USING ASPARTYL NITROBENZOTHIAZINE TO IMPROVE GLYCOLIGATION STRATEGY

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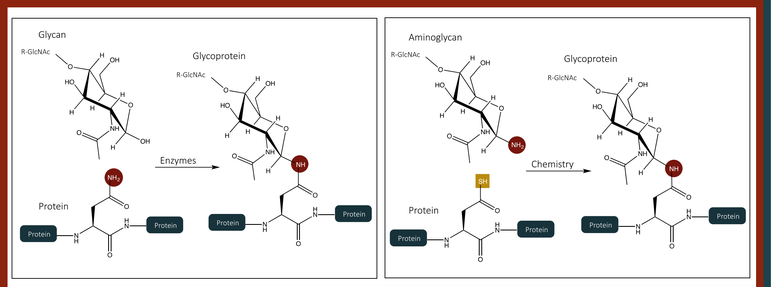
Background

Glycoproteins are important for cellular processes including cell recognition and protein cellular quality control [1]. They are estimated to make up > 50% of proteins [2]. Additionally, they may be the key to unlocking new therapeutic vaccines and cancer immunotherapy treatments [3]. However, glycoproteins remain understudied due to the difficulty of obtaining homogenous samples. A recently published glycoligation method may be a breakthrough if improved [1].

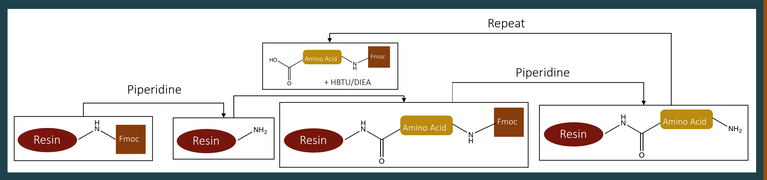
Glycosylation

Glycoligation

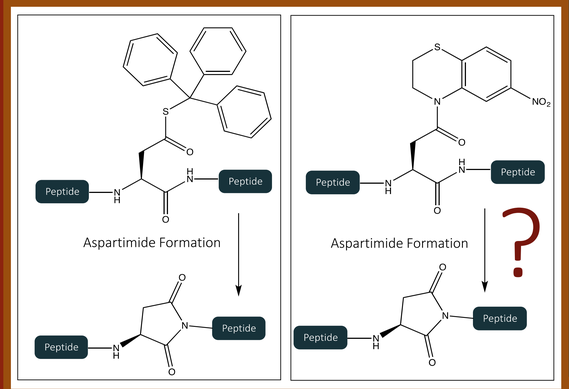


The glycoligation method required peptides to be created using solid phase peptide synthesis using trityl protected aspartic thioester (Asp(S-Trt)) residues at intended ligation sites [4].However, these thioacid precursors can induce an unwanted aspartimide formation [4]. A method to eliminate the aspartimide formation must be discovered to improve the glycoligation strategy. Aspartyl nitrobenzothiazine (Asp(NBT)) should be resistant to aspartimide formation, and work in place of Asp(S-Trt).

Solid Phase Peptide Synthesis



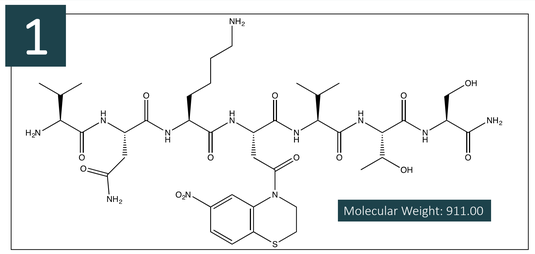
Asp(STrt) Asp(NBT)



The main objective of this study was producing and analyzing four peptides containing Asp(NBT) to test if Asp(NBT) can work in place of Asp(S-Trt) and avoid aspartimide formation. This was accomplished using the method of Solid Phase Peptide Synthesis [5].

Peptides Produced

1



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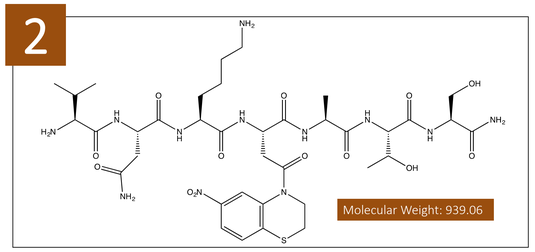

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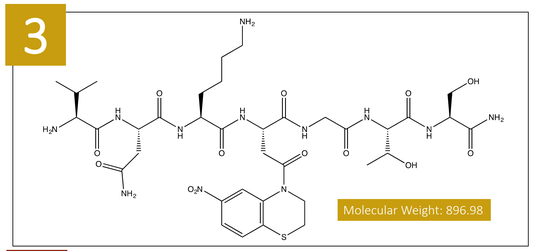

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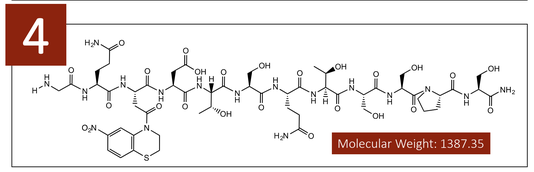
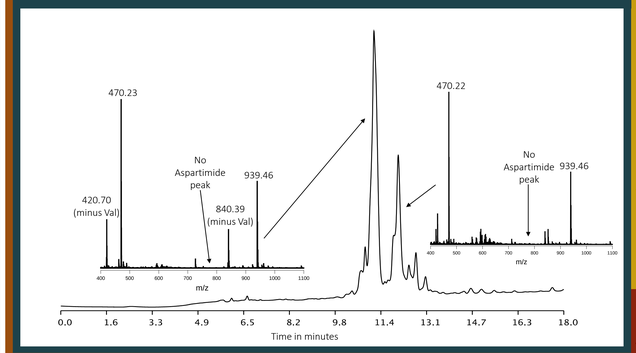


1-3 were chosen due to varying levels of aspartimide formation found using the Asp(S-Trt) versions [4]. 4 was chosen due to its medical significance. The glycosylated and GPI anchored version of 4 is the antigen to the therapeutic antibody Alemtuzumab, a commonly used drug to treat chronic lymphocytic leukemia [6]

Results:

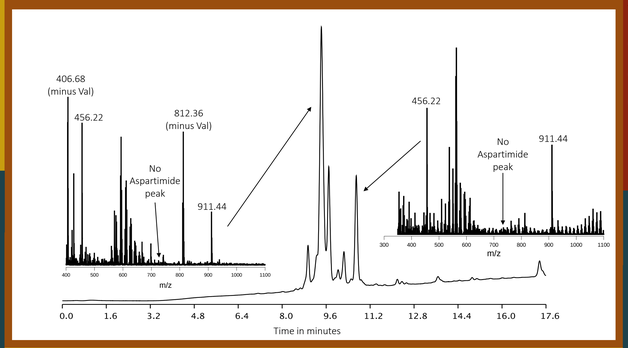
1. VNK-Asp(NBT)-VTS-NH_2

Gradient elution 0 – 50% Acetonitrile. 214 nm



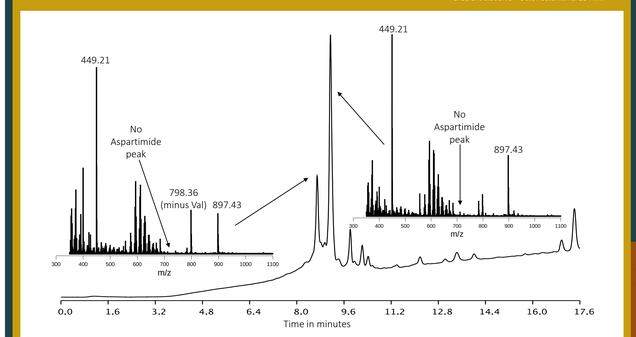
2. VNK-Asp(NBT)-ATS-NH_2

Gradient elution 5 – 50% Acetonitrile. 214 nm



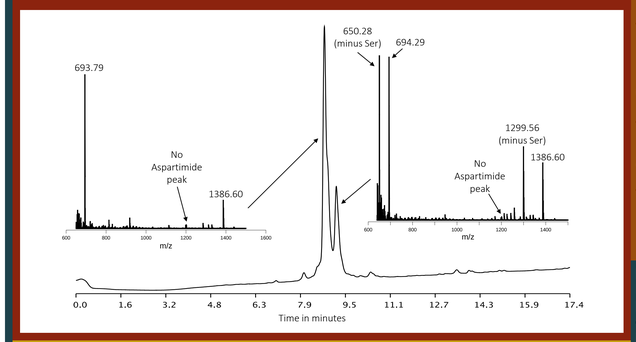
3. VNK-Asp(NBT)-GTS-NH_2

Gradient elution 5 – 50% Acetonitrile. 214 nm



4. GQ-Asp(NBT)-DTSQTSSPS-NH_2

Gradient elution 5 – 50% Acetonitrile. 214 nm



Control peptides were synthesized using aspartate in place of Asp(NBT).

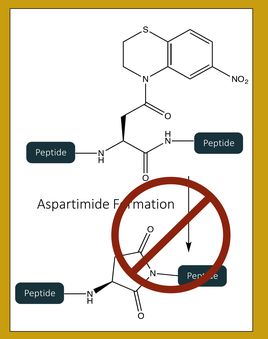
Gradient elution 0 – 30% Acetonitrile. 214 nm

	t_r (min)	mH^+ Calc. (g/mol)	mH^+ Obs. (g/mol)
1. VNKDVTS-NH_2	8.24	761.85	761.3
2. VNKDATS-NH_2	5.04	734.78	733.3
3. VNKDGTS-NH_2	5.55*	720.75	719.3
4. GQDDTSQTSSPS-NH_2	6.98	1209.16	1208.9

*100% H_2O isocratic elution. 214 nm

Conclusion

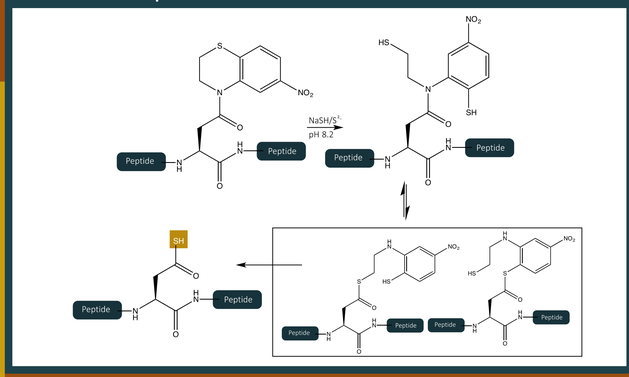
All four Asp(NBT) containing peptides were successfully synthesized without evidence of aspartimide formation. Analysis revealed secondary peaks containing peptide minus Val for 1-3 and peptide minus Ser for 4. These are consistent with control peptides and are likely a product of incomplete coupling.



Next Steps

The peptides synthesized in this study have been sent to the Garner lab at Washington State where the proposed method for turning Asp(NBT) peptide into glycoligation ready aspartic thioacid peptide will be tested. If successful, the amber suppression technique will be used to genetically encode proteins containing Asp(NBT) for use in the first protein glycosylation attempt using the new glycoligation method [7].

Proposed Asp(NBT) Peptide to Aspartic Thioacid Peptide Method



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