Electronic Supplementary Material (ESI) for Reaction Chemistry & Engineering.


# A continuous flow protocol to generate, regenerate, load and recycle chlorotrityl functionalised resins

## Electronic supporting information

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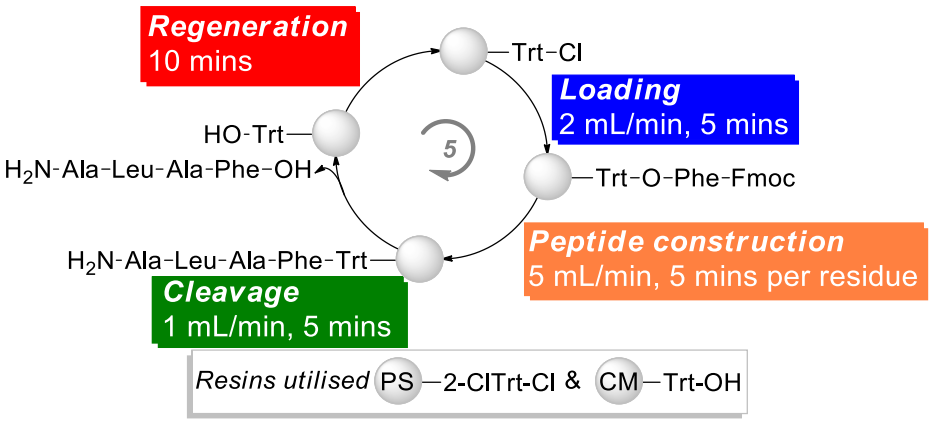
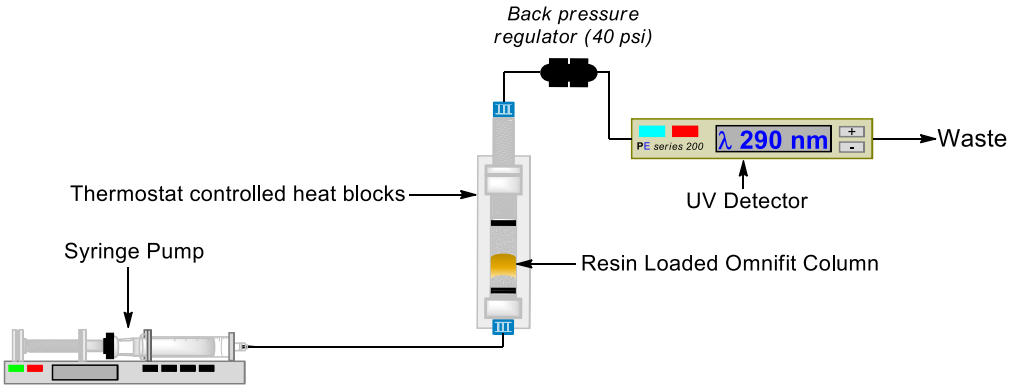



### Contents

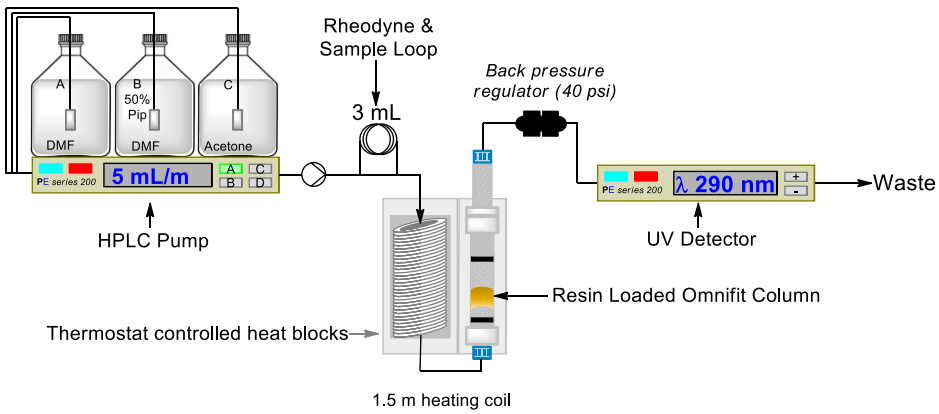
# 1. General information

Fmoc-protected amino acids were purchased from Auspep and used as received as was 2-chlorotrityl chloride polystyrene resin (0.98 mmol/g). Trityl-OH Chem Matrix Resin (0.47 mmol/g) was purchased from Biotage. All solvents were bulk, and distilled in glass prior to use. Materials were purchased from either Aldrich Chemical Co or ChemSupply. Peptide sequences were monitored using analytical RP-HPLC, performed using an Agilent instrument comprised of six modules; 1260 Bin Pump, 1260 HIP Degasser, 1260 ALS, 1260 TCC, 1260 DAD and 1260 FC-AS. Analytical RP-HPLC was performed using Phenomenex Onyx Monolithic reversed-phase C18 column (4.6 x 150 mm). Solvent A: 0.06% TFA in $H_2O$ and solvent B: 0.06% TFA in $CH_3CN$:$H_2O$ (9:1), flow rate of 1.0 mL/min, gradient 10-100 (%B), curve = 6, over 15.0 mins, and detection at 214 and 254 nm. Mass spectrometry data was obtained in the Western Sydney Mass Spectrometry Facility. Low resolution electrospray ionisation mass spectrometry (ESIMS) experiments were performed using a Waters TQ-MS triple quadrupole mass spectrometer fitted with an ESI source. Spectra were recorded in positive ion mode from analyte solutions injected (10 µL) into 0.1% formic acid in 50% aqueous methanol flowing at 0.1 mL min−1. A capillary voltage of 3.0 kV, cone voltage of 30 V, desolvation temperature of 300 °C and desolvation flow rate (nitrogen) of 500 L h−1 were employed. Spectra were collected over 1min with an m/z range of 100–1500. High resolution accurate mass spectra were obtained using a Waters Xevo QToF MS mass spectrometer. The instrument was fitted with an ESI probe and mass-corrected sample spectra were recorded in positive ion mode, incorporating leucine encephalin (200 pg/µL in 50% aqueous acetonitrile + 0.1% formic acid) as a lockmass compound. Prior to obtaining spectra, the m/z range of 50-2000 was calibrated against sodium iodide solution. Samples were prepared in 0.1% aqueous formic acid at concentrations of approximately 10 ng/mL and infused via syringe pump at 3 µL/min.

# 2. Apparatus set up




**ESI Figure 2.1**: Apparatus set up of flow loading using a syringe pump




**ESI Figure 2.2**: Apparatus set up for total flow synthesis utilising a HPLC pump, Reodyne injector with 3 mL injection loop, thermostat controlled heating block with heating coil, back pressure regulator and UV detector.

**ESI Figure 2.3**: Images of resin prior to regeneration (left) and post-regeneration (right)

## 3. Quantitation method of resin loading *via* UV titration

Three samples of loaded dried resin (< 10.0 mg each) were each added to a vial, freshly prepared solution of 20% piperidine in DMF (3.00 mL) was added to the dried resin. The resulting suspensions were gently agitated at rt for 0.5 h after which 300 µL of each resin suspension was transferred to a quartz UV cuvette and an additional 2.70 mL of 20% piperidine in DMF was added. A reference cell containing 3.00 mL of 20% piperidine was placed into a UV spectrophotometer and reference was set at 290 nm. The absorbance of the cuvettes containing the settled resin was then obtained (290 nm). The loading of the resin was then determined using the following equation (final loading = average of the three resin samples with associated error):

$$\text{Loading (mmol/g)} = [(\text{Abs}_{\text{sample}}) / (\text{mg of sample} \times 1.75)] \times 10$$

Theoretical loading was determined using the following equation:

$$A = B \times 1000 / [1000 + (B \times (M - X))]$$

A = theoretical substitution (mmol/g)
B = substitution of resin (mmol/g)
M = molecular weight of Amino Acid (with all protecting groups)
X = 36 (loss of Cl on amino acid loading)

## 4. General synthesis procedures

**General procedure (batch) for loading 2-chlorotrityl chloride resin:** 2-Chlorotritylchloride resin (100-200 mesh polystyrene), 1% DVB (100 mg, loading = 0.98 mmol/g) (AusPep) swelled with DCM (10 mL). The amino acid solution (Fmoc-AA-OH 4 eq, DIPEA 8 eq, in DCM 5 mL) was added to the suspension at 40 °C and agitated for 2 hours. The resin was filtered from the solution and washed with DCM (3 × 10 mL) and $Et_2O$ (3 × 10 mL). The resin was initially dried with a stream of $N_2$, and then subsequently under high vacuum for 18h. Three portions of the resin (<10 mg each) were taken for UV quantitation.

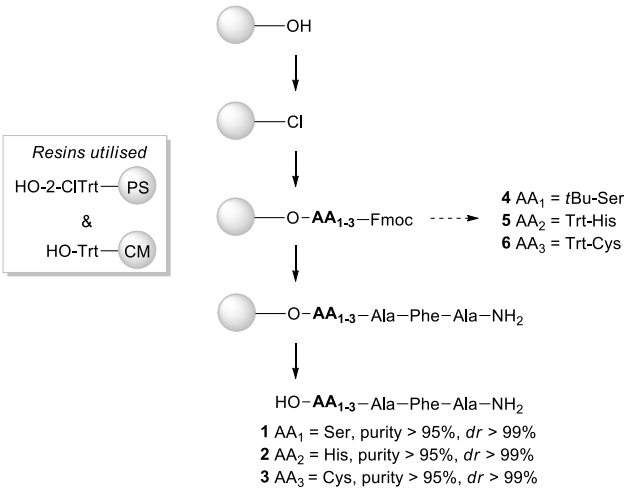
**General procedure (unoptimized flow) for loading 2-chlorotrityl chloride resin:** 2-Chlorotritylchloride resin (100-200 mesh polystyrene), 1% DVB (100 mg, loading = 0.98 mmol/g) (AusPep) was placed in a commercially available glass column (1 fixed end, 1 variable end, I.D. 10 mm L. 100 mm). The column was placed in-line with the apparatus according to ESI figure 2.1. To affect resin swelling, DCM (4 mL) was flowed through the resin (0.5 mL $min^{-1}$). The amino acid solution (Fmoc-AA-OH 4 eq, DIPEA 8 eq, in DCM 10 mL) was flowed through the resin (1 mL $min^{-1}$) at 40 °C. The resin was washed with DCM (10 mL) (5 mL

min$^{-1}$) and followed by $Et_2O$ (5 mL min$^{-1}$). The resin was initially dried with a stream on $N_2$, and then subsequently under high vacuum for 18h. Three portions of the resin (<10 mg each) were taken for UV quantitation.

**General procedure (optimized flow) for loading 2-chlorotrityl chloride resin:** 2-Chlorotritylchloride resin (100-200 mesh polystyrene), 1% DVB (100 mg, loading = 0.98 mmol/g) (AusPep) was placed in a commercially available glass column (1 fixed end, 1 variable end, I.D. 10 mm L. 100 mm). The column was placed in-line with the apparatus according to ESI figure 2.1. To affect resin swelling, DCM (4 mL) was flowed through the resin (0.5 mL min$^{-1}$). Acetyl chloride (3 M in DCM, 10 mL) was flowed through the resin (1 mL min$^{-1}$) at room temperature. The resin was washed with DCM (20 mL) at a flow rate of 5 mL min$^{-1}$. The amino acid solution (Fmoc-AA-OH 4 eq, DIPEA 8 eq, in DCM 10 mL) was flowed through the resin (1 mL min$^{-1}$) at 40 °C. To ensure all unreacted –Cl sites on the resin were hydrolyzed, MeOH (10 mL) was flowed through the resin (5 mL min$^{-1}$). The resin was washed with DCM (10 mL) (5 mL min$^{-1}$) and followed by $Et_2O$ (5 mL min$^{-1}$). The resin was initially dried with a stream on $N_2$, and then subsequently under high vacuum for 18h. Three portions of the resin (<10 mg each) were taken for UV quantitation.

**General procedure (optimized flow) for loading trityl-OH resin:** Trityl-OH resin (100-200 mesh Chem Matrix), (100 mg, loading = 0.47 mmol/g) (Biotage) was placed in a commercially available glass column (1 fixed end, 1 variable end, I.D. 10 mm L. 100 mm). The column was placed in-line with the apparatus according to ESI figure 2.1. To affect resin swelling, DCM (4 mL) was flowed through the resin (0.5 mL min$^{-1}$). Acetyl chloride (6 M in DCM, 10 mL) was flowed through the resin (1 mL min$^{-1}$) at room temperature. The resin was washed with DCM (20 mL) at a flow rate of 5 mL min$^{-1}$. The amino acid solution (Fmoc-AA-OH 4 eq, DIPEA 8 eq, in DCM 10 mL) was flowed through the resin (1 mL min$^{-1}$) at 40 °C. To ensure all unreacted –Cl sites on the resin were hydrolyzed, MeOH (10 mL) was flowed through the resin (5 mL min$^{-1}$). The resin was washed with DCM (10 mL) (5 mL min$^{-1}$) and followed by $Et_2O$ (5 mL min$^{-1}$). The resin was initially dried with a stream on $N_2$, and then subsequently under high vacuum for 18h. Three portions of the resin (<10 mg each) were taken for UV quantitation.

**General procedure for total flow synthesis.**




Initially, 100 mg of resin was placed within column and subsequently swelled in 3 mL of DCM. After standing at room temperature for 10 mins the excess solvent was drained and the adjustable column end-piece frit was fixed within ≈ 5 mm of the swollen resin surface. The column was then attached in-line according to ESI figure 2.2 before being placed within the heat-block cradle which was heated to 40 °C. The resin was then flushed with a continuous stream of DCM (10 mL) and the UV lamp was zeroed at 225 nm. Following equilibration, the Rheodyne injection loop was charged with a 2 mL DCM solution containing 0.83 mL of AcCl (6 M). Once loaded the flow of the continuous DCM solution was reduced 1.0 mL and the AcCl was injected. Upon the complete elution of AcCl, which was monitored via UV at 225 nm, the continuous DCM stream flow rate was increased to 10 ml/min until UV returned < 0.01 AU. Following equilibration, the DCM flow was reduced to 2 mL/min and the Rheodyne injection loop was charged with a 2 mL 1:1 DMF:DCM solution containing the C-terminal residue (4 equiv.) and DIPEA (8 equiv.). The loading solution was then injected and the DCM flow was maintained at 2 mL/min until the UV equilibrated. Upon complete elution of the loading solution (monitored *via* UV, 254 nm), which occurred in 5 mins, the resin was then capped (3 × 2mL, MeOH, injection, 5 mL/min, 40 °C). Following capping, the continuous solvent was switched to DMF, the flow rate was increased to 10 mL/min, temperature was increased to 60 °C, and the UV was re-zeroed at 290 nm. Fmoc-deprotection of the C-terminal residue was then effected with a 50% DMF piperidine solution which was flowed through the resin at 10 mL/min and 60

°C. Upon complete deprotection (monitored via UV, 290 nm) the solvent steam was switch to DMF and the flow rate was reduced to 5 mL/min. The injection loop was then loaded with coupling solution containing amino acid (AA) solution† (0.3 mmol), HATU (0.3 mmol), DMF (1 mL) and DIPEA (0.6 mmol) which was subsequently injected into the DMF solvent stream. After the elution of the coupling solution (UV <0.3 AU) the Fmoc protecting group was removed utilising 50% DMF piperidine at 10 mL/min and upon complete deprotection the solvent stream was switched to DMF and the flow rate reduced to 5 mL/min. The above steps of AA solution injection (step 1), Fmoc-deprotection (step 2) and washing (step 3) were repeated for all additional AA's in the sequence. After the final Fmoc-deprotection, the resin was removed from the heating block and washed with MeOH (2 × 10 mL), EtOAc (2 × 10 mL), hexanes (2 × 5.0 mL), DCM (2 × 5.0 mL) and $Et_2O$ (2 × 10 mL) and dried *in vacuo*. The peptide was cleaved from the resin using 10 mL cleavage cocktail (DCM:TFA:TIPS 98:1.5:0.5) The solution was evaporated to dryness for analysis .

†The AA solution was calculated based upon the final swollen resin volume; reagents were calculated accordingly for a 0.3 M solution based on the swollen volume (for 0.1g of PS-this was 1 $cm^3$).

# 5. Amino acid loading data

**ESI Table 5.1:** Results of loading using polystyrene 2-chlorotrityl chloride resin without optimisation

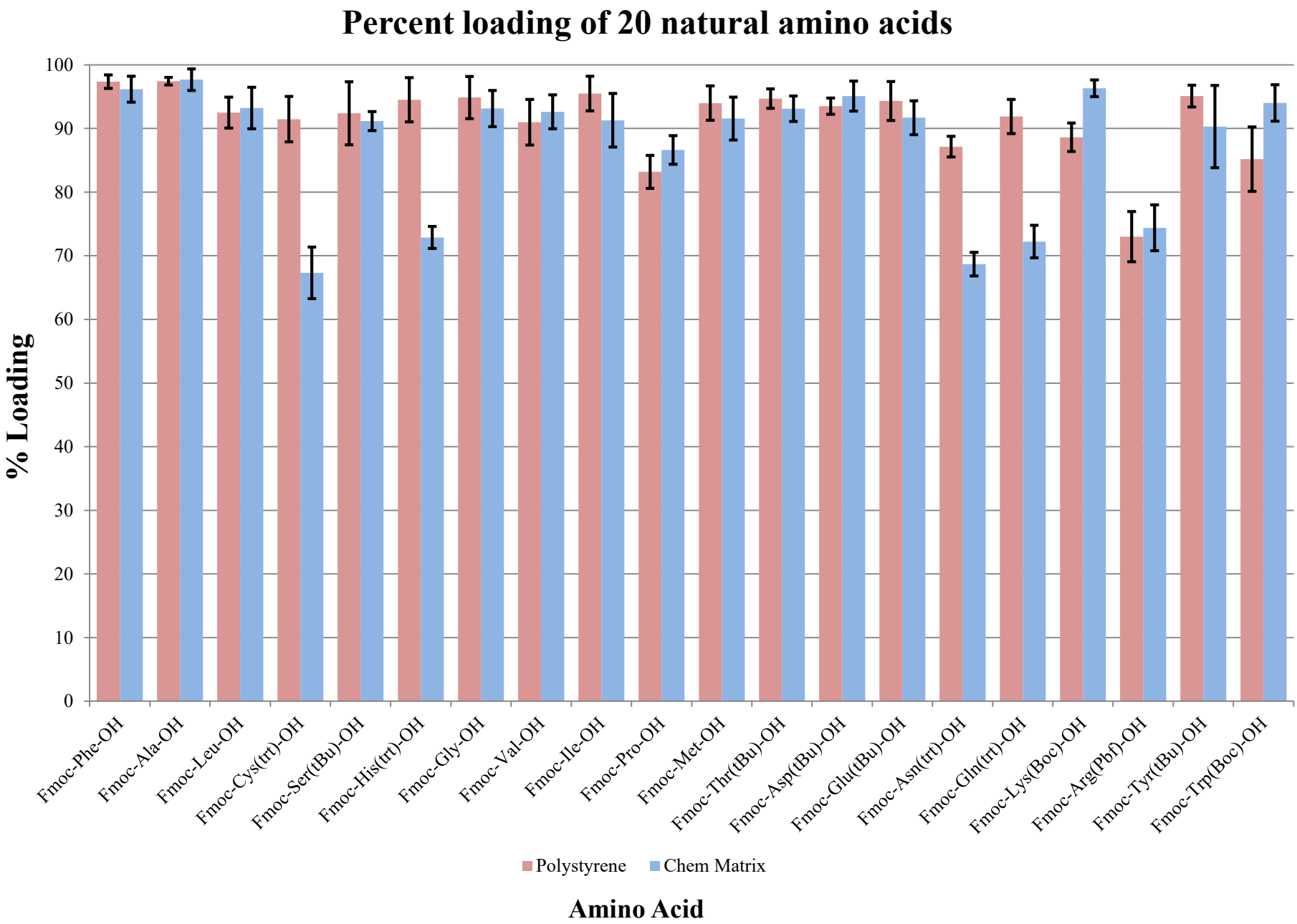
| | Flow rate of AA solution (mL $min^{-1}$)[a] | Mass of resin sample (mg) | Absorbance value (AU) | Loading (mmol/g)[b] | Loading (%)[c] | Mean Loading (% ± error) |
|---|---|---|---|---|---|---|
| Batch | N/A | 2.4 | 0.186 | 0.443 | 60.8 | **56.8 ± 3.4** |
| | | 2.6 | 0.183 | 0.402 | 55.2 | |
| | | 5.3 | 0.362 | 0.390 | 53.5 | |
| Flow | 0.13 | 2.5 | 0.237 | 0.542 | 74.3 | **66.7 ± 6.7** |
| | | 3.2 | 0.251 | 0.448 | 61.5 | |
| | | 6.3 | 0.517 | 0.469 | 64.3 | |

[a] 4 equivalents of AA and 8 equivalents of DIPEA in DCM.
[b] Calculated using standard UV quantitation method.
[c] Loading as a percentage of possible loading amount.

**ESI Table 5.2:** Optimisation of flow loading conditions treating resin with acetyl chloride, followed by amino acid.

| Resin | Acetyl chloride solution (M)[a] | Flow rate of acetyl chloride solution (mL $min^{-1}$) | Flow rate of AA solution (mL $min^{-1}$)[b] | Mass of resin sample (mg) | Absorbance value (AU) | Loading (mmol/g) | Loading (%) | Mean Loading (% ± error) |
|---|---|---|---|---|---|---|---|---|
| Polystyrene | 0.5 | 0.33 | 0.33 | 3.7 | 0.316 | 0.488 | 66.9 | 70.9 ± 5.0 |
| | | | | 4.9 | 0.432 | 0.504 | 69.1 | |
| | | | | 7.9 | 0.771 | 0.558 | 76.5 | |
| Polystyrene | 1 | 0.33 | 0.33 | 7.6 | 0.797 | 0.599 | 82.2 | 82.1 ± 3.0 |
| | | | | 2.3 | 0.232 | 0.576 | 79.1 | |
| | | | | 8.2 | 0.889 | 0.620 | 85.0 | |
| Polystyrene | 1 | 1 | 0.33 | 4.3 | 0.452 | 0.601 | 82.4 | 84.9 ± 3.3 |
| | | | | 9.1 | 1.03 | 0.647 | 88.7 | |
| | | | | 3.4 | 0.363 | 0.610 | 83.7 | |
| Polystyrene | 1 | 1 | 1 | 4.4 | 0.502 | 0.652 | 89.4 | 86.8 ± 2.5 |
| | | | | 9.0 | 0.993 | 0.630 | 86.5 | |
| | | | | 3.1 | 0.334 | 0.616 | 84.5 | |
| Polystyrene | 1 | 2.5 | 1 | 2.2 | 0.267 | 0.694 | 95.1 | 91.0 ± 3.7 |
| | | | | 3.9 | 0.439 | 0.643 | 88.2 | |
| | | | | 7.0 | 0.799 | 0.652 | 89.5 | |
| Polystyrene | 1 | 2.5 | 2.5 | 3.0 | 0.301 | 0.573 | 78.7 | 76.8 ± 1.9 |
| | | | | 6.2 | 0.592 | 0.546 | 74.9 | |
| | | | | 5.7 | 0.560 | 0.561 | 77.0 | |
| Polystyrene | 3 | 1 | 1 | 3.9 | 0.479 | 0.702 | 96.3 | **97.2 ± 1.1** |
| | | | | 6.0 | 0.753 | 0.717 | 98.4 | |
| | | | | 5.5 | 0.681 | 0.708 | 97.1 | |
| Polystyrene | 3 | 2.5 | 2.5 | 3.0 | 0.301 | 0.573 | 78.7 | 82.4 ± 4.1 |
| | | | | 6.2 | 0.647 | 0.596 | 81.8 | |
| | | | | 4.2 | 0.465 | 0.633 | 86.8 | |
| Chem Matrix | 3 | 1 | 1 | 7.0 | 0.377 | 0.308 | 74.9 | 76.3 ± 4.9 |
| | | | | 8.5 | 0.441 | 0.296 | 72.2 | |
| | | | | 3.9 | 0.229 | 0.336 | 81.7 | |
| Chem Matrix | 6 | 1 | 1 | 5.6 | 0.378 | 0.386 | 93.9 | **96.2 ± 2.1** |
| | | | | 7.1 | 0.499 | 0.402 | 97.8 | |
| | | | | 3.8 | 0.265 | 0.398 | 97.0 | |
| Chem Matrix | 10 | 1 | 1 | 3.7 | 0.239 | 0.369 | 89.9 | 83.2 ± 6.3 |
| | | | | 9.6 | 0.534 | 0.318 | 77.4 | |
| | | | | 6.1 | 0.361 | 0.338 | 82.3 | |

[a] Acetyl chloride in DCM. [b] 4 equivalents of AA and 8 equivalents of DIPEA in DCM.




**ESI Figure 5.1**: Comparison of loading percentages of 20 natural amino acids using the optimised loading method.

**ESI Table 5.3**: Exact weights and calculations for loading 20 natural amino acids on both PS-2-ClTrtCl and CM-ClTrt resins.

| Amino Acid | Molecular weight of AA including Protecting groups | Resin Type | Stated Resin Substitution (mmol/g) | Theoretical AA Resin Loading (mmol/g) | AA amount for 100mg load (mg)(4eq) | Mass of resin sample (mg) | Absorbance Measured (Au) | Loading (mmol/g) | Loading (%) | Mean Loading (% ± error) |
|---|---|---|---|---|---|---|---|---|---|---|
| Fmoc-Phe-OH | 387.43 | Polystyrene | 0.98 | 0.728 | 152 | 3.9 | 0.479 | 0.702 | 96.4 | |
| | | | | | | 6.0 | 0.753 | 0.717 | 98.5 | 97.3 ± 1.1 |
| | | | | | | 5.5 | 0.681 | 0.708 | 97.2 | |
| | | Chem Matrix | 0.48 | 0.411 | 75 | 5.6 | 0.378 | 0.386 | 93.8 | |
| | | | | | | 7.1 | 0.499 | 0.402 | 97.7 | 96.1 ± 2.0 |
| | | | | | | 3.8 | 0.265 | 0.398 | 97.0 | |
| Fmoc-Ala-OH | 311.33 | Polystyrene | 0.98 | 0.772 | 122 | 2.3 | 0.302 | 0.750 | 97.2 | |
| | | | | | | 7.4 | 0.981 | 0.758 | 98.1 | 97.4 ± 0.6 |
| | | | | | | 5.6 | 0.734 | 0.749 | 97.0 | |
| | | Chem Matrix | 0.48 | 0.424 | 60 | 3.2 | 0.234 | 0.418 | 98.6 | |
| | | | | | | 3.9 | 0.277 | 0.406 | 95.7 | 97.7 ± 1.7 |
| | | | | | | 6.7 | 0.491 | 0.419 | 98.8 | |
| Fmoc-Leu-OH | 353.4 | Polystyrene | 0.98 | 0.747 | 139 | 6.6 | 0.822 | 0.712 | 95.3 | |
| | | | | | | 8.1 | 0.961 | 0.678 | 90.8 | 92.5 ± 2.4 |
| | | | | | | 4.9 | 0.586 | 0.683 | 91.5 | |
| | | Chem Matrix | 0.48 | 0.417 | 68 | 9.9 | 0.698 | 0.403 | 96.6 | |
| | | | | | | 2.3 | 0.156 | 0.388 | 92.9 | 93.2 ± 3.3 |
| | | | | | | 4.0 | 0.263 | 0.376 | 90.1 | |
| Fmoc-Cys(trt)-OH | 585.7 | Polystyrene | 0.98 | 0.637 | 230 | 5.5 | 0.586 | 0.609 | 95.6 | |
| | | | | | | 7.4 | 0.741 | 0.572 | 89.8 | 91.5 ± 3.6 |
| | | | | | | 2.6 | 0.258 | 0.567 | 89.0 | |
| | | Chem Matrix | 0.48 | 0.38 | 112 | 4.7 | 0.197 | 0.240 | 63.0 | |
| | | | | | | 5.9 | 0.266 | 0.258 | 67.8 | 67.3 ± 4.1 |
| | | | | | | 6.6 | 0.312 | 0.270 | 71.1 | |
| Fmoc-Ser(tBu)-OH | 383.4 | Polystyrene | 0.98 | 0.731 | 150 | 1.9 | 0.234 | 0.704 | 96.3 | 92.4 ± 4.9 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 2.8 | 0.311 | 0.635 | 86.8 | |
| | | | | | | 7.3 | 0.879 | 0.688 | 94.1 | |
| | | | | | | 6.3 | 0.412 | 0.374 | 90.9 | |
| | | Chem Matrix | 0.48 | 0.411 | 74 | 5.0 | 0.323 | 0.369 | 89.8 | 91.2 ± 1.5 |
| | | | | | | 5.8 | 0.387 | 0.381 | 92.8 | |
| | | | | | | 4.9 | 0.522 | 0.609 | 97.7 | |
| | | Polystyrene | 0.98 | 0.623 | 243 | 5.3 | 0.549 | 0.592 | 95.0 | 94.5 ± 3.5 |
| | | | | | | 2.0 | 0.198 | 0.566 | 90.8 | |
| Fmoc-His(trt)-OH | 619.7 | | | | | | | | | |
| | | | | | | 9.2 | 0.451 | 0.280 | 74.7 | |
| | | Chem Matrix | 0.48 | 0.375 | 119 | 3.4 | 0.159 | 0.267 | 71.3 | 72.9 ± 1.7 |
| | | | | | | 6.5 | 0.310 | 0.273 | 72.7 | |
| | | | | | | 5.9 | 0.758 | 0.734 | 94.1 | |
| | | Polystyrene | 0.98 | 0.78 | 117 | 6.3 | 0.847 | 0.768 | 98.5 | 94.9 ± 3.3 |
| | | | | | | 7.9 | 0.992 | 0.718 | 92.0 | |
| Fmoc-Gly-OH | 297.3 | | | | | | | | | |
| | | | | | | 5.2 | 0.356 | 0.391 | 91.6 | |
| | | Chem Matrix | 0.48 | 0.427 | 57 | 5.8 | 0.396 | 0.390 | 91.4 | 93.1 ± 2.9 |
| | | | | | | 3.4 | 0.245 | 0.412 | 96.4 | |
| | | | | | | 6.8 | 0.783 | 0.658 | 87.2 | |
| | | Polystyrene | 0.98 | 0.755 | 133 | 5.7 | 0.710 | 0.712 | 94.3 | 91.0 ± 3.6 |
| | | | | | | 7.4 | 0.895 | 0.691 | 91.5 | |
| Fmoc-Val-OH | 339.4 | | | | | | | | | |
| | | | | | | 1.8 | 0.119 | 0.378 | 90.2 | |
| | | Chem Matrix | 0.48 | 0.419 | 65 | 5.3 | 0.371 | 0.400 | 95.5 | 92.6 ± 2.7 |
| | | | | | | 9.3 | 0.629 | 0.386 | 92.2 | |
| | | | | | | 5.7 | 0.721 | 0.723 | 96.8 | |
| | | Polystyrene | 0.98 | 0.747 | 139 | 3.6 | 0.458 | 0.727 | 97.3 | 95.5 ± 2.7 |
| | | | | | | 8.1 | 0.978 | 0.690 | 92.4 | |
| Fmoc-Ile-OH | 353.4 | | | | | | | | | |
| | | | | | | 5.9 | 0.412 | 0.399 | 95.7 | |
| | | Chem Matrix | 0.48 | 0.417 | 68 | 3.0 | 0.199 | 0.379 | 90.9 | 91.3 ± 4.2 |
| | | | | | | 1.9 | 0.121 | 0.364 | 87.3 | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | 4.0 | 0.451 | 0.644 | 85.1 | |
| Fmoc-Pro-OH | 337.4 | Polystyrene | 0.98 | 0.757 | 132 | 5.0 | 0.558 | 0.638 | 84.2 | 83.2 ± 2.6 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 7.8 | 0.829 | 0.607 | 80.2 | |
| | | | | | | 6.8 | 0.421 | 0.354 | 84.4 | |
| | | Chem Matrix | 0.48 | 0.419 | 65 | 6.1 | 0.387 | 0.363 | 86.5 | 86.6 ± 2.3 |
| | | | | | | 4.6 | 0.300 | 0.373 | 88.9 | |
| | | | | | | 4.6 | 0.563 | 0.699 | 94.8 | |
| | | Polystyrene | 0.98 | 0.738 | 146 | 5.6 | 0.658 | 0.671 | 91.0 | 94.0 ± 2.7 |
| | | | | | | 2.1 | 0.261 | 0.710 | 96.2 | |
| Fmoc-Met-OH | 371.5 | | | | | 9.8 | 0.65 | 0.379 | 91.8 | |
| | | Chem Matrix | 0.48 | 0.413 | 71 | 7.9 | 0.503 | 0.364 | 88.1 | 91.6 ± 3.4 |
| | | | | | | 6.1 | 0.418 | 0.392 | 94.8 | |
| | | | | | | 1.9 | 0.226 | 0.680 | 94.0 | |
| | | Polystyrene | 0.98 | 0.723 | 156 | 5.8 | 0.687 | 0.677 | 93.6 | 94.7 ± 1.5 |
| | | | | | | 6.4 | 0.781 | 0.697 | 96.4 | |
| Fmoc-Thr(tBu)-OH | 397.5 | | | | | 4.1 | 0.279 | 0.389 | 95.1 | |
| | | Chem Matrix | 0.48 | 0.409 | 76 | 2.9 | 0.189 | 0.372 | 91.1 | 93.1 ± 2.0 |
| | | | | | | 7.0 | 0.467 | 0.381 | 93.2 | |
| | | | | | | 6.8 | 0.802 | 0.674 | 94.1 | |
| | | Polystyrene | 0.98 | 0.716 | 161 | 4.6 | 0.544 | 0.676 | 94.4 | 93.5 ± 1.3 |
| | | | | | | 4.5 | 0.519 | 0.659 | 92.0 | |
| Fmoc-Asp(tBu)-OH | 411.5 | | | | | 6.9 | 0.458 | 0.379 | 93.2 | |
| | | Chem Matrix | 0.48 | 0.407 | 79 | 7.8 | 0.543 | 0.398 | 97.7 | 95.1 ± 2.4 |
| | | | | | | 1.8 | 0.121 | 0.384 | 94.4 | |
| | | | | | | 2.0 | 0.239 | 0.683 | 96.3 | |
| | | Polystyrene | 0.98 | 0.709 | 167 | 6.4 | 0.721 | 0.644 | 90.8 | 94.3 ± 3.1 |
| | | | | | | 4.7 | 0.559 | 0.680 | 95.9 | |
| Fmoc-Glu(tBu)-OH | 425.5 | | | | | 7.6 | 0.507 | 0.381 | 94.4 | |
| | | Chem Matrix | 0.48 | 0.404 | 82 | 5.1 | 0.321 | 0.360 | 89.0 | 91.7 ± 2.7 |
| | | | | | | 3.5 | 0.227 | 0.371 | 91.7 | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 6.8 | 0.657 | 0.552 | 87.4 | |
| | | Polystyrene | 0.98 | 0.632 | 234 | 7.6 | 0.718 | 0.540 | 85.4 | 87.1 ± 1.6 |
| | | | | | | 4.1 | 0.402 | 0.560 | 88.7 | |
| Fmoc-Asn(trt)-OH | 596.7 | | | | | 1.7 | 0.075 | 0.252 | 66.7 | |
| | | Chem Matrix | 0.48 | 0.378 | 115 | 6.9 | 0.321 | 0.266 | 70.3 | 68.7 ± 1.8 |
| | | | | | | 6.7 | 0.306 | 0.261 | 69.0 | |
| | | | | | | 5.5 | 0.546 | 0.567 | 90.5 | |
| | | Polystyrene | 0.98 | 0.627 | 239 | 6.6 | 0.688 | 0.596 | 95.0 | 91.9 ± 1.9 |
| | | | | | | 2.9 | 0.287 | 0.566 | 90.2 | |
| Fmoc-Gln(trt)-OH | 610.7 | | | | | 2.3 | 0.105 | 0.261 | 69.4 | |
| | | Chem Matrix | 0.48 | 0.376 | 117 | 2.7 | 0.132 | 0.279 | 74.3 | 72.2 ± 2.5 |
| | | | | | | 5.6 | 0.269 | 0.274 | 73.0 | |
| | | | | | | 4.0 | 0.423 | 0.604 | 87.8 | |
| | | Polystyrene | 0.98 | 0.688 | 184 | 7.4 | 0.812 | 0.627 | 91.1 | 88.6 ± 2.2 |
| | | | | | | 6.9 | 0.722 | 0.598 | 86.9 | |
| Fmoc-Lys(Boc)-OH | 468.6 | | | | | 5.1 | 0.343 | 0.384 | 96.8 | |
| | | Chem Matrix | 0.48 | 0.397 | 90 | 3.4 | 0.224 | 0.376 | 94.8 | 96.3 ± 1.3 |
| | | | | | | 8.4 | 0.568 | 0.386 | 97.3 | |
| | | | | | | 3.9 | 0.301 | 0.441 | 72.1 | |
| | | Polystyrene | 0.98 | 0.612 | 254 | 5.3 | 0.439 | 0.473 | 77.3 | 73.0 ± 3.9 |
| | | | | | | 4.8 | 0.358 | 0.426 | 69.6 | |
| Fmoc-Arg(Pbf)-OH | 648.8 | | | | | 8.6 | 0.425 | 0.282 | 76.1 | |
| | | Chem Matrix | 0.48 | 0.371 | 125 | 8.0 | 0.399 | 0.285 | 76.8 | 74.4 ± 3.6 |
| | | | | | | 4.1 | 0.187 | 0.261 | 70.2 | |
| | | | | | | 2.8 | 0.324 | 0.661 | 95.4 | |
| | | Polystyrene | 0.98 | 0.693 | 180 | 5.3 | 0.621 | 0.670 | 96.6 | 95.1 ± 1.7 |
| | | | | | | 6.8 | 0.769 | 0.646 | 93.2 | |
| Fmoc-Tyr(tBu)-OH | 459.5 | | | | | 6.9 | 0.460 | 0.381 | 95.5 | |
| | | Chem Matrix | 0.48 | 0.399 | 88 | 7.3 | 0.471 | 0.369 | 92.4 | 90.3 ± 6.5 |
| | | | | | | 3.5 | 0.203 | 0.331 | 83.1 | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 3.5 | 0.357 | 0.583 | 88.0 | |
| | | Polystyrene | 0.98 | 0.662 | 206 | 6.3 | 0.579 | 0.525 | 79.3 | 85.2 ± 5.1 |
| | | | | | | 6.9 | 0.705 | 0.584 | 88.2 | |
| Fmoc-Trp(Boc)-OH | 526.6 | | | | | | | | | |
| | | | | | | 7.0 | 0.448 | 0.366 | 94.0 | |
| | | Chem Matrix | 0.48 | 0.389 | 101 | 3.4 | 0.211 | 0.355 | 91.2 | 94.0 ± 2.9 |
| | | | | | | 6.2 | 0.409 | 0.377 | 96.9 | |

**ESI Table 5.4:** Optimisation and loading calculations of amino acids that had less than 90% loading in first loading protocol which were further transferred to total flow loading and synthesis.

| **AcCl** | **Resin PS-2-ClTrt-Cl** | **AcCl flow 1 mL/min** | **Loading solution 2 mL/min** | | | **Error** | |
|---|---|---|---|---|---|---|---|
| **Theoretical Loading** | 0.604435347 | Calculated loading | 0.588817789 | **Percent** | **97.4162** | 0.140274 | -0.69455 |
| **Fmoc-Arg(Pbf)-OH** | Amount | Average Abs | Loading | | | | |
| Sample A | 4.7 | 0.485 | 0.589665653 | 97.55644782 | | -0.14027 | |
| Sample B | 3.8 | 0.391 | 0.587969925 | 97.27590025 | | 0.140274 | |
| Sample C | 4.5 | 0.467 | 0.593015873 | 98.11072042 | | -0.69455 | |
| | Average | 0.588817789 | | | | | |

| **AcCl sample** | **Resin CM-Trt** | **AcCl flow 1 mL/min** | **Loading solution 2 mL/min** | | | Error | |
|---|---|---|---|---|---|---|---|
| **Theoretical Loading** | 0.362389799 | Calculated loading | 0.330935447 | **Percent** | **91.3203** | 2.717243 | -0.30654 |
| **Fmoc-Arg(Pbf)-OH** | Amount | Average Abs | Loading | | | | |
| **Sample A** | 3.7 | 0.215 | 0.332046332 | 91.62684301 | | -0.30654 | |
| **Sample B** | 5.7 | 0.329 | 0.329824561 | 91.01375439 | | 0.306544 | |
| **Sample C** | 4.2 | 0.236 | 0.321088435 | 88.60305572 | | 2.717243 | |
| | Average | 0.330935447 | | | | | |

| **AcCl** | **Resin PS-2-ClTrt-Cl** | **AcCl flow 1 mL/min** | **Loading solution 1 mL/min** | | | Error | |
|---|---|---|---|---|---|---|---|
| **Theoretical Loading** | **0.604435347** | **Calculated loading** | **0.582663151** | **Percent** | **96.3979** | 1.973982 | -2.19299 |
| **Fmoc-Arg(Pbf)-OH** | Amount | Average Abs | Loading | | | | |
| **Sample A** | 3.7 | 0.385 | 0.594594595 | 98.37190991 | | -1.97398 | |
| **Sample B** | 8.2 | 0.819 | 0.570731707 | 94.42394634 | | 1.973982 | |
| **Sample C** | 4.2 | 0.438 | 0.595918367 | 98.59091973 | | -2.19299 | |
| | **Average** | **0.582663151** | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **AcCl sample** | **Resin CM-Trt** | **AcCl flow 1 mL/min** | **Loading solution 1 mL/min** | | | Error | |
| **Theoretical Loading** | **0.362389799** | **Calculated loading** | **0.333654456** | **Percent** | **92.0706** | 3.579613 | -1.57404 |
| **Fmoc-Arg(Pbf)-OH** | Amount | Average Abs | Loading | | | | |
| **Sample A** | 2.3 | 0.132 | 0.327950311 | 90.49656244 | | 1.574036 | |
| **Sample B** | 4.9 | 0.291 | 0.339358601 | 93.64463396 | | -1.57404 | |
| **Sample C** | 6.7 | 0.376 | 0.320682303 | 88.49098507 | | 3.579613 | |
| | **Average** | **0.333654456** | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **AcCl** | **Resin PS-2-ClTrt-Cl** | **AcCl flow 1 mL/min** | **Loading solution 5 mL/min** | | | Error | |
| **Theoretical Loading** | **0.604435347** | **Calculated loading** | **0.455524387** | **Percent** | **75.3636** | 2.537245 | -0.52328 |
| **Fmoc-Arg(Pbf)-OH** | Amount | Average Abs | Loading | | | | |
| **Sample A** | 4.9 | 0.3879 | 0.452361516 | 74.84034787 | | 0.523277 | |
| **Sample B** | 3.7 | 0.297 | 0.458687259 | 75.88690193 | | -0.52328 | |
| **Sample C** | 9.1 | 0.701 | 0.440188383 | 72.82638012 | | 2.537245 | |
| | **Average** | **0.455524387** | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **AcCl sample** | **Resin CM-Trt** | **AcCl flow 1 mL/min** | **Loading solution 5 mL/min** | | | Error | |
| **Theoretical Loading** | **0.362389799** | **Calculated loading** | **0.252313109** | **Percent** | **69.6248** | 1.493651 | -2.03135 |
| **Fmoc-Arg(Pbf)-OH** | Amount | Average Abs | Loading | | | | |
| **Sample A** | 4.9 | 0.221 | 0.257725948 | 71.11843335 | | -1.49365 | |
| **Sample B** | 5.3 | 0.229 | 0.24690027 | 68.13113127 | | 1.493651 | |
| **Sample C** | 7.9 | 0.359 | 0.259674503 | 71.65612928 | | -2.03135 | |
| | **Average** | **0.252313109** | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **AcCl** | **Resin PS-2-ClTrt-Cl** | **AcCl flow 0.5 mL/min** | **Loading solution 2 mL/min** | | | Error | |
| **Theoretical Loading** | **0.604435347** | **Calculated loading** | **0.589091777** | **Percent** | **97.4615** | 1.429541 | -1.42954 |
| **Fmoc-Arg(Pbf)-OH** | Amount | Average Abs | Loading | | | | |
| **Sample A** | 5.7 | 0.579 | 0.580451128 | 96.03196291 | | 1.429541 | |
| **Sample B** | 6.3 | 0.659 | 0.597732426 | 98.89104429 | | -1.42954 | |
| **Sample C** | 2.9 | 0.297 | 0.585221675 | 96.8212197 | | 0.640284 | |
| | **Average** | **0.589091777** | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **AcCl sample** | **Resin CM-Trt** | **AcCl flow 0.5 mL/min** | **Loading solution2 mL/min** | | | Error | |
| **Theoretical Loading** | **0.362389799** | **Calculated loading** | **0.331492871** | **Percent** | **91.4741** | 0.32015 | -0.97164 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Fmoc-Arg(Pbf)-OH** | Amount | Average Abs | Loading | | | | |
| **Sample A** | 7.3 | 0.422 | 0.330332681 | 91.15396794 | | 0.32015 | |
| **Sample B** | 8.4 | 0.489 | 0.332653061 | 91.79426748 | | -0.32015 | |
| **Sample C** | 5.1 | 0.299 | 0.335014006 | 92.44576053 | | -0.97164 | |
| | **Average** | **0.331492871** | | | | | |
| | | | | | | | |
| **AcCl** | **Resin PS-2-ClTrt-Cl** | **AcCl flow 2.0 mL/min** | **Loading solution 2 mL/min** | | | Error | |
| **Theoretical Loading** | **0.604435347** | **Calculated loading** | **0.492808927** | **Percent** | **81.5321** | 3.398216 | -4.20519 |
| **Fmoc-Arg(Pbf)-OH** | Amount | Average Abs | Loading | | | | |
| **Sample A** | 3.4 | 0.281 | 0.472268908 | 78.13389972 | | 3.398216 | |
| **Sample B** | 6.1 | 0.548 | 0.513348946 | 84.93033193 | | -3.39822 | |
| **Sample C** | 2.9 | 0.263 | 0.518226601 | 85.73730903 | | -4.20519 | |
| | **Average** | **0.492808927** | | | | | |
| | | | | | | | |
| **AcCl sample** | **Resin CM-Trt** | **AcCl flow 2.0 mL/min** | **Loading solution2 mL/min** | | | Error | Error |
| **Theoretical Loading** | **0.362389799** | **Calculated loading** | **0.27755102** | **Percent** | **76.5891** | 2.25262 | -2.25262 |
| **Fmoc-Arg(Pbf)-OH** | Amount | Average Abs | Loading | | | | |
| **Sample A** | 6.7 | 0.335 | 0.285714286 | 78.84170213 | | -2.25262 | |
| **Sample B** | 3.5 | 0.165 | 0.269387755 | 74.33646201 | | 2.25262 | |
| **Sample C** | 4.9 | 0.243 | 0.283381924 | 78.1980964 | | -1.60901 | |
| | **Average** | **0.27755102** | | | | | |
| | | | | | | | |
| **HCTU DCM** | **Resin PS-2-ClTrt-Cl** | **AcCl flow 2.0 mL/min** | **Loading solution 2 mL/min** | | | Error | |
| **Theoretical Loading** | **0.362393739** | **Calculated loading** | **0.018290291** | **Percent** | **5.04708** | 3.179794 | -3.17979 |
| **Fmoc-Arg(Pbf)-OH** | Amount | Average Abs | Loading | | | | |
| **Sample A** | 7.6 | 0.009 | 0.006766917 | 1.867283171 | | 3.179794 | |
| **Sample B** | 2.3 | 0.012 | 0.029813665 | 8.226870781 | | -3.17979 | |
| **Sample C** | 9.1 | 0.014 | 0.008791209 | 2.425872153 | | 2.621205 | |
| | **Average** | **0.018290291** | | | | | |
| | | | | | | | |
| **AcCl sample** | **Resin CM-Trt** | **AcCl flow 2.0 mL/min** | **Loading solution2 mL/min** | | | Error | Error |
| **Theoretical Loading** | **0.362389799** | **Calculated loading** | **0.01977317** | **Percent** | **5.45633** | 2.155976 | -2.15598 |
| **Fmoc-Arg(Pbf)-OH** | Amount | Average Abs | Loading | | | | |
| **Sample A** | 4.3 | 0.009 | 0.011960133 | 3.300350322 | | 2.155976 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Sample B** | 2.9 | 0.014 | 0.027586207 | 7.612302274 | | -2.15598 | |
| **Sample C** | 4.3 | 0.011 | 0.01461794 | 4.033761504 | | 1.422565 | |
| | **Average** | **0.01977317** | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **AcCl** | **Resin PS-2-ClTrt-Cl** | **AcCl flow 1 mL/min** | **Loading solution 2 mL/min** | | | Error | |
| **Theoretical Loading** | **0.628390691** | **Calculated loading** | **0.589317659** | **Percent** | **93.782** | 0.748304 | -0.7483 |
| **Fmoc-Cys(Trt)-OH** | Amount | Average Abs | Loading | | | | |
| **Sample A** | 4.3 | 0.447 | 0.594019934 | 94.53035216 | | -0.7483 | |
| **Sample B** | 5.2 | 0.532 | 0.584615385 | 93.03374359 | | 0.748304 | |
| **Sample C** | 6.3 | 0.645 | 0.585034014 | 93.10036281 | | 0.681685 | |
| | **Average** | **0.589317659** | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **AcCl sample** | **Resin CM-Trt** | **AcCl flow 1 mL/min** | **Loading solution 2 mL/min** | | | Error | |
| **Theoretical Loading** | **0.370870417** | **Calculated loading** | **0.342318059** | **Percent** | **92.3013** | 0.145356 | -2.17715 |
| **Fmoc-Cys(Trt)-OH** | Amount | Average Abs | Loading | | | | |
| **Sample A** | 4.8 | 0.288 | 0.342857143 | 92.44661398 | | -0.14536 | |
| **Sample B** | 5.3 | 0.317 | 0.341778976 | 92.15590136 | | 0.145356 | |
| **Sample C** | 9.1 | 0.558 | 0.350392465 | 94.4784077 | | -2.17715 | |
| | **Average** | **0.342318059** | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **AcCl** | **Resin PS-2-ClTrt-Cl** | **AcCl flow 1 mL/min** | **Loading solution 2 mL/min** | | | Error | |
| **Theoretical Loading** | **0.604435347** | **Calculated loading** | **0.544371118** | **Percent** | **90.0628** | 4.682004 | -4.682 |
| **Fmoc-His(Trt)-OH** | Amount | Average Abs | Loading | | | | |
| **Sample A** | 3.2 | 0.289 | 0.516071429 | 85.3807494 | | 4.682004 | |
| **Sample B** | 4.6 | 0.461 | 0.572670807 | 94.74475818 | | -4.682 | |
| **Sample C** | 7.6 | 0.738 | 0.554887218 | 91.80257594 | | -1.73982 | |
| | **Average** | **0.544371118** | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **AcCl sample** | **Resin CM-Trt** | **AcCl flow 1 mL/min** | **Loading solution 1 mL/min** | | | Error | |
| **Theoretical Loading** | **0.362389799** | **Calculated loading** | **0.318188915** | **Percent** | **87.8029** | 3.705129 | -3.88703 |
| **Fmoc-His(Trt)-OH** | Amount | Average Abs | Loading | | | | |
| **Sample A** | 3.6 | 0.192 | 0.304761905 | 84.0978156 | | 3.705129 | |
| **Sample B** | 6.1 | 0.354 | 0.331615925 | 91.50807394 | | -3.70513 | |
| **Sample C** | 2.7 | 0.157 | 0.332275132 | 91.68997951 | | -3.88703 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Average** | **0.318188915** | | | | | |
| **AcCl** | **Resin PS-2-ClTrt-Cl** | **AcCl flow 1 mL/min** | **Loading solution 5 mL/min** | | | Error | |
| **Theoretical Loading** | **0.719907852** | **Calculated loading** | **0.693744485** | **Percent** | **96.3657** | 1.755571 | -1.75557 |
| **Fmoc-Ser(tBu)-OH** | Amount | Average Abs | Loading | | | | |
| **Sample A** | 6.2 | 0.739 | 0.681105991 | 94.61016283 | | 1.755571 | |
| **Sample B** | 4.7 | 0.581 | 0.706382979 | 98.12130496 | | -1.75557 | |
| **Sample C** | 7.1 | 0.852 | 0.685714286 | 95.25028571 | | 1.115448 | |
| | **Average** | **0.693744485** | | | | | |
| **AcCl sample** | **Resin CM-Trt** | **AcCl flow 1 mL/min** | **Loading solution 5 mL/min** | | | Error | |
| **Theoretical Loading** | **0.400952732** | **Calculated loading** | **0.380736014** | **Percent** | **94.9578** | 2.186881 | -2.18688 |
| **Fmoc-Ser(tBu)-OH** | Amount | Average Abs | Loading | | | | |
| **Sample A** | 4.9 | 0.334 | 0.389504373 | 97.14471112 | | -2.18688 | |
| **Sample B** | 5.3 | 0.345 | 0.371967655 | 92.77094913 | | 2.186881 | |
| **Sample C** | 7.9 | 0.529 | 0.382640145 | 95.43273164 | | -0.4749 | |
| | **Average** | **0.380736014** | | | | | |
| **AcCl** | **Resin PS-2-ClTrt-Cl** | **AcCl flow 0.5 mL/min** | **Loading solution 2 mL/min** | | | Error | |
| **Theoretical Loading** | **0.67834935** | **Calculated loading** | **0.658771827** | **Percent** | **97.1139** | 1.591126 | -1.59113 |
| **Fmoc-Lys(Boc)-OH** | Amount | Average Abs | Loading | | | | |
| **Sample A** | 4.6 | 0.539 | 0.669565217 | 98.70507246 | | -1.59113 | |
| **Sample B** | 5.3 | 0.601 | 0.647978437 | 95.5228212 | | 1.591126 | |
| **Sample C** | 2.3 | 0.265 | 0.658385093 | 97.05693582 | | 0.057011 | |
| | **Average** | **0.658771827** | | | | | |
| **AcCl sample** | **Resin CM-Trt** | **AcCl flow 0.5 mL/min** | **Loading solution2 mL/min** | | | Error | |
| **Theoretical Loading** | **0.387723199** | **Calculated loading** | **0.368511788** | **Percent** | **95.0451** | 0.651339 | -2.63066 |
| **Fmoc-Lys(Boc)-OH** | Amount | Average Abs | Loading | | | | |
| **Sample A** | 7.3 | 0.474 | 0.371037182 | 95.69641004 | | -0.65134 | |
| **Sample B** | 8.4 | 0.538 | 0.365986395 | 94.39373136 | | 0.651339 | |
| **Sample C** | 5.1 | 0.338 | 0.378711485 | 97.67573515 | | -2.63066 | |
| | **Average** | **0.368511788** | | | | | |

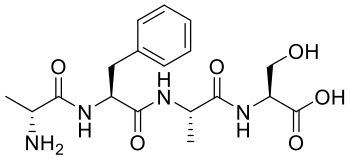
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **AcCl** | **Resin PS-2-ClTrt-Cl** | **AcCl flow 2.0 mL/min** | **Loading solution 2 mL/min** | | | Error | |
| **Theoretical Loading** | **0.744564678** | **Calculated loading** | **0.692550191** | **Percent** | **93.0141** | 0.543734 | -1.04976 |
| **Fmoc-Pro-OH** | Amount | Average Abs | Loading | | | | |
| **Sample A** | 4.2 | 0.512 | 0.696598639 | 93.55784127 | | -0.54373 | |
| **Sample B** | 4.1 | 0.494 | 0.688501742 | 92.47037398 | | 0.543734 | |
| **Sample C** | 3.9 | 0.478 | 0.7003663 | 94.06386325 | | -1.04976 | |
| | **Average** | **0.692550191** | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **AcCl sample** | **Resin CM-Trt** | **AcCl flow 2.0 mL/min** | **Loading solution2 mL/min** | | | Error | Error |
| **Theoretical Loading** | **0.408491797** | **Calculated loading** | **0.369936034** | **Percent** | **90.5614** | 3.669836 | -0.36538 |
| **Fmoc-Pro-OH** | Amount | Average Abs | Loading | | | | |
| **Sample A** | 6.7 | 0.432 | 0.368443497 | 90.19605767 | | 0.365378 | |
| **Sample B** | 6.8 | 0.442 | 0.371428571 | 90.92681277 | | -0.36538 | |
| **Sample C** | 5.2 | 0.323 | 0.354945055 | 86.89159918 | | 3.669836 | |
| | **Average** | **0.369936034** | | | | | |

# 6. Peptide synthesis and characterization data

### (S)-2-((S)-2-((S)-2-((R)-2-aminopropanamido)-4-methylpentanamido)propanamido)-3-(butoxy)propanoic acid

**Optimized procedure for the construction of 1 using PS-2ClTrtCl resin.** Compound **1** was synthesized utilising the general procedure for total flow synthesis using the following amino acids and reagent amounts†. Loaded with (Fmoc-Ser(*t*Bu)-OH (115 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1 (Fmoc-Ala-OH (93 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1 (Fmoc-Phe-OH (116 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1 (Fmoc-Ala-OH (93 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. After the final Fmoc-deprotection, the resin was removed from the heating block and washed with MeOH (2 × 10 mL), EtOAc (2 × 10 mL), hexanes (2 × 5 mL), DCM (2 × 5 mL) and $Et_2O$ (2 × 10 mL) and dried *in vacuo*. The peptide was cleaved from the resin using 10 mL cleavage cocktail (DCM:TFA:TIPS 98:1.5:0.5) The solution was evaporated to dryness for analysis. MS ($ESI^+$) *m/z* 395 (M + 1, 100 %). HRMS ($ESI^+$) for $C_{18}H_{27}N_4O_6$; calculated 395.1931, found, 395.1931. RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, $t_R$ 5.68 min. $^1H$ NMR (300 MHz, DMSO-$d_6$) δ 8.48 (d, *J* = 8.1 Hz, 1H), 8.28 (d, *J* = 7.6 Hz, 1H), 7.86 (d, *J* = 7.7 Hz, 1H), 7.28 - 7.24 (m, 4H), 7.23 – 7.14 (m, 1H), 4.55 (d, *J* = 8.6 Hz, 1H), 4.39 (p, *J* = 7.0 Hz, 1H), 4.33 – 4.24 (m, 1H), 3.62 (dd, *J* = 9.3, 4.4 Hz, 3H), 3.50 (dd, *J* = 9.3, 3.7 Hz, 3H), 3.11 – 2.99 (m, 2H), 2.77 (dd, *J* = 14.1, 10.1 Hz, 1H), 1.28 (d, *J* = 6.7 Hz, 3H), 1.22 (d, *J* = 7.0 Hz, 3H). $^{13}C$ NMR (75 MHz, DMSO-$d_6$) δ 181.48, 172.37, 170.83, 138.09, 129.62, 128.55, 126.80, 73.19, 62.01, 54.56, 50.64, 48.42, 48.41, 37.78, 27.62, 18.78.

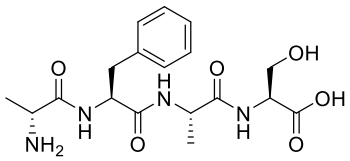


Chemical Formula: $C_{18}H_{26}N_4O_6$
Exact Mass: 394.19

†The AA solution was calculated based upon the final swollen resin volume; reagents were calculated accordingly for a 0.3 M solution based on the swollen volume (for 0.1g of PS this was 1 $cm^3$).

### (S)-2-((S)-2-((S)-2-((R)-2-aminopropanamido)-4-methylpentanamido)propanamido)-3-(butoxy)propanoic acid

**Optimized procedure for the construction of 1 using CM-trt-OH resin.** Compound **1** was synthesized utilising the general procedure for total flow synthesis using the following amino acids and reagent amounts†. Loaded with (Fmoc-Ser(*t*Bu)-OH (230 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1 (Fmoc-Ala-OH (186 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1 (Fmoc-Phe-OH (232 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1 (Fmoc-Ala-OH (186 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. After the final Fmoc-deprotection, the resin was removed from the heating block and washed with MeOH (2 × 10 mL), EtOAc (2 × 10 mL), hexanes (2 × 5 mL), DCM (2 × 5 mL) and $Et_2O$ (2 × 10 mL) and dried *in vacuo*. The peptide was cleaved from the resin using 10 mL cleavage cocktail (DCM:TFA:TIPS 98:1.5:0.5) The solution was evaporated to dryness for analysis. MS ($ESI^+$) *m/z* 395 (M + 1, 100 %). RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, $t_R$ 5.70 min.

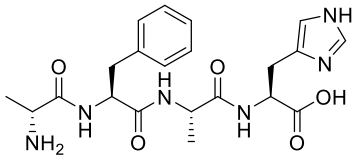


Chemical Formula: $C_{18}H_{26}N_4O_6$
Exact Mass: 394.19

†The AA solution was calculated based upon the final swollen resin volume; reagents were calculated accordingly for a 0.3 M solution based on the swollen volume (for 0.1g of CM-this was 2 $cm^3$).

**(S)-2-((S)-2-((S)-2-((R)-2-aminopropanamido)-4-methylpentanamido)propanamido)-3-(imidazol-4-yl)propanoic acid (2)**

**Optimized procedure for the construction of 2 using PS-2ClTrtCl resin.** Compound **2** was synthesized utilising the general procedure for total flow synthesis using the following amino acids and reagent amounts†. Loaded with (Fmoc-His(trt)-OH (185 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1 (Fmoc-Ala-OH (93 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1 (Fmoc-Phe-OH (116 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1 (Fmoc-Ala-OH (93 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. After the final Fmoc-deprotection, the resin was removed from the heating block and washed with MeOH (2 × 10 mL), EtOAc (2 × 10 mL), hexanes (2 × 5 mL), DCM (2 × 5 mL) and $Et_2O$ (2 × 10 mL) and dried *in vacuo*. The peptide was cleaved from the resin using 10 mL cleavage cocktail (DCM:TFA:TIPS 98:1.5:0.5) The solution was evaporated to dryness for analysis. MS ($ESI^+$) *m/z* 445 (M + 1, 100 %). HRMS ($ESI^+$) for $C_{21}H_{29}N_6O_5$; calculated 445.2199, found, 445.2197. RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, $t_R$ 2.58 min. $^1H$ NMR (300 MHz, DMSO-$d_6$) δ 8.91 (s, 1H), 8.51 (d, *J* = 8.1 Hz, 1H), 8.30 (dd, *J* = 7.6, 4.6 Hz, 2H), 8.00 (s, 3H), 7.36 (s, 1H), 7.33 – 7.11 (m, 5H), 4.63 – 4.46 (m, 2H), 4.24 (m, 1H), 3.75 (q, *J* = 7.0 Hz, 1H), 3.13 (dd, *J* = 15.2, 5.5 Hz, 1H), 3.10 – 2.91 (m, 2H), 2.72 (dd, *J* = 14.1, 10.3 Hz, 1H), 1.30 (d, *J* = 6.9 Hz, 3H), 1.20 (d, *J* = 7.0 Hz, 3H). $^{13}C$ NMR (75 MHz, DMSO-$d_6$) δ 172.64, 172.30, 170.96, 170.03, 138.13, 134.29, 129.84, 129.61, 128.56, 126.82, 117.44, 54.55, 51.52, 48.63, 48.38, 37.59, 26.75, 18.49, 17.67.

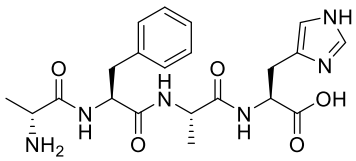


Chemical Formula: $C_{21}H_{28}N_6O_5$
Exact Mass: 444.21

†The AA solution was calculated based upon the final swollen resin volume; reagents were calculated accordingly for a 0.3 M solution based on the swollen volume (for 0.1g of PS this was 1 $cm^3$).

**(S)-2-((S)-2-((S)-2-((R)-2-aminopropanamido)-4-methylpentanamido)propanamido)-3-(imidazol-4-yl)propanoic acid (2)**

**Optimized procedure for the construction of 2 using CM-trt-OH resin.** Compound **2** was synthesized utilising the general procedure for total flow synthesis using the following amino acids and reagent amounts†. Loaded with (Fmoc-His(trt)-OH (371 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1 (Fmoc-Ala-OH (186 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1 (Fmoc-Phe-OH (232 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1 (Fmoc-Ala-OH (186 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. After the final Fmoc-deprotection, the resin was removed from the heating block and washed with MeOH (2 × 10 mL), EtOAc (2 × 10 mL), hexanes (2 × 5 mL), DCM (2 × 5 mL) and $Et_2O$ (2 × 10 mL) and dried *in vacuo*. The peptide was cleaved from the resin using 10 mL cleavage cocktail (DCM:TFA:TIPS 98:1.5:0.5) The solution was evaporated to dryness for analysis. MS ($ESI^+$) *m/z* 445 (M + 1, 100 %). RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, $t_R$ 2.59 min.

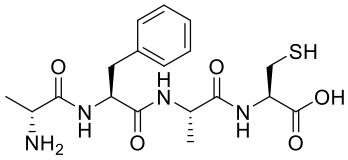


Chemical Formula: $C_{21}H_{28}N_6O_5$
Exact Mass: 444.21

†The AA solution was calculated based upon the final swollen resin volume; reagents were calculated accordingly for a 0.3 M solution based on the swollen volume (for 0.1g of CM-this was 2 $cm^3$).

**(R)-2-((S)-2-((S)-2-((R)-2-aminopropanamido)-4-methylpentanamido)propanamido)-3-(thio)propanoic acid (3)**

**Optimized procedure for the construction of 3 using PS-2ClTrtCl resin.** Compound **3** was synthesized utilising the general procedure for total flow synthesis using the following amino acids and reagent amounts†. Loaded with (Fmoc-Cys(trt)-OH (176 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1 (Fmoc-Ala-OH (93 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1 (Fmoc-Phe-OH (116 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1 (Fmoc-Ala-OH (93 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. After the final Fmoc-deprotection, the resin was removed from the heating block and washed with MeOH (2 × 10 mL), EtOAc (2 × 10 mL), hexanes (2 × 5 mL), DCM (2 × 5 mL) and $Et_2O$ (2 × 10 mL) and dried *in vacuo*. The peptide was cleaved from the resin using 10 mL cleavage cocktail (DCM:TFA:TIPS 98:1.5:0.5) The solution was evaporated to dryness for analysis. MS ($ESI^+$) *m/z* 411 (M + 1, 100 %). HRMS ($ESI^+$) for $C_{18}H_{27}N_4O_5S$; calculated 411.1702, found, 411.1703. RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, $t_R$ 4.35 min. $^1H$ NMR (300 MHz, DMSO-$d_6$) δ $^1H$ NMR (300 MHz, dmso) δ 8.40 (bs, 2H), 7.80 (d, $J$ = 7.7 Hz, 1H), 7.28 – 7.02 (m, 5H), 4.34 (dd, $J$ = 12.8, 7.7 Hz, 1H), 4.12 (s, 1H), 3.66 (t, $J$ = 6.2 Hz, 1H), 3.07 (dd, $J$ = 13.8, 5.0 Hz, 1H), 2.89 (dd, $J$ = 13.8, 8.1 Hz, 1H), 2.40 – 2.15 (m, 2H), 2.05 – 1.71 (m, 3H), 0.80 (d, $J$ = 6.5 Hz, 6H); $^{13}C$ NMR (75 MHz, DMSO-$d_6$) δ 13C NMR (75 MHz, dmso) δ = 174.82, 173.56, 170.90, 170.43, 138.68, 129.58, 128.32, 126.41, 58.65, 54.77, 53.16, 40.91, 40.64, 40.36, 40.08, 39.80, 39.52, 39.24, 37.77, 31.54, 30.97, 28.68, 19.61, 18.52.

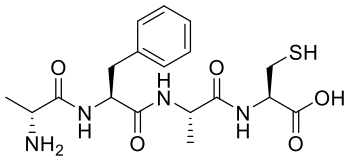


Chemical Formula: $C_{18}H_{26}N_4O_5S$
Exact Mass: 410.16

†The AA solution was calculated based upon the final swollen resin volume; reagents were calculated accordingly for a 0.3 M solution based on the swollen volume (for 0.1g of PS this was 1 $cm^3$).

**(R)-2-((S)-2-((S)-2-((R)-2-aminopropanamido)-4-methylpentanamido)propanamido)-3-(thio)propanoic acid (3)**

**Optimized procedure for the construction of 3 using CM-trt-OH resin.** Compound **3** was synthesized utilising the general procedure for total flow synthesis using the following amino acids and reagent amounts†. Loaded with (Fmoc-Cys(trt)-OH (351 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1 (Fmoc-Ala-OH (186 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1 (Fmoc-Phe-OH (232 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1 (Fmoc-Ala-OH (186 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. After the final Fmoc-deprotection, the resin was removed from the heating block and washed with MeOH (2 × 10 mL), EtOAc (2 × 10 mL), hexanes (2 × 5 mL), DCM (2 × 5 mL) and $Et_2O$ (2 × 10 mL) and dried *in vacuo*. The peptide was cleaved from the resin using 10 mL cleavage cocktail (DCM:TFA:TIPS 98:1.5:0.5) The solution was evaporated to dryness for analysis. MS ($ESI^+$) *m/z* 411 (M + 1, 100 %). RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, $t_R$ 4.34 min.

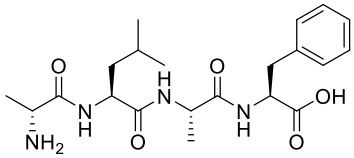


Chemical Formula: $C_{18}H_{26}N_4O_5S$
Exact Mass: 410.16

†The AA solution was calculated based upon the final swollen resin volume; reagents were calculated accordingly for a 0.3 M solution based on the swollen volume (for 0.1g of CM-this was 2 $cm^3$).

**(S)-2-((S)-2-((S)-2-((R)-2-aminopropanamido)-4-methylpentanamido)propanamido)-3-phenylpropanoic acid (7)**

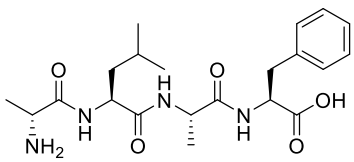
**Optimized procedure for the construction of 7 using PS-2ClTrtCl resin.** Compound **7** was synthesized utilising the general procedure for total flow synthesis using the following amino acids and reagent amounts†. Loaded with (Fmoc-Phe-OH (116 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1 (Fmoc-Ala-OH (93 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1 (Fmoc-Leu-OH (107 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1 (Fmoc-Ala-OH (93 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. After the final Fmoc-deprotection, the resin was removed from the heating block and washed with MeOH (2 × 10 mL), EtOAc (2 × 10 mL), hexanes (2 × 5 mL), DCM (2 × 5 mL) and $Et_2O$ (2 × 10 mL) and dried *in vacuo*. The peptide was cleaved from the resin using 10 mL cleavage cocktail (DCM:TFA:TIPS 98:1.5:0.5) The solution was evaporated to dryness for analysis. MS ($ESI^+$) *m/z* 421 (M + 1, 100 %), 443 (M + Na, 75%). HRMS ($ESI^+$) for $C_{21}H_{33}N_4O_5$; calculated 421.2451, found, 421.2452. RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, $t_R$ 5.69 min. $^1$H NMR (300 MHz, DMSO-$d_6$) δ 8.43 (d, *J* = 8.1 Hz, 1H), 8.02 (t, *J* = 8.0 Hz, 2H), 7.31 – 7.11 (m, 5H), 4.45 – 4.17 (m, 3H), 3.83 (q, *J* = 6.9 Hz, 1H), 3.03 (dd, *J* = 13.9, 5.2 Hz, 1H), 2.88 (dd, *J* = 13.9, 8.5 Hz, 1H), 1.58 (dq, *J* = 12.9, 6.6 Hz, 1H), 1.41 (dd, *J* = 8.6, 5.9 Hz, 2H), 1.31 (d, *J* = 6.9 Hz, 3H), 1.16 (d, *J* = 7.0 Hz, 3H), 0.91 – 0.78 (m, 6H). $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ 173.10, 172.40, 171.48, 169.76, 137.82, 129.57, 128.59, 126.87, 64.44, 51.46, 48.43, 48.32, 37.05, 24.48, 23.58, 21.84, 18.72, 17.67.




†The AA solution was calculated based upon the final swollen resin volume; reagents were calculated accordingly for a 0.3 M solution based on the swollen volume (for 0.1g of PS-this was 1 $cm^3$).

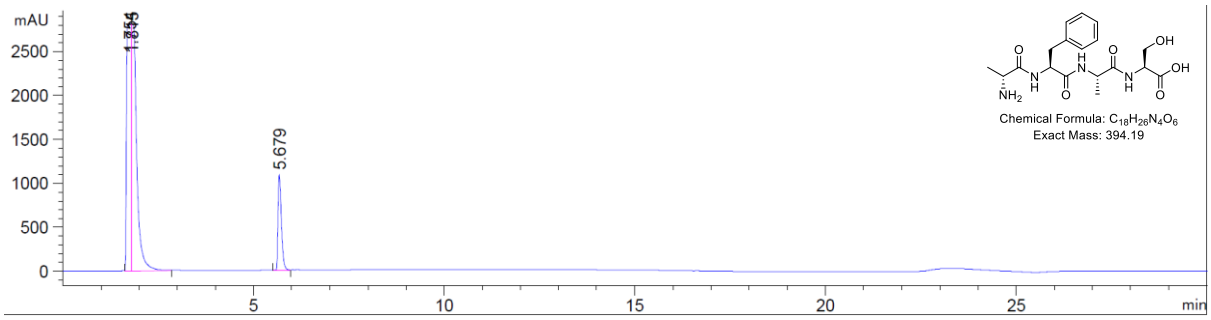
**(S)-2-((S)-2-((S)-2-((R)-2-aminopropanamido)-4-methylpentanamido)propanamido)-3-phenylpropanoic acid (7)**

**Optimized procedure for the construction of 7 using CM-trt-OH resin.** Compound **7** was synthesized utilising the general procedure for total flow synthesis using the following amino acids and reagent amounts†. Loaded with (Fmoc-Phe-OH (232 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1 (Fmoc-Ala-OH (186 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1 (Fmoc-Leu-OH (212 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1 (Fmoc-Ala-OH (186 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. After the final Fmoc-deprotection, the resin was removed from the heating block and washed with MeOH (2 × 10 mL), EtOAc (2 × 10 mL), hexanes (2 × 5 mL), DCM (2 × 5 mL) and $Et_2O$ (2 × 10 mL) and dried *in vacuo*. The peptide was cleaved from the resin using 10 mL cleavage cocktail (DCM:TFA:TIPS 98:1.5:0.5) The solution was evaporated to dryness for analysis. MS ($ESI^+$) *m/z* 421 (M + 1, 100 %), 443 (M + Na, 90%). RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, $t_R$ 5.71 min.

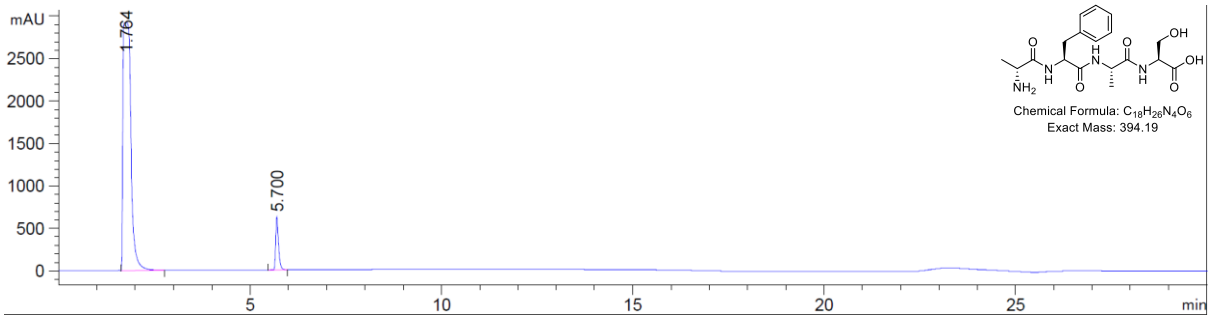



†The AA solution was calculated based upon the final swollen resin volume; reagents were calculated accordingly for a 0.3 M solution based on the swollen volume (for 0.1g of CM-this was 2 $cm^3$).

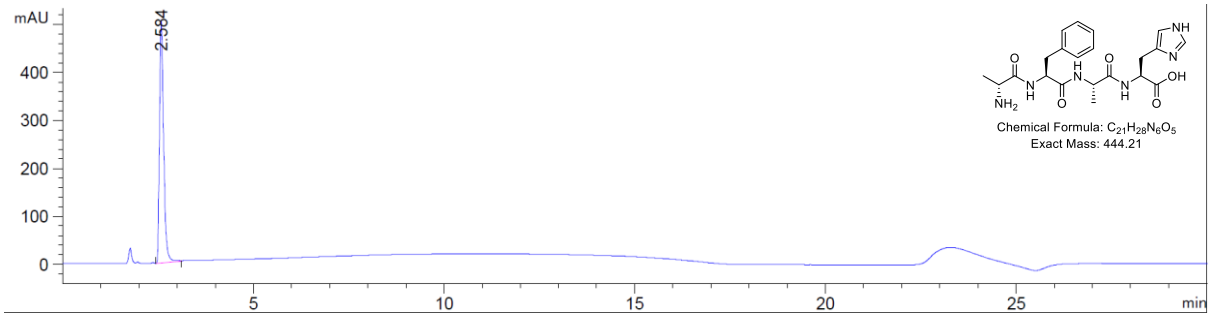
# 7. HPLC traces of final peptide sequences



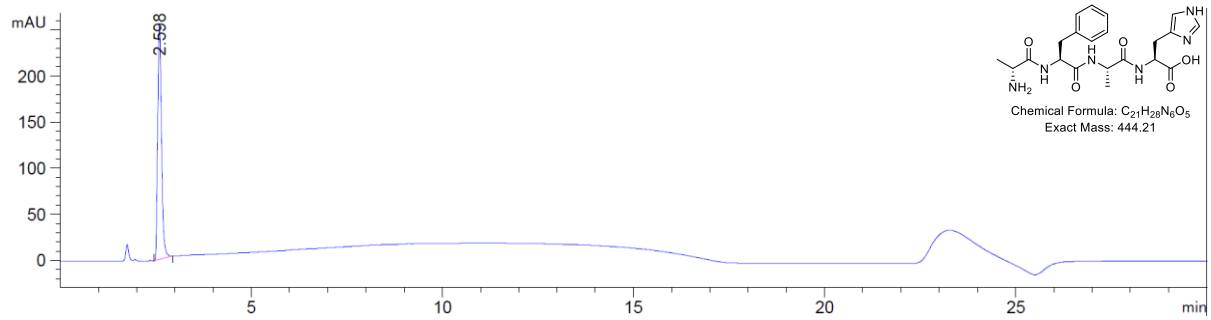

**ESI Figure 7.1**: HPLC Trace of PS-**1**.



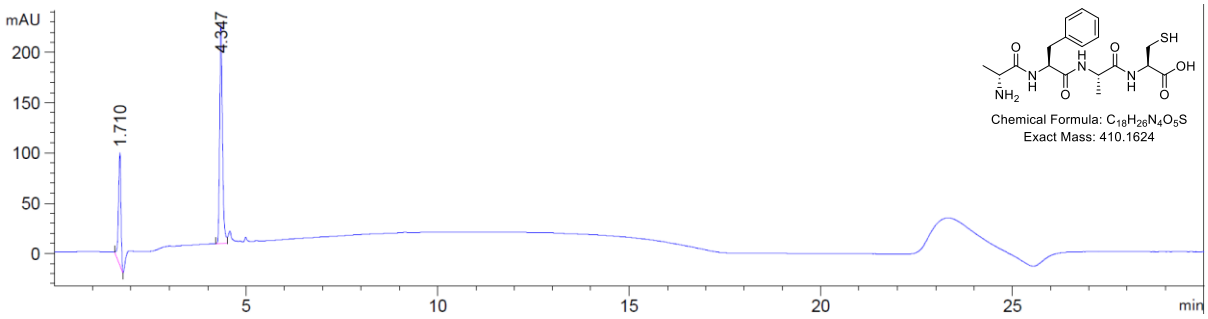

**ESI Figure 7.2**: HPLC Trace of CM-**1**.



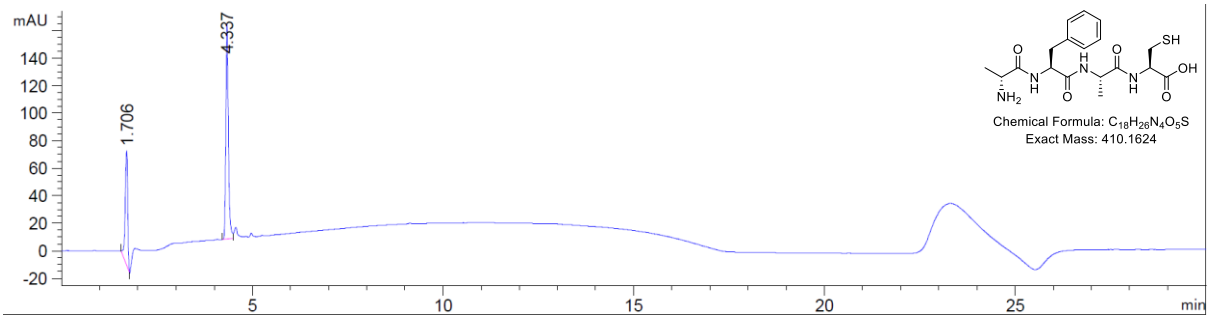

**ESI Figure 7.3**: HPLC trace of PS-**2**.



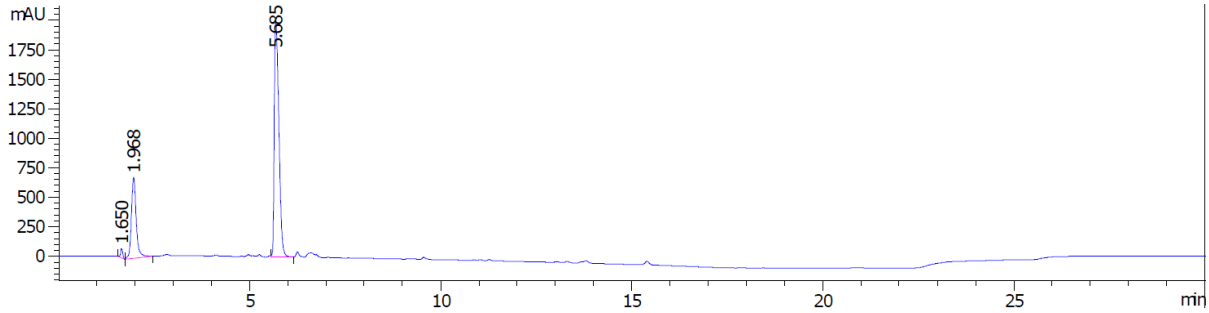

**ESI Figure 7.4**: HPLC trace of CM-**2**.



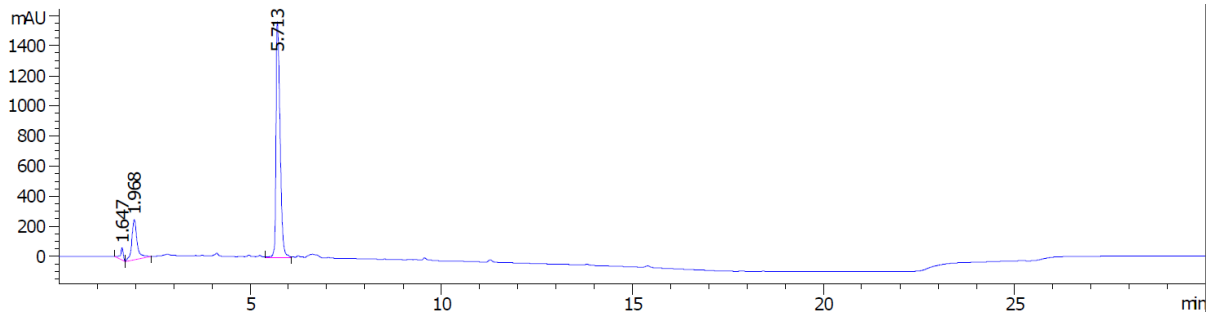

**ESI Figure 7.5**: HPLC trace of PS-**3**.




**ESI Figure 7.6**: HPLC trace of CM-**3**.

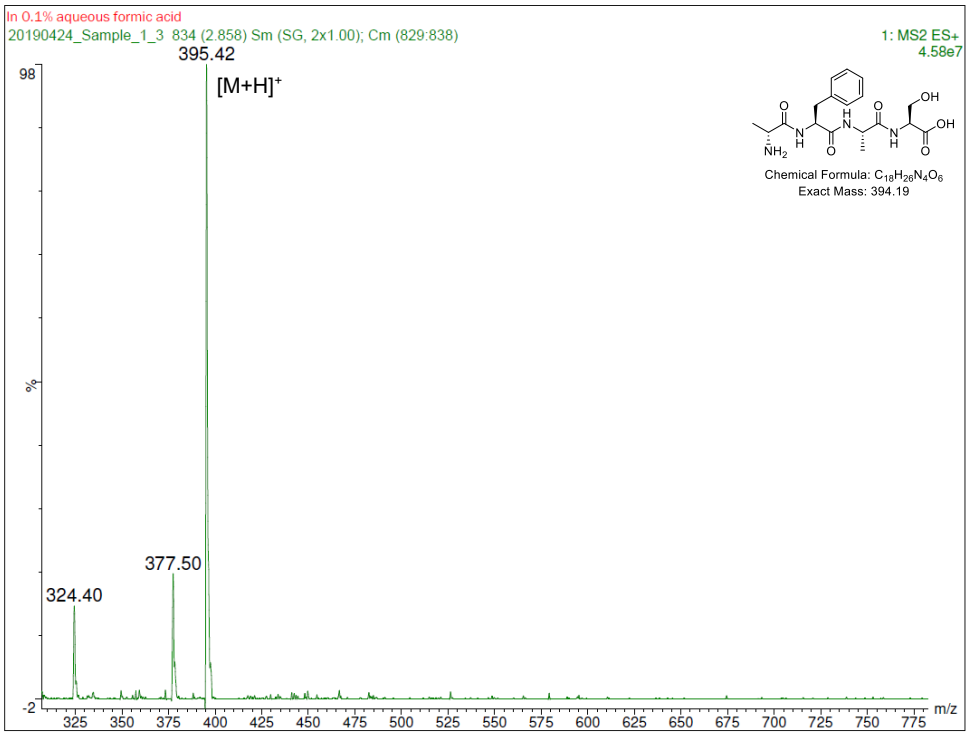



**ESI Figure 7.7**: HPLC Trace of PS-**7**.

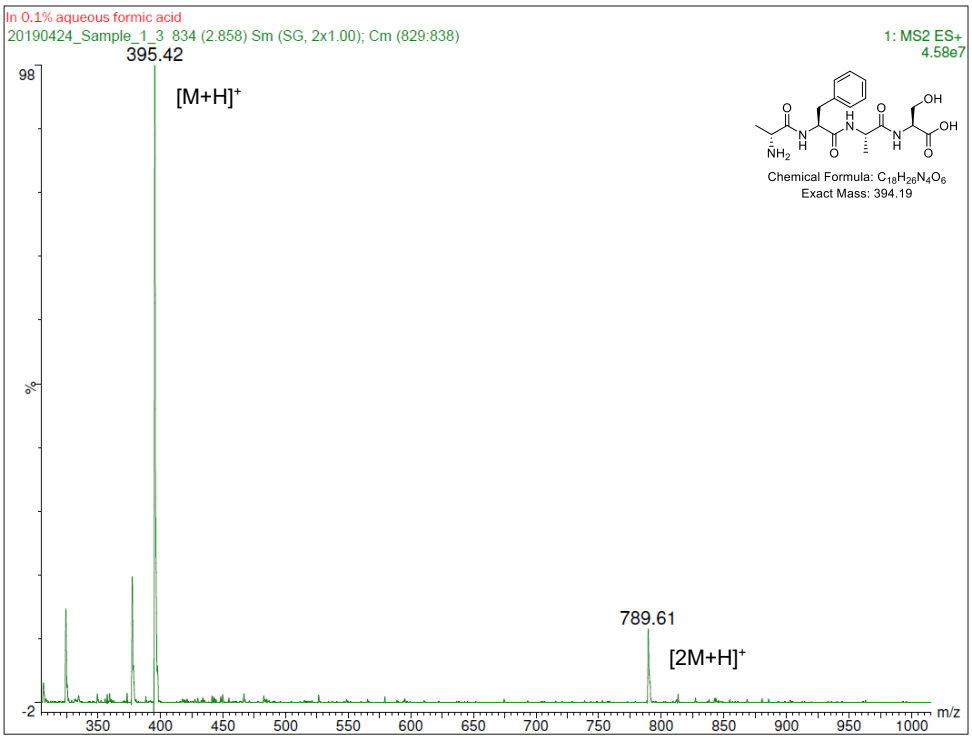



**ESI Figure 7.8**: HPLC trace of CM-**7**.

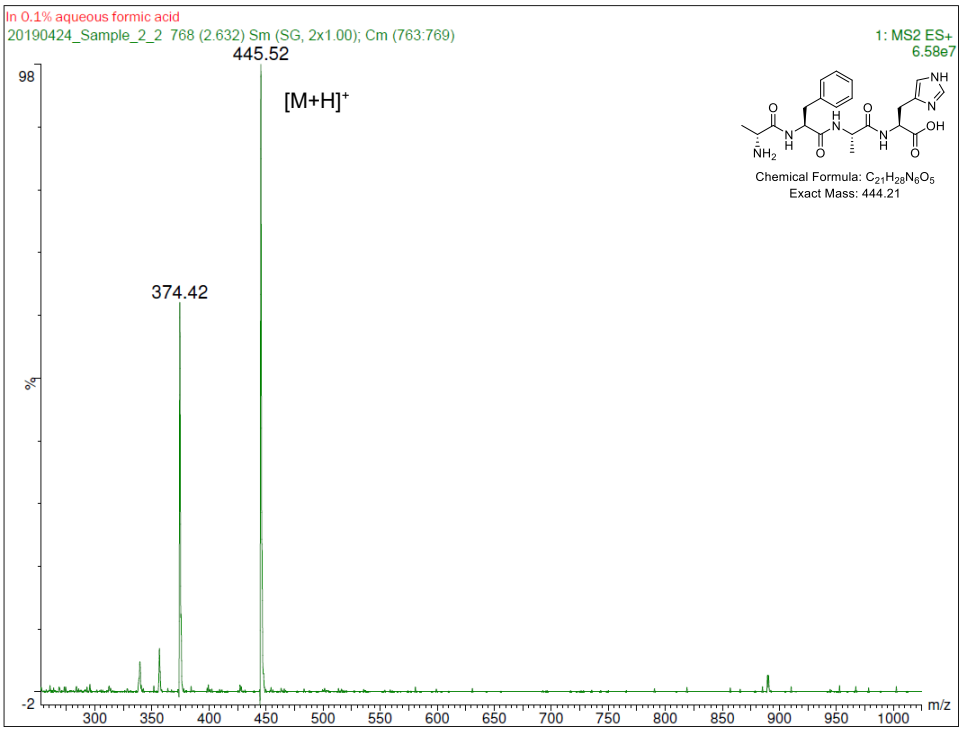
# 8. Mass spectra of peptide sequences



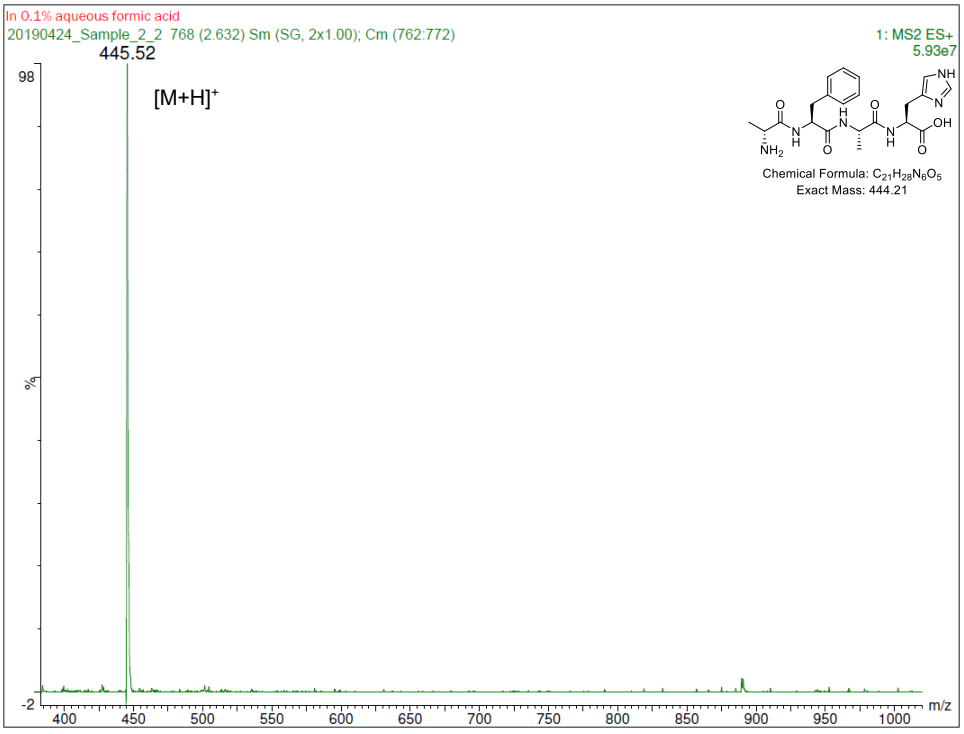

**ESI Figure 8.1**: LRMS of PS-**1**.



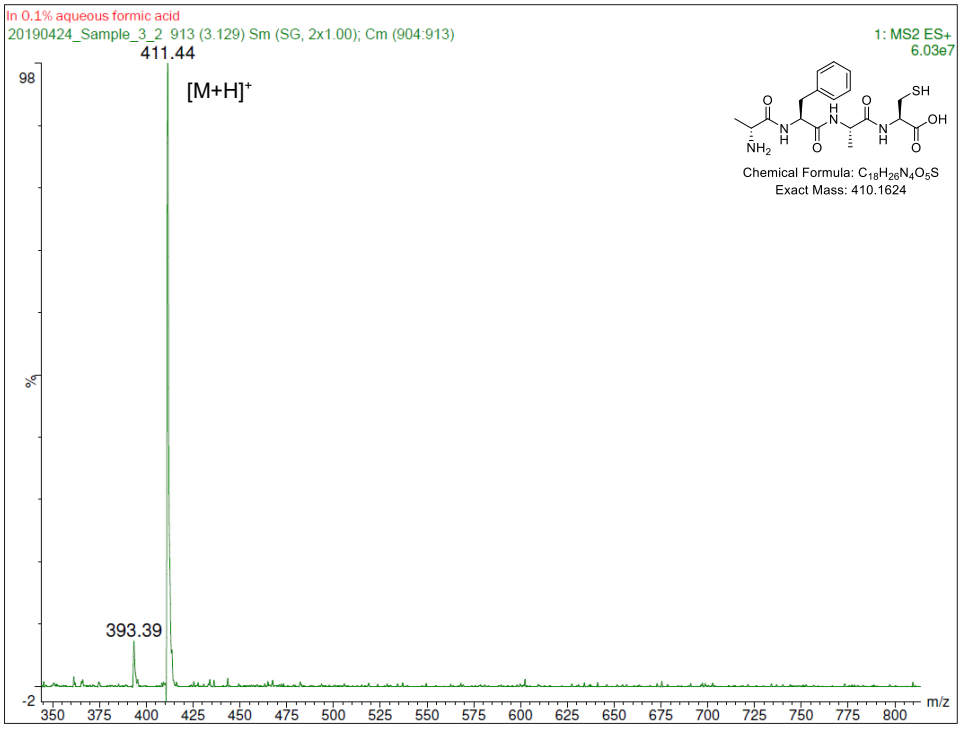

**ESI Figure 8.2**: LRMS of CM-**1**.



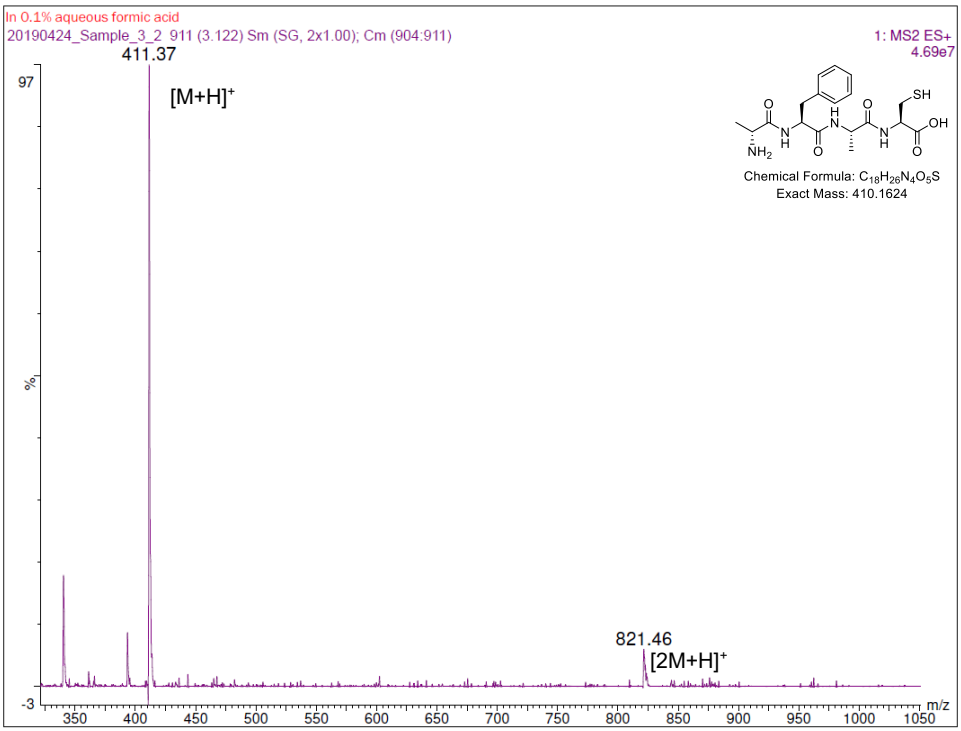

**ESI Figure 8.3**: LRMS of PS-**2**.



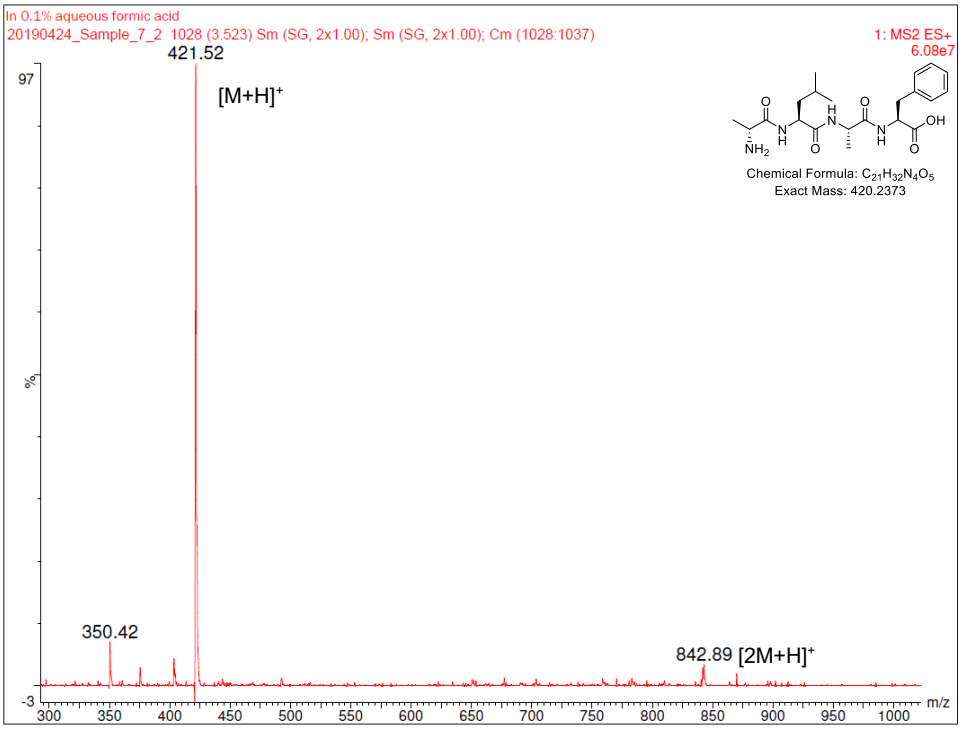

**ESI Figure 8.4**: LRMS of CM-**2**.



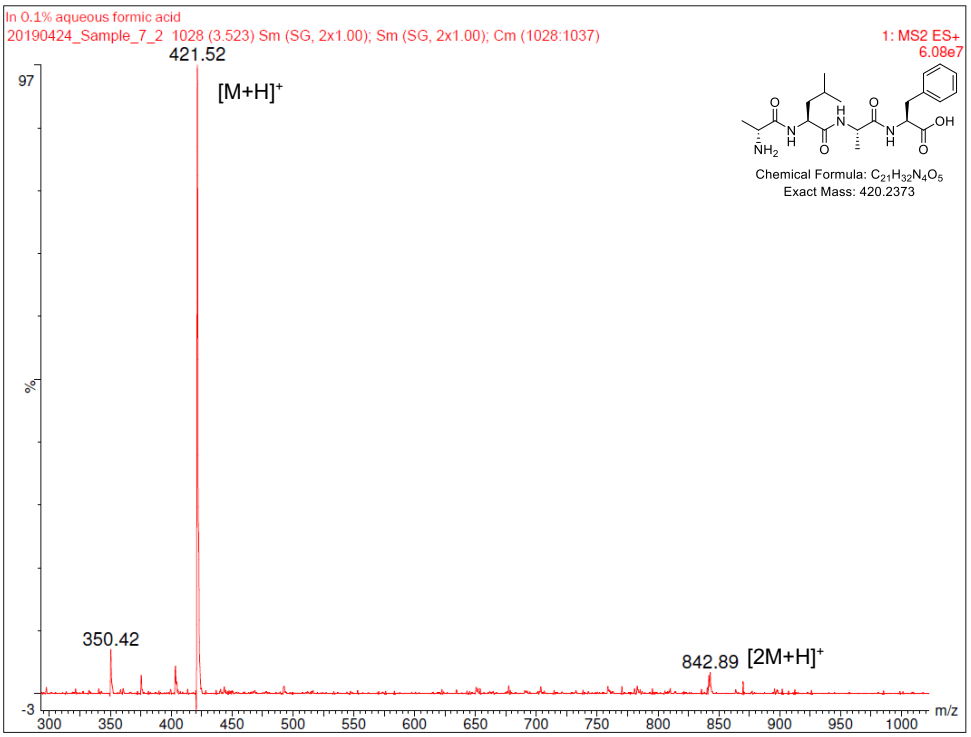

**ESI Figure 8.5**: LRMS PS-**3**.




**ESI Figure 8.6**: LRMS CM-**3**.




**ESI Figure 8.7**: LRMS PS-**7**.

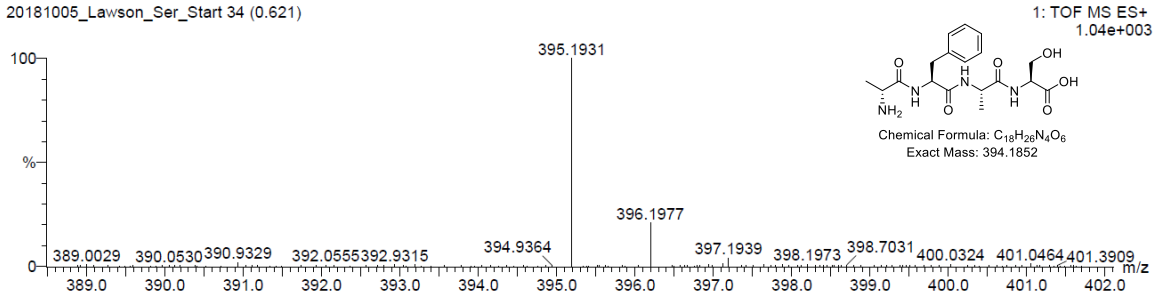



**ESI Figure 8.8**: LRMS of CM-**7**.

**Elemental Composition Report** **Page 1**

**Single Mass Analysis**
Tolerance = 10.0 PPM / DBE: min = 0.0, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 6

Monoisotopic Mass, Even Electron Ions
23 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 10-20 H: 20-30 N: 0-7 O: 2-6
20181005_Lawson_Ser_Start 34 (0.621)



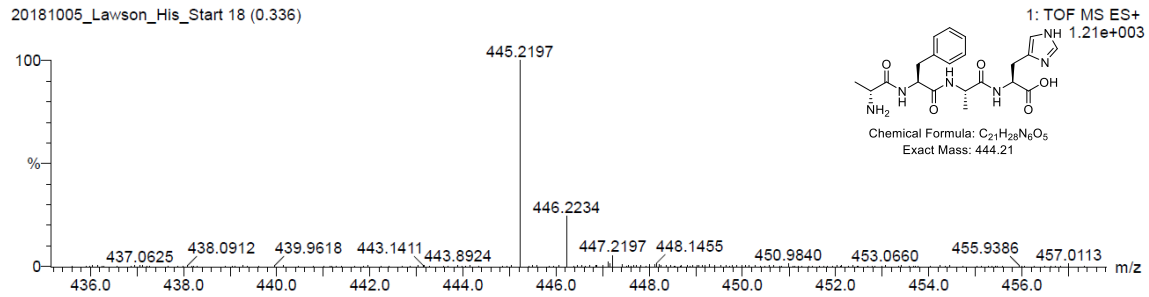

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Minimum: | | | | 0.0 | | | |
| Maximum: | | 5.0 | 10.0 | 50.0 | | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | i-FIT (Norm) | Formula |
| 395.1931 | 395.1931 | 0.0 | 0.0 | 7.5 | 165.2 | 0.0 | C18 H27 N4 O6 |

**ESI Figure 8.9**: HRMS of product **1**.

**Elemental Composition Report** **Page 1**

**Single Mass Analysis**
Tolerance = 10.0 PPM / DBE: min = 0.0, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 6

Monoisotopic Mass, Even Electron Ions
30 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 20-30 H: 20-30 N: 0-7 O: 2-6
20181005_Lawson_His_Start 18 (0.336)



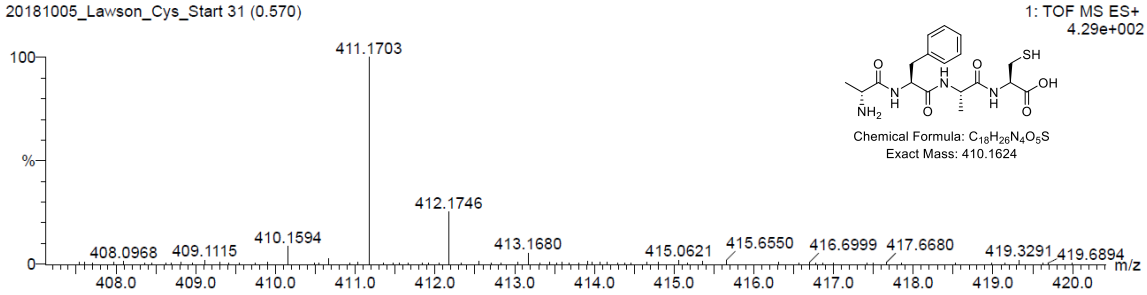

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Minimum: | | | | 0.0 | | | |
| Maximum: | | 5.0 | 10.0 | 50.0 | | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | i-FIT (Norm) | Formula |
| 445.2197 | 445.2199 | -0.2 | -0.4 | 10.5 | 184.8 | 0.0 | C21 H29 N6 O5 |
| | 445.2240 | -4.3 | -9.7 | 14.5 | 188.6 | 3.8 | C26 H29 N4 O3 |

**ESI Figure 8.10**: HRMS of Product **2**.

**Elemental Composition Report** **Page 1**

**Single Mass Analysis**
Tolerance = 10.0 PPM / DBE: min = 0.0, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 6

Monoisotopic Mass, Even Electron Ions
54 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 10-20 H: 20-30 N: 0-5 O: 2-6 S: 0-2
20181005_Lawson_Cys_Start 31 (0.570)







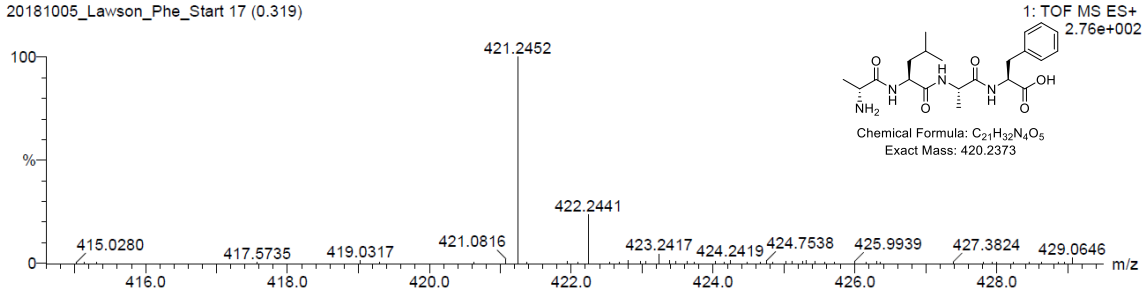
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Minimum: | | | | 0.0 | | | |
| Maximum: | | 5.0 | 10.0 | 50.0 | | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | i-FIT (Norm) | Formula |
| 411.1703 | 411.1702 | 0.1 | 0.2 | 7.5 | 85.0 | 0.0 | C18 H27 N4 O5 S |

**ESI Figure 8.11**: HRMS of Product **3**.

**Elemental Composition Report** **Page 1**

**Single Mass Analysis**
Tolerance = 10.0 PPM / DBE: min = 0.0, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 6

Monoisotopic Mass, Even Electron Ions
24 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 20-30 H: 30-40 N: 0-5 O: 2-6
20181005_Lawson_Phe_Start 17 (0.319)



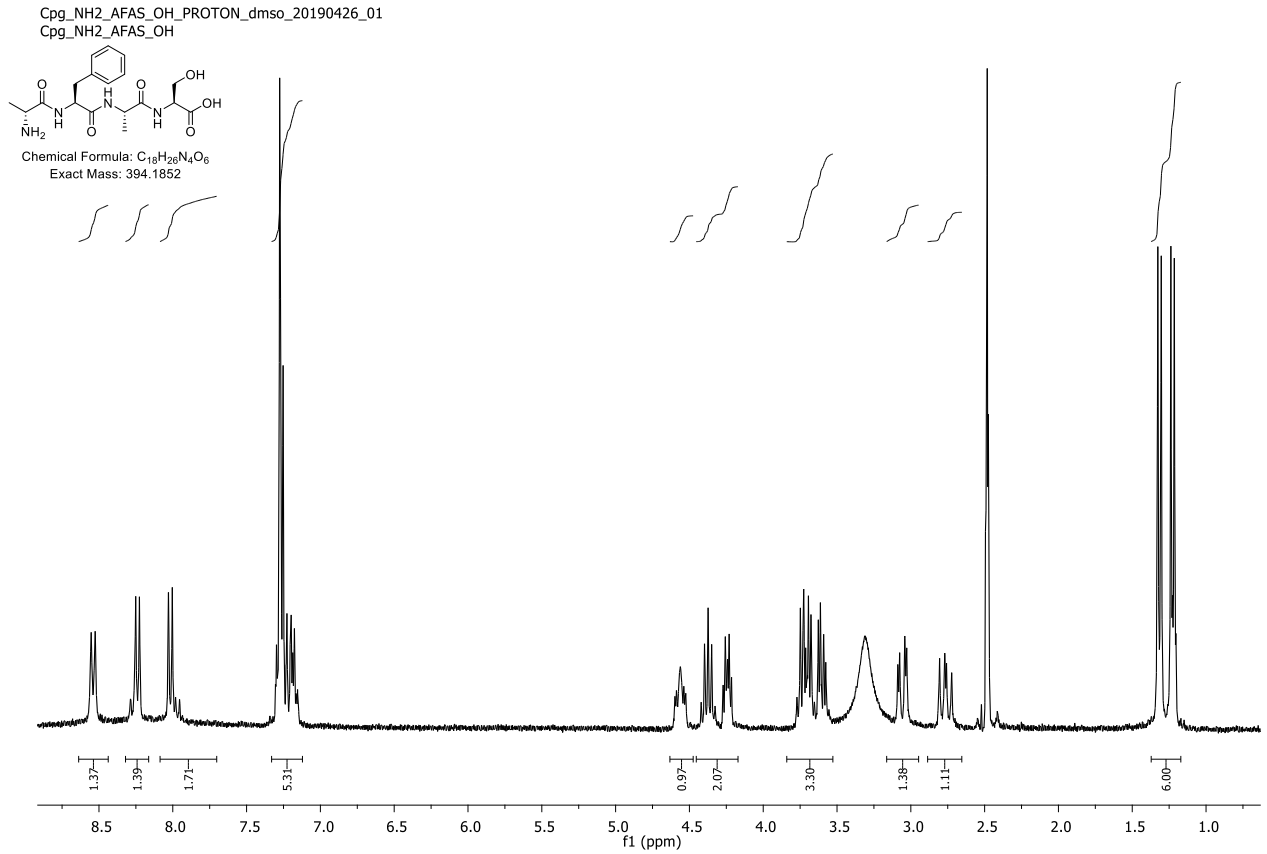




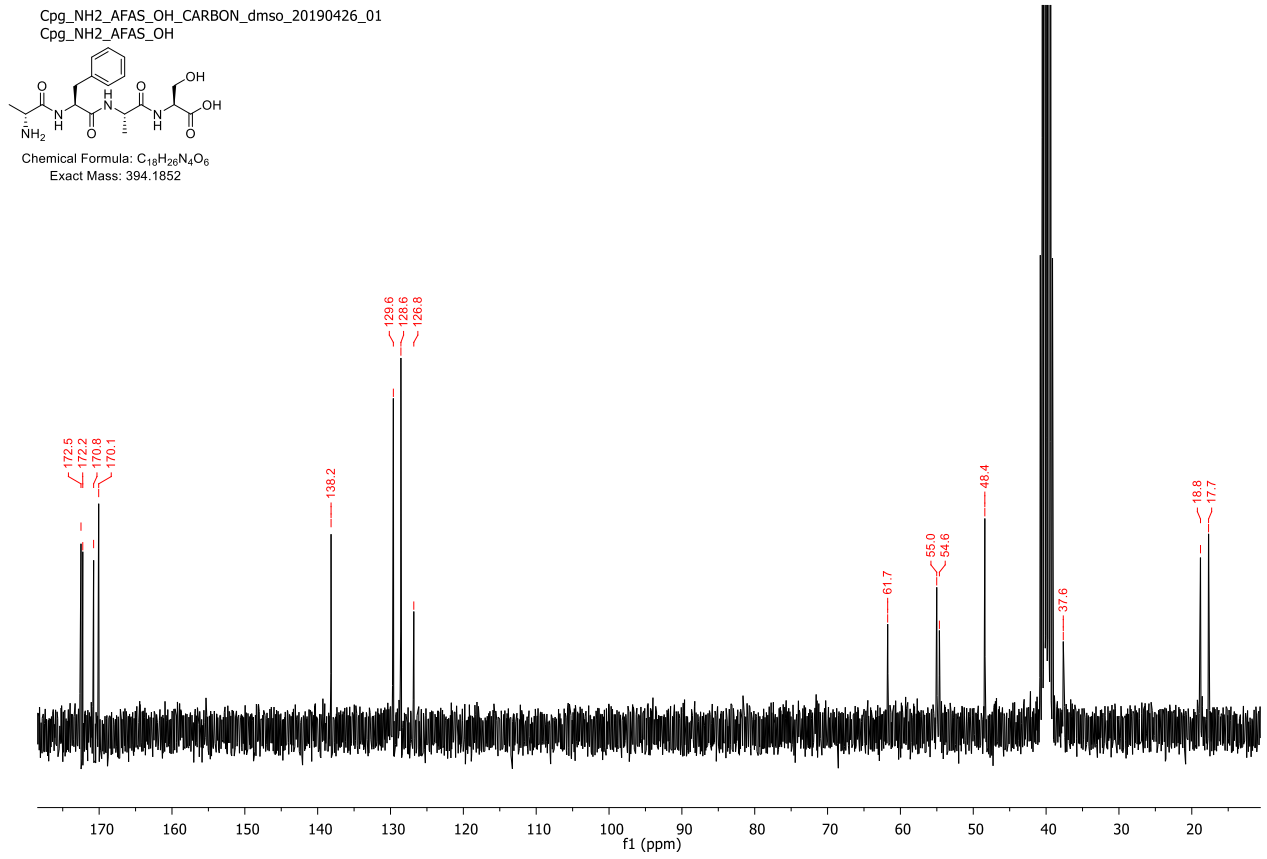
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Minimum: | | | | 0.0 | | | |
| Maximum: | | 5.0 | 10.0 | 50.0 | | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | i-FIT (Norm) | Formula |
| 421.2452 | 421.2451 | 0.1 | 0.2 | 7.5 | 60.3 | 0.1 | C21 H33 N4 O5 |
| | 421.2491 | -3.9 | -9.3 | 11.5 | 62.8 | 2.5 | C26 H33 N2 O3 |

**ESI Figure 8.12**: HRMS of Product **7**.

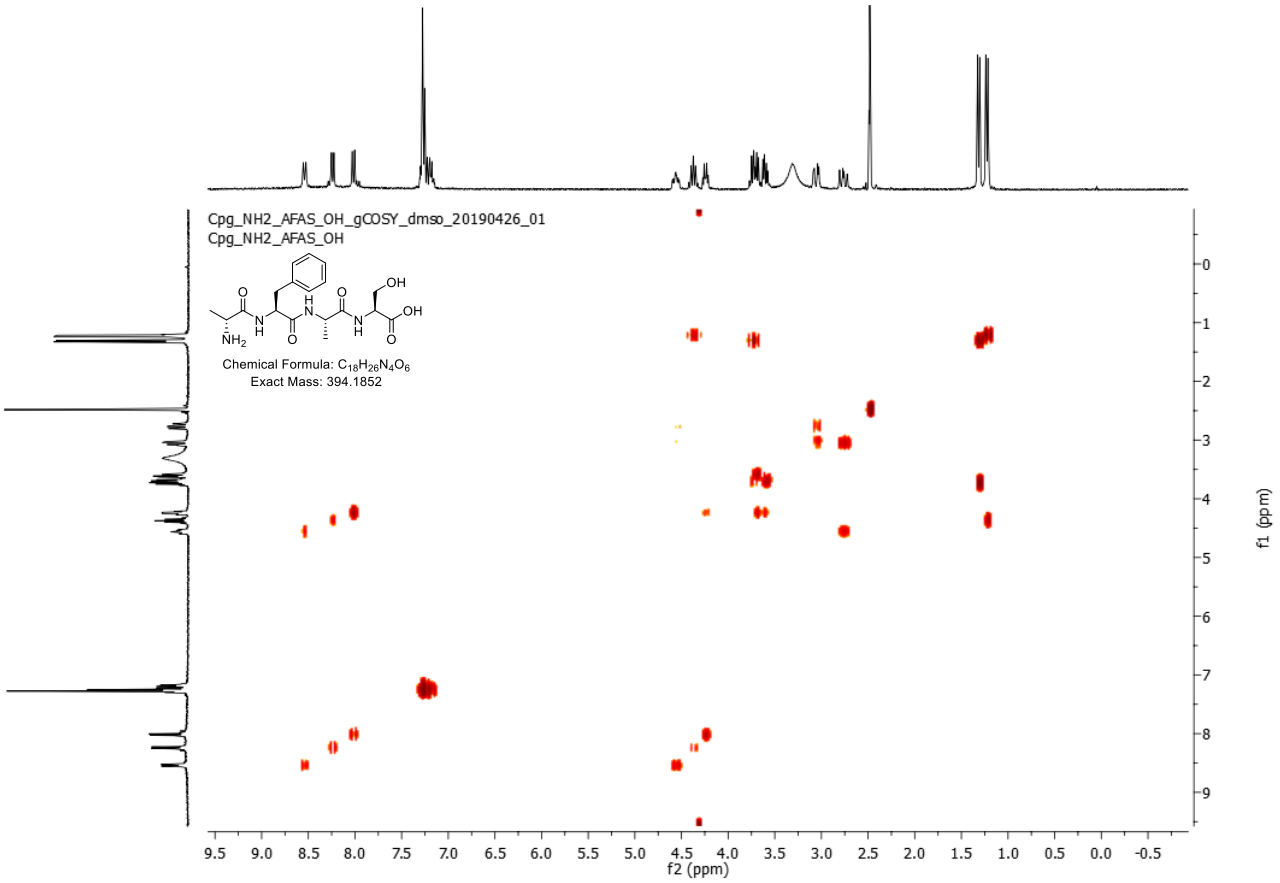
# 9. NMR Spectra of peptide sequences



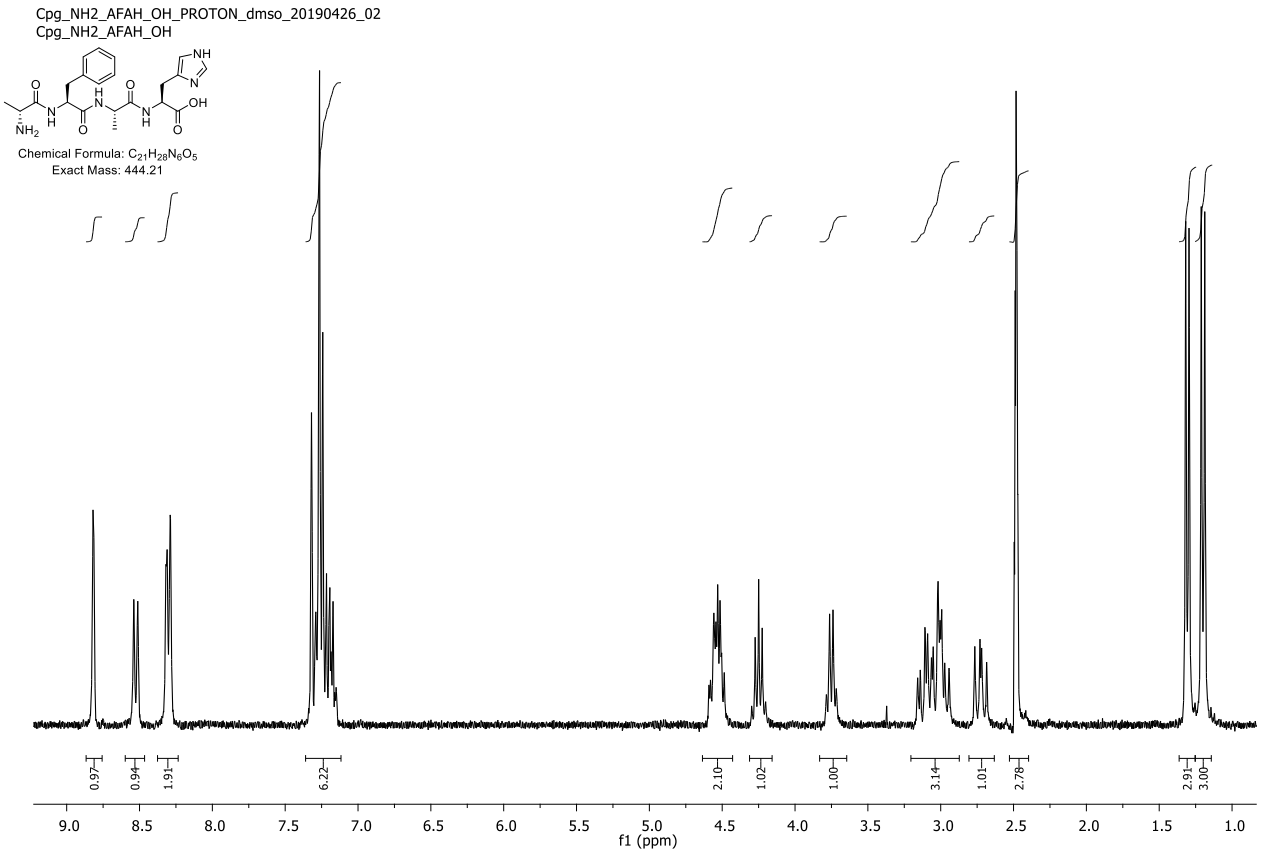

**ESI Figure 9.1**: $^{1}$H NMR of product **1**.



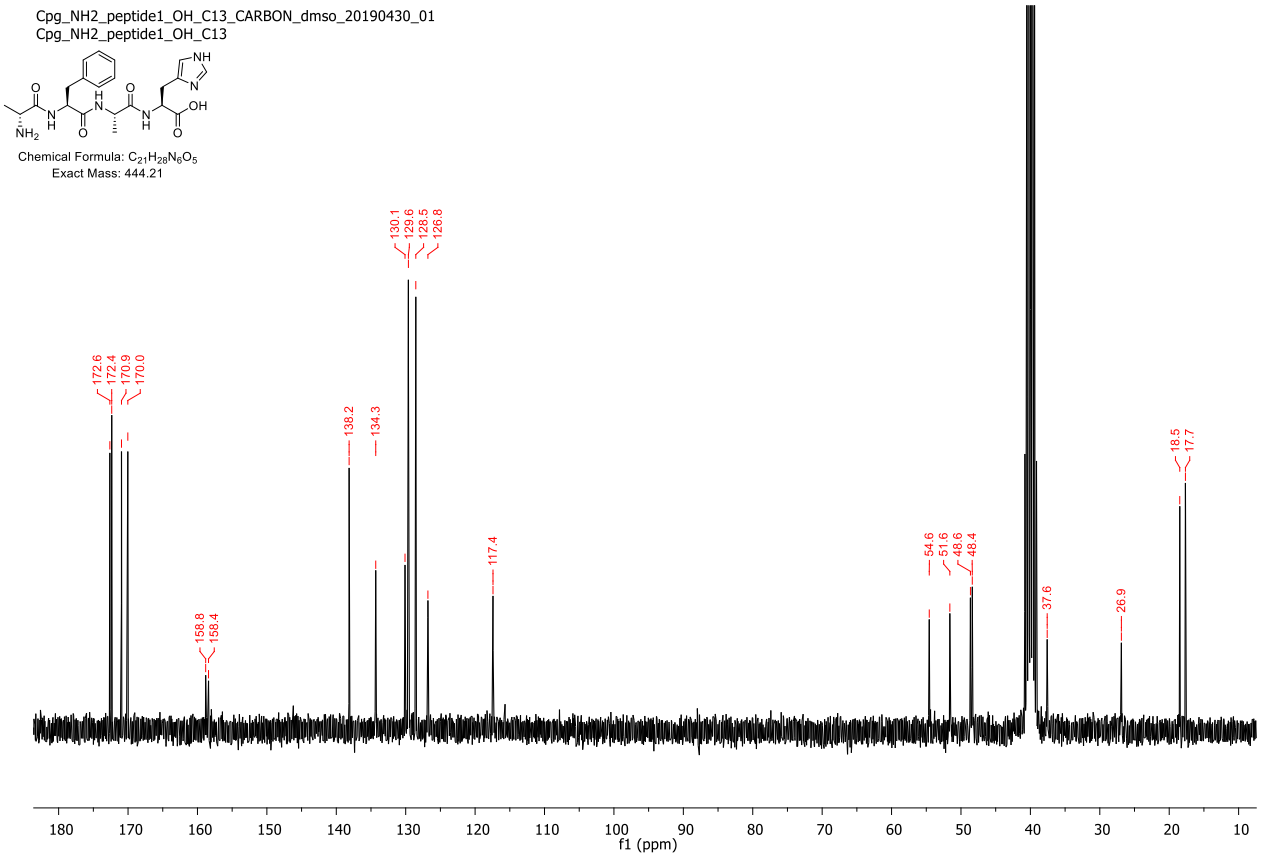

**ESI Figure 9.2**: $^{13}$C NMR of product **1**.



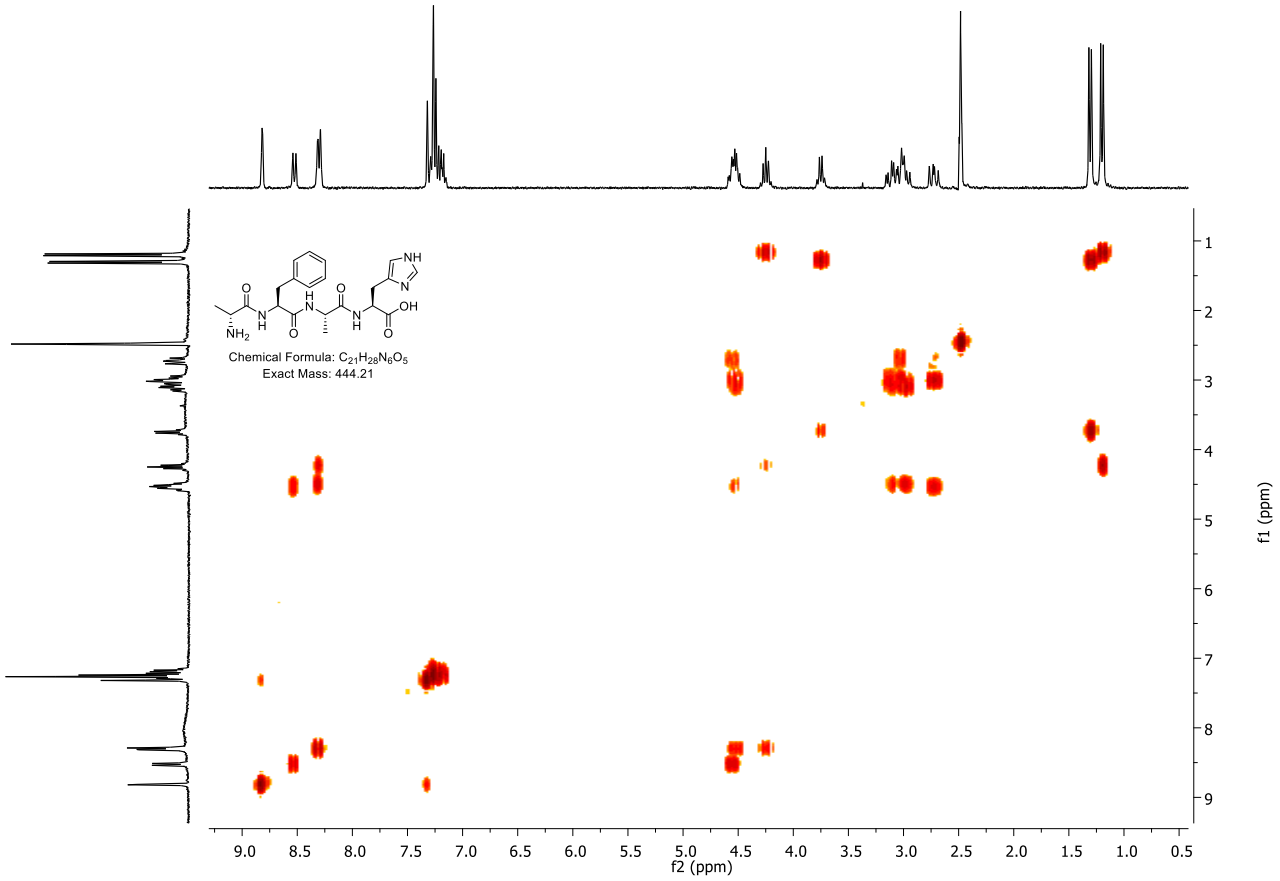

**ESI Figure 9.3**: 2D Cosy of product **1**.



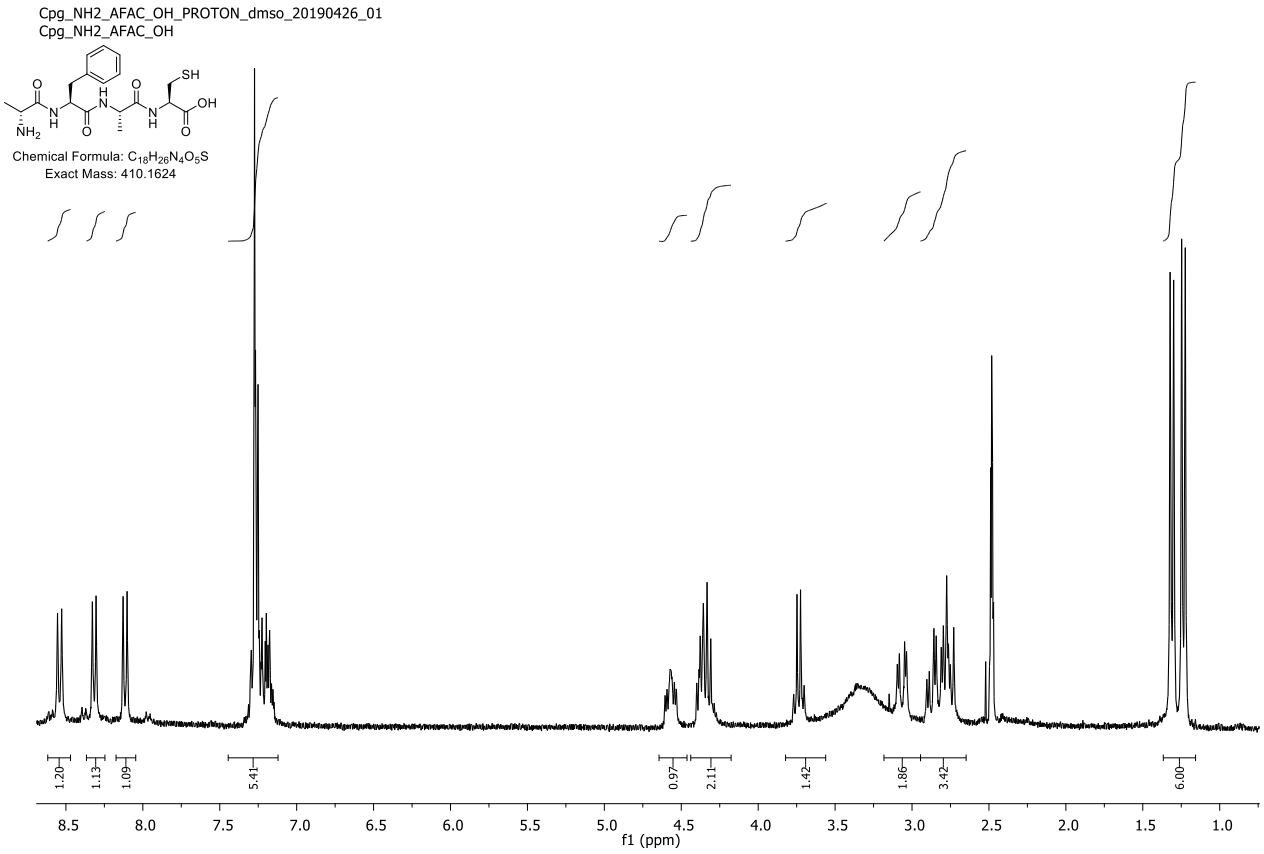

**ESI Figure 9.4**: $^{1}$H NMR of product **2**.



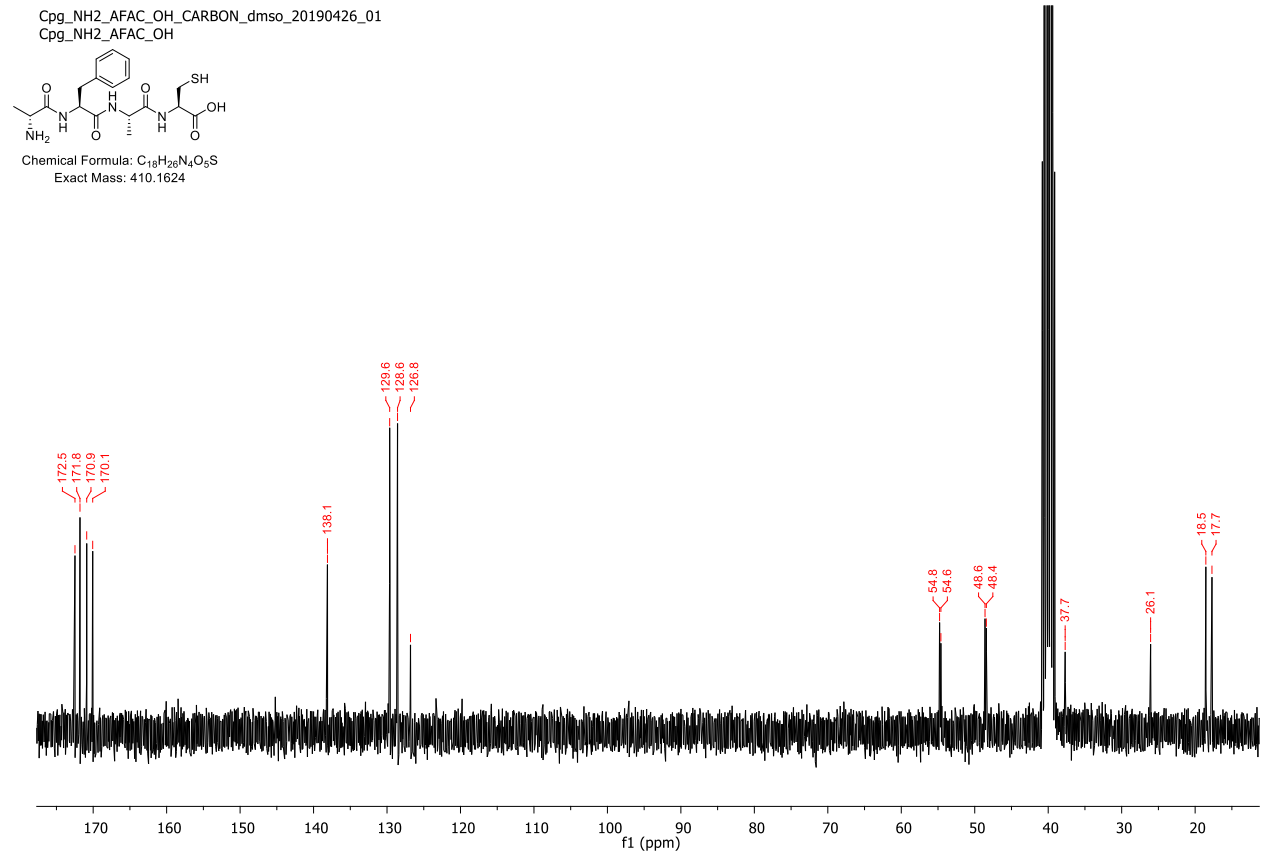

**ESI Figure 9.5**: $^{13}$C NMR of product **2**.



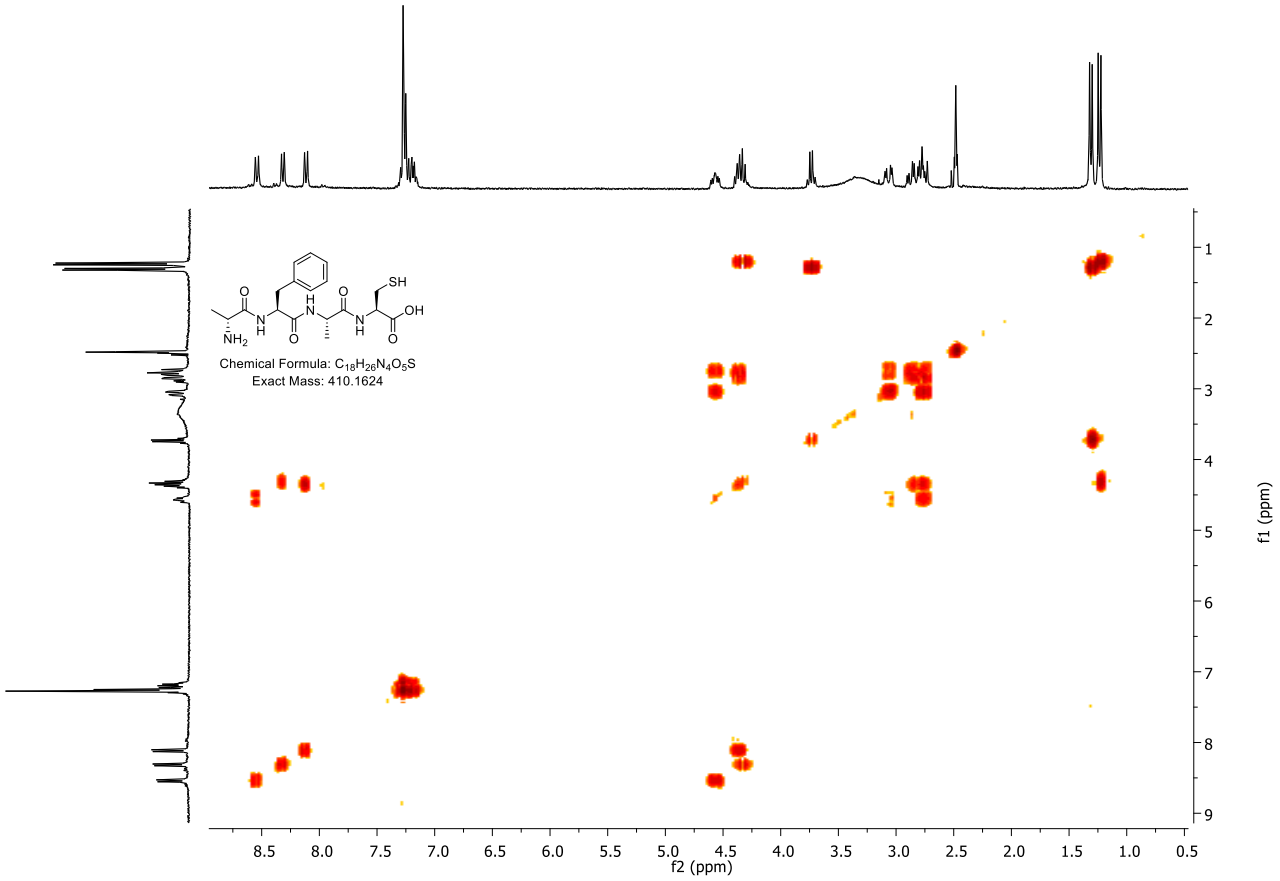

**ESI Figure 9.6**: 2D Cosy of product **2**.



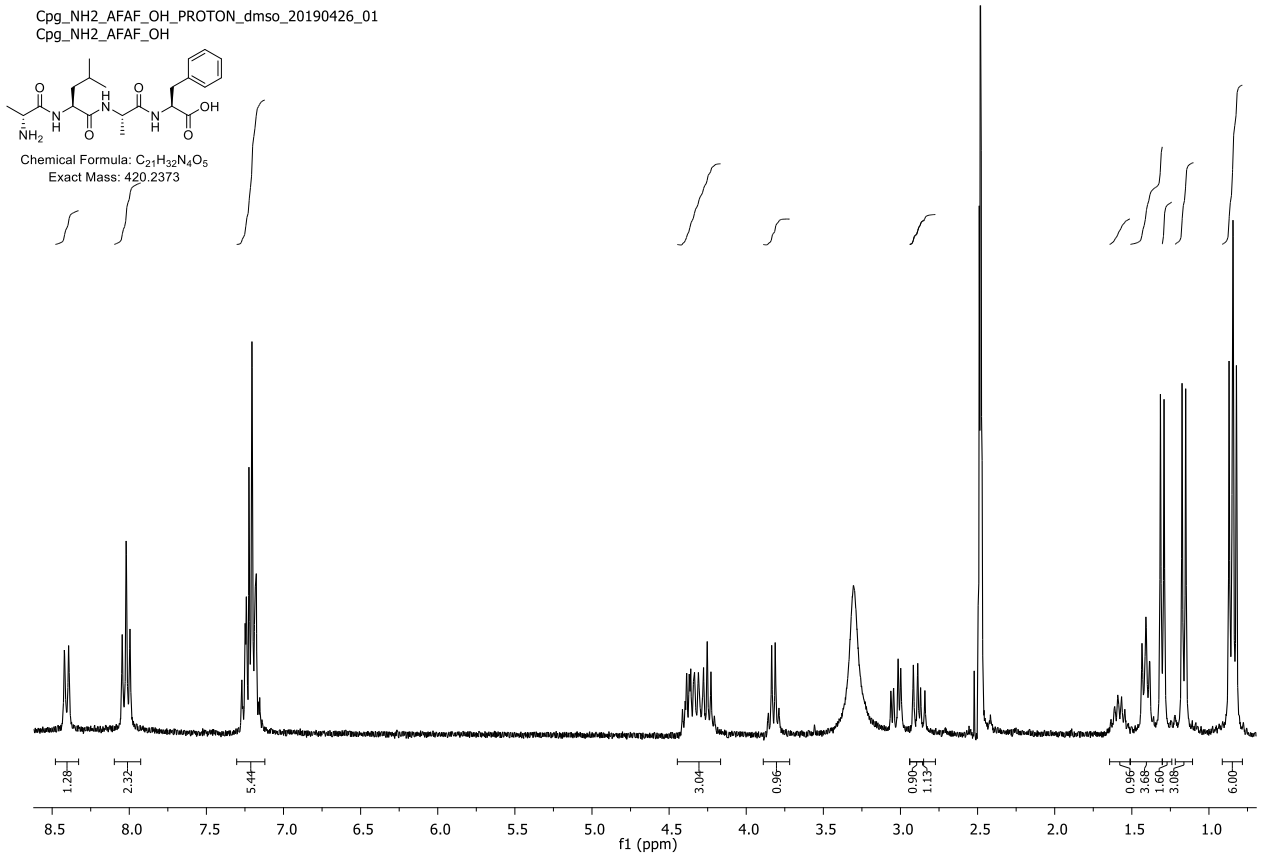

**ESI Figure 9.7**: $^{1}$H NMR of product **3**.



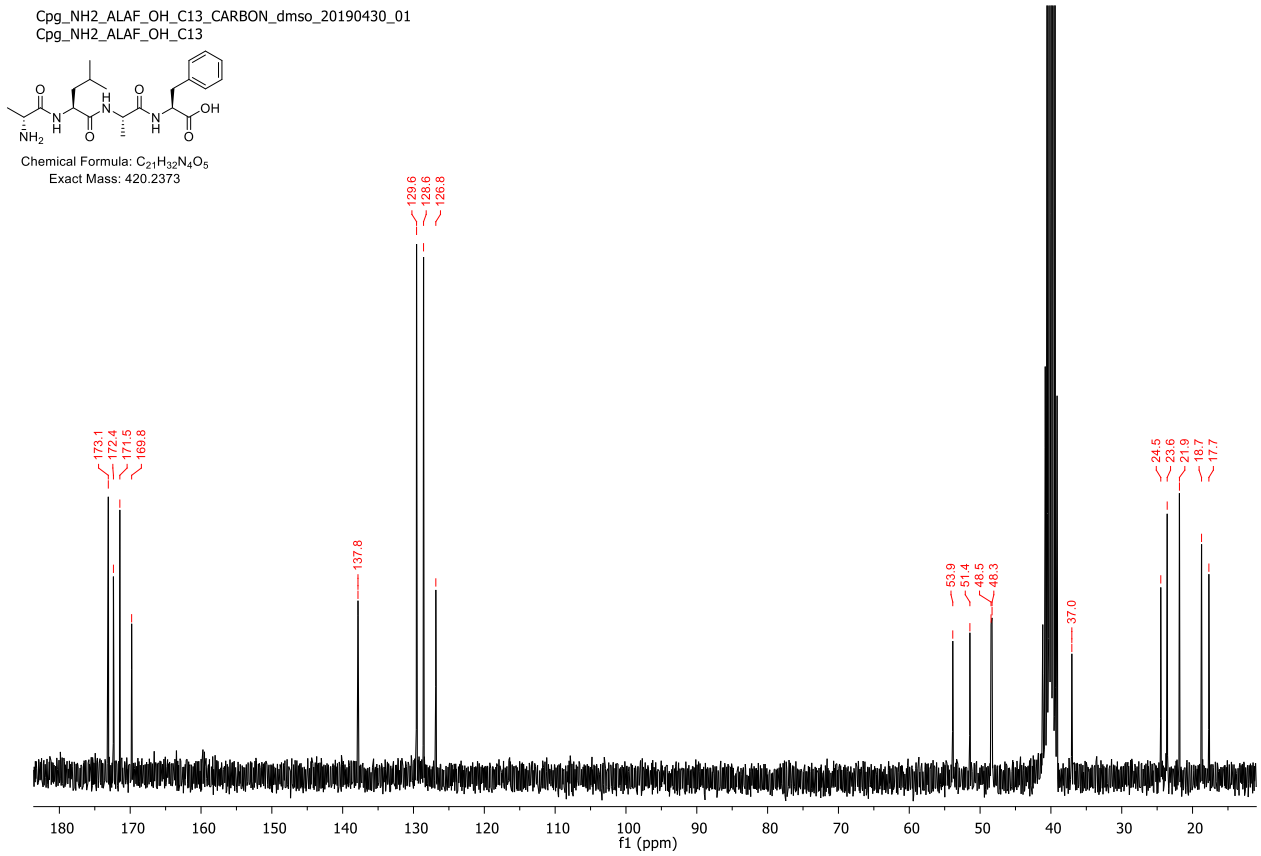

**ESI Figure 9.8**: $^{13}$C NMR of product **3**.



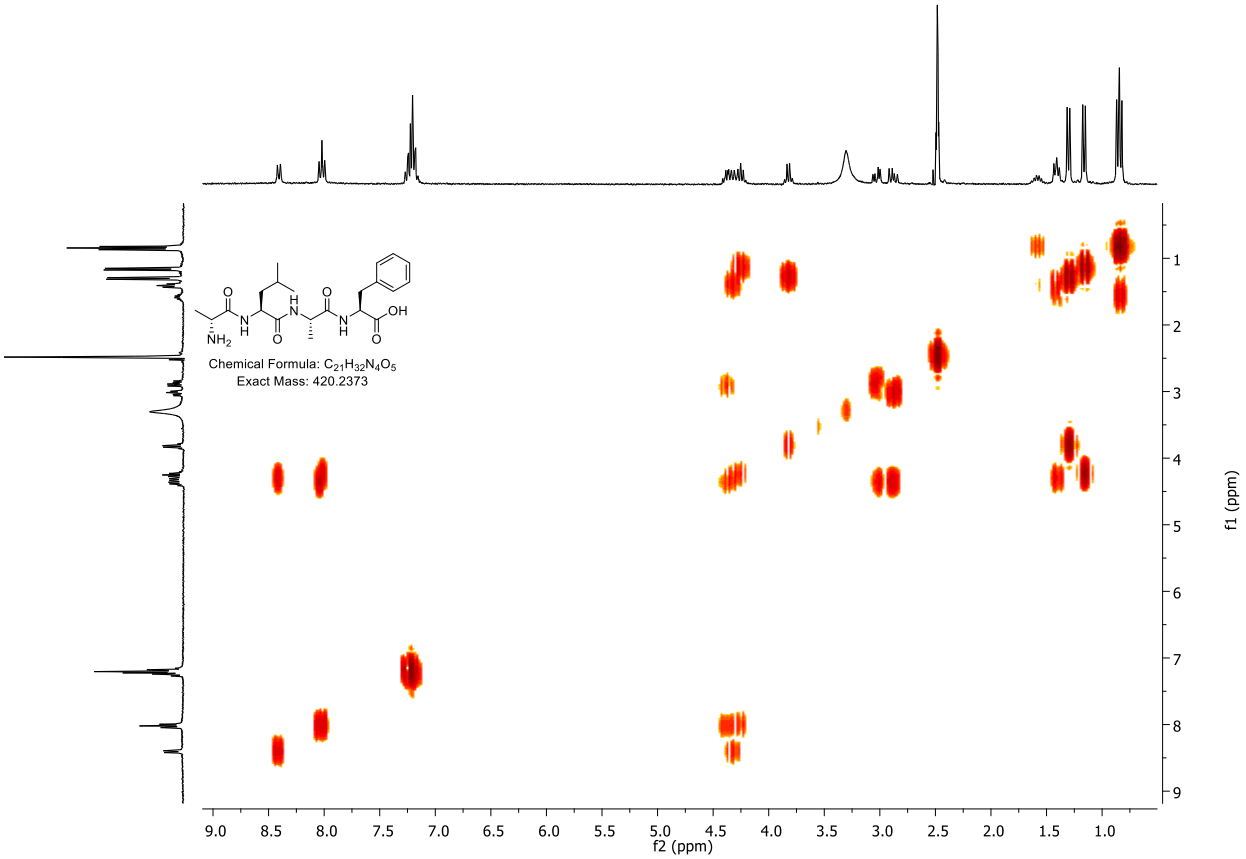

**ESI Figure 9.9**: 2D Cosy of product **3**




**ESI Figure 9.10**: $^{1}$H NMR of product **7**.




**ESI Figure 9.11**: $^{13}$C NMR of product **7**.

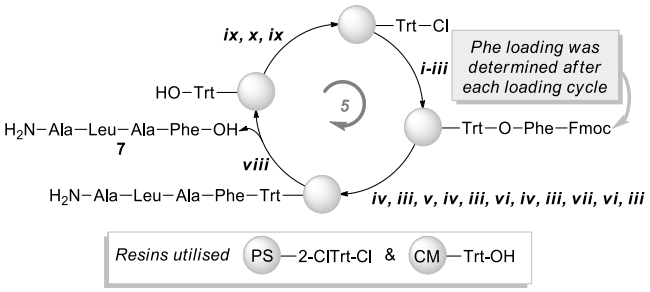



**ESI Figure 9.12**: 2D Cosy of product **7**.

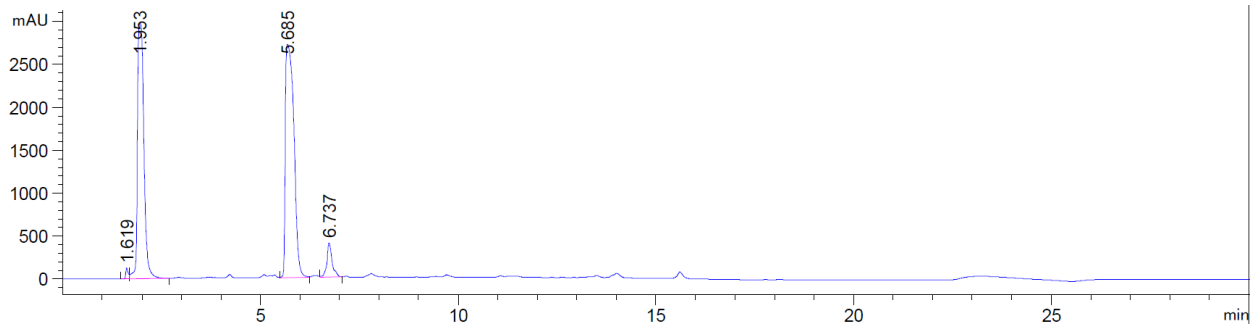
# 10. Recycled resin chromatographs

**ESI Table 10.1**: Loading percentages of the loaded, recycled and reloaded 2-CltrtCl resin using the general procedure for total flow synthesis of sequence **7**. The same resin was used subsequently for each sequence after previous sequence construction and cleavage (as shown in scheme 10.1).

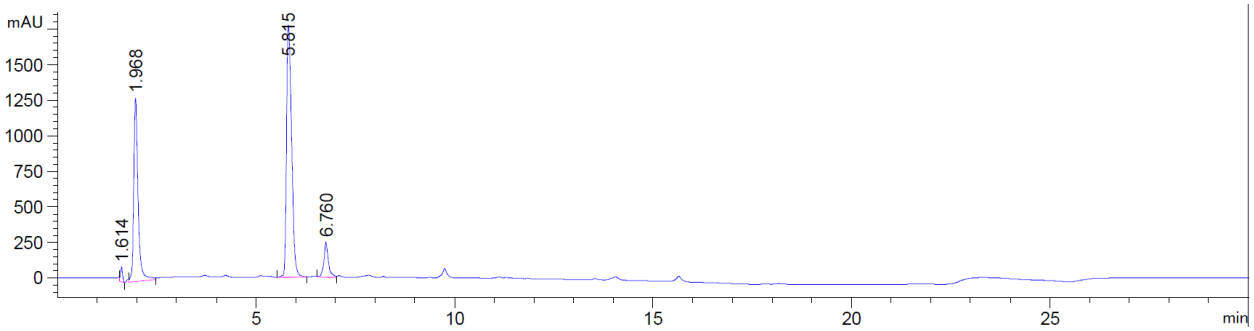
| | % Loading | | | | |
|---|---|---|---|---|---|
| Solid Support | Cycle 1 | Cycle 2 | Cycle 3 | Cycle 4 | Cycle 5 |
| Polystyrene | 97 (± 1) | 92 (± 1) | 91 (± 3) | 90 (± 2) | 88 (± 3) |
| Chem Matrix | 96 (± 3) | 94 (± 2) | 93 (± 2) | 92 (± 3) | 90 (± 4) |



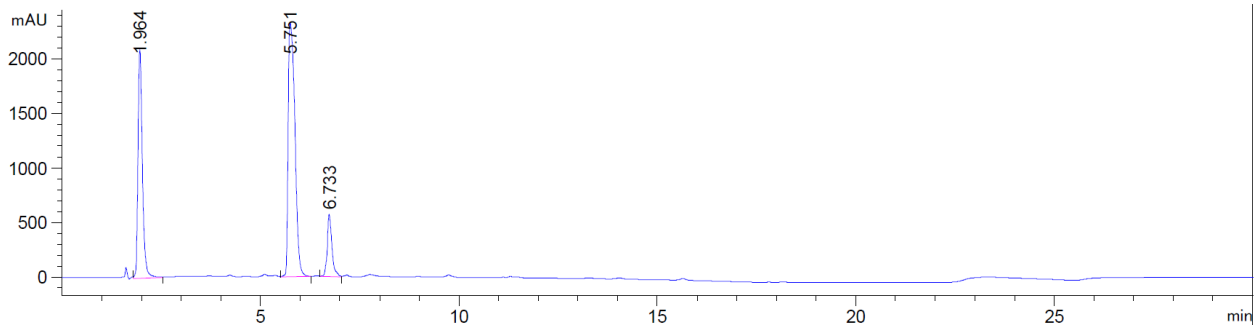

**ESI Scheme 10.1.** Continuous flow protocol to load, assemble, cleave, and recycle PS and CM trityl functionalised resin. *Reagents and condition*; ***i***) Fmoc-Phe-OH (4 eq.), DIPEA (8 eq.), DCM, 1.0 mL/min, 40 °C; ***ii***) MeOH (3 × 2mL, injections) 5 mL/min; ***iii***) DMF, 10 mL/min, 60 °C; ***iv***) 50% piperidine, DMF, 10 mL/min, 60 °C; ***v***) Fmoc-Ala-OH (0.3 M), HATU (0.3 M), DIPEA (0.6 M), DMF, 5 mL/min, 60 °C; ***vi***) Fmoc-Leu-OH (0.3 M), HATU (0.3 M), DIPEA (0.6 M), DMF, 5 mL/min, 60 °C; ***vii***) Fmoc-Ala-OH (0.3 M), HATU (0.3 M), DIPEA (0.6 M), DMF, 5 mL/min, 60 °C; ***viii***) DCM:TFA:$H_2O$ (18:1:1), 0.5 mL/min, 40 °C; ***ix***) DCM, 0.5 mL/min, 40 °C; ***x***) AcCl, 6.0 M, 40 °C, 0.5 mL/min.



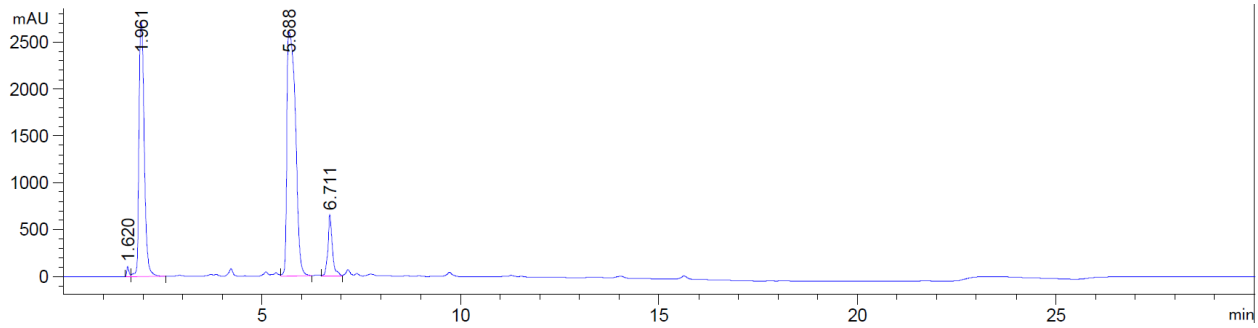

**ESI Figure 10.1**: HPLC Trace of cycle 1 of product **7** using PS-2-CltrtCl resin



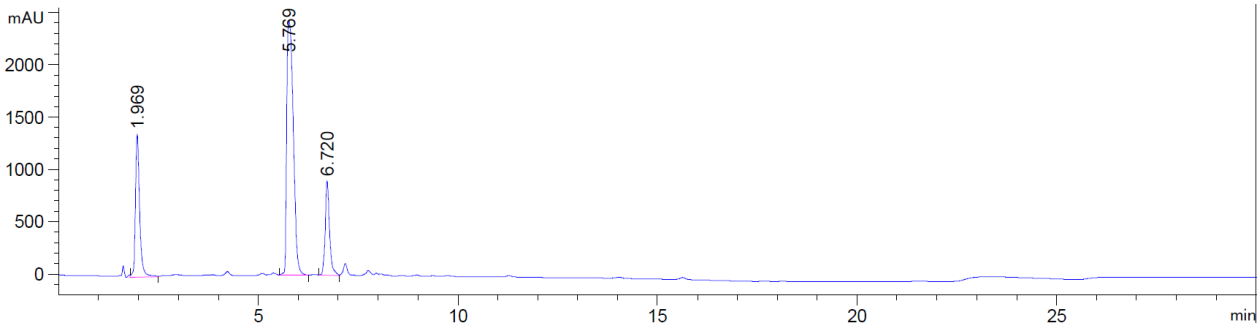

**ESI Figure 10.2**: HPLC Trace of cycle 2 of product **7** using PS-2-CltrtCl resin



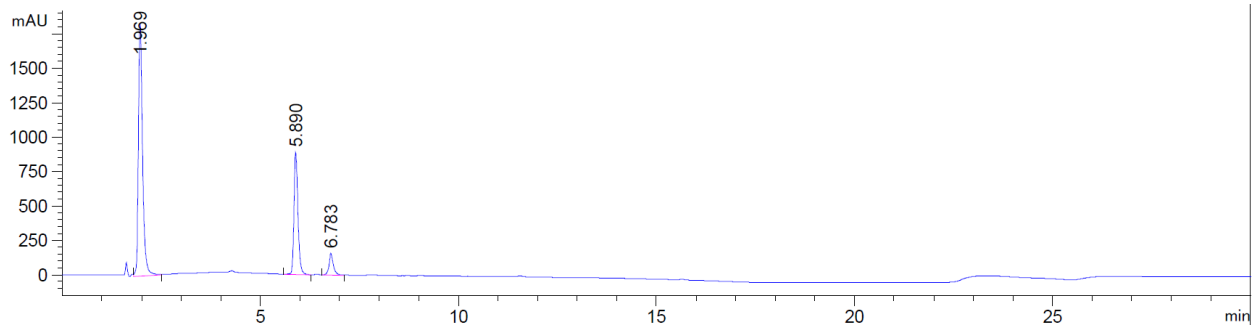

**ESI Figure 10.3**: HPLC Trace of cycle 3 of product **7** using PS-2-CltrtCl resin



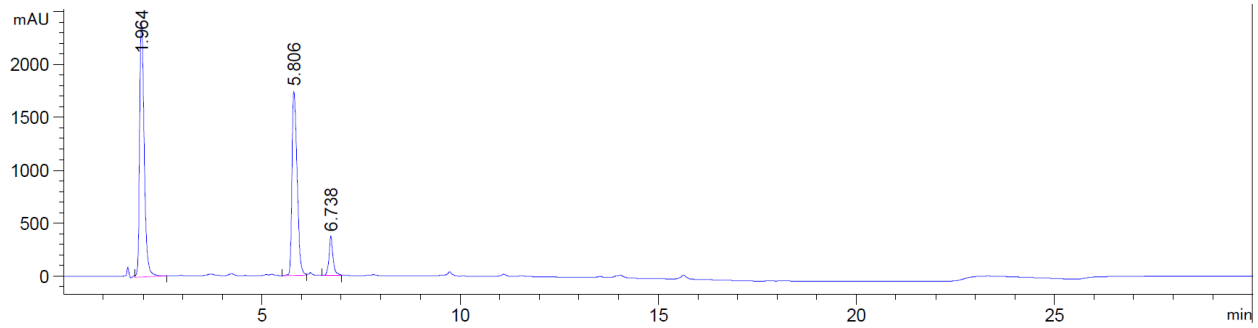

**ESI Figure 10.4**: HPLC Trace of cycle 4 of product **7** using PS-2-CltrtCl resin



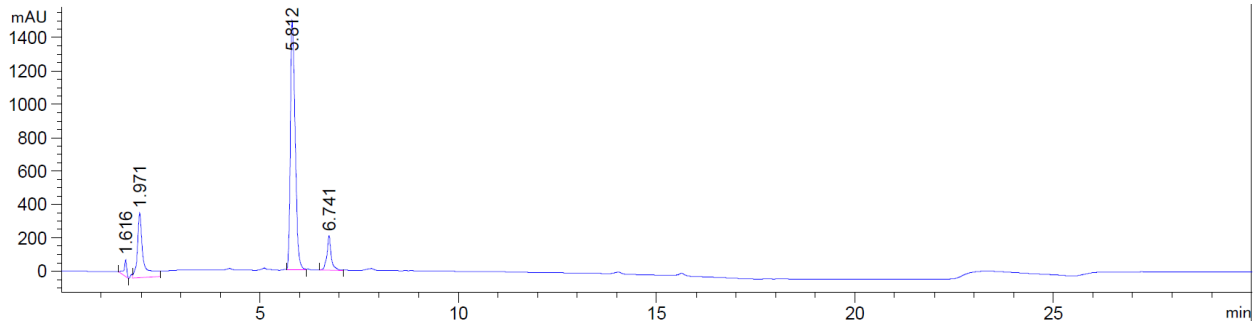

**ESI Figure 10.5**: HPLC Trace of cycle 5 of product **7** using PS-2-CltrtCl resin



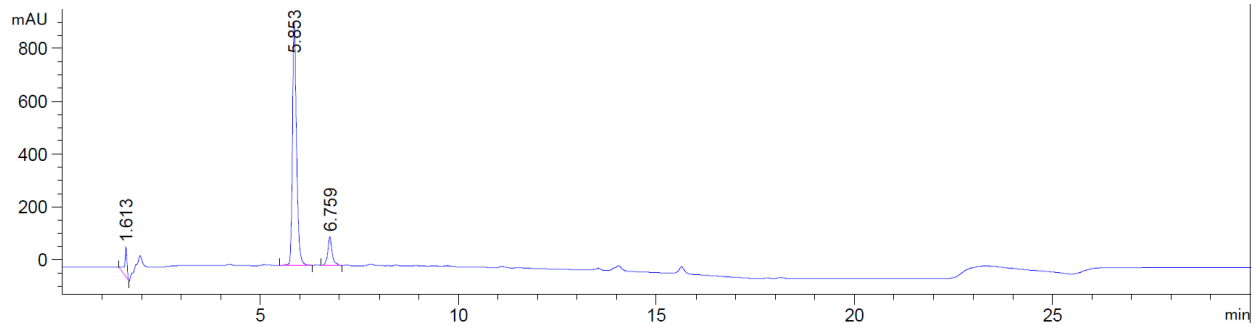

**ESI Figure 10.6**: HPLC Trace of cycle 1 of product **7** using CM 2-trtCl resin



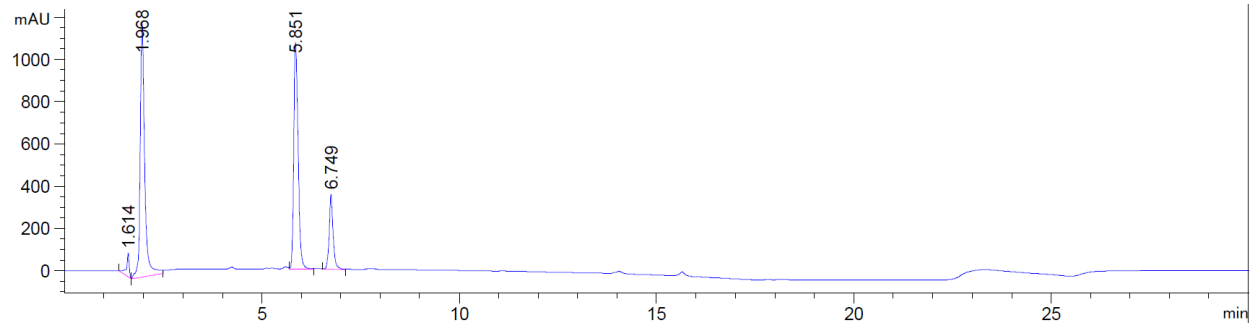

**ESI Figure 10.7**: HPLC Trace of cycle 2 of product **7** using CM 2-trtCl resin




**ESI Figure 10.8**: HPLC Trace of cycle 3 of product **7** using CM 2-trtCl resin




**ESI Figure 10.9**: HPLC Trace of cycle 4 of product **7** using CM 2-trtCl resin




**ESI Figure 10.10**: HPLC Trace of cycle 5 of product **7** using CM 2-trtCl resin