



Cite this: *New J. Chem.*, 2025, 49, 19418

Revised efficient and reproducible synthesis of an Fmoc-protected Tn antigen[†]

Peihan Xu, ^a Sabrina Piazza, [‡] Michael R. Reynolds, [‡] Ryan Gaudet, ^a Jonathan Chiaramonte, [§] Fabiola A. Chapa-Villarreal, ^a John J. Hayward ^{ab} and John F. Trant ^{*,abcd}

In 2021, our team reported a concise synthesis of Thomsen-Nouveau (Tn) antigen, a tumour-associated O-linked mucin glycopeptide. We have since realized that our characterization of the reported glycoside was mistaken. Instead of the intended ether-bonded α anomer, the β -anomer containing an ester glycosidic bond was formed using palladium catalysis and characterised incorrectly as the spectra are remarkably similar. We demonstrated this conclusively by repeating Danishefsky's synthesis, with some required modifications of the protocol, of Fmoc-Tn for confirmation. The error is too significant for a correction as it does affect the conclusions of the work, and the original paper has been retracted, though its work is included in this manuscript. In this replacement we report a successful synthesis of the Tn antigen using adjusted glycosylation conditions: a different glycosyl acceptor, N-Fmoc serine benzyl ester, using TMSOTf as the catalyst. This remains, to the best of our knowledge, the shortest Tn antigen synthesis reported from galactose, although it provides the Tn antigen now. Furthermore, this route gives ready access to an essential Tn antigen building block that can be used for large-scale solid phase peptide synthesis.

Received 9th June 2025,
Accepted 14th October 2025

DOI: 10.1039/d5nj02399h

rsc.li/njc

Introduction

Cancers are a leading cause of death globally. Despite constant advances in treatment, the percentage of cancer-related deaths is expected to increase in the developed world as other fatal conditions are continuously becoming better controlled.^{1,2} Although there is ever accelerating innovation in the treatment of cancer, progress remains agonizingly slow due to the enormous diversity of mutations responsible for causing cancer.³ The current primary triad of surgery and/or radiation/chemotherapy, both in tandem or individually, can be highly effective;⁴ but success is greatly dependent on a series of variables including (but not limited to): the location of the disease, metastasis status, types of mutations present, and

stage at detection. Therapeutic options are particularly lacking for late-stage cancers. Despite a few targeted therapies recently becoming available,⁵ especially for certain subtypes of breast⁶ and prostate⁷ cancer, in many cases these pharmaceutical interventions still often result in short term improvement, with disease recurrence emerging as the cancer mutates to resist the treatment. We consequently need orthogonal therapeutic modalities to complement this therapeutic triad. Cancer vaccines are a promising avenue as they could re-engage the body's immune system against malignancies.

For many purposes, cancer can be defined as uncontrolled cell growth that has evaded the immune system, and will eventually progress to the death of the host:⁸ the vast majority of precancerous clusters are hypothesized to be cleared by the immune system long before they can develop into a tumor.^{9,10} When looking to activate the immune systems, vaccines are particularly attractive;^{11,12} however, they require the identification of biomarkers correlated with cancer. Unfortunately, many oncogenes are simply upregulated in cancer, rather than unique to it. As they are also present on healthy cells, they are inappropriate vaccine targets as stimulation would initiate a systemic and very dangerous immune response. Tumor-associated carbohydrate antigens (TACAs) are different as they are not found on healthy adult tissue, yet are found on over 90% of biopsied carcinomas (e.g. breast, ovarian, colon, lung, and prostate cancers).¹³ This discrepancy likely arises because

^a Department of Chemistry and Biochemistry, University of Windsor, 401 Sunset Ave., Windsor, ON, N9B 3P4, Canada. E-mail: jtrant@uwindsor.ca

^b Department of Biomedical Sciences, University of Windsor, 401 Sunset Ave., Windsor, ON, N9B 3P4, Canada

^c Binary Star Research Services, LaSalle ON, N9J 3B, Canada

^d WE-Spark Research Institute, 401 Sunset Ave., Windsor, ON, N9B 3P4, Canada

[†] In memory of Jonathan Chiaramonte, friend and colleague.

[‡] These authors contributed equally to this work and the order is due to the arbitrary vagaries arising from the combination of the Latin alphabet and family name alone. Both authors, and those citing this work, may invert the order of the first two authors for any and all professional purposes. The preprint of this article inverts the names with the same statement to emphasize this point.

[§] Deceased.



these TACAs are found at the reducing-end of surface glycans, and are anchored to the amino acids or lipids of the membranes. In healthy tissue they are always further elaborated into larger glycostructures; however, in cancer the misregulation of glycosyl transferases responsible for this elaboration ensures that these TACA scaffolds remain naked, missing the additional residues. Their precise role in oncogenesis is unclear, but overexpression is generally correlated with a poor prognosis.^{8,14} These carbohydrate antigens have, when incorporated into glycopeptides or other immunogenic scaffolds, formed the basis of the development of anti-tumor immunotherapies through the induction of a specific immune response against cancer cells.¹⁵⁻¹⁸ However, ready access to these materials remains a significant challenge, and a simple scalable synthesis is required. As part of an extensive carbohydrate vaccine effort, we have needed a route to produce the Tn antigen (**1**)—an α -2-deoxy-2-N-acetylaminogalactose (GalNAc) *O*-linked to a serine residue. The simplest derivative of this appropriate for solid phase peptide synthesis (SPPS), for which we require it on multigram—and up to 100 g scale—is the Fmoc-protected, *per*-acetylated building block (**1A**). Although this molecule has been prepared in the past,¹⁹ we found many of the simple published routes were inconsistent batch-to-batch and couldn't always be reproduced if the chemist doing the reactions changed.²⁰⁻²⁴ Furthermore, the published routes often required complex protecting group strategies that increased the step and purification count, decreasing yield (Fig. 1). Finally, the NMR data provided, despite generally considered to be a critical piece of characterization data, was often reported without detailed assignment but instead with references to data that was obtained decades ago and lacking actual images of the spectra. We wish to report the route

we are using for our immediate needs, as well as up-to-date characterization data of all important products and byproducts.

Results and discussion

Our original synthetic approach began with a series of well-precedented steps (Scheme 1). Peracetylation of α -galactose (100 g scale) provided a 78% yield of the β anomer **2** after recrystallization from methanol. Subsequent bromination with HBr yielded the galactosyl bromide **3** in good yield after precipitation; the unstable bromosugar was then treated with zinc metal in acetonitrile at reflux,²⁶ providing bench stable galactal **4** in high yield. Although other approaches were explored,²⁰ this route proved to be the most reliable access to this material. We note that as of 2024, galactal **4** is now inexpensively available from many suppliers on scale, making it difficult to rationalize its synthesis from the parent sugar.

To introduce a nitrogen at C2, we found that Lemieux's ceric ammonium nitrate (CAN)-mediated azidonitration was highly effective and robust, providing crude **5**,²⁷ the anomeric nitrate can be readily hydrolysed to provide reducing sugar **6** as a mixture of two anomers. This hemiacetal was converted to a number of different activating groups (see references above) but yields for introducing the activating group were highly variable and the subsequent glycosylations inevitably inconsistent; Schmidt's trichloroacetimidates (TCA) proved the most promising.^{28,29} General protocols for the synthesis of TCAs call for the use of trichloroacetonitrile and potassium carbonate; however, we found that the more soluble caesium carbonate reduced reaction times from several hours to mere minutes with a concomitant reduction in product decomposition, leading to quantitative yields of the crude TCAs in a 10:1 mixture of α (**7a**) and β (**7b**) anomers. These compounds were used immediately without purification beyond simple isolation by filtration, to minimize decomposition.

TACAs are generally made to be incorporated into peptides. This means that the resulting product should have an Fmoc-protected amino group and a free acid. Fmoc amino acids are notoriously challenging to manipulate compared to Boc-protected analogues, but they are required for modern SPPS. The reason for this difference is not immediately clear, as Fmoc groups are stable to standard acid, base, and mild hydrogenation conditions, but any examination of the literature will always find many examples of a transformation being accomplished with Boc- or Cbz-protected amines, with many fewer examples, employing Fmoc.¶ This is often because the yields of reactions using Fmoc are simply lower, driven by a combination of factors including partial deprotection and often poor

¶ A SciFinder search of reactions of carbamate-protected α -amino esters (excluding amide formation) found a total of 59 495 reactions; only 11% used Fmoc-protected substrates (6726 reactions). Conversely, 58% used Boc (34 656 reactions) and 23% used Cbz (13 967 reactions) – even though the most common use of such products (i.e. SPPS) requires Fmoc protection on the nitrogen. This is borne out by the fact that it is much more common for carbamate-protection of α -amino acids to be switched to Fmoc (Boc \rightarrow Fmoc, 3305 reactions; Cbz \rightarrow Fmoc, 1485 reactions) rather than from Fmoc (Fmoc \rightarrow Boc, 284 reactions; Fmoc \rightarrow Cbz, 105 reactions).

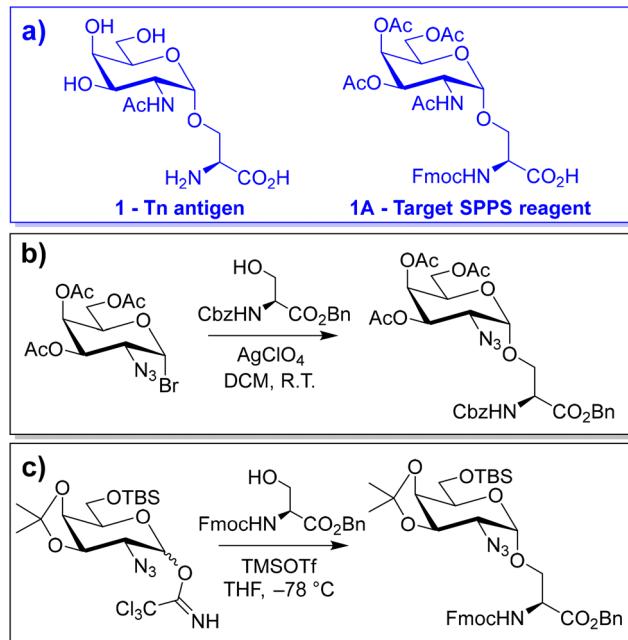
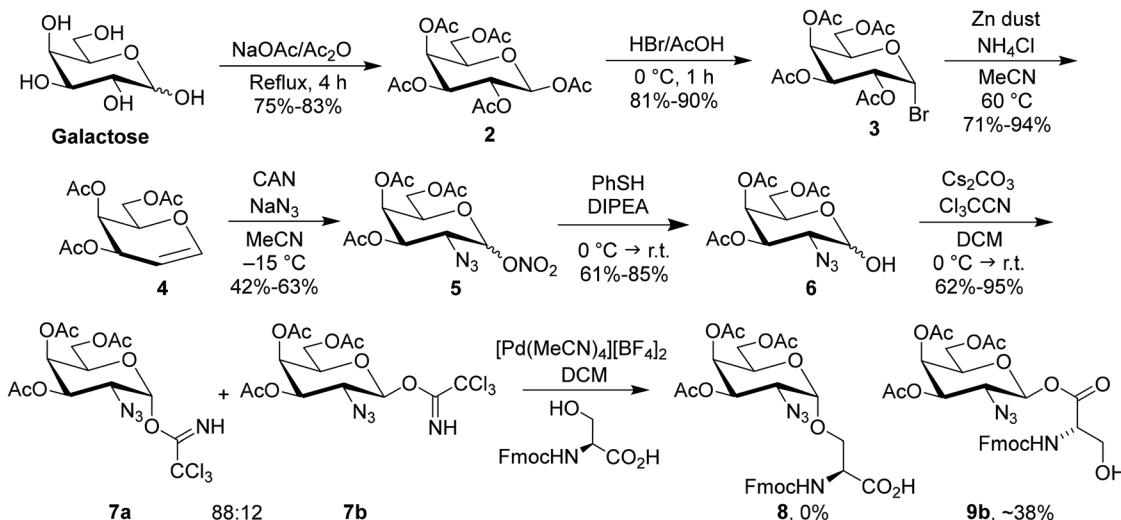


Fig. 1 Key previous syntheses of Tn building blocks for Fmoc-protected SPPS. (a) Tn antigen **1** and SPPS building block **1A**; (b) Paulsen and Hölk's synthesis of the Cbz-protected peracetylated Tn antigen;²⁵ (c) Danishefsky's synthesis of the Fmoc-protected Tn antigen.²⁰





Scheme 1 The original, erroneous, Trant group approach to the Tn Antigen protected for SPPS, with the correct glycosylation product provided. Yields are reported as ranges of yields from multiple trials and multiple operators, in many cases the mass balance includes significant starting material that can be recycled.

solubility of the fluorenyl group that might inhibit any desired reaction. However, Fmoc installation is often accompanied by the generation of hard-to-separate impurities—*e.g.* Fmoc- β -alanine-OH from the Lossen rearrangement of the Fmoc N -hydroxysuccinimide reagent, or the formation of activated esters during installation leading to undesired dipeptides (when using Fmoc-Cl).³⁰ Consequently, despite the potential difficulties with the use of such a substrate, we decided we needed to develop conditions appropriate for the Fmoc-protected amino acid. Finally, to shorten the number of steps further, we wanted to use an approach that avoided protection of the carboxylic acid at any point in the synthesis under the assumption that a protonated carboxylic acid is not a competitive nucleophile with the serine alcohol.

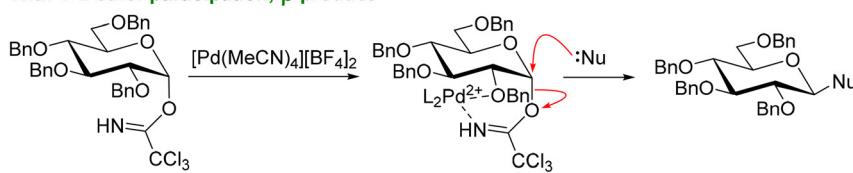
However, this approach proved to be more problematic than we expected. We tried various Lewis acids as catalysts, finally settling on the unusual palladium(II) catalyst, $[\text{Pd}(\text{MeCN})_4][\text{BF}_4]_2$ used by Nguyen,³¹⁻³³ as by far the most efficacious. While it was known that the $[\text{PdL}_n]^{2+}$ can complex to the oxygen of an appropriately oriented C-2 ether or the TCA nitrogen (Scheme 2a), we anticipated that it would not coordinate to the C-2 azide and so the reaction would pass through a standard oxocarbenium intermediate (Scheme 2b). This way the anomer of the initial TCA would not matter as the reaction would proceed through a planar intermediate (*pseudo-S_N1* mechanism), and the anomeric effect would ensure the formation of the desired α anomer. At first, the reaction was thought to be successful after comparing our NMR data with that of a similar compound from the literature (Compound 20, Fig. 2).²⁰ Compound 20 has an acetonide on C-3 and C-4, and a TIPS on C-6, instead of the acetate groups, but the NMR data of what we took to be the anomeric and amino acid signals largely agreed with each other. Combined with other characterization data such as a precise match by HRMS and a carbon spectrum that was consistent with the desired product, we believed that we had

successfully made the target compound 8. We published this result in the now retracted article.³⁴ As implied by that sentence, we were incorrect (Fig. 2).

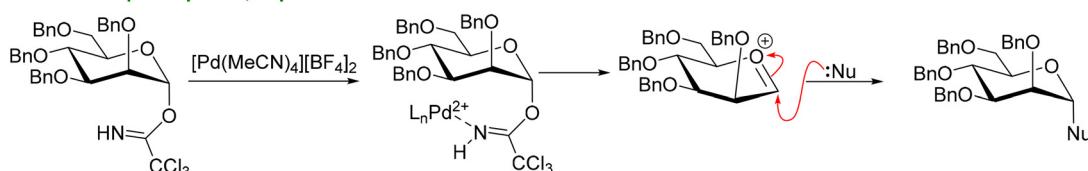
As noted, this study was conducted precisely so that we could generate large amounts of the Tn antigen to explore comparative TACA vaccine design. The yields of the glycosylation, although barely sufficient, were not acceptable for our process route, and we worked extensively on improving the reaction (including moving away from using large amounts of palladium as a Lewis acid), and continued to stock-pile material at what we believed was the stable azide 8. While doing this a new student attempted to assign all resonances in both the ¹³C and ¹H spectra, as an academic exercise, and observed some interactions in the 2D NMR that were not consistent with the anticipated structure. This led to the proposal that instead of compound 8, we had prepared the β anomer of the serine ester glycoside 9b. The evidence was that the proton signal at 5.57 ppm showed a large J-coupling of 8.5 Hz in the ¹H NMR spectrum. We had initially assigned this as the amide N-H proton from α -galactosamine, as we observed coupling to the C2-proton. However, it became apparent that this was not the case, and it was in fact the C1 anomeric proton. An α -anomer should have ³J_{1,2} coupling of around 3–4 Hz, whereas the 8.5 Hz observed is far more consistent with a β anomer. Further NMR experiments were conducted, and 1D NOE spectra showed interactions between this anomeric proton H_a and both H_c and H_e (Fig. 2). This interaction is unlikely to occur in α galactoside 8 as the anomeric proton is equatorial, therefore out of range of NOE effect from H_c and H_e, but is very reasonable in β galactoside 9b because of the 1,3-diaxial interactions present between these three protons. Furthermore, careful inspection of the ¹H-¹³C HMBC spectrum revealed an interaction between the anomeric proton and a carbonyl carbon, which would be highly unlikely had the galactose been connected to serine through the hydroxyl oxygen. However, if the glycosidic bond is made through the carboxylic oxygen to form an



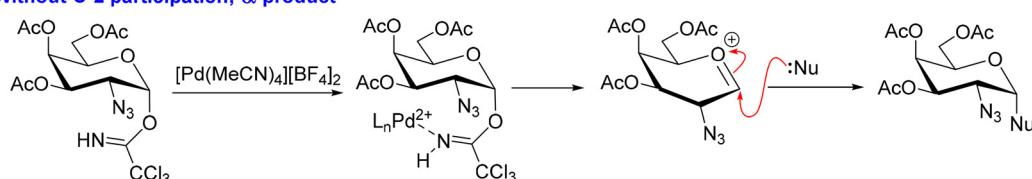
a) Nguyen's Precedent:
With C-2 ether participation; β -product



Without C-2 participation; α -product



b) Our hypothesis
Without C-2 participation; α -product



Scheme 2 Proposed mechanism for Nguyen's Palladium catalyzed glycosylation, and our proposed application of it to a C2 azido glycosyl donor.

ester, this unexpected correlation would be observed. Comparison of our ^1H NMR data on compounds 5, 6, and 8 showed that the anomeric proton's chemical shift in compound 8 (5.57 ppm) is closer to that of the β anomer of compound 5 (5.58 ppm), where it is next to an electron-withdrawing nitrate group, than to that of the β anomer of compound 6 (4.71 ppm), where it is next to an alcohol (Fig. 2). All these observations were only consistent with

the formation of compound 9b. In order to confirm this assignment, we wished to prepare the α -anomer 9a as well as Danishefsky *et al.*'s compound, 20 (Scheme 4).

We investigated other conditions (a full table can be found in the SI, experimental procedure of 9b) and found that BEt_3 and LiBF_4 in THF gave slightly higher yields of 9b, 58% and 46% respectively, than $[\text{Pd}(\text{MeCN})_4][\text{BF}_4]_2$ in DCM (up to 38%), but α -anomer was only observed in trace amount and could not be separated cleanly. The key determinant for the α/β selectivity seems to be the choice of base in the reaction of 6 to 7, as potassium carbonate favoured the formation of 7b ($7\text{a}:7\text{b} = 1:4-1:9$), whereas caesium carbonate favoured 7a (10:1). We were thus able to synthesise compound 9a, with only a trace amount of compound 9b, by using K_2CO_3 to form the acetimidate mixture and using BEt_3 in THF for the glycosylation (Scheme 3).

The inversion of stereochemistry suggests that this process is dominated by an $\text{S}_{\text{N}}2$ mechanism instead of going through an oxocarbenium intermediate. The fact that with the palladium Lewis acid we only obtained 9b with (retrospectively) only trace evidence of 9a is likely due to the attenuated reactivity of this Lewis Acid (passing through the $\text{S}_{\text{N}}2$ rather than oxocarbenium pathway), and a combination of the relative underactivity and limited amount of the β -TCA under the reaction conditions. This need for an $\text{S}_{\text{N}}2$ -capable nucleophile makes the engagement of a supposedly protonated carboxylic acid more surprising.³⁵

One of the factors leading to our mistakenly identifying 9b as 8 was that the NMR data of similar compounds—8 itself is novel and has no exact precedent—is from the older literature; it therefore lacks spectral support (*i.e.* a reproduction of the

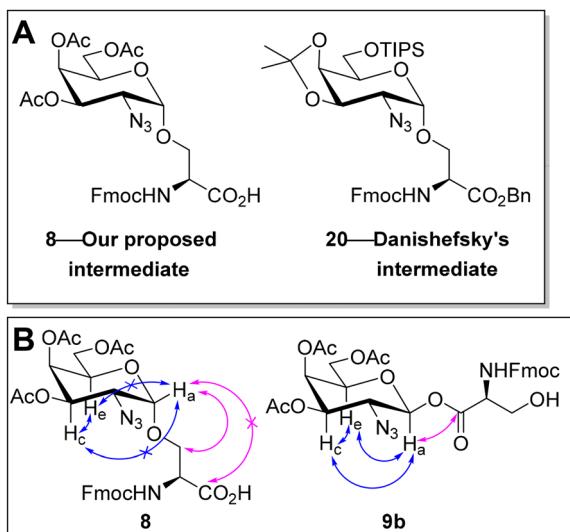
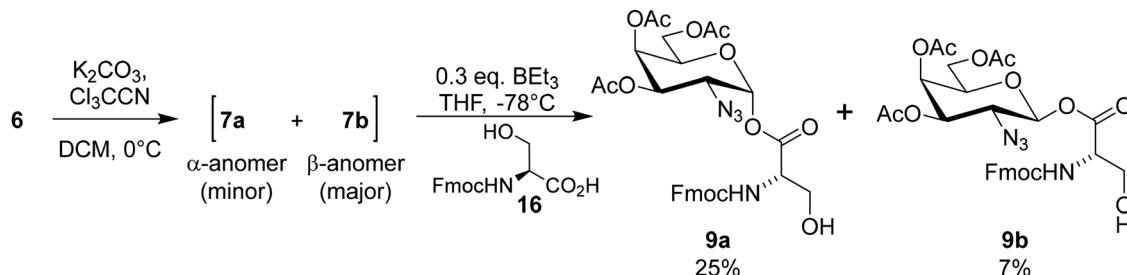


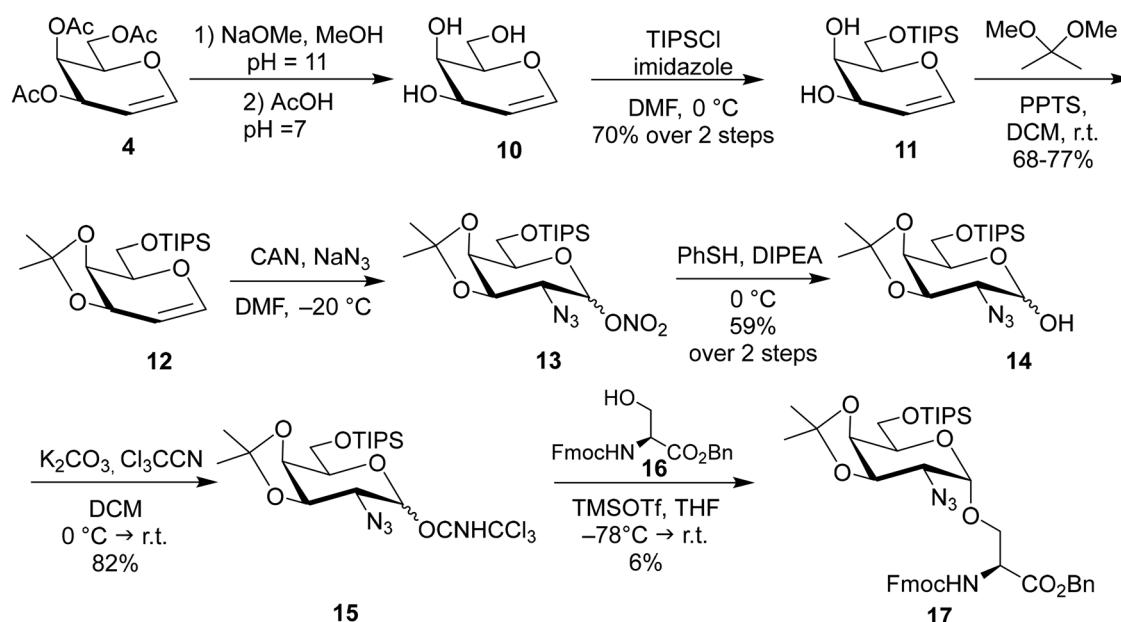
Fig. 2 Comparison of our intermediate with the most relevant comparator and structural revision. (A) The structure of our proposed intermediate 8 and the equivalent structure from Danishefsky and colleagues' 1998 synthesis.²⁰ (B) Expected NOE (blue) and HMBC (pink) correlations in compound 8 and observed correlations in compound 9b.

Scheme 3 The synthesis of the α anomer of ester glycoside 9a.

spectrum), the data is unassigned, and was collected on lower resolution spectrometers. This meant that it was easier for us to convince ourselves that the interpretation of our own data was for our desired compound; we saw what we wanted to see. We also did not consider that ester formation could be competitive; after all the product 9b has never been reported. We thus wished to reproduce Danishefsky's synthesis to confirm their data—and our own—and provide the spectra to ourselves and others, while providing a comparison of their approach to this glycoside to our own (Scheme 4).

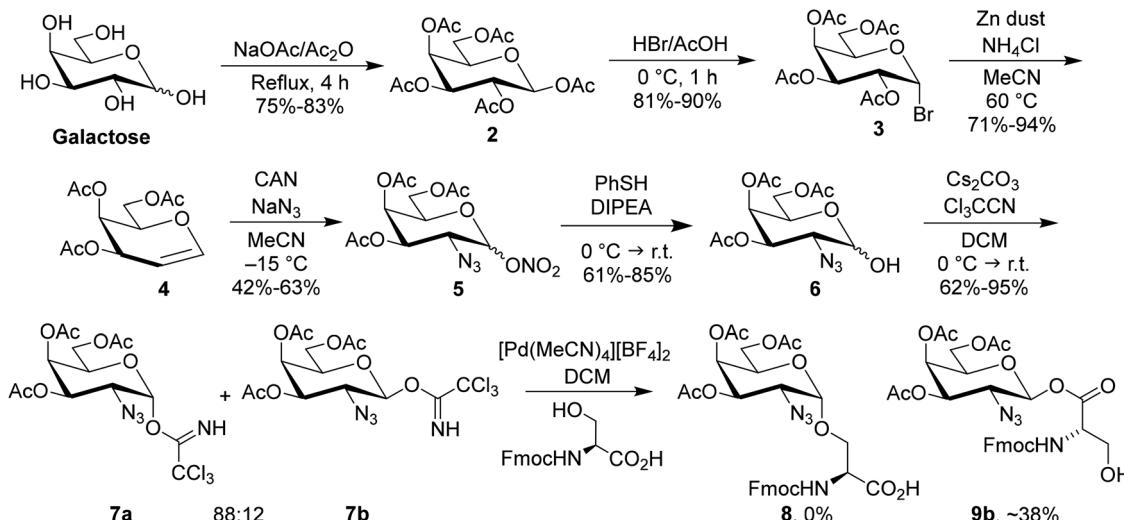
The reported synthesis started with compound 16, but its preparation was not described.³⁶ Thus we prepared 16 from galactal 4, following the most obvious synthesis (likely the same route used by Danishefsky). Deacetylation of compound 4 using NaOMe gave compound 14. It is worth noting that our standard protonation quench of Zemplén conditions employing DOWEX (always regenerated at least once)³⁷ caused the enol ether in the product to undergo various side reactions and eventually led to full decomposition, as the crude NMR did not show the presence of the desired compound but instead a complex mixture of what we took to be oligomers. We could successfully obtain 14 using 50% acetic acid instead of DOWEX during the

quench, titrating the acid until the reaction mixture reached a neutral pH (monitored using litmus paper). C6-selective TIPS protection of compound 14 was conducted at room temperature according to literature protocols,³⁸ and the product, 15, could be used without purification for the next step, although over-protection was observed. We note that modifying the conditions by conducting this reaction at a lower temperature (0 °C) significantly improved the yield of 15 as it effectively prevents the addition of a second TIPS group. The acetonide protection to fully-protected 16 was straightforward following the reported procedure.³⁹ The last three reactions were conducted as described by Danishefsky,²⁰ except for a solvent change to DMF in the reaction from compound 16 to 17 after attempts with the reported MeCN failed. With these modifications, we were able to access Danishefsky's glycoconjugate intermediate 20. The data acquired is an excellent match for what was published (with improved resolution arising from our higher field magnet), and we provide full assignment for the convenience of future readers (see either the SI or the deposited NMR raw data). Re-synthesis and assignment of compound 20 helped confirm our characterization of compounds 9a and 9b; however, the overall yield using this approach is unsatisfactory,



Scheme 4 Our modified approach of Danishefsky's synthesis of glycoconjugate 17.



Scheme 5 Modified synthesis of **1A** and **1** employing Fmoc-Ser-OBn, **16**.

partially arising from the use of acid-sensitive protecting groups in a silica-chromatography intense sequence. In fairness, Danishefsky's protecting group strategy was designed precisely so that **20** could be selectively deprotected at C-6 (while keeping C-3 and C-4 protected) for F1 α antigen synthesis; however, for Tn antigen synthesis we do not need to discriminate between these positions and thus we can use a more unified protection strategy.

We thus needed to develop an alternative route to the Tn-SPPS building block **1A**. We began with an analysis of what had gone wrong: a protonated carboxylic acid (the reaction occurs in the presence of a Lewis Acid without exogenous Brønsted base) outcompeting an alcohol in gross nucleophilicity was surprising. However, a potential explanation for this comes from a consideration of the relative acidities of the species involved. While we were unable to find a value for trichloroacetamide itself, in DMSO the pK_a of the related trifluoroacetamide is 17.2, whereas an aliphatic carboxylic acid is significantly lower ($\text{AcOH} = 12.6$).³⁵ We thus hypothesize that the trichloroacetamide anion acts as a base to deprotonate the carboxylic acid moiety of the serine. Consequently, we had unwittingly produced the carboxylate and that was competitive with the alcohol.

To avoid this carboxylate and the concomitant formation of ester glycoside, we reluctantly protected the offending functional group as a benzyl ester (Scheme 5). The benzyl ester is well precedented, easy to install and the deprotection conditions (Pd/C and H_2) would not affect other protecting groups in the compound.^{20,24} We kept the trichloroacetimidate strategy as it showed promising reactivity and started the new route with compound **11** and LiBF_4 , but no reaction was observed. This agrees with our hypothesis that $\text{Cl}_3\text{CCONH}^-$ may have been acting as a base in the presence of carboxylic acid, generating carboxylate as the nucleophile attacking trichloroacetimidate in S_N2 fashion—the neutral alcohol is simply not competitive and with no possible acid, no reaction can occur (other than the TCA rearrangement to the more stable *N*-glycoside⁴⁰). We then changed the Lewis acid to TMSOTf (0.3 equiv.) and it resulted in

satisfactory yield. Both anomers were isolated from the crude product (a 10 : 1- α : β ratio). Compound **12a**'s anomeric proton is at 4.87 ppm, $J = 3.6$ Hz, and shows no interaction with either the C3 or C5-H in the NOE experiment. The characterization data is in line with literature data and what is expected for α -galactoside. Full spectral data and assignments are provided in the SI. Compound **12a** was then subjected to reduction and acetylation to give **13** in high yield. The benzyl ester can be reduced to the acid under H_2 and Pd/C to give compound **1A**, which is ideal for SPPS. Under the same conditions but with a higher amount of Pd/C (1 equiv.) present, the Fmoc group can also be cleaved off to give compound **22**, which can be easily deacetylated to give the parent Tn antigen **1** should that be desired.

Conclusion

We consequently report a readily scalable total synthesis of a suitably protected Tn antigen for SPPS as well as the correct interpretation of previously misidentified products. We also reproduced Danishefsky's Tn antigen synthesis and provide both a moderately improved synthesis of the differentially protected building block as well as NMR data with full assignment and additional characterization data.

Author contributions

Conceptualization, JFT; funding acquisition JFT; investigation, PX, RG, SP, JC, MRR; methodology, PX, RG, SP, MRR, JJH; project administration, JFT; supervision, JFT; writing –original draft, PX, RG, SP, MRR; writing – review and editing, PX, SP, MRR, JJH, JFT.

Conflicts of interest

JFT is the CEO of Binary Star Research Services. BSRS has no business interest in the subject of this article. BSRS had no

input into the results, methodology, or conclusions of this article and provided no funding and received no benefit. The other authors have no conflicts to declare.

Data availability

Supplementary information (SI): All synthetic methods, characterization listings, and spectra are provided. See DOI: <https://doi.org/10.1039/d5nj02399h>.

Additionally, we have deposited all of the raw data obtained directly from the spectrometer organized by compound as presented in the supplementary information as a.zip archive in the Borealis Repository at: <https://doi.org/10.5683/SP3/XZA7PC>.

Acknowledgements

The authors would like to thank Iraj Sadraei, and Dina Dilinaer of the Trant Team for attempting the glycosylation reaction, the Windsor Cancer Centre Foundation Seeds 4 Hope program (2018-01 to JFT); the Natural Sciences and Engineering Research Council of Canada Discovery Grants Program (NSERC, 2018-06338 to JFT) the Canadian Foundation for Innovation and Ontario Research Fund (37425 to JFT); the Banting Research Foundation (2018-1403 to JFT); the NSERC Undergraduate Student Research Award (USRA-2017 to SP); both a 2024 & a 2025 BioCanRx Indigenous Student Summer Internship to RG; and the Ontario Early Researcher Award Program (ER18-14-114 to JFT) for funding this research. Finally, JFT, MRR, and SP would like to acknowledge the Chiaramonte family for sharing J. Chiaramonte with us for too short a time, we share their grief and we continue to miss his participation in our lives every day.

References

1. Soerjomataram and F. Bray, *Nat. Rev. Clin. Oncol.*, 2021, **18**, 663–672.
2. L. Rahib, M. R. Wehner, L. M. Matrisian and K. T. Nead, *JAMA Netw. Open*, 2021, **4**, e214708.
3. American Cancer Society, *Cancer Facts & Figures*, American Cancer Society, Atlanta, 2020.
4. R. L. Siegel, K. D. Miller and A. Jemal, *Ca-Cancer J. Clin.*, 2020, **70**, 7–30.
5. C. L. Arteaga, M. X. Sliwkowski, C. K. Osborne, E. A. Perez, F. Puglisi and L. Gianni, *Nat. Rev. Clin. Oncol.*, 2012, **9**, 16.
6. T. N. Schumacher and R. D. Schreiber, *Science*, 2015, **348**, 69–74.
7. M. C. Maia and A. R. Hansen, *Crit. Rev. Oncol. Hematol.*, 2017, **113**, 292–303.
8. S. I. Sadraei, M. Reynolds and J. F. Trant, *Adv. Carbohydr. Chem. Biochem.*, 2017, **74**, 137–237.
9. M. M. Tu, M. M. A. Rahim, C. Sayed, A. B. Mahmoud and A. P. Makrigiannis, *Cancer Immunol. Res.*, 2017, **5**, 1016–1028.
10. A. Corthay, *Front. Immunol.*, 2014, **5**, DOI: [10.3389/fimmu.2014.00197](https://doi.org/10.3389/fimmu.2014.00197).
11. H. Sultan, V. I. Fesenkova, D. Addis, A. E. Fan, T. Kumai, J. Wu, A. M. Salazar and E. Celis, *Cancer Immunol. Immunother.*, 2017, **66**, 203–213.
12. Z. Hu, P. A. Ott and C. J. Wu, *Nat. Rev. Immunol.*, 2017, **18**, 168.
13. G. F. Springer, *Science*, 1984, **224**, 1198–1206.
14. T. Kieber-Emmons, B. Monzavi-Karbassi, L. F. Hutchins, A. Pennisi and I. Makhoul, *Hum. Vaccines Immunother.*, 2017, **13**, 323–331.
15. S. F. Slovin, S. J. Keding and G. Ragupathi, *Immunol. Cell Biol.*, 2005, **83**, 418–428.
16. D. Feng, A. S. Shaikh and F. Wang, *ACS Chem. Biol.*, 2016, **11**, 850–863.
17. M. Glaffig, B. Palitzsch, S. Hartmann, C. Schüll, L. Nuhn, B. Gerlitzki, E. Schmitt, H. Frey and H. Kunz, *Chem. – Eur. J.*, 2014, **20**, 4232–4236.
18. R. M. Wilson and S. J. Danishefsky, *J. Am. Chem. Soc.*, 2013, **135**, 14462–14472.
19. G. Arsequell and G. Valencia, *Tetrahedron: Asymmetry*, 1997, **8**, 2839–2876.
20. X.-T. Chen, D. Sames and S. J. Danishefsky, *J. Am. Chem. Soc.*, 1998, **120**, 7760–7769.
21. S. D. Kuduk, J. B. Schwarz, X.-T. Chen, P. W. Glunz, D. Sames, G. Ragupathi, P. O. Livingston and S. J. Danishefsky, *J. Am. Chem. Soc.*, 1998, **120**, 12474–12485.
22. B. Liebe and H. Kunz, *Tetrahedron Lett.*, 1994, **35**, 8777–8778.
23. T. Reipen and H. Kunz, *Synthesis*, 2003, 2487–2502.
24. A. Miermont, H. Barnhill, E. Strable, X. Lu, K. A. Wall, Q. Wang, M. G. Finn and X. Huang, *Chem. – Eur. J.*, 2008, **14**, 4939–4947.
25. H. Paulsen and J.-P. Hölk, *Carbohydr. Res.*, 1982, **109**, 89–107.
26. This molecule is stable in the freezer under argon, but rapidly decomposes under ambient conditions and should not be stored on the bench overnight or longer.
27. R. U. Lemieux and R. M. Ratcliffe, *Can. J. Chem.*, 1979, **57**, 1244–1251.
28. X. Zhu and R. R. Schmidt, in *Handbook of Chemical Glycosylation*, ed. A. V. Demchenko, Wiley, Weinheim, 2008, vol. 1, pp. 143–184.
29. R. R. Schmidt and X. Zhu, in *Glycoscience*, ed. B. Fraser-Reid, K. Tatsuta and J. Thiem, Springer-Verlag, Berlin, 2008, vol. 1, pp. 452–524.
30. R. Behrendt, P. White and J. Offer, *J. Pept. Sci.*, 2016, **22**, 4–27.
31. J. Yang, C. Cooper-Vanossell, E. A. Mensah and H. M. Nguyen, *J. Org. Chem.*, 2008, **73**, 794–800.
32. M. J. McKay, B. D. Naab, G. J. Mercer and H. M. Nguyen, *J. Org. Chem.*, 2009, **74**, 4705–4711.
33. E. A. Mensah, J. M. Azzarelli and H. M. Nguyen, *J. Org. Chem.*, 2009, **74**, 1650–1657.
34. M. R. Reynolds, S. Piazza, J. Chiaramonte, P. Xu, F. A. Chapa-Villarreal and J. F. Trant, *New J. Chem.*, 2021, **45**, 19224–19227.
35. F. G. Bordwell, *Acc. Chem. Res.*, 1988, **21**, 456–463.
36. Personal Communication: Danishefsky to Trant, July 2017. In 2017 in the preparation of our comprehensive review of the subject (Sadraei *et al.*, 2017—ref. 7), JFT had contacted



- Dr Danishefsky for the details to be included in the article. Dr Danishefsky apologized for not being able to provide that data as his lab was shutting down and he no longer had access to that information.
- 37 A. Peyret, J. F. Trant, C. V. Bonduelle, K. Ferji, N. Jain, S. Lecommandoux and E. R. Gillies, *Polym. Chem.*, 2015, **6**, 7902–7912.
- 38 S. Medina, M. J. Harper, E. I. Balmond, S. Miranda, G. E. M. Crisenzia, D. M. Coe, E. M. McGarrigle and M. C. Galan, *Org. Lett.*, 2016, **18**, 4222–4225.
- 39 C. M. Woo, L. Lu, S. L. Gholap, D. R. Smith and S. B. Herzon, *J. Am. Chem. Soc.*, 2010, **132**, 2540–2541.
- 40 M. G. Hoffmann and R. R. Schmidt, *Liebigs Ann. Chem.*, 1985, 2403–2419.

