Peptide Synthesis Report

MOFFITT CANCER CENTER

PROTEOMICS

Project Title: Internal standard for Bcl-2 homologous antagonist/killer (BAK_HUMAN)

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Design and Synthesis:

MPS#	Peptide Sequence	Residues	Modification	MWT Monoisotopic
p-0060	QLGIIGDDINR	77-87	A3 to G	1212.646

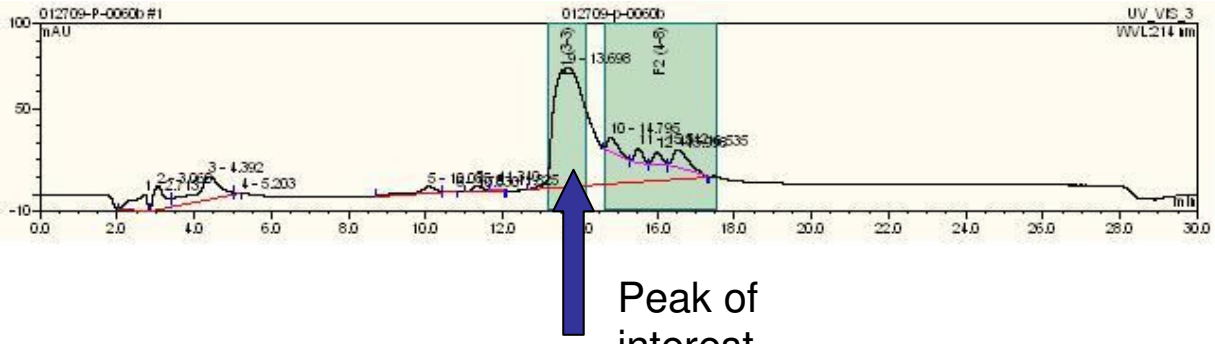
Solid state peptide synthesis (Symphony, Protein Technologies, Tucson, AZ) is used to make standards at the 25 micromole scale using standard FMOC chemistry. Briefly, Wang resin (Novabiochem, Germany) serves as the support to initiate peptide formation. Activator solvents for the amino acid coupling consist of 150 mM N-Methylmorpholine in N-methylpyrrolidone (NMP) with 100 mM of O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU). Deprotection between couplings is achieved using 20% piperidine in NMP with 2% 1,8-diazabicyclo [5,4,0] undec-7-ene. Upon sequence completion, cleavage from the resin is performed using 95% TFA, 2.5% water, and 2.5% triisopropylsilane. Peptides are then precipitated in ice cold ethyl ether, washed twice, and resuspended in water prior to lyophilization.

Glycine substituted for Alanine for more facile coupling of the last amino acids (see below).

Difficult, intermediate, easy synthesis coupling map:
QLAIIGDDINR to QLGIIGDDINR

Semi-preparative HPLC:

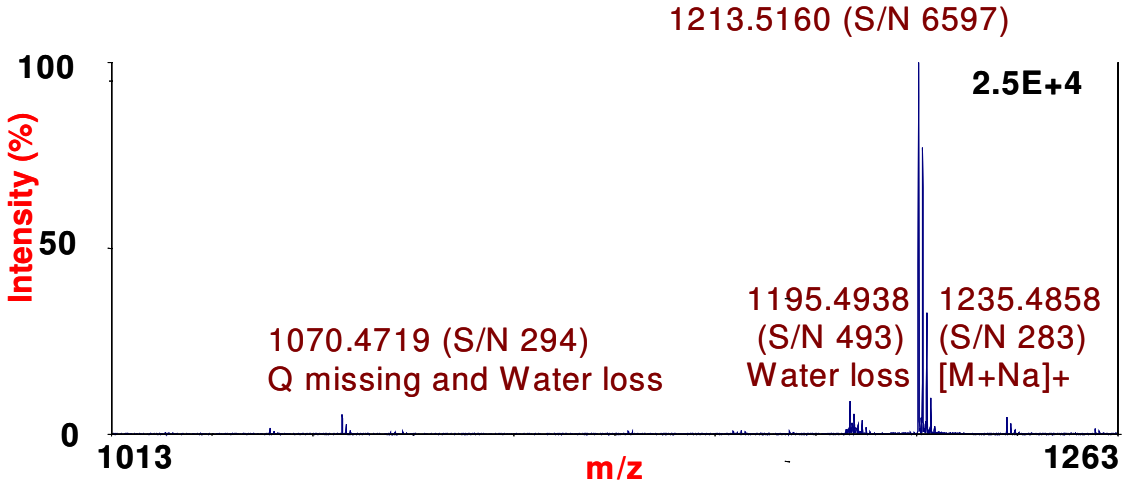
HPLC purification is performed on a semi-preparative system (U3000, Dionex, Sunnyvale, CA) using a C18 reverse phase column (TP 238, 250 x 10mm, 10-15 µm particles, Grace Vydac, Deerfield, IL). Twenty minute gradients are run from 5% to 60% B solvent (A: 2% acetonitrile/0.1% formic acid; B: 100% acetonitrile/0.1% formic acid). Eluted peptides are measured at 214 nanometer wavelength and collected with automatic triggering (Foxy Jr, ISCO).



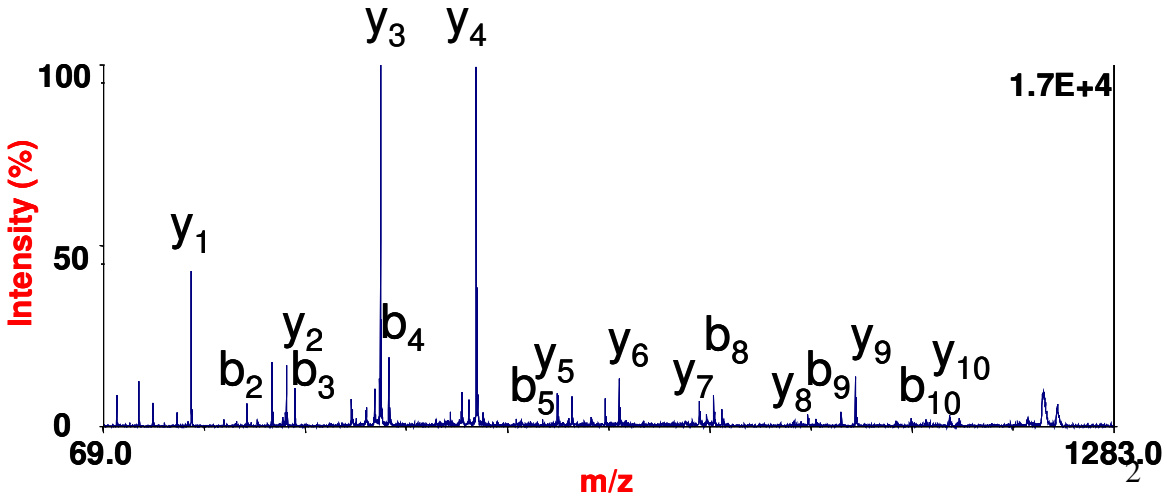
MALDI:

Purified peptides are analyzed with MALDI MS to verify purity and sequenced with MS/MS (4700, Applied Biosystems, Framingham, MA). Peptides in 2% ACN with 0.1% formic acid are mixed 1:1 with a-cyano-4-hydroxycinnamic acid (10 mg/ml) in 50% ACN and deposited in 1 microliter aliquots on the MALDI target.

MS Spectrum: 95.7% purity



MS/MS spectrum:



Fragment Ion table with Identified peaks:

SEQ	#	B	Y	#
Q	1	129.0665	1213.654	11
L	2	242.1505	1085.596	10
G	3	299.172	972.5115	9
I	4	412.256	915.49	8
I	5	525.3401	802.406	7
G	6	582.3616	689.3219	6
D	7	697.3885	632.3004	5
D	8	812.4155	517.2735	4
I	9	925.4995	402.2465	3
N	10	1039.542	289.1625	2
R	11	1195.644	175.1196	1