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Stereoselective Synthesis of Sialylated Tumor-Associated Glycosylamino Acids

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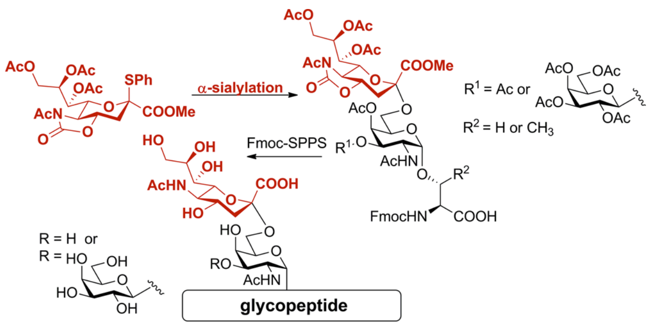
ABSTRACT



Suitably protected sialyl T_N and 2,6-sialyl T tumor-associated carbohydrate antigen-derived amino acids have been prepared stereoselectively using an oxazolidinone-derived sialoside donor. These glycosylamino acids can be employed directly in the solid-phase synthesis of glycopeptides, as demonstrated by the efficient preparation of tumor-associated MUC1 glycopeptide fragments.

Aberrant glycosylation of cell surface glycoproteins is a common feature on numerous tumor cell types.[1] These often truncated carbohydrate structures, called tumor-associated carbohydrate antigens (TACAs), are a result of the downregulation of glycosyltransferase enzymes which occurs with concomitant overexpression of sialyltransferases upon tumor progression (Figure 1).[1,2] Four common TACAs include *N*-acetylgalactosamine (GalNAc) and Gal-β-1,3-GalNAc, commonly termed the T_N and T antigens, as well as the sialylated antigens, sialyl T_N (ST_N) and 2,6-sialyl T (ST).[1,3] An example of a protein that is highly overexpressed on epithelial tumor cells and bears these TACAs is the mucin glycoprotein MUC1. Specifically, the extracellular domain of MUC1 displays numerous copies of these truncated glycans on serine and threonine residues within a 20 amino acid repeat called the variable number tandem repeat (VNTR) region.[2,4] This leads to the exposure of peptide epitopes which can be targeted by the immune system, a property that has been exploited in the development of synthetic glycopeptide cancer vaccines.[3,4]

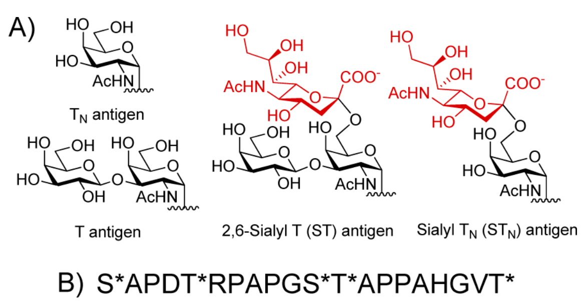


Figure 1. (A) Structures of TACAs and (B) the primary sequence of the VNTR of MUC1 (* indicates glycosylation sites).

One strategy for the synthesis of vaccine candidates (and other glycopeptides) involves the preparation of suitably protected glycosylamino acid cassettes followed by incorporation into targets through Fmoc-strategy solid-phase

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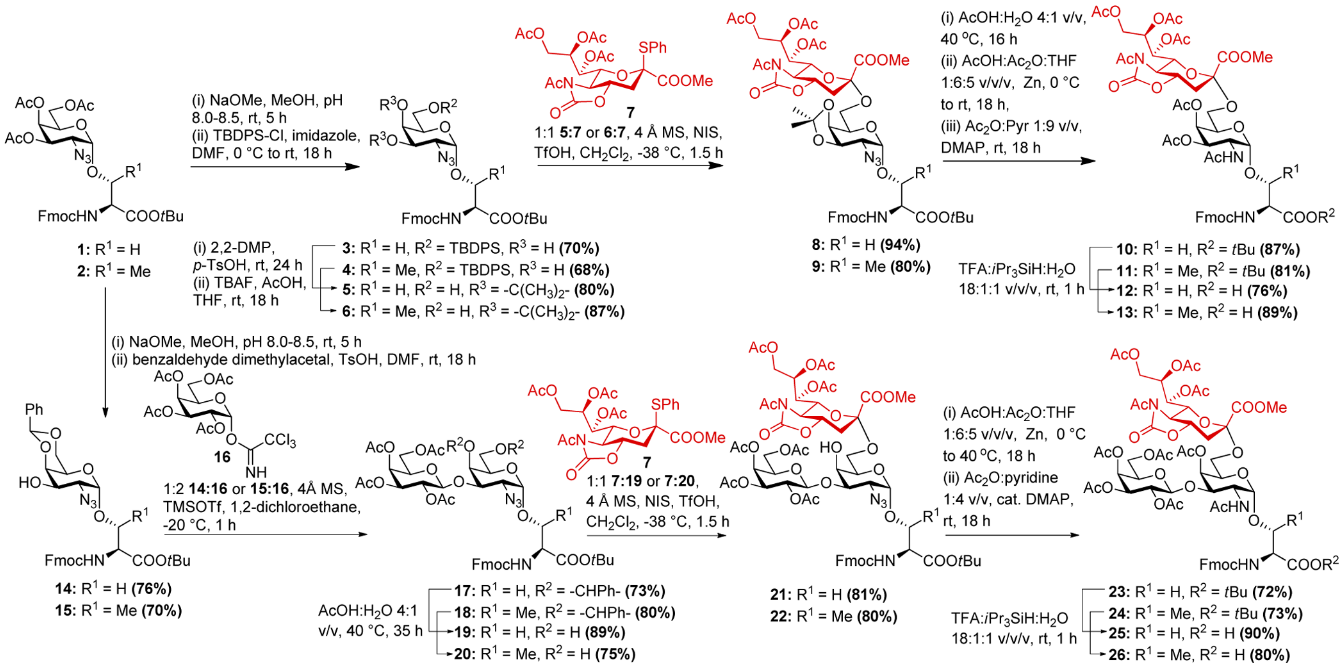
Scheme 1. Synthesis of Sialyl T_N and 2,6-Sialyl T-Derived Ser and Thr Residues



peptide synthesis (Fmoc-SPPS).[5] Despite the enormous interest and activity in the areas of glycosylamino acid synthesis[3a] and sialylated cancer vaccines,[3] as well as the wealth of literature available on sialylation chemistry, there remains only a small number of methods for accessing sialyl T_N and sialyl T-bearing glycosylamino acids for incorporation into SPPS.[3a,5a] This is due, in major part, to difficulties in both accessing a single anomer and competing 2,3-elimination reactions on the sialyl donor during activation.[6]

A common method for the construction of sialylated glycosylamino acids makes use of a stereodirecting nitrile solvent to promote α-selectivity with a range of sialyl donors.[7] However, in most cases these reactions do not exclusively afford the α-anomer. A number of chemoenzymatic methods employing sialyltransferases have also been utilized in glycosylamino acid and glycopeptide assembly,[8] but these often prove prohibitively expensive to scale up. An alternative strategy for the induction of complete α-selectivity in sialylation reactions involves the use of directing auxiliaries within the sialyl donor.[9] A recently employed class of auxiliaries are the unsubstituted and *N*-acylated 5*N*,4*O* oxazolidinone moieties[10] which have provided α-sialosides exclusively in the Neu5Ac-α(2,6)-GalNAc linkage found in TACAs.[11,10i] Herein, we report the first use of an oxazolidinone directing auxiliary in the stereoselective synthesis of glycosylamino acids bearing the ST_N and ST antigens, as well as their rapid and efficient incorporation into glycopeptides.

Synthesis of suitably protected ST_N-derived amino acid building blocks began with 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-D-galactose α-linked to Fmoc-protected serine (Ser, **1**) and threonine (Thr, **2**) which were prepared in four linear steps from D-galactosamine, as described previously (Scheme 1).[12] The glycosylamino acids were first deacetylated under Zemplén conditions before regioselective 6-silyl

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protection *via* treatment with *tert*-butyldiphenylsilyl chloride (TBDPS-Cl) and imidazole to form **3** and **4**. Isopropylidene protection of the 3- and 4-hydroxyl groups, followed by buffered fluoridolysis of the silyl ether, afforded acceptors **5** and **6** in good yield over the two steps. Gratifyingly, glycosylation of both acceptors with a stoichiometric quantity of *N*-acetyl-5*N*,4*O*-carbonyl protected phenylthiosialoside **7**[10b] under *N*-iodosuccinimide/triflic acid (NIS/TfOH) promotion conditions at −38 °C furnished the sialylated glycosyl amino acids **8** and **9** in 94% and 80% yield, respectively, with only the α-sialoside produced in both cases. The α-stereochemistry was confirmed through extraction of the $^3J_{C1,H3ax}$ coupling constants (see Supporting Information (SI)).[13] It is important to note that only 0.4 equiv of TfOH was employed, so as to prevent acidolysis of the isopropylidene. Rather than removing the oxazolidinone auxiliary at this stage, we anticipated that it could be removed following solid-phase assembly of the glycopeptides. Accordingly, hydrolysis of the isopropylidene acetals of **8** and **9** with aqueous AcOH, followed by reductive acetylation of the C2-azido moiety with nanoparticle Zn, Ac_2O, and AcOH, and acetylation of the free hydroxyl groups at the 3- and 4-positions with Ac_2O, pyridine, and DMAP, gave acetamides **10** and **11** in 87% and 81% yield over 3 steps. Finally, acidolysis of the *tert*-butyl ester with TFA, triisopropylsilane, and water provided the peracetylated ST_N-derived Ser and Thr cassettes **12** and **13** in good overall yields.

Next, we turned our attention to the synthesis of suitably protected 2,6-ST Ser and Thr cassettes. Deacetylation of **1** and **2** followed by treatment with benzaldehyde dimethylacetal/TsOHprovided benzylidene acetals **14** and **15**. Schmidt glycosylation between acceptors **14** and **15** and tetraacetyl-D-galactose trichloroacetimidate (**16**) afforded T-antigen core structures **17** and **18** in good yield with complete *β*-selectivity due to the neighboring group effect. The benzylidene acetal was next removed by hydrolysis with aqueous AcOH to provide diols **19** and **20**. Regioselective glycosylation of the 6-hydroxyl of acceptors **19** and **20** with a stoichiometric quantity of donor **7** under NIS/TfOH promotion conditions afforded the corresponding 2,6-ST core structures **21** and **22** in 81% and 80% yield, respectively. Small quantities of the doubly sialylated tetrasaccharides were detected by LC-MS (< 5%); however, these could be easily separated from the desired products by flash chromatography. The stereo- and regiochemistry of the ST glycosylamino acids **21** and **22** were confirmed by NMR spectroscopic analysis (see SI). From here, reductive acetylation of the C2-azide was achieved by treatment with nanoparticle Zn in a mixture of AcOH and Ac_2O at elevated temperature (40 °C). The crude *N*-acetylated glycosylamino acids were subsequently treated with Ac_2O, pyridine, and DMAP to acetylate the remaining 4-hydroxyl group to provide **23** and **24** in 72% and 73% yield, respectively. Finally, acidolytic cleavage of the *tert*-butyl ester with TFA/iPr$_3$SiH/H_2O gave the target 2,6-ST amino acids **25** and **26** in excellent yields.

Having synthesized the requisite glycosyl-Ser and glycosyl-Thr building blocks we were next interested in demonstrating their utility in the synthesis of glycopeptides *via* direct incorporation into Fmoc-SPPS protocols. Specifically, we targeted four tumor-associated MUC1 glycopeptides **27**−**30** which were chosen with a view to incorporation into synthetic cancer vaccine candidates in future work (Scheme 2). Glycopeptides **27** and **28** possess an ST_N- or ST-derived Ser moiety, respectively, within the amino acid sequence GSTAPPAHGVT which embodies the immunostimulatory GSTA epitope of MUC1.[14] In contrast, glycopeptide targets **29** and **30** correspond to an entire copy of the MUC1 VNTR region (SAPDTRPAPGSTAPPAHGVT) with a key Thr residue within the PDTRP immunodominant epitope[14b,c,15] derivatized with an ST_N (**29**) or ST (**30**) moiety. Synthesis of **27** and **28** began from preloaded 2-chlorotrityl chloride resin **31** which was elongated *via* Fmoc-SPPS to provide resin bound nonapeptide **32**. From here glycosyl-Ser building blocks **12** and **25**, bearing either the ST_N or ST antigen respectively, were coupled to the resin bound peptide. It is worth noting that due to the propensity of glycosyl-Ser residues to epimerize upon coupling, a slight excess of the precious glycosylamino acid (1.2 equiv) was used in the presence of 2-(1*H*-7- azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate (HATU, 1.2 equiv), *sym*-collidine (1.2 equiv), and 1-hydroxy-7-azabenzotriazole (HOAt, 1.5 equiv).[16] Pleasingly, these conditions almost completely prevented epimerization (< 5% detected by LC-MS) and facilitated quantitative coupling to afford resin bound **33** and **34**. Upon N-terminal Fmoc-deprotection we discovered that the oxazolidinone moiety was susceptible to nucleophilic attack with piperidine, generating resin-bound *N*-acetyl piperidyl urea and piperidyl urea byproducts (see SI). As such, we set out to identify improved conditions for the *en bloc* removal of the oxazolidinone moiety prior to further elongation of the glycopeptide chain. The optimized conditions involved treating the resin bound peptides **33** and **34** with DTT and DBU in DMF which led to clean opening of the oxazolidinone and concomitant Fmoc-deprotection (see SI). The resulting peptide was subsequently elongated by a further glycine residue to provide **35** and **36**. Having prepared the desired resin bound target, the glycopeptides were deprotected and cleaved from the resin by treating with an acidic cocktail. The crude glycopeptides were next subjected to Zemplén deacetylation conditions followed by saponification of the

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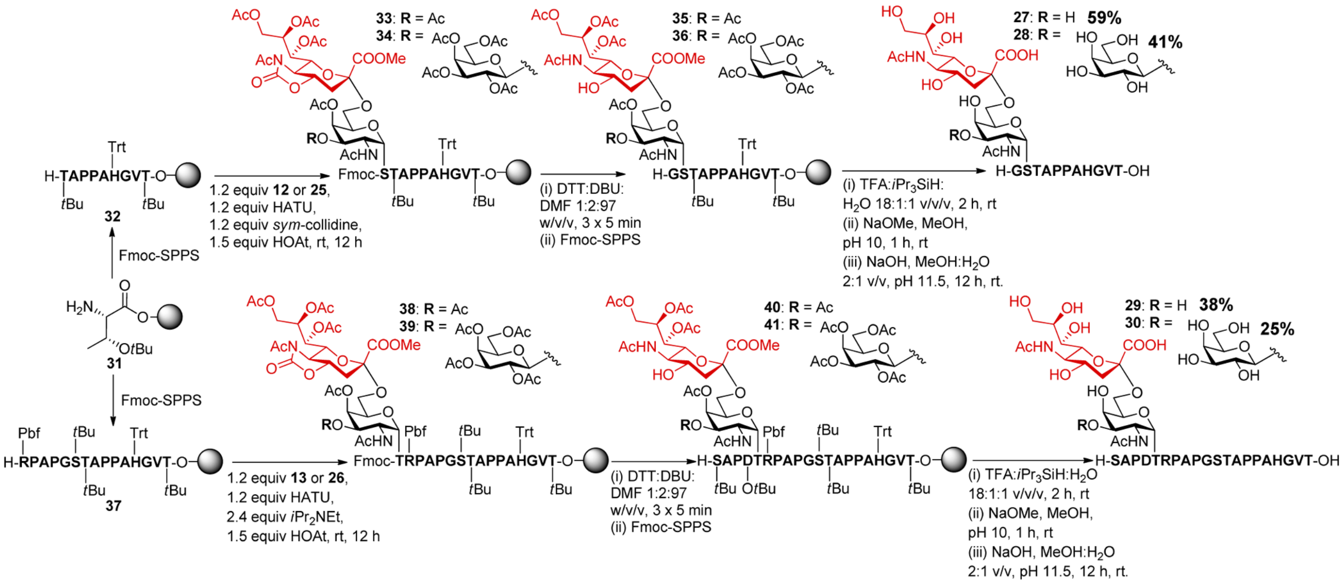
Scheme 2. Solid-Phase Assembly of ST_N- and ST-Derived MUC1 Glycopeptides



remaining methyl ester on the sialic acid moiety. Gratifyingly, following purification by reversed-phase HPLC, the desired MUC1 glycopeptides **27** and **28** were isolated in excellent yields (59% and 41%, respectively) based on the original resin loading. Having successfully demonstrated the utility of the sialylated glycosyl-Ser residues in the solid-phase synthesis of glycopeptides, we next turned our attention to the synthesis of MUC1 VNTR glycopeptides **29** and **30** bearing ST_N and ST antigen-derived Thr residues, respectively. Synthesis of resin bound peptide **37** was achieved by Fmoc-SPPS. Coupling of sialylated glycosyl-Thr residues **13** and **26** was carried out using slightly modified conditions than those employed for **12** and **25** (HATU, HOAt, *N,N*-diisopropylethylamine in DMF) to afford resin bound glycopeptides **38** and **39**, respectively. The oxazolidinone and N-terminal Fmoc group of **38** and **39** were removed using the optimized DTT and DBU deprotection conditions before the peptide chain was elongated by a further four amino acids by Fmoc-SPPS to provide resin bound eicosaglycopeptides **40** and **41**. Acidolytic side chain deprotection and cleavage of these peptides from the resin, followed by deacetylation of the carbohydrate units and saponification of the C-1 methyl ester, provided the crude glycopeptides. Following purification by reversed-phase HPLC the MUC1 VNTR glycopeptides **29** and **30** were isolated in 38% and 25% yields respectively, based on the original resin loading.

In summary, we have successfully developed high yielding and stereoselective routes to suitably protected Ser and Thr residues bearing the ST_N and ST antigens. These glycosylamino acid cassettes containing the α-directing *N*-acyl oxazolidinone group could be utilized directly in the Fmoc-SPPS of glycopeptides as highlighted by the synthesis of four MUC1 tumor-associated targets. Future work in our laboratories will focus on the use of this synthetic methodology to assemble a range of glycopeptides for incorporation into cancer vaccine candidates.

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Supporting Information Available. Full characterization of all novel compounds, ^{1}H and ^{13}C NMR spectra, and analytical HPLC chromatograms. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.