Published in final edited form as:

Org Biomol Chem. 2014 March 21; 12(11): 1699–1702. doi:10.1039/c4ob00128a.

Synthesis of the tumor associative α -aminoxy disaccharide of the TF antigen and its conjugation to a polysaccharide immune stimulant

Jean Paul Bourgault^a, Kevin R. Trabbic^a, Mengchao Shi^a, and Peter R. Andreana^a

Peter R. Andreana: peter.andreana@utoledo.edu

^aDepartment of Chemistry and School of Green Chemistry and Engineering, The University of Toledo, 2801 W. Bancroft St, Toledo, OH 43606

Abstract

The α -aminoxy derivative of the Thomsen-Friedenriech tumor associated carbohydrate antigen has been synthesized in 11 steps utilizing a D-GalN₃ acceptor carrying a pre-installed α -*N*-hydroxysuccinimide moiety. The natural α linkage was prepared in high selectivity employing a suitably protected D-GalN₃-thioglycoside donor with *N*-hydroxysuccinimide. With access to α -TF-OH₂, the preparation of the TF-PS A1 vaccine candidate ensued smoothly through oxime bond formation.

The Thomsen Friedenriech (TF or T) antigen [β -D-Gal-(1,3)- α -D-GalNAc-Ser/Thr], (**1a/1b**), (Figure 1) is an important cancer antigen/biomarker found in high density on human breast, colon, stomach, bladder, prostate, and liver tumor cells.¹ TF can be found on the large transmembrane mucin protein MUC1, which is overexpressed in epithelial cancers.² Modifications of TF include non-reducing end sialylation, *N*-acetylglucosinylation or fucosylation creating a host of *O*-glycans on healthy tissues.³ Under normal metabolic conditions with saccharide “caps” the TF is “masked” from immune surveillance, however in various carcinoma cells this *O*-linked tumor disaccharide is revealed. The presence of TF could occur through increased T synthase activity or through decreased subsequent modification.⁴ Alternatively increased cellular concentrations of UDP-Gal and the TF precursor, the Thomsen-nouveau antigen (Tn antigen, α -D-GalNAc Ser/Thr) could also play a role.¹ TF was discovered by the combined efforts of Thomsen and Friedenriech.⁵ The initial observation occurred during an attempt to determine the ABO blood type of bacterial contaminated red blood cells. The bacteria neuraminidases exposed the disaccharide and the resulting desialylated TF caused agglutination on addition of all human sera (ABO). Two significant facts were determined from the discovery namely: 1) that antibodies against the TF antigen were already 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.¹

The TF disaccharide has attracted considerable attention from the scientific community^{1, 6} due to expression on numerous carcinomas. TF is also believed to play a direct role in adhesion and metastasis through interaction with galectin-3.^{2, 7} Verification of the tumor relevance for TF has encouraged our group to investigate the preparation of an aminooxy derivative of the TF antigen (**2**) (Figure 1) for subsequent conjugation to the immunogenic zwitterionic polysaccharide PS A1 (**15**).⁸ 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.^{8d}

An important component of this conjugation strategy, in the same fashion as our earlier report with the conjugation of Tn,^{8b} involves the use of anomeric aminooxy sugars first prepared by Roy and then later by Dumy and Bertozzi.⁹ To the best of our knowledge the α -aminooxy TF antigen (**2**) has been 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 D-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**.¹² 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 protection^{10–11} at the anomeric position during the required glycosylation, an acceptor was envisioned that would carry a preinstalled α -NHS group for the duration of the synthetic strategy. There are numerous reports of reductions,^{9a, 9b, 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 the aminooxy STn antigen. In order for the Bertozzi approach to be realized, a suitable protecting group was required; removal of which would occur under mild conditions, leaving the α -NHS moiety intact at the anomeric position.

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 TBDPSCl 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 NIS 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 **9** 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 carboxylic acid as observed by Andersson and Roy.^{9a, 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 **6a** or **6b**, (Scheme 3) the chloroester **11** was prepared in 84% yield by treating the respective secondary alcohol with ClAcCl/pyr.¹⁹ The masked thioglycoside **11a** or **11b** 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 labile with decomposition occurring under both NEt₃/MeOH¹⁹ and saturated NaHCO₃ 5:1 MeOH/H₂O²¹ 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 **12**²³ (Scheme 4). Recent reports noted such reactions as being hampered by competing orthoester formation.^{11b, 24} Fortunately the major product of this pivotal glycosylation had the required β 1–3 linkage found in the TF antigen (Figure 1) and provided disaccharide **13**, which was isolated in 56% yield. The characteristic anomeric doublet observed at 4.80 ppm with a J_{H1-H2} of 8.00 Hz confirmed that the desired β linkage was formed. This conclusion was further supported by COSY and HMQC.

With protected disaccharide **13** in hand, subsequent steps focused on removal of the benzylidene acetal and reduction of the azide. First, activated zinc dust, in 3:2:1 THF:AcOH:Ac₂O, was used to reveal the *N*-acetyl^{11a, 25} moiety at the 2-position. The crude reaction mixture, obtained from the azide reduction, was then treated with 80% aqueous acetic acid at 80 °C to remove the benzylidene acetal and provide diol **14** in 56% yield over two steps.²⁴ The product exhibited no overlap of signals in the ¹³C NMR, and therefore all of the carbonyls were precisely accounted for.²⁶ This spectrum confirmed that the β 1–3 linkage had been made and that **14** was not the unwanted orthoester.

In the final deprotection step, hydrazine hydrate was utilized to remove the remaining acetyl groups as well as to displace the succinimidyl protecting group to reveal the α -aminoxy TF antigen **2**. As earlier reports noted^{9a, 10}, the two byproducts from this reaction, acetohydrazide and tetrahydropyridazine-3,6-dione, complicate the purification of the respective aminoxy sugar. After several attempts at crystallizing the crude reaction mixture from methanol, we turned our attention to size exclusion chromatography. The disparity in MW allowed excellent separation on a Bio-gel P-2 column using water as the eluent giving rise to pure **2** in 64% yield from protected disaccharide **14**; NMR data matched the previously reported values.¹⁰

Following the preparation of **2**, the key one pot oxidation and conjugation between PS A1 and the α -aminoxy TF was conducted (Scheme 5). This was accomplished by cleaving the vicinal diols of galactofuranose residues present on PS A1 with NaIO₄, creating strategically placed aldehydes along the tetrasaccharide repeating unit.^{8a, 27} After 90 mins of PS A1 (**15**) exposure to NaIO₄, KCl was introduced to precipitate any remaining periodate as KIO₄.²⁸ Finally **2** was added to oxidized 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.^{8b} The ¹H 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 -aminoxy TF antigen and its conjugation to PS A1 has been described. The synthesis of **2** is highlighted by successful α selective glycosylation 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.^{9a, 13, 16} This study further establishes the utility of NHS glycosides in the synthesis of aminoxy sugars, thereby making conjugations of nucleophilic sugars to appropriate carbonyls possible through physiologically stable oxime formation.²⁹ Future studies are concomitantly underway for the evaluation of the TF-PS A1 conjugate as a potential cancer vaccine candidate utilizing murine models.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the National Institute of Health (NCI-1R01 CA156661-01).

Notes and references

1. Heimburg-Molinaro J, Lum M, Vijay G, Jain M, Almogren A, Rittenhouse-Olson K. Vaccine. 2011; 29:8802–8826. [PubMed: 21964054]

2. Yu LG, Andrews N, Zhao Q, McKean D, Williams JF, Connor LJ, Gerasimenko OV, Hilken J, Hirabayashi J, Kasai K, Rhodes JM. *J Biol Chem.* 2007; 282:773–781. [PubMed: 17090543]
3. Ju T, Otto VI, Cummings RD. *Angew Chem Int Ed.* 2011; 50:1770–1791.
4. Yu LG. *Glycoconjugate J.* 2007; 24:411–420.
5. (a) Thomsen O. *Z Immunitäts Forsch.* 1927; 52:85–90. (b) Friedenreich, V. *The Thomsen hemagglutination phenomenon.* Copenhagen; Denmark: 1930.
6. (a) Kuduk SD, Schwarz JB, Chen XT, Glunz PW, Sames D, Ragupathi G, Livingston PO, Danishefsky SJ. *J Am Chem Soc.* 1998; 120:12474–12485. (b) Sabbatini PJ, Ragupathi G, Hood C, Aghajanian CA, Juretzka M, Iasonos A, Hensley ML, Spassova MK, Ouerfelli O, Spriggs DR, Tew WP, Konner J, Clausen H, Abu Rustum N, Dansiehefsky SJ, Livingston PO. *Clin Cancer Res.* 2007; 13:4170–4177. [PubMed: 17634545] (c) Zhu J, Wan Q, Lee D, Yang G, Spassova MK, Ouerfelli O, Ragupathi G, Damani P, Livingston PO, Danishefsky SJ. *J Am Chem Soc.* 2009; 131:9298–9303. [PubMed: 19518111] (d) Hoffmann-Röder A, Kaiser A, Wagner S, Gaidzik N, Kowalczyk D, Westerlind U, Gerlitzki B, Schmitt E, Kunz H. *Angew Chem Int Ed.* 2010; 49:8498–8503. (e) Springer GF, Desai PR, Spencer BD, Tegtmeyer H, Carlstedt SC, Scanlon EF. *Cancer Detect Prev.* 1995; 19:374–380. [PubMed: 7553680] (f) Brinäs RP, Sundgren A, Sahoo P, Morey S, Rittenhouse-Olson K, Wilding GE, Deng W, Barchi JJ. *Bioconjugate Chem.* 2012; 23:1513–1523.
7. (a) Glinsky VV, Glinsky GV, Rittenhouse-Olson K, Huflejt ME, Glinskii OV, Deutscher SL, Quinn TP. *Cancer Res.* 2001; 61:4851–4857. [PubMed: 11406562] (b) Rhodes JM, Campbell BJ, Yu LG. *Biochem Soc Trans.* 2008; 36:1482–1486. [PubMed: 19021580] (c) Heimbürg J, Yan J, Morey S, Glinskii O, Huxley V, Wild L, Klick R, Roy R, Glinsky V, Rittenhouse-Olson K. *Neoplasia.* 2006; 8:939–948. [PubMed: 17132226]
8. (a) Cobb BA, Wang Q, Tzianabos AO, Kasper DL. *Cell.* 2004; 117:677–687. [PubMed: 15163414] (b) De Silva RA, Wang Q, Chidley T, Appulage DK, Andreana PR. *J Am Chem Soc.* 2009; 131:9622–9623. [PubMed: 19552408] (c) Silva R, Appulage D, Pietraszkiewicz H, Bobbitt K, Media J, Shaw J, Valeriote F, Andreana P. *Cancer Immunol Immunother.* 2012; 61:581–585. [PubMed: 22297566] (d) Surana NK, Kasper DL. *Immunol Rev.* 2012; 245:13–26. [PubMed: 22168411]
9. (a) Cao S, Tropper FD, Roy R. *Tetrahedron.* 1995; 51:6679–6686. (b) Renaudet O, Dumy P. *Tetrahedron Lett.* 2001; 42:7575–7578. (c) Hudak JE, Yu HH, Bertozzi CR. *J Am Chem Soc.* 2011; 133:16127–16135. [PubMed: 21866965]
10. Renaudet O, Dumy P. *Tetrahedron Lett.* 2004; 45:65–68.
11. (a) Svarovsky SA, Barchi JJ Jr. *Carbohydr Res.* 2003; 338:1925–1935. [PubMed: 14499569] (b) Cato D, Buskas T, Boons GJ. *J Carbohydr Chem.* 2005; 24:503–516. (c) Jiaang WT, Chang MY, Tseng PH, Chen ST. *Tetrahedron Lett.* 2000; 41:3127–3130. (d) Keding SJ, Endo A, Danishefsky SJ. *Tetrahedron.* 2003; 59:7023–7031.
12. Goddard-Borger ED, Stick RV. *Org Lett.* 2007; 9:3797–3800. [PubMed: 17713918]
13. Marcaurelle LA, Shin Y, Goon S, Bertozzi CR. *Organic Letters.* 2001; 3:3691–3694. [PubMed: 11700114]
14. Hosokawa S, Ogura T, Togashi H, Tatsuta K. *Tetrahedron Lett.* 2005; 46:333–337.
15. Crich D, de la Mora M, Vinod AU. *J Org Chem.* 2003; 68:8142–8148. [PubMed: 14535796]
16. Andersson M, Oscarson S. *Glycoconjugate J.* 1992; 9:122–125.
17. Smith AB, Ott GR. *J Am Chem Soc.* 1996; 118:13095–13096.
18. The excess TBDPSCl was hydrolyzed to TBDPSOH, regrettably TBDPSOH and 9 had the same rf ethyl acetate/hexanes, requiring the usage of toluene for the separation
19. Lin CC, Lin NP, Sahabuddin LS, Reddy VR, Huang LD, Hwang KC, Lin CC. *J Org Chem.* 2010; 75:4921–4928. [PubMed: 20578749]
20. The yield of this reaction was not calculated b/c of the presence of an inseparable impurity
21. Wong CH, Ye XS, Zhang Z. *J Am Chem Soc.* 1998; 120:7137–7138.
22. Lefebvre DJ, Kamerling JP, Vliegenthart JFG. *Org Lett.* 2000; 2:701–703. [PubMed: 10814414]
23. Schmidt RR, Michel J. *Angew Chem Int Ed.* 1980; 19:731–732.
24. Dziadek S, Brocke C, Kunz H. *Chem Eur J.* 2004; 10:4150–4162. [PubMed: 15352098]
25. Tanaka H, Adachi M, Takahashi T. *Chem Eur J.* 2005; 11:849–862. [PubMed: 15580651]

26. See supporting info Carbon NMR data for 14
27. Hermanson, GT. Bioconjugate Techniques. Academic Press; San Diego: 1996.
28. Odom OW, Robbins DJ, Lynch J, Dottavio-Martin D, Kramer G, Hardesty B. Biochemistry. 1980; 19:5947–5954. [PubMed: 6162473]
29. Kalia J, Raines RT. Angew Chem Int Ed. 2008; 47:7523–7526.

Figure 1.

TF antigen is found alpha linked to Ser/Thr on cancer mucins such as MUC1 (**1a/1b**). α -TF-OH₂ (**2**).

Scheme 1.
Synthesis of thioglycoside **6**

Scheme 2. Initial attempt at preparing NHS acceptor **10**.

Scheme 3.

Preparation of α -NHS acceptor **10** from chloroacetyl thioglycoside **11 α** or **11 β** .

Scheme 4.
Preparation of α -aminooxy TF (2).

Scheme 5.
TF-PS A1 (16).