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Solving the Convergence Problem in the Synthesis of Triantennary N -Glycan Relevant to Prostate-Specific Membrane Antigen (PSMA)

Maciej A. Walczak[1] and **Samuel J. Danishefsky**[1,2]

[1]Laboratory for Bioorganic Chemistry, Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, New York 10065, United States

[2]Department of Chemistry, Columbia University, 3000 Broadway, New York, New York 10027, United States

Abstract

The first total synthesis of triantennary, fully sialylated *N*-glycan of complex type is described. Two strategies for installation of sialylated antennae are explored and both approaches converge on a global glycosylation step that delivers the desired tetradecasaccharide in good yields.

Introduction

It is well established that the post-translational attachment of glycan domains can play a central role in mediating protein stability, function, and structure.[1] Interestingly, changes in protein glycosylation patterns may signal the onset of aberrant biochemical processes. This phenomenon has been exploited as a means by which to monitor disease progression.[2] Of particular interest to our laboratory is the finding that transformed tumor cells often exhibit characteristic levels and types of cell surface carbohydrate expression. Theoretically, it should be possible to design vaccine constructs bearing these tumor-associated carbohydrate antigens, which, upon presentation to the immune system, would induce a selective immune response, leading to the eradication of tumor cells expressing these carbohydrates. A major research program underway in our laboratory is devoted to the de novo synthesis and immunological evaluation of complex carbohydrate-based anticancer vaccine candidates.[3] A number of our most promising constructs have been advanced to clinical trials.

In the context of this research program, we identified Prostate Specific Membrane Antigen (PSMA) as an attractive target for a potential vaccine against prostate cancer.[4] PSMA, a membrane-bound glycoprotein possessing carboxypeptidase activity, is apparently localized in the prostate sac and is heavily over-expressed in prostate cancer.[5] Additionally, PSMA is upregulated in an androgen-independent stage.[6] Moreover, this glycoprotein is found on the neovasculature of a panel of solid tumors, including renal cells, breast, and colonic carcinomas.[7] The immunogenic properties of the peptidic domain of PSMA, as well as the enzymatic activity of the glycoprotein,[8] have been harnessed for the development of anti-tumor therapies.[4] However, less well understood is the role of the oligosaccharide domain of PSMA, and its potential application for the development of prostate cancer vaccines.[9]

*Corresponding Author: s-danishefsky@ski.mskcc.org.

ASSOCIATED CONTENT

Supporting Information General experimental procedures, including spectroscopic and analytical data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

PSMA possesses ten sites of *N*-linked glycosylation, and the glycans comprise about 20-25% of the total molecular weight of the glycoprotein.[10] Based on the crystal structure of truncated PSMA, eight asparagine sites are exposed to the extracellular environment[11] and certain glycosylation positions (e.g. Asn638) can play a critical role in maintaining enzymatic function of this homodimeric glycoprotein.[12] A consensus structure of PSMA glycan, isolated from prostate tumor cells, is depicted in Figure 1. This complex-type oligosaccharide is characterized by the presence of sialic acid residues (Neu5Ac) at each of its three antennae. The sialic moieties play an important role in recognition processes, through mediation of glycoprotein conformation and binding of positively charged compounds.[13] In addition, resident sialic acid motifs can complicate immune surveillance of potential antigenic sites.[14] Synthetic access to homogeneous **1** would enable studies on the biological role of the glycan expression in PSMA and its potential role in the etiology and pathogenesis of prostate cancer. We previously accomplished the synthesis of a related core biantennary sialylated glycan structure often found in the mammalian glycoproteins.[15] Since the highly branched tetradecasaccharide **1** has also been identified in other glycoproteins of biological interest,[16] studies on the role of glycosylation in these systems could deepen our understanding of how the phenomenon of altered carbohydrate expression can be harnessed for therapeutic purposes.

As depicted in Figure 1, glycan **1** is composed of a pentasaccharide core (saccharides 1-5), equipped with three trisaccharide antennae. Our general synthetic strategy envisioned the three-fold glycosylation of the partially protected pentasaccharide with excess sialylated trisaccharide donor. The pentasaccharide precursor itself would be accessed through a sequence featuring coupling of the “reducing” chitobiose end (saccharides 1 and 2) with a mannose donor (saccharide 3). The resultant core system would then be further elaborated *via* α-mannosylation, with building blocks orthogonally protected at the 2′ and 4′-OH (saccharide 4) and at the 2′-OH (saccharide 5). At the outset of these studies, we were mindful of the difficulties typically associated with glycosylation of sterically hindered 4′-OH mannose acceptors, which could well pose a significant challenge to the successful execution of the plan.[15]

Results and Discussion

We first sought to gain access to core pentasaccharide **14**, bearing three free hydroxyl groups, in order to examine the feasibility of installing all three sialylated antennae in a single step. The synthesis of key intermediate **14** commenced with the coupling of two protected glucosamine monosaccharides, **2**[17] and **3**,[18] to generate disaccharide **4** in nearly quantitative yield (Scheme 1). Removal of the acetyl group, under Zemplén conditions, afforded the chitobiose acceptor, **5**, properly functionalized for the requisite β-mannosylation. Thus, under Crich β-mannosylation conditions,[19] **5** and **6**[20] were efficiently coupled with high stereoselectivity (*dr*>10:1). The crude mixture was then treated with a buffered solution of DDQ to provide **8** in 56% yield over two steps. Coupling of **8** and **9**, with subsequent reductive cleavage of the benzylidene linkage, proceeded in 88% yield to afford only the desired isomer, **11**. Next, attachment of the mannose thioethyl donor **12** to tetrasaccharide **11** provided pentasaccharide **13** in 90% yield. Finally, liberation of the acyl functionalities under basic conditions afforded triol **14** in 83% yield. Use of the 2,5-difluorobenzoyl (dFBz)[21] protecting group was very helpful in allowing for a high yield in this step. Similar attempts to perform removal of unsubstituted benzoyl groups led to incomplete conversion and eventual cleavage of the phthalimidyl functionalities.[22]

We next prepared a series of trisaccharide derivatives (Scheme 2). Thioether trisaccharide **15** (itself prepared in 25 steps from commercially available materials),[23] was converted to anomeric fluoride **16** through exposure to HF/pyridine. Alternatively, the Schmidt type

donor, **19**, could be prepared through coupling of **15** with 2-(trimethylsilyl)ethanol, followed by acidic cleavage of the anomeric protecting group and formation of the corresponding trichloroacetimidate. With a range of glycosyl donors in hand, we were now in a position to evaluate the feasibility of the proposed global three-fold glycosylation of pentasaccharide **14**.

The results of the attempted global glycosylation with pentasaccharide **14** are summarized in Table 1. Thus, when a five-fold excess of donor **14** was exposed to Sinaÿ radical conditions,[24] only 10% of the desired product, **20**, was observed, along with unidentified side-products (entry 1). A survey of coupling protocols revealed thioether **15** to be an efficient donor under NIS/TfOH-mediated conditions (entry 3). The yield of this transformation was further improved when the reaction was conducted with AgOTf as a stoichiometric additive (entry 4). It is also of note that, when the reaction was carried out under more dilute conditions (<0.025M), no triglycosylated product was observed (MS-ESI) and only partially substituted intermediates were detected. Thus, under the optimized conditions (0.04 M), the desired saccharide **20** was formed in 21% yield, which translates to an average yield of 60% per glycosyl acceptor coupling. In a related glycosylation event with a disaccharide donor, we observed an improved yield of triantennary oligosaccharide, indicating a detrimental steric impact of the sialic acid moiety on global glycosylation reactions.[22] Saccharide **20** was isolated following extensive chromatographic purifications on silica gel and gel filtrations (Sephadex LH-20 and Bio-Gel S-X1). We were also able to isolate 20% of biantennary products from this reaction, which, upon re-exposure to the above glycosylation conditions, afforded **20** in 10% yield. Attempts to achieve coupling with fluoride donor **16** (under $Cp_2HfCl_2/AgClO_4$ or $BF_3{*}Et_2O$ conditions), or through sulfoxide-mediated glycosylation protocols,[25] led to clean activation of the donor, but only decomposition of pentasaccharide **14** (entries 5 and 6). Interestingly, Schmidt donor **19** was found to be a suitable reagent for this transformation, delivering **20** in 19% yield, with only trace amounts of diglycosylated intermediates (entry 7).

Based on the findings summarized in Table 1, we hypothesized that the efficiency of the synthetic route toward **1** could be greatly improved if the sialylated antenna at the most sterically hindered position (ring 4, 4′-OH) were installed first. To this end, trisaccharide **8** was coupled with orthogonally protected mannose donor **21** and the resultant tetrasaccharide was then exposed to reductive conditions to provide **22** in 53% yield (Scheme 3). The top mannose branching was introduced *via* reaction with donor **23** and removal of the silyl functionality. When **25** was coupled with **15** under the optimized conditions (Table 1, entry 4), octasaccharide **26** was obtained in 60% yield. The subsequent reductive removal of the 2′-OH protective groups proceeded uneventfully. We were pleased to find that installation of the remaining two trisaccharide antennae proceeded in 50% yield (>70% per glycosyl acceptor), which represents a significant improvement in overall efficiency, in comparison to the global glycosylation strategy.

With compound **20** in hand, we next proceeded to the stage of global deprotection. As shown in Scheme 4, the ester functionalities were cleaved through exposure to NaOMe/MeOH, and the resultant crude triacid was treated with 1,2-ethylenediamine in *n*-butanol and PhMe, to liberate the five amine functionalities.[26] This mixture was then exposed to selective *N*-acetylation conditions to deliver triacid **28**. The best yield for this sequence was obtained when the excess of diamine was removed following initial cleavage of the phthalimidyl ring and the deprotection was completed in refluxing toluene. Although we were unable to remove the benzyl groups through hydrogenolysis, exposure of **28** to dissolving metal conditions provided clean deprotection of the 24 benzyl functionalities in one step to deliver the target glycan **1**, in moderate overall yield.

Conclusion

In summary, we have disclosed the first total synthesis of fully sialylated triantennary *N*-linked glycan, **1**. In toto, oligosaccharide **1** was reached in 71 steps from commercially available materials, through a synthetic route featuring an optimized convergent installation of the terminal sialylated antennae. While a large number of steps were required to reach this complex target, very little material is needed to allow for evaluation of the underlying biological question. Moreover, the synthetic program outlined in this paper will surely provide guidance for the synthesis of other highly branched members of this family of complex PSMA-related *N*-linked glycodomains. Studies on the biological and immunological properties of glycan **1** are currently underway.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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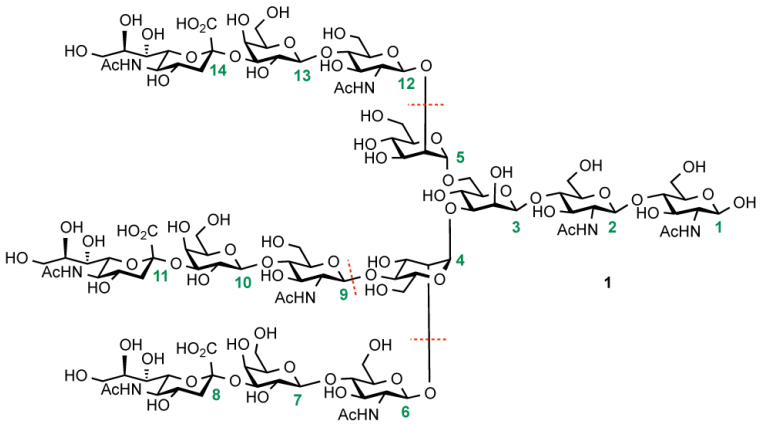
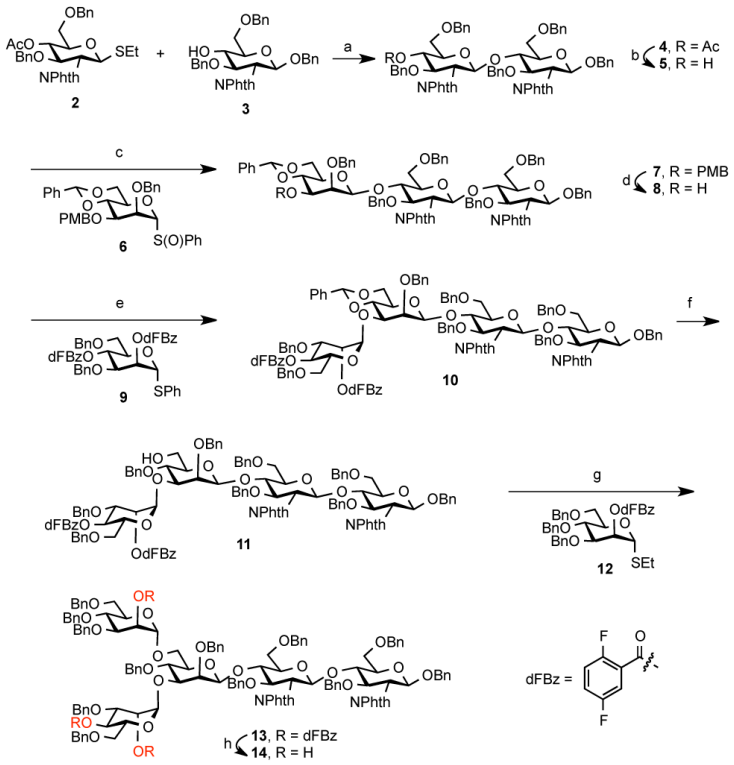
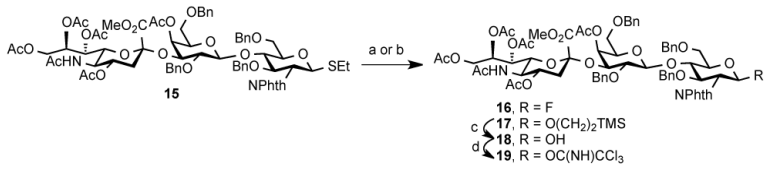


Figure 1.



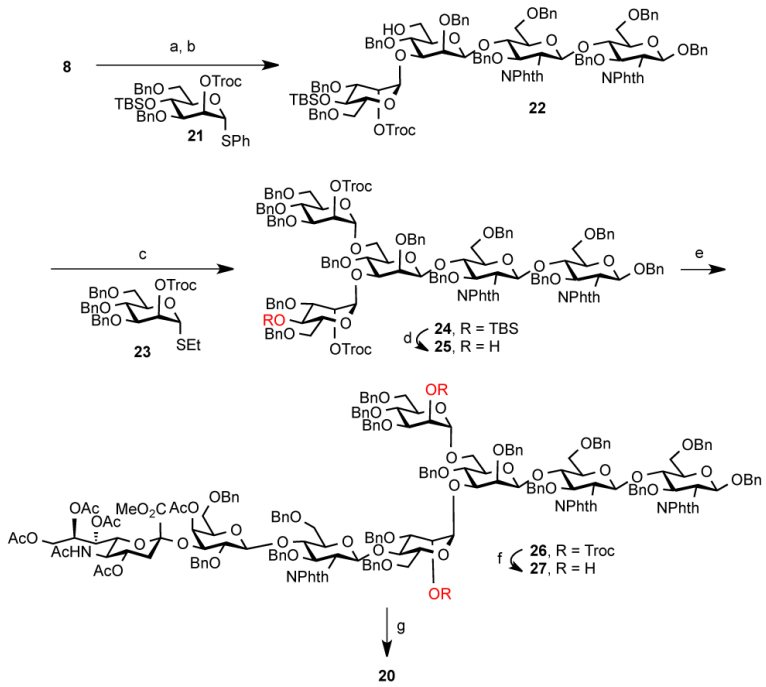
Scheme 1. Reagents and Conditions

(a) NIS, AgOTf, CH_2Cl_2, 3Å MS, −25 °C, quant.; (b) NaOMe, MeOH, THF, rt, 1 h, 91%; (c) **6**, Tf_2O, DTBMP, CH_2Cl_2, 4A MS; (d) DDQ, CH_2Cl_2, phosphate buffer pH 7, 56% (over 2 steps); (e) **9**, NIS, AgOTf, CH_2Cl_2, 3Å MS, 90%; (f) BH_3/THF, *n*-Bu_2BOTf, CH_2Cl_2, 0 °C, 4 h, 88%; (g) **12**, NIS, AgOTf, CH_2Cl_2, −50 to −25 °C, h, 90%; (h) NaOMe, MeOH, THF, rt, 1.5 h, 83%.



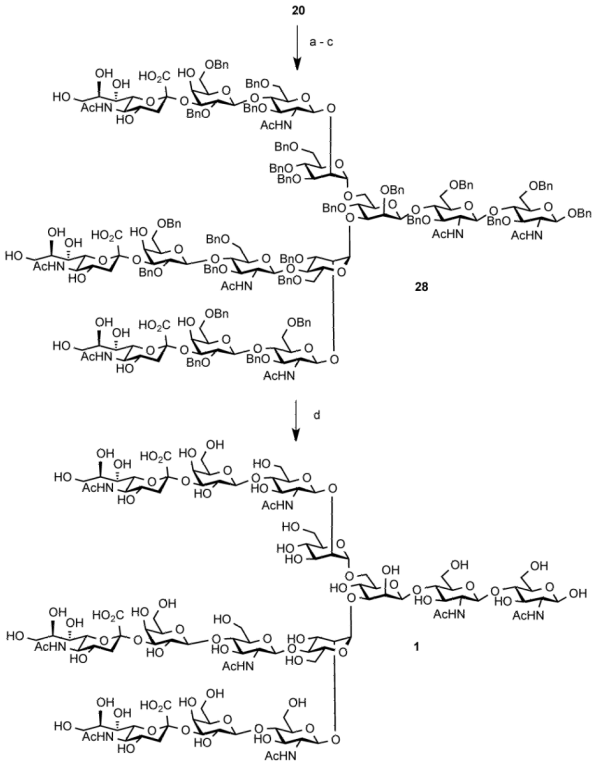
Scheme 2. Reagents and Conditions

(a) NBS, HF/pyridine, CH_2Cl_2, rt, 91%; (b) $TMSCH_2CH_2OH$, NIS, AgOTf, CH_2Cl_2, 3Å MS, −25 °C, 94%; (c) TFA, CH_2Cl_2, H_2O, rt, 5 h, 67%; (d) CCl_3CN, DBU, CH_2Cl_2, 0 °C, 87%.



Scheme 3. Reagents and Conditions

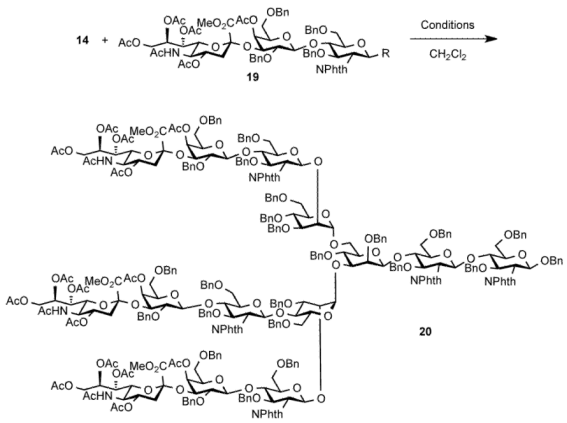
(a) **21**, NIS, AgOTf, CH_2Cl_2, 3Å MS, 2 h; (b) BH_3·THF, *n*-Bu_2BOTf, CH_2Cl_2/THF, 0 °C, 3 h, 53% (over 2 steps) (c) **23**, NIS, AgOTf, CH_2Cl_2, 3Å MS, −20 °C, 3 h, 78%; (d) HF/pyridine, THF, rt, 44 h, 58%; (e) **15**, NIS, AgOTf, CH_2Cl_2, 3 Å MS, −20 °C, 36 h, 60%; (f) nano-Zn, AcOH, THF, rt, 20 min, 85%; (g) **15**, NIS, AgOTf, CH_2Cl_2, 3 Å MS, −20 °C, 36 h, 50%.



Scheme 4. Reagents and Conditions

(a) NaOMe/NaOH, MeOH/H_2O, rt, 20 h; (b) 1,2-ethylenediamine, *n*-BuOH, PhMe, 90 °C, 24 h; (c) Ac_2O, Et_3N, MeOH, rt; (d) Na/NH_3, THF, −78 °C, 11-25% (over 4 steps).

Table 1



Entry	R	Conditions	Yield [%]
1	SEt (**15**)	$(4\text{-}BrC_6H_4)_3NSbCl_6$, 0 °C → rt	10%
2	SEt (**15**)	BSP, Tf_2O, TTBP, −60 °C	ND
3	SEt (**15**)	NIS, TfOH, −20 °C	16%
4	SEt (**15**)	NIS, AgOTf, −20 °C	21%
5	F (**16**)	Cp_2HfCl_2, $AgClO_4$, 0 °C	<5%
6	OH (**18**)	Ph_2SO, Tf_2O, −60 °C	ND
7	$OC(NH)CCl_3$ (**19**)	TMSOTf, −30 °C	19%

[a]Key: ND=None Detected