Teledyne ISCO Studentship Prep HPLC Report

TELEDYNE ISCO
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Chromatography Application Note AN128

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Abstract

Segments of the human leptin peptides have shown promising results in the treatment of Alzheimer's disease. Various peptides have been synthesized and have shown good pharmacokinetic viability in bio-assay studies. The purification of these peptides was of utmost importance for the experiment's success and, ergo, the ACCQ*Prep*® equipment from Teledyne ISCO was used. Modified peptides were synthesized using the CEM Liberty Blue synthesizer and then cleaved from the amino acid resin. Following solvent removal using the Genevac evaporator, the peptide was then purified on the ACCQ*Prep* system. Characterization, purity, and yield calculations were later conducted. Using solvents of 0.05% TFA in water and 0.05% TFA in acetonitrile in the ACCQ*Prep* system, the final peptide purities obtained were incredibly successful (see Table 1). On most accounts, the chromatogram obtained was clear and displayed exactly the pure peptide desired. However, on some occasions, the yields of the obtained pure peptides were less than the expected literature values. The two main theories for these unexpected results are as follows. At times, certain peptides did not crash out as expected during the experimental procedure, and so a different procedure was created for those cases. Secondly, the method used on the ACCQ*Prep* system produced some chromatograms with multiple significant peaks, rather than one clear peak. The desired peptide may have been found in some of the other peaks too, which may have increased the overall yield. Overall, the experiment was successful in showing that the ACCQ*Prep* system provided by Teledyne ISCO produced high quality pure peptides that would later be used in bio-assay studies for the treatment of Alzheimer's disease. With a few modifications to the experimental method of peptide synthesis as well as modifying the solvents used for the ACCQ*Prep* system, pure peptides can easily and successfully be made for future studies.

Experimental

All reagent positions on the Liberty Blue (LB) microwave peptide synthesizer were back-flushed and back-purged before the reaction was started. The relevant amino acids were dissolved in respective amounts of DMF before being added to the tubes on the LB synthesizer in the correct positions. The resin was placed inside the reaction vessel and the desired peptide sequence method was used. After reaction completion, the resin was washed with DCM, and a frit separated the resin from the DCM. A cleavage solution of 94% TFA, 2% TIPS, 2% EDT and 2% water (5 mL) was added to the peptide and centrifuged at 1000 rpm for 2 hours at room temperature. The solution was then filtered using a frit into ice-cold diethyl ether (15 mL), where it precipitated out to form a white solid.[1] The peptide was placed in the refrigerator overnight. The peptide was then centrifuged for 20 min at 4000 rpm to separate the peptide from the diethyl ether. The diethyl ether was decanted, and any remaining diethyl ether was removed under N_2. The peptide was then placed in a freezer overnight.

A 50/50 solution of H_2O and ACN was added to the peptide until it dissolved. This solution was then transferred to a test tube before being added to the prep HPLC system. This separated impurities from the peptide using a column. A sample of each test tube containing the peptide was added to HPLC vials and added to the analytical HPLC system. The purities of the fractions were obtained, and pure fractions were combined and freeze dried. After drying, the yield, final purity, NMR spectrum, and mass spectrum were obtained.

Note: This experimental method was carried out for every peptide sequence made. Changes were made to the method for the LB synthesizer depending on the identity of the sequence used.

1 If the peptide did not crash out after this step, additional steps were undertaken – detailed in Appendix 1.

Generalized Reaction Scheme

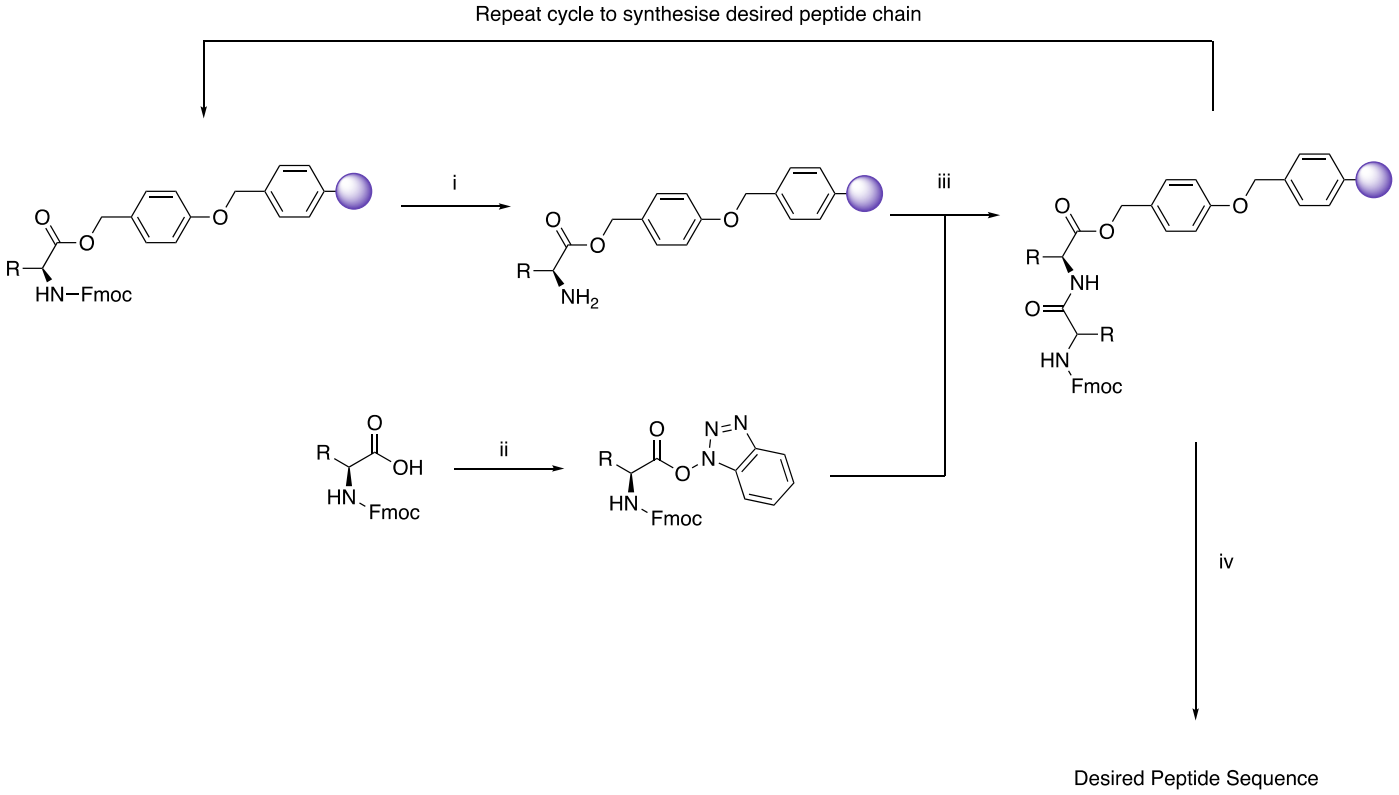

Figure 1: Reaction Scheme for Solid-phase peptide synthesis. i) piperidine/DMF 20:80, µW, 90 °C; ii) DIC, DMF; iii) Oxyma pure, DMF; iv)) piperidine/DMF 20:80, µW, 90 °C

Reagents

Target: Control Hexapeptide (random) — AA1

Amino Acid	Mass / g	Volume DMF / mL
Fmoc-Met-OH	0.21	2.8
Fmoc-Lys(Boc)-OH	0.27	2.8
Fmoc-Asp(O^{t}Bu)-OH	0.24	2.8
Fmoc-Leu-OH	0.20	2.8
Fmoc-Ser(^{t}Bu)-OH	0.22	2.8
Trp-PS Resin	0.33	N/A

Table 1: Reagents Used for Peptide Synthesis

Target: Control Nonapeptide (random) — AA2

Amino Acid	Mass / g	Volume DMF / mL
Fmoc-Met-OH	0.21	2.8
Fmoc-Lys(Boc)-OH	0.27	2.8
Fmoc-Asp(O^{t}Bu)-OH	0.24	2.8
Fmoc-Leu-OH	0.39	5.5
Fmoc-Ser(^{t}Bu)-OH	0.22	2.8
Fmoc-Thr(^{t}Bu)-OH	0.23	2.8
Fmoc-Ile-OH	0.20	2.8
Trp-PS Resin	0.33	N/A

Table 2: Reagents Used for Peptide Synthesis

Target: Nonapeptide used for bio-assays — AA3

Amino Acid	Mass / g	Volume DMF / mL
Fmoc-Ala-OH	0.35	5.5
Fmoc-Gly-OH	0.66	11
Fmoc-Leu-OH	0.20	2.8
Fmoc-Trp(Boc)-OH	0.30	2.8
Leu-PS Resin	0.32	N/A

Table 3: Reagents Used for Peptide Synthesis

Target: Halogenated hexapeptide (7FW) — AA4

Amino Acid	Mass / g	Volume DMF / mL
Fmoc-Cys(Trt)-OH	0.65	5.5
Fmoc-His(Boc)-OH	0.53	5.5
Fmoc-Leu-OH	0.20	2.8
Fmoc-Pro-OH	0.19	2.8
Fmoc-7-F-Trp-OH	0.25	2.8
Ala-PS Resin	0.19	N/A

Table 4: Reagents Used for Peptide Synthesis

Target: Ala-substituted hexapeptide — AA5

Amino Acid	Mass / g	Volume DMF / mL
Fmoc-Ala-OH	0.18	2.8
Fmoc-His(Trt)-OH	0.35	2.8
Fmoc-Leu-OH	0.20	2.8
Fmoc-Pro-OH	0.19	2.8
Fmoc-Ser(^{t}Bu)-OH	0.22	2.8
Trp-PS Resin	0.33	N/A

Table 5: Reagents Used for Peptide Synthesis

Target: Halogenated hexapeptide (6FW) — AA6

Amino Acid	Mass / g	Volume DMF / mL
Fmoc-Cys(Trt)-OH	0.65	5.5
Fmoc-His(Boc)-OH	0.53	5.5
Fmoc-Leu-OH	0.20	2.8
Fmoc-Pro-OH	0.19	2.8
Fmoc-6-F-Trp-OH	0.25	2.8
Ala-PS Resin	0.19	N/A

Table 6: Reagents Used for Peptide Synthesis

Target: Ala-substituted hexapeptide — AA7

Amino Acid	Mass / g	Volume DMF / mL
Fmoc-Ala-OH	0.18	2.8
Fmoc-Cys(Trt)-OH	0.65	5.5
Fmoc-His(Boc)-OH	0.53	5.5
Fmoc-Leu-OH	0.20	2.8
Fmoc-Ser(^{t}Bu)-OH	0.22	2.8
Trp-PS Resin	0.33	N/A

Table 7: Reagents Used for Peptide Synthesis

Target: Ala-substituted hexapeptide — AA8

Amino Acid	Mass / g	Volume DMF / mL
Fmoc-Ala-OH	0.18	2.8
Fmoc-His(Trt)-OH	0.53	5.5
Fmoc-Leu-OH	0.20	2.8
Fmoc-Pro-OH	0.19	2.8
Fmoc-Trp(Boc)-OH	0.30	2.8
Ala-PS Resin	0.14	N/A

Table 8: Reagents Used for Peptide Synthesis

Results

Prep HPLC Method

Sample: AA8
Column: Prep HPLC
Flow Rate: 18.9 mL/min
Equilibriation Volume: 153.2 mL
Initial Waste: 0.0 mL
Solvent: A1 Waste 0.05% TFA
Solvent: B1 Acetonitrile 0.05% TFA

HP 150
Peak Tube Volume: 12 mL
Non-Peak Tube Volume: Max.
Loading Type:
Wavelength 1 (red): 230nm
Threshold: 0.02 AU
Wavelength 2 (purple): 254nm

Run Notes:
Prep HPLC Column: C18 20x150mm Dimensions: 20mm x 150 mm 5µm
Sample: 1.60 mL from tube 8

Duration	%B	Solvent A	Solvent B
0.8	5.0	A1 Water 0.05% T	B1 Acetonitrile 0.0
1.8	5.0	A1 Water 0.05% T	B1 Acetonitrile 0.0
5.5	20.0	A1 Water 0.05% T	B1 Acetonitrile 0.0
26.7	26.0	A1 Water 0.05% T	B1 Acetonitrile 0.0
9.4	35.0	A1 Water 0.05% T	B1 Acetonitrile 0.0
0.4	80.0	A1 Water 0.05% T	B1 Acetonitrile 0.0
1.7	80.0	A1 Water 0.05% T	B1 Acetonitrile 0.0
0.4	50.0	A1 Water 0.05% T	B1 Acetonitrile 0.0

Target: Hexapeptide

Yield: 65%
Purity: >99%

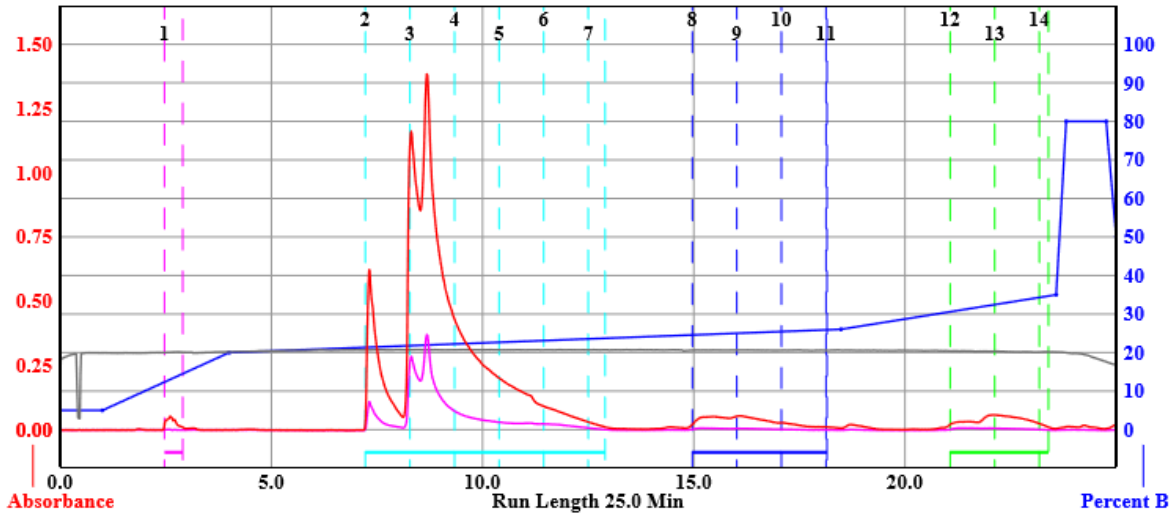

Figure 2: Prep HPLC Chromatogram

Target: Nonapeptide

Yield: No yield measured.
Purity: >99%

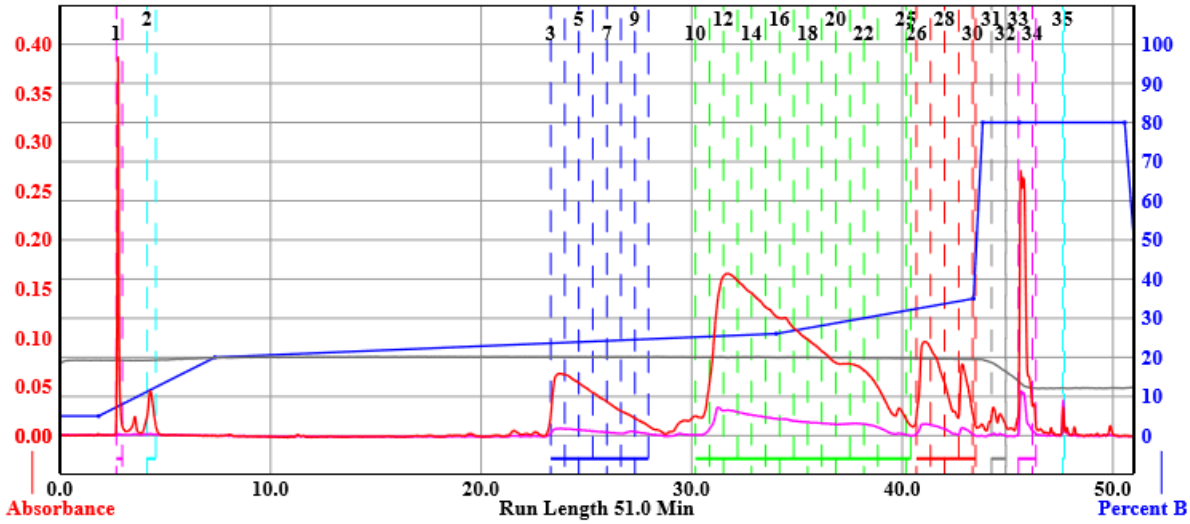

Figure 3: Prep HPLC Chromatogram

Target: Nonapeptide used for bio-assays

Yield: 8%
Purity: >99%

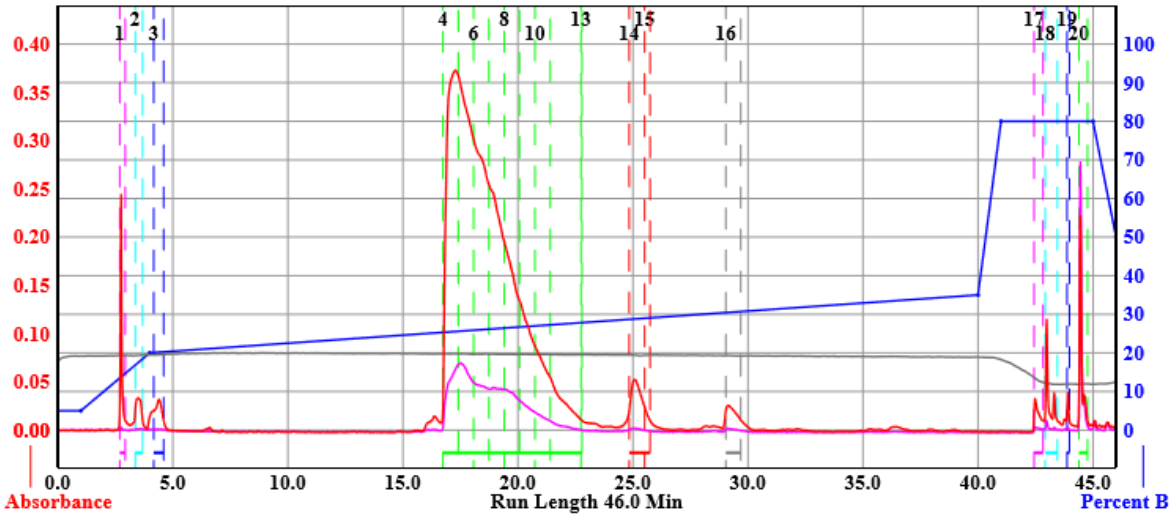

Figure 4: Prep HPLC Chromatogram

Target: Halogenated hexapeptide (7FW)

Yield: 78%
Purity: 98%

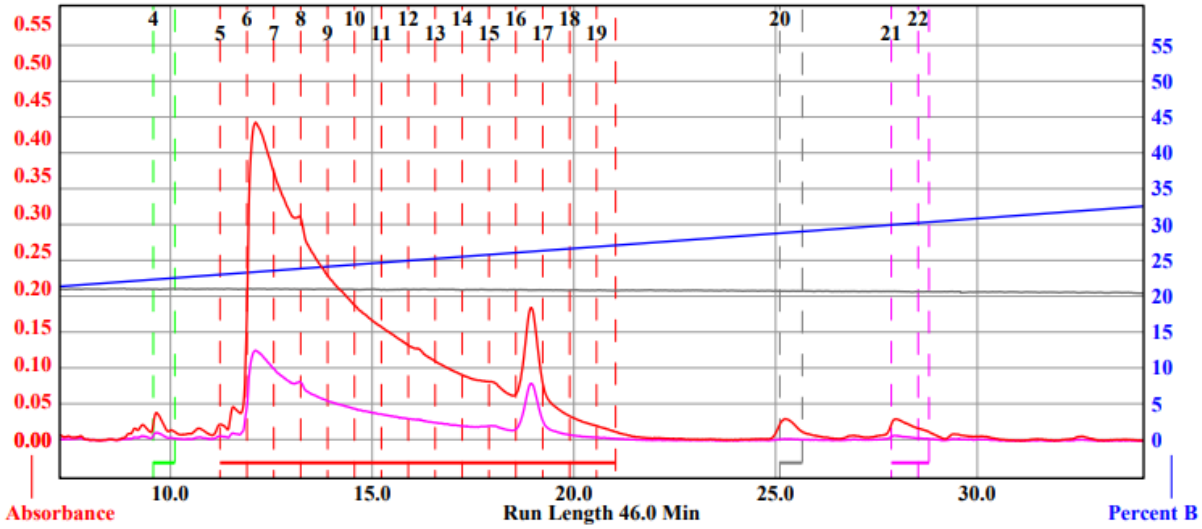

Figure 5: Prep HPLC Chromatogram

Target: Ala-substituted hexapeptide

Yield: 13%
Purity: >99%

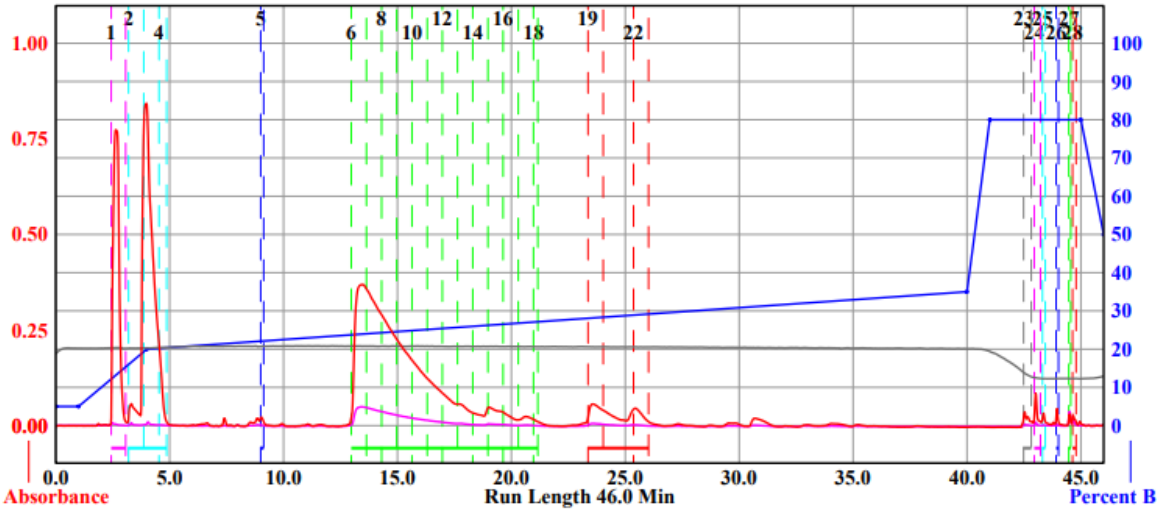

Figure 6: Prep HPLC Chromatogram

Target: Halogenated hexapeptide (6FW)

Yield: 10% (Fractions 6-12 were the desired peptide. Yield may be low due to the other significant peak.)
Purity: >99%

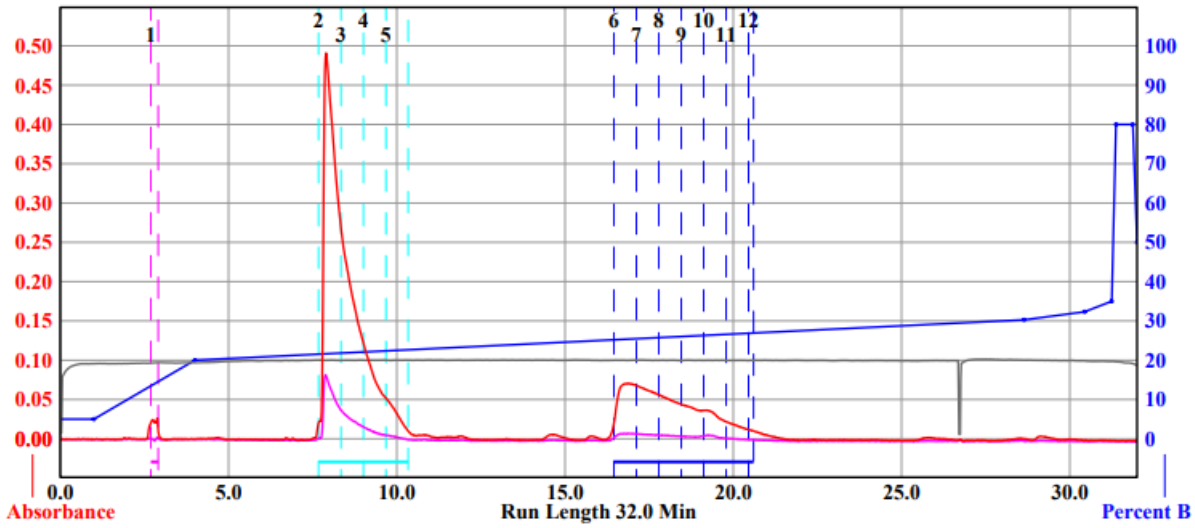

Figure 7: Prep HPLC Chromatogram

Target: Ala-substituted hexapeptide

Yield: 27% (Fractions 10 and 11 were impure and therefore not used.)
Purity: 83% Impure (Prep HPLC purification process to be repeated by research group.)

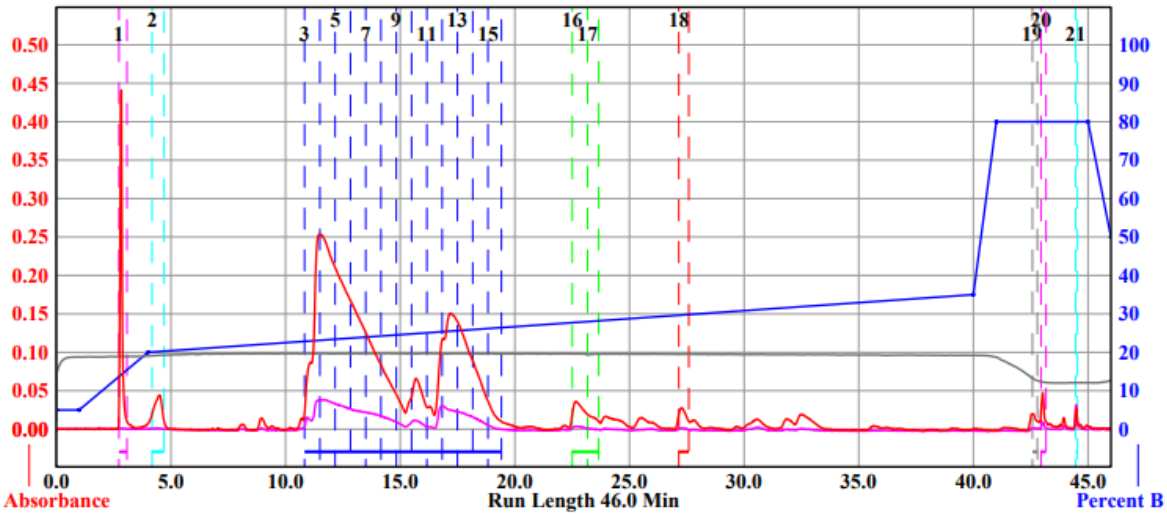

Figure 8: Prep HPLC Chromatogram

Target: Ala-substituted hexapeptide

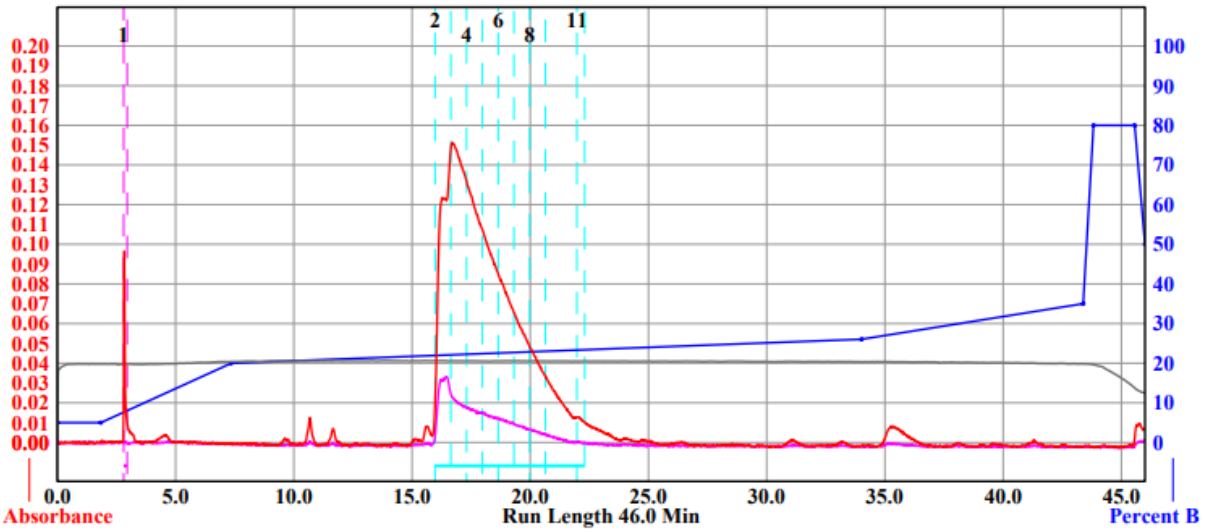

Figure 9: Prep HPLC Chromatogram

Target:

Yield: 55 %
Purity: > 99 %

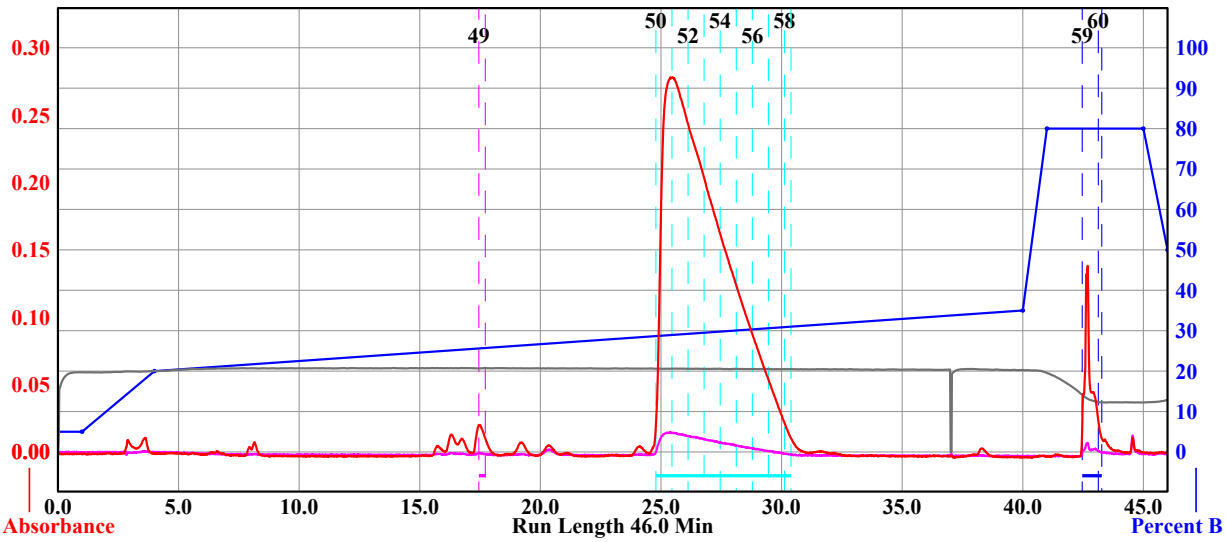

Figure 10: Prep HPLC Chromatogram, where 0.1% TFA was used for solvent

Conclusion

The purities of the different peptides synthesized were high. However, on some counts, their respective yields were much lower than expected. This may have been due to precipitation during the synthesis procedure that led to some product being lost throughout work-up procedures. Increasing the TFA concentration to 1% resulted in chromatograms displaying only one peak corresponding to the product, resulting in easier determination of the product peak. An ammonia buffer could also be used to produce cleaner chromatograms, and the PKa of the various peptides synthesized could also be calculated to assess exactly how to use the buffer most effectively.

Appendix

If no peptide formed, the remaining solvent was evaporated on the rotary evaporator. A solution of water (with 10% acetic acid), 10 mL was made and added to the round bottom flask. This solution was then extracted using a separating funnel and washed with 10mL DCM three times. The aqueous layer was kept and was placed on the rotary evaporator again to remove the solvent. The steps were followed as in “Experimental” after this.

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