Development of a Stereodivergent Route to Glycosylated Amino Acids

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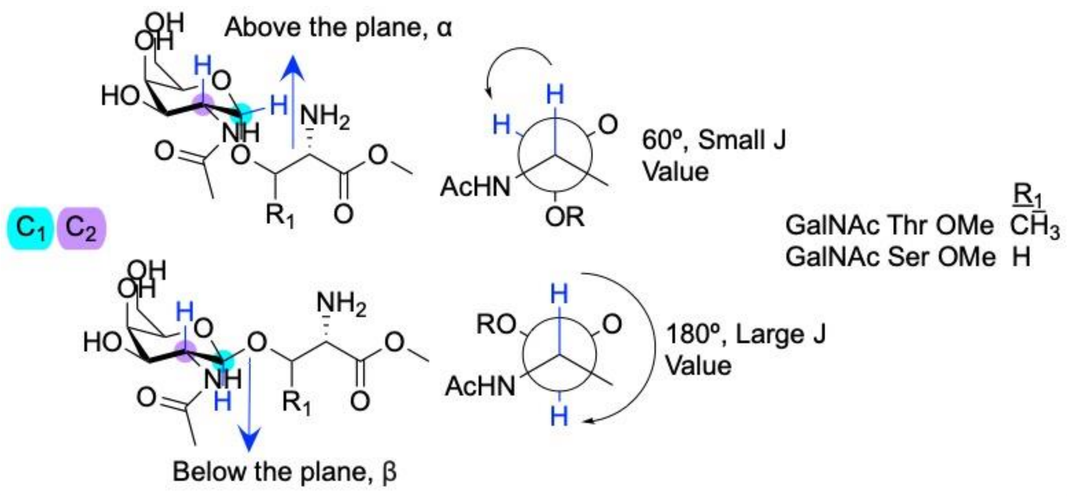
What is Glycosylation?

Glycosylation is a common post-translational modification that is characterised by the addition and extension of carbohydrates on proteins and lipids.[1] Protein glycosylation is abundant. Protein glycosidic bonds linked to amino acid residues by *C*-, *O*-, *N*- and *S*- are observed, with *O*-glycosylation most commonly occurring through covalent attachment to serine (Ser) and threonine (Thr) residues.[2]
Glycosylation can result in the formation of either an α or β glycosidic bond, as the attachment of a residue at the C_1 position can be in two orientations: axial or equatorial. The orientation of amino acid residues attached at the C_1 position of GalNAc can be determined from the coupling constants (*J*) between C_1 and C_2 positions, measured in ^{1}H NMR.

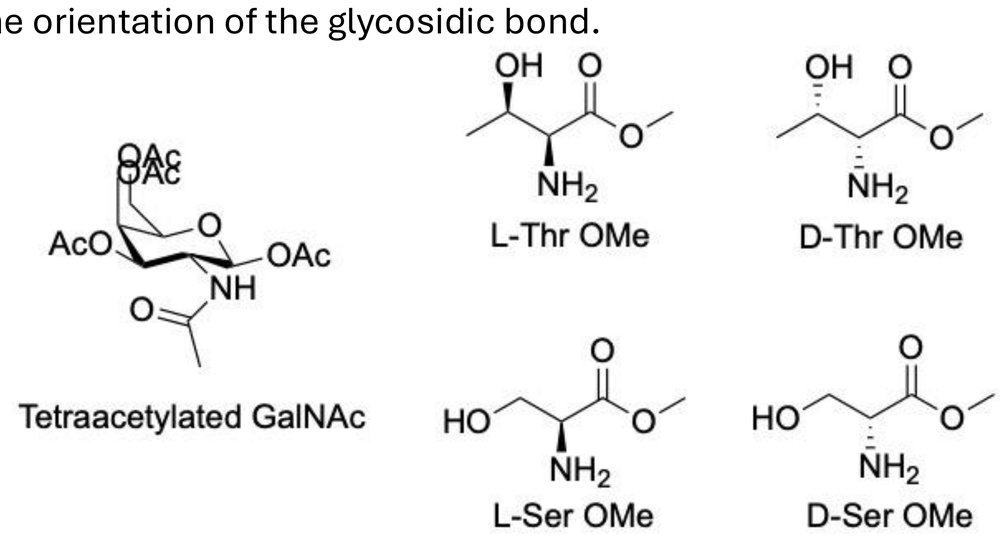


Analytical Methods

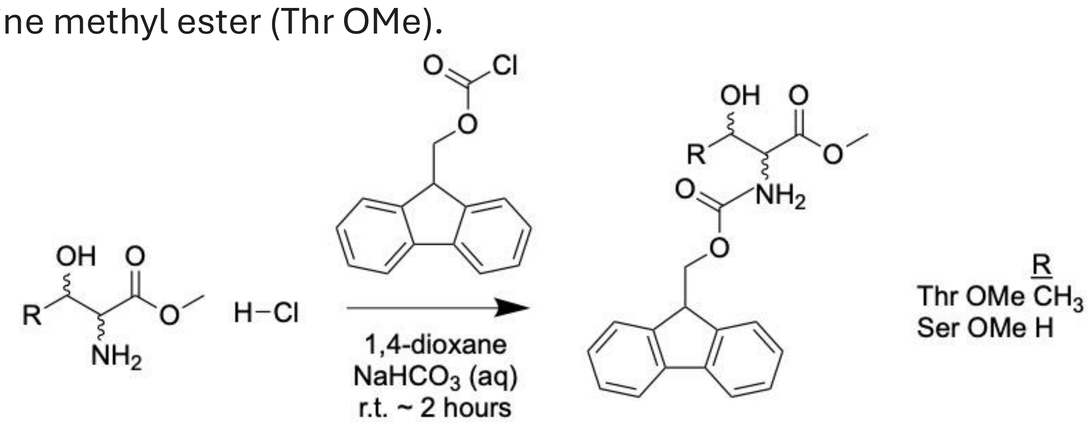
The formation of each product was confirmed using ^{1}H NMR and this, along with ^{13}C NMR and 2D NMR was used to assign peaks. ^{1}H NMR was used to determine coupling constants (*J*) between the H atoms on C_1 and C_2, allowing for determination of whether an α or β glycosidic bond had been formed.
LCMS and HRMS was also used to confirm the correct product had been made as well as to help identify ratios of α and β glycosidic bond formation.

Synthetic Route

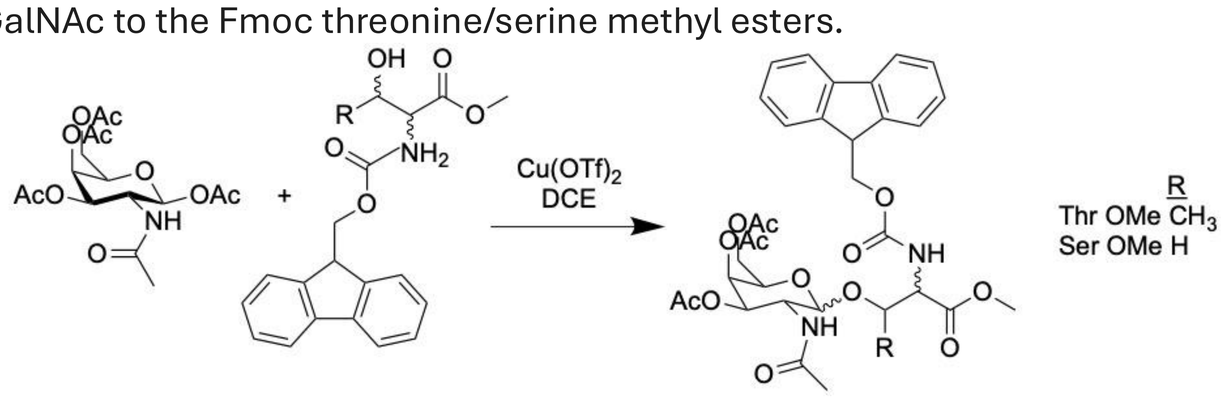
O-glycosylation of proteins is poorly understood. Therefore, glycosylation reactions between tetraacetylated GalNAc and L and D isomers of serine (Ser) and threonine (Thr) derivatives was undertaken as a means to investigate how the stereochemistry of amino acids and reaction times may affect the orientation of the glycosidic bond.



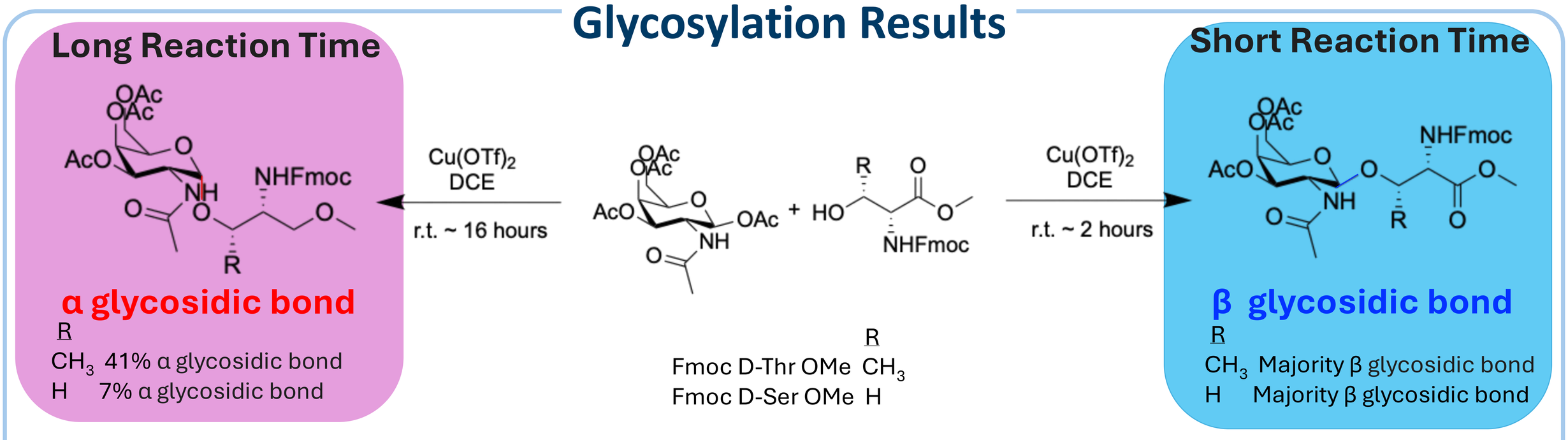
Based on a synthetic route developed by Frank,[2] an Fmoc protecting group was added to L and D isomers of serine methyl ester (Ser OMe) and threonine methyl ester (Thr OMe).



Glycosylation reactions were undertaken via the addition of tetraacetylated GalNAc to the Fmoc threonine/serine methyl esters.



Glycosylation Results



Glycosylation results showed that both Fmoc D-Thr OMe and Fmoc D-Ser OMe favoured formation of the β glycosidic bond, especially with a short reaction time (2h). However, with an increased reaction time (16h), formation of the α glycosidic bond increased for both amino acids.

Conclusion

Results indicate that the use of either the D or L isomers of threonine (Thr) and serine (Ser) did not affect whether an α or β glycosidic bond was formed, showing that the glycosidic bonds formed with the D isomers were consistent with previous findings with the L isomers.[2] However, to further investigate the stereochemical effect of amino acids on glycoside bond formation, other stereocentres (e.g. allo isomers) should be explored.

References

1.H. Wu, and J. Kohler, *Curr. Opin. Chem. Biol.,* 2019, **53**, 173-182.

2. F. Frank, R. Lawson, and T. McAllister, *RSC Chem. Biol.*, 2025, **6**, DOI: 10.1039/D5CB00076A.