Supporting Information

Rigid Peptide Macrocycles via On-Resin Glaser Stapling

Philip A. Cistrone, Anthony P. Silvestri, Jordi C. J. Hintzen and Philip E. Dawson*

Departments of Chemistry and Cell and Molecular Biology,
The Scripps Research Institute
La Jolla, California 92037

Table of Contents

Materials and Methods S-2

Synthesis of Fmoc-L-Ser(propargyl)-OH S-3

Synthesis of BCL9 Peptides S-4

Synthesis of Bpy-diol. S-5

Procedure for On-Resin Glaser Stapling S-6

ESI-MS Characterization of Purified BCL9 Peptides S-7

Materials and Methods

All reagents were used directly from commercial suppliers: DMF (dimethylformamide), DMSO (dimethylsulfoxide), MeCN (acetonitrile), DCM (dichloromethane), and Et_2O (diethyl ether) from Fisher; TFA (trifluoroacetic acid), DIPEA (N,N-diisopropylethylamine) and DMS (Dimethylsulfide) from Sigma Aldrich; 4-methylpiperidine and CuCl (copper(I) chloride) from Alfa Aesar; HATU (1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate) from Chem-Impex Int'l.; Fmoc-protected amino acids from Bachem; Rink Amide ChemMatrix resin (0.48 mmol/gram) from Novabiochem. Water was purified using a Millipore Milli-Q water purification system.

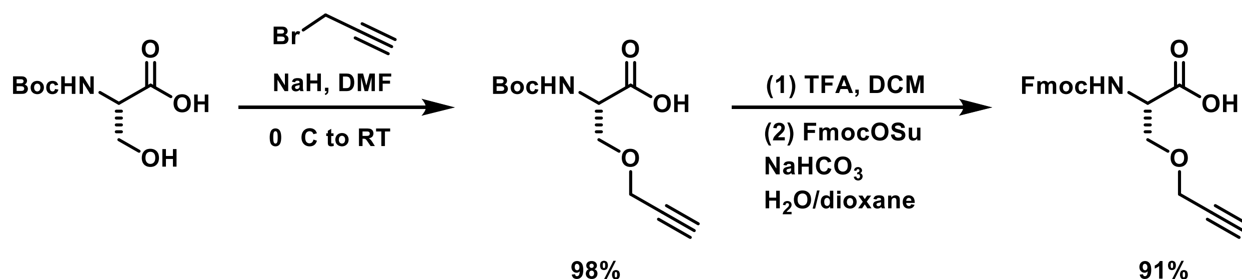
All BCL9 *i*→*i*+4 peptides were purified by reversed-phase HPLC (RP-HPLC). Analytical RP-HPLC was carried out on an Agilent 1100 Series HPLC on a Phenomenex Jupiter Proteo column (4 µm, 90 Å, 150 × 4.6 mm) at a flow rate of 1 mL/min. Analytical injections were monitored at 214 nm. Preparative RP-HPLC was performed on a Waters Delta Prep 4000 equipped with a Waters UV detector model 486 and a Phenomenex Jupiter Proteo column (10 µm, 90 Å, 250 × 21.20 mm) at a flow rate of 15 mL/min. Preparative injections were monitored at 220 nm. Buffer A: H_2O (0.05% TFA); Buffer B: MeCN/H_2O (9:1) (0.05% TFA).

All BCL9 *i*→*i*+4 peptides were characterized using electrospray ionization MS on a LC/MS API 2000 Plus triple quadrupole mass spectrometer (Sciex). Peptides masses were calculated from the experimental mass to charge (m/z) ratios from all of the observed protonation states of a peptide by using the onboard analyst software package (Sciex). Samples were injected via electrospray into the TOF reflectron analyzer at an ESI voltage of 4000V and a flow rate of 200 microliters/minute.

All BCL9 *i*→*i*+5/6/7 peptides were purified by reverse-phase HPLC (RP-HPLC) on a Waters 2545 Quaternary Gradient Module equipped with an Acquity QDa Detector and a Waters 2767 Sample Manager. Preparative injections were monitored at 280 nm. Buffer A: H_2O (0.1% Formic Acid); Buffer B: MeCN/H_2O (9:1) (0.1% Formic Acid).

Circular dichroism spectroscopy was performed on an Aviv Circular Dichroism Spectrometer, Model 62DS, using a quartz cuvette of 1 mm path length at 25°C.

Synthesis of Fmoc-L-Ser(propargyl)-OH†



In a 1 L round-bottom flask equipped with a stir bar, Boc-Ser-OH (5.00 g, 24.4 mmol, 1.0 equiv.) was dissolved in anhydrous DMF (150 mL) and cooled to 0 °C. To this solution was added NaH (2.34 g, 58.5 mmol, 2.4 equiv.; 60% wt. dispersion in mineral oil) in portions. The reaction was protected with a $CaCl_2$ guard tube and stirred at 0 °C for 2 hours. Propargyl bromide (3.80 mL, 34.1 mmol, 1.4 equiv.; 80% wt. solution in PhMe) was added dropwise over ~10 minutes and the reaction stirred at 0 °C for 1 hour. The ice-bath was removed and the reaction allowed to stir for 20 hours at room temperature. EtOH (~20 mL) was added to destroy excess NaH and the reaction concentrated. Water (~100 mL) was added, washed with Et_2O (4 x 50 mL), and acidified with 3 M HCl. The protonated carboxylic acid was then extracted from aqueous with EtOAc (4 x 75 mL), dried over Na_2SO_4, and concentrated to yield Boc-L-Ser(propargyl)-OH in 98% yield (5.79 g, 23.8 mmol).

In a 1 L round-bottom flask, TFA (40 mL) was added to a solution of Boc-L-Ser(propargyl)-OH (5.79 g, 23.8 mmol, 1.0 equiv.) in DCM (50 mL). This reaction was stirred at RT for 6 hours and then concentrated. Saturated, aqueous $NaHCO_3$ solution (40 mL) was slowly added to the resultant TFA-rich oil. Solid $NaHCO_3$ was then added until gas evolution ceased and their remained a small amount of residual solid $NaHCO_3$ (~750 mg) on the bottom of the flask. This suspension was poured into a vigorously stirring solution of FmocOSu (8.83 g, 26.2 mmol, 1.1 equiv.) in dioxane (75 mL) and maintained for 24 hours at room temperature. The reaction was concentrated, water (enough for full dissolution; sonication may be necessary) was added, and the aqueous washed with Et_2O (4 x 100 mL). The aqueous layer was made acidic with 3M HCl and extracted with EtOAc (4 x 100 mL). The organic layer was dried over Na_2SO_4 and concentrated to yield Fmoc-L-Ser(propargyl)-OH as a tan solid in 91% yield (7.90 g, 21.6 mmol).

Spectral properties matched those previously reported in *Org. Biomol. Chem.* **2015**, 9398.

†Procedure adapted from *Org. Biomol. Chem.* **2015**, 9398.

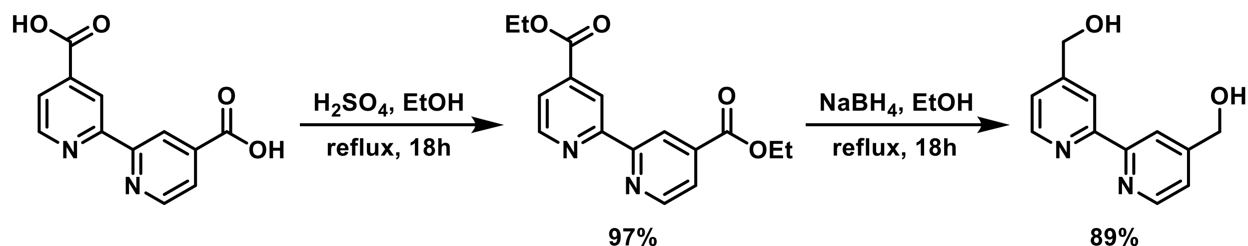
Synthesis of BCL9 Peptides

All peptides were chain assembled on Rink Amide ChemMatrix resin (0.48 mmol/gram) using a CSBio Fmoc-peptide synthesizer, model CS336X. All amino acid couplings were carried out with the equivalent ratio of [5]:[5]:[7.5] of [Fmoc-protected amino acid]:[0.4 M HATU in DMF]:[DIPEA] for 20 minutes following standard SPPS protocol with N-terminal Fmoc-protection. Propargyl serine (PraS) was coupled by hand using the equivalent ratio of [2]:[2]:[3.2] of these components for 40 minutes, and followed by a qualitative ninhydrin test to ensure complete coupling.

Unstapled/linear peptides proceeded to standard cleavage from resin using 125 μL TIPS, 125 μL H_2O, 500 μL DMS and 4.25 mL conc. TFA, the TFA was blown off using N_2 and the resultant residue suspended in cold Et_2O. After this suspension was subjected to centrifugation for 15 minutes at 4000 rpm the organic supernatant was decanted into the waste and the remaining white solid was dissolved in 10% MeCN in H_2O and lyophilized. The crude peptide thus obtained was purified via preparative reversed-phase HPLC (RP-HPLC) using a gradient of 100% buffer A to 80% buffer A / 20% buffer B over 40 minutes.

On-resin stapling reactions were performed prior to cleavage, as described on page S-6.

Synthesis of Bpy-diol†



Concentrated sulfuric acid (5 mL) was added to a 250 mL round-bottom flask containing a stir bar, carboxylic acid (3.00 g, 12.3 mmol), and absolute EtOH (75 mL). The slightly pink suspension was refluxed for 18 hours, cooled, and dumped into ice-water (~300 mL) whereupon a white precipitate formed and was isolated by filtration. The filter cake was washed with water and subsequently dried to yield diester (3.58 g, 11.9 mmol, 97% yield).

The diester (3.36 g, 11.2 mmol, 1.0 equiv.) was dissolved in absolute EtOH (180 mL) in a 1 L round-bottom flask and $NaBH_4$ (8.46 g, 223.6 mmol, 20.0 equiv.) was added in portions. The mixture was maintained at reflux with vigorous stirring for 18 hours and then carefully quenched with a saturated, aqueous solution of NH_4Cl. The EtOH was removed under reduced pressure and the aqueous extracted with EtOAc (4 x 75 mL). The organic layer was dried over Na_2SO_4 and concentrated to give the title compound as a white solid in 89% yield (2.16 g, 9.99 mmol).

Spectral properties matched those previously reported in *Inorg. Chem.* **2001**, 6073.

†Procedure adapted from *Inorg. Chem.* **2001**, 6073.

Procedure for On-Resin Glaser Stapling

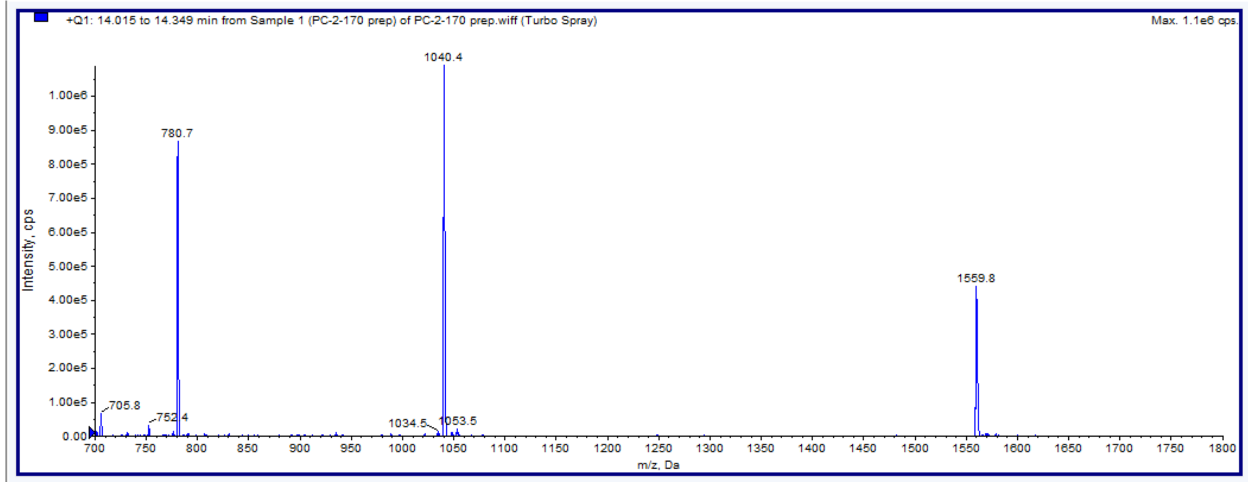
To a 15 mL Falcon tube there was added CuCl (100 mg, 1 mmol, 10 equiv.), bipyridine diol ligand (330 mg, 1.5 mmol, 15 equiv.), and 10 mL DMSO. The mixuture was briefly vortexed to solubilize the Cu and ligand. To this was added 0.1 mmol scale of washed Fmoc-protected BCL9 peptide resin, followed by 350 µL DIEA (2 mmol, 20 equiv.). The tube was parafilmed and placed on a benchtop shaker apparatus for the desired reaction time, typically 3 days (72 hrs).

Following the stapling reaction, the resin was DMF-washed, Fmoc-deprotected by standard procedure, and subjected to identical TFA cleavage conditions as the linear peptides as described on page S-4.

ESI-MS Characterization of Purified BCL9 Peptides

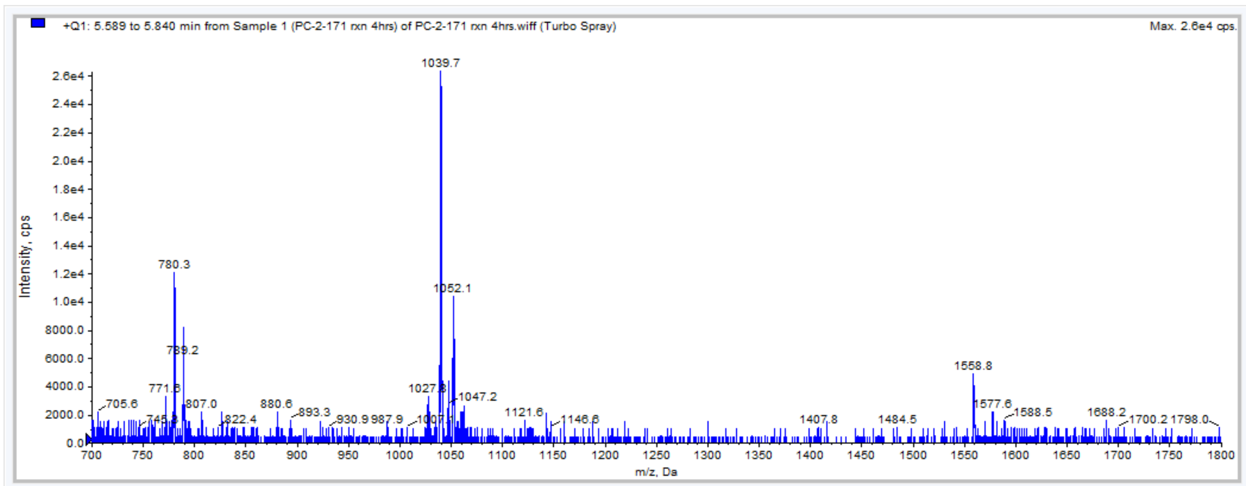
BCL9 *i*→*i*+4: LWQEQLEHR(PraS)RSL(PraS)TLRDIQRMLF-NH_2 (24 amino acids)

Linear/Unstapled:



MW=3118.62
Expected (m+2)/2 = 1560.1
Found (m+2)/2 = 1559.8
Expected (m+3)/3 = 1040.5
Found (m+3)/3 = 1040.4

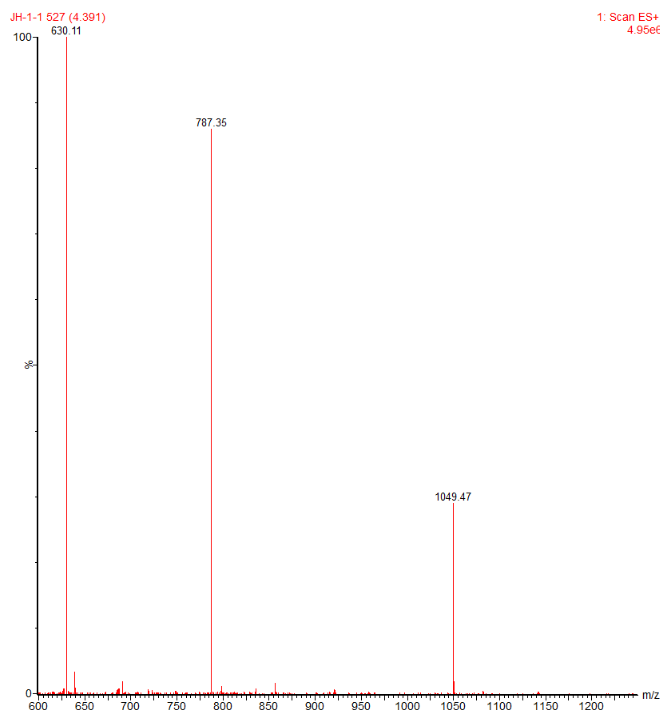
Stapled:



MW=3116.61
Expected (m+2)/2 = 1559.1
Found (m+2)/2 = 1558.8
Expected (m+3)/3 = 1039.8
Found (m+3)/3 = 1039.7

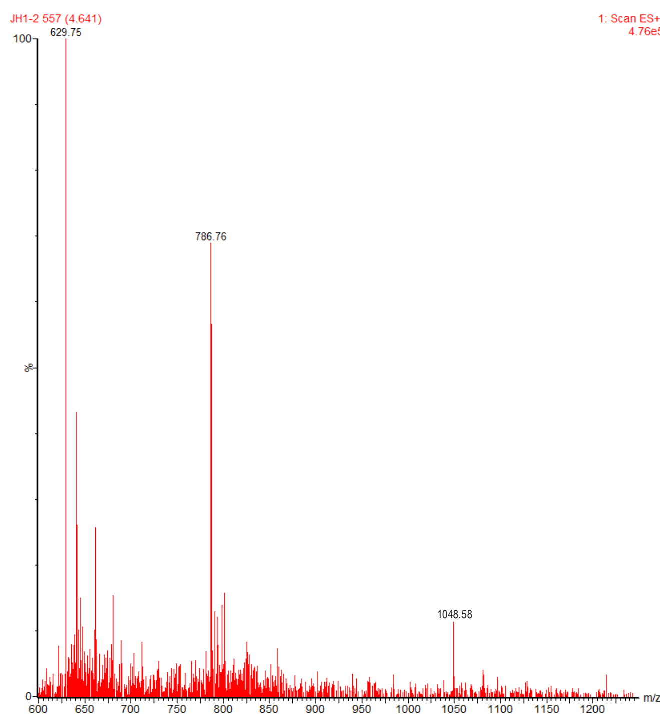
<u>BCL9 *i*→*i*+5:</u> LWQEQLEHR(PraS)RSLQ(PraS)LRDIQRMLF-NH_2 (24 amino acids)

Linear/Unstapled:



MW=3145.65
Expected (m+3)/3 = 1049.55
Found (m+3)/3 = 1049.47
Expected (m+4)/4 = 787.41
Found (m+4)/4 = 787.35

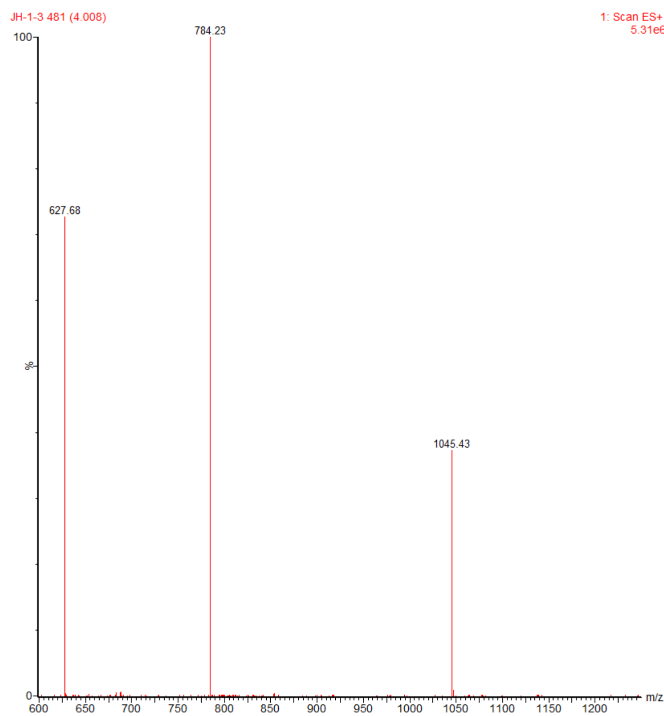
Stapled:



MW=3143.63
Exp. (m+3)/3 = 1048.87
Found (m+3)/3 = 1048.58
Exp. (m+4)/4 = 786.90
Found (m+4)/4 = 786.76

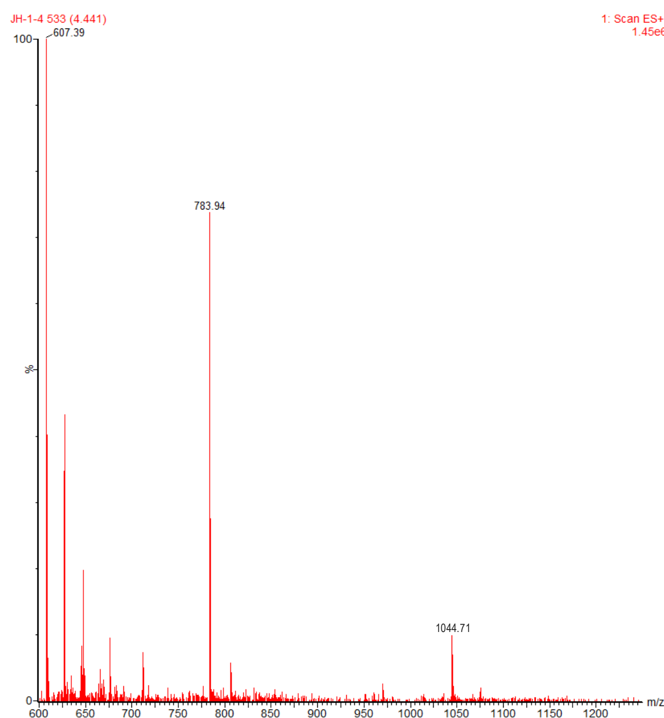
<u>BCL9 *i*→*i*+6:</u> LWQEQLEHR(PraS)RSLQT(PraS)RDIQRMLF-NH_2 (24 amino acids)

Linear/Unstapled:



MW=3133.59
Expected (m+3)/3 = 1045.53
Found (m+3)/3 = 1045.43
Expected (m+4)/4 = 784.39
Found (m+4)/4 = 784.23

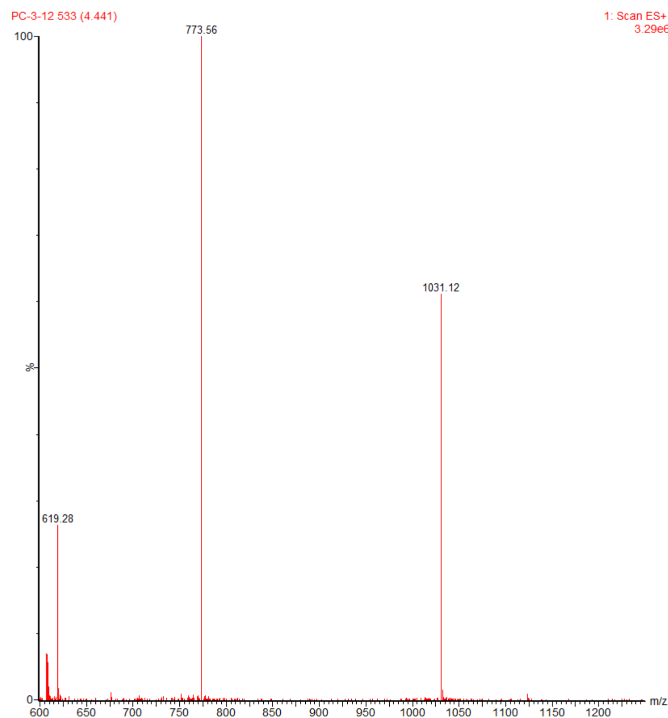
Stapled:



MW=3131.58
Expected (m+3)/3 = 1044.86
Found (m+3)/3 = 1044.71
Expected (m+4)/4 = 783.90
Found (m+4)/4 = 786.94

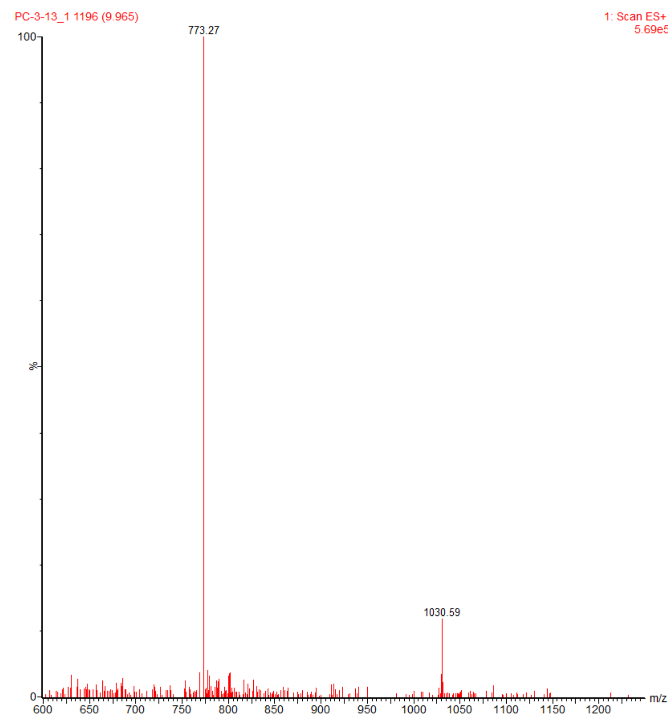
BCL9 *i*→*i*+7: LWQEQLEHR(PraS)RSLQTL(PraS)DIQRMLF-NH_2 (24 amino acids)

Linear/Unstapled:



MW=3090.56
Expected (m+3)/3 = 1031.18
Found (m+3)/3 = 1031.12
Expected (m+4)/4 = 773.64
Found (m+4)/4 = 773.56

Stapled:



MW=3088.55
Expected (m+3)/3 = 1030.52
Found (m+3)/3 = 1030.59
Expected (m+4)/4 = 773.13
Found (m+4)/4 = 773.27