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Synthesis of a C-linked Antifreeze Glycoprotein (AFGP) Mimic: Probes for Investigating the Mechanism of Action

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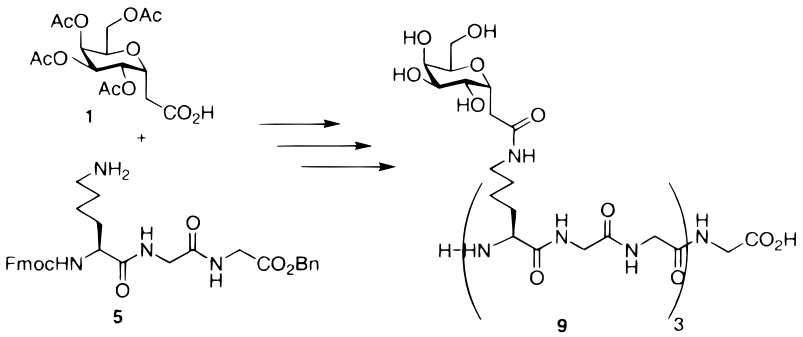
ABSTRACT



A general convergent synthesis has been developed to afford a low molecular weight C-linked antifreeze glycoprotein (AFGP) mimic (9). Structural mimics of AFGPs have tremendous potential as probes to better understand how native AFGPs inhibit ice crystal growth in organisms that inhabit subzero environments.

Biological antifreezes[1] are a diverse class of proteins found in fish, amphibians, plants, and insects. These compounds have the ability to inhibit *in vivo* ice crystal growth and, consequently, allow these organisms to survive subzero temperatures. This is a noncolligative phenomenon attributed only to biological antifreezes.

One class of biological antifreezes, the antifreeze glycoproteins (AFGPs) are isolated from Arctic and Antarctic teleost fish. These proteins range in molecular weight from 2.4 to 34 kDa and are composed of a tripeptide repeating unit (L-threonyl-L-alanyl-L-alanyl) where the L-threonine residue is glycosylated with the disaccharide β-D-galactosyl-(1,3)-α-D-*N*-acetylgalactosamine (Figure 1).[1e] The AFGPs of

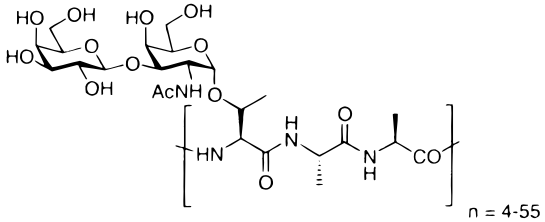


Figure 1. A typical antifreeze glycoprotein (AFGP).

2.4−2.7 KDa may have the threonine residue substituted with arginine and/or alanine substituted with proline.[2]

During the past decade, much effort has been devoted to understanding the mechanism by which AFGPs and other

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biological antifreezes function.[3] One reason for this is the potential medical, commercial, and industrial applications of these compounds.[4] Before such applications can be fully realized, a detailed understanding of ice-binding specificity and affinity must be achieved. Unfortunately, this issue continues to generate intense debate and a general mechanism has failed to emerge. Key to understanding the mechanism of action and rationally designing biological antifreezes with enhanced stability and activity are detailed structure−activity relationship studies. The AFGPs are ideal candidates for such studies since they possess a well-conserved primary and secondary structure.

Despite the many advances in the field of oligosaccharide and glycoconjugate chemistry[5] complex glycans require lengthy and costly syntheses.[6] Two reasons for this are the high lability of the anomeric carbon−oxygen bond under various reaction conditions[7] and the need to employ orthogonal protecting group strategies. Only four papers describing the synthesis of AFGPs and related analogues have appeared over the past decade. These syntheses have employed solution-phase[8] or continuous flow solid-phase techniques[9] and involve direct glycosylation of the peptide backbone or a stepwise elongation of the peptide backbone using a glycoconjugate. More recently, a diphenylphosphoryl azide (DPPA)-mediated polymerization of a glycosylated tripeptide has been developed.[10] To date, none of these AFGPs and AFGP analogues have been tested for antifreeze protein-specific activity.

As part of our continuing efforts toward the rational design of AFGP mimics possessing enhanced stability and activity, we have developed a general synthetic strategy to afford structural mimics of AFGPs (Figure 2).

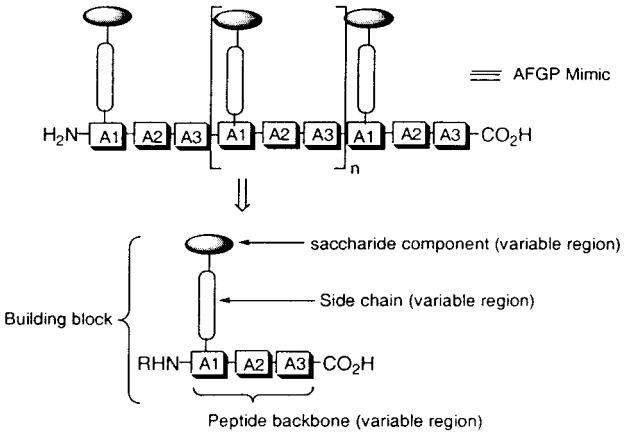

Figure 2. Model for AFGP structure−activity relationship studies.

The approach is centered on the synthesis of glycosylated tripeptide building blocks that are assembled using conventional solid-phase synthesis to furnish structural mimics of AFGPs. Our approach differs from previous ones in that C-linked glycoconjugates are utilized.[11] As a consequence, enhanced stability is expected since C-linked glycoconjugates are not susceptible to acid/base or enzyme-mediated hydrolysis. While this may seem like a dramatic structural modification, recent studies have demonstrated that C-linked glyconjugates bind substrates with nearly identical conformations and affinities as natural O-linked glycoconjugates.[12] These studies seem to preclude the notion that hydrogen bond involvement by the intersaccharidic oxygen atoms is essential in binding and establish a precedent for C-linked oligosaccharides as therapeutic agents and biological probes.

Synthesis of the glycosylated tripeptide building block is convergent in that saccharide and tripeptide components are covalently attached in a final step. Since early chemical and enzymatic modification of native AFGP[13] demonstrated that the terminal galactose residue was crucial for activity, our efforts have been focused on AFGP mimics that possess a

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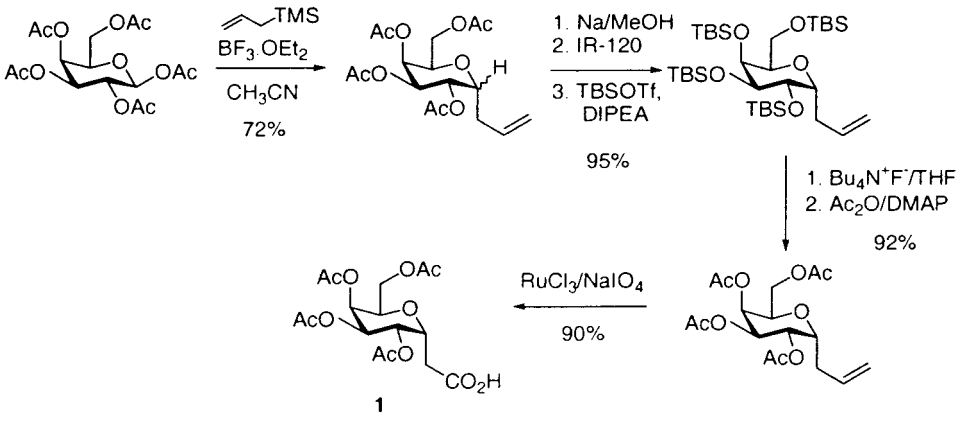
Scheme 1. Synthesis of C-Linked Saccharide Component



truncated saccharide. This approach is often utilized when studying carbohydrate−protein interactions in biological systems[14a−f] where in most cases, only the terminal residue(s) of a complex oligosaccharide are necessary for tight interactions with a receptor.[14g]

Scheme 1 outlines the synthesis of the saccharide component. C-allylation of β-D-galactose pentaacetate with allyltrimethylsilane produced 3-[2,3,4,6-tetra-*O*-acetyl-D-galactopyranosyl]propene as a 80:20 mixture of α- and β-anomers.[11j]

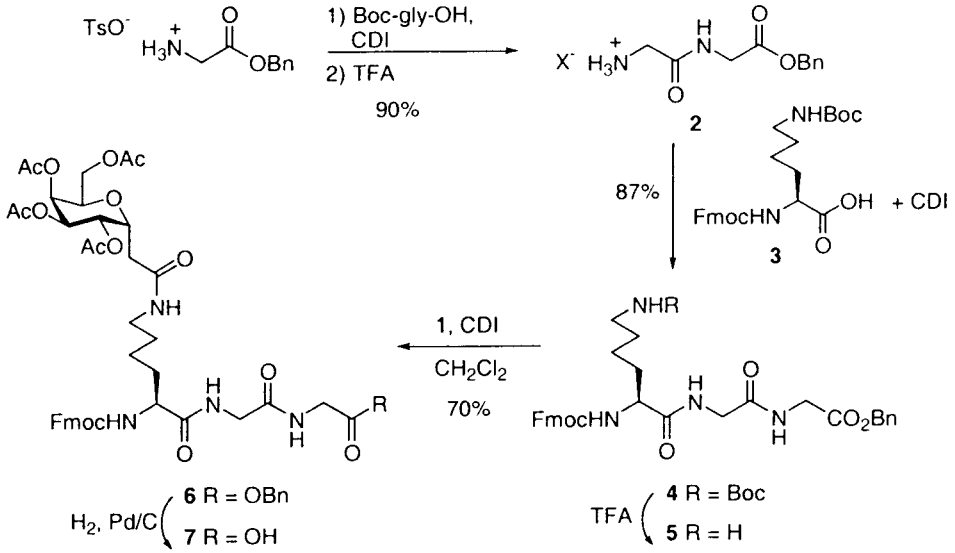
This mixture proved difficult to separate by column chromatography. Previous work[11i] led us to consider replacing the acetate groups with less polar *tert*-butyldimethylsilyl groups. This was accomplished using standard literature procedures and as anticipated, the α- and β-anomers were easily separated by column chromatography. Desilylation and reacetylation of the α-anomer was accomplished as a one-pot procedure with near quantitative yields and the resulting olefin was oxidized to furnish **1**.

The tripeptide component was prepared as outlined in Scheme 2. Dipeptide **2** was synthesized by reacting commercially available Boc-glycine and glycine benzyl ester with 1,1′-carbonyldiimidazole (CDI) as a coupling agent.

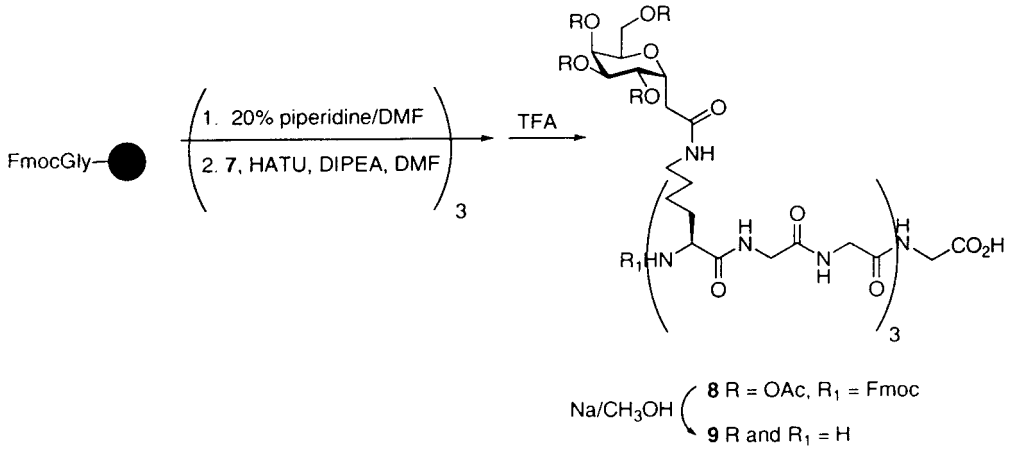
The dipeptide N-terminus was deprotected and then coupled to the commercially available lysine derivative (**3**) to give **4** in 87% yield. Removal of the *tert*-butylcarbamate and coupling of **1** to the ϵ-amino terminus produced **6**. Upon hydrogenolysis, carboxylic acid **7**, a structural analogue of the glycosylated L-arginine-L-alanine-L-alanine tripeptide unit found in lower molecular weight AFGP was produced.

The C-linked AFGP mimic was assembled from building block **7** by conventional solid-phase synthesis using a Wang resin preloaded with Fmoc-glycine (Scheme 3). Successive couplings using *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluourphophate (HATU) as coupling agent followed by cleavage from the resin resulted in glycoconjugate **8**. After removal of the N-terminus protecting group and acetates, **9** was obtained in 55% yield. This

Scheme 2. Synthesis of Glycosylated Tripeptide Component



Scheme 3. Solid-Phase Synthesis of C-Linked AFGP Mimic



C-linked AFGP mimic is presently being tested for antifreeze protein-specific activity.

This paper describes the synthesis of a C-linked AFGP analogue. The synthesis is general in nature and, thus, is amenable to the preparation of other C-linked AFGP mimics necessary to conduct structure−activity relationship studies aimed at elucidating the mechanism by which AFGPs bind to the surface of an ice crystal and inhibit growth. Concurrently, other structurally diverse C-linked AFGP mimics are being prepared in our laboratory. These mimics will elucidate how structure of the saccharide, amino acid side-chain and polypeptide backbone affect ice-binding specificity and affinity.

Acknowledgment. The authors thank the National High Magnetic Field Laboratory (NHMFL) in Tallahassee, FL, for access to high-field NMR and FT-ICR mass spectrometry.

Supporting Information Available: Complete characterization data (^{1}H and ^{13}C NMR and mass spectral data) is available for all new compounds. This material is free of charge via the Internet at http://pubs.acs.org.

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