Mucin Peptides-Antibody Binding Study by NMR Spectroscopy

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Abstract

Mucin, a component of mucus, is a class of large, heavily glycosylated proteins that are found on epithelial surfaces such as respiratory, gastrointestinal, and reproductive tracts. Mucin serves as a lubricant, acts as a protective barrier to soft tissues, and binds to pathogens to protect the body from infection. MUC-1 mucin is a type of mucin encoded by the gene MUC-1; it consists of three domains: the extracellular, transmembrane and intracellular domain. On normal cells, mucins are highly glycosylated and are found on the apical surfaces of cells. In tumor cells, MUC-1 mucin can be found all over the cell surfaces. Tumor mucin has carbohydrate chains that are shortened compared to the normal one. The shortening of carbohydrate side chains exposes MUC-1 epitopes to the immune system, which allows for antibody production against it. MUC-1 mucin is a growing topic of research in vaccination against breast cancer using synthetic carbohydrates and peptides. In this study we will present the results for the synthesis of mucin peptides by solid-phase peptide synthesis method, characterization of the peptide structures, and mucin peptide binding to antibody by NMR spectroscopy.

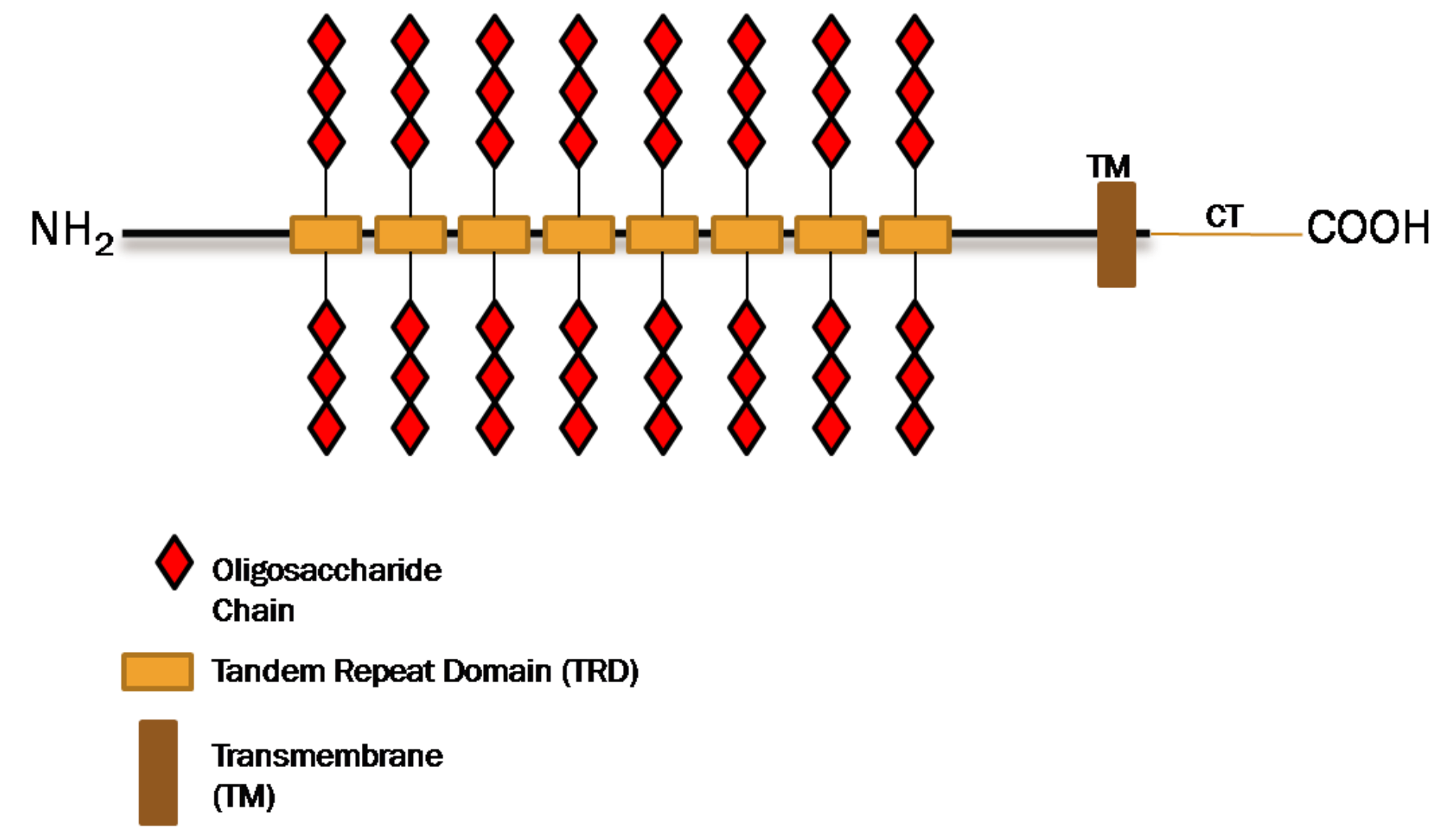
Background

Mucin is a class of large, heavily glycosylated proteins found on the apical surfaces of epithelial cells. The heavy glycosylation of the protein provides a hydrophilic environment which aids in hydration and lubrication. MUC-1 mucin is a transmembrane protein containing three domains coded by the gene MUC-1. The extracellular portion extends farther than most extracellular cell receptors and is larger than the transmembrane and intracellular portions; it extends far from the surface of the cells and disrupts cell adhesions of pathogens and other cells thereby protecting the epithelial cells from invasion (1).

The central region of the mucin protein core consists of a tandem repeat of 20 amino acids (AHGVTSAPDSRPAPGSTAPA) saturated with O-linked oligosaccharides (2). On normal cells, MUC-1 mucins are rich with carbohydrate side chains and are moderately expressed throughout the cell surface. In cancer cells, MUC-1 mucins are overexpressed and are found all over the cell surface (3). The carbohydrate side chains on the tumor mucin are shortened. Hypoglycosylation of MUC-1 mucin exposes the polypeptide backbone to the immune system, which allows for antibody production against it. MUC-1 mucin is a growing topic of research for vaccination against breast cancer using synthetic carbohydrates or peptides. In order to generate these synthetic carbohydrates and peptides, researchers have to understand what region on the MUC-1 mucin antibodies bind to.

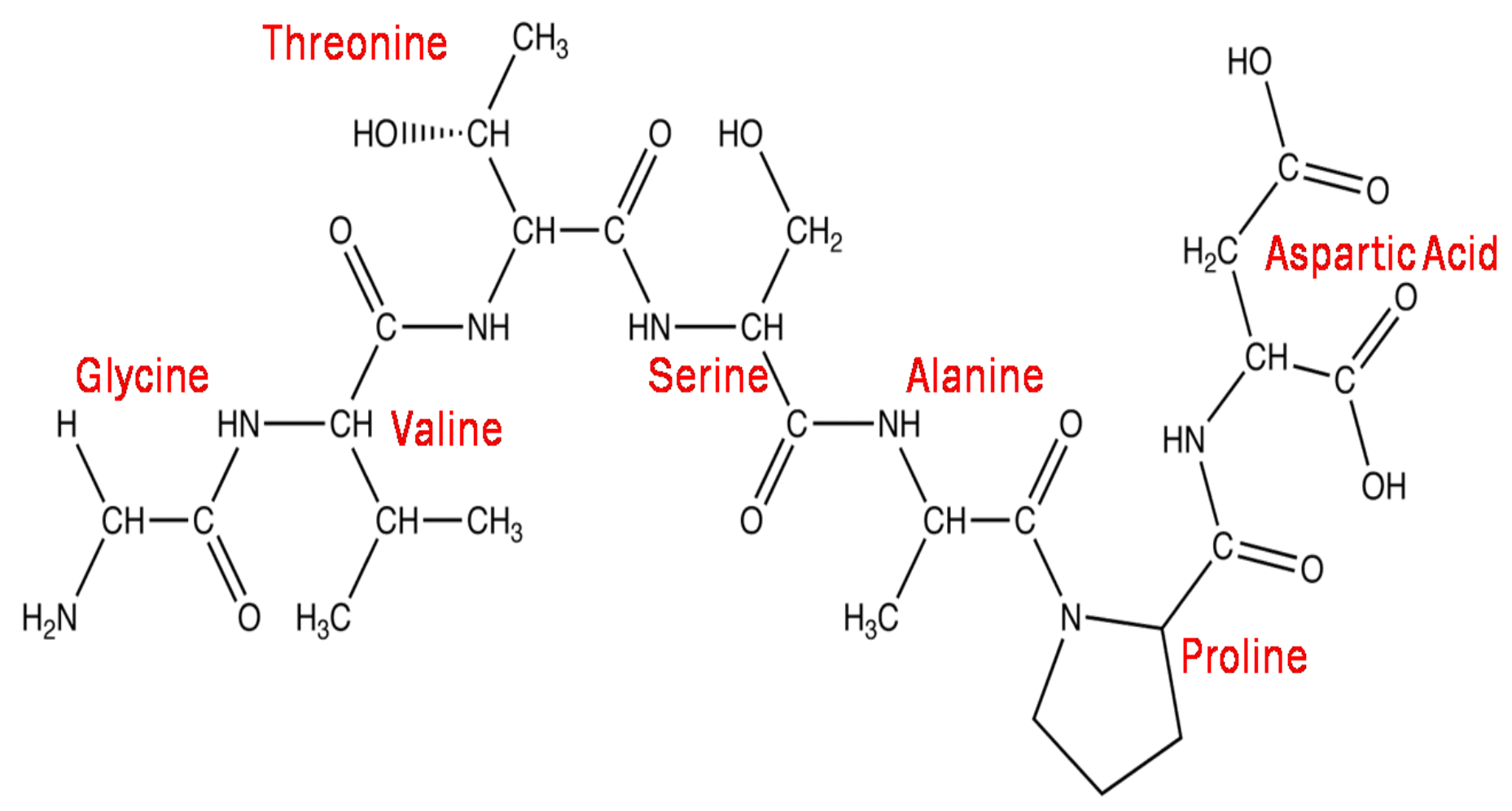
In this study, a small mucin peptide with seven residues (GVTSAPD) will be studied using NMR. We will present the results for the synthesis of mucin peptide by solid-phase peptide synthesis method, characterization of the peptide structures, and mucin peptide binding to antibody by NMR spectroscopy. NMR data will be presented and assignments of all hydrogens on the peptide will be accomplished by 1-D and 2-D NMR spectroscopy.

General Structure:

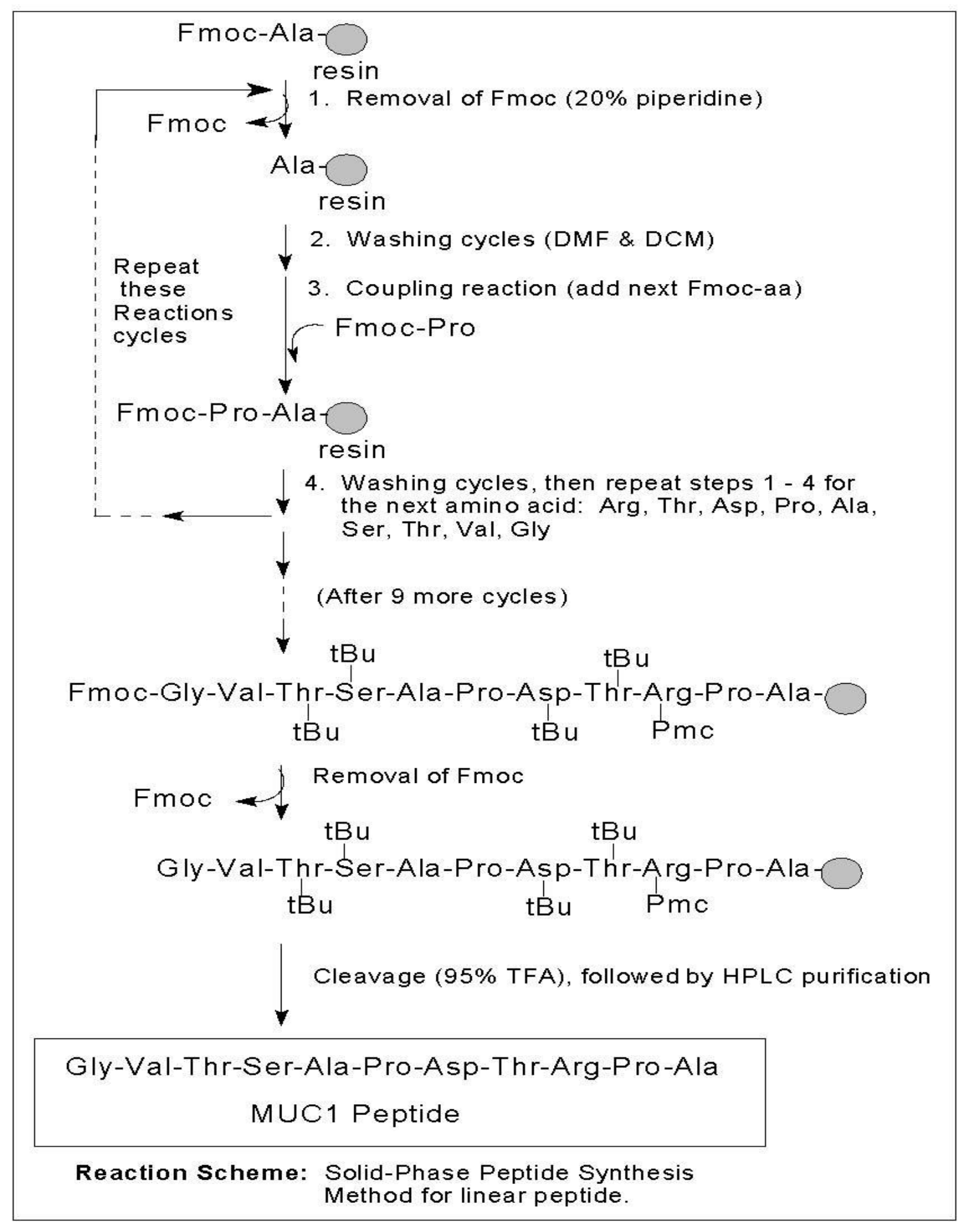


The structure of the MUC-1 mucin has three domains. The extracellular domain consists of a 20-100 tandem repeat of 20 amino acids and is mainly Serine, Proline, and Threonine. The transmembrane portion of the protein consists of hydrophobic residues and the intracellular domain contains the cytoplasmic tail which consists of 72 amino acids.

Structure of peptide (GVTSAPD) being studied in this project:



Methods



Reaction Scheme: Solid-Phase Peptide Synthesis Method for linear peptide.

Synthesis:
Synthesis of the MUC-1 peptide was carried out manually by FMOC-chemistry, using the Solid-Phase Peptide Synthesis technique. The synthesis began with a solid resin (Wang Resin), from which the peptide chain is lengthened by coupling with subsequent FMOC-amino acids that formed the sequence of the peptide. Once the desired sequence is obtained, the peptide chain is cleaved off from the resin by 95% TFA, leaving the desired crude product in solution, from which HPLC was used to isolate and purify the desired peptide (11-mer, MUC-1 peptide).

Results and Discussion

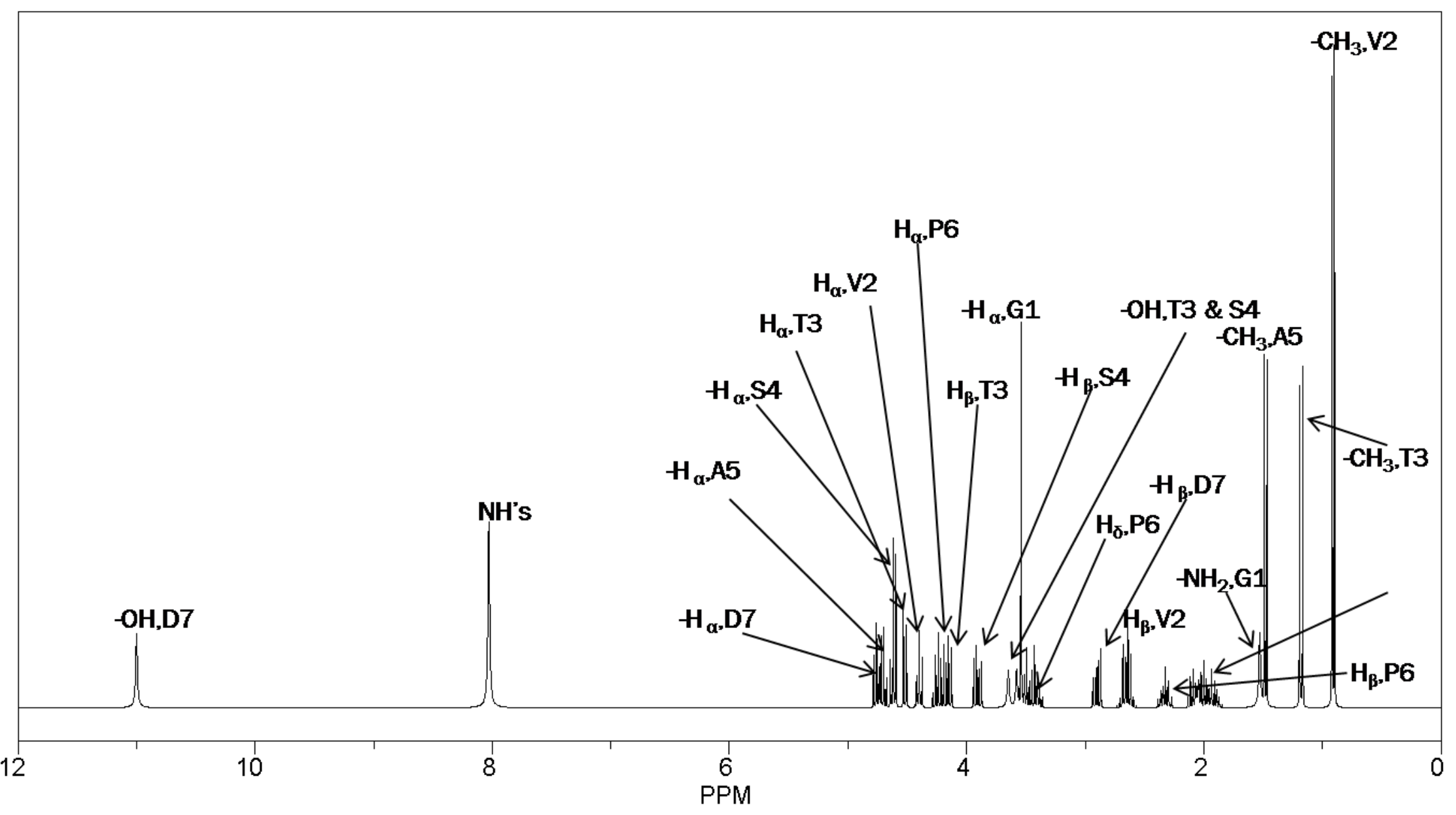


Fig. 1: Theoretical 1-D NMR spectrum of peptide (GVTSAPD) using Chemdraw with calculated assignments of hydrogens.

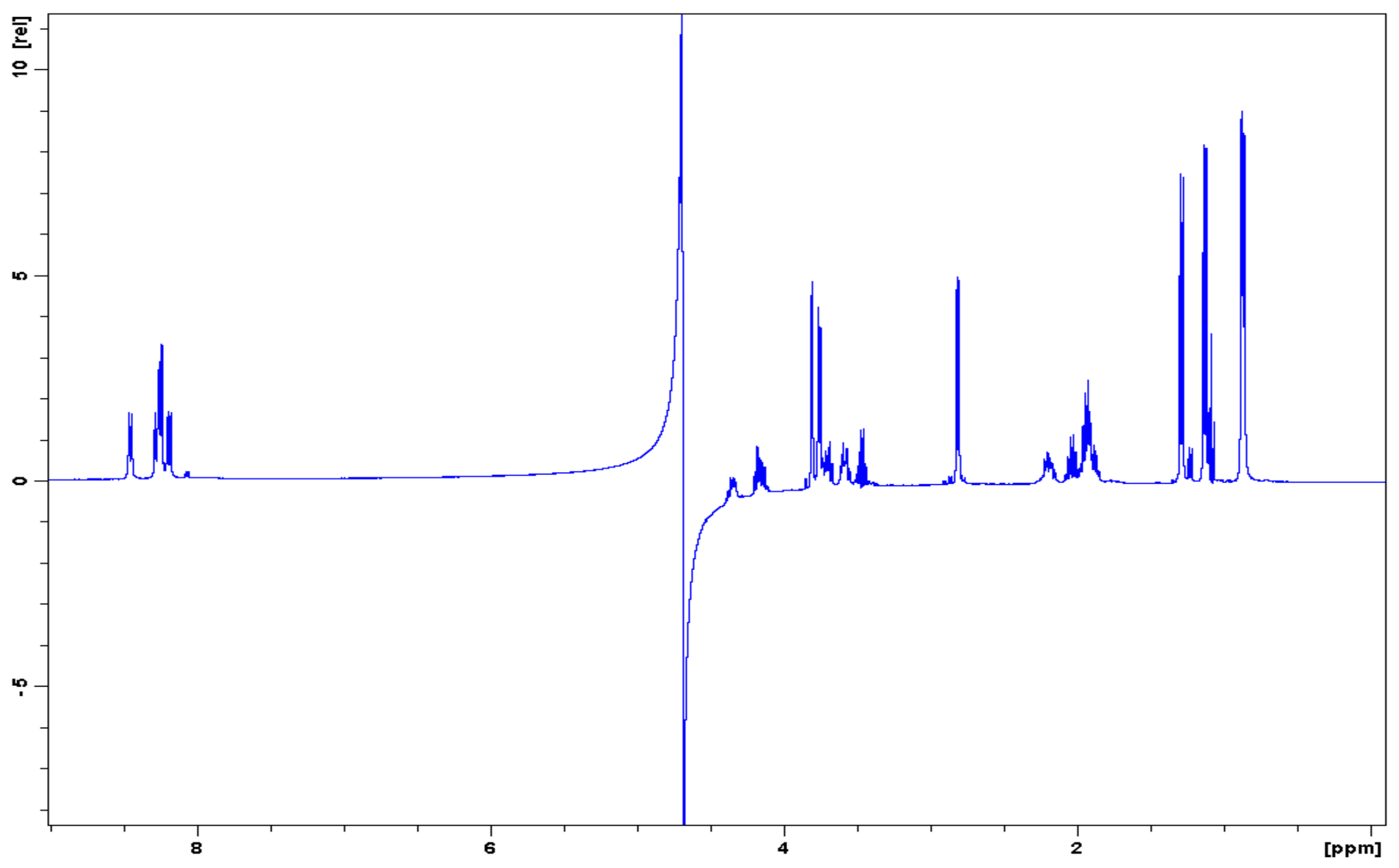


Fig. 2: 1-D NMR spectrum of the peptide (GVTSAPD) in 20 mM of Phosphate buffer, in 90% H_2O and 10% D_2O, pH 5 at 298 K. The HDO peak at 4.7 ppm is suppressed. The theoretical spectrum of the peptide help identify the H's in the experimental 1-D NMR spectrum. Most calculated peaks appear in the same region as the experimental spectrum. In the theoretical spectrum, all the NH's show up as one peak; however, in the experimental spectrum, the NH's appear at different frequency.

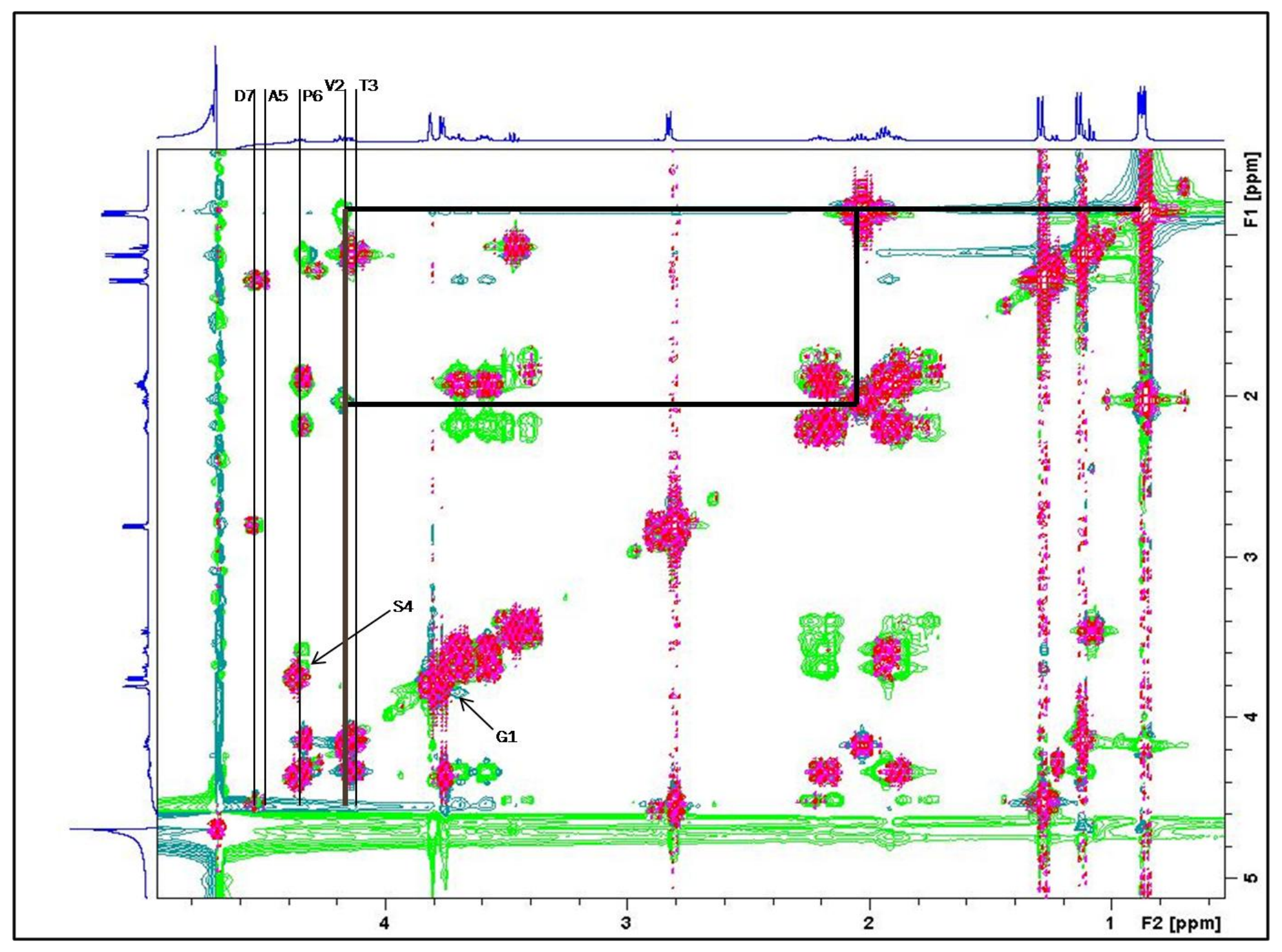


Fig. 3: Results of peptide using 2-D NMR spectroscopy showing assignments of all hydrogens using the 2D COSY and TOCSY NMR data for the GVTSAPD peptide being studied. The red contours (COSY cross-peaks) show the correlation of two hydrogens within three bonds, and the green contours (TOCSY data) show the correlation between all hydrogens within one amino acid. The bolded line shows the spin system of Valine.

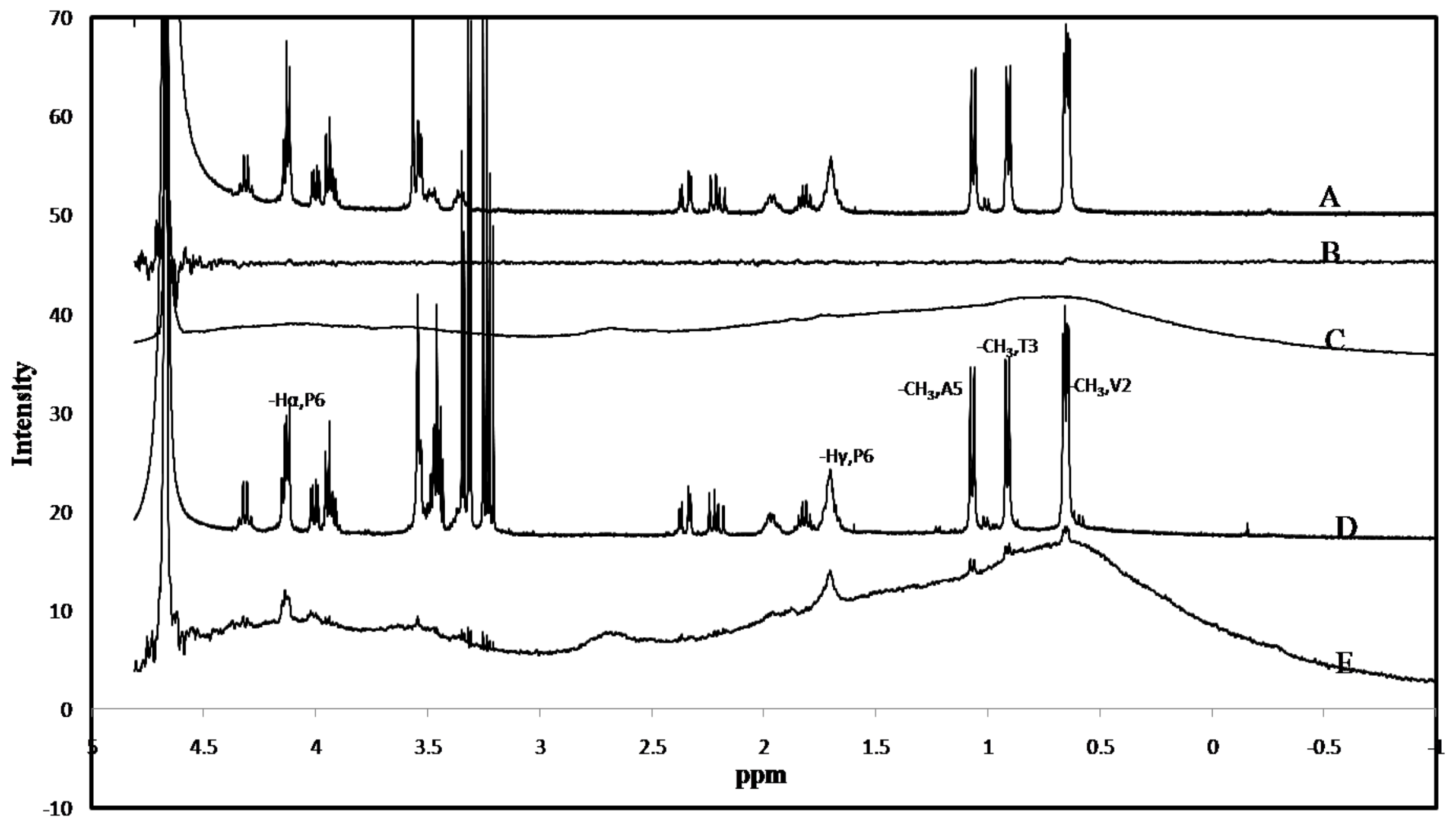


Fig. 4: Results of STD NMR and 1-D NMR on the peptide and antibody molecule to show where antibodies bind. (A) 1-D NMR spectrum of peptide; (B) STD NMR of peptide without antibody molecule; this spectrum is flat because there is no binding due to the absence of antibody molecule; (C) STD NMR spectrum of antibody molecule without peptide; this spectrum has a broad hump due to the antibody molecule, but there is no sharp peak because the peptide is absence so no binding occurs; (D) 1-D NMR spectrum of peptide and antibody molecule mixture. The peaks of the H's of the peptide are on top of the antibody spectrum, which is broaden. The ratio of peptide to antibody is 100:1; (E) STD NMR spectrum of peptide and antibody molecule mixture. The peaks in E shows that $-CH_3$ groups of V2, T3, and A5, and the Hγ and Hα of P6 bind to the antibody molecule.

Conclusion

- ➢ The peptide has been synthesized correctly through solid-phase peptide synthesis.
- ➢All of the hydrogens on the peptide are assigned. The 2-D NMR makes it easier to assign the protons as indicated by Fig. 3.
- ➢The STD NMR is a powerful technique to detect binding groups of the peptide.
- ➢Based on the STD NMR of peptide and antibody molecules, we conclude that the antibodies bind to $-CH_3$,V2; $-CH_3$,T3; $-CH_3$,A5; Hγ and Hα of P6.
- ➢Peptide with amino acid sequence based on the tandem repeat domain can bind to the antibody produced against MUC-1 mucin.

References

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Acknowledgments

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