Electronic Supplementary Information

Identification of protease substrates by combinatorial profiling on tentagel beads

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Experimental section

General. Reagents were purchased in the highest quality available from Fluka, Sigma, Bachem, Novabiochem, NeoMPS or Aldrich. All solvents used in reactions were bought in *p.a.* quality or distilled and dried prior to use. Solvents for extractions were distilled from technical quality. Sensitive reactions were carried out under nitrogen or argon, the glassware being heated under high vacuum. Preparative RP-HPLC (flow rate 100 mL min^{-1}) was performed with a Waters Delta Prep 4000 system with a Waters Prepak Cartridge (500 g) as column and Waters 486 Tunable Absorbance Detector. Semi-preparative RP-HPLC (flow rate 4 mL min^{-1}) was performed with a Water 510 Pump operated with a Waters Automated Gradient Controller and Jasco UV-2075 Plus Detector on a Vydac 218 TP (1.0 cm × 25 cm) column. Analytical RP-HPLC (flow rate 4 mL min^{-1}) was performed on Waters 600E systems with Waters Atlantis (4.6 mm × 100 mm, dC18, 5 mm) column, UV detection with Waters 996 photodiode array detector). Data recording and processing was done with Waters Empower2 software. Eluents for all systems were: A: water and 0.1% TFA; D: acetonitrile, water and TFA (3/2/0.1%). Analytes were quantified using external standards. MS and HRMS analyses were provided by the mass spectrometry service of the Department of Chemistry and Biochemistry, University of Berne. ^{1}H and ^{13}C NMR spectra were recorded on

Bruker AC 300 (300 MHz) and DRX 500 or Avance 500 (500 MHz) instruments. Chemical shifts (*d*) are given in ppm referring to solvent residual peak, coupling constants (*J*) in Hertz (Hz). Solid phase peptide chemistry was performed in polypropylene syringes fitted with a polyethylene frit and a teflon stopcock and stopper.

Coupling of the Fmoc-protected amino acids. The resin was washed and swollen inside the reactor with DCM (2 × 5 mL) and DMF (1 × 5 mL). The appropriate resin (library: TentaGel HL (0.65 mmol g^{-1}), single sequences: resin was acylated with 2.5 equivalents of *N*-Fmoc amino acid in the presence of 2.5 equivalents of DIPCDI and 2.5 equivalents of HOBt in DMF. After 1 hr the resin was washed (3 × each) with DMF, DCM, and MeOH, and controlled with the TNBS (trinitrobenzenesulfonic acid) or chloranil test followed by acetylation.

Cleavage of the Fmoc protecting group. The Fmoc protecting group was removed with 5 mL of a solution of DMF-piperidine (4:1, *v*/*v*) for 10 min. After filtration, the procedure was repeated and then washed (3 × each) with DMF, DCM and MeOH.

***N*-Acetylation.** The resin was acetylated with a solution of acetic acid anhydride-DCM (1:1, *v*/*v*) for 10 min. After filtration, the procedure was repeated and then washed (3 × each) with DMF, DCM and MeOH.

TFA cleavage. The cleavage was carried out using TFA-H_2O-TIS (triisopropylsilane) as a (95:2.5:2.5, *v*/*v*) solution for 6 hrs.

Resin mixing and splitting. The resin was suspended in DMF-DCM (2:1, *v*/*v*), and mixed *via* nitrogen bubbling for 15 min, and then distributed in four equal portions.

4-Carboxybenzaldehyde 2-{ethyl-[4-(4{nitrophenylazo)phenyl]-amino}ethyl ester (3). A solution of 4-carboxybenzaldehyde (**1**) (100 mg, 0.67 mmol), Disperse Red 1 (**2**) (210 mg, 0.67 mmol) and DMAP (81 mg, 0.67 mmol) in DCM was cooled in an ice-bath before EDC (141 mg, 0.74 mmol) was added. The reaction was allowed to heat up to rt overnight. The reaction mixture was extracted with brine and evaporated to dryness. The red residue was flash chromatographed (n-hexane-ethyl acetate (2:1, v/v)) to yield 3 as a red waxy solid (30 mg, 10%). Rf = 0.21 (n-hexane-ethyl acetate (2:1, v/v)). IR (neat) $\tilde{\nu}$ = 2973, 1718, 1703, 1596, 1585, 1512, 1382, 1333, 1310, 1266, 1198, 1131, 1100, 1071, 1015, 856, 824, 756 cm-1. 1H NMR (300 MHz, CDCl3) □ = 10.01 (s, 1H), 8.27 (d, 2H, J = 7.16 Hz), 8.04 (d, 2H, J =

6.60 Hz), 7.76 (m, 6H), 6.81 (d, 2H, J = 9.42 Hz), 4.49 (t, 2H, J = 6.62 Hz), 3.72 (t, 2H, J = 6.62 Hz), 3.51 (q, 2H, J = 6.97 Hz), 1.12 (t, 3H, J = 6.97 Hz) ppm. 13C NMR (300 MHz, CDCl3) □ = 191.4, 169.9, 155.9, 153.5, 150.7, 142.9, 141.9, 135.0, 130.3, 129.6, 124.7, 122.6, 111.6, 62.4, 58.5, 42.3, 12.4 ppm. ESI MS(+): calcd for C24H24N4O5+ 447.17, found 447.19.

On-bead proteolytic assays. 50 mg library resin was swollen overnight in 100 mM bis-tris buffer at the indicated pH (see below). The swelling solution was removed by filtration. 1 mL of a solution of the protease of interest (1 mg/ml)1 in 100 mM bis-tris buffer was added and resin was shaken for 18 hrs and washed extensively with bis-tris buffer, DMSO, DMF, MeOH, DCM, MeOH, DMF and finally with bis-tris buffer again (3 × each). Then 1 mL of 10 mM solution of **3** in THF/H_2O/AcOH (90:5:5, *v*/*v*/*v*) was added and the resin was shaken for 1 hrs. Afterwards $NaBH_3CN$ (6 mg, 0.09 mmmol) was added and the resin was shaken for 1 hrs. The resin was washed extensively with bis-tris buffer, DMSO, DMF, MeOH, DCM, MeOH, DMF and finally with bis-tris buffer again (3 × each) and a suspension of the resin in DMF was transferred to a silica gel plate and the beads were observed under a microscope. Single red colored beads were transferred via a syringe needle to amino acid analysis vials. The following commercial enzymes were used: Trypsin from pig pancreas 1645 U mg^{-1} (Fluka 82495); Subtilisin from *Bacillus licheniformis* 10.5 U mg^{-1} (Fluka 85968); α-Chymotrypsin from bovine pancreas 74.6 U mg^{-1} (Fluka 27270); Pepsin from porcine stomach mocusa 4500 U mg^{-1} (SIGMA p-6887).

Peptide Synthesis. The peptides were re-synthesized on Rink amide NovaGel™ (high-loading PEG-PS resin 0.63 mmol g^{-1}) using the same coupling conditions as described for the linear library synthesis. After TFA cleavage the peptides were precipitated with methyl *tert*-butyl ether and dissolved in water-acetonitrile mixture.

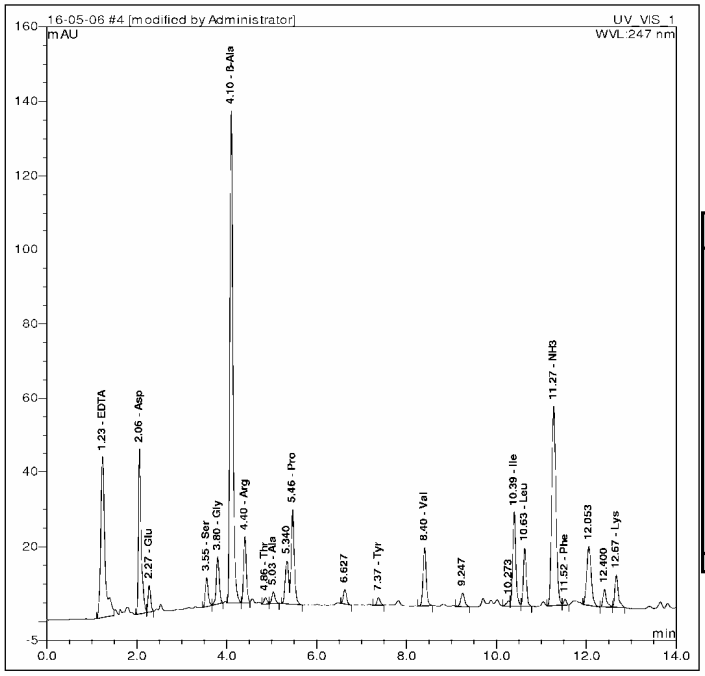
Solution phase proteolytic cleavage. Peptides **10**-**17** were conditioned as 2 mM stock solutions in water. The proteolysis was started by adding 5 μL of a freshly prepared 1 mg mL^{-1} stock solution2 of the protease in 100 mM bis-tris buffer to a mixture of 50 μL peptide stock solution and 45 μL 100 mM bis-tris buffer. The assay concentration under these conditions was 1 mM for the peptide substrate and 50 μg mL^{-1} for the protease. The following pH were used: Trypsin: pH 8, Subtilisin: pH 6.5, α-Chymotrypsin: pH 8, Pepsin: pH 4. The reactions were analyzed by RP-HPLC. Flow rate: 3.0 mL min^{-1}, 100% A, 0%D to 100% D, 0% 0 in 15

min. The crude proteolytic mixture was also subjected to ESI MS(+) analysis. The peptide fragments were identified using a computer generated database of possible mass fragments. For the Meprin-alpha the provided stock solution was diluted appropriately.

Bead analysis. The beads were transferred *via* a sterile syringe needle to amino acid analysis vial and hydrolyzed with aqueous HCl (6 M) at 110°C for 22 hrs. Quantitative AAA was performed by HPLC after derivatization with phenyl isothiocyanate. The detection limit for such analysis is usually 50 pmoles. We used tentagel beads of 90 micrometer diameter with 0.63 mmol/g loading. Sequences were assigned from AAA using the TAGSFREE program available from the author's website (http://www.dcb.unibe.ch/groups/reymond/). The user provides an input file <name>.csv describing the library as follows: Each line lists a different amino acid (in 3 letter code) followed by the usage vector as a series of 1's and 0's for each position up to the planned number of variable positions all separated by semicolons. The program handles "unique pair" designs or any other design. An excel file listing unique pairs is provided as a help for library design. Running the TAGSFREE program on the input file <name>.csv generates an output file <name>LIB.txt in which all possible AAA and the corresponding sequences are listed in the order of AAA and an output file <name>STAT.txt which contains the analysis of the generated library. The AAA of any bead is written in form of a series of integers (0, 1, 2) indicating the relative amounts of each building block in the order used in the input file. Decoding is carried out by typing an AAA in the search window of a text editor capable of handling the large library file. The search function automatically goes to the line(s) containing the AAA, which shows the corresponding sequence(s).

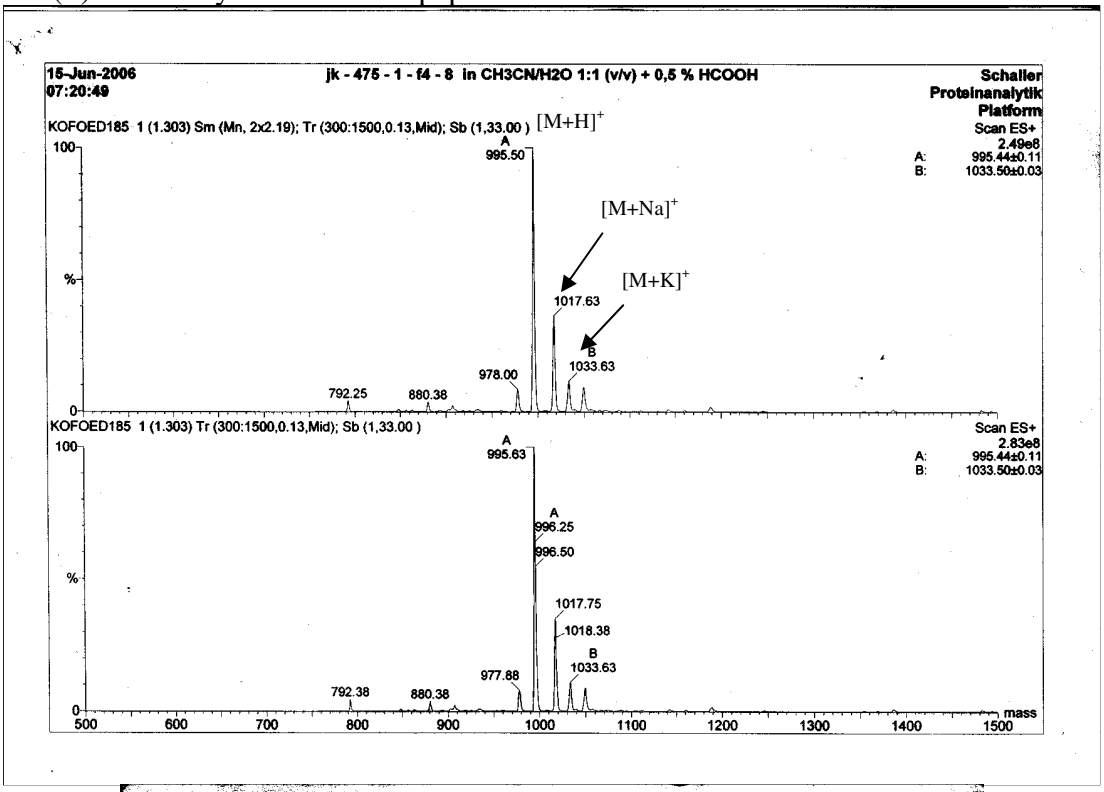
Ac-Pro-Asp-Gly-Leu-Arg-Ile-Val-Asp-Bla-NH_2 (4). From NovaGel^{TM}(160 mg, 0.63 mmol g^{-1}), **4** was obtained as colorless foamy solid after preparative HPLC purification (35 mg, 31%, as TFA-salt); anal. RP-HPLC (80% A, 20% D to 20% A, 80% D in 15 min): t_R = 6.68 min; ESI MS(+): calcd for $C_{43}H_{73}N_{13}O_{14}$: 995.54, found: 995.50.

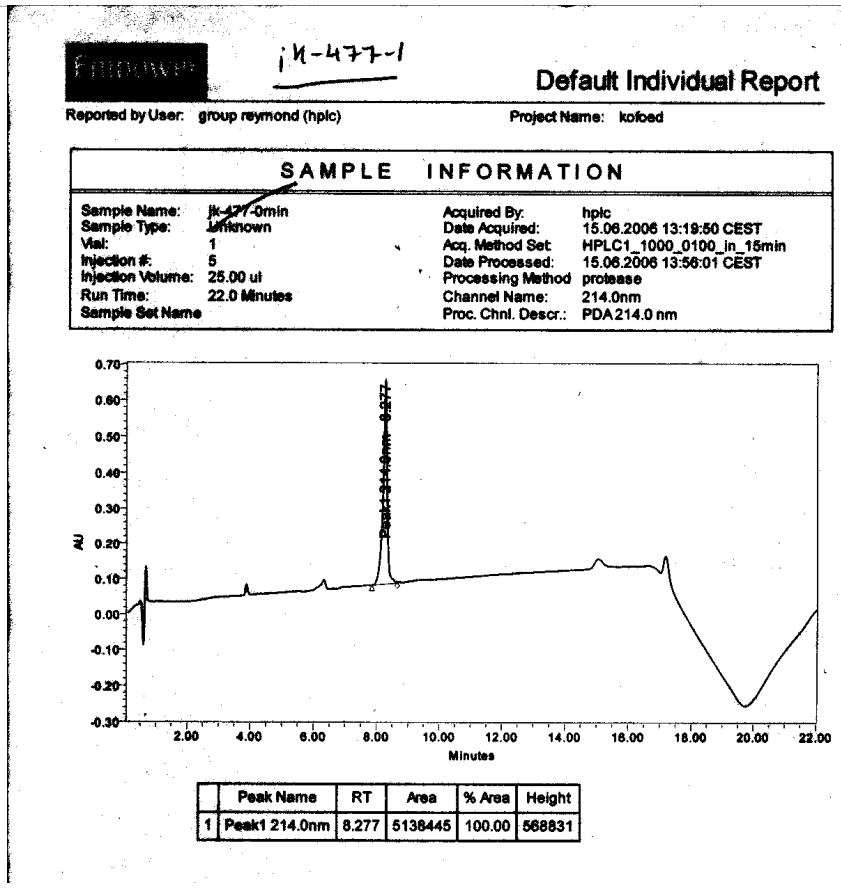
Amino acid analysis of the bead corresponding to peptide **4**



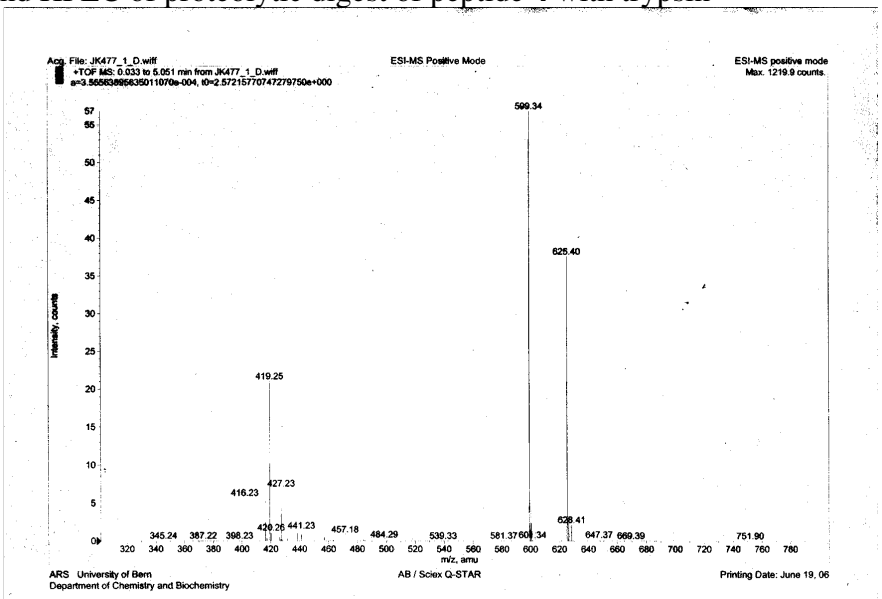
RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	Amount pmol	Peak Name
1,23	1,22	0,085	4,67	43,07	462,1	EDTA
2,06	2,06	0,059	3,19	44,28	197,0	Asp
2,27	2,27	0,062	0,47	7,10	33,8	Glu
3,55	3,55	0,068	0,57	7,76	40,6	Ser
3,80	3,79	0,068	0,96	12,55	65,6	Gly
4,10	4,09	0,070	10,32	132,38	715,9	ß-Ala
4,40	4,40	0,065	1,25	17,62	88,9	Arg
4,86	4,85	0,074	0,14	1,74	10,3	Thr
5,03	5,03	0,073	0,26	3,16	15,7	Ala
5,46	5,46	0,071	2,05	25,34	108,9	Pro
7,37	7,36	0,074	0,17	2,04	9,9	Tyr
8,40	8,39	0,073	1,27	15,53	74,0	Val
10,39	10,38	0,072	2,04	25,13	119,1	Ile
10,63	10,62	0,071	1,20	15,56	76,7	Leu
11,27	11,27	0,100	5,83	53,62	859,4	NH3
11,52	11,52	0,066	0,11	1,53	7,8	Phe
12,67	12,67	0,076	0,76	8,58	24,1	Lys
Total:			35,249	416,977	2909,60	

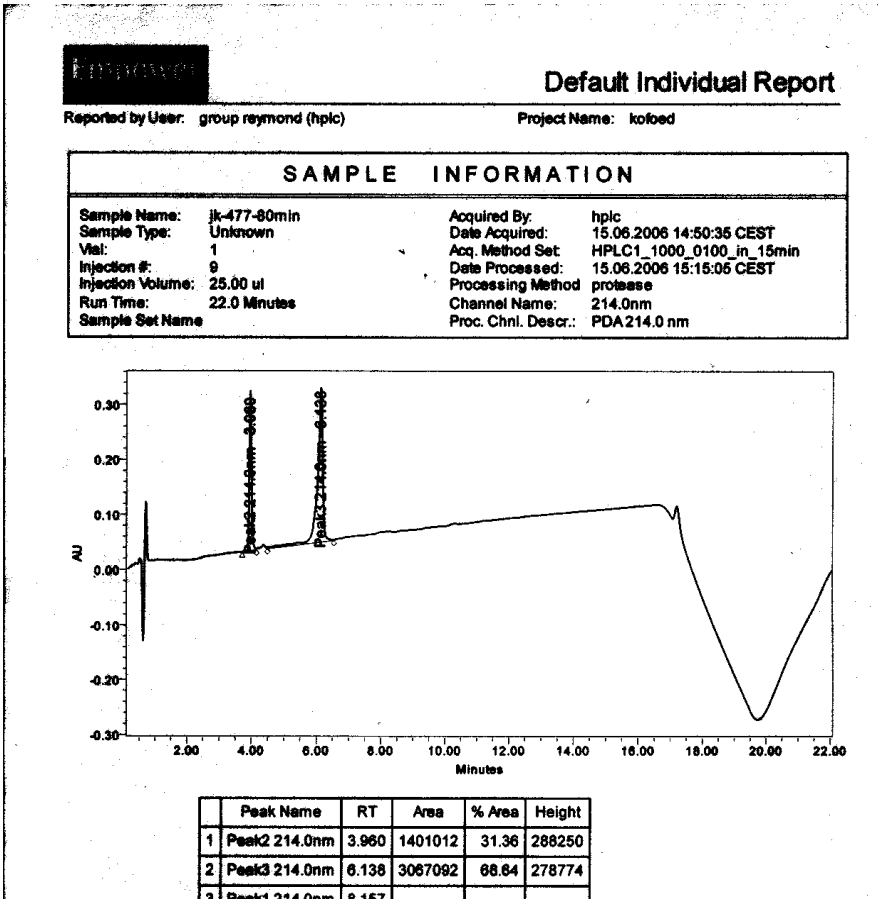
ESI-MS(+) and analytical HPLC of peptide **4**





ESI MS(+) and HPLC of proteolytic digest of peptide **4** with trypsin

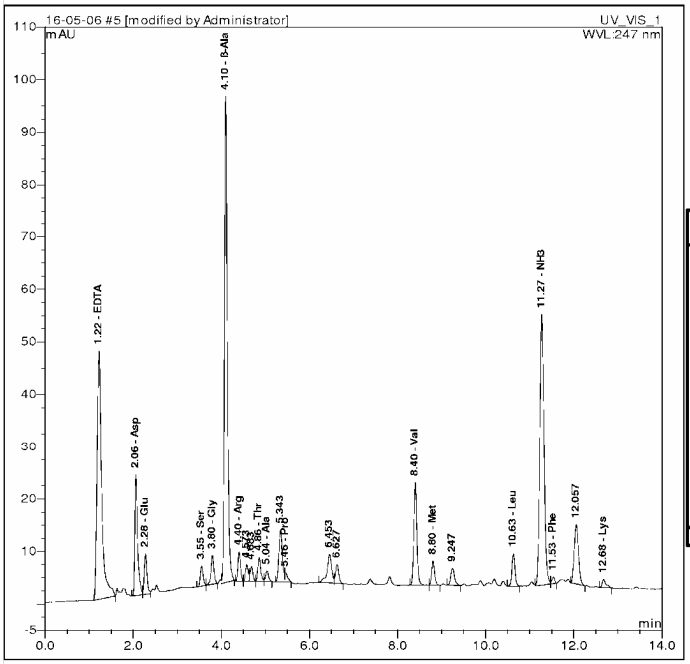




76.03992 G	191.06686 DG	345.22509 GLR	557.37756 GLRIV	787.43144 DGLRIVD
89.07157 B(AMID)	204.09851 DB(AMID)	346.19787 IVD	572.35209 RIVDB(AMID)	811.46786 P(ACET)DGLRIV
118.08687 V	231.17093 IV	387.27204 RIV	573.33609 DGLRI	857.48455 DGLRIVDB(AMID)
132.10252 I	233.11381 VD	401.28769 LRI	599.31539 P(ACET)DGLR	926.49480 P(ACET)DGLRIVD
132.10252 L	273.10876 P(ACET)D	416.25098 IVDB(AMID)	615.38304 LRIVD	996.54791 P(ACET)DGLRIVDB(AMID)
134.04540 D	288.20363 LR	443.21428 P(ACET)DGL	672.40450 DGLRIV	
134.04540 D	288.20363 RI	458.30915 GLRI	672.40450 GLRIVD	
158.08182 P(ACET)	303.16692 VDB(AMID)	460.25203 DGLR	685.43615 LRIVDB(AMID)	
175.11957 R	304.15092 DGL	500.35610 LRIV	712.39945 P(ACET)DGLRI	
189.12398 GL	330.13022 P(ACET)DG	502.29898 RIVD	742.45761 GLRIVDB(AMID)	

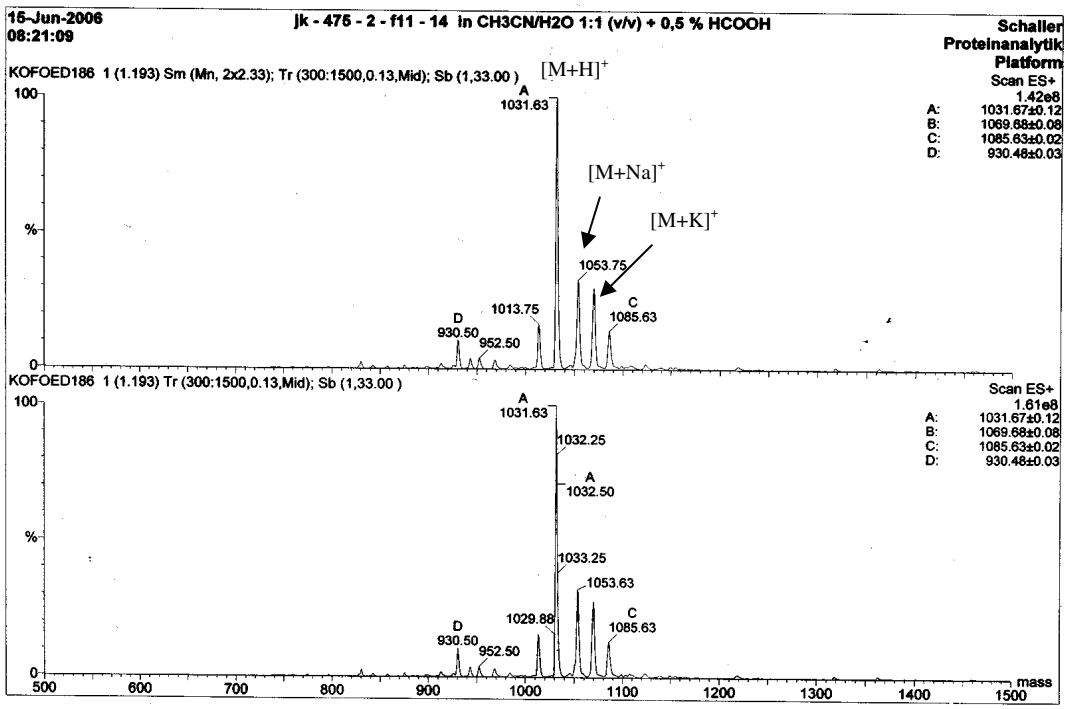
Ac-Thr-Asp-Gly-Arg-Glu-Met-Val-Leu-Bla-NH_2 (5). From NovaGel^{TM}(160 mg, 0.63 mmol g^{-1}), **5** was obtained as colorless foamy solid after preparative HPLC purification (27 mg, 31%, as TFA-salt); anal. RP-HPLC (80% A, 20% D to 20% A, 80% D in 15 min): t_R = 5.04 min; ESI MS(+): calcd for $C_{42}H_{73}N_{13}O_{15}S$: 1031.51, found: 1031.63.

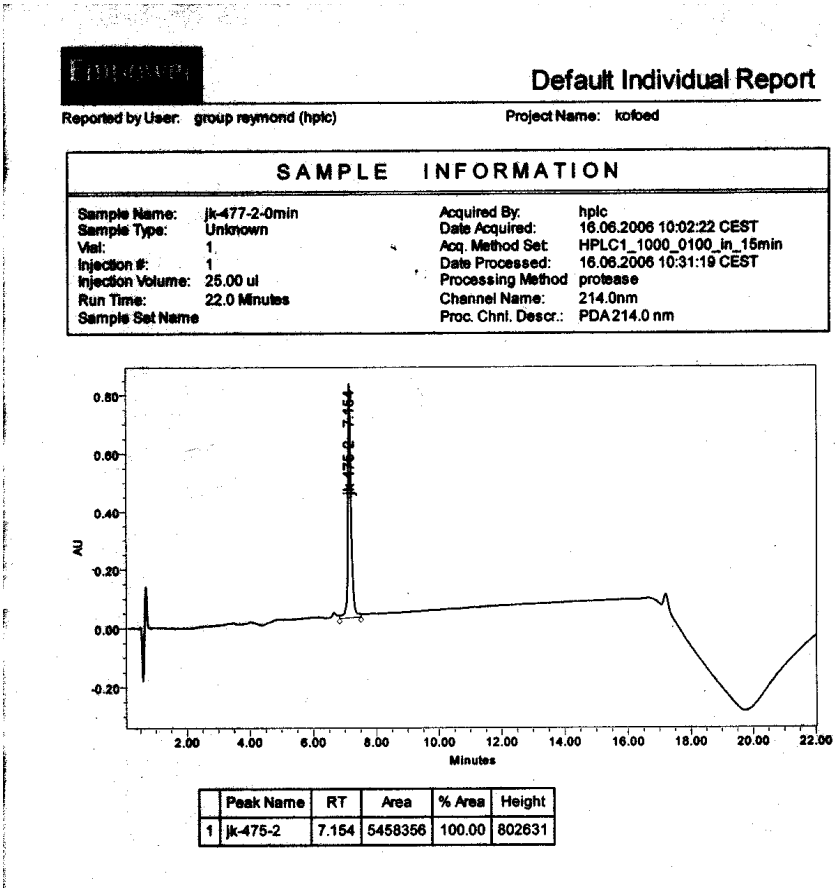
Amino acid analysis of the bead corresponding to peptide **5**



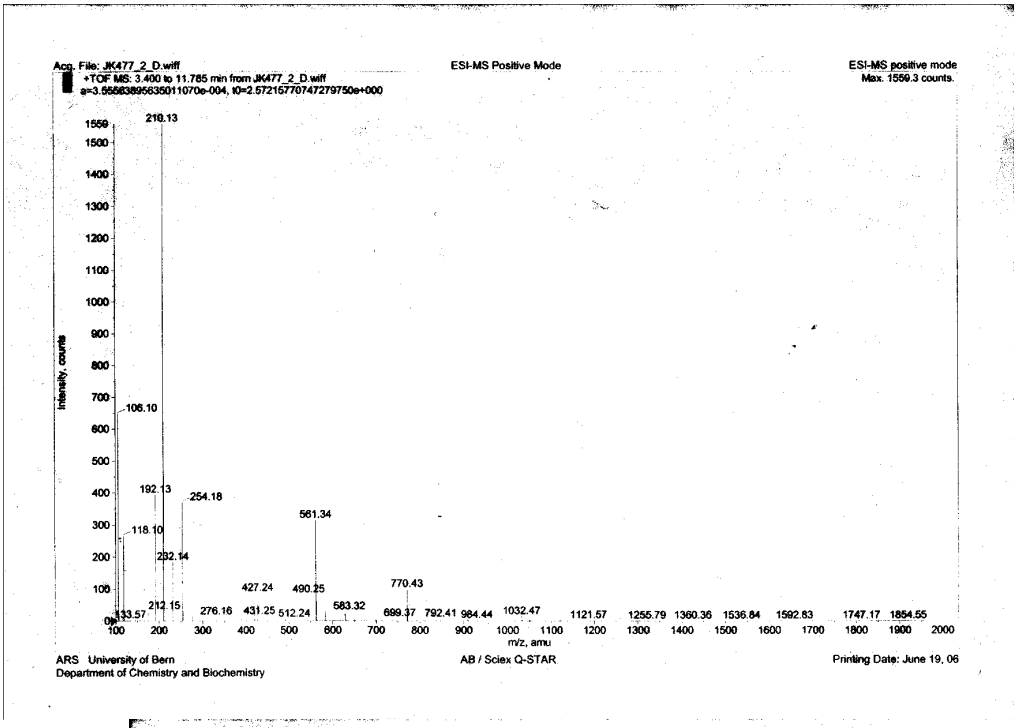
RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	Amount pmol	Peak Name
1,22	1,22	0,099	5,73	47,35	508,1	EDTA
2,06	2,06	0,059	1,66	23,02	102,4	Asp
2,28	2,27	0,064	0,56	7,71	36,7	Glu
3,55	3,55	0,067	0,28	3,84	20,1	Ser
3,80	3,79	0,069	0,43	5,48	28,6	Gly
4,10	4,09	0,071	7,29	92,75	501,6	ß-Ala
4,40	4,40	0,064	0,38	5,54	27,9	Arg
4,86	4,85	0,069	0,35	4,53	26,9	Thr
5,04	5,03	0,069	0,15	1,98	9,9	Ala
5,46	5,46	n.a.	0,13	1,70	7,3	Pro
8,40	8,39	0,072	1,58	19,53	93,0	Val
8,80	8,79	0,072	0,36	4,58	24,1	Met
10,63	10,62	0,071	0,48	6,18	30,4	Leu
11,27	11,27	0,101	5,68	51,62	827,3	NH3
11,53	11,52	0,073	0,10	1,27	6,4	Phe
12,68	12,67	0,078	0,16	1,60	4,5	Lys
Total:			25,310	278,685	2255,33	

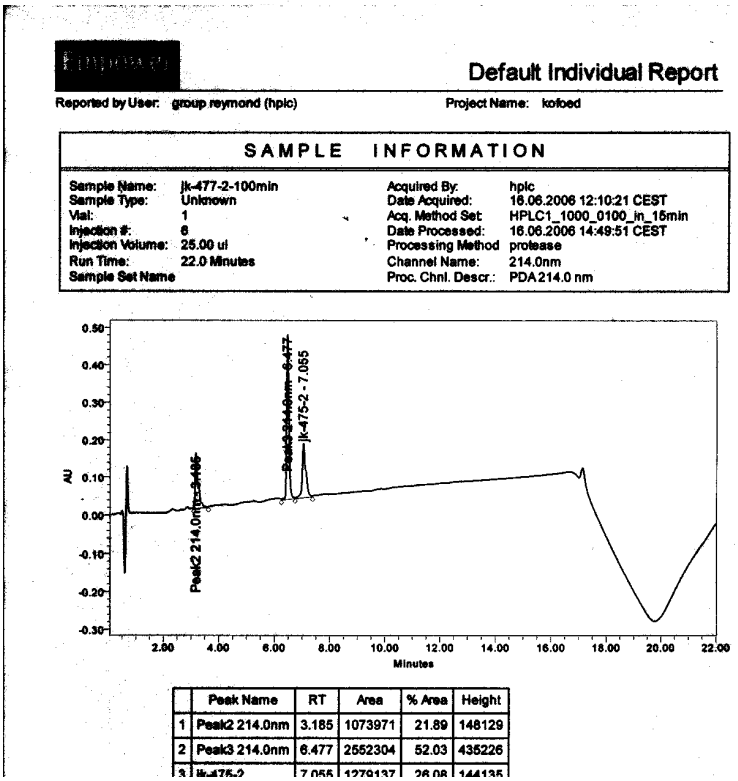
ESI-MS(+) and analytical HPLC of peptide **5**





ESI MS(+) and HPLC of proteolytic digest of peptide **5** with trypsin

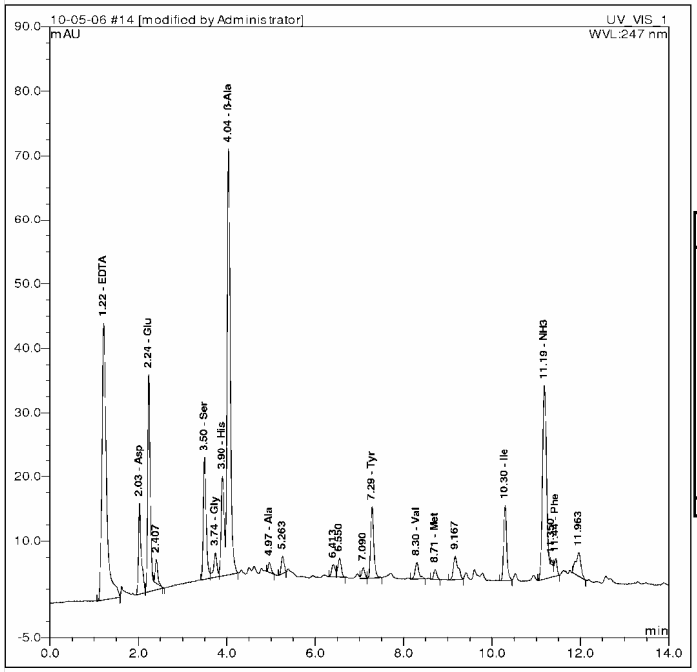




76.03992	G	202.15563	LB(AMID)	361.18362	GRE	561.30712	EMVLB(AMID)	819.40352	DGREMVL
89.07157	B(AMID)	231.17093	VL	362.21142	MVL	591.29252	GREMV	849.37774	T(ACET)DGREMV
118.08687	V	232.14103	GR	378.16995	EMV	607.25105	DGREM	889.45663	DGREMVLB(AMID)
132.10252	L	249.12736	MV	432.26453	MVLB(AMID)	619.26884	T(ACET)DGRE	962.46180	T(ACET)DGREMVL
134.04540	D	277.10368	T(ACET)D	435.20265	REM	647.35512	REMVL	1032.51491	T(ACET)DGREMVLB(AMID)
148.06105	E	279.10154	EM	476.21056	DGRE	704.37658	GREMVL		
150.05895	M	301.22404	VLB(AMID)	490.22625	T(ACET)DGR	706.31946	DGREMV		
162.07674	T(ACET)	304.16216	RE	491.25401	EMVL	717.40823	REMVLB(AMID)		
175.11957	R	334.12514	T(ACET)DG	492.22411	GREM	750.30933	T(ACET)DGREM		
191.06686	DG	347.16797	DGR	534.27106	REMV	774.42969	GREMVLB(AMID		

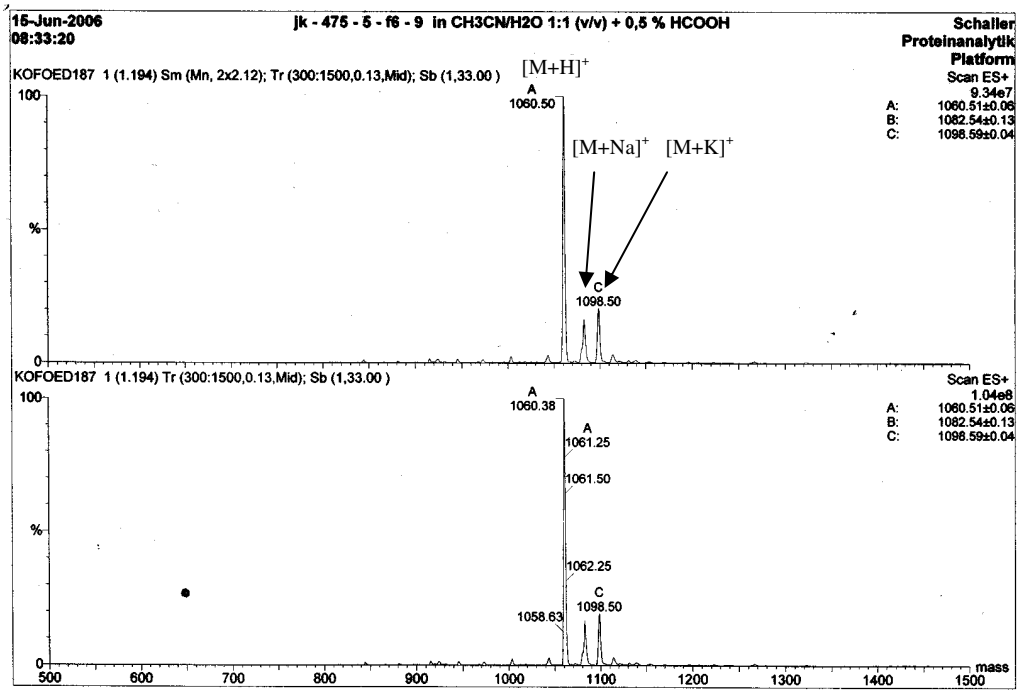
Ac-His-Asp-Glu-Ser-Glu-Ile-Tyr-Gly-Bla-NH_2 (6). From NovaGel^{TM} (160 mg, 0.63 mmol g^{-1}), **6** was obtained as colorless foamy solid after preparative HPLC purification (51 mg, 43%, as TFA-salt); anal. RP-HPLC (80% A, 20% D to 20% A, 80% D in 15 min): t_R = 2.90 min; ESI MS(+): calcd for $C_{45}H_{64}N_{12}O_{18}$: 1060.45, found: 1060.50.

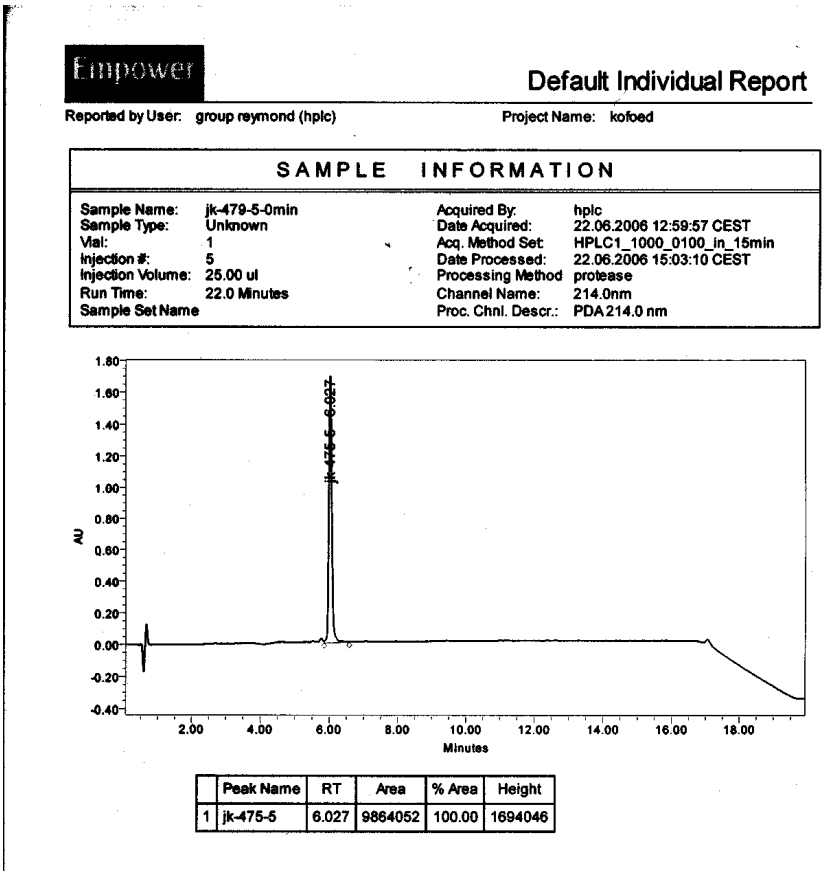
Amino acid analysis of the bead corresponding to peptide **6**



RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	Amount pmol	Peak Name
1,22	1,22	0,096	5,14	43,08	349,8	EDTA
2,03	2,03	0,060	1,06	14,13	57,8	Asp
2,24	2,24	0,063	2,51	33,81	151,5	Glu
3,50	3,50	0,069	1,45	18,94	93,0	Ser
3,74	3,74	0,070	0,33	3,82	19,2	Gly
3,90	3,90	0,076	1,19	15,51	79,5	His
4,04	4,04	0,074	5,50	66,33	315,0	ß-Ala
4,97	4,97	0,063	0,11	1,57	7,6	Ala
7,29	7,28	0,073	0,90	11,07	52,7	Tyr
8,30	8,31	0,082	0,25	2,53	11,8	Val
8,71	8,71	0,071	0,12	1,50	9,7	Met
10,30	10,29	0,071	0,94	11,71	55,8	Ile
11,19	11,18	0,099	3,40	30,15	354,7	NH3
11,44	11,42	n.a.	0,20	2,73	14,5	Phe
Total:			23,098	256,888	1572,72	

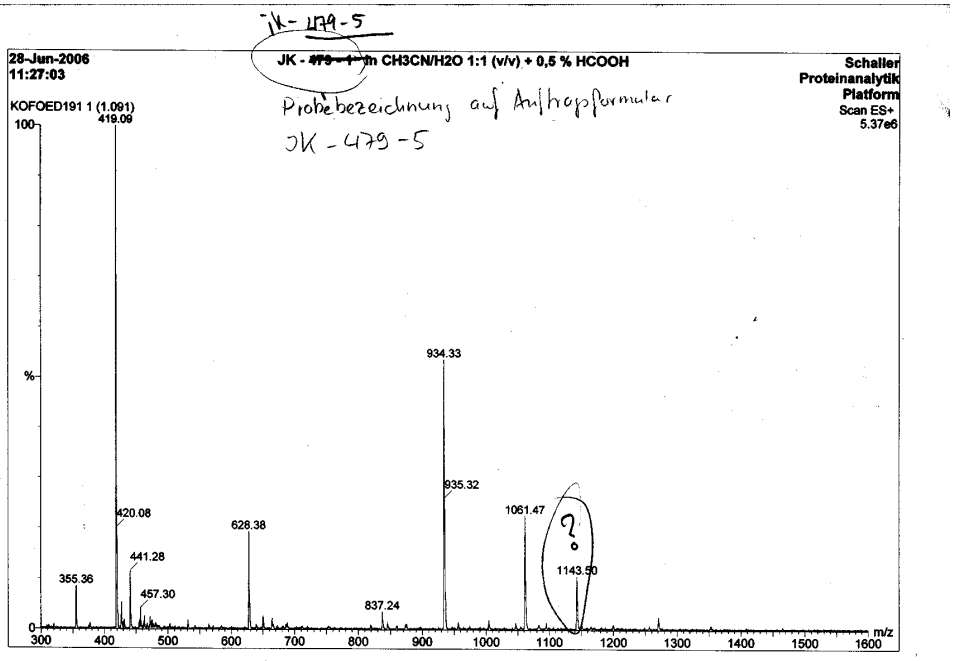
ESI-MS(+) and analytical HPLC of peptide **6**

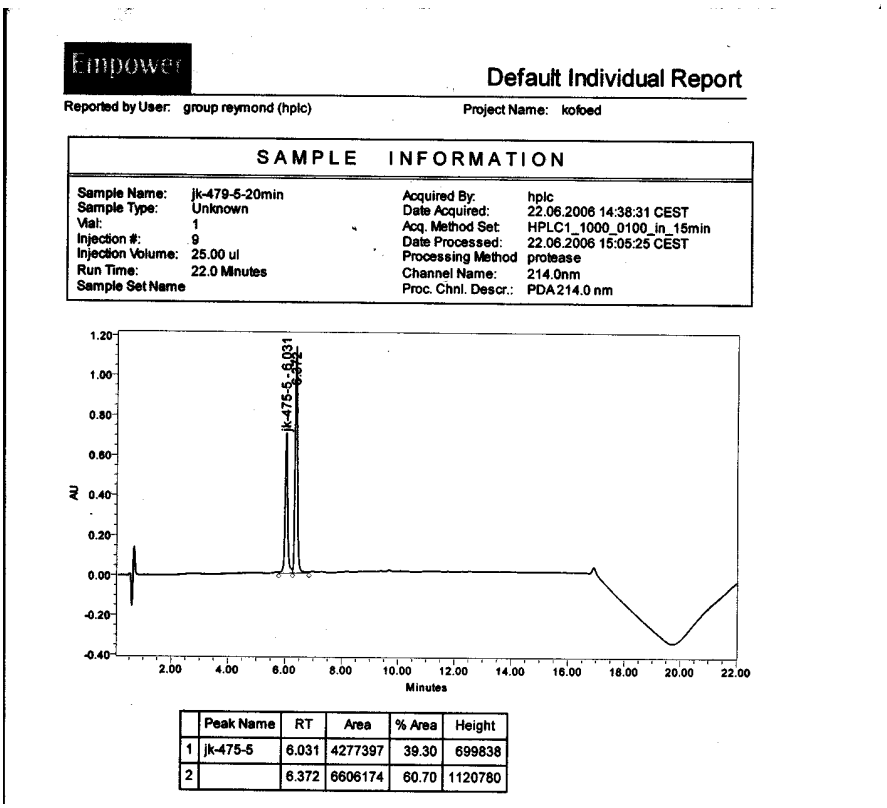




	Peak Name	RT	Area	% Area	Height
1	jk-475-5	6.027	9864052	100.00	1694046

ESI MS(+) and HPLC of proteolytic digest of peptide **6** with α-Chymotrypsin



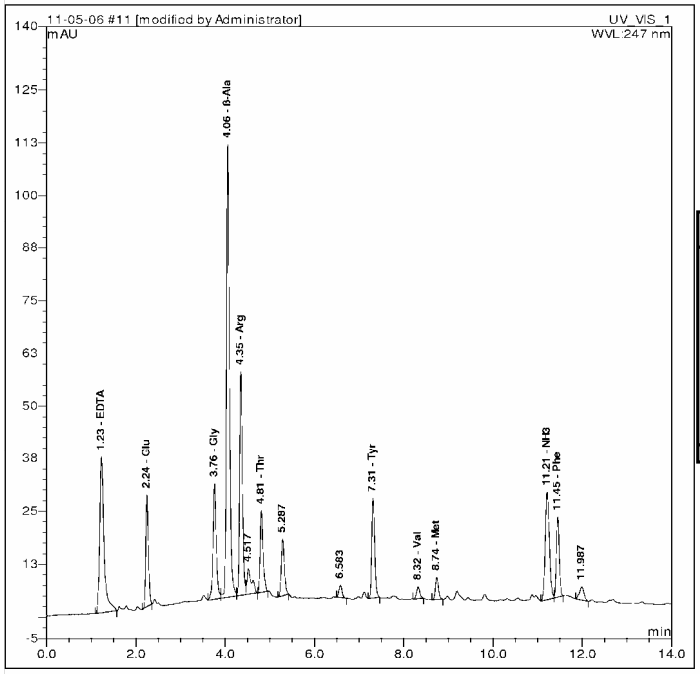


	Peak Name	RT	Area	% Area	Height
1	jk-475-5	6.031	4277397	39.30	699838
2		6.372	6606174	60.70	1120780

76.03992	G	198.08797	H(ACET)	348.17714	SEI	481.22990	EIYG	697.30452	ESEIYG
89.07157	B(AMID)	235.09308	ES	350.12002	DES	511.24047	SEIY	755.31000	DESEIY
106.05049	S	235.09308	SE	352.18731	IYG	529.18953	H(ACET)DES	767.35763	ESEIYGB(AMID)
132.10252	I	239.10325	YG	364.13567	ESE	551.28301	EIYGB(AMID)	771.31618	H(ACET)DESEI
134.04540	D	261.14511	EI	422.24042	IYGB(AMID)	568.26193	SEIYG	812.33146	DESEIYG
146.09303	GB(AMID)	263.08799	DE	424.20844	EIY	592.24667	DESEI	882.38457	DESEIYGB(AMID)
148.06105	E	295.16585	IY	442.15750	H(ACET)DE	638.31504	SEIYGB(AMID)	934.37951	H(ACET)DESEIY
148.06105	E	309.15636	YGB(AMID)	477.21973	ESEI	640.28306	ESEIY	991.40097	H(ACET)DESEIYG
182.08179	Y	313.11491	H(ACET)D	479.16261	DESE	658.23212	H(ACET)DESE	1061.45408	H(ACET)DESEIYGB(AMID)

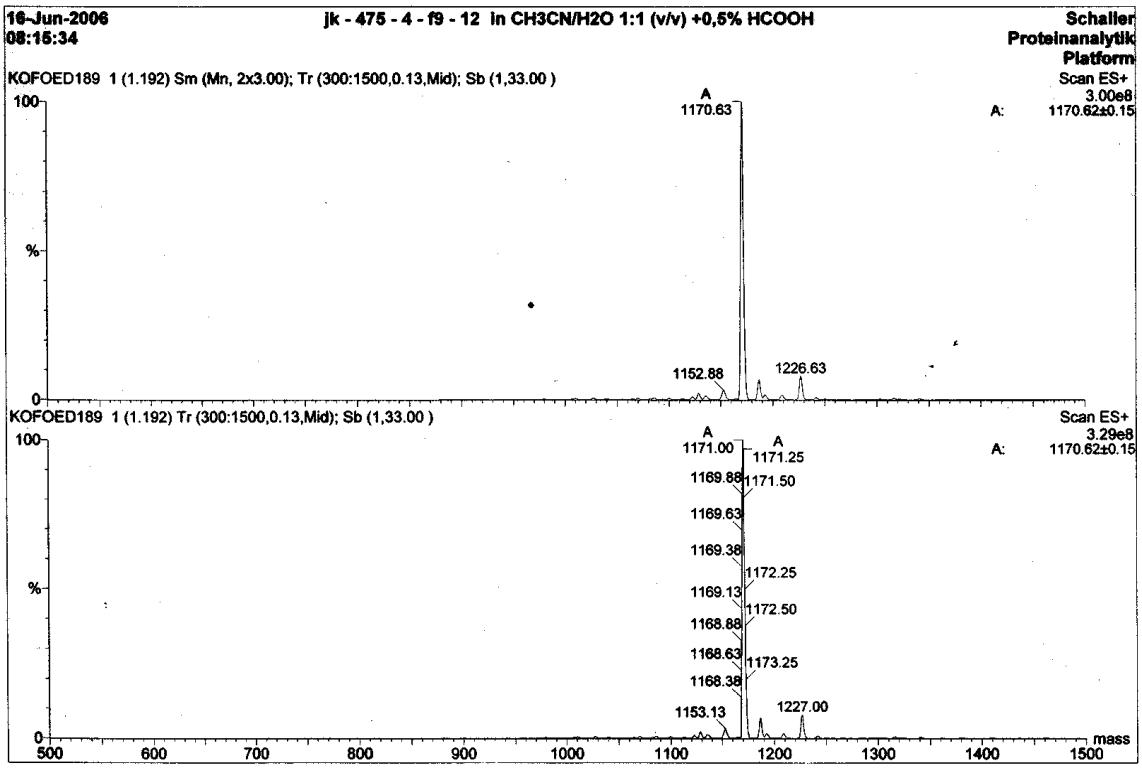
Ac-Thr-Phe-Glu-Arg-Arg-Met-Tyr-Gly-Bla-NH_2 (7). From NovaGel^{TM} (160 mg, 0.63 mmol g^{-1}), **7** was obtained as colorless foamy solid after preparative HPLC purification (31 mg, 22%, as TFA-salt); anal. RP-HPLC (80% A, 20% D to 20% A, 80% D in 15 min): t_R = 4.45 min; ESI MS(+): calcd for $C_{51}H_{78}N_{16}O_{14}S$: 1170.56, found: 1170.63.

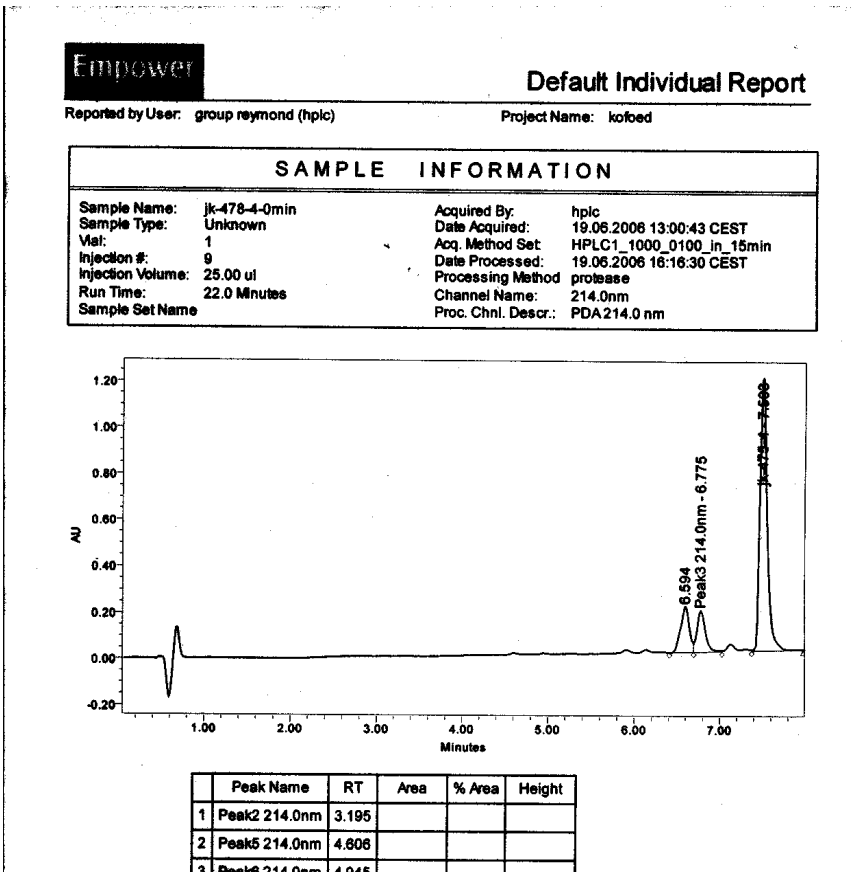
Amino acid analysis of the bead corresponding to peptide **7**



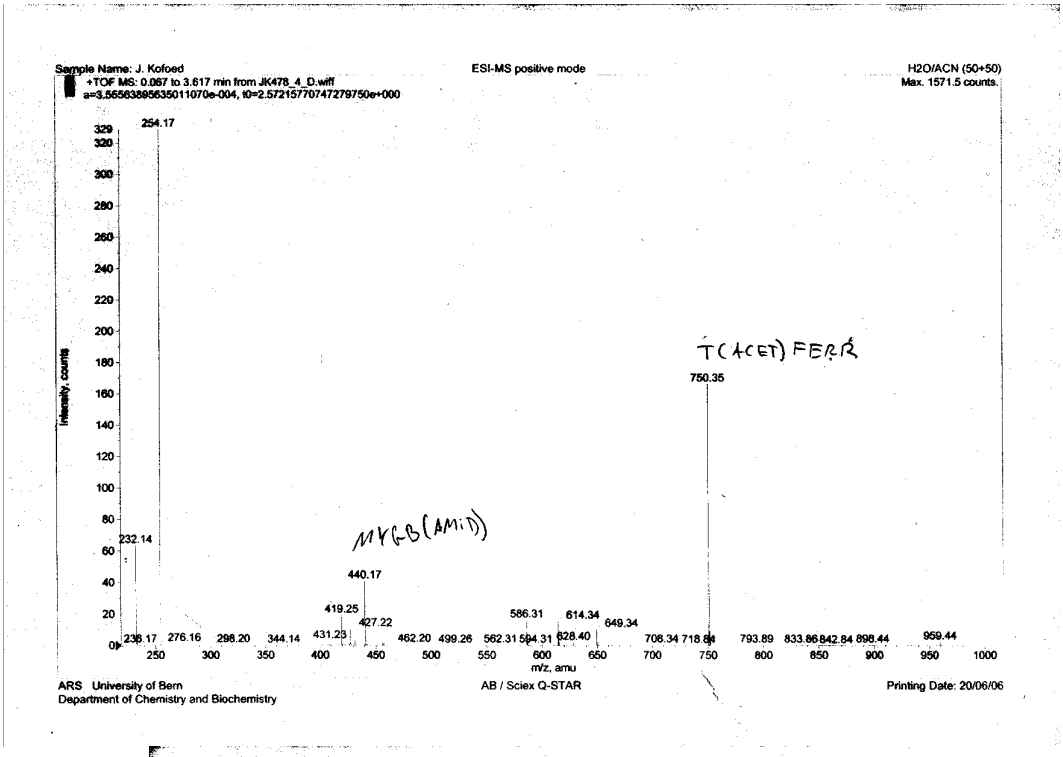
RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	Amount pmol	Peak Name
1,23	1,22	0,091	4,13	36,82	267,0	EDTA
2,24	2,25	0,064	1,81	26,25	134,7	Glu
3,76	3,76	0,071	2,25	27,23	148,7	Gly
4,06	4,06	0,070	8,55	107,34	573,4	ß-Ala
4,35	4,35	0,066	3,86	53,02	275,8	Arg
4,81	4,81	0,069	1,49	19,24	116,8	Thr
7,31	7,31	0,069	1,78	23,44	120,7	Tyr
8,32	8,34	0,080	0,24	2,77	13,9	Val
8,74	8,74	0,071	0,40	5,21	31,6	Met
11,21	11,21	0,100	2,72	25,34	336,6	NH3
11,45	11,44	0,071	1,43	18,92	102,7	Phe
Total:			28,669	345,563	2121,92	

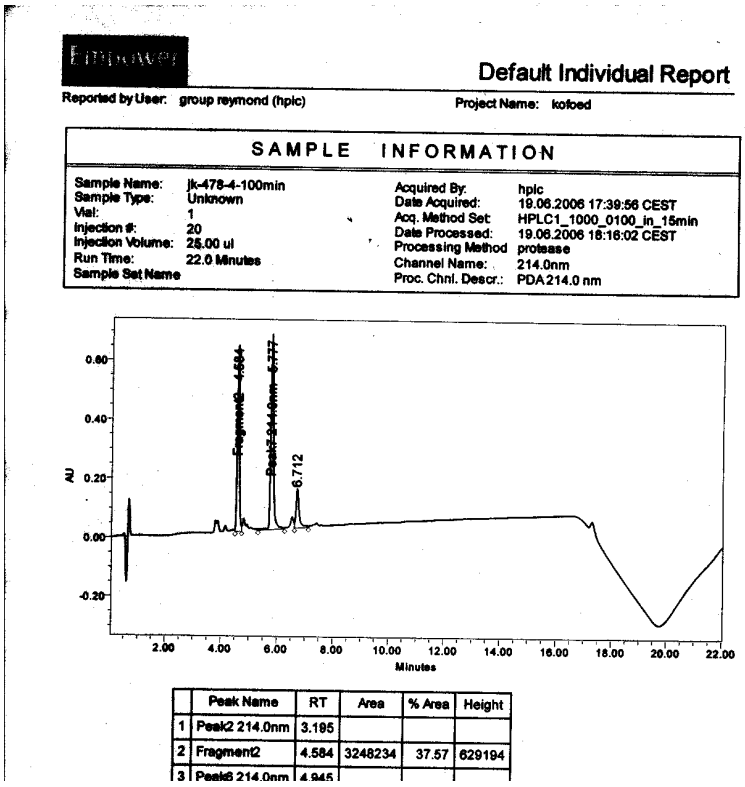
ESI-MS(+) and analytical HPLC of peptide **7**





ESI MS(+) and HPLC of proteolytic digest of peptide **7** with Subtilisn

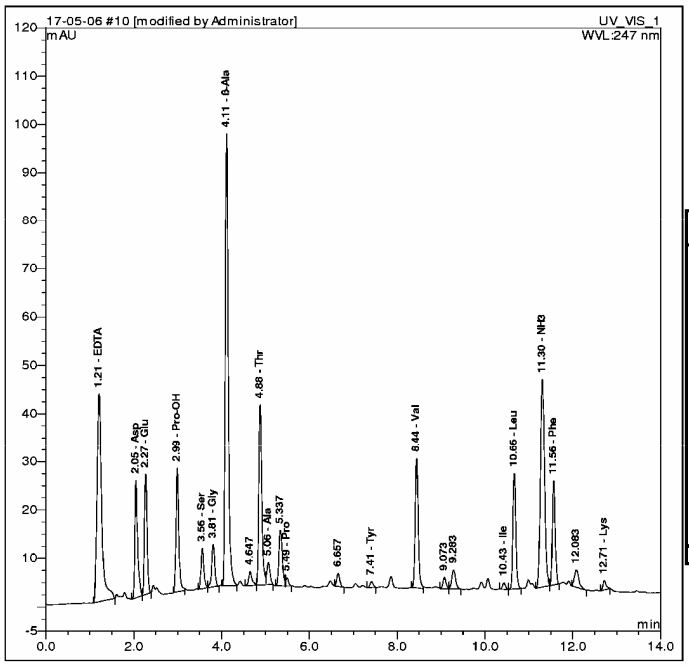




76.03992	G	182.08179	Y	370.14374	MYG	594.28885	T(ACET)FER	811.38855	ERRMYG
89.07157	B(AMID)	239.10325	YG	438.18774	T(ACET)FE	596.29796	RMYGB(AMID)	881.43045	T(ACET)FERRM
146.09303	GB(AMID)	295.12946	FE	440.19685	MYGB(AMID)	607.33168	FERR	881.44166	ERRMYGB(AMID)
148.06105	E	304.16216	ER	451.23057	FER	625.32450	RRMY	901.43550	FERRMY
150.05895	M	306.16006	RM	460.26327	ERR	682.34596	RRMYG	958.45696	FERRMYG
162.07674	T(ACET)	309.14515	T(ACET)F	462.26117	RRM	738.37217	FERRM	1028.51007	FERRMYGB(AMID)
166.08687	F	309.15636	YGB(AMID)	469.22339	RMY	750.38996	T(ACET)FERR	1044.49378	T(ACET)FERRMY
175.11957	R	313.12228	MY	526.24485	RMYG	752.39907	RRMYGB(AMID)	1101.51524	T(ACET)FERRMYG
175.11957	R	331.22068	RR	591.30376	ERRM	754.36709	ERRMY	1171.56835	T(ACET)FERRMYGB(AMID)

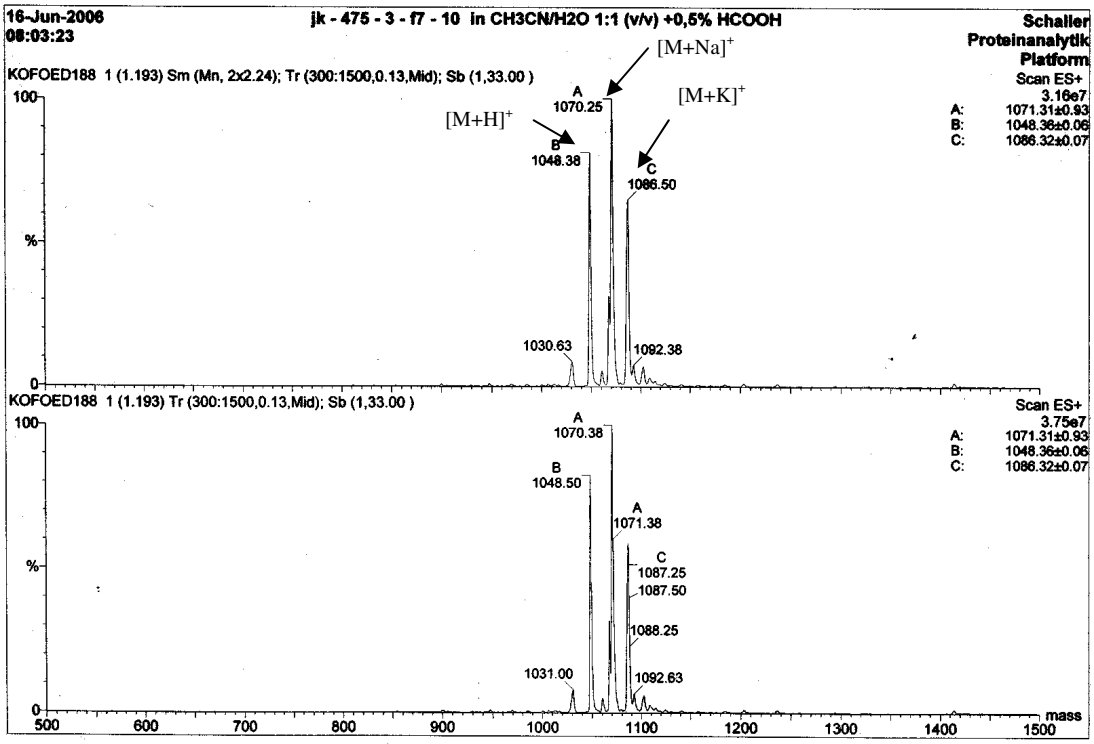
Ac-Thr-Asp-Glu-Leu-Phe-Hyp-Val-Thr-Bla-NH_2 (8). From NovaGel^{TM} (160 mg, 0.63 mmol g^{-1}), **8** was obtained as colorless foamy solid after preparative HPLC purification (53 mg, 50%, as TFA-salt); anal. RP-HPLC (80% A, 20% D to 20% A, 80% D in 15 min): t_R = 6.41 min; ESI MS(+): calcd for $C_{47}H_{72}N_{10}O_{17}S$: 1048.51, found: 1048.38.

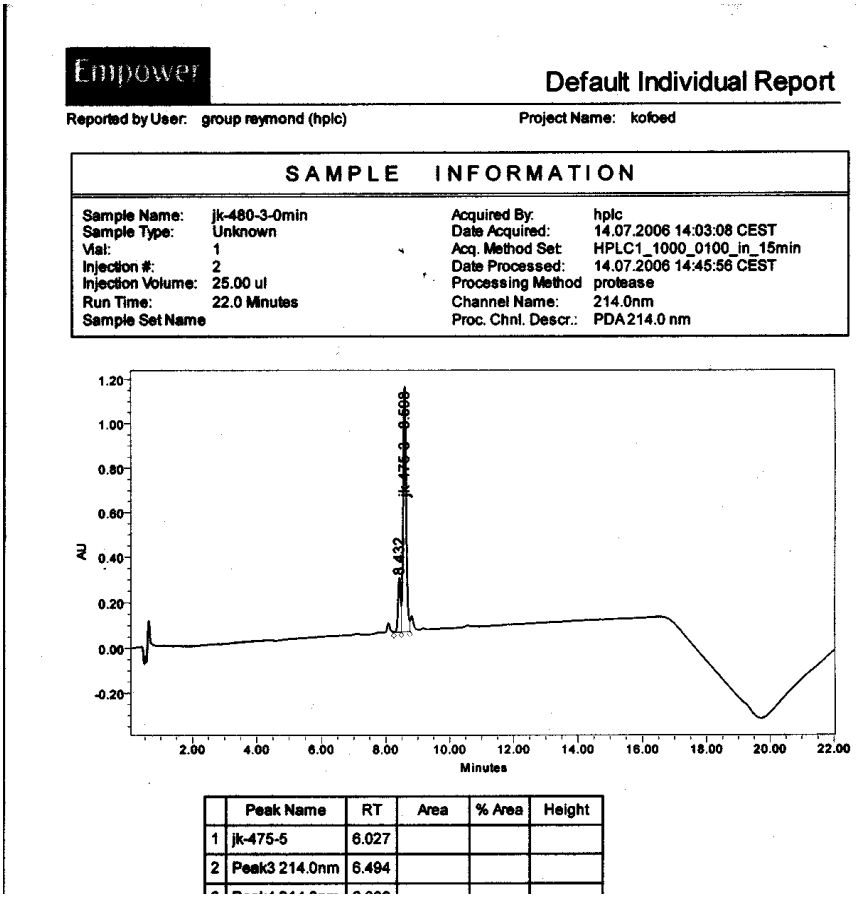
Amino acid analysis of the bead corresponding to peptide **8**



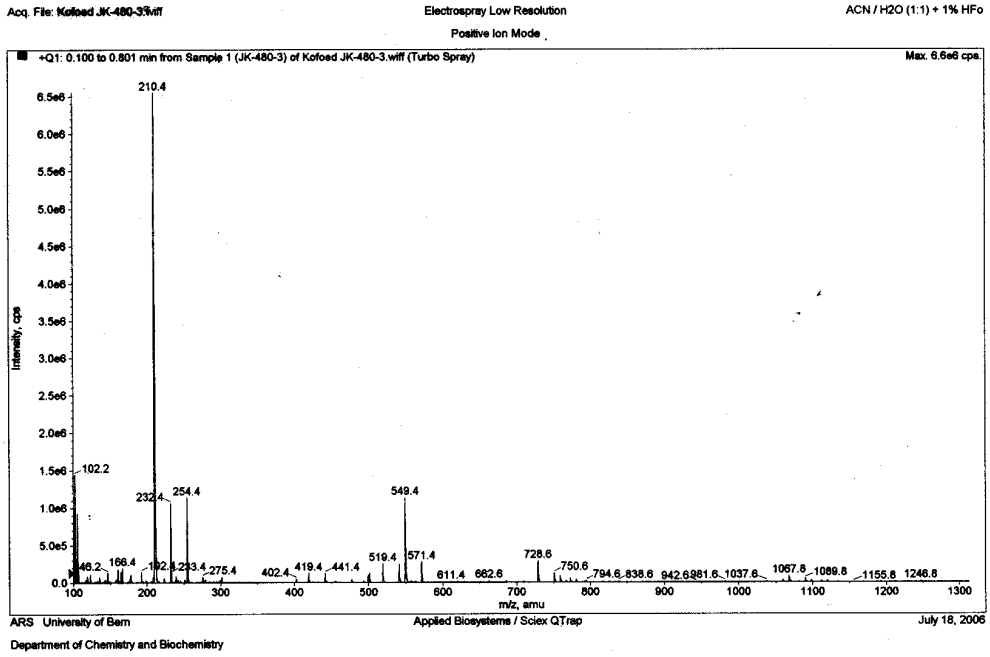
RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	Amount pmol	Peak Name
1,21	1,22	0,103	5,28	43,00	453,2	EDTA
2,05	2,08	0,062	1,78	24,22	99,1	Asp
2,27	2,29	0,065	1,73	24,77	107,4	Glu
2,99	3,00	0,067	1,92	25,53	94,9	Pro-OH
3,56	3,57	0,069	0,63	8,30	38,9	Ser
3,81	3,82	0,071	0,70	8,68	41,9	Gly
4,11	4,12	0,072	7,51	93,70	455,6	ß-Ala
4,88	4,88	0,070	2,94	37,34	208,0	Thr
5,06	5,06	0,081	0,38	4,52	21,0	Ala
5,49	5,49	n.a.	0,12	1,67	6,6	Pro
7,41	7,40	0,078	0,10	1,16	5,3	Tyr
8,44	8,43	0,072	2,15	26,73	118,4	Val
10,43	10,42	0,069	0,10	1,32	5,9	Ile
10,66	10,66	0,071	1,84	23,82	111,2	Leu
11,30	11,30	0,100	4,65	43,03	435,5	NH3
11,56	11,55	0,070	1,60	21,49	102,0	Phe
12,71	12,70	0,073	0,16	1,91	5,1	Lys
Total:			33,587	391,208	2310,21	

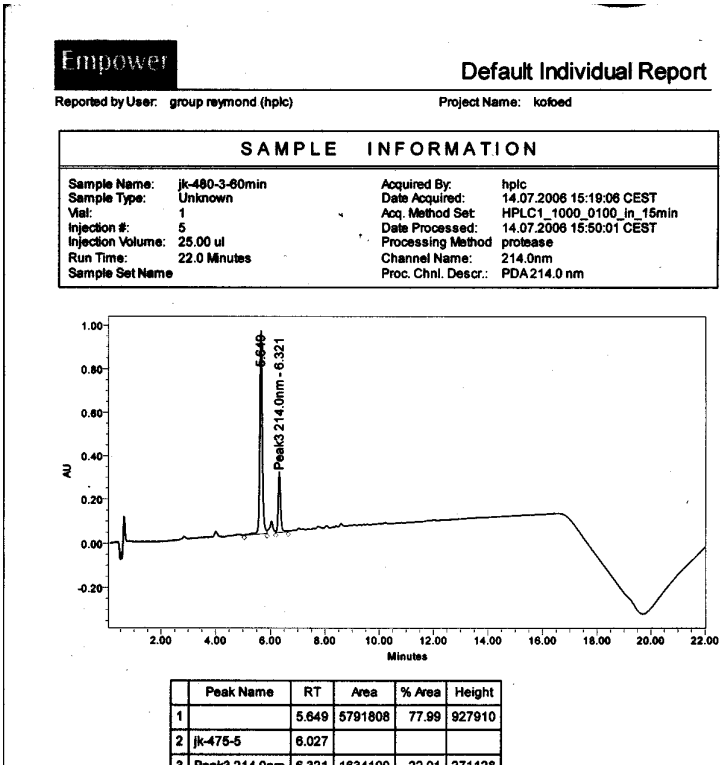
ESI-MS(+) and analytical HPLC of peptide **8**





ESI MS(+) and HPLC of proteolytic digest of peptide **8** with Pepsin

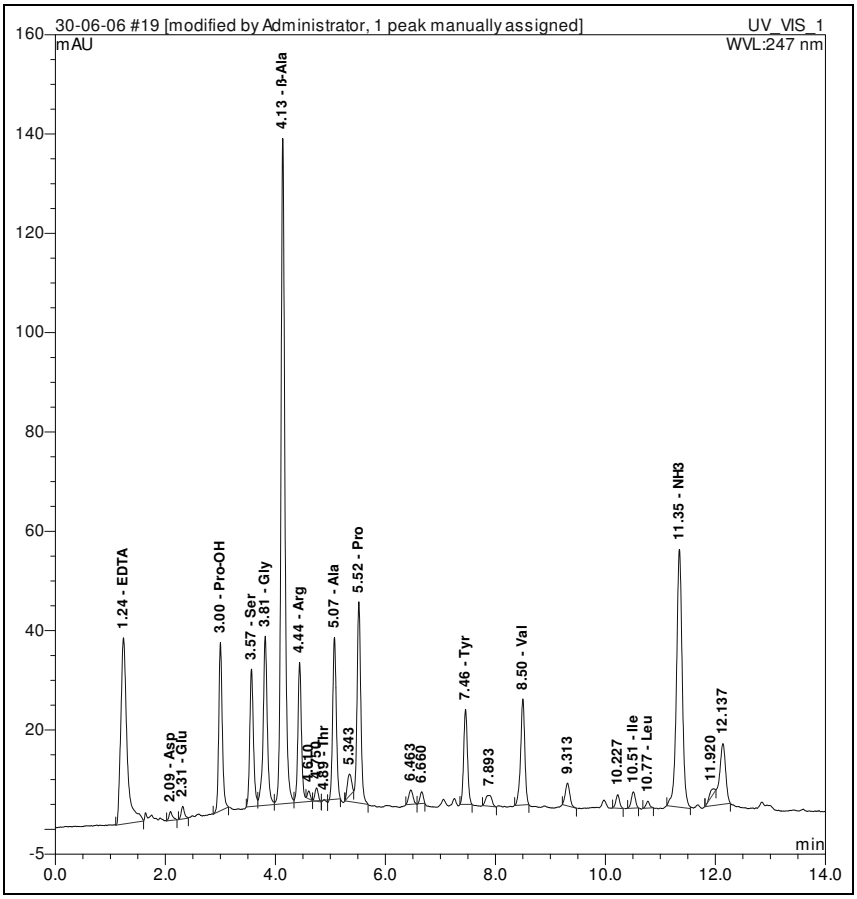




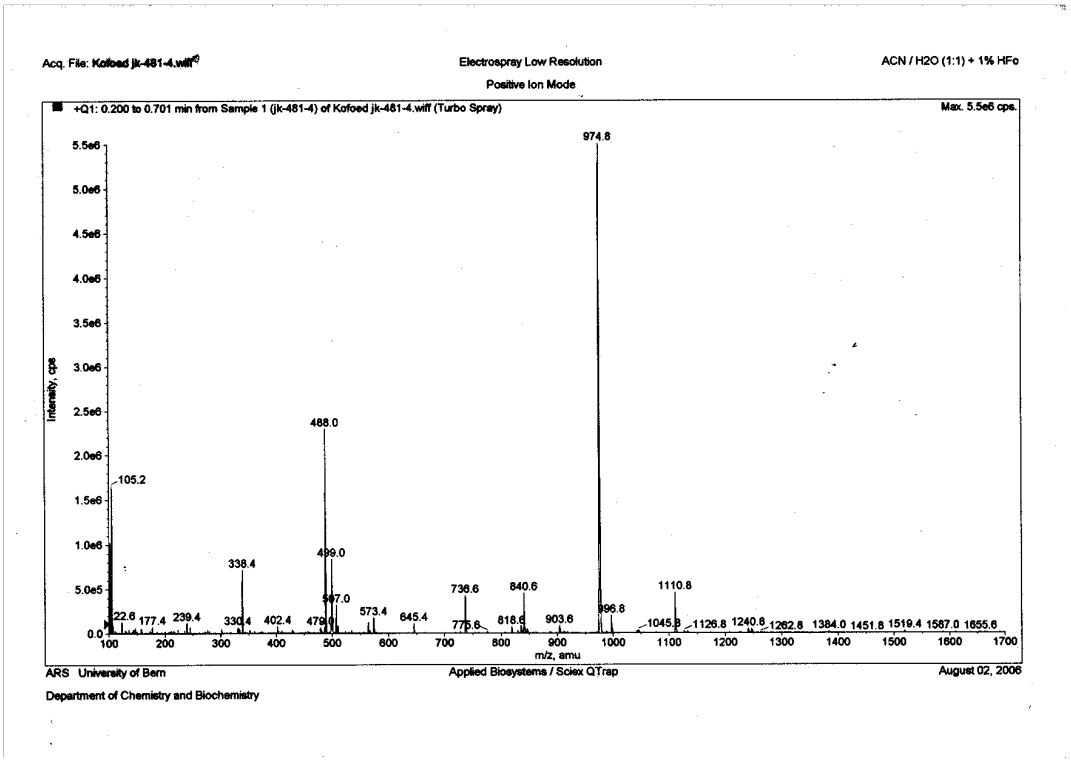
89.07157 B(AMID)	219.13455 VT	378.20296 FXV	549.30375 FXVTB(AMID)	836.40423 DELFXVT
118.08687 V	231.13455 XV	392.21861 LFX	592.33470 LFXVT	878.41483 T(ACET)DELFXV
120.06614 T	261.14511 EL	402.23534 XVTB(AMID)	620.32961 ELFXV	906.45734 DELFXVTB(AMID)
132.06614 X	263.08799 DE	406.14627 T(ACET)DE	636.28814 DELFX	979.46251 T(ACET)DELFXVT
132.10252 L	277.10368 T(ACET)D	408.21352 ELF	662.38781 LFXVTB(AMID)	1049.51562 T(ACET)DELFXVTB(AMID)
134.04540 D	279.13455 FX	479.25064 FXVT	666.29874 T(ACET)DELF	
148.06105 E	279.17093 LF	491.28702 LFXV	721.37729 ELFXVT	
162.07674 T(ACET)	289.18766 VTB(AMID)	519.23033 T(ACET)DEL	735.35655 DELFXV	
166.08687 F	332.18223 XVT	521.26120 ELFX	779.34642 T(ACET)DELFX	
190.11925 TB(AMID)	376.17205 DEL	523.24046 DELF	791.43040 ELFXVTB(AMID)	

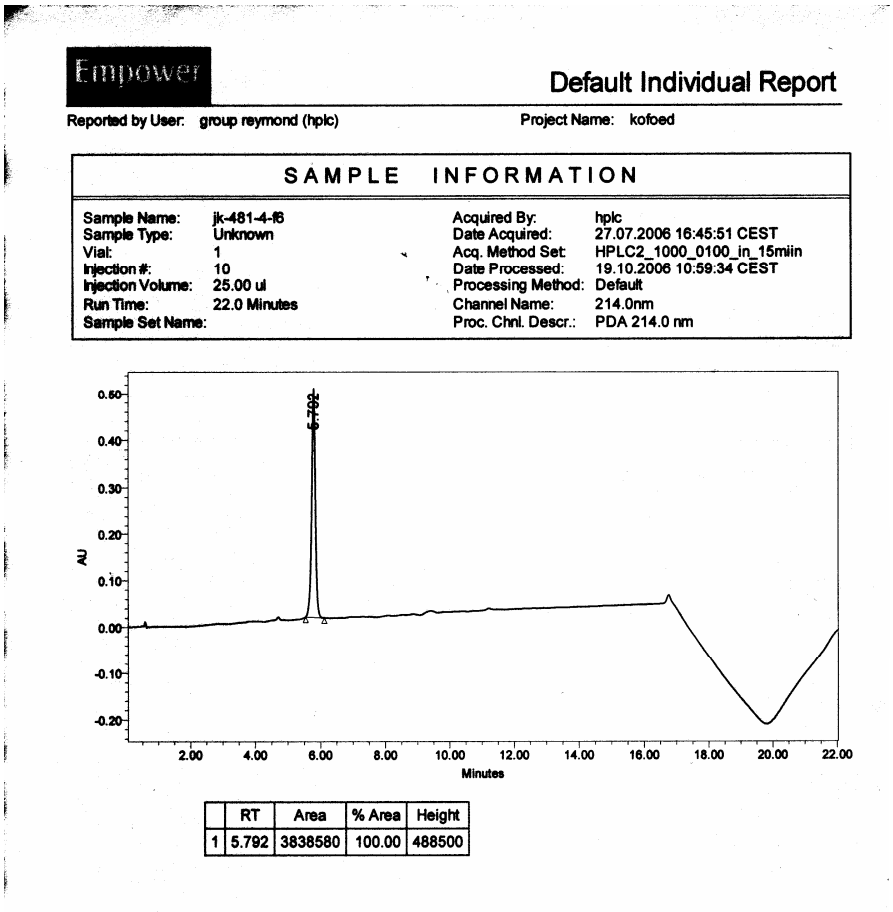
Ac-Pro-Val-Tyr-Ser-Arg-Hyp-Ala-Gly-Bla-NH_2 (9). From NovaGel^{TM} (160 mg, 0.63 mmol g^{-1}), **9** was obtained as colorless foamy solid after preparative HPLC purification (59 mg, 53%, as TFA-salt); anal. RP-HPLC (100% A, 0% D to 0% A, 100% D in 15 min): t_R = 5.79 min; ESI MS(+): calcd for $C_{43}H_{67}N_{13}O_{13}$: [M+H]+ 974.5, found: 974.8.

Amino acid analysis of the bead corresponding to peptides **9** and **10**



ESI-MS(+) and analytical HPLC of peptide **9**





Ac-Hyp-Val-Tyr-Arg-Pro-Ser-Ala-Gly-Bla-NH_2 (10). From NovaGel^{TM} (160 mg, 0.63 mmol g^{-1}), **10** was obtained as colorless foamy solid after preparative HPLC purification (74 mg, 67%, as TFA-salt); anal. RP-HPLC (100% A, 0% D to 0% A, 100% D in 15 min): t_R = 5.76 min; ESI MS(+): calcd for $C_{43}H_{67}N_{13}O_{13}$: [M+H]+ 974.5, found: 974.8.

ESI-MS(+) and analytical HPLC of peptide **10**