# Peptide Synthesis Report

MOFFITT CANCER CENTER

PROTEOMICS

**Project Title: Internal standard for Glucocorticoid receptor (GR) (Nuclear receptor subfamily 3 group C member 1) (GCR_HUMAN)**

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**Design and Synthesis:**

| MPS# | Peptide Sequence | Residues | Modification | MWT Monoisotopic |
|---|---|---|---|---|
| p-0125 | SQDLFDEIR | 682-690 | E3 to D | 1121.53534 |

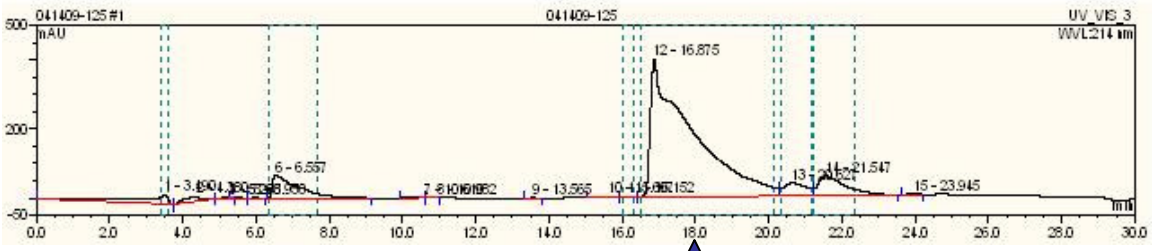
Solid state peptide synthesis (Symphony, Protein Technologies, Tucson, AZ) is used to make standards at the 25 micromole scale using standard FMOC chemistry. Briefly, Wang resin (Novabiochem, Germany) serves as the support to initiate peptide formation. Activator solvents for the amino acid coupling 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.

Glutamic acid was substituted for aspartic acid for more facile coupling.

Difficult, intermediate, easy synthesis coupling map
SQELFDEIR to SQDLFDEIR

**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).

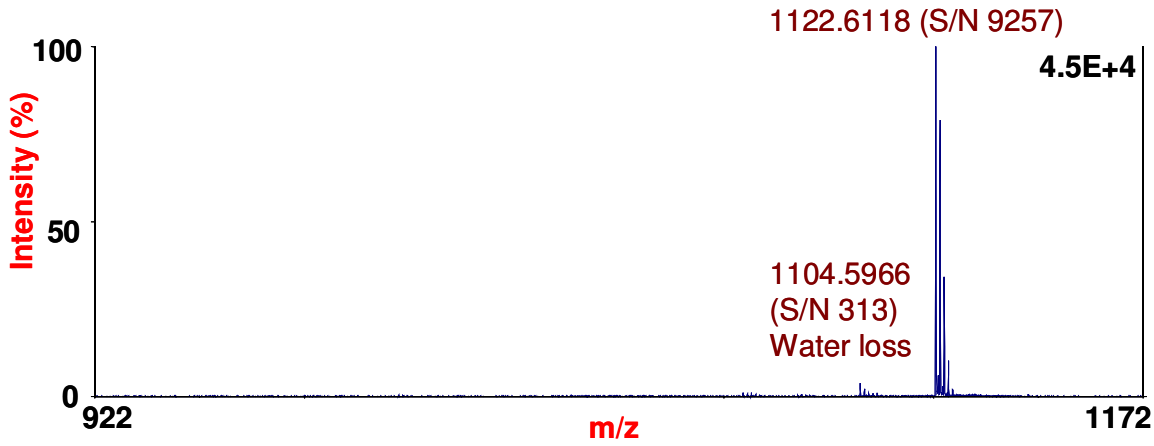



Peak of interest

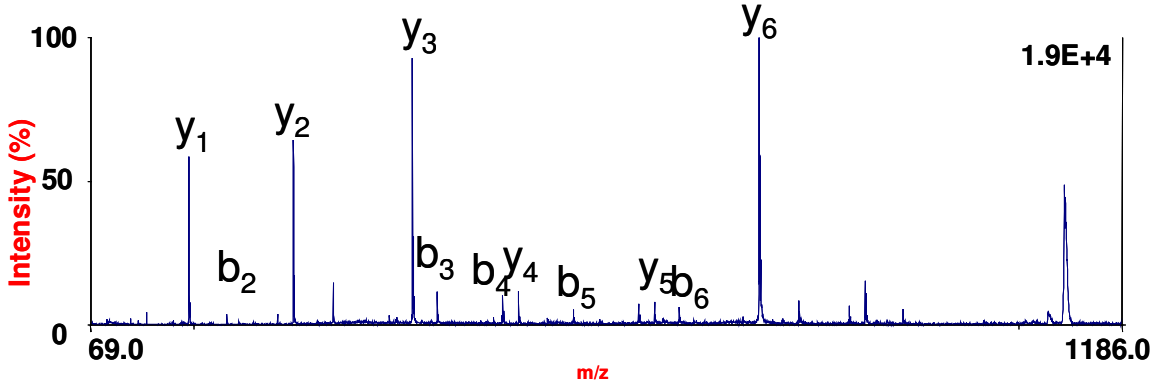
**MALDI:**

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: ~100 % purity**




**MS/MS spectrum:**



**Fragment Ion table with Identified peaks:**

| SEQ | # | B | Y | # |
|---|---|---|---|---|
| S | 1 | 88.0399 | 1122.543 | 9 |
| Q | 2 | 216.0985 | 1035.511 | 8 |
| D | 3 | 331.1254 | 907.4526 | 7 |
| L | 4 | 444.2095 | 792.4256 | 6 |
| F | 5 | 591.2779 | 679.3416 | 5 |
| D | 6 | 706.3048 | 532.2731 | 4 |
| E | 7 | 835.3474 | 417.2462 | 3 |
| I | 8 | 948.4315 | 288.2036 | 2 |
| R | 9 | 1104.533 | 175.1196 | 1 |