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Building Biologics by Chemical Synthesis: Practical Preparation of Di- and Triantennary *N*-Linked Glycoconjugates

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Supporting Information

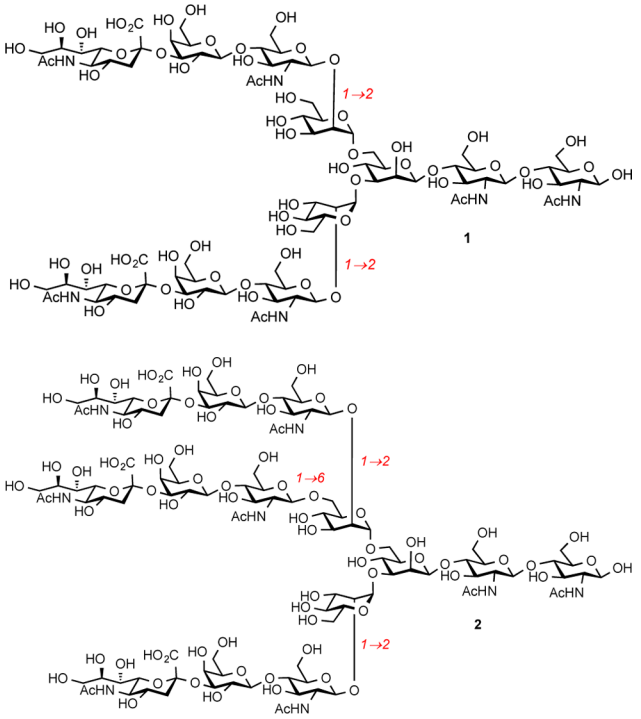
ABSTRACT: A unified strategy for the syntheses of bi- and triantennary fully sialylated *N*-glycans is described. The synthesis capitalizes on a global glycosylation strategy that delivers the desired undeca- and tetradecasaccharide in excellent yields. Finally, conjugation of the glycan to PSMA oligopeptide is described.

Remodeling of glycan composition in glycoproteins often results in critical changes in the (inter)cellular environment, and may signal metabolic imbalance.[1] These changes can induce functional modifications of glycoproteins by altering their structure, stability, and site of localization.[2] Given the importance of glycoproteins as potential therapeutic and diagnostic assets, their chemical synthesis could well prove to be of paramount importance in providing viable avenues for the investigations of covalent posttranslational modifications.[3] A great deal of structural variability comes from *N*-linked glycans, which are linked to the peptidic backbone through a strategic asparagine residue. The parent sialylated glycan is believed to be a biantennary structure containing terminal *N*-acetylneuraminic acid residues connected via an α-2,6- and/or α-2,3-linkage (**1**, Chart 1). The nature of these glycosyl bonds depends on the origin of protein; many glycoproteins contain a mixture of both linkage regioisomers. The first chemical synthesis of an undecasaccharide containing an α-2,6-linked sialic acid was reported by Ogawa.[4] However, it was found that this glycan can be easily obtained in substantial quantities from natural sources through isolation from egg yolk.[5] Another typical regioisomer, containing α-2,3-linked bonds (**1**), was prepared via synthetic means by Ito.[6] Not surprisingly, the chemical synthesis of this structure required lengthy sequences. Synthetic surrogates containing unnatural linkages have also been prepared.[7] A higher order structure of *N*-linked glycan contains additional trisaccharide branching linked via the 6-OH position of a mannose residue (**2**, Chart 1). The structure **2** and its fucosylated analog have been identified in a panel of glycoproteins, including erythropoietin (EPO),[8] follicle-stimulating hormone (FSH),[9] and human chorionic gonadotropin (hCG),[10] rendering synthesis of these oligosaccharides a worthwhile pursuit. Here, we present a practical approach for the synthesis of dibranched (undecasaccharide) and tribranched (tetradecasaccharide) structures containing terminal Neu5Ac residues.

As a part of our overall program directed toward the development of novel therapeutic agents and diagnostic strategies against cancer, we investigated the role of glycoproteins in the emergence and progression of prostate cancer.[11] We have shown that the extent of *N*-glycan branching in prostate-specific antigen (PSA) can be differentiated with synthetic glycoconjugates containing di-, tri-, and tetraantennary glycans. However, these systems did not contain terminal sialic acid residues, which are known to be critical components in cell signaling and promotion of immune response.[12] Access to glycans **1** and **2** would allow for validation of the processing of the glycans in biological systems and analysis of the impact of various glycoforms on the structure and function of glycoproteins. Additionally, given the complexity of these systems, chemical synthesis would provide ultimate validation of the synthetic strategies for the preparation of branched oligosaccharides.

At the retrosynthetic level, we envisioned that each antenna could be installed in a single glycosylation event using a properly

Chart 1



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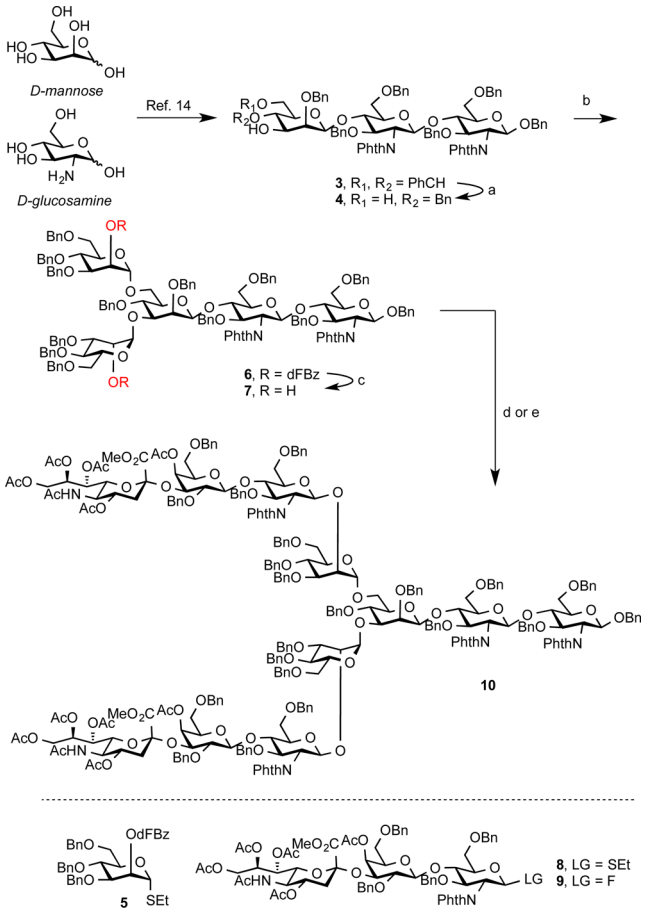
functionalized donor with Neu5Ac residues already in place.[13] We were mindful of potential hurdles associated with this approach since incorporation of three sialylated antennae in a related system presented a challenging synthetic step requiring careful orchestration to achieve practical yields.[14] However, because of the convergent nature of our synthetic plan, these critical steps seemed an attractive possibility to allow for a rapid assembly of the glycan core in a minimal number of synthetic operations.

The synthesis began with trisaccharide **3**,[14] which was reductively opened to provide diol **4** in an excellent yield (Scheme 1). This intermediate was then coupled with mannose

Scheme 1. Synthesis of Protected Undecasaccharide 10[a]



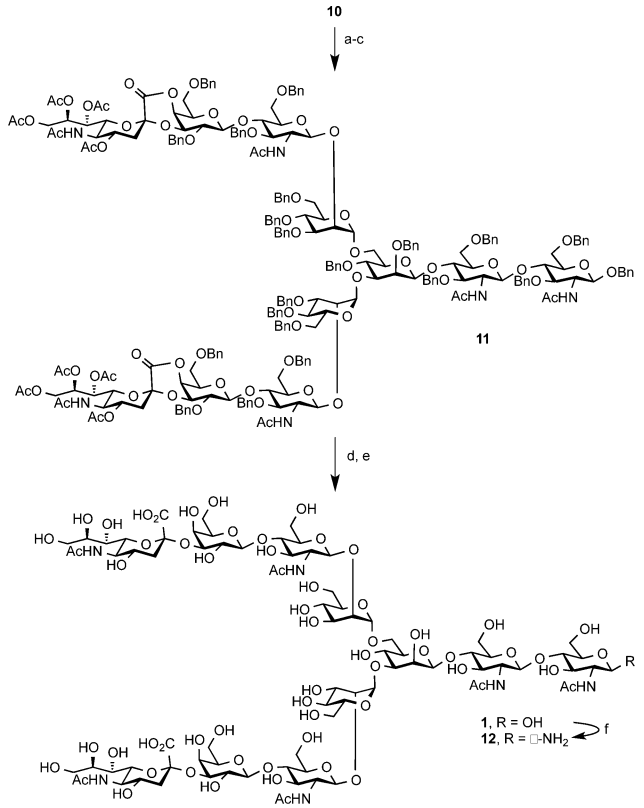
[a]Reagents and conditions: (a) BH_3·THF, *n*-Bu_2BOTf, CH_2Cl_2, 0 °C, 3 h, 83%; (b) **5**, NIS, AgOTf, CH_2Cl_2, −20 °C, 2 h, 89%; (c) NaOMe, MeOH, THF, rt, 1 h, 99%; (d) **8**, NIS, AgOTf, CH_2Cl_2, −20 °C, 24 h, 75%; (e) **9**, $Cp_2Zr(OTf)_2$·THF, CH_2Cl_2, 0 °C, 2 h, 74%.

thioether **5**[14] in 89% yield and the alcohol functionalities were liberated under basic conditions to afford diol **7**. It is well to note the importance of the dFBz (2,5-difluorobenzoyl) group in this step. Attempts to remove unsubstituted benzoyl groups in an analogous pentasaccharide led only to 40% yield of **7**, and a mixture of products arising from a partial cleavage of the phthalimide group was observed.

At this point we were ready to evaluate the feasibility of conducting a double glycosylation.[15] Accordingly, we investigated the utility of two trisaccharide donors in this transformation. Thioether **8**,[15] under NIS/TfO^- conditions, did indeed provide the expected undecasaccharide **10** in 75% isolated yield. Similarly, glycosylation with anomeric fluoride proceeded very efficiently and reached completion in 1.5 h at 0 °C to deliver **10** in a similar yield (86% average yield per glycosyl acceptor).[16] It is noteworthy that the reaction did not seem to produce, at least to the levels of our detection, transient monoglycosylated products. The stereochemistry of all of the glycosidic bonds was confirmed by analysis of $^1J_{CH}$ of anomeric carbon atoms.

With sufficient quantities of undecasaccharide **10** in hand, we advanced to the deprotection sequence (Scheme 2). First, the

Scheme 2. Deprotection Sequence for Glycan 1[a]



[a]Reagents and conditions: (a) NaOMe/NaOH, MeOH/H_2O, CH_2Cl_2, rt, 24 h; (b) 1,2-ethylenediamine, EtOH, 80 °C, 24 h; (c) Ac_2O, pyridine, rt, 24 h, 35% (over three steps); (d) NaOMe, MeOH, CH_2Cl_2 then H_2O, rt, 24 h; (e) Na, NH_3, THF, −78 °C, 4 h; (f) Ac_2O, $NaHCO_3$ (aq), 0 °C, 77% (over 3 steps); (g) NH_4HCO_3, H_2O, 40 °C, 3 d, 99%.

acetate groups were removed with NaOMe/MeOH and the methyl ester functionalities were saponified with NaOH. The resultant diacid was then used in a crude state to remove four phthalimide groups with 1,2-ethylenediamine in refluxing ethanol. We found that this set of conditions proved to be a more robust protocol for the deprotection of nitrogen functionalities in comparison with the conditions reported previously,[14] which required higher reaction temperatures. The resultant crude material was then subjected to acetylation conditions to provide bislactone **11** in 35% yield over a three-step sequence. The peracetylation step allows for purification of the oligosaccharide material before the reductive removal of benzyl groups. To complete the synthesis of **1**, saponification of **11** with NaOMe/NaOH furnished diacid, which was then subjected to Birch-type reduction to reveal undecasaccharide **1** in an appreciable yield of 77%. Occasionally, we found that some *N*-

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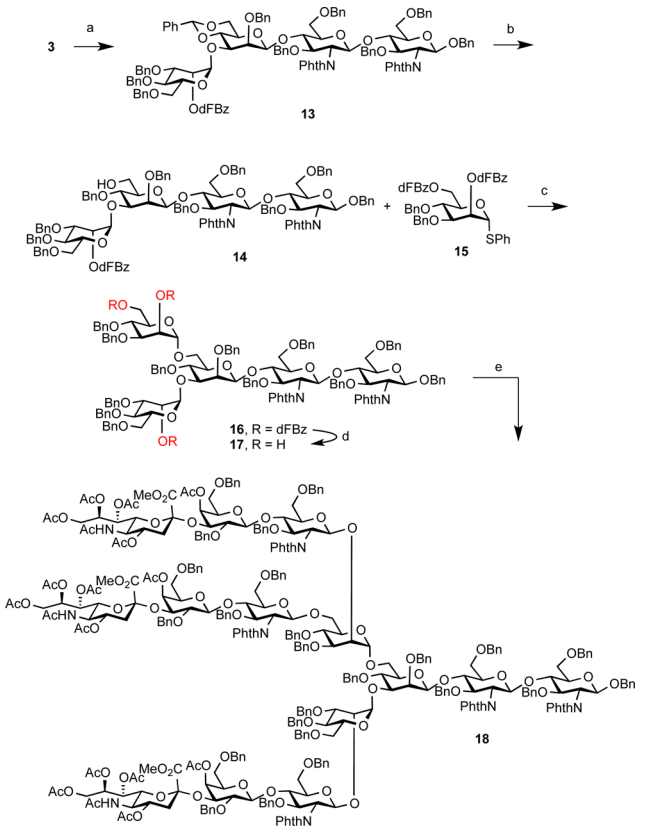
acetamide groups were cleaved under the reductive step, and an additional acetylation step (Ac_2O, sat. $NaHCO_3$(aq), 0 °C) was necessary to provide homogeneous material. Unlike deprotection of fucosylated biantennary glycan, which was shown to lead to a partial cleavage of the oligosaccharide chain, we observed only clean removal of the benzyl groups without any truncated saccharide fragments and/or over-reduction products.[15] Compared to the synthesis of **11** reported previously,[6] our approach capitalizes on a more convergent assembly of the core undecasaccharide and it displays significantly improved yields. The analytical data for glycan **1** match the previous reports.[6,10,17]

Having established a synthetic approach for the construction of the biantennary saccharide, we turned our attention to tetradecasaccharide **2** (Scheme 3). The synthesis started by

Scheme 3. Synthesis of Protected Tetradecasaccharide 18[a]

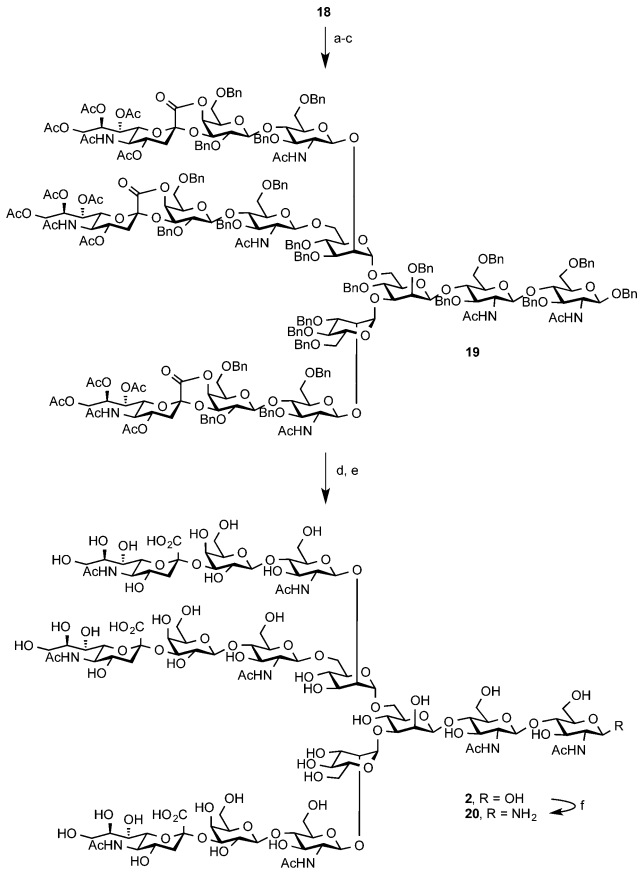


[a]Reagents and conditions: (a) **5**, NIS, AgOTf, CH_2Cl_2, 4 Å MS, −50 to −20 °C, 1.25 h, 91%; (b) BH_3·THF, *n*-Bu_2BOTf, CH_2Cl_2, 0 °C, 4 h, 99%; (c) **15**, NIS, AgOTf, CH_2Cl_2, −10 °C, 2 h, 87%; (d) NaOMe, MeOH/THF, rt, 1.7 h, 93%; (e) **9**, $Cp_2Zr(OTf)_2$·THF, CH_2Cl_2, 0 °C, 1.5 h, 71%.

coupling of **3** to a mannose thioether donor **5**, which proceed in an excellent yield. Regioselective benzylidene opening revealed the desired 6-OH acceptor, as confirmed by 2D NMR analysis. The alcohol functionality served as the glycosyl acceptor site to the mannose donor **15** bearing the corresponding protective groups at positions 2 and 6. Finally, deprotection under basic conditions allowed for a rapid construction of triol **17** in excellent yield.

Unlike the case in the synthesis of triantennary system described previously,[14] it could be anticipated that the glycosylation reaction with the corresponding trisaccharide donors should be a rather more facile process. Indeed, coupling of **17** with anomeric fluoride donor **9** under the conditions optimized for the synthesis of biantennary glycan **1** provided the desired triantennary glycan **18** in a surprisingly high yield (89% per glycosyl acceptor). Notably, we were again unable to detect biantennary oligosaccharide intermediates upon completion of the reaction. However, thioether donor **8** afforded **18** in a significantly diminished yield (42%) and a significant amount of diglycosylated intermediate was observed. Completion of the synthesis of **2** is depicted in Scheme 4. A sequence of steps used

Scheme 4. Deprotection Sequence for Glycan 2[a]



[a]Reagents and conditions: (a) NaOMe/NaOH, MeOH/H_2O, rt, 24 h; (b) 1,2-ethylenediamine, EtOH, 80 °C, 24 h; (c) Ac_2O, pyridine 33% (over 3 steps); (d) NaOMe, MeOH/CH_2Cl_2 then H_2O, rt, 24 h; (e) Na/NH_3, THF, −78 °C, 71% (over 2 steps); (f) NH_4HCO_3, H_2O, 40 °C, 3 d, 82%.

in the preparation of **1** was used to provide tris-lactone **19**. This compound was taken further to finish the deprotection sequence under the Birch conditions furnishing free oligosaccharide **2** in 71% yield.

In order to evaluate the feasibility of chemical ligation of saccharides **1** and **2** to a peptide chain, a selected motif of prostate-specific membrane antigen (PSMA)[18] **21** was used. This peptide corresponds to Gly332-Gln340 native sequence of PSMA, and it was chosen on the basis of the X-ray structure as the fragment containing one of the most exposed glycosylation sites in this glycoprotein.[19] Thus, Kochetkov amination[20] of **1** (Scheme 2) followed by repeated lyophilizations afforded anomeric amine **12** in an almost quantitative yield. The subsequent ligation to **21** was carried out under the Lansbury conditions[21] to afford the PSMA glycoconjugate **22** in 54%

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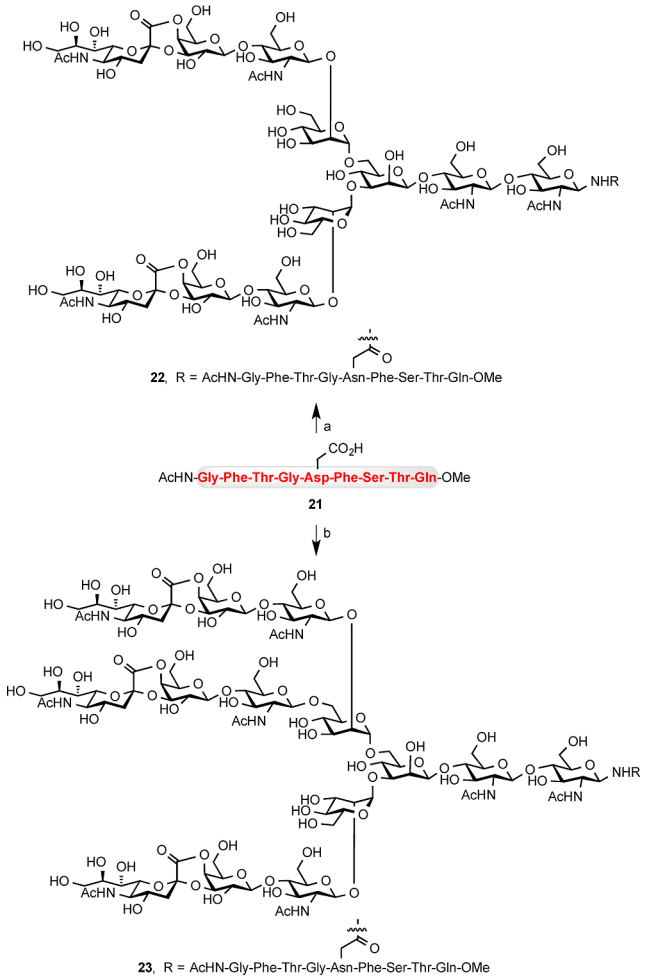
Scheme 5. Conjugation of Glycans 1 and 2 to PSMA Oligopeptide[a]



[a]Reagents and conditions: (a) **12**, HATU, DIPEA, DMSO, rt, 1.5 h, 54%; (b) **20**, HATU, DIPEA, rt, 1 h, 10%.

(Scheme 5). Similarly, triantennary amine **20** was coupled with peptide **21** to furnish glycoconjugate **23** in 10% yield.

In summary, we have described here a unified strategy for the synthesis of sialylated di- and tribranched *N*-linked glycans based on multiple glycosylations using very efficient anomeric fluoride donor. This approach allowed for preparation of fully sialylated glycans in excellent yields in a minimal number of steps. Finally, conjugation of the saccharides **1** and **2** to a native peptidic sequence of PSMA was demonstrated, supporting the feasibility of preparing diagnostic and therapeutic tools based on tumor-associated carbohydrate antigens related to prostate cancer.

■ 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.

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Notes

The authors declare no competing financial interest.

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