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Synthesis of an Isostere of an O-Linked Glycopeptide

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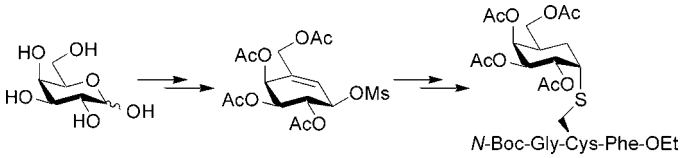
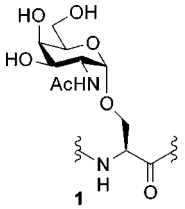
ABSTRACT



A route for the synthesis of an electrophilic, carbocyclic galactose equivalent from D-galactose is described. The strategy utilizes ring-closing metathesis with Grubbs's second-generation catalyst as the key step. The galactose-derived electrophile reacted in an S_N2 fashion with *N*-Boc-cysteine methyl ester to provide an α-galactosylserine isostere. The method was extended to the synthesis of a glycopeptide isostere.

The attachment of carbohydrate chains to proteins is a ubiquitous post- and cotranslational modification known as glycosylation. Glycosylation is linked to several biological processes such as protein transport, cell adhesion, and signal transduction.[1] The conformations of proteins can be altered by the attachment of carbohydrate residues,[2] which may be important for protein folding. Aberrant glycosylation is associated with several diseases, including cancerous and inflammatory conditions.[3] As a result of the role glycosylation plays in these biologically relevant issues, there is a considerable amount of interest in glycoproteins. This in turn creates a requirement for glycoproteins with a defined composition in order to correlate structure and function of specific glycoprotein motifs. However, glycoproteins are biosynthesized as complex mixtures of proteins with varying degrees of glycosylation at specific sites, each component of which is called a glycoform. The presence of mixtures of glycoforms complicates isolation of homogeneous glycoproteins from natural sources. As a result, the development of efficient methods for synthesizing homogeneous glycoproteins is a goal for organic chemists.

A common way in which oligosaccharides are attached to proteins is through the hydroxyl groups of serine and threonine residues, as in the α-*N*-acetylgalactosaminylserine unit **1**. Although solid-phase peptide synthesis (SPPS) can be adapted to allow incorporation of glycosylated amino acids,[4] the synthesis of glycopeptides using SPPS is not entirely routine due to size limitations and problems with deglycosylation during peptide synthesis. However, one method that has potential to become routine is the chemoselective ligation of a carbohydrate chain to a full-length protein.



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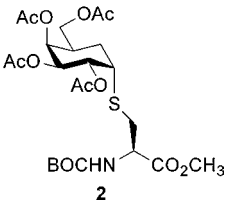

2

Progress toward the development of chemoselective ligation strategies for this purpose was reported by Bertozzi,[5] Flitsch,[6] Jones,[7] Davis,[8] and Schmidt.[9] These methods all

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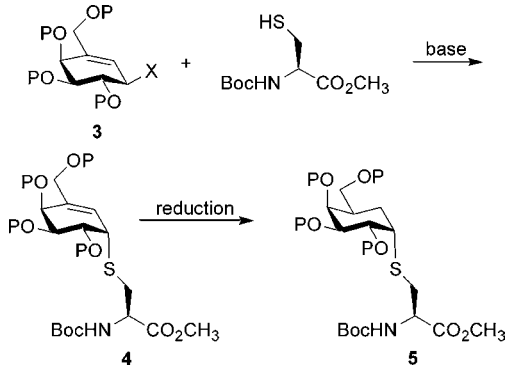
use two functional groups that exclusively react with each other under mild conditions to produce the neoglycoproteins. Although some functional glycoproteins were synthesized using some of these methods, many of the new linkages are substantially different from those found in wild-type glycoproteins. To overcome this obstacle, we propose new chemistry for the ligation of the carbohydrate and peptide components using techniques that construct linkages more similar to those found in wild-type glycoproteins (Scheme 1).

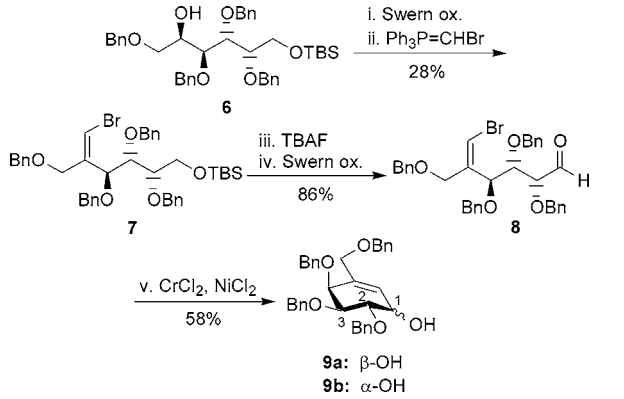
Scheme 1. Synthesis Plan for α-Galactosylserine Isostere **5**



To surmount the difficulty in constructing an acetal, we propose a chemoselective S_N2 reaction between activated cyclohexene **3** and a thiolate derived from cysteine. This would generate unsaturated thioether **4**, whose olefin would be reduced to provide glycosyl amino acid isostere **5**.[10] Isostere **5** differs in two atoms from wild-type structure **1**, but these substitutions are not expected to alter the conformation significantly. However, the stability of **5** to chemical and enzymatic hydrolysis should be higher. We chose to demonstrate the principle of this strategy with the synthesis of α-galactosylserine isostere **2**.

The conversion of carbohydrates to functionalized cyclohexanes or cyclopentanes has been investigated by Ferrier and other researchers.[11] These methods do not provide access to the cyclohexene scaffold of **3** with the correct substitution pattern around the cyclohexene ring. Furthermore, methods for the synthesis of highly substituted cyclohexenes such as **3** from non-carbohydrate starting materials often require the use of microbial oxidation[12] or produce mixtures of stereoisomers.[13] As a result, the synthesis of isostere **2** began from galactose-derived alcohol **6** (Scheme 2).[14] Following a

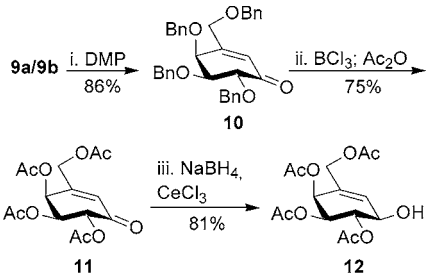
Scheme 2. Synthesis of Galactose-Derived Cyclohexenols **9a**/**9b**



literature procedure,[15] oxidation under Swern conditions[16] provided the ketone, which was then converted to vinyl bromide **7** as a 6:1 *Z*:*E* mixture. Removal of the TBS ether unveiled the alcohol, which underwent smooth conversion to aldehyde **8** under Swern conditions. Upon treatment with $CrCl_2$ and $NiCl_2$, intramolecular Nozaki−Hiyama−Kishi cyclization[17] occurred to provide cyclohexenols **9a** and **9b** in a 6:1 **9a**:**9b** ratio. Cyclohexenols **9a** and **9b** were separated by HPLC for characterization, and assignment of the relative configuration of each was accomplished by ^{1}H NMR analysis.[18]

Early experiments showed that the steric bulk of the multiple benzyl ether protecting groups affected the planned olefin reduction, so a route was devised to replace them with acetates (Scheme 3). Oxidation of **9a**/**9b** with Dess−Martin

Scheme 3. First-Generation Synthesis of Cyclohexenol **12**



periodinane[19] produced perbenzylated cyclohexenone **10**. The benzyl ethers were removed using boron trichloride, and the free hydroxyls were reprotected as acetates to yield per-

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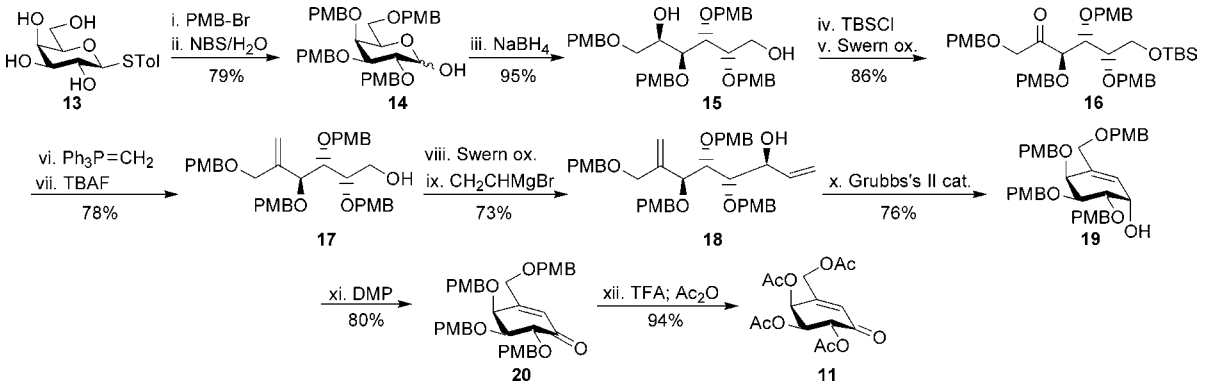
(10) Reduction of the olefin to the saturated carbocycle followed by mesylation and reaction with a thiolate nucleophile failed. The reaction of a mesylate related to compound **3** (P = Bn, X = OMs) with *N*-acetylserine methyl ester did not undergo the substitution reaction to produce an oxygen-linked analogue.

Scheme 4. Synthesis of Enone **11** Using Ring-Closing Metathesis

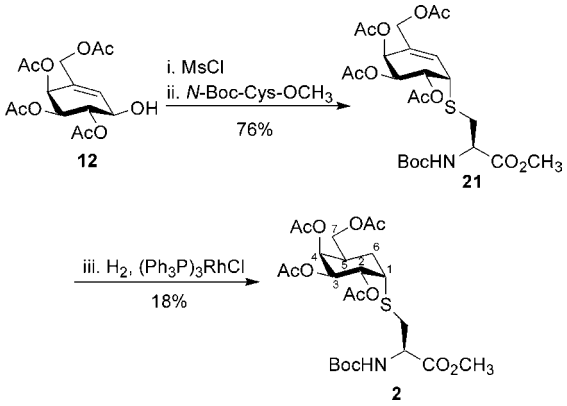


acetylated cyclohexenone **11**. Reduction of the ketone under Luche conditions[20] provided pseudoequatorial cyclohexenol **12** as a 30:1 ratio of diastereomers.

The first-generation synthesis of **12** possessed several low yields, which led us to develop a second-generation synthesis (Scheme 4) using a ring-closing metathesis inspired by the work of Madsen[21] and Lowary.[22] Starting from *p*-tolyl 1-thio-*β*-D-galactopyranoside **13**,[23] the free hydroxyls of **13** were protected as *p*-methoxybenzyl ethers and the anomeric position deprotected with *N*-bromosuccimide in wet acetone[24] to provide pyranose **14**. Sodium borohydride reduction of **14** furnished diol **15**. Selective protection of the primary alcohol of **15** as a TBS ether followed by Swern oxidation of the secondary alcohol provided ketone **16**. Wittig olefination of the ketone and deprotection of the TBS ether occurred smoothly to give alcohol **17**. After Swern oxidation of **17** to the aldehyde, addition of vinylmagnesium bromide yielded a 4:1 mixture of allylic alcohols **18** (major isomer shown). Ring-closing metathesis of the diastereomeric mixture of alcohols **18** was carried out with 10 mol % Grubbs's second-generation catalyst[25] in refluxing toluene to provide the cyclohexenols **19** (major isomer shown). The mixture of diastereomers was oxidized to the enone **20** with Dess−Martin periodinane. Removal of the PMB ethers with trifluoroacetic acid followed by acetylation of the free hydroxyls furnished enone **11**.

With suitable amounts of **11** available, α-galactosylserine isostere **2** was completed (Scheme 5). Conversion of **12** to the allylic mesylate was accomplished cleanly with methanesulfonyl chloride.

Scheme 5. Synthesis of α-Galactosylserine Isostere **2**



Reaction of the crude mesylate with *N*-Boc cysteine methyl ester occurred with complete inversion at the mesylate-bearing stereocenter to provide axial allylic sulfide **21**. Reduction of **21** with 750 psi H_2 in the presence of Wilkinson's catalyst gave isostere **2** as the major isomer. Following purification by preparative thin-layer chromatography, no other isomers could be detected. Under these conditions, an equal amount of carbon−sulfur bond reduction was observed.

The synthesis of α-galactosylserine isostere **2** demonstrated the proof-of-principle for this technique. Extension to the synthesis of a glycopeptide isostere broadened the scope of the method and required a peptide possessing a free cysteine.

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(18) In **9a**, $J_{\text{vinyl proton,1}} = 2.5$ Hz and $J_{1,2} = 7$ Hz, suggesting a diaxial relationship between H-1 and H-2. In **9b**, both of these coupling constants are 4 Hz, suggesting that there is an axial−equatorial relationship between H-1 and H-2. In addition, NOE interactions between H-1 and H-3 were observed for **9a** and between H-1 and H-2 for **9b**.

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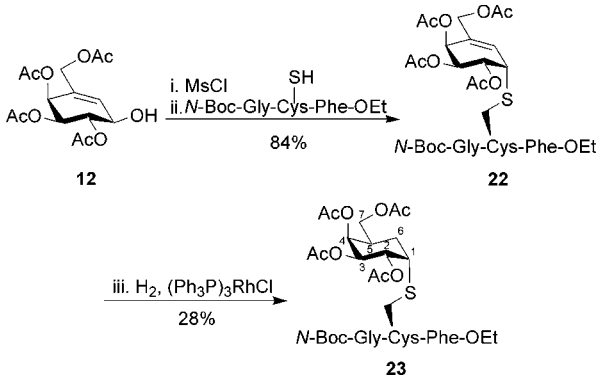
The tripeptide *N*-Boc-Gly-Cys(SH)-Phe-OEt was chosen as a representative example (synthesis in Supporting Information). Following mesylation of **12**, reaction of *N*-Boc-Gly-Cys(SH)-Phe-OEt with the crude mesylate occurred with complete inversion of configuration at the mesylate-bearing carbon to provide axial allylic thioether **22** (Scheme 6).

Scheme 6. Synthesis of Glycopeptide Isostere **23**

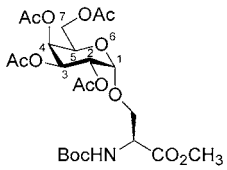


Reduction of **22** with 500 psi H_2 in the presence of Wilkinson's catalyst proceeded with an equal amount of carbon−sulfur bond reduction. Flash chromatography of the reaction mixture provided **23** as a single isomer.

^{1}H NMR analysis of **2** provided strong evidence that the cyclohexane ring possessed a $^4C_1$-like chair conformation. Using automated difference SPT-NMR spectroscopy,[26] several coupling constants of the cyclohexane ring protons of **2** were obtained for comparison with the acetylated α-galactosylserine (GalSer, Table 1). For **23**, the same data was obtained using a double-quantum-filtered COSY experiment. Comparison of $J_{1,2}$, $J_{2,3}$, and $J_{3,4}$ for **2**, **23**, and GalSer suggests that the conformations of the carbocycles in each compound are similar. However, this is a comparison of only three coupling constants. Furthermore, as **2**, **23**, and GalSer still possess protecting groups, this does not accurately reflect their conformations in aqueous solution of the corresponding deacylated compounds, which may be different. The large $J_{5,6ax}$ values for **2** and **23** confirmed the stereochemistry at C-5 resulting from the olefin reductions.

In summary, two routes to a carbocyclic galactose equivalent were developed, one using an intramolecular Nozaki−Hiyama−Kishi cyclization, the second using a ring-closing metathesis. An allylic pseudoequatorial alcohol at C-1 of this carbocycle was converted to an electrophilic mesylate that reacted with the free thiols of cysteine and a cysteine-containing peptide in an S_N2 fashion to yield allylic thioethers. Hydrogenation of the trisubstituted alkenes under high pressure in the presence of Wilkinson's catalyst provided glycosyl amino acid and glycopeptide isosteres.

Table 1. ^{1}H NMR Analysis of α-Galactosylserine Isostere **2**, Glycopeptide Isostere **23**, and Wild-Type α-Galactosylserine



α-Galactosylserine (GalSer)

	2	23	GalSer
$J_{1,2}$ (Hz)	1-2	3.1	3.7
$J_{2,3}$	12.1	10.2	10.0
$J_{3,4}$	1-2	2.8	3.3
$J_{6ax,6eq}$	14.1	13.5	N/A
$J_{6ax,1}$	3.6	6.6	N/A
$J_{6eq,1}$	1-2	3.1	N/A
$J_{6ax,5}$	13.3	9.9	N/A

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Supporting Information Available: Experimental details and full characterization of all new compounds; ^{1}H and ^{13}C NMR spectra of selected compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0490779

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