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β-Glycosides of Hydroxyproline via an Umpolung Approach

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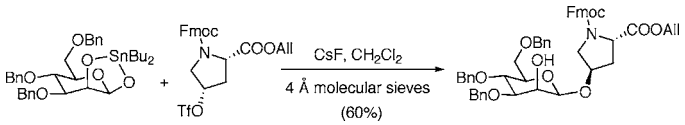
ABSTRACT



Reaction of 1,2-*O*-dibutylstannylene-3,4-6-tri-*O*-benzyl-β-D-mannopyranose with *N*α-fluorenylmethoxycarbonyl-*cis*-4-trifluoromethanesulfonyl-oxyproline allyl ester led to formation of a β-mannoside of *trans*-4-hydroxyproline. Subsequent manipulation of the C2 hydroxy group gave rise to β-D-Glc and β-D-GlcNAc derivatives.

Glycosides of *trans*-4-hydroxyproline (Hyp) have been known for a long time in plants, and these hydroxyproline-rich plant glycoproteins (HRGPs) have been reviewed.[1] Some more recent discoveries are depicted in Figure 1. West and co-workers have elucidated the structure of the pentasaccharide attached to Pro143 in SKP1, a protein associated with ubiquitination in the slime mould *Dictyostelium*.[2] The stereochemistry between the GlcNAc residue and Hyp is presumed to be α, by analogy to α-GlcNAc-Ser/Thr linkages formed by enzymes of the GT27 family in the Golgi. This is a feature which might be clarified by chemical synthesis of relevant model compounds. Leonard et al. recently described the characterization of novel *O*-glycans in Art v 1, the major allergen of mugwort (*Artemisia vulgaris*).[3] Two types of linkage were identified: single β-L-arabinosides and complex arabinogalactans linked via a β-D-Gal residue. In this letter, we describe the development of new methodology that we hope will be useful in the chemical synthesis of prototypical compounds to be used as standards in the identification of natural glycopeptides.

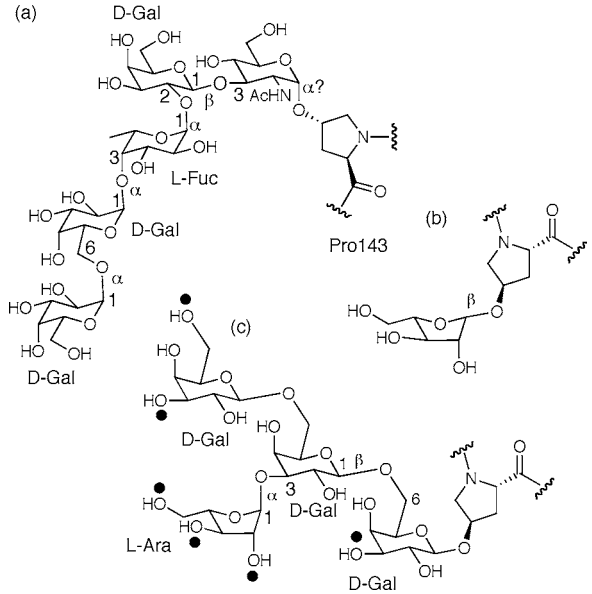


Figure 1. Hyp glycosides in nature: (a) *Dictyostelium discoideum*; (b) and (c) *Artemisia vulgaris*; ● = possible point of further arabinosylation.

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(1) (a) Sommer-Knudsn, J.; Bacic, A.; Clarke, A. E. *Phytochemistry* **1998**, *47*, 483−497. (b) Kieliszewski, M. J. *Phytochemistry* **2001**, *57*, 319−323. (c) Khashimova, Z. S. *Chem. Nat. Comput.* **2003**, *39*, 229−236.

(2) West, C. M. *Cell. Mol. Life Sci.* **2003**, *60*, 229−240 and references therein.

(3) Leonard, R.; Petersen, B. O.; Himly, M.; Kaar, W.; Wopfner, N.; Kolarich, D.; van Ree, R.; Ebner, C.; Duus, J. Ø.; Ferreira, F.; Altmann, F. *J. Biol. Chem.* **2005**, *280*, 7932−7940.

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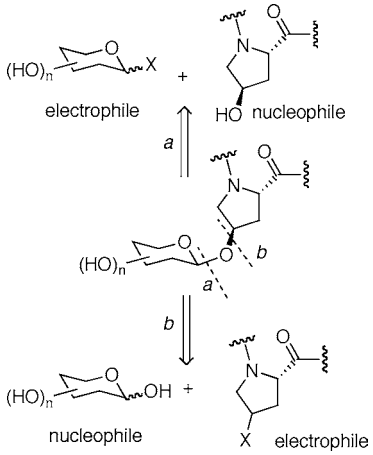
The chemical and enzymatic synthesis of glycosides of Hyp is plagued by low yields.[4] Retrosynthetically, there are two potential disconnections of the glycosidic linkage (designated *a* and *b*, Scheme 1). While approach *a* is

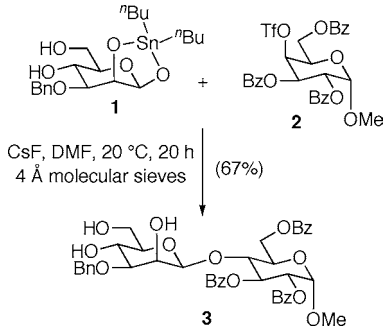
Scheme 1. Retrosynthetic Analysis



conventional, it is only partially successful because the axial hydroxy group of *trans*-4-hydroxyproline is a poor nucleophile. Switching the roles of the sugar and the amino acid in *O*-glycoside formation (route *b*)—that is, umpolung—presents an alternative approach.

Hodosi and Kovác have formed β-glycosides of mannose (Scheme 2) and rhamnose, utilizing 1,2-*O*-stannylene acetals[5]

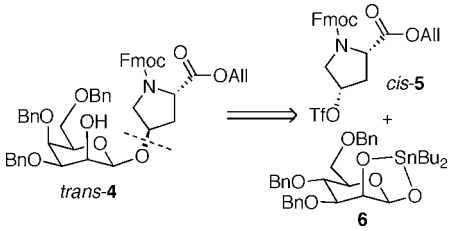
Scheme 2. Precedent for Stannylene Acetals in Glycosylation Reactions



of these sugars and carbohydrate-based triflate reaction partners.[6] This approach "locks" the configuration at the anomeric center, ensuring formation of a β-linkage. Darwish et al. have reported their efforts to apply this chemistry to the synthesis of biologically relevant β-arabinofuranoside derivatives.[7]

The reaction proceeds with inversion of configuration at the electrophilic center. A successful extension of their work seemed an attractive approach to the synthesis of Hyp glycosides (e.g., *trans*-**4**). However, we felt that protection of the alcohols in the glycosyl donor, as benzyl ethers, would be beneficial. This would prevent any side reactions arising from migration of the tin; the electron-donating protecting groups would improve the nucleophilicity of the oxygens in the tin acetal, and intermediates would be less polar and more easily purified. Our immediate targets thus became a suitable Hyp electrophile (e.g., **5**) and the known stannylene acetal **6**[8] (Scheme 3). While β-mannosides of Hyp have not been

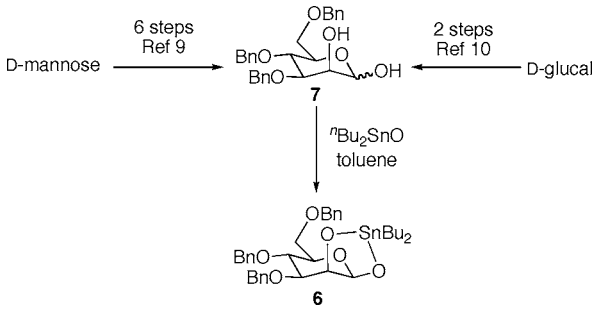
Scheme 3. Specific Details of Retrosynthetic Analysis



identified to date in nature, we saw this as a good test case for the reaction chemistry. Moreover, we believed that manipulation of the C2−OH, with inversion of configuration, might afford access to a range of β-glucosides.

We prepared the 1,2-diol precursor to the stannylene acetal from D-mannose via the 1,2-orthoacetate (Scheme 4).[9] This

Scheme 4. Synthesis of Stannylene Acetal



compound is also available via the more technically demanding route of Gin and co-workers.[10] The latter approach holds appeal since the corresponding stannylene acetal of D-talose ought to be accessible from D-galactal, and this would lead us into the suite of compounds with the D-galacto configuration.

(4) Taylor, C. M.; Weir, C. A.; Jørgensen, C. G. *Aust. J. Chem.* **2002**, *55*, 135−140 and references therein.

(5) For a review of tin chemistry in the field of carbohydrates, see: Grindley, T. B. *Adv. Carbohydr. Chem. Biochem.* **1998**, *53*, 17−142.

(6) (a) Hodosi, G.; Kovác, P. *J. Am. Chem. Soc.* **1997**, *119*, 2335−2336. (b) Hodosi, G.; Kovác, P. *Carbohydr. Res.* **1998**, *308*, 63−75.

(7) Darwish, O. S.; Callam, C. S.; Hadad, C. M.; Lowary, T. L. J. *Carbohydr. Chem.* **2003**, *22*, 963−981.

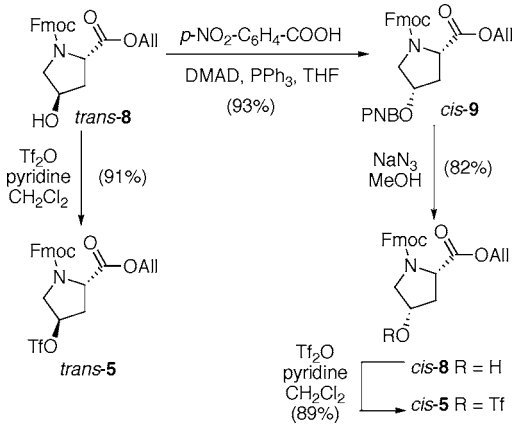
(8) (a) Srivastava, V. K.; Schuerch, C. *Tetrahedron Lett.* **1979**, *35*, 3269−3272. (b) Nicolaou, K. C.; van Delft, F. L.; Conley, S. R.; Mitchell, H. J.; Jin, Z.; Rodríguez, R. M. *J. Am. Chem. Soc.* **1997**, *119*, 9057−9058.

(9) (a) Mazurek, M.; Perlin, A. S. *Can. J. Chem.* **1965**, *43*, 1918−1923. (b) Beignet, J.; Tiernan, J.; Woo, C. H.; Kariuki, B. M.; Cox, L. R. *J. Org. Chem.* **2004**, *69*, 6341−6356.

(10) Kim, J.-Y.; Di Bussolo, V.; Gin, D. Y. *Org. Lett.* **2001**, *3*, 303−306.

On the basis of Hodosi and Kovác's work, a triflate leaving group was the obvious choice. Unfortunately, the triflate of Boc-Hyp-OMe has been described as unstable,[11] so we had some reservations about the viability of **5** (Scheme 5). The

Scheme 5. Synthesis of Hyp and hyp Derivatives

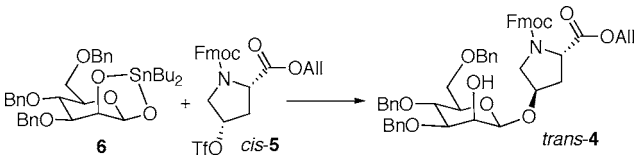


other issue that needed to be addressed was that, in fact, we required a derivative of *cis*-4-hydroxyproline (hyp) since C4 was expected to undergo inversion of configuration during the glycosylation reaction. We prepared hyp derivative *cis*-**5** by analogy to the work of Gomez-Vidal and Silverman.[12]

Glycosylation reactions were conducted under a variety of conditions (Table 1). We began with DMF since this had been the solvent of choice for Hodosi and Kovác.[6] We isolated no glycoside, but obtained the corresponding formate ester **10** in good yield.[13] Dichloromethane ultimately gave the best results since both reaction partners were soluble and stable. Employing 2 equiv of the stannylene acetal **6** relative to triflate *cis*-**5** was beneficial, but increasing the stoichiometry further gave no advantage.

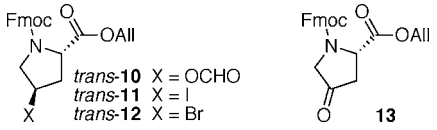
In the alkylation and acylation of carbohydrate-derived stannylene acetals, it is conventional to include a tetrabutylammonium halide salt.[5] We were reluctant to include such a nucleophilic species since it might displace the triflate. Indeed, inclusion of tetrabutylammonium iodide and tetrabutylammonium bromide led to formation of the corresponding 4-halo-prolines **11** and **12**, respectively (entries 5 and 6). Fortunately, better results were forthcoming with the inclusion of fluoride salts. Fluoride is less nucleophilic than the larger halide ions, and we believe it does more than "solubilize the tin complex", as stated by Hodosi and Kovác.[6] In fact, it may serve as a fifth ligand in the monomeric complex[14] or a more reactive dimeric complex.[15] That it does

Table 1. Glycosylation Reactions[a]



entry	ratio 6:5	solvent	additives (equiv)	yield **4** (%)
1	1:1	DMF		*b*
2	1:1	CH_2Cl_2		37
3	2:1	CH_2Cl_2		45
4	6:1	CH_2Cl_2		44
5	2:1	CH_2Cl_2	$^nBu_4NI$ (2)	*b*
6	2:1	CH_2Cl_2	$^nBu_4NBr$ (2)	*b*
7	2:1	CH_2Cl_2	$^nBu_4NF$ (1)	37
8	2:1	CH_2Cl_2	$^nBu_4NF$ (2)	27
9	2:1	CH_2Cl_2	CsF (1)	55
10	2:1	CH_2Cl_2	CsF (2)	60
11	2:1	$CHCl_3$	CsF (2)	59
12	2:1	DMSO	CsF (2)	*b*
13	2:1	CH_2Cl_2	CsF (2) 18-crown-6 (2)	37

[a] 4 Å molecular sieves added to all reactions. [b] Other products isolated:



not harm the triflate, or the Fmoc protecting group,[16] is circumstantial evidence that it is actively complexed with the tin. Replacing the tetrabutylammonium counterion with cesium improved the yield. Disappointingly, inclusion of a crown ether, in a bid to make the fluoride anion more "naked", did not help.

Earlier reports indicated that the rate of reaction is faster in more polar solvents.[6] Chloroform led to no improvement, and DMSO resulted in the isolation of **13**, presumably formed via a Swern-type mechanism.[17]

The NMR spectra of glycoside *trans*-**4** were very complex. To fully assign the spectra and unambiguously confirm stereochemical issues, we removed the Fmoc protecting group (Scheme 6). NMR spectra of secondary amine, *trans*-**14**, were of a single species (not a mixture of rotamers as was the case for *trans*-**4**). The anomeric proton in *trans*-**14** showed nOe's to both H3 and H5, indicating that all three protons are on the same face of the pyranose ring (Figure 2). This is good evidence for the β-stereochemistry of the glycosidic linkage.

It was also necessary to address the stereochemistry at Cγ of the Hyp residue. Since *trans*-**5** was readily available, we glycosylated it, presumably forming *cis*-**4**, the glycoside of hyp (Scheme 6). Following N-deprotection, a single compound was formed (*cis*-**14**) which was distinct from *trans*-

(11) Lowe, G.; Vilaivan, T. *J. Chem. Soc., Perkin Trans. 1* **1997**, 539−546.

(12) Gomez-Vidal, J. A.; Silverman, R. B. *Org. Lett.* **2001**, *3*, 2481−2484.

(13) DMF has been shown to behave as a formate anion equivalent and displace tosylates: Suri, S. C.; Rodgers, S. L.; Radhakrishnam, K. V.; Nair, V. *Synth. Commun.* **1996**, *26*, 1031−1039.

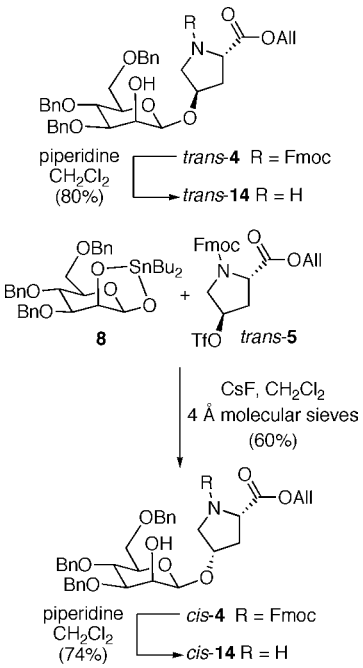
(14) David, S. In *Preparative Carbohydrate Chemistry*; Hanessian, S., Ed.; Marcel Dekker: New York, 1997; pp 69−86.

(15) Bredenkamp, M. W.; Spies, H. S. C.; van der Merwe, M. J. *Tetrahedron Lett.* **2000**, *41*, 547−550.

(16) The Fmoc group can be cleaved by dilute TBAF in DMF: Ueki, M.; Amemiya, M. *Tetrahedron Lett.* **1987**, *28*, 6617−6620.

(17) Tidwell, T. T. *Org. React.* **1990**, *39*, 297−572.

Scheme 6. Fmoc Removal and Synthesis of the Corresponding Glycoside of *cis*-4-Hydroxyproline (hyp)



14. The glycosylation reaction is thus stereospecific with respect to Cγ of the Hyp residue. The assumption that the reaction proceeds with inversion of configuration is supported by nOe experiments. These indicate a weak correlation between Hα and Hγ of the Pro residue in *cis*-**14**; there is no nOe between these protons in *trans*-**14**. The Cγ-exo conformation of the pyrrolidine ring, proposed in Figure 2, is consistent with nOe data.

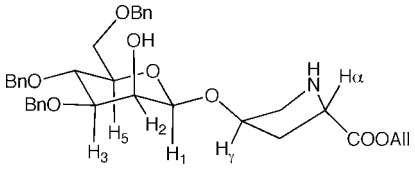
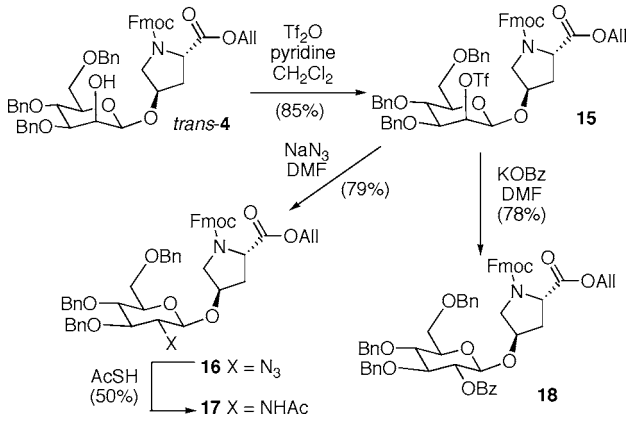


Figure 2. Proposed conformation of β-glycoside *trans*-**14** (after removal of Fmoc group).

While β-mannosides are notoriously difficult to construct,[18] such Hyp glycosides are not known to occur naturally. The real value of compound **4** lies in the "handle" presented at C2. To illustrate this, we have converted the axial alcohol to an equatorial *N*-acetamide, viz. *N*-acetylglucosamine derivative **17** (Scheme 7). We were also able to displace the

Scheme 7. Synthesis of Hyp Derivatives



intermediate triflate with the benzoate anion to give β-D-glucoside **18**.[19]

In summary, we have glycosylated hydroxyproline via a novel approach, employing a carbohydrate nucleophile and an amino acid electrophile. The yield of the glycosylation reaction did not live up to our expectations;[4] perhaps due to partial instability of protecting groups to the reaction conditions. While the method is restricted to the synthesis of β-glycosides, we believe that modification of the functionality and stereochemistry at C2 (Scheme 7) makes our approach competitive. Moreover, extension of this work to compounds with the D-galacto configuration will give rise to many biologically relevant glycosides. Inclusion of these glycosylated amino acid building blocks into oligopeptides can be expected to provide insight into the structure and role of these conjugates in nature.

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Supporting Information Available: Experimental procedures and ^{1}H and ^{13}C NMR spectra for compounds *trans*-**4**, *cis*-**4**, *trans*-**5**, *cis*-**5**, *cis*-**9**, *trans*-**10**, *cis*-**10**, *trans*-**14**, *cis*-**14**, **15**, **16**, **17**, and **18**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL061424M

(18) Gridley, J. J.; Osborn, H. M. I. *J. Chem. Soc., Perkin Trans. 1* **2000**, 1471−1491.

(19) For the displacement of a mesylate using potassium benzoate, see: Miljkovic, M.; Gligorijevic, M.; Glisin, D. *J. Org. Chem.* **1974**, *39*, 3223−3226.