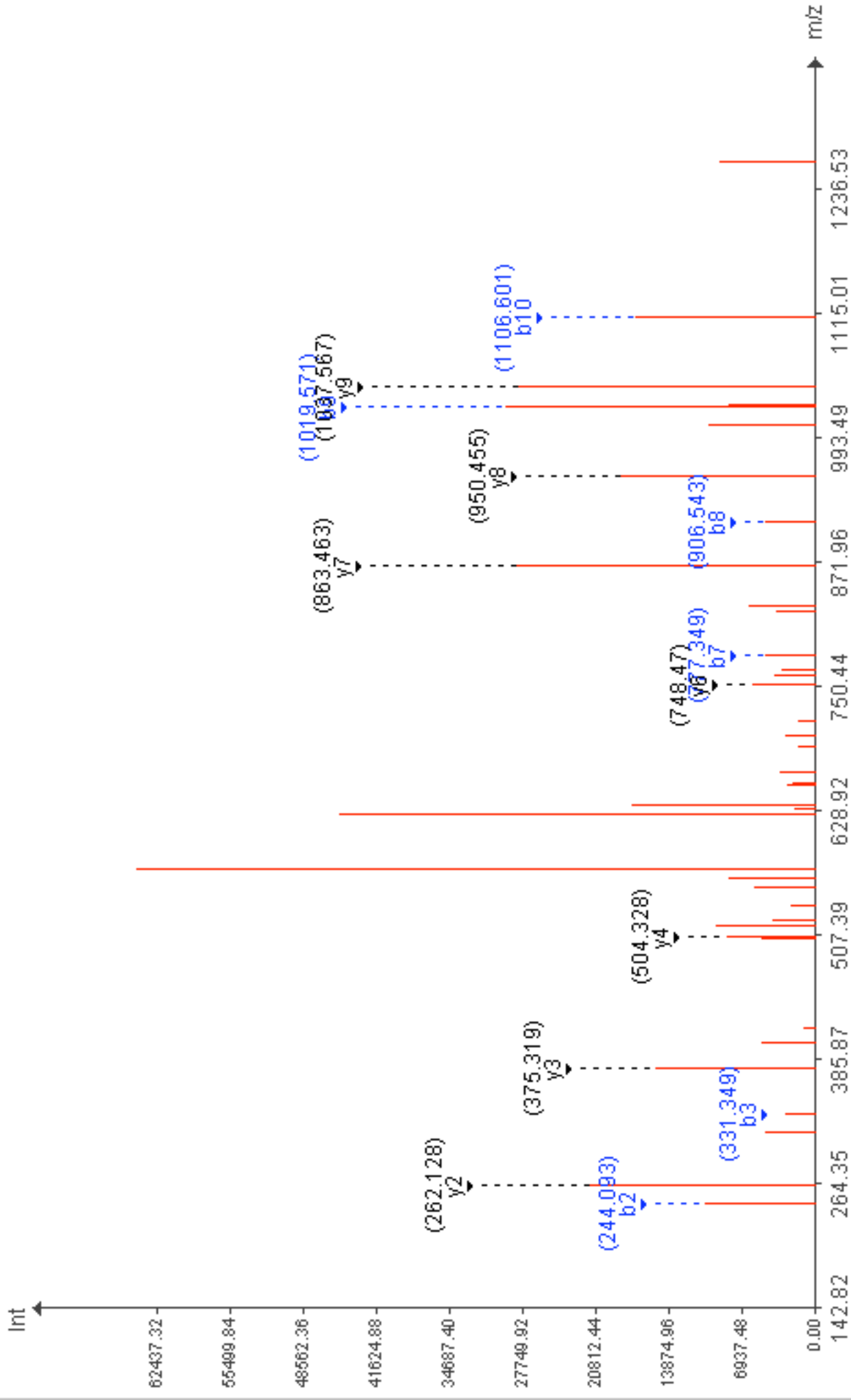


**AcD3-VVSSDDEELSR-COOH**

**precursor mass: 640.8980 Da (charge: +2) protein: Q9QZS2 (96-106)**

**score (threshold): 51.0 (42.0)**

**instrument: Agilent Esquire HCT**

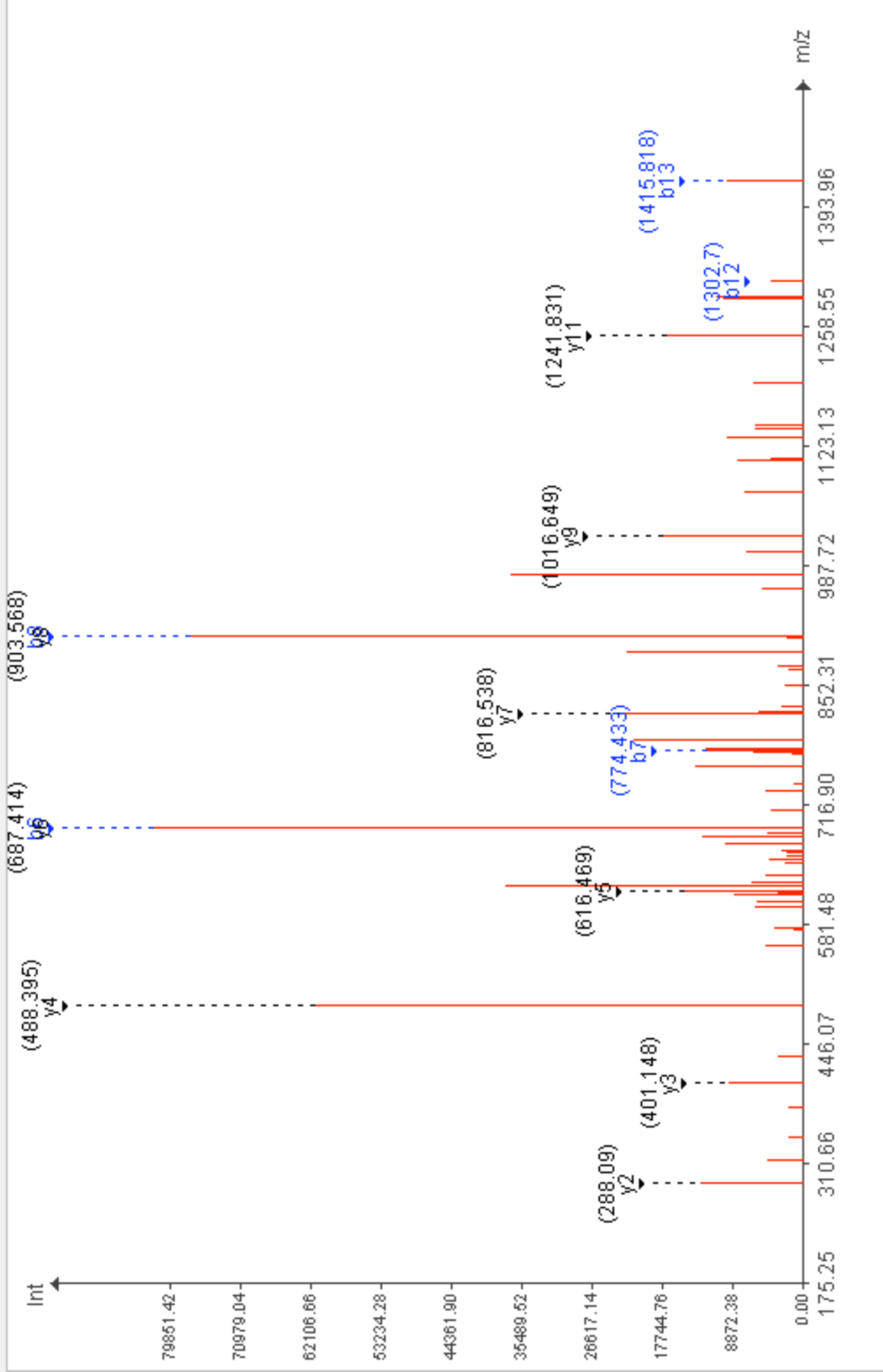


**AcD3-STDPQLSEALR-COOH**

precursor mass: 795.4170 Da (charge: +2) protein: Q60710 (445-458)

score (threshold): 76.0 (42.0)

instrument: Agilent Esquire HCT



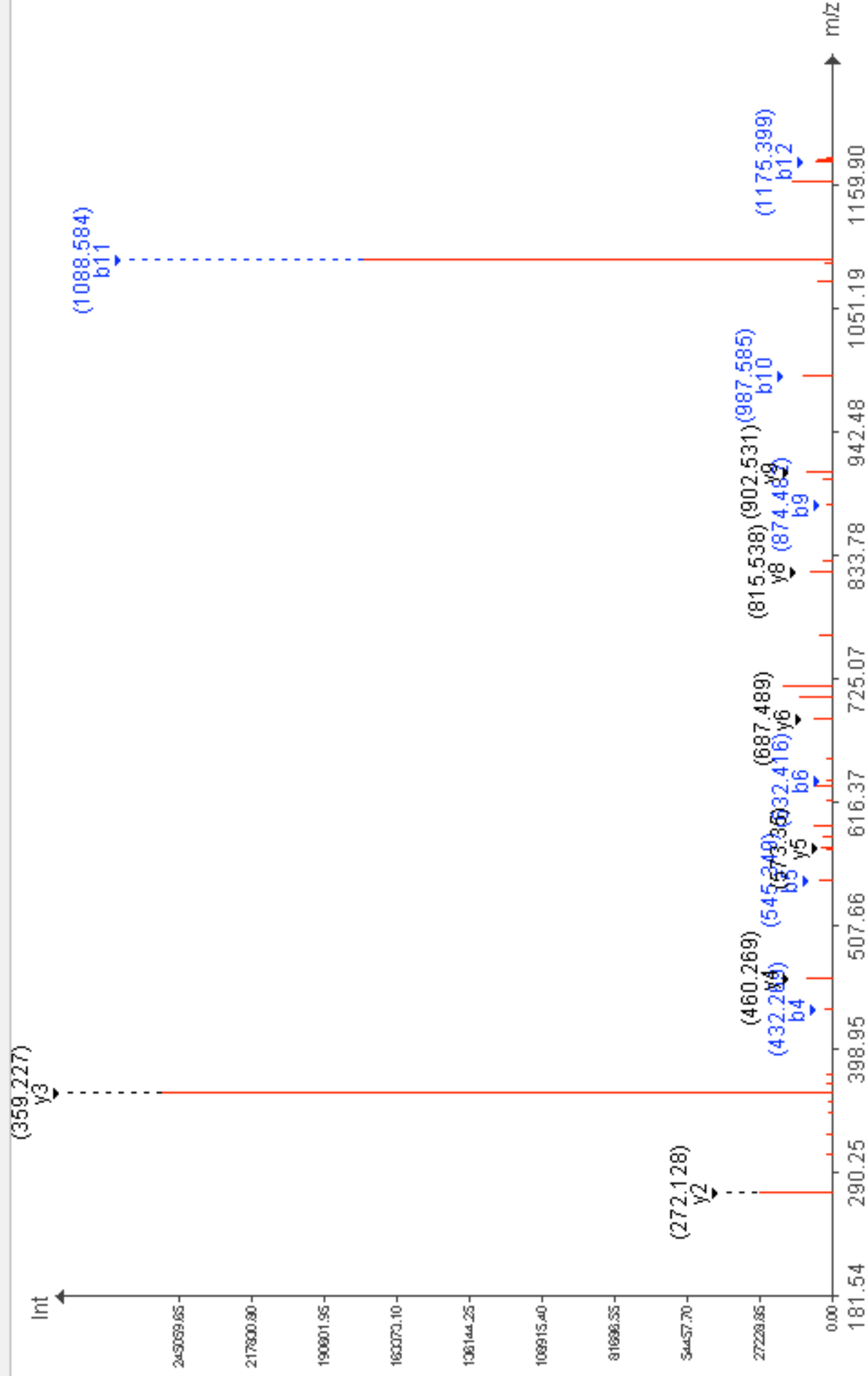


**AcD3-M(Mox)APAL.SGANL.TSPR-COOH**

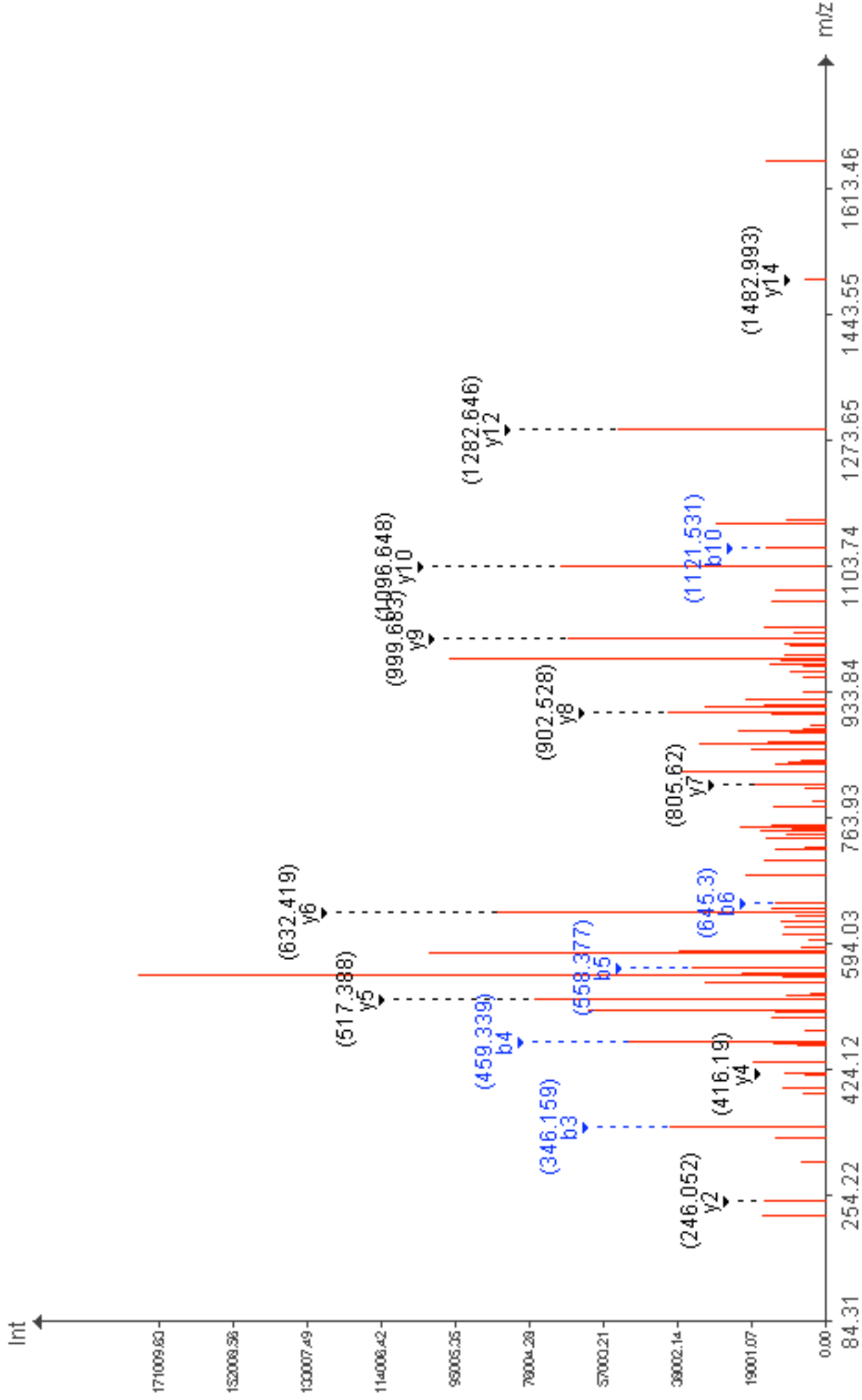
precursor mass: 723.9500 Da (charge: +2) protein: Q8BTI8 (2324-2337)

score (threshold): 45.0 (30.0)

instrument: Agilent Esquire HCT



**AcD3-GENLVSM(Mox)TVEGPPPK(AcD3K\*)DTGIAR-COOH**  
precursor mass: 758.7280 Da (charge: +3) protein: P27048 (74-94)  
score (threshold): 62.0 (46.0)  
instrument: Agilent Esquire HCT

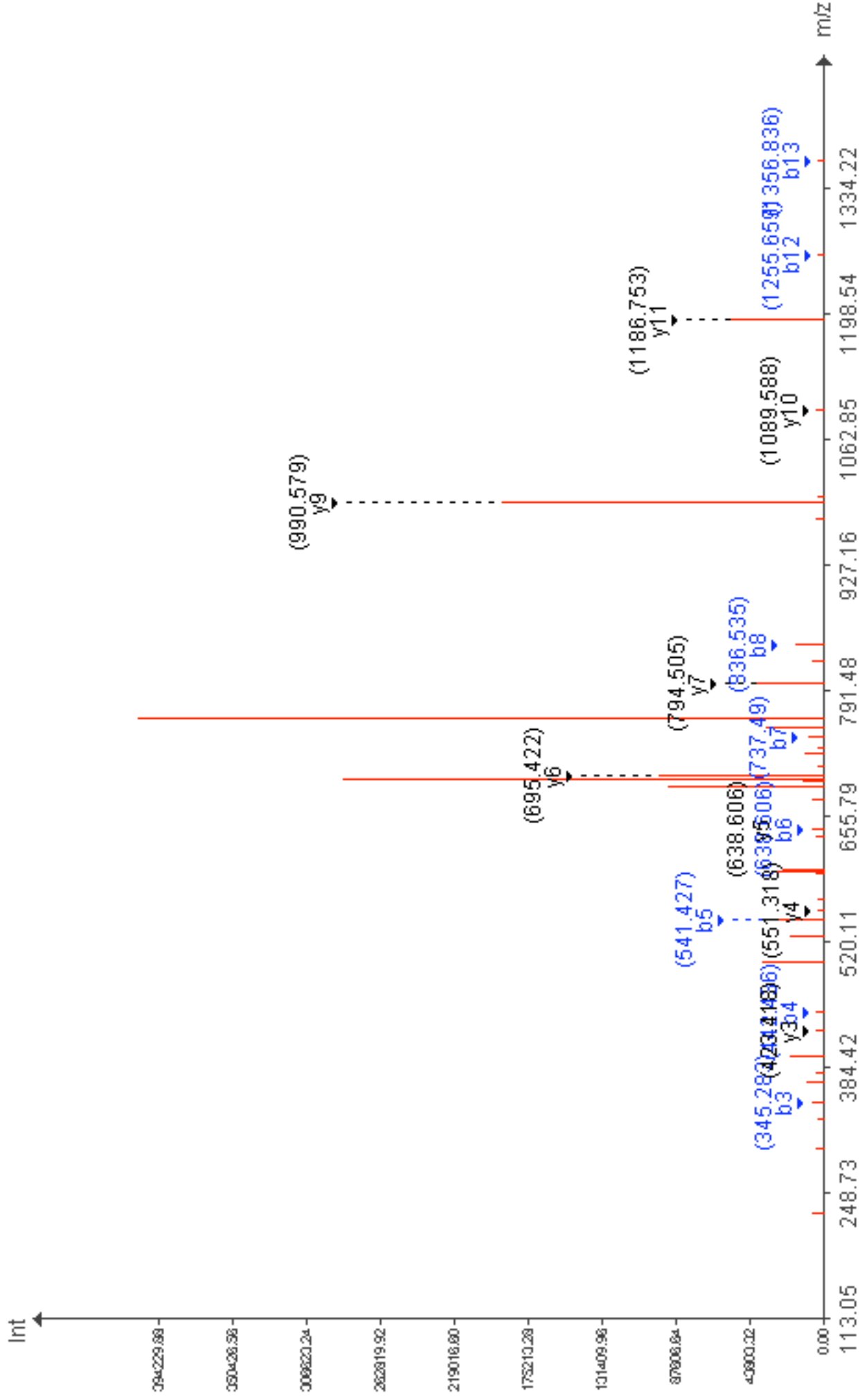


**AcD3-TPTPVPVGSQM(Mox)TR-COOH**

precursor mass: 765.9100 Da (charge: +2) protein: P26369 (133-146)

score (threshold): 68.0 (42.0)

instrument: Agilent Esquire HCT

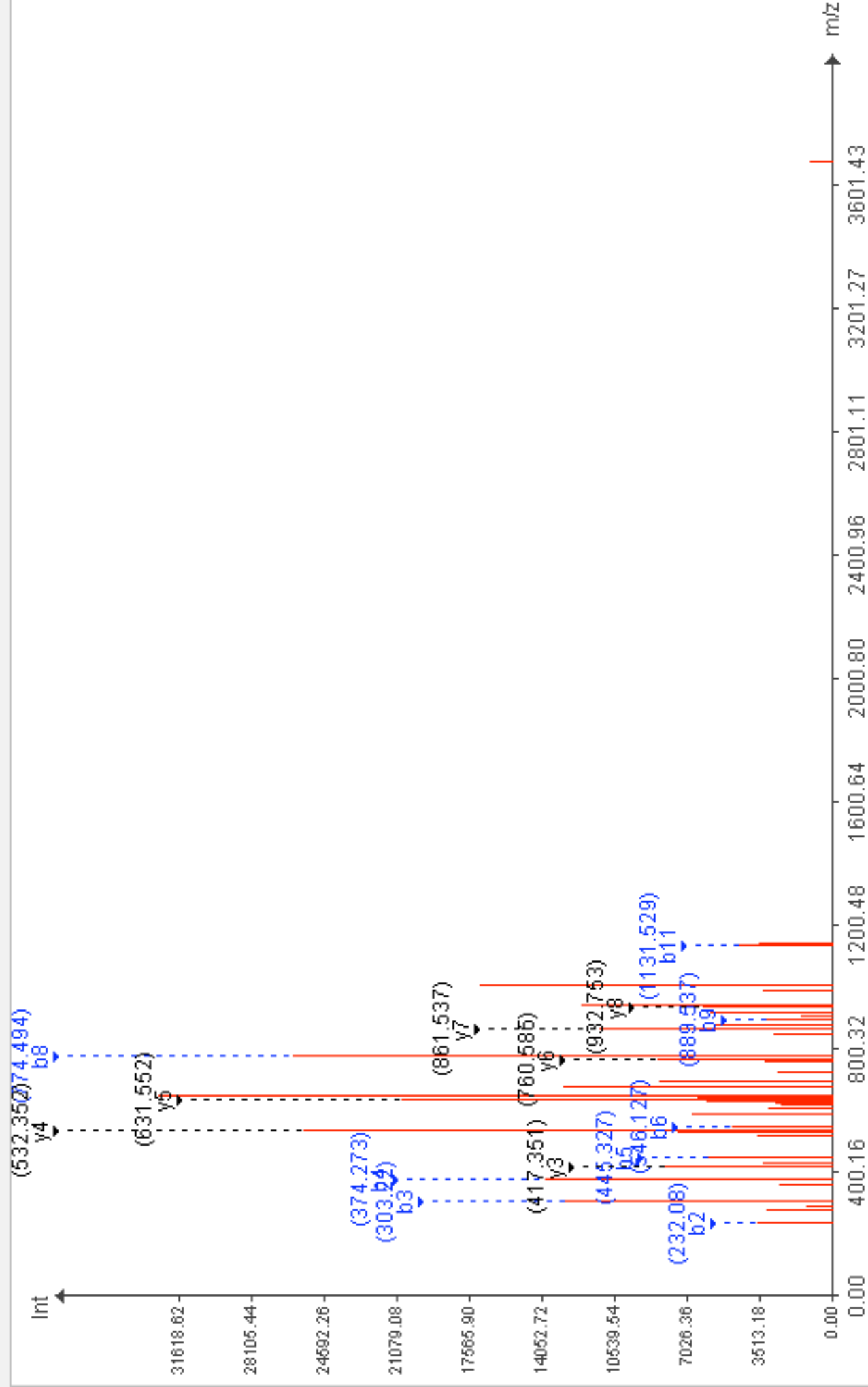


AcD3-GEAAATEVDLER-COOH

precursor mass: 653.1680 Da (charge: +2) protein: O54988 (668-679)

score (threshold): 52.0 (45.0)

instrument: Agilent Esquire HCT

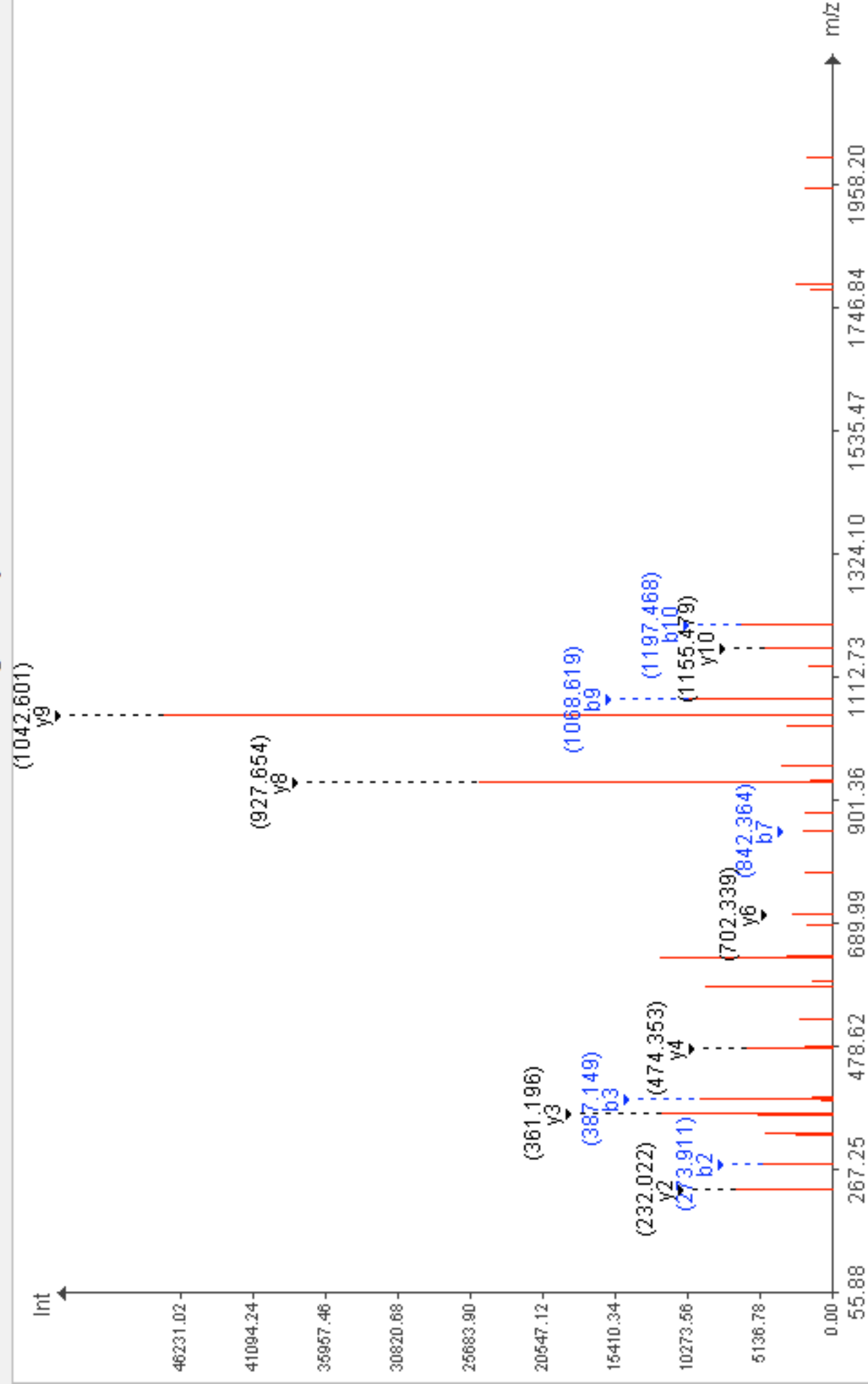


**AcD3-EVIDPQDLLEGR-COOH**

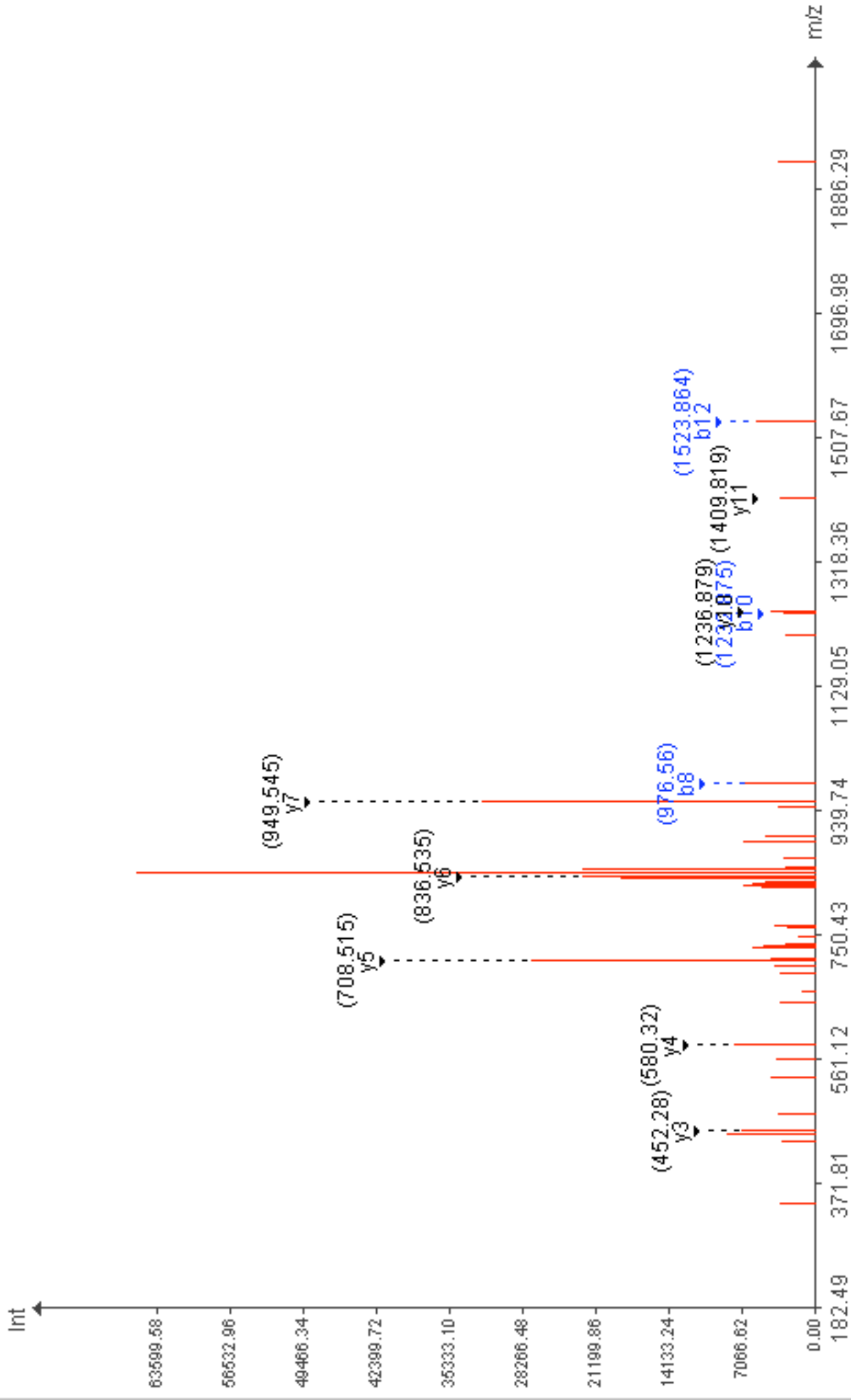
precursor mass: 714.8560 Da (charge: +2) protein: O35988 (30-41)

score (threshold): 62.0 (42.0)

instrument: Agilent Esquire HCT



**AcD3-SPK(AcD3K\*)K(AcD3K\*)STVLQQQYNR-COOH**  
precursor mass: 906.5190 Da (charge: +2) protein: P26039 (425-438)  
score (threshold): 53.0 (42.0)  
instrument: Agilent Esquire HCT

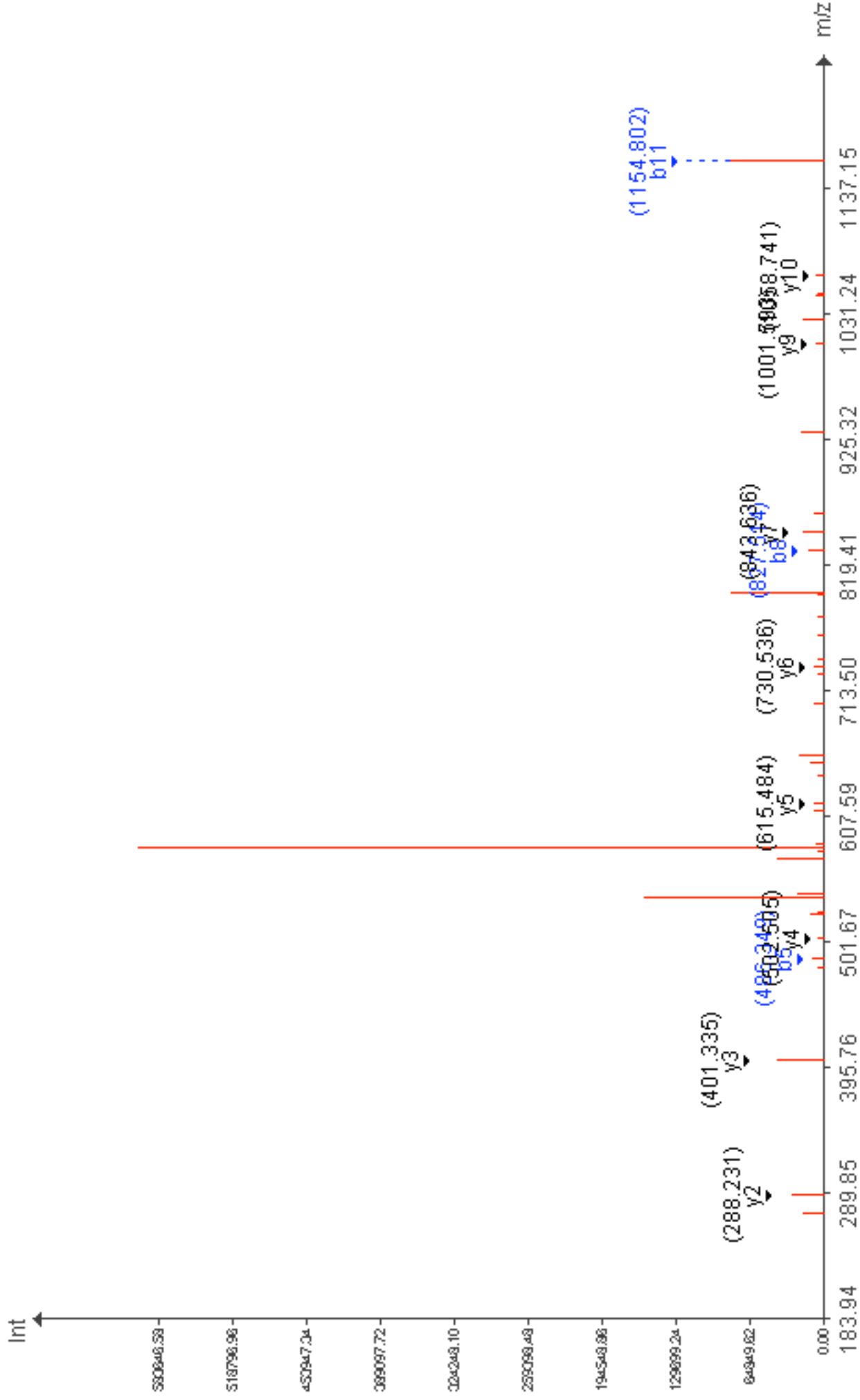


**AcD3-QPGGTLDLILR-COOH**

**precursor mass: 664.9490 Da (charge: +2) protein: Q62318 (732-743)**

**score (threshold): 42.0 (42.0)**

**instrument: Agilent Esquire HCT**

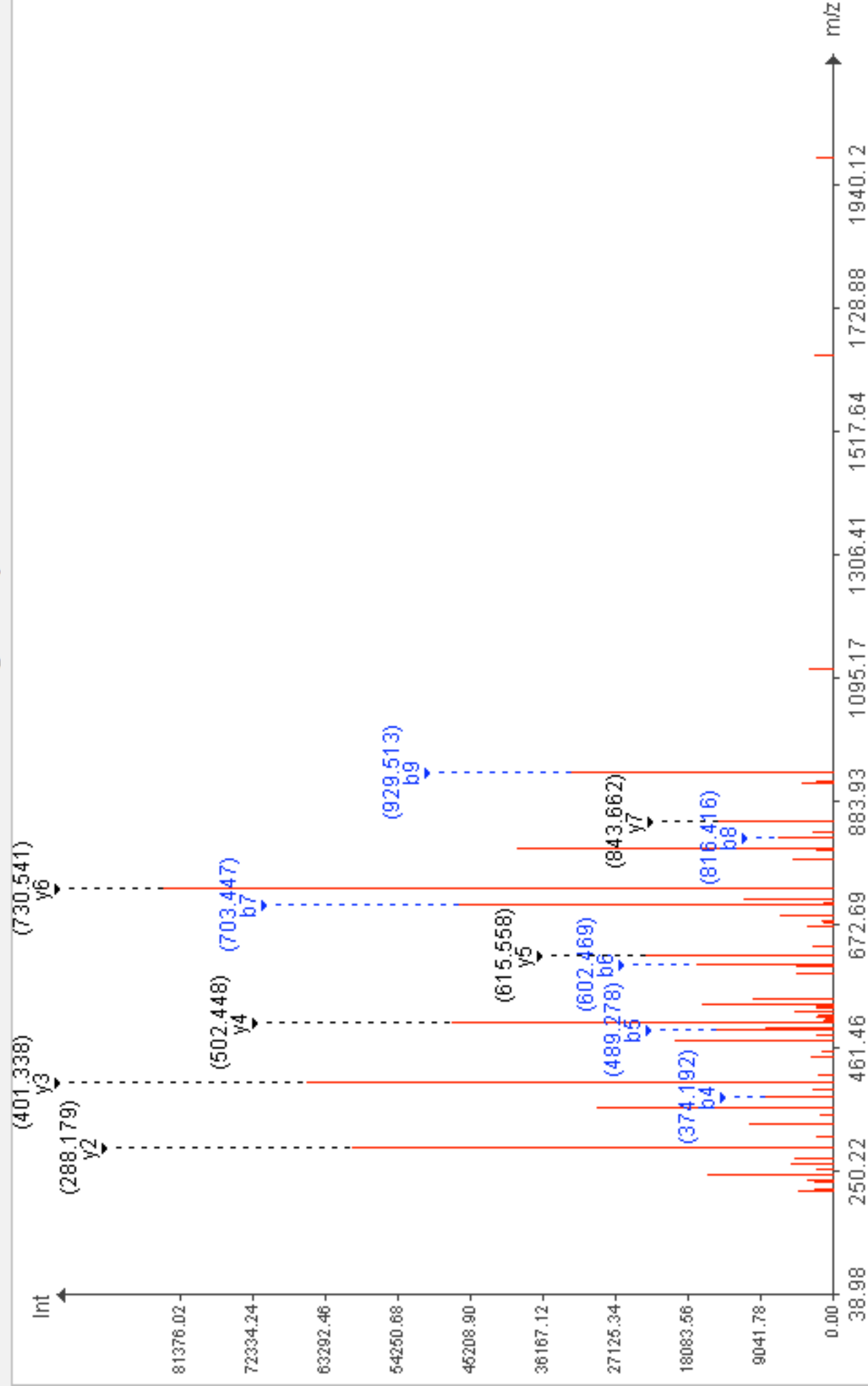


AcD3-GGTLDLTLIR-COOH

precursor mass: 552.3440 Da (charge: +2) protein: Q62318 (734-743)

score (threshold): 65.0 (42.0)

instrument: Agilent Esquire HCT



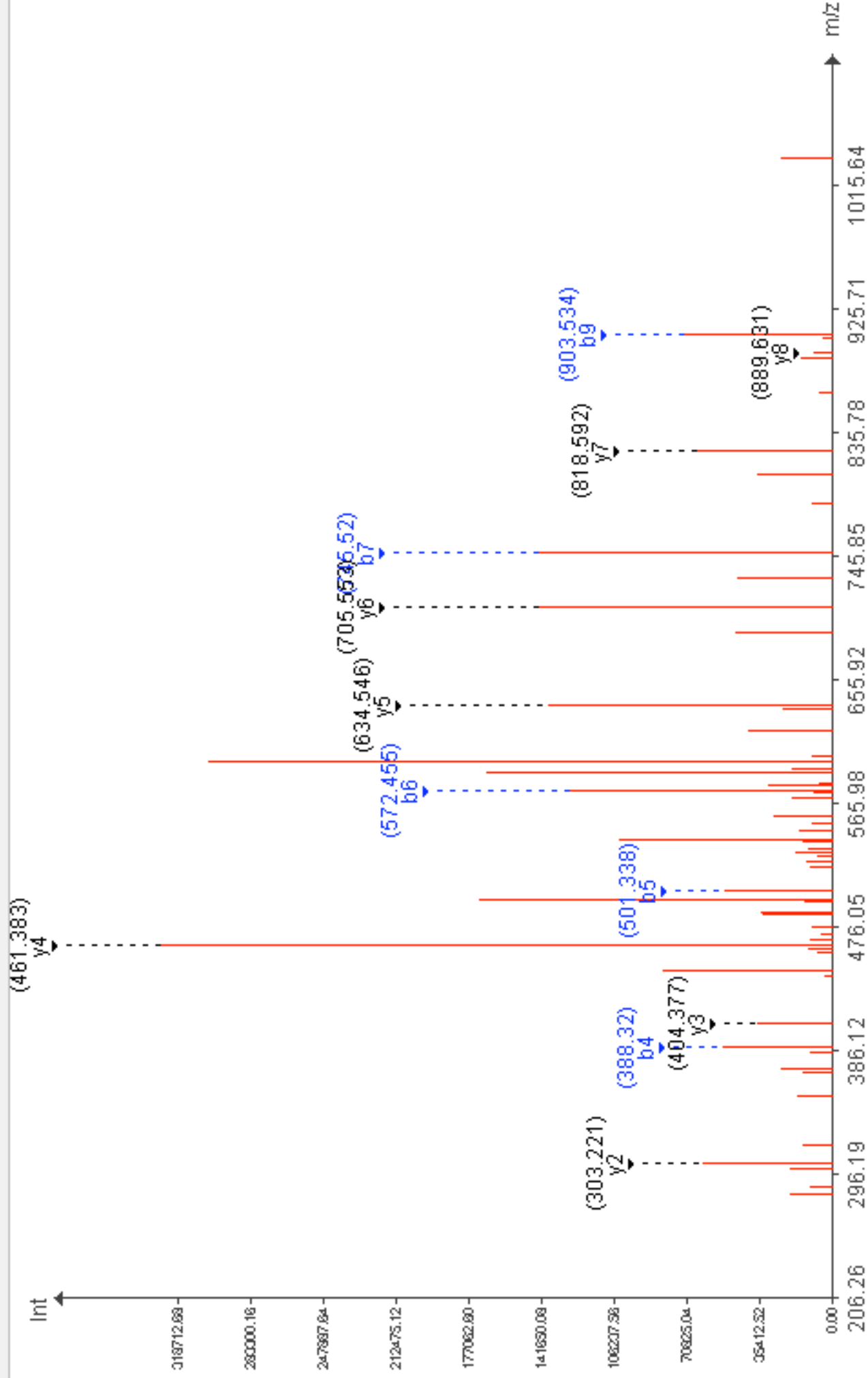


**AcD3-SSPALAK(AcD3K\*)GTQR-COOH**

precursor mass: 603.4240 Da (charge: +2) protein: O08784 (592-602)

score (threshold): 59.0 (42.0)

instrument: Agilent Esquire HCT

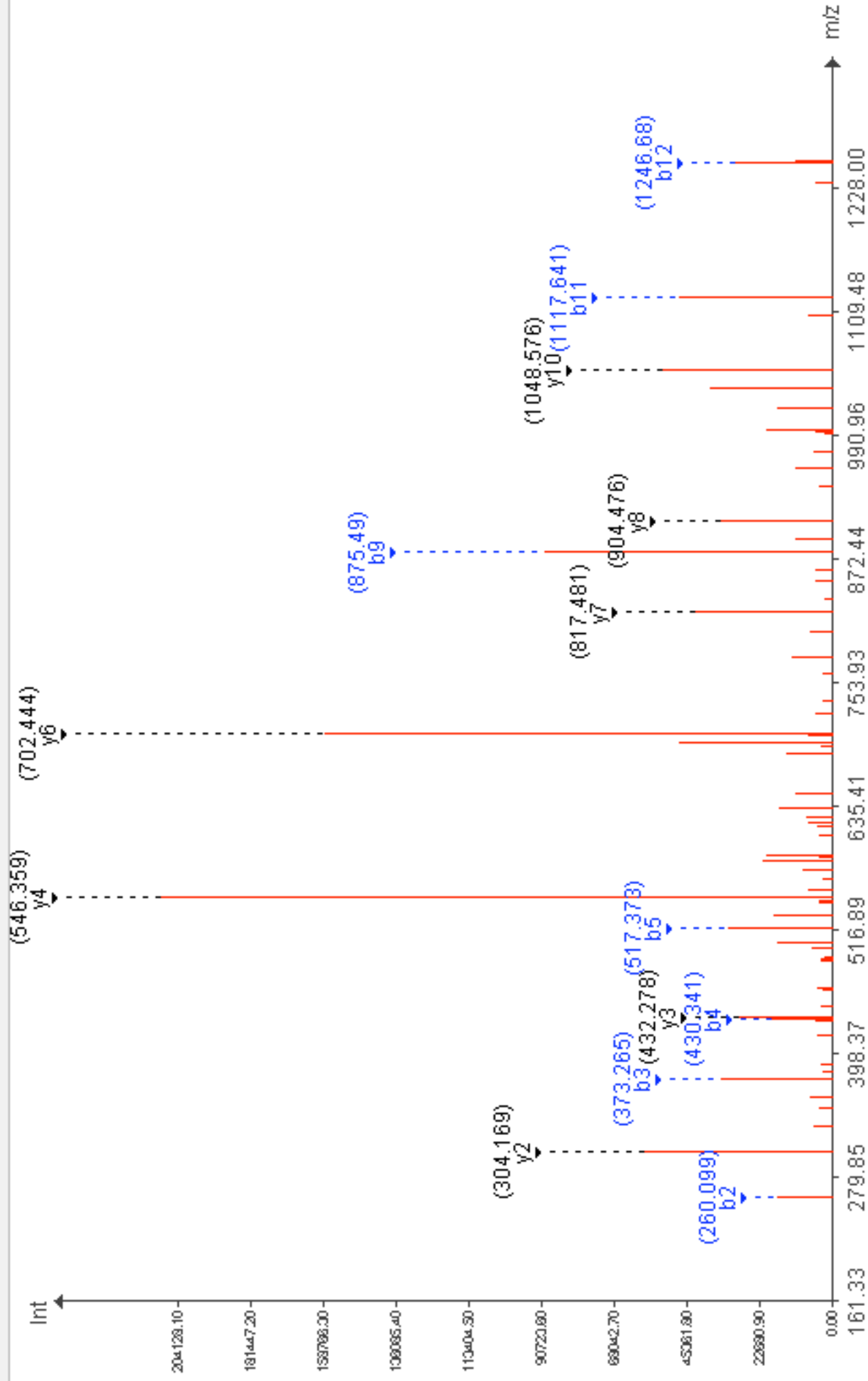


**Acd3-DVLGSSN(Dam)GVNQR-COOH**

precursor mass: 710.8760 Da (charge: +2) protein: Q9JHJ0 (151-163)

score (threshold): 76.0 (42.0)

instrument: Agilent Esquire HCT



**AcD3-ALPFSQM(Mox)SELLTR-COOH**

precursor mass: 777.4160 Da (charge: +2) protein: Q8R5H6 (254-266)

score (threshold): 58.0 (47.0)

instrument: Agilent Esquire HCT

