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Synthesis of a Dimer of β-(1,4)-L-Arabinosyl-(2*S*,4*R*)-4-hydroxyproline Inspired by Art v 1, the Major Allergen of Mugwort

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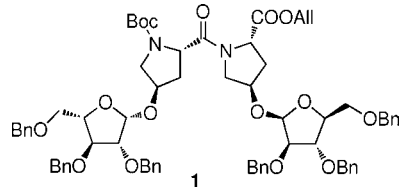
ABSTRACT



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***N*α-*tert*-Butoxycarbonyl-L-trans-4-hydroxyproline allyl ester (Boc-Hyp-OAll) was glycosylated with 2,3,5-tri-*O*-benzyl-L-arabinose *p*-cresylthioglycoside in 60% yield with 4:1 β:α stereoselectivity. Deprotection of *N*- and *C*-terminii independently gave a prolyl amine and prolyl carboxylate respectively that were coupled under standard conditions with 1-[*bis*-(dimethylamino)methylene]-1H-1,2,3-triazolo-[4,5,b]-pyridininium hexafluorophosphate 3-oxide (*N*-HATU) to give the dimer 1 in 46% yield. These results represent the first steps toward the production of homogeneous oligomers to determine the minimal epitope of the Art v 1 allergen.**

The pollen of mugwort (*Artemisa vulgaris*) is a significant contributor to hay fever in Europe and North America. Altmann and co-workers have proposed a model for the structure of the major allergen, Art v 1 (Figure 1). Detailed degradation studies, in conjunction with molecular modeling[1] and characterization by NMR and mass spectrometry,[2] revealed two novel *O*-glycosides linked via *trans*-4-hydroxyproline (Hyp). One of these motifs, characterized by clusters of contiguous β-L-arabinofuranosides of Hyp, was found to be a key recognition element for antibodies generated in response to the natural protein. The identification of the minimal epitope and more detailed characterization requires access to homogeneous glycopeptide fragments. Exceedingly small quantities of Art v 1 glycoprotein can be isolated from pollen. Alkaline hydrolysis of the protein leads to complex mixtures of compounds containing the β-Araf-Hyp motif.

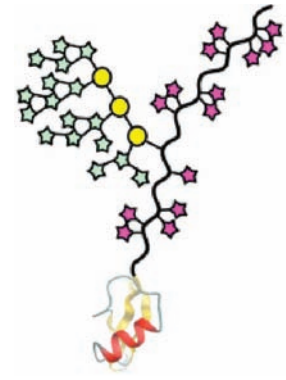

Figure 1. Model for the Art v 1 glycoprotein. Monosaccharide units are represented by: β-D-Gal (yellow circle), α-L-Ara (turquoise star), β-L-Ara (pink star). Copyright The American Society for Biochemistry and Molecular Biology, 2005.

The heterogeneous nature of this digest renders the isolation of even miniscule amounts of pure amino acids and oli-

(1) Himly, M.; Jahn-Schmid, B.; Dedic, A.; Kelemen, P.; Wopfner, N.; Altmann, F.; Van Ree, R.; Briza, P.; Richter, K.; Ebner, C.; Ferreira, F. *FASEB J.* **2003**, *17*, 106–18.

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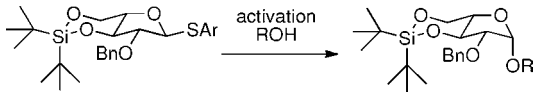
gopeptides an unrealistic goal. The key to accessing useful amounts of high-purity oligomers is via chemical synthesis.

The repetitive β-Ara*f*-Hyp motif of Art v 1 is unprecedented and poses a challenge to synthesis for several reasons. First, it is difficult to control the stereoselectivity of glycosylation with an arabinofuranosyl donor.[3] The anomeric effect is weak due to multiple low energy conformations for furanoses and neighboring group participation favors an α (1,2-*trans*) arabinoside. Second, the hydroxy group of Hyp is a poor nucleophile, in part because of its axial orientation in the preferred Cγ-*exo* conformation of the pyrrolidine ring, and glycosylation rarely proceeds in good yield.[4] Moreover, the best precedents for the assembly of such a glycoclustered peptide lie in the synthesis of the mucins, wherein glycosylated threonine residues have been coupled.[5] The amalgamation of two glycosylated prolines is inherently more difficult since the prolyl amine is secondary and cyclic.

Fortunately, significant progress has been made in recent years vis-à-vis the synthesis of β-arabinosides. Ito's group has used intramolecular aglycone delivery (IAD) to good effect[6] and Lowary has employed 2,3-anhydro sugars.[7] We quickly focused our attention on the independent reports of conformationally restricted glycosyl donors from the Boons[8] and Crich[9] groups (Scheme 1). The 3,5-*O*-di-*tert*-butylsilyl

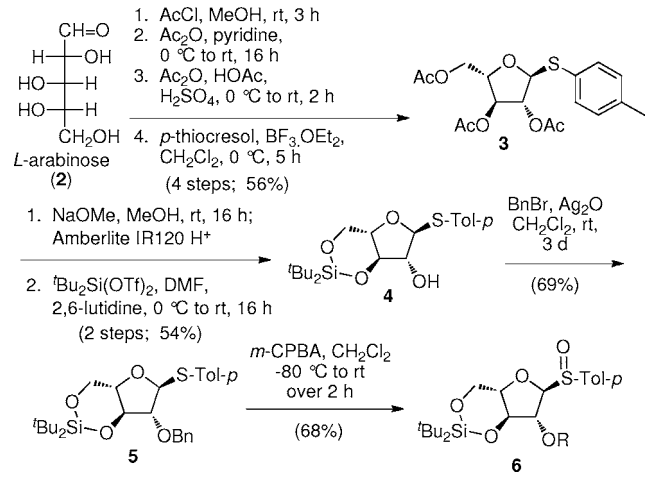
Scheme 1. Conformationally-Restricted Glycosyl Donor



acetal protecting group locks the arabinofuranosyl ring in a conformation[10] that favors nucleophilic attack from the β-face (Scheme 1). A number of subsequent oligosaccharide syntheses have utilized such donors, reporting excellent stereoselectivity in some instances[11] and modest results in other cases.[12] To-date glycosyl acceptors have been predominantly primary and secondary alcohols of monosaccharides. Differences in the stereoselectivity of D- and L-donors has been explored by Zhu.[13]

Thioglycoside and sulfoxide glycosyl donors were prepared according to Scheme 2. We chose to work with the

Scheme 2. Synthesis of Glycosyl Donors



thiocresyl glycoside, as employed by Crich.[9] We obtained mediocre yields of the silyl acetal **4** and experienced considerable difficulty forming the benzyl ether at C2 according to the originally reported, standard conditions (NaH, BnBr, DMF). We found that the nonbasic conditions reported more recently by Zhu's group[13] gave more reproducible results. Oxidation of the thioglycoside gave the sulfoxide **6** as a mixture of diastereomers. We prepared this additional glycosyl donor, since Crich had shown that better β-selectivity was obtained via the sulfoxide method.[9]

Glycosylation of Boc-Hyp-OAll (**7**) with thioglycoside **5** and sulfoxide **6** gave complex mixtures of products (Scheme 3). Isolation of pure **8*β***, for characterization, required HPLC purification. Evidence for the stereochemistry of the glycosidic linkage was afforded by the coupling constant (J = 5.2 Hz) for H1 of the arabinose unit. The plethora of other products made it difficult to determine yields and α/β ratios. The outcome of the reaction was unsatisfactory in both regards.

Our hypothesis was that the poor results of the glycosylation reactions were due to the instability of the silyl acetal to the reaction conditions. To test this theory, we prepared perbenzylated thioglycoside **9** and the corresponding sul-

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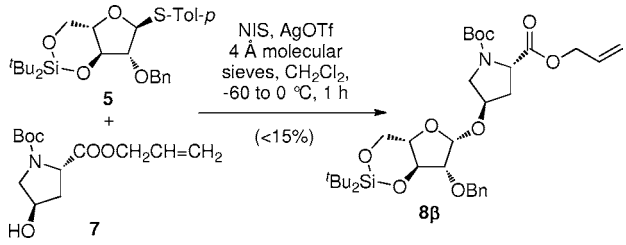
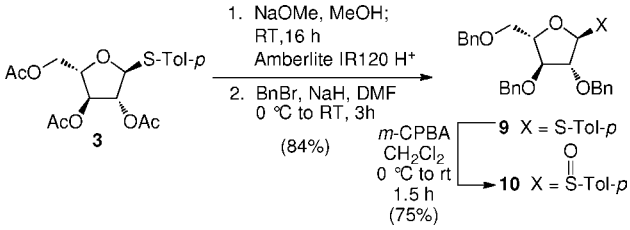
Scheme 3. Glycosylation with Donor **5**



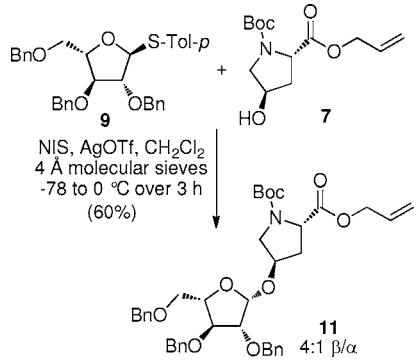
foxide **10** (Scheme 4). Lowary had previously obtained reasonable β-selectivity with *ent*-**9**.[14]

Scheme 4. Preparation of Perbenzylated Donors



To our delight, reaction of thioglycoside **9**[12] with hydroxyproline acceptor **7**, under standard conditions, gave a reasonable yield of glycoside **11** (Scheme 5) with a

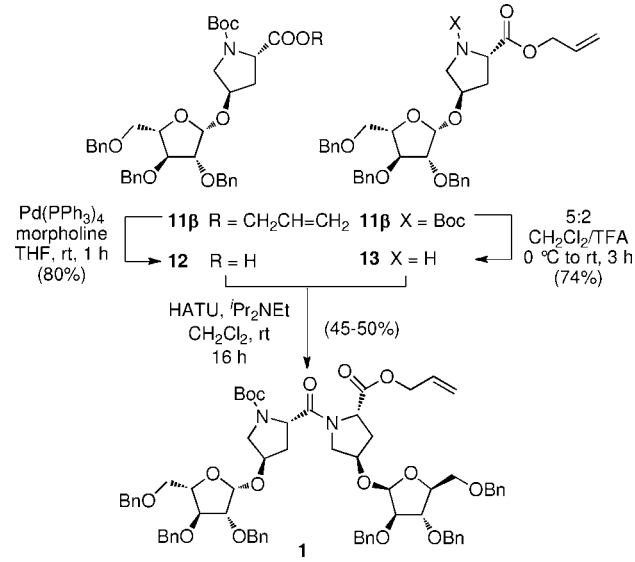
Scheme 5. Formation of Glycoside **11*β***



typical β/α ratio of 4:1. The product mixtures were less complex and the yields significantly improved relative to Scheme 3. Moreover, pure β-glycoside **11*β*** could be obtained by flash chromatography on gram-scale. There are further advantages to this route. The glycosyl donor **9** is available in two steps from **3** in 84% yield (compared to three steps in only 37% yield for donor **5**). The glycoside **11** is more robust than **8** and, downstream, a single hydrogenolytic step will be required to remove all carbohydrate protecting groups. The use of the sulfoxide donor **10** gave similar yields and superior β-selectivity (25:1), as judged by integration of the anomeric proton signals in the ^{1}H NMR spectrum. Unfortunately, the chromatography to separate pure β-glycoside from assorted byproducts was more tedious. This practical consideration, in combination with the extra step to produce the sulfoxide donor **10**, led us to favor the thioglycoside donor **9** for large-scale preparations of **11*β***.

With a practical synthesis of monomer **11*β*** in hand, the next objective became formation of the dimer (Scheme 6).

Scheme 6. Dimerization of Glycoside **11*β***



Selective cleavage of the Boc-group afforded secondary amine **12**. Flash chromatography of amine **12**, prior to the coupling reaction, was beneficial. Deallylation with Pd(PPh$_3$)$_4$ in the presence of morpholine generated the acid **13**, but we were unable to remove traces of the catalyst.

We investigated a number of peptide coupling reagents, renowned for their effectiveness in hindered couplings,[15] including HATU,[16] bromo-*tris*-pyrrolidino-phosphonium hexafluorophosphate (PyBroP)[17] and tetramethylfluoroformamidinium hexafluorophosphate (TFFH).[18] Our best results to-date are given in Scheme 6. The doubly glycosylated dipeptide exists as four conformations, presumably varying about the two central peptide bonds, in approximately equal

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amounts. Further efforts to improve the efficiency of dimer formation, the assembly of larger oligomers, and conformational studies are underway.

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Supporting Information Available: Experimental procedures and ^{1}H and ^{13}C NMR spectra for compounds **1** and **3**−**11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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