

# First synthesis of a C-glycoside analogue of a tumor-associated carbohydrate antigen employing samarium diiodide promoted C-glycosylation

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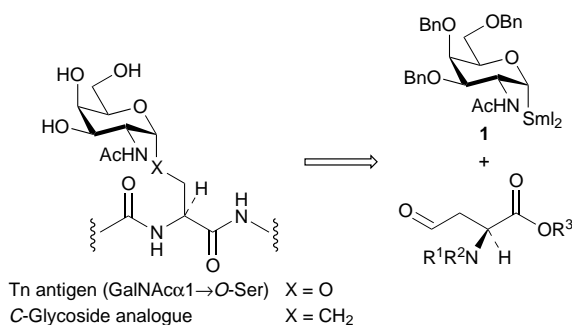
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**A hydrolytically stable analogue of the Tn antigen has been efficiently synthesized for the first time employing a SmI<sub>2</sub>-promoted coupling of the pyridyl sulfone of N-acetylgalactosamine with an aldehydamino acid derivative.**

The Tn (GalNAcα1→O-Ser/Thr) and sialyl Tn (NeuAcα2→6GalNAcα1→O-Ser/Thr) epitopes are major tumor-associated O-linked glycopeptide motifs of cell surface glycoproteins which are expressed in over 70% of human epithelial cancers such as lung, colon, stomach and breast carcinomas.<sup>1</sup> In normal cells, however, the Tn antigen is cryptic, where it is further glycosylated giving rise to complex carbohydrates of the mucin-type glycoproteins.<sup>2</sup> This antigen has also been identified as a partial structure of the HIV envelope glycoprotein gp120.<sup>3</sup> We are particularly interested in preparing analogues of these epitopes as molecular components of potential small molecular weight synthetic vaccines,<sup>4</sup> with high immunogenicity and *in vivo* stability against various carcinoma. A C-glycoside analogue in which the interglycosidic oxygen is replaced with a methylene group<sup>5</sup> may well conform to such desired properties. Here we present for the first time the synthesis of one such mimic of these tumor-associated antigens, namely that of O-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-serine (Scheme 1).

Several recent syntheses of C-glycosidic amino acids have now appeared in the literature, although only one has been directly applied to 2-hexosamine derivatives. In this approach, Kessler reported the stereoselective synthesis of a C-glycoside mimic of N<sup>4</sup>-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-asparagine, an important constituent of N-glycopeptides, via the coupling of a glycosyl dianion with a modified aspartic acid derivative as the key step.<sup>6,7</sup>

We have recently shown that reductive samarium of glycosyl pyridyl sulfones in the presence of carbonyl substrates leads to a viable and rapid route to C-glycosides.<sup>8</sup> In particular, we have demonstrated that the anomeric organosamarium of N-acetylgalactosamine affords predominantly α-C-glycosides upon condensation with simple aldehydes and ketones.<sup>8c,f</sup> We hypothesized that with a suitable aldehyde precursor of an amino acid derivative, its coupling with **1** could lead to the



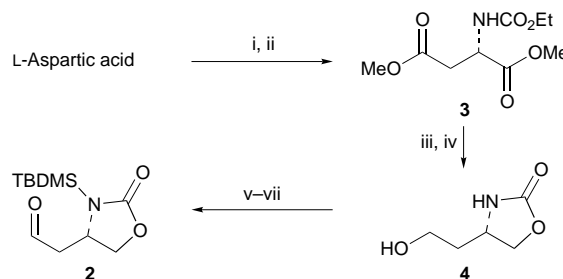
Scheme 1

required C-glycosidic amino acid after a subsequent deoxygenation step (Scheme 1). Preliminary results in this direction showed that the carbon chain of the aldehyde precursor was of ideal length for the coupled product to undergo *in situ* lactonization upon addition of the C<sup>1</sup>-glycosyl anion to the aldehyde C=O bond, thus complicating the following deoxygenation.<sup>8f</sup> To prevent this event, we decided to employ the cyclic carbamate derivatives of the type **2**, the synthesis of which is outlined in Scheme 2.

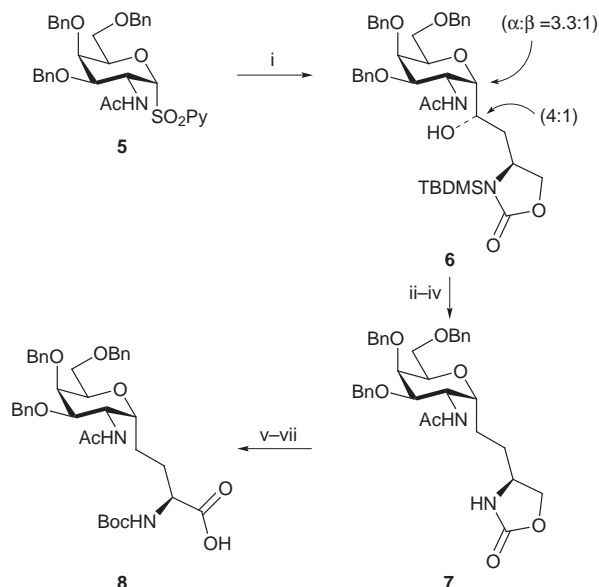
L-Aspartic acid was easily converted to alcohol **4** in four steps adopting the protocol described by McKillop for the conversion of amino acids to their corresponding cyclic carbamates.<sup>9</sup> Hence, esterification in acidic MeOH and treatment with ClCO<sub>2</sub>Et led to the dimethyl ester **3**. Subsequent reduction to the diol and cyclisation upon treatment with 2 equiv. of NaH afforded the cyclic carbamate **4** in an overall yield of 81%. Bis(silylation) was accomplished upon treatment of **4** with TBDMSOTf in collidine, after which the primary alcohol could be selectively liberated with HF in MeCN.<sup>10</sup> Oxidation employing the Swern conditions then gave crystalline aldehyde **2** in high yield.

The key coupling step, outlined in Scheme 3, was performed by adding a THF solution of SmI<sub>2</sub> (2 equiv.) to the previously described glycosyl pyridyl sulfone **5**<sup>8c,f</sup> and aldehyde (1.2 equiv.) at room temperature. An immediate reaction ensued, as monitored by the instantaneous consumption of the one-electron reducing agent, leading after work up to the isolation of the desired C-glycosides in high yield (82%) in favor of the α-anomers **6**‡ (α:β, 3.3:1). We note again the success of this anionic C-glycosylation reaction even though pyridyl sulfone **5** possesses an acidic NH proton. It was necessary to install a protecting group at the carbamate nitrogen in **2** for the coupling reaction to succeed. In its absence (*e.g.* replacing the TBDMS group by H), protonation at C1 of the sugar unit was the sole product observed.§

Whereas treatment of **6** (major isomer) with thiocarbonyl diimidazole¶ surprisingly led to the formation of a cyclic oxazoline involving the C2 acetamido group, use of the traditional Barton deoxygenation method proved effective for the removal of the newly created C7 hydroxy group. Thus



**Scheme 2** Reagents and conditions: i, AcCl, MeOH; ii, ClCO<sub>2</sub>Et, NaHCO<sub>3</sub>, 93% (2 steps); iii, NaBH<sub>4</sub>, CaCl<sub>2</sub>, EtOH-THF (2:1); iv, NaH, THF, 87% (2 steps); v, TBDMSOTf, collidine; vi, HF, MeCN, 71% (2 steps); vii, (COCl)<sub>2</sub>, Et<sub>3</sub>N, DMSO, 99%



**Scheme 3** Reagents and conditions: i, **2** (1.2 equiv.),  $\text{SmI}_2$  (2.2 equiv.) THF, 20 °C, 82%; ii, NaH,  $\text{CS}_2$ , MeI, THF, 83%; iii,  $\text{HSnBu}_3$  (1.5 equiv.), AIBN (0.05 equiv.), toluene, 110 °C; iv, TBAF (3.5 equiv.), THF, 95% (2 steps); v,  $(\text{Boc})_2\text{O}$  (1.5 equiv.),  $\text{Et}_3\text{N}$  (2.0 equiv.), DMAP (cat.), THF; vi,  $\text{Cs}_2\text{CO}_3$  (cat.), MeOH, 83% (2 steps); vii, Jones oxidation, 70%

formation of the methyl xanthate from alcohol **6**, followed by tin hydride promoted radical deoxygenation, afforded **7** after desilylation in 79% (three steps). A three-step procedure involving introduction of a Boc group, selective hydrolysis of the cyclic carbamate<sup>11</sup> and oxidation of the primary alcohol to its corresponding carboxylic acid<sup>11</sup> then completed the synthesis of the desired *C*-glycosyl amino acid.<sup>||</sup>

In conclusion, we have successfully prepared a hydrolytically stable Tn antigen mimic *via* a  $\text{SmI}_2$ -promoted *C*-glycosylation protocol. This important amino acid building block is now ready to be incorporated into small peptide chains in order to test them as potentially viable synthetic peptide vaccines against various human epithelial cancers, the results of which will be reported in due course.

## Notes and References

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‡ Selected data: for **6**  $\delta_{\text{H}}$  (250 MHz,  $\text{CDCl}_3$ ) (major isomer) 6.18 (d, 1 H,  $J$  8.4, NH), 4.37 (ddd, 1 H,  $J$  9.4, 6.5, 2.9,  $\text{H}_5$ ), 4.18 (dd, 1 H,  $J$  11.2, 9.4,  $\text{H}_{6a}$ ), 4.16 (ddd, 1 H,  $J$  8.4, 3.1, 2.2,  $\text{H}_2$ ), 3.88 (dd, 1 H,  $J$  3.1, 3.1,  $\text{H}_3$ ), 3.76 (dd, 1 H,  $J$  6.5, 3.1,  $\text{H}_4$ ), 3.74 (dd, 1 H,  $J$  3.5, 2.2,  $\text{H}_1$ ), 3.69 (dd, 1 H,  $J$  11.2, 2.9,  $\text{H}_{6b}$ ).

§ The protecting group on the carbamate nitrogen also plays a pivotal role for obtaining high coupling yields of the *C*-glycoside as exemplified by the use of the Boc group, where the *C*-glycosylation yield was reduced by one half. Another advantage is that it provides easily separable isomers.

¶ We have recently applied this reagent successfully for the deoxygenation of a *C*-disaccharide prepared *via* the  $\text{SmI}_2$  route [see ref. 8(b)].

|| It is interesting to note that *C*-glycoside **8** does not occupy the normally expected <sup>4</sup>*C*<sub>1</sub> chair conformation, as is seen from the coupling constants ( $J_{2,3} = 4.0$ ,  $J_{3,4} = 2.9$ ,  $J_{4,5} = 5.6$  Hz). This has also been observed for other *N*-acetyl- $\alpha$ -*C*-galactosamines (ref. 12) and monosaccharides (ref. 13) derivatives possessing benzyl protecting groups at the C3, C4 and C6 hydroxy groups. However, the corresponding deprotected or peracetylated *C*-glycosides were found to possess the normal chair conformation.

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