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Semisynthesis of Intact Complex-Type Triantennary Oligosaccharides from a Biantennary Oligosaccharide Isolated from a Natural Source by Selective Chemical and Enzymatic Glycosylation

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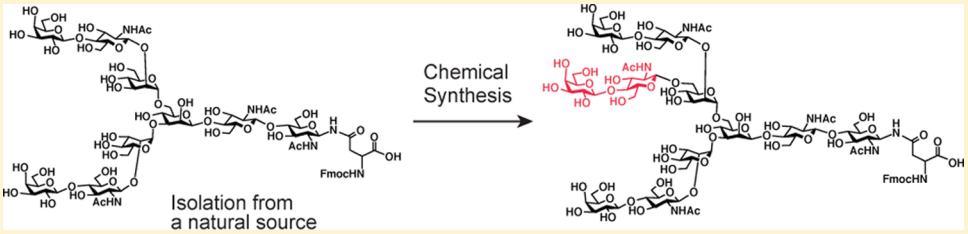
Supporting Information



ABSTRACT: Attachment of oligosaccharides to proteins is a major post-translational modification. Chemical syntheses of oligosaccharides have contributed to clarifying the functions of these oligosaccharides. However, syntheses of oligosaccharide-linked proteins are still challenging because of their inherent complicated structures, including diverse di- to tetra-antennary forms. We report a highly efficient strategy to access the representative two types of triantennary oligosaccharides through only 9- or 10-step chemical conversions from a biantennary oligosaccharide, which can be isolated in exceptionally homogeneous form from egg yolk. Four benzylidene acetals were successfully introduced to the terminal two galactosides and two core mannosides of the biantennary asialononasaccharide bearing 24 hydroxy groups, followed by protection of the remaining hydroxy groups with acetyl groups. Selective removal of one of the benzylidene acetals gave two types of suitably protected glycosyl acceptors. Glycosylation toward the individual acceptors with protected Gal-β-1,4-GlcN thioglycoside and subsequent deprotection steps successfully yielded two types of complex-type triantennary oligosaccharides.

Modification of proteins on cell surfaces and in body fluids with oligosaccharides is one of the major post-translational modifications.[1,2] Oligosaccharides not only regulate functions of glycoproteins but also act as ligands to receptors and contribute in several biological events such as immune responses, activation of cell−cell interactions, and endocytosis.[3,4]

However, as functional studies have been lacking because of the scarcity of oligosaccharides as well as considerable heterogeneities in the structures of oligosaccharides isolated from natural sources,[5] chemical syntheses have contributed greatly to provide homogeneous oligosaccharides.[6] Previously established regioselective protection/deprotection methods for multiple hydroxy groups of sugars and highly efficient glycosylation reactions have enabled the synthesis of numerous oligosaccharides and glycoconjugates, such as glycolipids and glycoproteins.[7−10] These well-established chemical methods, including chemoenzymatic syntheses, afford homogeneous oligosaccharides to reveal their roles in biological events at the molecular level.[11] For example, oligosaccharide arrays prepared using chemically synthesized oligosaccharides are employed to elucidate interactions between an oligosaccharide and a protein.[12,13]

The chemical synthesis of an oligosaccharide requires an efficient synthetic strategy,[6] as it is unfortunately a time-consuming protocol because of the need for repetitive protection/deprotection of hydroxy groups as well as the stepwise construction and purification of stereoselective glycosidic linkages.

Frequently found on mature glycoproteins, a complex-type oligosaccharide is a representative oligosaccharide form on glycoproteins and exhibits unique antennary structures. The reducing terminal oligosaccharide links with the nitrogen atom of an asparagine side chain. It is known that changing the number of antennae alters the physiological properties and biological activities of glycoproteins, as seen in the case of erythropoietin (EPO).[14] In the case of cancer cells, it is known that the number of branches increases depending on the tumor stage.[15] However, specific details of the functions of antennary structures still remain unclear.

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Syntheses of complex-type tri- and tetra-antennary oligosaccharides have been reported by the Danishefsky,[16,17] Wong,[18] Boons,[19] and Unverzagt[20] groups; however, these syntheses needed many conversion steps and largely relied on the sophisticated synthetic abilities of the researchers. The preparation of complex-type tri- and tetra-antennary oligosaccharides is a very difficult task, as to the best of our knowledge even biantennary complex-type oligosaccharides have never been synthesized in less than 20 synthetic steps.[21,22]

Under these circumstances, we have been studying an alternative concept for the semisynthesis of naturally occurring complex-type multiantennary oligosaccharides starting from biantennary asialononasaccharide **3**, which can be isolated in sufficient amounts from egg yolk.[23,24] Biantennary oligosaccharide **3** contains two terminal $Gal_{f,i}$-β-1,4-$GlcNAc_{e,h}$ linked to the two mannoside ends of the core pentasaccharide Man_g-α-1,3-(Man_d-α-1,6)-Man_c-β-1,4-$GlcNAc_b$-β-1,4-$GlcNAc_a$-Asn via β-1,2 linkages that are a basic structure of human complex-type oligosaccharides (Figure 1). There are two forms of the natural triantennary structure, **1** and **2**, which have an additional Gal-β-1,4-GlcNAc branch connected to a terminal mannoside of the core pentasaccharide through either a β-1,6 or β-1,4 linkage, respectively (Figure 1). We envisioned that the desired triantennary forms could be synthesized by a single glycosylation reaction of a specific hydroxy group of the biantennary structure. Once such a semisynthesis of triantennary oligosaccharides from the biantennary oligosaccharide is performed, the number of chemical synthetic steps will be dramatically reduced compared with that of the conventional stepwise chemical syntheses, and we will be able to easily obtain practical amounts of the triantennary oligosaccharides. In order to accomplish this semisynthesis, however, we need to develop selective protection/deprotection protocols toward 24 hydroxy groups in biantennary oligosaccharide **3**.

To date, the concept of semisynthesis has been demonstrated in the synthesis of many useful compounds,[25,26] while large oligosaccharides bearing many hydroxy groups as well as other functional groups have never been used as substrates in semisynthesis. Semisyntheses of antibiotics and modified proteins usually employ regioselective reactions toward a specific functional group exposed on a substrate that is isolated from a natural source.[27]

In terms of sugar derivatives, semisynthesis has been performed on mono- and disaccharide units.[28−32] The specific protection/deprotection protocols for mono- and disaccharides have been developed, and this chemistry has enabled us to perform the stepwise construction of large oligosaccharides.

Here we describe the new semisynthesis of the two naturally occurring triantennary complex-type oligosaccharides **1** and **2** by 9- and 10-step conversions, respectively, involving manipulation of 24 hydroxy groups as well as other functional groups of the biantennary oligosaccharide **3** isolated from a natural source.

RESULTS AND DISCUSSION

Our synthesis began with the sequential selective protection of 24 hydroxy groups of asialononasaccharide **3**, which was prepared from chicken egg yolk as previously reported.[23] A specific hydroxy group needs to be retained by selectively protecting other hydroxy groups, thus allowing the conversion of oligosaccharide **3** into suitable glycosyl acceptors. Such acceptors can then be used for a glycosylation reaction in order to yield a triantennary oligosaccharide.

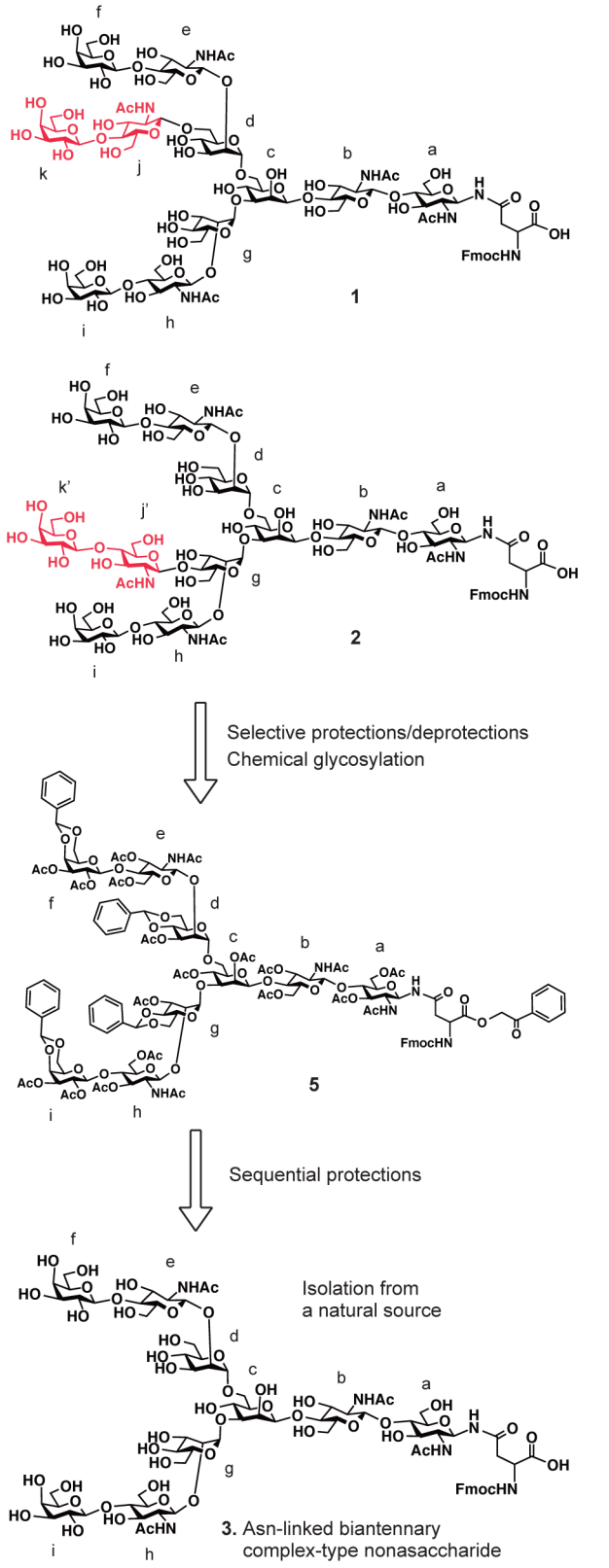

Figure 1. Synthetic strategy for typical human-type triantennary oligosaccharides.

First, we examined the selective protection of primary hydroxy groups, but these attempts were not successful because of the low solubility of **3** in organic solvents and the similar reactivities of the many hydroxy groups of the molecule. Trityl, *tert*-butyldimethylsilyl, and *tert*-butyldiphenylsilyl groups were

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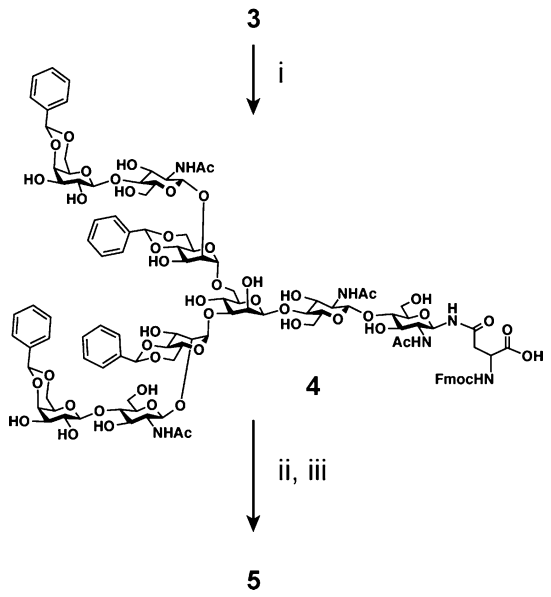
tested for 6-OH protection (Figures S15-1 and S15-2). When these reactions were conducted with a few equivalents of reagents to primary alcohols, they did not yield suitable results. Although most of the substrate remained as a precipitate, a small amount of the substrate dissolved into the solvent, allowing the reaction to proceed. Diluting the solvent did not improve the conditions. Furthermore, using an excess of reagents caused overprotection of the substrate dissolved in the solvent. As a result, we could not regulate any selective protection of primary and secondary hydroxy groups among the 24 hydroxy groups.

Next, we examined selective protections with benzylidene acetal toward the primary 6-OH and secondary 4-OH groups of both $Man_{d,g}$ and $Gal_{f,i}$ of oligosaccharide **3**. After intensive optimization of the reaction conditions, we succeeded in obtaining tetrabenzylidene derivative **4** in which eight hydroxy groups out of 24 were selectively protected, including two hydroxy groups that we intended to use for future glycosylation (Man_d-6-OH and Man_g-4-OH; Scheme 1 and Figures S1 and S2). The benzylidenation reaction usually promotes an equilibrium state, giving a thermodynamically stable product with the protection of a specific 1,3-diol among multiple hydroxy groups. In the case of the oligosaccharide, we presumed that 1,3-diol protection with an equilibrium system was more applicable than the protection of primary alcohols among the 24 hydroxy groups.

Scheme 1[a]



[a]Reagents and conditions: (i) $PhCH(OMe)_2$, CSA, DMF, rt, 17 h. (ii) Phenacyl bromide, $^iPr_2NEt$, DMF, rt, 3 h. (iii) Ac_2O/pyridine (1:1), DMAP, rt, 3 h, 18% (over three steps).

Subsequent phenacyl esterification of the carboxylic acid in the asparagine moiety followed by peracetylation of residual hydroxy groups afforded fully protected oligosaccharide **5** (Figure 2a) in 18% yield over the three steps. In order to determine the positions of the benzylidene acetals, two-dimensional (2D) NMR experiments were conducted. The heteronuclear single-quantum coherence (HSQC) and heteronuclear multiple-bond correlation (HMBC) spectra of oligosaccharide **5** are shown in Figure 2b. HMBC correlation signals between the benzyl protons of the benzylidene acetals and C-4 and C-6 of $Gal_{f,i}$ were observed. Similar correlation signals were found in $Man_{d,g}$ as well. These correlation patterns indicated that the benzylidene acetals were exclusively introduced at the 4- and 6-OH of $Gal_{f,i}$ and $Man_{d,g}$. In terms of the phenacyl ester of asparagine, this ester was found to form a cyclic product (aspartimide) via nucleophilic attack of the N-glycosylated side-chain nitrogen under basic conditions and upon purification with ammonium acetate buffer. This aspartimide formation was also observed during glycopeptide syntheses using oligosaccharylasparagine derivatives.[33] To prevent this side reaction, fractions of purified oligosaccharide **5** were kept on ice during the HPLC purification step and were then lyophilized as soon as possible. Moreover, we took care to keep the reaction from strongly basic conditions in order to prevent aspartimide formation.

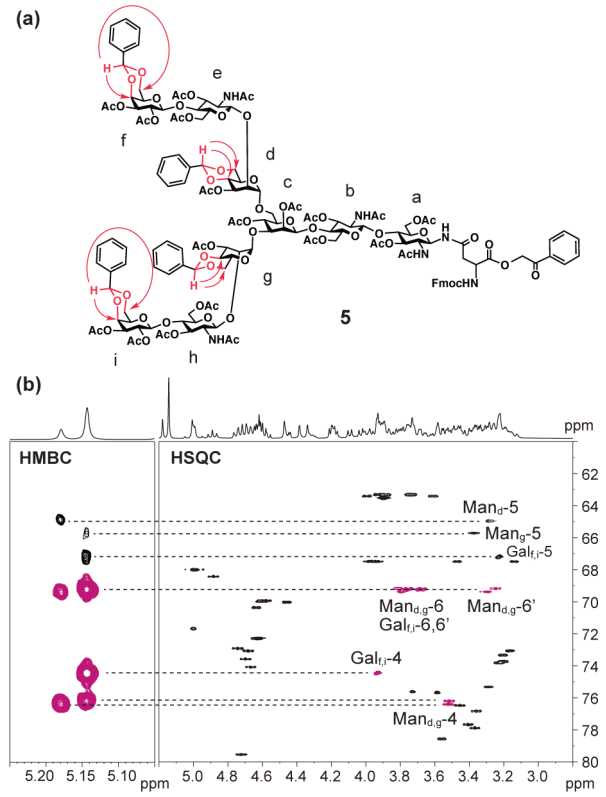


Figure 2. (a) Structure of fully protected oligosaccharide **5**. The observed HMBC correlation signals are shown by red arrows. (b) Selected regions of the HMBC (left square) and HSQC (right square) spectra of **5**. HMBC correlation signals between the benzyl protons of the benzylidene acetals and C-4 and C-6 were observed in $Man_{d,g}$, and similar correlation signals were also confirmed in $Gal_{f,i}$.

In addition to this protection protocol yielding oligosaccharide **5**, in which $Gal_{f,i}$ and $Man_{d,g}$ were protected with benzylidene acetals, we also examined the selective protection of only $Man_{d,g}$ with benzylidene acetals in order to yield suitable glycosyl acceptors. We conducted this step because the third Gal-β-1,4-GlcNAc antenna links with Man_d-6-OH or Man_g-4-OH in the target triantennary oligosaccharides **1** and **2**, but we could not find any selective protection protocol toward Man_d and Man_g residues. Multiple products were formed during benzylidenation of **5** (Figure S2a).

Therefore, we examined the selective deprotection of benzylidene acetals of fully protected oligosaccharide **5** in order to convert it into suitable acceptors for the syntheses of triantennary oligosaccharides. During the extensive investiga-

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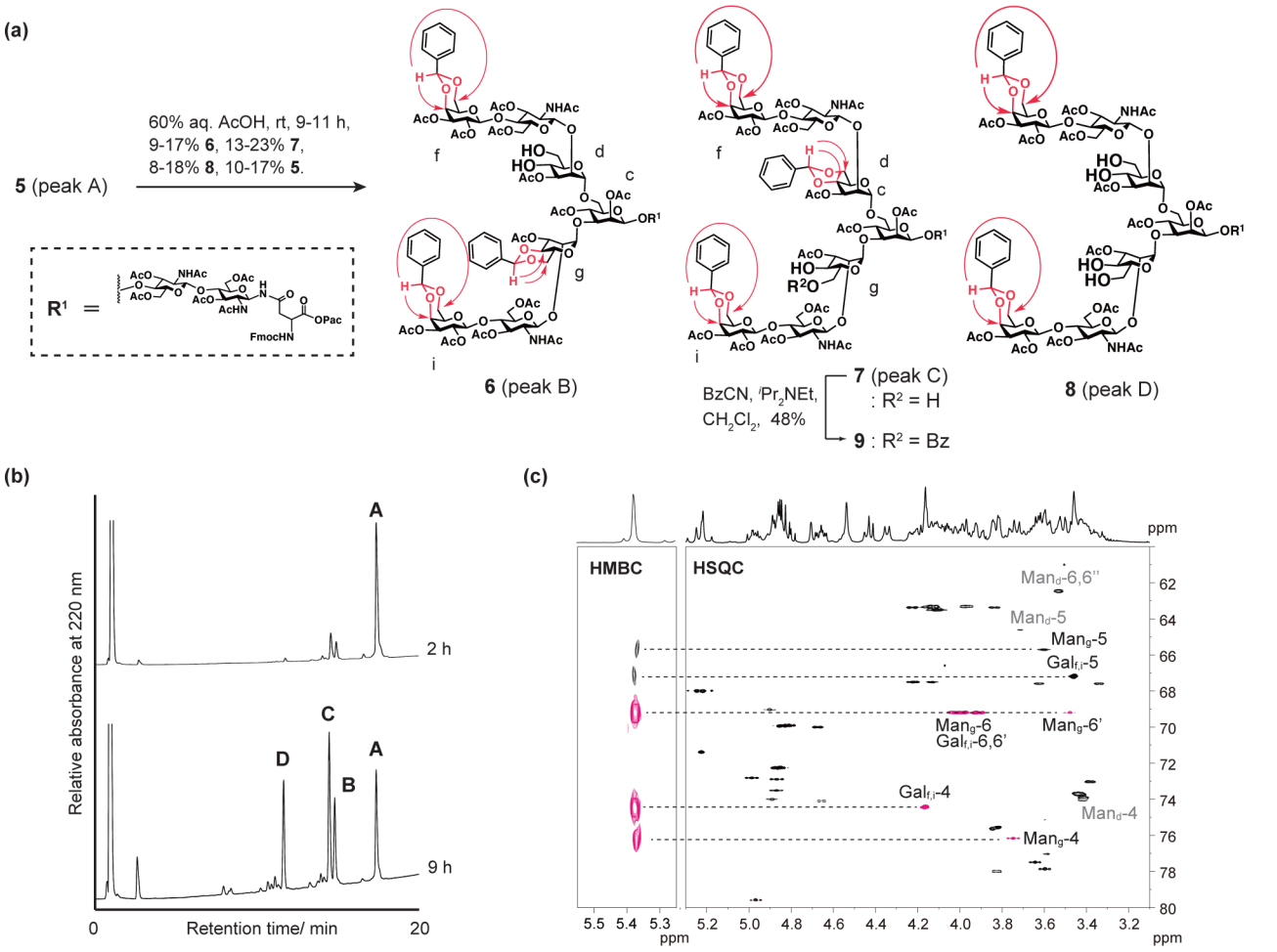

Figure 3. (a) Structures of partially debenzylidenated oligosaccharides **6**, **7**, and **8**. The observed HMBC correlation signals are shown by red arrows. Man_g-6-OH of diol **7** was further protected with BzCN in CH_2Cl_2 to afford glycosyl acceptor **9** (48%). (b) Monitoring of the selective debenzylidenation reaction by RP-HPLC analysis. (c) Characterization of glycosyl acceptor **6**. Selected regions of the HMBC (left square) and HSQC (right square) spectra of **6** are shown.

tion of the selective removal of benzylidene groups, we found that the two benzylidene acetals of the $Man_{d,g}$ residues are prone to deprotection in comparison with those of the $Gal_{f,i}$ residues under mild acidic conditions. This unexpected finding enabled us to obtain biantennary oligosaccharyl acceptors **6** and **7** that were suitable for the semisyntheses of triantennary oligosaccharides **1** and **2**, respectively (Figure 3a,b). As shown in Figure 3b, this deprotection of the benzylidene groups in the mannosides gradually proceeded under the optimized conditions using 60% aqueous acetic acid. The increase of two tribenzylidene derivatives (peaks B and C) and concomitant decrease of tetrabenzylidene derivative **5** (peak A) were observed by HPLC/mass spectrometry. When reaction time was extended, all four benzylidene acetals were removed. The maximum conversion yield of a mixture of two tribenzylidene derivatives was found to be 46% based on the peak areas of the HPLC chromatogram, and individual isomers were isolated in yields of 9−17% for **6** (peak B, 12% on average) and 13−23% for **7** (peak C, 17% on average) by reversed-phase HPLC (RP-HPLC).

In order to confirm which hydroxy groups were made free under acidic removal of a benzylidene acetal, the structures of these two isomers (peaks B and C) were individually determined by 2D NMR experiments (Figures 3c and S4). According to these NMR assignments, we concluded that the structures corresponding to peaks B and C were tribenzylidene derivatives **6** and **7**, respectively (Figure 3a). We also checked for the possibility of acetyl migration to the newly formed hydroxy groups under these acidic conditions, and on the basis of NMR and HPLC analyses we concluded that acetyl rearrangement did not occur. For the synthesis of triantennary oligosaccharide **1**, we decided to use diol **6** as a glycosyl acceptor to examine selective glycosylation toward primary Man_d-6-OH, which is more reactive than Man_d-4-OH. For the synthesis of triantennary oligosaccharide **2**, primary Man_g-6-OH in diol **7** was selectively benzoylated by treatment with BzCN to afford a 48% yield of glycosyl acceptor **9**, which has a free Man_g-4-OH hydroxy group. This structure was also confirmed by NMR experiments, in which we observed that the chemical shifts of H-6 and C-6 of Man_g changed (Figure S10).

To confirm the dependence of the benzylidene group on the sugar stereochemistry for selective deprotection, we investigated the deprotection of benzylidene groups of monosaccharides (Figure 4). Six types of methyl 4,6-*O*-benzylidene-α-D-glycosides with and without Ac groups were prepared for this study. In both cases, the reactions using glucosides and mannosides were faster than those using galactosides. We also

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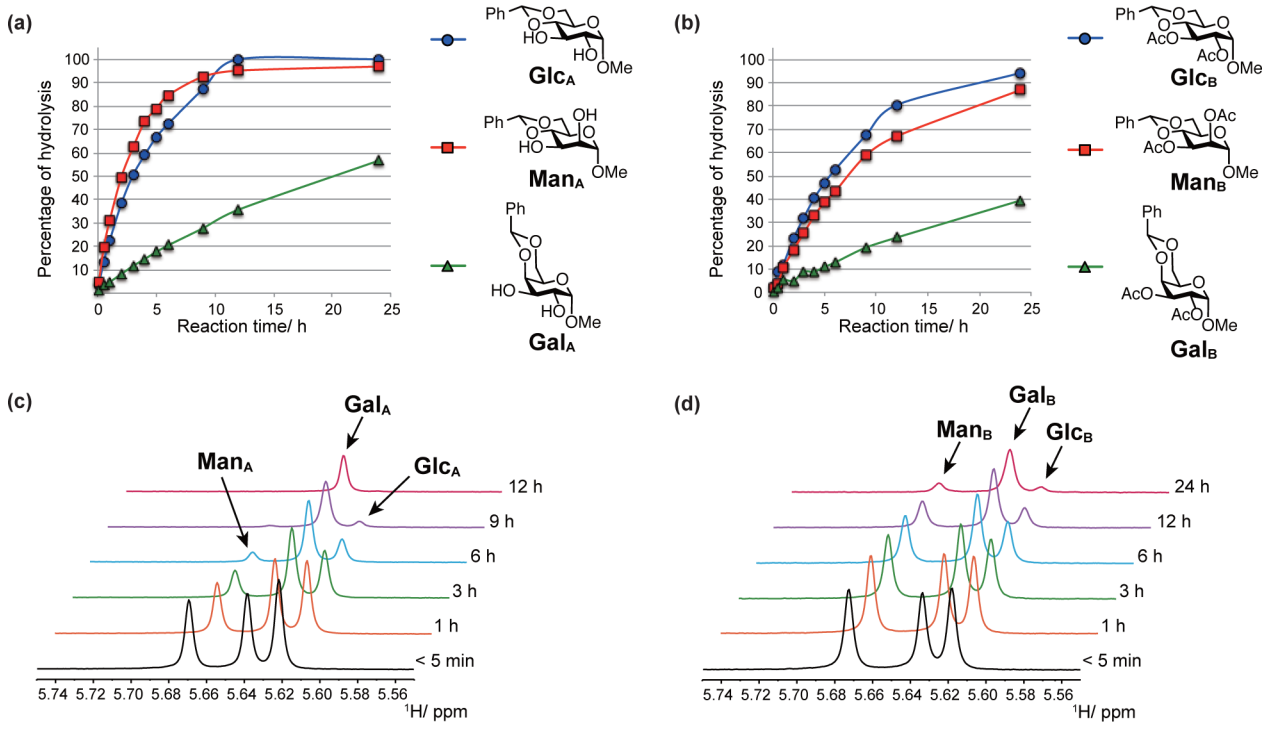
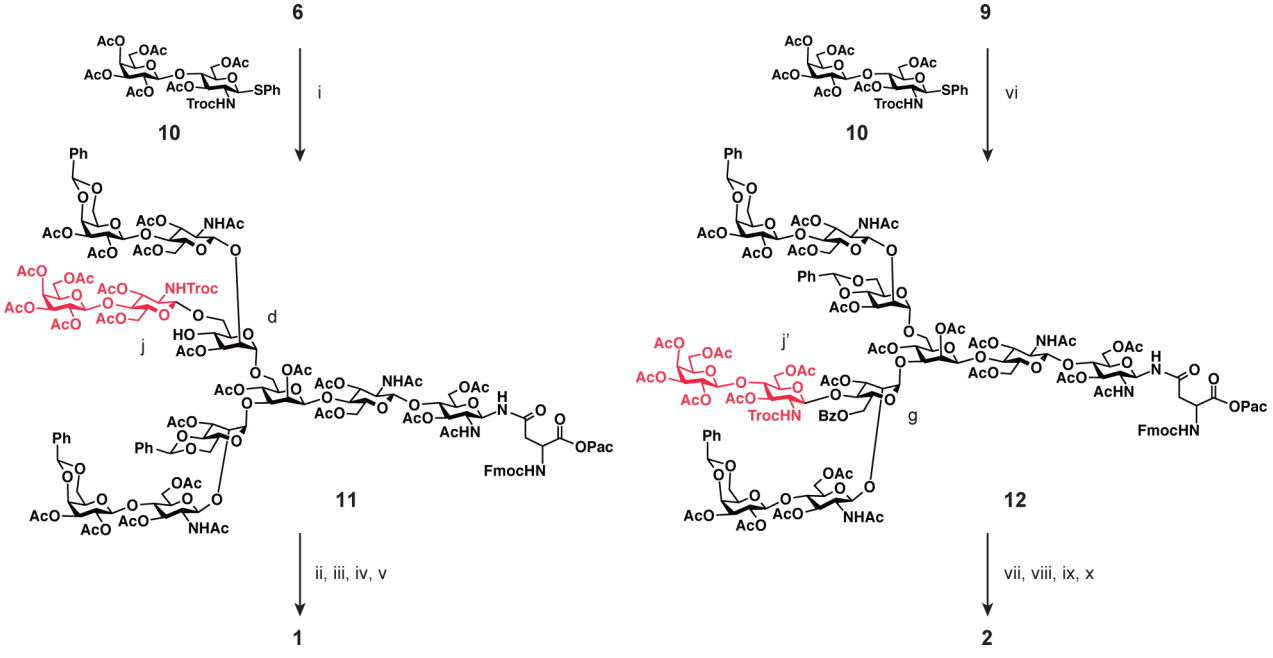


Figure 4. (a) Hydrolysis rates of benzylidene groups of methyl 4,6-*O*-benzylidene-α-D-glycosides. (b) Hydrolysis rates of benzylidene groups of methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-α-D-glycosides. (c) Monitoring of the competitive reaction using a mixture of methyl 4,6-*O*-benzylidene-α-D-glycosides. Glc_A and Man_A show faster decreases of the resonance for the benzyl proton of the benzylidene group. (d) Monitoring of the competitive reaction using a mixture of methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-α-D-glycosides. Glc_B and Man_B show faster decreases of the resonance for the benzyl proton of the benzylidene group.

Scheme 2[a]



[a]Reagents and conditions: (i) **10**, NIS, TfOH, CH_2Cl_2, 0 °C, 1 h, 47%. (ii) Zn, THF/AcOH/Ac_2O (3:2:1), 0 °C to rt, 16 h. (iii) MeOH/aq. NaOH, 0 °C to rt, 2.5 h. (iv) FmocOSu, aq. $NaHCO_3$, acetone, 0 °C to rt, 3.5 h. (v) aq. TFA, 0 °C, 10 min, 63% (over four steps). (vi) NIS, TfOH, CH_2Cl_2, 0 °C to rt, 2 h, 44%. (vii) Zn, THF/AcOH/Ac_2O (3:2:1), 0 °C to rt, 18 h. (viii) MeOH/aq. NaOH, 0 °C to rt, 2 h. (ix) FmocOSu, aq. $NaHCO_3$, acetone, 0 °C to rt 3 h. (x) aq. TFA, 0 °C, 10 min, 44% (over four steps).

tested competitive hydrolysis reactions using an acidic solution containing three benzylidene derivatives. These competitive reactions also showed the same selectivity (Figures 4c,d, S13, and S14). We presumed that the selectivity toward oligosaccharide **5** was due to the difference in stereochemistry at the 4-position.

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We then examined the glycosylation of suitably protected biantennary oligosaccharyl acceptors **6** and **9** with thioglycoside **10** (Scheme 2), which was prepared from lactose in eight steps via an azidonitration reaction.[34] For the synthesis of triantennary oligosaccharide **11**, diol **6** was coupled with glycosyl donor **10** by TfOH/NIS activation. We found the best conditions to be acceptor **6** (5 mM in CH_2Cl_2) and excesses of donor **10** (10 equiv) and *N*-iodosuccinimide (NIS) (10 equiv) with a reaction time of 1 h at 0 °C. These conditions yielded the desired triantennary oligosaccharide **11** in 47% yield, while small amounts of byproducts, such as the diglycosylated product, were also observed. The formation of this diglycosylated product was accelerated when the amount of NIS was increased. In addition, this glycosylation reaction was prone to form the aspartimide derivative through intramolecular cyclization during the long reaction time. For example, using donor (2 equiv with respect to acceptor) and NIS (2 equiv with respect to donor) at −20 °C for 3.5 h did not complete the reaction and gave aspartimide (Figure S15-3).

Moreover, in this reaction, we also observed another byproduct exhibiting the same mass as the desired product **11** by HPLC/MS analysis. This byproduct was thought to possibly be an α-linked isomer or a regioisomer, but because of its negligible amount we could not isolate and assign this byproduct.

Contrary to the glycosylation of Man_d-6-OH for the synthesis of triantennary oligosaccharide **11**, the glycosylation of 4-OH of Man_g for the synthesis of triantennary oligosaccharide **12** was slow (Scheme 2). Although the desired triantennary oligosaccharide **12** was obtained, acceptor **9** (5 mM in CH_2Cl_2) still remained even when an excess of donor **10** (20 equiv) was employed and the reaction temperature was elevated to room temperature. Unreacted acceptor **9** was recovered during the purification step and used for the glycosylation reaction repeatedly. After optimization of this reaction, the desired triantennary oligosaccharide **12** was obtained in 44% yield after HPLC purification.

The structures of triantennary oligosaccharides **11** and **12** were confirmed by 2D NMR experiments. An HMBC correlation between the anomeric proton of $GlcN_j$ and C-6 of Man_d was observed in **11** (Figure 5, dotted line). Similarly, an HMBC correlation between the anomeric proton of $GlcN_{j'}$ and C-4 of Man_g was also observed in **12** (Figure S6b, dotted line). These HMBC correlation signals clearly indicate that we indeed obtained the protected triantennary oligosaccharides **11** and **12** by the semisynthesis methods.

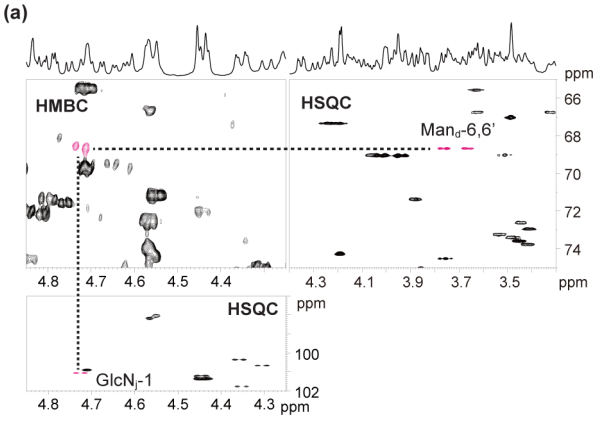
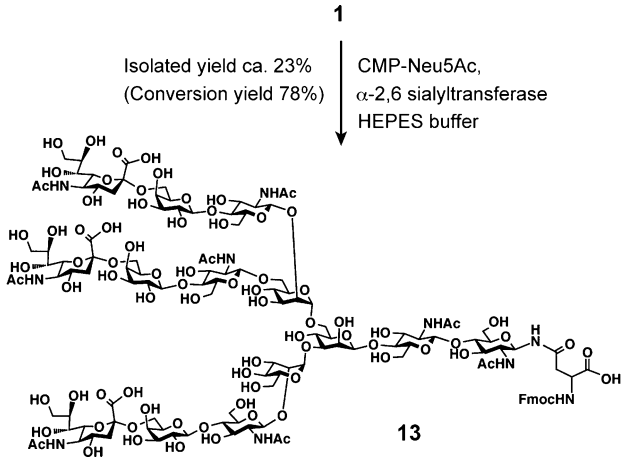


Figure 5. Selected regions of the HMBC and HSQC spectra of **11**. An HMBC correlation signal between C-6 of Man_d and H-1 of $GlcN_j$ (dotted line) was observed. The newly synthesized β-linkage was determined by the coupling constant observed for H-1 and H-2 in $GlcN_j$ (8.5 Hz).

Finally, we examined deprotection reactions of **11** and **12** in order to obtain the desired intact triantennary asialoundecasaccharides **1** and **2**, respectively (Scheme 2). First, the Troc group was removed by treatment with Zn powder, after which acetylation yielded the acetamide derivatives. The phenacyl group was also removed during this zinc treatment. Subsequently, all of the acyl groups and the Fmoc group were removed by treatment with NaOH in MeOH, and Fmoc groups were reintroduced to the asparagine moieties. The removal of the remaining benzylidene acetals was carefully conducted by means of a brief treatment of 90% aqueous trifluoroacetic acid (TFA) at 0 °C, and the desired triantennary oligosaccharides **1** and **2** were obtained in 63% and 44% yield, respectively. High-resolution mass spectral and 2D NMR analyses were conducted (see the Supporting Information) and established beyond doubt that homogeneous triantennary oligosaccharides were obtained.

In addition, we examined enzymatic sialylation toward triantennary oligosaccharide **1** (Scheme 3) because mature complex-type oligosaccharides have sialic acid residues at the nonreducing termini and sialic acid is known to be involved in many biological events. Enzymatic sialylation usually proceeds in a stereo- and regioselective manner in a single step.[35] Because α-2,3 and α-2,6 sialyl linkages with the terminal galactoside are found in the structures of natural oligosaccharides, enzymatic sialylation by specific α-2,3 or α-2,6 sialyltransferase seems to be effective in making the desired sialyl oligosaccharides. Wong and co-workers reported a combined enzymatic and chemical strategy for the syntheses of sialylated multiantennary complex-type oligosaccharides with their regeneration system of sugar nucleotides.[18] In our application, sialic acid residues were installed on **1** using α-2,6 sialyltransferase (*Photobacterium damsela*, [EC 2.4.99.1])[36] in the presence of CMP-Neu5Ac. This sialylation reaction proceeded in 78% conversion yield. The structure of triantennary sialyl oligosaccharide **13** was also confirmed by NMR experiments and mass spectrometry.

Scheme 3



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CONCLUSION

We have established a strategy for the semisynthesis of complex-type triantennary oligosaccharides from a biantennary oligosaccharide isolated from a natural source. The sequential protection and deprotection of 24 hydroxy groups of this biantennary oligosaccharide successfully yielded two glycosyl acceptors bearing free hydroxy groups at the desired positions, which are essential for the semisyntheses of two types of natural triantennary oligosaccharides. Because the selective introduction of a protecting group is sometimes difficult for a complex molecule, the idea to regulate the reactivity during the deprotection step was critical.

This new strategy significantly reduced the number of synthetic steps and overcame limitations of traditional oligosaccharide syntheses that rely on repetitive protection/deprotection and multiple glycosylation steps. In terms of the synthesis of triantennary oligosaccharide **1** having three Gal-β1,4-GlcNAc antennae through a β-1,6 and two β-1,2 linkages with the terminal mannosides of the core, more than 50 synthetic steps were eliminated compared with the previous conventional chemical oligosaccharide synthesis.[18]

Because the biantennary oligosaccharide can be isolated on multigram scales and the synthetic routes of the triantennary oligosaccharides have been established once on the basis of rigorous structural analyses, the synthesis of triantennary oligosaccharides can be repeatedly performed to promptly provide sufficient amounts, such as dozens of milligrams. Our current experiments demonstrated that biantennary nonasaccharide **3** (ca. 600 mg) could be converted into triantennary undecasaccharides **1** (4 mg) and **2** (2 mg). Isolation of biantennary nonasaccharide **3** from egg yolk can be easily performed on over a 20 g scale, and we consider there to be no limitation on the synthesis of triantennary undecasaccharides. Work on syntheses of glycoproteins bearing multiantennary oligosaccharides prepared by the semisynthesis reported here is currently in progress.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b13098.

All experimental details of the syntheses of the disaccharide donor and triantennary oligosaccharides and investigation of the debenzylidenation reactions, including general procedures and spectroscopic and analytical data (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) *Essentials of Glycobiology*; Varki, A., Cummings, R. D., Esko, J. D., Freeze, H. H., Stanley, P., Bertozzi, C. R., Hart, G. W., Etzler, M. E., Eds.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 2009.

(2) Apweiler, R.; Hermjakob, H.; Sharon, N. *Biochim. Biophys. Acta, Gen. Subj.* **1999**, *1473*, 4.

(3) Lowe, J. B. *Cell* **2001**, *104*, 809.

(4) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683.

(5) Rudd, P. M.; Dwek, R. A. *Crit. Rev. Biochem. Mol. Biol.* **1997**, *32*, 1.

(6) Boltje, T. J.; Buskas, T.; Boons, G. J. *Nat. Chem.* **2009**, *1*, 611.

(7) Guo, J.; Ye, X. S. *Molecules* **2010**, *15*, 7235.

(8) Toshima, K.; Tatsuta, K. *Chem. Rev.* **1993**, *93*, 1503.

(9) Zhu, X.; Schmidt, R. R. *Angew. Chem., Int. Ed.* **2009**, *48*, 1900.

(10) Unverzagt, C.; Kajihara, Y. *Chem. Soc. Rev.* **2013**, *42*, 4408.

(11) Schmaltz, R. M.; Hanson, S. R.; Wong, C.-H. *Chem. Rev.* **2011**, *111*, 4259.

(12) Blixt, O.; Head, S.; Mondala, T.; Scanlan, C.; Huflejt, M. E.; Alvarez, R.; Bryan, M. C.; Fazio, F.; Calarese, D.; Stevens, J.; Razi, N.; Stevens, D. J.; Skehel, J. J.; van Die, I.; Burton, D. R.; Wilson, I. A.; Cummings, R.; Bovin, N.; Wong, C. H.; Paulson, J. C. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 17033.

(13) Stevens, J.; Blixt, O.; Paulson, J. C.; Wilson, I. A. *Nat. Rev. Microbiol.* **2006**, *4*, 857.

(14) Higuchi, M.; Oh-eda, M.; Kuboniwa, H.; Tomonoh, K.; Shimonaka, Y.; Ochi, N. *J. Biol. Chem.* **1992**, *267*, 7703.

(15) Dennis, J. W.; Laferte, S.; Waghorne, C.; Breitman, M. L.; Kerbel, R. S. *Science* **1987**, *236*, 582.

(16) Walczak, M. A.; Hayashida, J.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2013**, *135*, 4700.

(17) Walczak, M. A.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2012**, *134*, 16430.

(18) Shivatare, S. S.; Chang, S. H.; Tsai, T. I.; Ren, C. T.; Chuang, H. Y.; Hsu, L.; Lin, C. W.; Li, S. T.; Wu, C. Y.; Wong, C. H. *J. Am. Chem. Soc.* **2013**, *135*, 15382.

(19) Wang, Z.; Chinoy, Z. S.; Ambre, S. G.; Peng, W.; McBride, R.; de Vries, R. P.; Glushka, J.; Paulson, J. C.; Boons, G. J. *Science* **2013**, *341*, 379.

(20) Unverzagt, C.; Gundel, G.; Eller, S.; Schuberth, R.; Seifert, J.; Weiss, H.; Niemietz, M.; Pischl, M.; Raps, C. *Chem. - Eur. J.* **2009**, *15*, 12292.

(21) Ogawa, T.; Sugimoto, M.; Kitajima, T.; Sadozai, K. K.; Nukada, T. *Tetrahedron Lett.* **1986**, *27*, 5739.

(22) Wu, B.; Hua, Z.; Warren, J. D.; Ranganathan, K.; Wan, Q.; Chen, G.; Tan, Z.; Chen, J.; Endo, A.; Danishefsky, S. J. *Tetrahedron Lett.* **2006**, *47*, 5577.

(23) Kajihara, Y.; Suzuki, Y.; Yamamoto, N.; Sasaki, K.; Sakakibara, T.; Juneja, L. R. *Chem. - Eur. J.* **2004**, *10*, 971.

(24) Seko, A.; Koketsu, M.; Nishizono, M.; Enoki, Y.; Ibrahim, H. R.; Juneja, L. R.; Kim, M.; Yamamoto, T. *Biochim. Biophys. Acta, Gen. Subj.* **1997**, *1335*, 23.

(25) Kirschning, A.; Hahn, F. *Angew. Chem., Int. Ed.* **2012**, *51*, 4012.

(26) Altmann, K.-H.; Gaugaz, F.; Schiess, R. *Mol. Diversity* **2011**, *15*, 383.

(27) Muir, T. W. *Annu. Rev. Biochem.* **2003**, *72*, 249.

(28) Kumar H. V., R.; Naruchi, K.; Miyoshi, R.; Hinou, H.; Nishimura, S.-I. *Org. Lett.* **2013**, *15*, 6278.

(29) Lopin, C.; Jacquinet, J.-C. *Angew. Chem., Int. Ed.* **2006**, *45*, 2574.

(30) Vibert, A.; Lopin-Bon, C.; Jacquinet, J.-C. *Chem. - Eur. J.* **2009**, *15*, 9561.

(31) Kurita, K.; Matsumura, Y.; Takahara, H.; Hatta, K.; Shimojoh, M. *Biomacromolecules* **2011**, *12*, 2267.

DOI: 10.1021/jacs.5b13098
J. Am. Chem. Soc. 2016, 138, 3461−3468

(32) Laezza, A.; Iadonisi, A.; Castro, C. D.; De Rosa, M.; Schiraldi, C.; Parrilli, M.; Bedini, E. *Biomacromolecules* **2015**, *16*, 2237.
(33) Yamamoto, N.; Takayanagi, A.; Sakakibara, T.; Dawson, P. E.; Kajihara, Y. *Tetrahedron Lett.* **2006**, *47*, 1341.
(34) Kajihara, Y.; Kodama, H.; Wakabayashi, T.; Sato, K.-i.; Hashimoto, H. *Carbohydr. Res.* **1993**, *247*, 179.
(35) Izumi, M.; Wong, C.-H. *Trends Glycosci. Glycotechnol.* **2001**, *13*, 345.
(36) Yamamoto, T.; Nakashizuka, M.; Kodama, H.; Kajihara, Y.; Terada, I. *J. Biochem.* **1996**, *120*, 104.

DOI: 10.1021/jacs.5b13098
J. Am. Chem. Soc. 2016, 138, 3461−3468