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A New Approach for the Synthesis of Hyperbranched *N*-Glycan Core Structures from Locust Bean Gum

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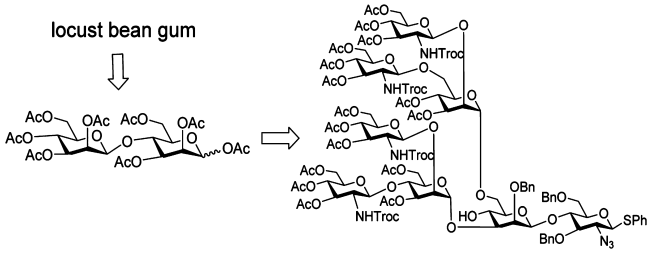
ABSTRACT



A novel protocol for the synthesis of general *N*-glycan core structures was established by means of Manβ(1→4)Man peracetate derived from a naturally abundant locust bean gum as a key starting material. Phenyl (2-*O*-benzyl-4,6-*O*-benzylidine-β-D-mannopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-azido-2-deoxy-1-thio-β-D-glucopyranoside facilitated the synthesis of key intermediates leading to hyperbranched *N*-glycan core structures.

Asparagine-linked type oligosaccharides (*N*-glycans) of glycoproteins play essential roles in the maintenance and regulation of many functions such as cell differentiation, cell adhesion, and homeostatic immune balance.[1] Recent studies on large-scale *N*-glycan analysis have revealed the importance of structural alteration of human serum/cellular *N*-glycans during disease progression. Many such glycans serve as potent biomarkers for the early diagnosis of disease and as new agents for therapeutic antibodies.[2] In addition, as expression levels of highly branched *N*-glycans are enhanced distinctly during cancer cell proliferation and metastasis,[3,4] it is clear that the construction of *N*-glycan compound libraries is of growing importance for gaining insights into the functions of cell surface glycoproteins. However, the relationship between structure and function of *N*-glycans remains mostly unclear due to the structural complexity and heterogeneity in the dynamic posttranslational modification at the potential *N*-glycosylation sites.[5] Recently, chemical and enzymatic syntheses of glycopeptides and glycoproteins having *N*-glycans have been accelerated conspicuously by using various endo-β-*N*-acetyl-D-glucosaminidases and oligosaccharide oxazolines as

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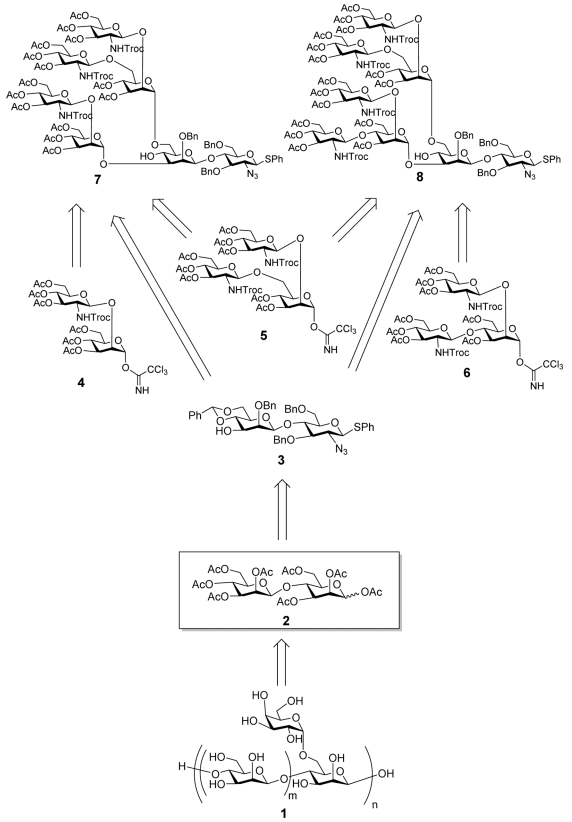
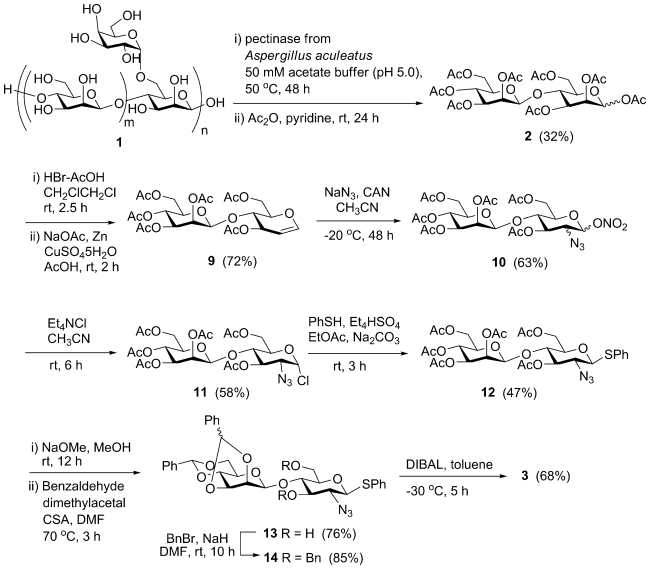
sophisticated donor substrates.[6] However, it is thought that the feasibility of this potential approach depends strongly on the availability of the complex *N*-glycans found ubiquitously in Nature. Although extensive efforts have been devoted to the synthesis of complicated hyperbranched *N*-glycans,[7] the syntheses generally entail tedious procedures for the preparation of many designated monosaccharide synthons and multistep stereoselective glycosidation reactions. Especially, it should be noted that formation of the Manβ(1→4)GlcNAc unit present in the *N*-glycan core moiety is one of the most difficult steps in stereoselective glycoside synthesis[8] due to both the anomeric effect and neighboring group participation in the mechanism of glycosidation reactions using D-mannosyl donors, which are not beneficial for the stereoselective generation of the β1,4-mannoside linkages.[9]

We hypothesized that the β1,4-mannobiose derivative **2**, distributed often in some natural polysaccharides (**1**), might become an ideal starting material[10] for the synthesis of a key intermediate **3**, phenyl (2-*O*-benzyl-4,6-*O*-benzylidine-β-D-mannopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-azido-2-deoxy-1-thio-β-D-glucopyranoside, to facilitate the construction of tri- and tetra-antennary derivatives **7** and **8** when employed in combination with compounds (**4**, **5**, and **6**) as outlined in Scheme 1. To demonstrate the feasibility of this synthetic strategy, we decided to establish the standardized synthetic protocols for accessing compounds **2**–**6** and to assess their potential for the synthesis of the above-mentioned hyperbranched *N*-glycan core structures.

Scheme 1. Synthesis of Hyperbranched *N*-Glycan Core Structures Using Galactomannan As a Key Starting Material



Scheme 2. Synthetic Route to the Key Intermediate **3** from Locust Bean Gum **1**



Our previous finding that the β1,4-mannobiose octaacetate **2**,[11] obtainable through the acid hydrolysis of guar

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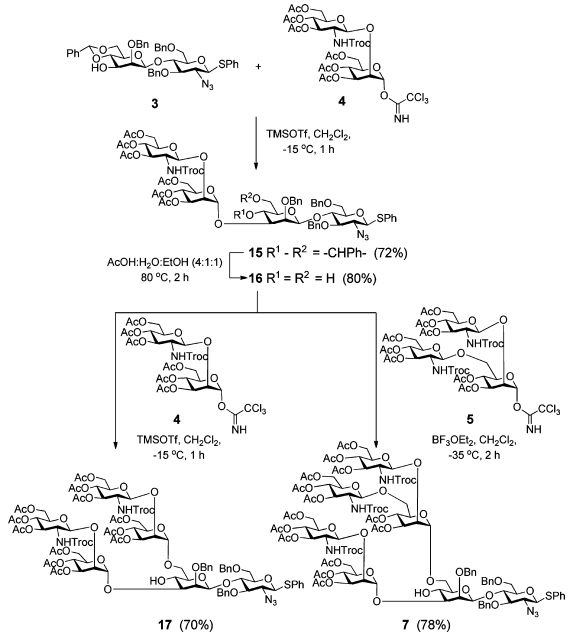
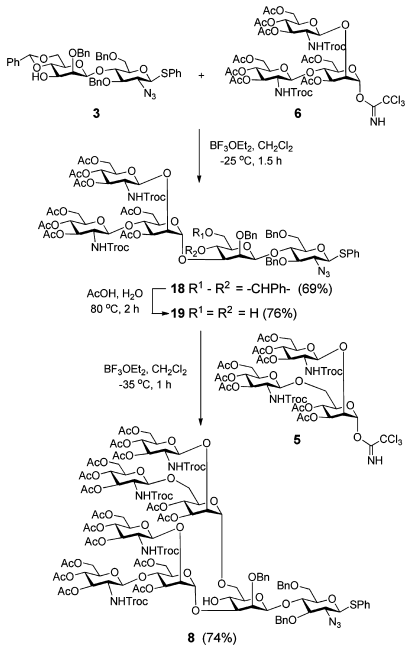
Scheme 3. Synthesis of Compounds **17** and **7** from **3**, **4**, and **5**

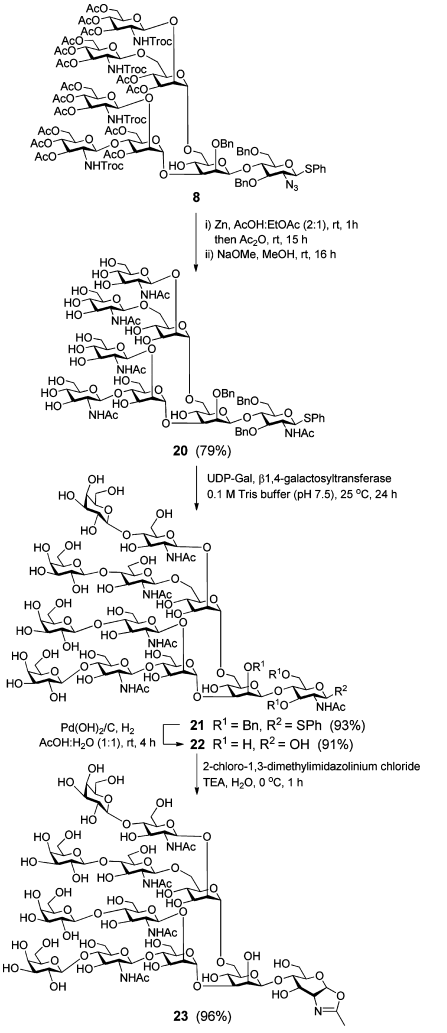


Scheme 4. Synthesis of Compound **8** from **3**, **5**, and **6**



gum, can be converted into compounds corresponding to the disaccharide unit, Manβ(1→4)GlcNAc, encouraged us to improve and optimize the procedure for the preparation of this important compound **2** from the most suited material among some abundant polysaccharides containing the common formula of galactomannan shown in Scheme 1. In the present study, we investigated the degradation products of two abundant polysaccharides, guar gum and locust bean gum, under various hydrolytic

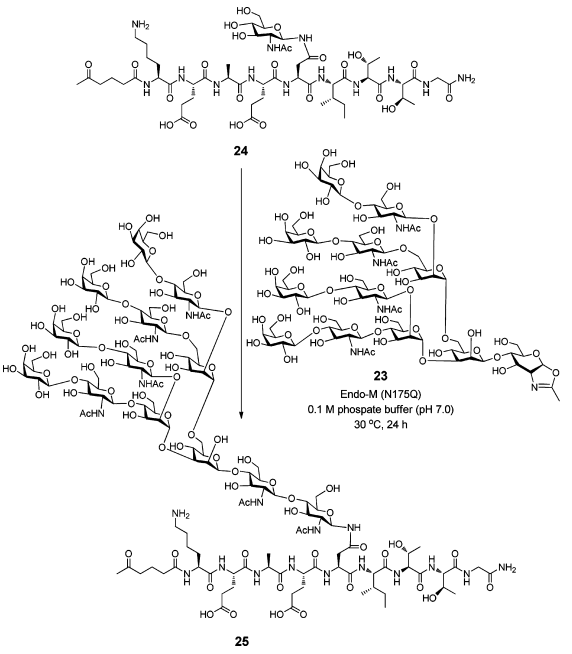
Scheme 5. Synthesis of Oxazoline Derivative **23** from **8**



conditions as listed in Table S1. Consequently, we discovered that the degradation of locust bean gum, by treatment with pectinase from *Aspergillus aculeatus* at 50 °C for 48 h in 50 mM acetate buffer (pH 5.0), gave dominantly a disaccharide Manβ(1→4)Man, and subsequent per-*O*-acetylation of the crude product facilitated the isolation of β1,4-mannobiose octaacetate **2** by silica gel chromatography in 32% overall yield (51 g from 100 g of crude locust bean gum) (Scheme 2).

As anticipated, per-*O*-acetate **2** allowed for the highly efficient synthesis of the key intermediate **3** by a general procedure as shown in Scheme 2. 2-Azido derivative **10** obtained readily by azidonitration of glycal **9** was treated with tetrabutylammonium chloride in acetonitrile to make the purification of the gluco configured chloride **11** possible. Next, β-thioglycoside **12** derived from the nucleophilic substitution of **11** with thiophenol was subjected to de-*O*-acetylation, benzylidenation, and benzylation. It afforded regioselectively protected disaccharide **14** as a mixture of stereoisomers at the 2′,3′-*O*-benzylidene group. However, it was revealed that a reductive ring-opening reaction of compound **14** occurred at the 2′,3′-*O*-benzylidine ring[12]

Scheme 6. Synthesis of EPO-Related Peptide (20−28) Having Tetra-antennary *N*-Glycan **25** Using **23** as a Glycosyl Donor



selectively in the *endo* isomer in the presence of DIBAL and gave the desired disaccharide intermediate **3** having a 3′-OH group in high yield.

We tested the feasibility of the intermediate **3** for the synthesis of di-, tri-, and tetra-branched derivatives. To achieve a systematic and streamlined synthesis of such hyperbranched *N*-glycan structures from disaccharide acceptor **3**, we prepared glycosyl donors **4**, **5**, and **6** by conventional procedures[13−15] (see Supporting Information). As indicated in Schemes 3 and 4, an advantage of the present strategy is evident because tetrasaccharide **15**/**16** derived by coupling **3** with **4** could be employed directly for the synthesis of key intermediates **17** and **7** using a suited donor **4** or **5**, respectively. Similarly, the pentasaccharide **18**/**19** obtained by coupling **3** with **6** becomes the glycosyl acceptor for the coupling with the imidate **5** to lead efficiently to the targeted octasaccharide derivative **8** in high yield.

Next, our interest was focused on the feasibility of using **8** in the synthesis of glycopeptides via an endoglycosidase-catalyzed reaction with peptides carrying a GlcNAc residue. Scheme 5 indicates a process for the preparation of

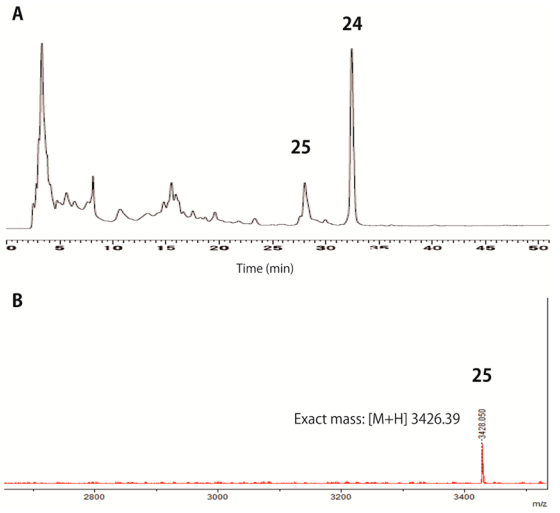


Figure 1. EPO-related peptide having tetra-antennary *N*-glycan **25** characterized by HPLC (A) and MALDI-TOF-MS (B).

oxazoline **23** from **8**. After galactosylation of partially protected **20** by GalT with UDP-Gal, dodecasaccharide **22** was converted readily into the oxazoline **23** under conditions reported previously.[16] As shown in Figure 1, it was demonstrated, for the first time, that the reaction between **23** and **24** conducted by recombinant endo-M (N175Q)[6b] proceeded significantly and afforded the desired glycopeptide **25** in moderate yield (Scheme 6). Further optimization will be needed for the practical, large-scale synthesis.

In conclusion, we developed a novel synthetic strategy toward various multivalent *N*-glycan core structures by using β1,4-mannobiose octaacetate **2** derived efficiently from abundant locust bean gum as a key starting material. Versatility of the intermediate **3** was demonstrated by synthesizing bi-, tri-, and tetra-antennary *N*-glycan core derivatives **17**, **7**, and **8** in high yields. It should be emphasized that the present strategy should allow for the synthesis of a variety of glycopeptides and glycoproteins having homogeneous and highly complicated *N*-glycans when combined with endoglycosidase-catalyzed *trans* glycosylation protocols.[6]

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Supporting Information Available. General procedure, characterization data of all new compounds, and ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.