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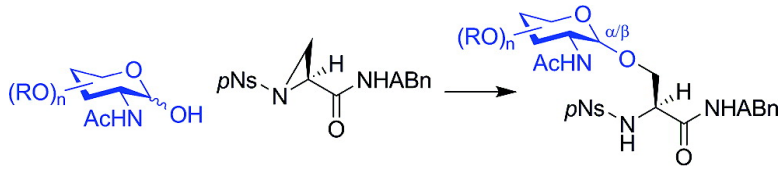

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Ring-Opening of Aziridine-2-Carboxamides with Carbohydrate C1-*O*-Nucleophiles. Stereoselective Preparation of α- and *β*-*O*-Glycosyl Serine Conjugates

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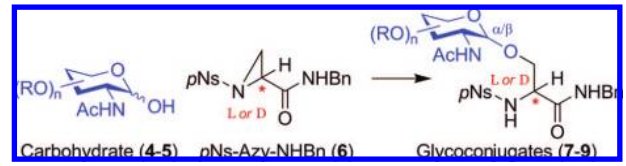
O-Glycosylation of Ser/Thr residues within glycoproteins are common post-translational modifications, often serving as key determinants of biological function. α-D-GalNAc (**1**, Chart 1, found in mucins, blood group determinants, tumor antigens, antifreeze glycoproteins),[1−3] *β*-D-GlcNAc (**2**, present in eukaryotic regulatory proteins),[4] and α-D-Man (**3**, identified in yeasts, molds, and mammals)[5] comprise some of the more common natural carbohydrate anchor residues. Of these, the α-D-GalNAc-Ser/Thr connection (**1**) is the most difficult to construct by chemical glycosylation, as it incorporates a pyranosyl C2-acetamido group in a 1,2-cis configuration that directly opposes traditional neighboring group participatory effects. Current approaches employ either electrophilic glycosyl donors with nonparticipatory C2-aza groups, such as azide[6] and oxazolidinone[7] for α-glycosylation of Ser/Thr nucleophiles, or *O*-conjugate addition to C2-nitro-glycals.[8] These methods require subsequent C2-*N*-functional group interchange to access an appropriate glycosyl amino acid for use in solution or solid phase peptide synthesis. Remarkably, the complementary coupling approach employing pyranose C1-*O*-hemiacetal nucleophiles with Ser-derived electrophiles has not been reported. We describe herein the stereoselective formation of the α-GalNAc-Ser, *β*-GlcNAc-Ser, and α-Man-Ser linkages via the ring opening of aziridine-2-carboxamides with pyranose C1-*O*-nucleophiles.

Reports of regioselective ring-opening of aziridine-2-carboxylate derivatives with oxygen nucleophiles are uncommon.[9] Although $BF_3{\cdot}OEt_2$[10,11] and CuOAc/DBU[12] have been shown to be effective aziridinolysis promoters with alkyl and aryl alcohol nucleophiles, the analogous process involving hemiacetal nucleophiles, characteristic of carbohydrates, remains elusive. Initial attempts (Table 1, entries 1 and 2) at regioselective aziridinolysis of *N*-benzyl-1-*p*-nosyl-L-aziridine-2-carboxamide (L-**6**) with 2-acetamido-2-deoxy-3,4,6-tri-*O*-benzyl-D-glucopyranose (**4**) by applying these existing methods proved ineffective, requiring the development of an alternate coupling procedure.

It was found that the use of alkali metal salts of carbohydrate hemiacetals provided rapid regio- and stereoselective aziridine opening. For example (entries 3 and 4), treatment of a mixture of the protected GlcNAc hemiacetal **4** and aziridine L-**6**, with either KH or NaH in DMF, led to good yields of the glycoconjugate **7**, favoring the contrasteric α-anomer. Conversely, when THF solvent was employed (entries 5 and 6), high *β*-anomeric selectivities in **7** were achieved with similar yields.[13] Application of these coupling conditions to 2-acetamido-2-deoxy-3,4,6-tri-*O*-benzyl-D-galactopyranose (**5**, entries 7 and 8) with aziridine L-**6** proceeded with comparable efficiencies, obtaining either high α-selectivity (KH/DMF) to directly afford the Tn-tumor antigen α-GalNAc-Ser linkage (**8α**, entry 7), or high *β*-selectivity (NaH/THF) to furnish its anomeric counterpart (**8*β***, entry 8). Importantly, the analogous couplings (KH/DMF or NaH/THF) of protected GalNAc hemiacetal **5** with the D-aziridine carboxamide D-**6** (entries 9 and 10), led to the α- and *β*-GalNAc-D-Ser diastereomers **9α** and **9*β***, with high anomeric selectivity. These latter examples not only indicate the suitability of aziridine electrophiles of the D-configuration in this reaction, but also implicitly verify the absence of amino acid epimerization during the process. Taken together, these initial experiments signal the versatility of anomeric stereocontrol in C1-*O*-alkylation with judicious selection of solvent and metal counterion,[14−16] effects which have received limited attention for the C2-NHAc class of carbohydrates[17−20] and heretofore have remained unexplored for the synthesis of natural glycopeptide conjugates.

Chart 1

Table 1



Entry	Carbohydrate + Aziridine	Reagent	Solvent	Glycoconjugate	Yield (α:β)
1	4 + L-6	$BF_3{\cdot}OEt_2$	CH_2Cl_2	Bn_3-D-GlcNAc-pNs-L-Ser-NHBn **7α/β**	< 5%
2		CuOAc/DBU	PhMe		< 5%
3		KH	DMF		74% (8:1)
4		NaH	DMF		71% (8:1)
5		KH	THF		78% (1:4)
6		NaH	THF		79% (1:20
7	5 + L-6	KH	DMF	Bn_3-D-GalNAc-pNs-L-Ser-NHBn **8α/β**	73% (10:1
8		NaH	THF		77% (1:20
9	5 + D-6	KH	DMF	Bn_3-D-GalNAc-pNs-D-Ser-NHBn **9α/β**	67% (5:1)
10		NaH	THF		76% (1:20

However, this coupling reaction of **4/5** with **6** was found to be best applied to secondary amides of aziridine-2-carboxamides.[21] Adhering to this constraint, a new secondary amide protective group for carboxylic acids was developed to expand the versatility of this coupling reaction. Consideration of an *o*-allylbenzyl derivative (ABn) as a *C*-terminus amide protective group proved fruitful in this regard, both in terms of its compatibility with the aziridine opening process, as well as its ability to undergo multifaceted functionalization.

In establishing the former criterion (Chart 2), the ABn-derivatized *p*-Ns-aziridine-2-carboxamide (**11**, *p*Ns-Azy-NHABn) was coupled with various carbohydrate hemiacetals **10**, using either optimized conditions "A" (KH, 18-cr-6, DMF) or "B" (NaH, PBu_3,[22] 1,4-

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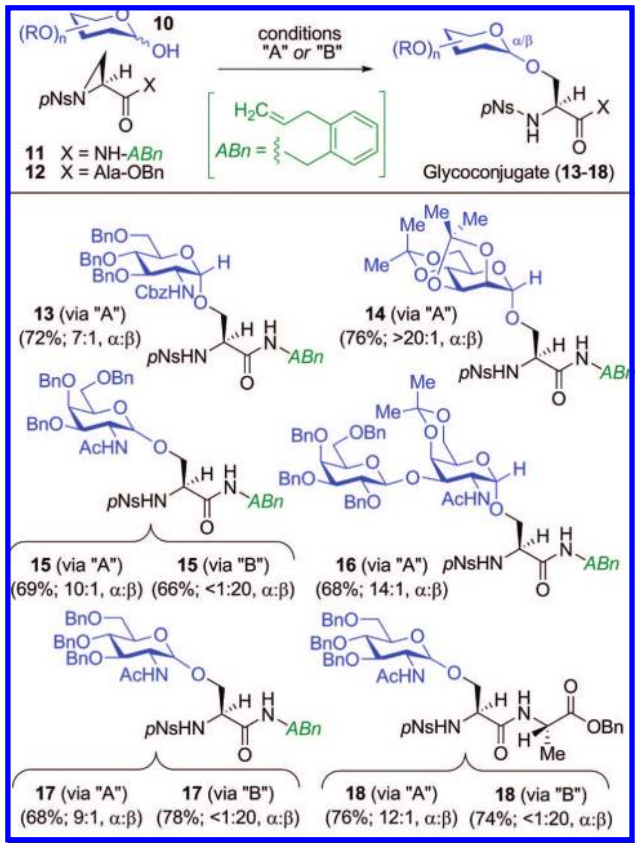
Chart 2[a]



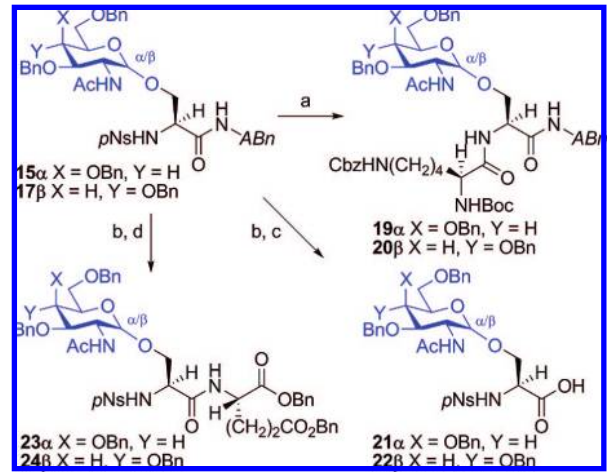
[a] Reagents and conditions: (A) KH, 18-cr-6, DMF, 0 °C; (B) NaH, PBu_3, 1,4-dioxane, 23 °C.

dioxane). Anomeric stereoselective couplings were effected with aziridinyl amides **11** or **12** to furnish the conjugates **13**, **14**, **15α/β** (**15α** = Tn-antigen), **16** (T-antigen), **17α/β**, and **18α/β**. Notably, the glycosyl-adducts **16** and **18** illustrate aziridinolysis with a disaccharide nucleophile and a dipeptide-derived electrophile, respectively.

The utility of *p*Ns-Azy-NHABn (**11**), beyond its role as a suitable electrophile, lies in its ability to undergo useful *N*- and *C*-terminal derivatization following aziridinolysis (Scheme 1). Thiol-mediated *N*-*p*Ns deprotection is a standard procedure;[23,24] however, modification of this protocol by performing it in the presence of a suitable thioester leads to in situ *N*-acylation.[25] For example, a direct *N*-terminus extension of GalNAc adduct **15** (Scheme 1) to dipeptide **19** was accomplished by treatment with PhSH and Boc-Lys(Cbz)-SPh. Conversely, selective liberation of the *C*-terminus of **15** is possible via oxidative alkene cleavage[26] of the ABn protective group and Schiff base formation to generate the cyclic *N*-acyl-enamide. Subsequent Ce^{IV}-mediated benzylic oxidation[27] provides the transient *N*-acyl-isoquinolinium species, which can either (1) undergo in situ hydrolysis to provide the carboxylic acid **21** as a monomer for solution or solid-phase peptide synthesis, or (2) engage in direct *C*-terminus extension via acyl transfer to an amino group, such as that in H-Glu(Bn)-OBn, to furnish dipeptide **23**. Similar outcomes for amino acid functionalization can be seen with the protected *β*-GlcNAc-Ser conjugate (**17**→**20**, **22**, **24**).

The feasibility of aziridine 2-carboxamide ring-opening with pyranose C1-*O*-nucleophiles has been established. The process provides good levels of anomeric selectivity and is tolerant to the native C2-NHAc group. This, in addition to novel methods for *N*- and *C*-terminus extension of amino acids, should provide access

Scheme 1[a]



[a] Reagents and conditions: (a) Boc-Lys(CBz)-SPh, PhSH, K_2CO_3, DMSO, CH_3CN, 23→90 °C, 80% (**19**), 76% (**20**); (b) OsO_4, $(nBu_4N)_2IO_4$, 2,6-lutidine, acetone, H_2O, 23 °C; TFA, CH_3CN, 23 °C, 70% (from **15**), 82% (from **17**); (c) $(py\text{-}H)_2CeCl_6$, THF, H_2O, 23 °C, 83% (**21**), 79% (**22**); (d) H-Glu(Bn)-OBn, Et_3N, $(py\text{-}H)_2CeCl_6$, CH_2Cl_2, 23 °C, 72% (**23**), 74% (**24**).

to new building blocks for glycopeptide synthesis, with particular attention to the challenging α-GalNAc-Ser motif.[28]

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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