C-Glycosyl α-Amino Acids as Structural Encoders of Peptide Conformation

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INTRODUCTION

Inspired by Nature's own biopolymers, where folding unlocks function, and by expanding the pool of 20 amino acids with non-canonical ones, numerous synthetic oligomers have been shaped to carry on different functions. Non-canonical amino acids have become not just tools, but keystones of modern science; they are indispensable in drug discovery, impart useful functional properties to catalysts and materials, and provide diverse hinges for bioconjugation reactions. *C*-glycosyl amino acids represent a privileged class of non-canonical amino acids; they occur in nature as products of bacterial metabolism and display wide spectrum of biological activities.

The *C*-glycoside linkage is inherently resistant to acidic, basic and enzymatic hydrolysis; therefore *C*-glycosylation is a well-established strategy for improving the pharmacokinetic properties of peptides. However, the influence of chiral *C*-glycosyl amino acid incorporation on peptide conformation remains insufficiently explored. Most existing synthetic approaches restrict *C*-glycosyl amino acid placement to the N-terminus, C-terminus, or specific residues containing pre-installed reactive groups.[1,2] Here, we present a more versatile strategy based on the design and synthesis of customized C-glycosyl amino acids (Figure 1).[3]

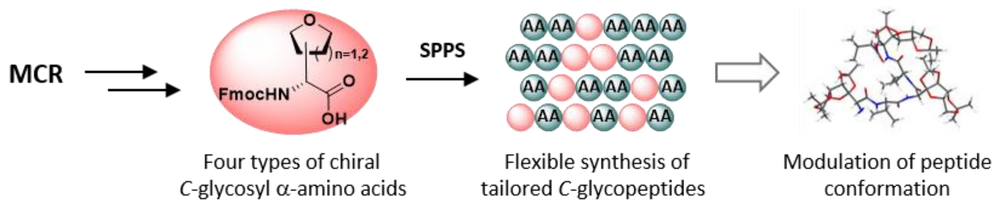
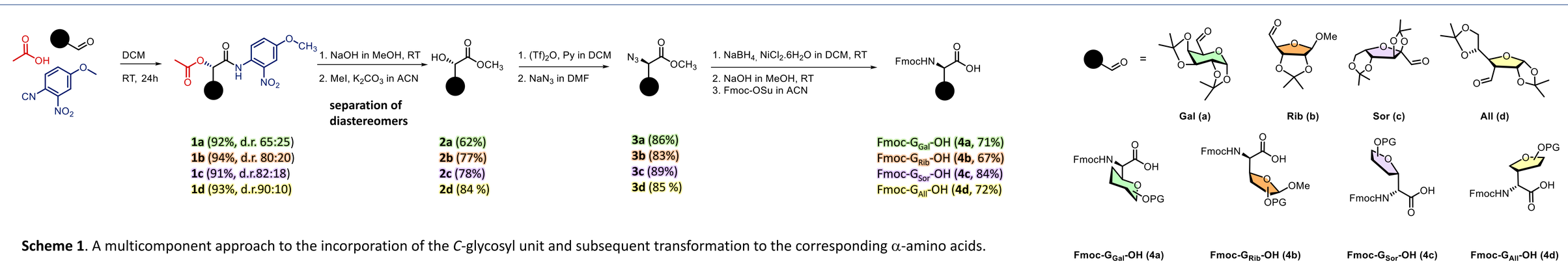


Figure 1. General approach to customized *C*-glycosyl α-amino acids for a flexible *C*-glycopeptide synthesis.

RESULTS

Four α-amino acids bearing protected galactopyranose, ribofuranose, sorbofuranose, or allofuranose side chains were synthesized by modification of multicomponent reaction (MCR) products (Scheme 1), and incorporated into peptides using a solid-phase methodology, enabling substitution at diverse sequence positions (Figure 2). Solid-phase synthesis was carried out with 2-chlorotrityl chloride resin, which can be cleaved under mild acidic conditions that leave isopropylidene groups in place. Thus, protected peptides were obtained after cleavage from the resin and purified by a combination of precipitation and RP-HPLC.



Scheme 1. A multicomponent approach to the incorporation of the *C*-glycosyl unit and subsequent transformation to the corresponding α-amino acids.

Conformational analysis of the model peptide **5** and *C*-glycopeptides was done by combination of NMR spectroscopy (in DMSO) and geometry optimization based on the collected NOE distance restraints from the 2D 1H–1H NOESY spectra. Typically, 7-15 NOEs were identified and used for constrained geometry optimizations. All calculations were performed in Gaussian 16 program package, Revision C.01. Detailed NMR analyses revealed that each *C*-glycosyl α-amino acid promotes distinct conformational preferences, primarily stabilized by hydrogen-bonding networks between backbone amides and carbohydrate side chains (Figure 3).[3]

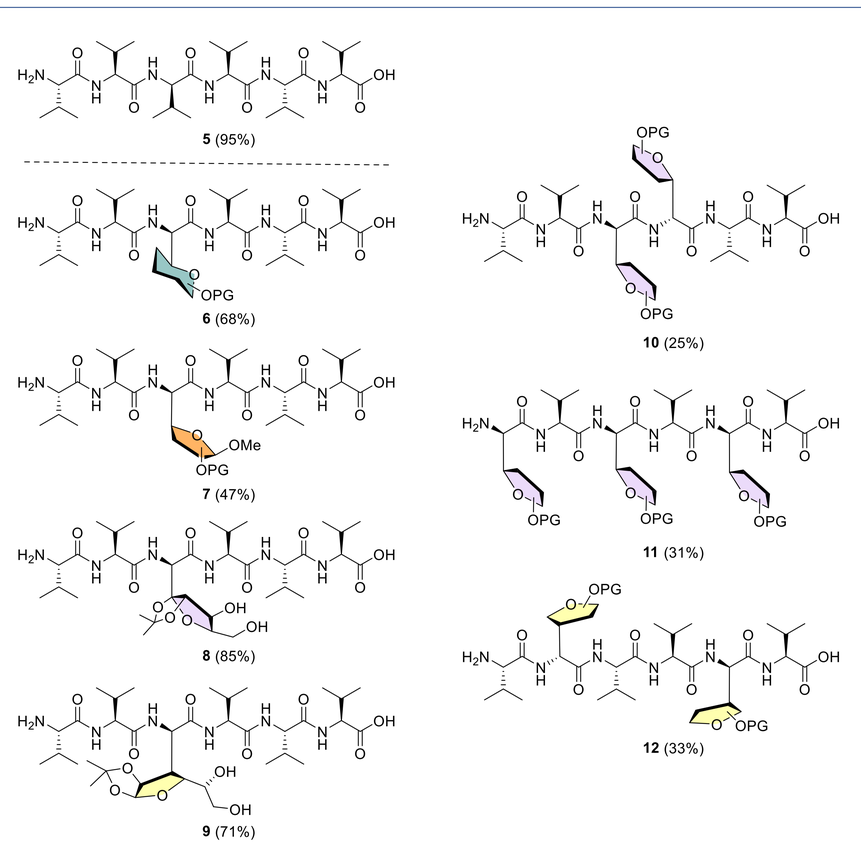


Figure 2. Model peptide **5** and *C*-glycopeptides obtained by SPPS

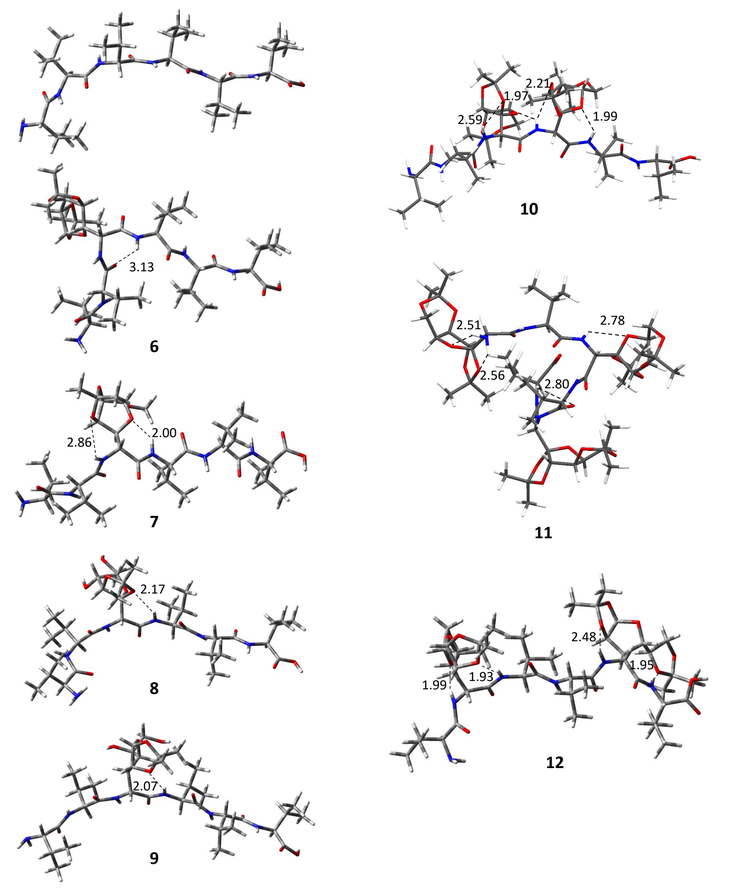


Figure 3. Conformational analysis of selected *C*-glycopeptides

CONCLUSIONS

While *C*-glycosylation represents a powerful strategy to overcome the limitations of *O*-glycosides and peptides in the drug discovery process, exploiting *C*-glycosyl amino acids as structural modulators is impeded by limited knowledge of the influence of carbohydrate ring size, stereochemistry, and substituents on the conformational preferences of peptides. We demonstrated that a flexible strategy comprising customized *C*-glycosyl α-amino acids enables synthesis of tailored *C*-glycopeptides with site-specific, consecutive or alternating positioning of these non-canonical amino acids.[3] NMR analysis uncover conformational information encoded within four non-canonical *C*-glycosyl α -amino acids, offering new molecular tools for catalysis, materials development and drug discovery.

References:

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