The α-aminooxy derivative of the Thomsen Friedenriech tumor associated carbohydrate antigen has been synthesized in 11 steps utilizing a α-GalN₃ acceptor carrying a pre-installed α-N-hydroxysuccinimidyl moiety. The natural α linkage was prepared in high selectivity employing a suitably protected α-GalN₃-thioglycoside donor with N-hydroxysuccinimide. With access to α-TF-ONH₂, the preparation of the TF-PS A1 vaccine candidate ensued smoothly through oxime bond formation.

The Thomsen Friedenriech (TF or T) antigen [α-β-Gal-(1,3)-α-N-GalNAc-Ser/Thr], (1a/1b) (Fig. 1), is an important cancer antigen/biomarker found in high density on human breast, colon, stomach, bladder, prostate, and liver tumor cells. TF can be found on the large transmembrane mucin protein MUC1, which is overexpressed in epithelial cancers. Modifications of TF include non-reducing end sialylation, N-acetylgalcosaminylation or fucosylation creating a host of carbohydrates only eliciting a T-cell independent immune response. PS A1 consist of a tetrasaccharide repeating unit, has a MW of approximately 110 kD and consists of a right handed helical structure with two repeating units per turn. The T-cell dependent activity of this polysaccharide has been shown to arise from the zwitterionic nature of the polymer. Previous work has revealed that when this alternating charge character is chemically neutralized the T-cell dependent response is as well.

The TF disaccharide has attracted considerable attention from the scientific community due to expression on numerous carcinomas. TF is also believed to play a direct role in adhesion and metastasis through interaction with galectin-3. Verification of the tumor relevance for TF has encouraged our group to investigate the preparation of an aminooxy derivative of the TF antigen (2) (Fig. 1) for subsequent conjugation to the immunogenic zwitterionic polysaccharide PS A1 (15). This capsular polysaccharide is isolated from the commensal anaerobic bacteria B. fragilis and has challenged the paradigm concerning carbohydrates only eliciting a T-cell independent immune response. PS A1 consist of a tetrasaccharide repeating unit, has a MW of approximately 110 kD and consists of a right handed helical structure with two repeating units per turn. The T-cell dependent activity of this polysaccharide has been shown to arise from the zwitterionic nature of the polymer. Previous work has revealed that when this alternating charge character is chemically neutralized the T-cell dependent response is as well.

An important component of this conjugation strategy, in the same fashion as our earlier report with the conjugation of Tn, involves the use of anomeric aminooxy sugars first prepared by Roy and then later by Dumy and Bertozzi. To the best of our knowledge the α-aminooxy TF antigen (2) has been present in normal human sera and (2) humans are intolerant to this antigen and hence application thereof in vaccine design and development could prove promising.

The TF disaccharide is found alpha linked to Ser/Thr on cancer mucins such as MUC1 (1a/1b). α-TF-ONH₂ (2).

Fig. 1 TF antigen is found alpha linked to Ser/Thr on cancer mucins such as MUC1 (1a/1b). α-TF-ONH₂ (2).
synthesized previously by only one other group.10 TF linked to Ser/Thr has also been prepared by various methods as a building block for solid phase peptide synthesis.6a,11

Alternative to the previous synthesis of α-TF-ONH₂,10 α-galactosamine-HCl (3) was chosen as a suitable starting material (Scheme 1). First, 3 was treated with imidazole-1-sulfonyl azide hydrochloride, followed by acetic anhydride to provide peracetylated azide 4. Phenyl thioglycoside 5 was then prepared through treatment of 4 with BF₃·OEt₂ and PhSH. Next the acetyl groups of 5 were removed using the Zemplén method and the benzylidene acetal was installed to provide thioglycoside acceptor 6 in an α/β ratio of 1.7:1 (readily separable by silica gel column chromatography). Inspired by previous uses of alkoxy protection10,11 at the anomeric position during the required glycosylation, an acceptor was envisioned that would carry a preinstalled α-NHS moiety intact at the anomeric position.

There are numerous reports of reductions,9α,8,10,13 and also a single example of TBS protection14 being conducted in the presence of NHS glycosides. The only previous example found of a glycosylation involving a succinimidyl protecting group was reported by Bertozzi and co-workers13 for the preparation of α-NHS glycosides. The only previous example found of α-NHS group for the duration of the synthetic strategy. There are numerous reports of reductions,9α,8,10,13 and also a single example of TBS protection14 being conducted in the presence of NHS glycosides.

In our initial attempts, along the lines of the aforementioned work,13 we elected to pursue TBDPS for 3-OH protection. Compound 7 was prepared in 79% yield by treatment of 6 with excess TBDPSCI and imidazole in DMF (Scheme 2). The protected donor 7 was then activated with NIS- TfOH in the presence of 1.1 eq. of NHS. Unfortunately under these conditions, the succinimidyl anion, the by-product from the NHS activation, out competed NHS for the donor. This unexpected reaction pathway gave a 2 : 1 - α/β mixture of compound 8 in 73% yield. However we were able to isolate α-NHS glycoside 9 in low yield. In an attempt to avoid the unwanted C-N bond formation, the reaction was conducted with excess (4 equiv.) NHS. These conditions proved sufficient for the formation of the α-NHS product in 75% yield, 8 : 1 - α/β.15

With protected NHS acceptor 9 in hand, we next turned our attention to the removal of the TBDPS group. Compound 9 was treated with TBAF in THF at r.t. which completely converted 9 to 10 as observed by thin layer chromatography, however, we were only able to isolate 10 in <40% yield. The low yield was attributed to opening of the NHS ring by hydroxide, forming the glycosyl carbonylic acid as observed by Andersson and Roy.9α,16 The presence of a hydroxide is believed to arise from the strong basicity of TBAF and that moisture entered our reaction vessel. This alternate reaction pathway might be avoided by adjusting the pH of the commercial TBAF before addition.13,17 This strategy could provide a viable pathway to 10, but reported deprotections utilizing this method in the presence of NHS continually suffer from low yields.13 In light of the fact that excess reagent and a subsequent laborious purification step were needed we decided to pursue an alternative strategy to prepare compound 10.18

In a similar manner as reported in a synthesis of TF-O-Ser/Thr,11c the chloroacetyl group (ClAc) was examined as an alternative to TBDPS. Starting from 6α or 6β (Scheme 3) the chloroester 11 was prepared in 84% yield by treating the respective secondary alcohol with ClAcCl/pyr.19 The masked thioglycoside 11α or 11β was treated with NIS-TfOH and 4 equiv. NHS providing the α-NHS glycoside as the major product.20 Determining the α/β ratio for this reaction was complicated by the formation of the aforementioned succinimidyl by-product; our reported yield is based solely on the isolated α-NHS. The NHS group proved to be base liable with decomposition occurring under both NEt₃-H₂O and saturated NaHCO₃ conditions. The successful deprotection was achieved through the use of the non-basic nucleophile, thiourea, in 5 : 1 EtOH-pyr22 at room temperature giving rise to the deprotected NHS acceptor 10 in 65% yield over two steps.

With access to appreciable quantities of our desired α-NHS acceptor 10, we attempted the glycosylation using trichloroacetimidate donor 1223 (Scheme 4). Recent reports noted such...
NaIO₄, creating strategically placed aldehydes along the tetra-vicinal diols of galactofuranose residues present on PS A1 with conducted (Scheme 5). This was accomplished by cleaving the 2 using water as the eluent giving rise to pure MW allowed excellent separation on a Bio-gel P-2 column attention to size exclusion chromatography. The disparity in ing the crude reaction mixture from methanol, we turned our respective aminooxy sugar. After several attempts at crystalliz- ing the byproducts from this reaction, acetohydrazide and tetrahydro- pyridazine-3,6-dione, complicate the purification of the yield from protected disaccharide PS A1 and the reaction mixture was allowed to stir overnight in the dark at r.t. to provide the TF-PS A1 oxime conjugate, with a percent loading assumed to be similar to our previous report with conjugated Tn. The ³H NMR spectrum of 16 showed a distinct oxime doublet centered at 8.00. In addition a new singlet could be observed at 2.29 (N-acetyl of TF) and 1D TOCSY of anomeric protons at 4.61 and 5.46 displayed spin systems comparable to the TF-ONH₂ (2) proton spectra.

Conclusions

In summary, the synthetic preparation of the α-aminooxy TF antigen and its conjugation to PS A1 has been described. The synthesis of 2 is highlighted by successful α selective glycosyla- tion and deprotection in the presence of the succinimidyl protecting group. New evidence is presented that the succinimidyl group can be carried through a number of transformations provided that basic conditions be avoided. This study further establishes the utility of NHS glycosides in the syn- thesis of aminooxy sugars, thereby making conjugations of nucleophilic sugars to appropriate carbonyls possible through physiologically stable oxime formation. Future studies are concomitantly underway for the evaluation of the TF-PS A1 conjugate as a potential cancer vaccine candidate utilizing murine models.

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Notes and references
