# Synthesis of Peptides and Bioassay for Anticancer Activity

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## Abstract

Solid-phase peptide synthesis was used to produce milligram quantities of Glycine-Cysteine-Glycine-Asparagine-Serine or GCGNS and GGGNS, GGGGS, GGGGG, GCGGS, and GGCNS. These pentapeptides were examined for anti-cancer activity against MCF-7 breast cancer cells. Despite previous results to the contrary, all unpurified and three of the five C18 purified peptides decreased breast cancer cell viability significantly. This indicates that GGGGS, GGGGG, and GGCNS demonstrate anti-cancer activity against MCF-7.

## Introduction

Cancer is a globally recognized conditioned that is defined as the rapid, uncontrolled division of abnormal cells that invade the tissues that it develops in and some associated tissues. Peptides are short compositions of amino acids that are the building blocks of proteins and have shown to have effects on cell signaling including, in some cases, apoptosis causing cell death. Previous work has shown that peptides can have both anticancer and antimicrobial properties.[1]

Computational chemistry has developed bioinformatics programs that are able to predict a peptide's anticancer activity based on structural features. The program AntiCP predicted high potential for Glycine-Cysteine-Glycine-Asparagine-Serine or GCGNS, while other programs such as ACPP, CallPPD and ToxinPred had mild to moderate predictions of anticancer effects.[2] These computational predictions led to this study, with GCGNS being the first target for synthesis.

Solid-phase peptide synthesis (SPPS) is a standard laboratory technique for building specific peptide sequences in the lab. The solid-phase resin allows for excess reagents and protective groups to be rinsed away as the peptide is built in a stepwise fashion with relatively good stability and purity. Variations in the sequence were made by systematically substituting glycine for all other amino acids or rearranging amino acids in initial sequence GGCNS.

Once the pentapeptides were synthesized, their anticancer activities were evaluated. The breast cancer cell line, MCF-7, was first exposed to the peptides and then examined for viability using an MTT bioassay. A reduction in cell viability would indicate that a peptide decreased proliferation of MCF-7 breast cancer cells, while no reduction would indicate that the MCF-7 cells survive treatment with peptide(s) for 24-48 hours. Additionally, complete loss of viability could indicate cell death as an artifact of residual solvents in the peptides that are toxic to cells.

## Materials and Methods

**Solid-State Peptide Synthesis**

SSPS, a standard synthetic method for synthesizing peptides with relatively high purity, was used to produce peptides attached to solid-state Wang resin.[3]

**Spin Column Peptide Purification**

Relatively quick purification was achieved using C18 spin columns which allows peptides to bind to a resin in the column while contaminants are spun away from the samples via centrifugation.

**Cell Viability Assay**

The crude peptides were tested by against MCF-7 breast cancer cells for their tendency to change cell viability. Assay details and media components were prepared as described by Siegfried and Schroeder.[4]

## Procedure

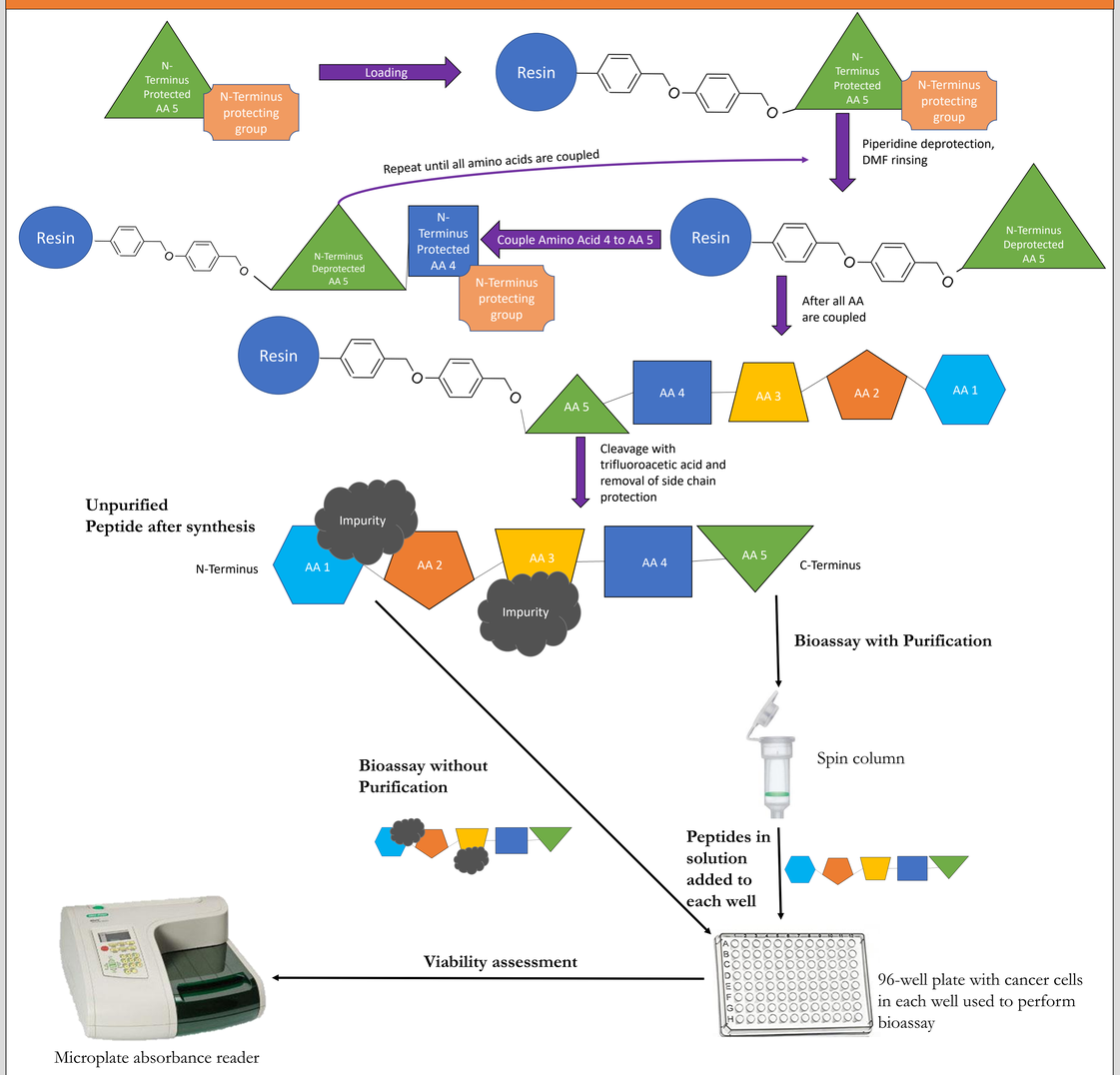
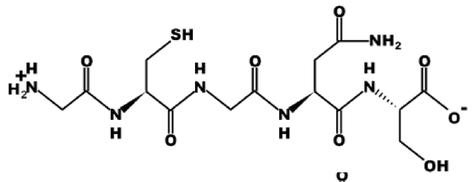
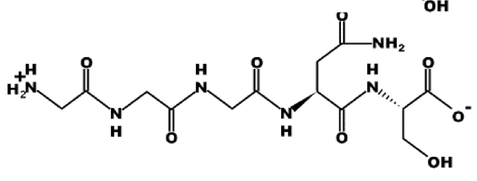
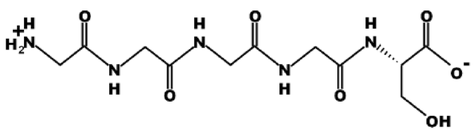
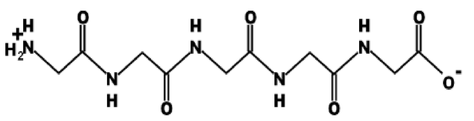
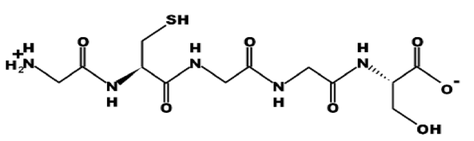
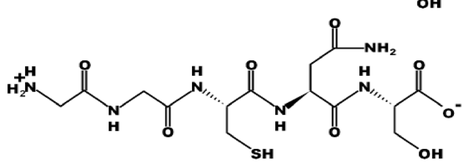



Figure 1. Overall procedure for synthesis and analysis of peptides using a bioassay with cancer cells.

## Results

Table 1. The sequences, line structures and yields of six synthesized peptides.

| Peptide | Structure | Yield |
|---|---|---|
| GCGNS |  | 46.1 mg |
| GGGNS |  | 100.0 mg |
| GGGGS |  | 176.1 mg |
| GGGGG |  | 151.3 mg |
| GCGGS |  | 2.7 mg |
| GGCNS |  | 40.6 mg |

## Results

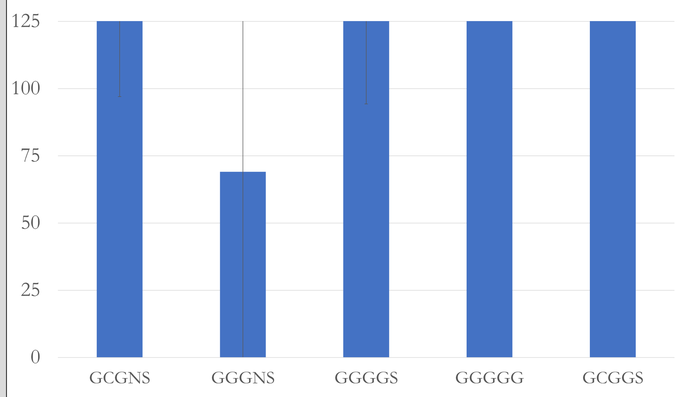



Figure 3. Cell viability for unpurified peptides against MCF-7 breast cancer cells.

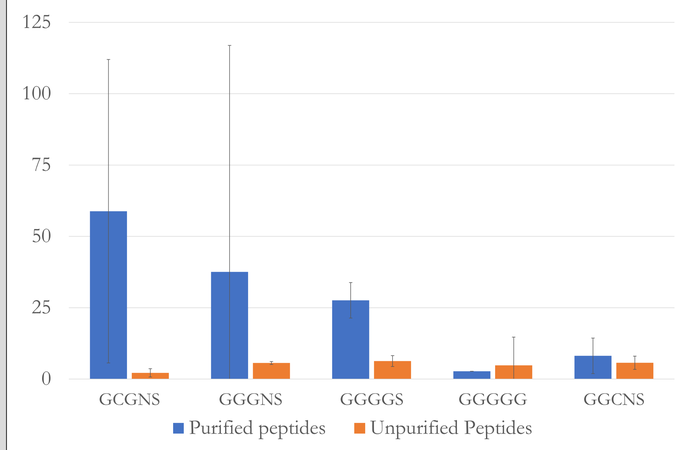



Figure 4. Cell viability for purified peptides and unpurified peptides against MCF-7 breast cancer cells.

## Discussion

Figure 3 shows that all the peptides significantly increased the viability of the MCF-7 cancer cells. Previous research supports that free amino acids allow cancer cells to survive longer and proliferate at greater rates.[5] This suggested that storing the unpurified peptides in the freezer for months led to degradation into amino acids.

This year, the SPSS protocol was modified to include DIC activation to enhance coupling and synthesize the peptides more rapidly. In addition, the MCF-7 bioassay was completed as quickly as possible after the peptides were made.

The data in Figure 4 suggests that while the unpurified peptides maintained their structures, the lack of purification was likely causing a massive reduction in cell viability. Residual solvents, like trifluoroacetic acid (TFA) and dimethylformamide (DMF), remained with the peptides during the MCF7 bioassay. Both TFA and DMF are known to be highly toxic to cells.

The peptides were synthesized again and purified with a C18 spin column to remove impurities. Figure 4 demonstrates that MCF-7 viability was improved after C18 purification compared to crude peptides, indicating improved peptide purity via removal of cytotoxic TFA and DMF. In this bioassay, both GGGGS and GGGNS showed significantly decreased cell viability. This suggests that the peptide sequences GGGGS, GGGGG and GGCNS have some anti-cancer activity against MCF-7 breast cancer.

## References

[1]M. R. Felicio, O. N. Silva, S. Goncalves, N. C. Santos, and O. L. Franco. Peptides with Dual Antimicrobial and Anticancer Activities. *Frontiers in Chemistry*. **2017,** 5(5).
[2]T. E-kobon, P. Thongararm, S. Roytrakul, L. Meesku, and P. Chumnanpuen. Prediction of anticancer peptides against MCF-7 breast cancer cells from the peptidomes of *Achatina fulcia* mucus fractions. *Computation and Structural Biotechnology*. **2016,** 14:49-57.
[3]M . Stawikowski and G. B. Fields. Introduction to Peptide Synthesis. *Current protocols in protein science*. **2002**, 18(1).
[4]S. A. Siegfried and J. R. Schroeder. Toxicity of thieves oils to mcf-7 and mda-mb-231 breast cancer cells. *American Journal of Essential Oils and Natural Products*. **2018,** 6(1): 01-08.
[5]Krall, A., Xu, S., Graeber, T. et al. Asparagine promotes cancer cell proliferation through use as an amino acid exchange factor. Nat Commun. **2016,** 7: 11457 .