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# Manual and Automated Solid Phase Synthesis of Peptides for Breast Cancer Cell Targeting

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# Manual and Automated Solid Phase Synthesis of Peptides for Breast Cancer Cell Targeting

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## Abstract

Four peptides were synthesized using solid phase peptide synthesis. The two target peptides synthesized were based off peptide 18-4 and its negative analog. Each were synthesized once manually, and once using an automatic peptide synthesizer. Peptide 18-4 has a high affinity for breast cancer cells, allowing it to be used to detect circulating tumor cells (CTCs) in blood (Kaur et. al, 2015). Manual solid phase peptide synthesis is performed by anchoring the first amino acid to Wang resin and coupling each Fmoc protected amino acid individually until the target sequence is achieved. To synthesize the peptides automatically, the automated Tribute Synthesizer was used. To analyze the success of the peptide synthesis, mass spectrometry was used to determine if the synthesized peptide has the correct molecular mass. Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) was used to analyze the relative amount of the correct peptide in the synthesized sample. Due to greater potential for human error, the two peptides synthesized manually were missing one or two amino acids. Using the Tribute synthesizer, peptide 18-4 and its negative analog were successfully synthesized. Automated peptide synthesis was found to be faster and more successful than manual peptide synthesis.

## Introduction

The goal of this research was to successfully synthesize two peptides, peptide 18-4 ($NH_2$ - WxEAAYQrFLC - COOH) and its negative analog ($NH_2$ - WxEAAYQrFLC - COOH ). The peptides synthesized are intended for use in real-time detection of breast cancer cells. Peptide 18-4 has high affinity for breast cancer cells, and can thus be used with a microcantilever array system to selectively detect circulating tumor cells in blood. This peptide was discovered by Kaur and her research team at the University of Alberta (Kaur et. al, 2015).

To synthesize a peptide using solid-phase synthesis, the C terminal amino acid is bound to resin and then each successive amino acid is coupled to the N-terminus. In solid phase synthesis, the basic procedure is as follows:

1. Coupling of the first amino acid to resin
2. Deprotection of the first amino acid
3. Activation and coupling of next amino acid
4. Elongation of the peptide (deprotection, activation, coupling)
5. Cleavage of the peptide from the resin

To analyze whether or not the correct amino acids are present in a peptide during or after synthesis, mass spectrometry is used. The mass ($[M+H]^{+1}$) of the cleaved peptide is determined using Matrix-Assisted Laser Desorption/ionization - Time of Flight (MALDI-TOF) mass spectrometry. To determine the relative amount of the target peptide in a sample or purity of the sample, reversed-phase high performance liquid chromatography (RP-HPLC) is used. Table 1 outlines the four peptides synthesized.

**Table 1. Description of Peptides Synthesized**

| Peptide | Method of Synthesis | Target Sequence | Calculated Molecular Weight |
|---|---|---|---|
| Peptide 1 | Manual Synthesis by Alicia Cuber | $NH_2$ - WxEAAYQrFL - COOH | 1296 Da |
| Peptide 2 | Manual Synthesis | $NH_2$ - EPAAYQRFT - COOH | 1082 Da |
| Peptide 3 | Tribute Automatic Synthesizer | $NH_2$ - WxEAAYQrFL - COOH | 1296 Da |
| Peptide 4 | Tribute Automatic Synthesizer | $NH_2$ - EPAAYQRFT - COOH | 1082 Da |

## Materials and Methods

**Manual Solid Phase Synthesis**: Peptides 1 and 2 were synthesized manually. The 1st amino acid activated using Oxyma and DIC, was coupled to Wang resin. The amino acid bound to resin was deprotected from Fmoc using 20% piperidine. The 2nd amino acid activated by dissolving in DMF with HCTU and NMM, was then added to the resin bound 1st amino acid for coupling. The peptide was elongated by repeating the deprotection and coupling steps until the peptide was complete. Complete peptide was cleaved from the resin and purified.

**Synthesis Using Tribute Synthesizer:** Peptides 3 and 4 were synthesized using the Tribute Synthesizer. Each amino acid was added to a separate labeled tube with HTCU. Each tube was loaded into the Tribute Synthesizer and left to synthesize overnight. The peptide was then cleaved and purified as above.

**MALDI-TOF and RP-HPLC of Peptide Samples**: The calculated molecular weight of the peptide was found using the website pepcalc.com. After cleavage, the sample was mixed with MALDI matrix and then spotted onto the MALDI plate. The plate was inserted into the MALDI-TOF and, using the computer program provided, an appropriate mass range was selected for analysis.

For RP-HPLC of Peptides 3 and 4, 100-500 µL of peptide solution (in DMF and water) was injected. Each large peak, apart from the very first and very last was collected in a separate tube. Each peak was analyzed using MALDI-TOF to determine the peak that corresponds to pure peptide.

## Results and Discussion

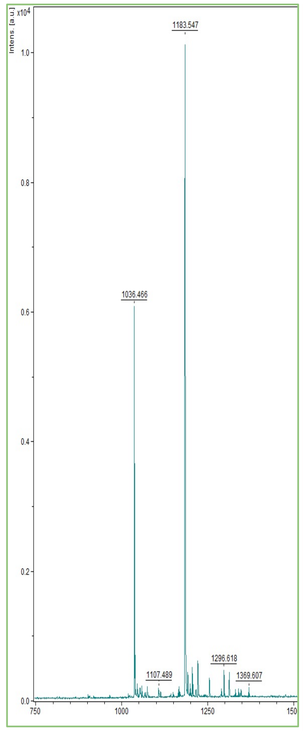


**Figure 1. Mass Spec of Peptide 1**
The mass $[M+H]^{+1}$ calculated was 1296 Da and the masses found were 1036 Da and 1183 Da.

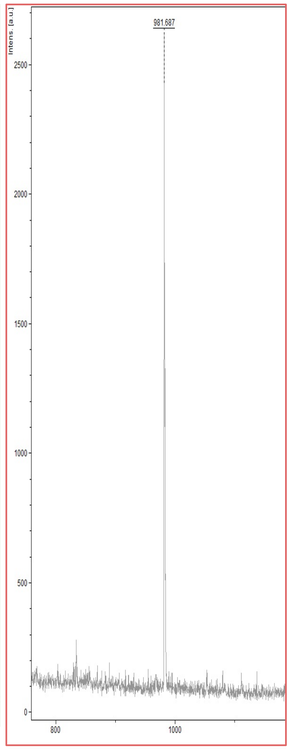


**Figure 2. Mass Spec of Peptide 2**
The mass calculated was 1082 Da and the mass found was 981.46 Da.

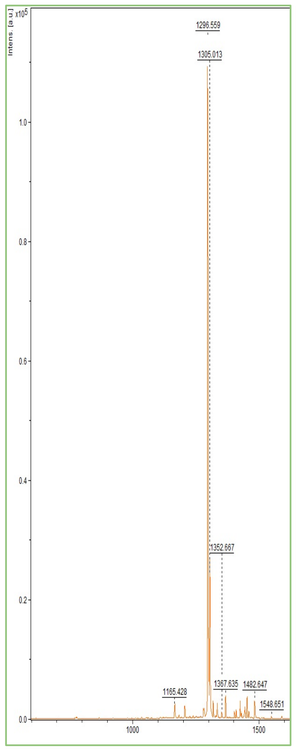


**Figure 3. Mass Spec of Peptide 3**
The mass calculated was 1296 Da and the mass found was 1296 Da.

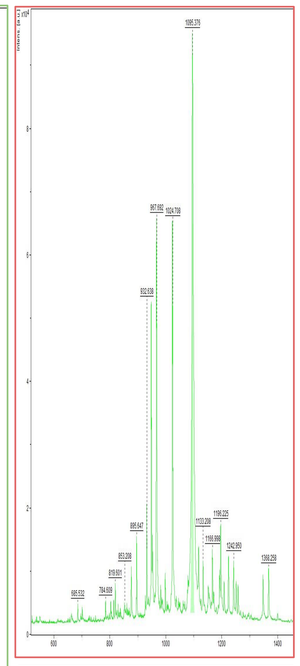


**Figure 4. Mass Spec of Peptide 4 with norleucine coupled**
The mass calculated was 1094 Da and three masses were found. The major peak shows a mass of 1095 Da. The minor peaks show masses of 967 Da, 932 Da and 1024 Da.

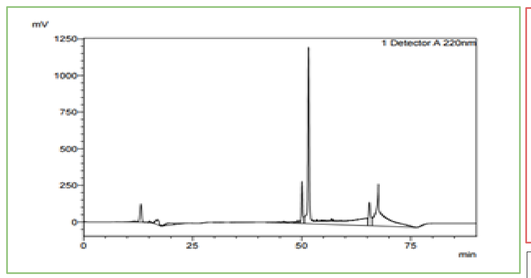


**Figure 5. RP-HPLC of Peptide**
The peak that eluted at 51 minutes contained a peptide with a found mass of 1296 Da, indicating presence of Peptide 3.

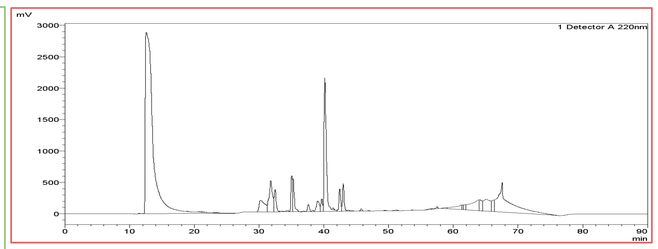


**Figure 6. RP-HPLC of Peptide 4 with norleucine manually coupled.**
Three major peaks eluted, one at 31 minutes, 40 minutes, and 42 minutes. The 31-minute peak contained a peptide with a mass of 947 Da and the 42-minute peak contained a peptide with a mass of 967 Da. The 40-minute elution peak contained a peptide with the mass of 1095 Da, indicating the presence of Peptide 4 with norleucine coupled.

**Table 2. Summary of Peptides Synthesized**

| Peptide Name | Target Sequence | Calculated Molecular Weight | Found Molecular Weight | Found Sequence |
|---|---|---|---|---|
| Peptide 1 | $NH_2$ - WxEAAYQrFL - COOH | 1296 Da | a. 1036 Da<br>b. 1183 Da | a. NH2 - WxAYQrFL - COOH [M + Na]<br>b. NH2 - WxEAYQrFL - COOH [M + Na] |
| Peptide 2 | $NH_2$ - EPAAYQRFT - COOH | 1082 Da | 981.46 Da | NH2 - EPAAYQRF - COOH |
| Peptide 3 | $NH_2$ - WxEAAYQrFLC - COOH | 1296 Da | 1296 Da | NH2- WxEAAYQrFLC - COOH |
| Peptide 4 w/ norleucine | $NH_2$ - xEPAAYQRF - COOH | 1094 Da | Major: 1095 Da<br>Minor A: 947<br>Minor B: 967 Da | Major Pep: $NH_2$ - xEPAAYQRF - COOH<br>Minor A: $NH_2$ - xEPAAYQR - COOH<br>Minor B: $NH_2$ - xEPAAYRF - COOH |

## Conclusions

**Peptide 1**

- Peptide 1 has two found molecular weights, which indicates that Peptide 1 consists of two peptides: one missing glutamate and alanine, and the other missing alanine from the target sequence.
- Peptide 1 will not be sufficient at binding to CTC's, so manual synthesis of Peptide 18-4 was unsuccessful.

**Peptide 2**

- Peptide 2 is missing the amino acid threonine.

**Peptide 3**

- Peptide 3 was successfully synthesized and should be able to selectively bind to CTC's.
- The RP-HPLC of Peptide 3 indicates that a clean, mostly pure peptide is present.

**Peptide 4**

- Synthesis of Peptide 4 was at first unsuccessful, but after manually coupling norleucine, it can be used as a negative analog of Peptide 4.
- The HPLC of Peptide 4 coupled to norleucine indicates three peptides are present.
- Because Peptide 4 is to be used as a negative analog, the presence of two minor peptides will not interfere with future work.

In conclusion, manual synthesis was unsuccessful at synthesizing Peptides 1 and 2. Automated synthesis was successful at synthesizing Peptides 3 and 4 to be used as Peptide 18-4 and its negative analog. These peptides are to be used in future research involving selective binding to CTC's.

## References

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