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Studies Related to Norway Spruce Galactoglucomannans: Chemical Synthesis, Conformation Analysis, NMR Spectroscopic Characterization, and Molecular Recognition of Model Compounds

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Abstract: Galactoglucomannan (GGM) is a polysaccharide mainly consisting of mannose, glucose, and galactose. GGM is the most abundant hemicellulose in the Norway spruce (*Picea abies*), but is also found in the cell wall of flax seeds, tobacco plants, and kiwifruit. Although several applications for GGM polysaccharides have been developed in pulp and paper manufacturing and the food and medical industries, attempts to synthesize and study distinct fragments of this polysaccharide have not been reported previously. Herein, the synthesis of one of the core trisaccharide units of GGM together with a less-abundant tetrasaccharide fragment is described. In addition, detailed NMR spectroscopic characterization of the model compounds, comparison of the spectral data with natural GGM, investigation of the acetyl-group migration phenomena that takes place in the polysaccharide by using small model compounds, and a binding study between the tetrasaccharide model fragment and a galactose-binding protein (the toxin viscumin) are reported.

Keywords: conformation analysis · galactoglucomannan · molecular recognition · NMR spectroscopy · oligosaccharide synthesis

Introduction

Galactoglucomannan (GGM) is the most abundant hemicellulose in the Norway spruce (*Picea abies*).[1] GGM has also been found in several other plants and fruits including club mosses, horsetails, whisk ferns, kiwifruit, and tobacco plants.[2] The utilization of water-soluble polysaccharides, in particular GGM, in mechanical pulping and papermaking has traditionally been of great importance.[3] Recently, methods for the large-scale isolation of acetylated GGM (AcGGM) from *P. abies* have been developed, thus simultaneously sparking new interest in the applications and chemical properties of these compounds. Polysaccharide material suited for such work can now be isolated on the kilogram scale, thus providing sufficient amounts of material for screening and extending the applications of these compounds.[1a,4] Consequently, a deeper understanding of the role of GGM in pulp and paper manufacturing has been achieved together with emerging applications of GGM in immunology.[5] GGM has also been shown to be a promising substitute of the widely utilized gum arabic (arabinogalactan) as an emulsifier of hydrophobic beverages.[1a]

Several earlier studies have focused on the structural elucidation of water-soluble AcGGM from *P. abies*.[6] It has been suggested that the main chain consists of randomly distributed β-(1→4)-linked mannopyranosyl and β-(1→4)-linked glucopyranosyl units. Furthermore, the presence of α-(1→6)-linked galactopyranosyl residues has been confirmed in several studies.[6a,7] In all previous investigations, the galactopyranosyl units were found to be linked to the mannopyranoside residues of the linear backbone of GGM. These monosaccharides have been suggested to be present in a mannose/glucose/galactose (Man/Glc/Gal) ratio of 4:1:0.1, although this ratio is strongly influenced by the isolation conditions.[8] In addition, the acetyl-group pattern of AcGGM from *P. abies* with an acetylation degree of 0.3 has been analyzed, and the acetyl groups are, according to the currently accepted view, mainly present at the O2 and O3 atoms of the mannopyranosyl units (Figure 1).[1a,6a]

Although AcGGM can be readily isolated on a large scale, several ambiguities concerning the structure and acetylation pattern of this material remain. In fact, determination of structure and size of AcGGM has been very difficult due to the heterogeneous nature of the native material. This behavior is reflected in the wide range of molecular weights (i.e., 20–78 kDa) reported for these polysaccharides.[1a,4] In

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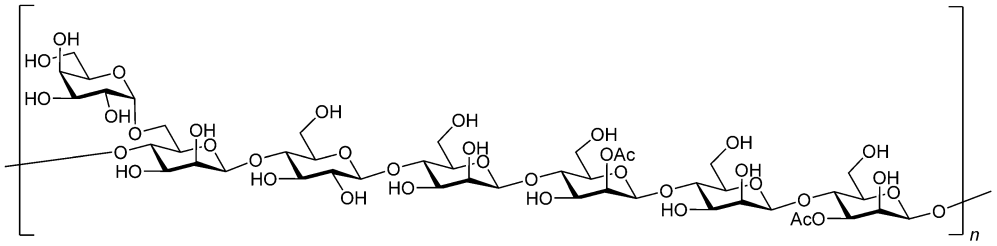


Figure 1. One of the possible chemical structures of the AcGGM backbone.

addition, acetyl-group migration has been proposed to occur from O2 and O3 to even the O6 position in the mannopyranosyl residues.[1a] An investigation of such phenomena is difficult to perform on the heterogeneous polysaccharide samples available. Therefore, we became interested in the synthesis and possible utilization of model compounds. Herein, the synthesis of one of the trisaccharide repeating units of AcGGM, a less-abundant tetrasaccharide fragment containing an α-(1→6)-linked galactopyranosyl residue and some model substrates for acetyl-group migration studies are reported. In addition, a complete NMR spectroscopic characterization and conformation analysis of the model compounds and a comparison of the NMR spectroscopic data of model compounds with natural GGM polysaccharide are presented. The tetrasaccharide fragment bearing an α-(1→6)-linked galactopyranosyl residue was further utilized in a binding study with the galactose-binding protein viscumin, an AB toxin, to test bioaffinity.

Results and Discussion

Synthesis of model compounds: The applications of AcGGM have in recent years rapidly expanded from its traditional roles in pulp- and paper-making processes to applications in the food and medicinal industries.[1a,3,5] From a biochemical standpoint, the roles of the individual fragments of the GGM polysaccharide would be interesting to study as such, particularly in view of immunological applications. Previously, this investigation would not have been possible due to the heterogeneous nature of the native samples. In the case of AcGGM, the individual fragments contain challenging glycosidic linkages, including β-mannopyranosyl- and α-galactopyranosyl-linked sugar units, which for a long time were considered difficult to construct by chemical methods. In the present study, the synthesis of two oligosaccharide fragments found in AcGGM was planned to provide material for such studies (Figure 2).

For the initial attempt on the synthesis of trisaccharide **1**, a linear strategy was devised that started from the three monosaccharide building blocks **3**–**5** (Figure 3). The synthesis of donor **3** has been addressed previously and was based on previously reported procedures.[9] Acceptor **4** was prepared by the route shown in Scheme 1. The commercially available methyl mannopyranoside **6** was converted into **7**

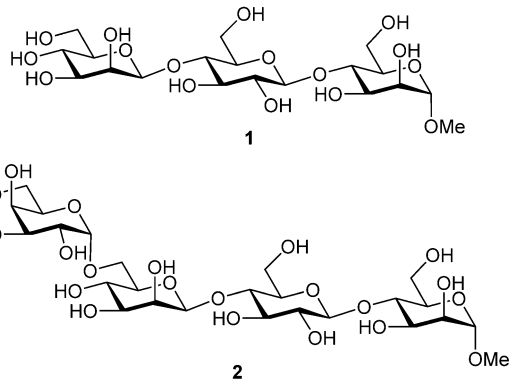


Figure 2. The synthetic targets representing one of the trisaccharide repeating units of GGM **1** (top) and a less-abundant tetrasaccharide fragment **2** (bottom).

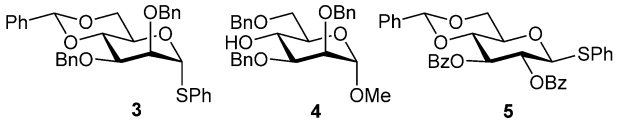
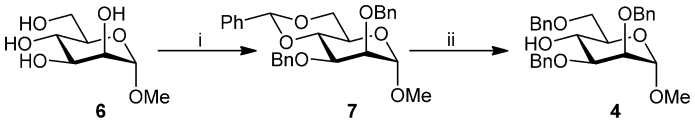


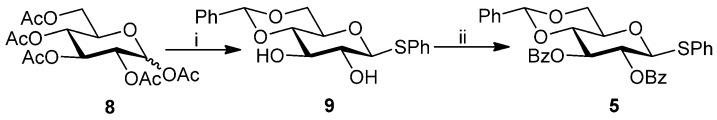
Figure 3. Building blocks prepared for the synthesis of trisasaccharide **1**.



Scheme 1. Synthesis of acceptor **4**. i) 1. PTSA, $C_6H_5OCH(OMe)_2$, 2 h, 60 °C, 200 mbar (60 %); 2. BnBr, NaH, DMF, 3 h, RT (88 %); ii) TFA, Et_3SiH, CH_2Cl_2, 2 h, RT (50 %). PTSA = *para*-toluenesulfonic acid, TFA = trifluoroacetic acid.

by utilizing one equivalent of benzaldehyde dimethyl acetal under slightly acidic conditions, in analogy to the procedures described by McGowan and Berchtold in 1982.[10a] The remaining hydroxyl groups were benzylated by using the standard protocol involving NaH and BnBr. Next, the benzylidene acetal was partially cleaved by the reductive methodology of DeNinno et al.[11a] and Arasappan and Fraser-Reid[11b] to give the 4-OH/6-OBn-containing acceptor **4** in moderate yield. A similar efficiency was also witnessed previously in the partial cleavage of benzylidene-protected mannopyranosides under these conditions.[12]

With building blocks **3** and **4** in hand, attention was turned to the preparation of building-block donor **5**, which was synthesized according to the synthetic route shown in Scheme 2. Peracetylated D-glucopyranose was subjected to



Scheme 2. Synthesis of donor **5**. i) 1. SHPh, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 24 h, RT (96%; α/β 1:9); 2. NaOMe, MeOH/THF 3:1, 24 h, RT (95%); 3. PTSA, $C_6H_5OCH(OMe)_2$, DMF, 60°C, 200 mbar (78%; pure β anomer); ii) BzCl, pyridine, 5 h, RT (90%). BzCl = benzoyl chloride.

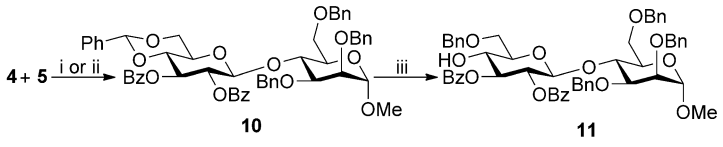
$BF_3{\cdot}OEt_2$-promoted glycosylation followed by deacetylation under conditions developed by Zemplén et al.[13] The benzylidene-protected thioglycoside **9** was synthesized according to previously described procedures.[10] As expected, the yields were significantly higher in the benzylidene formation with the glucopyranoside relative to those of the mannopyranosides. This outcome is mainly due to the *trans* relationship of the 2- and 3-hydroxyl groups in the glucose moiety, which significantly decreases formation of the dibenzylidene side product formed with mannopyranosides. The remaining hydroxyl groups were benzoylated by means of standard methods by using BzCl in pyridine. With all of the building blocks prepared, the focus was next shifted to assembly of trisaccharide **1**.

Following a linear pathway, donor **5** was first coupled with acceptor **4** in the presence of the conventional NIS/TMSOTf promoter/activator system (Scheme 3).[14] Several difficulties were encountered during the first attempts of this glycosylation reaction. In our initial approach, by following the standard activation protocol with 0.12 equivalents of TMSOTf, a moderate 47% yield of the desired product was obtained with the formation of several side products. When the amount of TMSOTf was decreased to 0.06 equivalents, the yield of the desired disaccharide **10** increased. The main difficulty encountered during the synthesis was the highly crystalline nature of the benzoylated glucose donor **5** and disaccharide **10**. The characteristic feature of these compounds, namely, the formation of solids/crystals, posed a major concern during chromatographic purification because the products partially solidified in the column (despite being highly soluble in the solvent system) and were lost, thereby lowering the amount of isolable material. Furthermore, the crystallization of disaccharide **10** in the presence of donor **5** was impossible due to the small scale of the synthesis. In addition, the partial reductive cleavage, following the protocol described by Arasappan and Fraser-Reid, resulted in a poor yield of the desired disaccharide **11**.[11b]

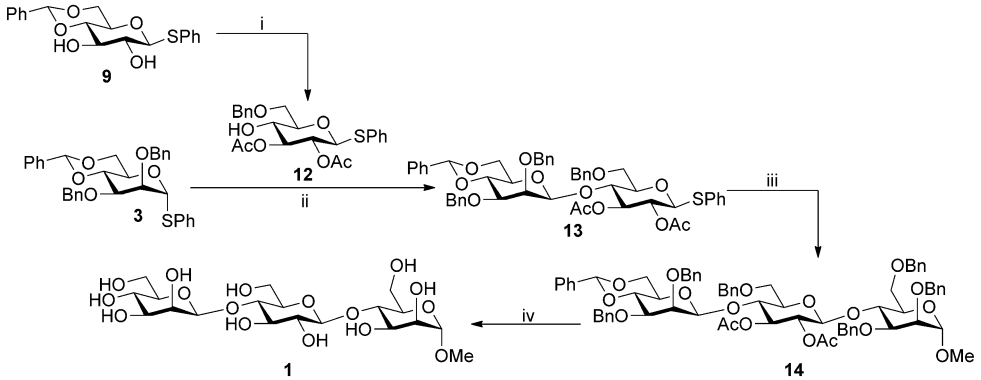
Consequently, the synthetic strategy required revision. As the selective ring-opening reaction of the benzylidene acetal moiety in disaccharide **10** turned out to be difficult, the synthetic approach was changed from linear to convergent. The convergent route would add two potential benefits to the synthetic pathway: Importantly, the β-mannopyranosyl linkage could be constructed first, followed by convergent coupling of disaccharide to acceptor **4**.

In addition, the selective ring-opening reaction of the benzylidene acetal unit could now be performed on the glucose building block instead of the disaccharide. To avoid the problematic crystalline nature displayed by the dibenzoylated glucoside, the benzoyl groups were simultaneously replaced by acetyl groups. The synthesis of trisaccharide **1** is shown in Scheme 4. Acceptor **12** was synthesized from **9** by acetylation of the free hydroxyl group followed by a selective ring-opening reaction of the benzylidene acetal moiety by the use of methods described previously.[11] In this approach, the yield proved to be excellent. The large variation in the efficiency of the methodology described by Arasappan and Fraser-Reid was highly dependent on the stereochemistry of the individual sugars involved in the reactions.[11b] Similar results have been reported previously.[12] Next, donor **3** was activated following the β-mannosylation protocol of Crich et al. and then coupled with acceptor **12**.[15] The glycosylation reaction proceeded with moderate efficiency and high selectivity (β/α = 10:1). The moderate efficiency observed is most likely due to the sluggish reaction and the small amounts (~15% each) of starting material/hydrolyzed donor present in the reaction mixture. Although not optimized further and explored in the present study, the yield of this individual glycosylation reaction could possibly be enhanced by the addition of 1-octene to the reaction mixture after the activation of donor **3**. In earlier reports, 1-octene was shown to be a mild reagent capable of trapping the thioadducts formed as side products during the activation and conversion of donor **3** into the activated glycosyl triflate.[16] Donor **13** was then coupled with acceptor **4** under the reaction conditions optimized in the earlier attempt displayed in Scheme 4 (0.06 equivalents of TMSOTf). The convergent glycosylation proceeded smoothly to give the protected trisaccharide **14** in 77% yield of isolated product. A two-step deprotection sequence that consists of deacetylation[13b] followed by hydrogenolysis gave the trisaccharide **1** in 84% overall yield.

With the trisaccharide repeating unit synthesized, the focus was shifted toward the assembly of the less-abundant tetrasaccharide fragment **2**.



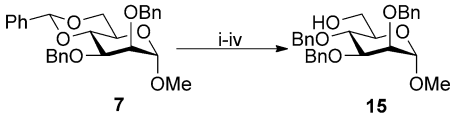
Scheme 3. Synthesis of disaccharide **11**. i) NIS (1.2 equiv), TMSOTf (0.12 equiv), CH_2Cl_2, 1.5 h, −25°C (47%); ii) NIS (1.2 equiv), TMSOTf (0.06 equiv), CH_2Cl_2, 1.5 h, −25°C (76%); iii) TFA, Et_3SiH, CH_2Cl_2, RT, 1–5 h (<15%). NIS = *N*-iodosuccinimide, TMSOTf = trimethylsilyl trifluoromethanesulfonate.



Scheme 4. Synthesis of GGM core trisaccharide **1**. i) 1. Ac_2O, pyridine, RT, 5 h (95%); 2. Et_3SiH, TFA, 0°C→RT, 3 h (85%); ii) 1. BSP, TTBP, Tf_2O, CH_2Cl_2, −60°C, 0.5 h: 2. **12**, −78°C, 2 h (53%); iii) **4**, NIS, TMSOTf, CH_2Cl_2, −40°C, 1.5 h (77%); iv) 1. NaOMe, MeOH/THF (1:1), RT, 19 h (84%); 2. Pd/C, H_2 (2.8 bar), MeOH, RT, 19 h (quant.). BSP = 1-(phenylsulfinyl)piperidine, TTBP = 2,4,6-tri-*tert*-butyl pyrimidine, Tf_2O = triflic anhydride.

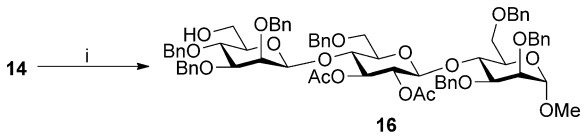
Trisaccharide **14** was chosen as a suitable starting material for the synthesis of tetrasaccharide **2**. Once again, selective ring opening of the benzylidene acetal was required as a key transformation in the preparation of trisaccharide acceptor **16**. In this approach, however, the reverse selectivity in the ring-opening reaction was desired (6-OH/4-OBn) as the galactopyranosyl residue in **2** is α-(1→6)-linked to the sugar backbone. For this purpose, several procedures have been reported based on, for example, $PhBCl_2/Et_3SiH$,[17] $LiAlH_4/AlCl_3$,[18] $TMSOTf/BH_3{\cdot}THF$,[19] and $Cu(OTf)_2/BH_3{\cdot}THF$.[20]

Instead of trisaccharide **14**, the appropriately protected monosaccharide **7** was utilized in the initial screening and optimization of reaction conditions (the results are summarized in Scheme 5). In our hands, several of the previously reported procedures did not work with the efficiencies reported.[17–19] In the initial attempts, both the $PhBCl_2/Et_3SiH$[17] and $LiAlH_4/AlCl_3$[18] protocols were quickly excluded from further use due to poor efficiencies. By considering the moderate efficiency obtained by the $TMSOTf/BH_3{\cdot}THF$ protocol,[19] a continued investigation into borane-based systems was launched. The selectivity in the partial cleavage of benzylidene acetals is generally a combination of steric and electronic factors. It has been suggested that 6-OBn derivatives are formed when borane is activated by the Lewis acid, thereby coordinating to the more nucleophilic 6-OH group. In cases in which the borane moiety is not activated under the reaction conditions employed, the Lewis acid becomes the most electrophilic species in the reaction mixture and coordinates to the 6-OH group of the sugar, thus providing the 4-OBn derivative as the product.[21] Consequently, in the present study, it became important to investigate different Lewis acids under similar conditions. A procedure using $Cu(OTf)_2$ as the Lewis acid in the selective ring-opening reaction of benzylidene acetals has been described recently by Shie et al.[20] Initial attempts that utilized this protocol resulted in complete cleavage of the benzylidene acetal moiety. The solvent was identified as the major source of the problems in this reaction, and monosaccharide **15** could be isolated in high yield after switching from THF to CH_2Cl_2. Although this procedure appeared to function well for monosaccharides, only a few reports on the applications of similar protocols to oligosaccharides have been described previously.



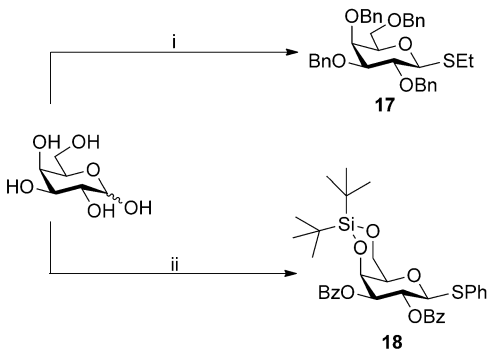
Scheme 5. Screening of methodologies for the selective ring-opening reaction of benzylidene acetals. i) $PhBCl_2$, Et_3SiH, −78°C, CH_2Cl_2, 2–4 h (35%); ii) $LiAlH_4$, $AlCl_3$, CH_2Cl_2/Et_2O 4:1, RT→50°C, 1 h (35%); iii) TMSOTf, $BH_3{\cdot}THF$, CH_2Cl_2, RT, overnight (50%); iv) $Cu(OTf)_2$, $BH_3{\cdot}THF$, CH_2Cl_2, RT, overnight (82%).

When applying similar conditions to the protected trisaccharide **14**, the trisaccharide acceptor **16** was obtained in excellent yield (Scheme 6). This outcome is, to our knowledge, one of the first times in which a similar procedure has been applied to an oligosaccharide. The high efficiency and selectivity observed under the conditions explored are encouraging for future applications of this methodology.



Scheme 6. Synthesis of the trisaccharide acceptor **16**. i) $Cu(OTf)_2$, $BH_3{\cdot}THF$, CH_2Cl_2, RT, overnight (85%).

With the trisaccharide acceptor prepared, the challenge concerning α-galactosylation remained. For this purpose, donors **17** and **18** were prepared according to reported procedures by using the routes shown in Scheme 7.[22] Both of these donors have in recent years been applied to the α-galactosylation of various acceptors and were therefore also screened in the present study. Donor **17** has previously been shown to result in reactions with high α-selectivity and effi-



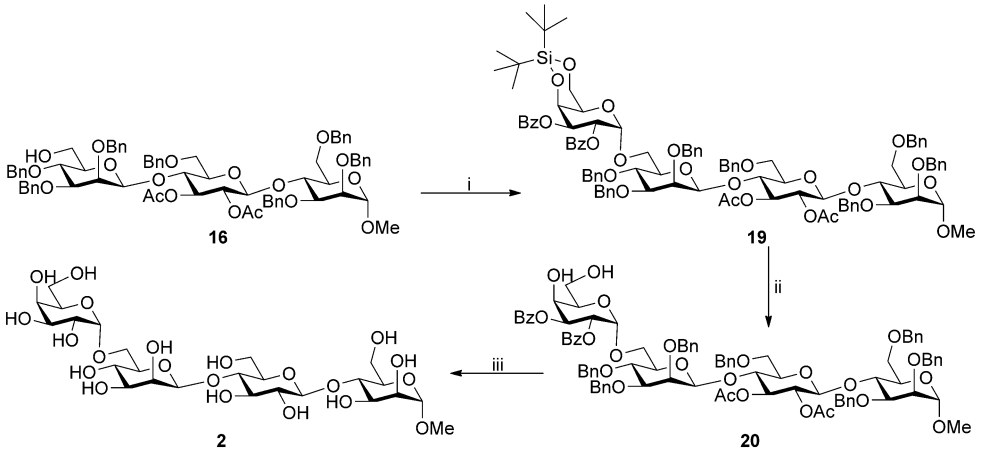
Scheme 7. Synthesis of donors **17** and **18**. i) 1. Ac_2O, NaOAc, reflux, 2 h (50%; pure β isomer); 2. SHEt, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0°C, 4 h (95%); 3. NaOMe, MeOH/THF (4:3), RT, overnight (quant.); 4. BnBr, NaH, DMF, RT, 20 h (80%); ii) 1. Ac_2O, NaOAc, reflux, 2 h (50%; pure β isomer); 2. SHPh, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0°C, 4 h (95%); 3. NaOMe, MeOH/THF (1:1), RT, overnight (quant.); 4. 1) Di-TBS$(OTf)_2$, pyridine, RT, 3 h; 2) BzCl, RT, 2 h (57%). Di-TBS$(OTf)_2$ = di-*tert*-butylsilyl ditriflate.

ciency in ethereal solvents including Et_2O.[23] As a result, these conditions were first screened in the α-galactosylation of acceptor **16** by using the NIS/TMSOTf promoter system.[14] However, only moderate-to-low selectivities (α/β=4:1) were achieved under these conditions because the separation of the two diastereomers by column chromatography proved to be challenging. Although several variations in solvent proportions (Et_2O/CH_2Cl_2) and the activation/promoter systems were tried, the selectivity of the glycosylation could not be improved. Therefore, another methodology using $CuBr_2$, TBAB, AgOTf in dichloroethane and DMF, utilized successfully earlier by Mukherjee and Misra for α-galactosylation of a tetrasaccharide acceptor, was tested.[24a] In the present study, however, this protocol failed to yield the desired product.

The given experience caused us to turn our attention to donor **18**. This donor has been applied previously by Ando and co-workers for the α-selective galactosylation of several oligosaccharides and aglycones.[22b,c,25] In the present study, the methodology was applied to the α-selective galactosylation of **16**, thus providing tetrasaccharide **19** in good yield as shown in Scheme 8. Whereas the yield was 68% and the selectivity excellent, the formation of a second tetrasaccharide was also observed. Based on the $J_{H1,H2}$ coupling constant in the ^{1}H NMR spectrum of this tetrasaccharide byproduct, it appeared to be α-linked,[26] as it was most likely an orthoester of the product. With the glycosylation efficiency already in the desired range, further attempts to optimize the experimental conditions were not made. The origin of the high α-selectivity in the glycosylation reaction is puzzling because ester-protecting groups are present at both the O2 and O3 positions of donor **18**. A likely explanation for the high α-selectivity may, at least in part, rest on the steric and electronic properties induced by the 4,6-silylene acetal moiety, as also suggested previously by Kiso and co-workers.[27] For the synthesis of the deprotected tetrasaccharide **2**, the silylene acetal of **19** was cleaved in 68% yield by using a HF/pyridine complex.[28] Next, the ester groups were demasked under conditions similar to the ones developed by Zemplén et al.[13b] with NaOMe in a MeOH/THF mixture followed by hydrogenolysis of the benzyl groups in 92% overall yield over two steps.

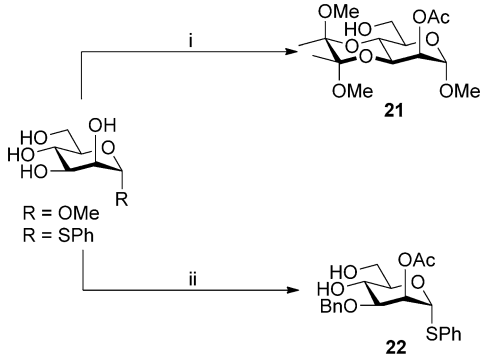
With one of the core trisaccharide repeating units (i.e., **1**) and a less-abundant tetrasaccharide unit (i.e., **2**) synthesized, our attention could be turned to the investigation of the acetyl-group migration phenomena that occurs during the isolation of native GGM.

Acetyl-group migration in GGM: The acetylation degree of GGM is approximately 0.3, which varies slightly depending on the methods used in the isolation process.[1a,6] The acetyl groups are mainly located at the O2 and O3 positions of the mannopyranosyl residues. It has been proposed that acetyl groups may migrate either directly from axial O2 to O6 or from O3 to O6 in the mannopyranosyl units.[1a] Migration between O2 and O3 is known to take place in substrates



Scheme 8. Synthesis of tetrasaccharide **2**. i) **18**, NIS, TfOH (or TMSOTf), CH_2Cl_2, 0°C, 1.5 h (68%); ii) HF/pyridine, THF, 0°C→RT, 6 h (68%); iii) 1. NaOMe, MeOH/THF (1:1), RT, overnight (96%); 2. Pd/C, H_2 (2.8 bar), MeOH, RT, overnight (96%).

that contain acetyl protecting groups and will not be discussed in more detail in the present context. The migration from O2 and O3 to O6 is unique as it would require the acetyl group to "leap" across the molecule in space. This phenomenon is difficult to study on the native polysaccharide due to a severe overlap of the signals in the NMR spectra. Consequently, small molecular models were synthesized to study this process. Mannopyranosides are known to exist mainly in the energetically favored $^4C_1$ conformation. On the basis of this assumption, a few differently acetylated mannopyranoside model compounds were synthesized (Scheme 9). The synthesis of these compounds followed standard protecting-group manipulations and is thus not presented in more detail.[29]



Scheme 9. i) 1. Butanedione, trimethyl orthoformate, CSA, MeOH, reflux, 24 h (79%); 2. TBDMSCl, imidazole, DMF, 0°C→RT, 24 h, 90%; 3. Ac_2O, pyridine, dichloromethane, 48 h (92%); 4. 70% HF/pyridine, THF, 0°C→RT, 24 h (93%); ii) 1. PTSA, $C_6H_5OCH(OMe)_2$, DMF, 60°C, 200 mbar, 2 h (53%); 2. a) Bu_2SnO, toluene, 120°C, 3 h; 2) TBAB, CsF, BnBr, 120°C, 3 h (85%); 3. Ac_2O, pyridine, RT, 3 h (93%); 4. 80% AcOH, 70°C, 1.5 h (87%). CSA = camphorsulfonic acid, TBAB = tetrabutylammonium bromide, TBDMSCl = *tert*-butyldimethylsilyl chloride.

In this initial study, the possibilities of acetyl-group migration from O2 to O6 in $^4C_1$-mannopyranosides were evaluated. Compound **21**, locked in the $^4C_1$-conformation by the diacetal protecting group between positions 3 and 4, was selected as a suitable model compound to investigate the O2→O6 acetyl-group migration. No migration was observed when this compound was subjected to conditions similar to the ones used for the isolation of native GGM (75°C, D_2O/DMSO (dimethyl sulfoxide) 1:1). One possible reason for the absence of migration could be the low degree of flexibility in this model molecule. Mannoside **22** was synthesized for verification of the hypothesis. Although **22** exhibits a much higher degree of flexibility, migration did not take place in this compound either.

On the basis of these results, it seems quite unlikely that migration would occur in the GGM polysaccharide from O2 to O6 as has been suggested previously.[1a] More model compounds will, however, be needed to investigate the acetyl-group migration from O3 to O6. Appropriately protected and "locked" high-energy conformations (such as those depicted in Figure 4) should be synthesized and studied under

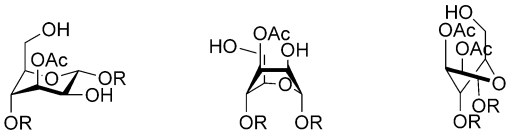


Figure 4. High-energy conformations of mannopyranosides in which acetyl-group migration could be possible. Left→right: $^1C_4$, $B_{1,4}$, $^5H_4$.

similar conditions to explore the possibility of acetyl-group migration comprehensively. In these conformers, the acetyl groups are significantly closer to the O6 atom and migration could be more favored than in the $^4C_1$ conformation. Such an approach should further be combined with molecular modeling to create appropriate models to investigate the phenomenon. The synthesis of such molecules for further studies on the acetyl-migration behavior of GGM is currently planned in our laboratories. The acetylated trisaccharide fragment representing the second repeating unit in the GGM backbone might prove to be an additional probe utilizable in studies on the acetyl-group migration/cleavage patterns of GGM.

NMR spectroscopic comparison of the model compounds versus natural GGM: The complete ^1H and ^{13}C NMR spectroscopic characterization of both **1** and **2** was required to compare the NMR spectroscopic data of the synthetic model structures and natural GGM (Figure 2). The NMR spectroscopic characterization of oligosaccharides can be difficult due to severe signal overlap in the ^1H NMR spectra. Previously, we have described the complete NMR spectroscopic characterization of a β-(1→2)-linked mannotetraose by using standard NMR spectroscopic experiments (^1H; ^{13}C, 1D-TOCSY, COSY, HSQC (coupled and decoupled), and HMBC) in combination with spectral simulations with the PERCH software.[30] A similar set of NMR spectroscopic experiments was used for the spectral assignment reported herein. The NMR spectra were recorded at 308 K (in D_2O) to shift the water peak, which would otherwise overlap with the anomeric protons. The ^1H NMR spectrum of **1** displays three well-separated anomeric protons at $\delta = 4.76$ (d, H1), 4.74 (d, H1′), and 4.52 ppm (d, H1′). These signals were also targeted by selective excitation with 1D-TOCSY[31] with a spin-lock time of 300 ms. The results of these experiments are visualized in Figure 5. By use of COSY, HSQC (coupled and decoupled), and HMBC both the ^1H and ^{13}C NMR spectra of **1** could be completely assigned.

This information, however, was not sufficient to define accurate coupling constants. For this purpose, the NMR spectral simulation software PERCH (Peak Research)[32] was utilized, thus leading to accurate ^1H NMR spectral simulation, coupling constants, and coupling patterns (Figure 6).

The discussion of the NMR spectroscopic characterization of oligosaccharides is exemplified and limited to the complete assignment of **1**. A similar set of NMR spectroscopic techniques in combination with spectral simulations was applied to yield the NMR spectroscopic characterization of all

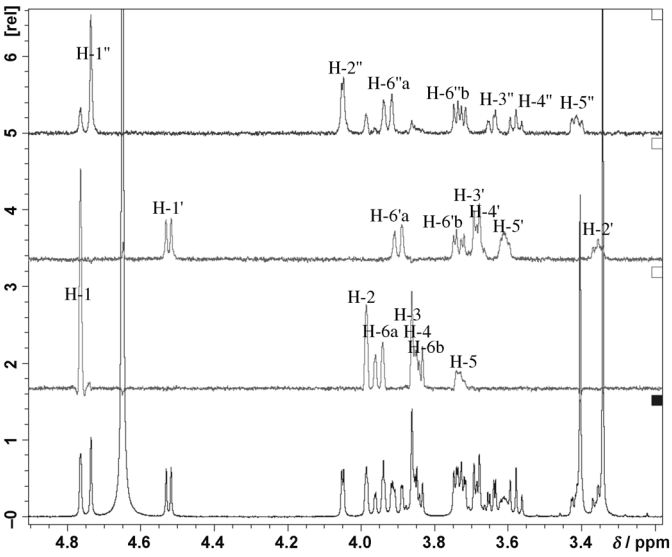


Figure 5. 1D-TOCSY spectra of the different residues in **1**. Top→bottom: Man*p″*, Glc*p′*, Man*p*, and the entire ^{1}H NMR spectrum of **1**.

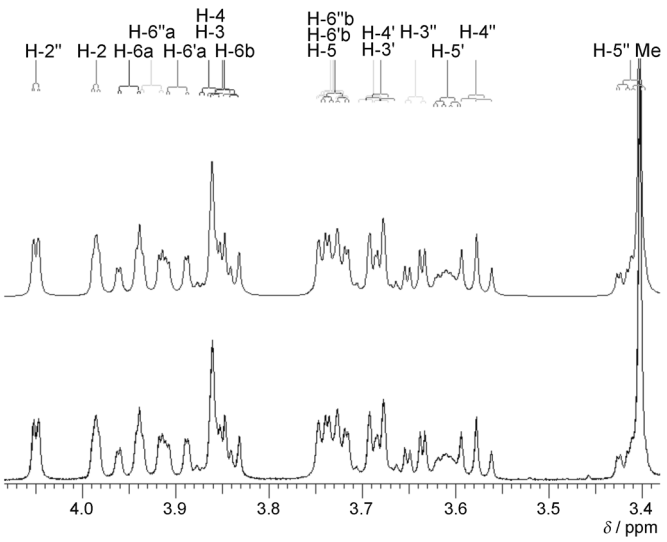


Figure 6. Spectral simulation of the ^{1}H NMR spectrum of **1** (δ = 4.08–3.38 ppm region) with the PERCH NMR software. Top: simulated spectrum, bottom: observed spectrum.

the other compounds presented herein. With the NMR spectra of model compounds **1** and **2** solved, our attention was turned to the comparison of the chemical shifts obtained with those of natural GGM. The ^{1}H and ^{13}C NMR chemical shifts of **1** and **2** together with reported values of the chemical shifts of GGM[6a,8a,33] are summarized in Table 1. The coupling constants and patterns observed in the ^{1}H NMR spectra of **1** and **2** and the $J_{C,H}$ coupling constants for the anomeric centers are given in the experimental section.

Many of the chemical shifts for the individual signals in **1** and **2** correlate relatively well with the values reported for natural GGM (Table 1).[6a,8a,33] For certain signals, significant chemical-shift differences have been reported. One of the main signals for which large deviations have been reported is the H2 signal of the β-(1→4)-linked mannopyranoside residue. This signal has been reported to appear in natural GGM both at δ = 3.08 and 4.08 ppm.[6a,8a,33] In both model compounds **1** and **2**, the chemical shift for H2 was close to δ = 4.08 ppm, which suggests this value to be the correct one. In fact, all the other signals from this residue are found at similar frequencies in both the model compounds and GGM. These data suggest either an assignment error or typographical mistake in the reports for the chemical shift of H2 in GGM reported by Hannuksela and Hervé du Penhoat.[6a] The chemical shifts for H6a, H6b, and C6 of the β-(1→4)-linked glucopyranoside residues have not been reported for GGM, but differed by less than δ = 0.2 ppm in both model substances and could, therefore, be assumed to be similar in the polysaccharide structure. The H5 and C5 chemical shifts of the β-(1→4)-linked glucopyranoside residues of GGM appeared at a higher frequency than in model compounds **1** and **2**. As the values are almost exactly similar for all the other signals in this residue, there might be some uncertainties in the chemical shifts reported for GGM previously. Surprisingly, there is very little reported data available for the β-(1→4)-linked mannopyranoside residue bearing an α-(1→6)-linked galactopyranosyl unit. These chemical shifts were easily identified in the model compounds, thus complementing the available reported data. Interestingly, the H5 proton is shifted toward a higher frequency and the C5 carbon atom toward a lower frequency when the β-(1→4)-linked mannopyranosyl unit is substituted by an α-(1→6)-linked galactopyranosyl residue. A further notable difference in the chemical shifts of the α-(1→6)-galactosylated mannopyranosyl unit is the large difference for the C6 carbon atom, which is located at δ = 66.8 ppm in the substituted mannopyranosyl residue versus δ = 61.5 ppm in the unsubstituted residue. These values should provide a convenient reference point for future determinations of the amount of 6-substituted β-(1→4)-linked mannopyranosyl units in the GGM backbone.

Although slight deviations were found in the NMR spectroscopic characterization of GGM relative to results of respective studies on model tri- and tetrasaccharides, most signals were found at positions that were in good agreement with previously reported spectral data. These results indicate that it is indeed possible to infer the composition of polysaccharides by characterizing small molecular models of limited size. To further evaluate and continue the study on the GGM polymer, several other small molecular fragments should be synthesized and analyzed. The most important among these fragments should be the synthesis and characterization of the second repeating unit bearing acetyl groups.

Conformation analysis: Studies on the three-dimensional conformation of polysaccharides are important for several reasons. In the case of GGM, the natural shape and function of the molecule plays an important role in any application. Accordingly, the conformation analysis of model compound

Table 1. Chemical shifts of natural GGM and model substances **1** and **2**.[a]

Sugar	H1 C1	H2 C2	H3 C3	H4 C4	H5 C5	H6a,b C6
Native GGM						
β-Man*p*-(1→	4.75	4.08 (or 3.08)[6a]	3.66	3.58	3.45	3.95, 3.74
	101.2	71.12	72.50	77.63	76.1	61.9
→4)-β-Glc*p*-(1→	4.53	3.364	3.702	3.63	3.78	–
	103.6	73.87	74.98	79.519	77.5	
α-Gal*p*-(1→	5.038	3.83	3.958	4.01	3.92	3.77
	99.84	69.46	70.31	70.26	72.3	62.29
→6)-β-Man*p*-(1→	4.79	–	–	–	3.70	4.03–3.94,
	–	–	–	–	–	3.84–3.79
Model substrate 1						
β-Man*p*-(1→	4.73	4.05	3.65	3.58	3.41	3.93, 3.73
	100.6	71.1	73.4	67.3	77.0	61.5
→4)-β-Glc*p*-(1→	4.52	3.35	3.69	3.68	3.61	3.90, 3.73
	103.0	73.5	74.6	79.2	75.3	60.8
→4)-α-Man*p*	4.76	3.99	3.86	3.86	3.73	3.95, 3.84
	101.2	70.1	69.8	77.2	71.8	60.9
Model substrate 2						
→6)-β-Man*p*-(1→	4.75	4.07	3.65	3.68	3.61	3.96, 3.78
	100.8	71.1	73.5	67.2	75.1	66.8
→4)-β-Glc*p*-(1→	4.53	3.35	3.69	3.67	3.62	3.89, 3.73
	103.0	73.5	74.7	79.6	75.1	60.8
→4)-α-Man*p*	4.76	3.98	3.86	3.85	3.73	3.95, 3.84
	101.2	70.1	69.9	77.3	71.8	60.9
α-Gal*p*-(1→	5.00	3.81	3.95	3.99	3.95	3.74, 3.74
	98.9	69.1	71.6	69.9	70.0	61.8

[a] Chemical shifts are expressed in δ (ppm) with CD_3OD/D_2O as internal standards. The chemical shifts for GGM are reported values.[6a,8a,33]

2 was performed by using the MacroModel program as integrated in the MAESTRO[34] graphical interface and the MM3* force field.[35] By applying a combined molecular mechanics/NMR spectroscopic approach, the conformation around every glycosidic linkage was elucidated.[36] The analysis of the potential-energy surfaces calculated for every Φ/Ψ glycosidic torsion angle suggested the existence of one well-defined minimum-energy conformer for the two β-(1→4)-linkages (defined as H1-C1-O5-C4 and C1-O5-C4-H4), thus corresponding to the typical *syn* Φ/Ψ geometry, in agreement with the *exo*-anomeric effect. Two different potential-energy maps were calculated for the α-(1→6)-linkage, by considering either the *gg* or *gt* rotamers at the C5–C6 hydroxymethyl torsion angle. In this case, the corresponding Φ angle was in the *exo*-anomeric region, whereas the Ψ angle (defined as C1-O1-C6-C5) was predicted to be in the *anti* region. Therefore, two model tetrasaccharides were constructed with either *gg* or *gt* geometries at the O6-substituted Man residue and submitted to molecular-dynamics simulations with the same force field. No interconversions to other energy regions were detected and the observed NMR spectroscopic data (NOE interactions and *J* couplings) were consistent with the found geometries. Indeed, the measured small- and medium-size *J* coupling values ($J_{6''a,5''}=6.7$, $J_{6''b,5''}=2.1$ Hz) for the C5–C6 linkage indicate the simultaneous existence of both *gg* and *gt* rotamers in equilibrium. Additionally, for the two β-Man*p*-(1→4)-β-Glc*p* and β-Glc*p*-(1→4)-α-Man*p* linkages, the presence of NOE interactions between the anomeric proton of the substituted pyranose and H4 of its corresponding aglycon strongly argued for the presence of the theoretical global minima. Based on the coupling constants obtained from the spectral simulations, all of the residues adopt the expected $^4C_1$ conformation. The NOE correlations between noncontiguous residues were not observed, thus suggesting the backbone of GGM to be linear. The α-(1→6)-linked galactopyranosyl residue does not influence the linearity of the structure and can, therefore, be considered to be a substituent. Because this moiety is spatially readily accessible and could thus serve as contact point for molecular recognition with lectins,[37] we tested this assumption by using a model protein with specificity to galactosides.[38]

Molecular recognition of tetrasaccharide 2 by a model lectin: The applications of GGM have in recent years expanded from the traditional roles in pulping and papermaking to new areas including immunology. To delineate the potential for interactions and the binding mode of GGM at the molecular level, NMR spectroscopic experiments between tetrasaccharide **2** and a model lectin, the toxic *Viscum album* agglutinin (VAA), were performed.[38] This lectin belongs to the AB-type family of toxin plant lectins and targets terminal galactose residues because mannose/glucose have an IC_{50} value that is higher than galactose by more than 100-fold.[39] With the complete NMR spectroscopic characterization and conformation analysis of **2** done, the prerequisites for such a study were available. First, evidence of any interaction was deduced from the analysis of the STD patterns[35,40] for a ligand/lectin molar ratio of 25:1, which also permitted us to deduce the binding epitope of the interacting ligand. Expectedly, VAA recognizes mainly the galactopyranosyl unit in the tetrasaccharide, which showed the highest saturation (Figure 7). In addition, signifi-

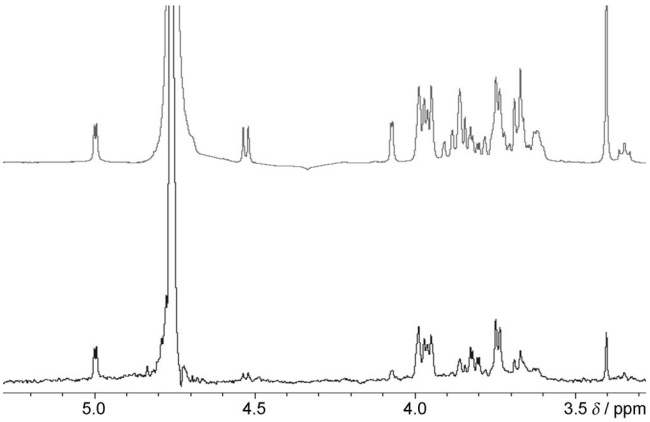


Figure 7. Bottom: saturation transfer difference (STD)-NMR spectrum of the viscumin/tetrasaccharide **2** system. Top: the corresponding off-resonance spectrum. The concentration of the lectin was 59 μM (pH 7.5, D_2O, PBS, 300 K) and the ligand/lectin = 25:1 (mol/mol). PBS = phosphated-buffered saline.

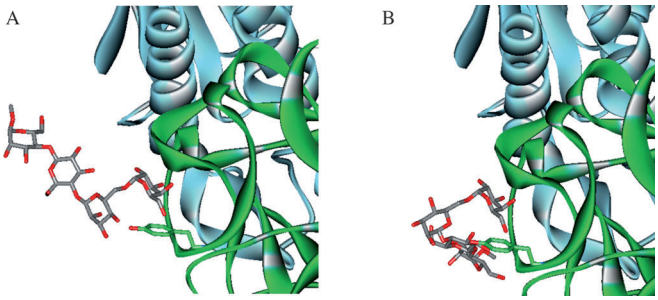


Figure 8. A) Structural model of the complex of VAA (PDB code: 1PUM, tyrosine (Tyr) site) with the global-minimum conformation of **2** (*gg* rotamer around the α-Gal*p*-(1→6)-β-Man*p* linkage) based on docking analysis and the experimental input from STD NMR spectroscopic analysis. Only the galactose unit and the contiguous Man residue make contact with the lectin. B) Structural model of the complex of VAA (PDB code: 1PUM, Tyr site) with the global-minimum conformation of **2** (*gt* rotamer around the α-Gal*p*-(1→6)-β-Man*p* linkage). Important steric clashes between the ligand and lectin take place, thus precluding an interaction.

cantly smaller STD values were measured for the mannopyranosyl residue closest to the galactose moiety. The STD signals observed were similar when the saturation was set in the aromatic and aliphatic regions. These experimental observations suggest that the binding mode of **2** mainly involves the terminal galactose unit. To translate this experimental information into a 3D structural model, a docking protocol was applied.[41]

A model of the molecular-recognition mode was then obtained through a simple docking protocol.[39] The deposited structure of VAA in the Protein Data Bank (PDB) was employed as a template for the protein, whereas both possible conformers of the ligand, with either the *gg* or *gt* geometries around the α-(1→6)-glycosidic linkage, were used for the ligand. Because the STD experiments had unequivocally demonstrated that the major epitope of **2** involved the galactose residue, manual docking of the terminal galactose on the preferred Tyr249 site[42] was performed. The nonreducing galactose residue of the bound lactose in the crystallographic structure (PDB code: 1PUM) was employed as a template for the manual docking. Interestingly, the *gg* rotamer led to a complex (Figure 8A). Besides major contacts between the galactose unit and lectin, proximity to VAA was also found for the β-Man*p* residue. In contrast, the reducing end in the *gt* rotamer will make major steric clashes with VAA (Figure 8B). Therefore, the modeling results suggest a conformational-selection process, with exclusive binding of only one of the two conformers present in solution (the *gg* rotamer around the (α-Gal*p*-(1→6)-β-Man*p*) linkage on binding of **2** to VAA).

Conclusion

Fundamental studies related to the nature of the GGM polysaccharide found in *P. abies* have been described. In detail, a synthetic route to one of the trisaccharide repeating units and a less-abundant tetrasaccharide fragment has been devised. During the development of the synthetic route, several methodologies for the selective ring-opening reaction of benzylidene acetals[11,12,17–20] and α-galactosylations[23,24] were evaluated. On the basis of our findings, several of the previously reported procedures for various reactions proceeded with less than satisfactory efficiency in the current synthesis. Nevertheless, high-yielding procedures were found, such as the partial cleavage of benzylidene acetal moieties by using the methodology reported by Shie et al.[20] and the α-galactosylation protocol reported by Ando and co-workers.[22b,c,25]

In addition to the synthesis of the GGM backbone structures, a few model substrates were prepared to study the acetyl-group migration patterns reported for GGM. The acetyl-group migration from O2 to O6 was not observed in these compounds, thus suggesting that similar migrations do not take place in mannopyranosides in the $^4C_1$ conformation.

With the backbone structures synthesized, a short comparison between the NMR chemical shifts of the model compounds and the GGM polymer was performed. Although many of the chemical shifts were in good agreement with each other, several uncertainties were also found by the use of the small molecular models. The data presented herein can prove valuable in the future as a reference when analyzing the NMR spectra of the GGM polysaccharide.

New applications for the GGM polymer are being developed, including those from biomedical perspectives. Consequently, a brief conformation analysis of the model compounds and a binding study between a lectin (viscumin) and tetrasaccharide **2** was conducted. Binding was confirmed by STD and docking experiments. Furthermore, the existence of a conformational-selection process, with exclusive binding of only one of the two major conformers present in solution, namely, the *gg* conformer, was deduced. Obviously, GGM can have the potential to protect cells from the toxicity of this potential biohazard.

Experimental Section

Reaction solvents were dried and distilled prior to use when necessary. All the reactions containing moisture- or air-sensitive reagents were carried out under argon. The NMR spectra were recorded on a Bruker Avance NMR spectrometer operating at 600.13 MHz (^{1}H: 600.13 MHz, ^{13}C: 150.90 MHz). The probe temperature during the experiments was kept at 25 °C, unless indicated otherwise. All the products were fully characterized by using the following 1D techniques ^{1}H, ^{13}C, and TOCSY in combination with the following 2D techniques DQF-COSY, NOESY, HSQC, and HMBC by using pulse sequences provided by the manufacturer. Chemical shifts are expressed on the δ scale (in ppm) using tetramethylsilane (TMS) or residual chloroform or methanol as internal standards. Coupling constants are given in Hz and provided only once, when first encountered. Coupling patterns are given as singlet (s), doublet (d), triplet (t), and so forth. The computational analysis of the ^{1}H NMR spectroscopic analysis of all the compounds was achieved by using the PERCH NMR software with starting values and spectral parameters obtained from the various NMR spectroscopic techniques used. HRMS was recorded using Bruker Micro Q-TOF with ESI operated in the positive mode. Optical rotations were measured at 23 °C, unless otherwise stated, with a PerkinElmer polarimeter equipped with a Na lamp (λ = 589 nm). TLC analysis was performed on aluminum sheets precoated with silica gel 60 F254 (Merck). Flash chromatography was carried out on silica gel 60 (0.040–0.060 mm; Merck). Spots were visualized by UV radiation followed by charring with H_2SO_4/MeOH (1:4) and heating.

Thiophenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-2,3-di-*O*-acetyl-6-*O*-benzyl-β-D-glucopyranoside (13): BSP (54 mg, 0.26 mmol, 1.2 equiv), TTBP (80 mg, 0.32 mmol, 1.5 equiv), and Tf_2O (45 μL, 0.28 mmol, 1.3 equiv) were added to a solution of donor **3** (116 mg, 0.24 mmol) in dry dichloromethane (2.5 mL) at −60 °C (acetone + dry ice). The resulting mixture was stirred for 0.5 h followed by cooling to −78 °C and addition of acceptor **12** (110 mg, 0.25 mmol, 1.15 equiv) dissolved in dichloromethane (1.5 mL) over a period of 15 min. The reaction mixture was stirred for 2 h and quenched by the addition of triethylphosphite (170 μL). The reaction mixture was stirred for 1 h at −78 °C, brought to RT, diluted with dichloromethane (30 mL), and washed with saturated aqueous $NaHCO_3$. The water phase was separated and extracted with dichloromethane (3×20 mL). The combined organic phases were washed with brine (30 mL), dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography (hexane/EtOAc 2:1), thus providing **13** as a white foam (100 mg, 53 %). R_f = 0.54 (hexane/EtOAc 3:2); $[\alpha]_D$ = −46.2 (c = 0.2 in $CHCl_3$); ^{1}H NMR (600.13 MHz, $CDCl_3$): δ = 7.51–7.24 (m, 25 H; arom. *H*), 5.55 (s, 1 H; C*H*′Ph), 5.23 (dd, $J_{3,2}$ = 9.3, $J_{3,4}$ = 9.4 Hz, 1 H; H3), 4.92 (dd, $J_{2,1}$ = 10.1 Hz, 1 H; H2), 4.78 and 4.67 (each d, J = −12.1 Hz, each 1 H; 2′-CH_2Ph), 4.71 and 4.57 (each d, J = −12.4 Hz, each 1 H; 3′-CH_2Ph), 4.68 (d, 1 H; H1), 4.57 and 4.38 (each d, J = −11.9 Hz, each 1 H; 6-CH_2Ph), 4.38 (d, $J_{1',2'}$ = 1.0 Hz, 1 H; H1′), 4.26 (dd, $J_{6'a,5'}$ = 4.8, $J_{6'a,6'b}$ = −10.2 Hz, 1 H; H6′a), 4.07 (dd, $J_{4',5'}$ = 9.3, $J_{4',3'}$ = 9.8 Hz, 1 H; H4′), 3.89 (dd, $J_{4,5}$ = 9.9 Hz, 1 H; H4), 3.78 (dd, $J_{6'b,5'}$ = 10.0 Hz, 1 H; H6′b), 3.68 (dd, $J_{6a,5}$ = 1.9, $J_{6a,6b}$ = −11.2 Hz, 1 H; H6a), 3.65 (dd, $J_{2',3'}$ = 3.1 Hz, 1 H; H2′), 3.52 (dd, $J_{6b,5}$ = 3.5 Hz, 1 H; H6b), 3.46 (ddd, 1 H; H5), 3.40 (dd, 1 H; H3′), 3.13 (ddd, 1 H; H5′), 2.08 (s, 3 H; 2-OCOCH_3), 2.08 ppm (s, 3 H; 3-OCOCH_3); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 170.0 (3-O*C*OCH$_3$), 169.5 (2-O*C*OCH$_3$), 138.6–126.0 (arom. *C*), 101.9 ($^1J_{C1',H1'}$ = 157.4 Hz; C1′), 101.4 (*C*′HPh), 85.6 ($^1J_{C-1,H-1}$ = 157.1 Hz; C1), 78.9 (C5), 78.6 (C4′), 78.0 (C3′), 76.4 (C2′), 75.3 (C4), 74.6 (2′-*C*H$_2$Ph), 74.2 (C3), 73.6 (6-*C*H$_2$Ph), 72.4 (3′-*C*H$_2$Ph), 70.2 (C2), 68.6 (C6′), 68.3 (C6), 67.4 (C5′), 20.9 (3-OCO*C*H$_3$), 20.8 ppm (2-OCO*C*H$_3$); HRMS: *m*/*z* calcd for $C_{50}H_{52}O_{12}SNa$: 899.3077 $[M+Na]^+$; found: 899.3080.

Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-2,3-di-*O*-acetyl-6-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (14): Preactivated molecular sieves (4 Å) were added to a solution of donor **13** (81 mg, 0.09 mmol, 1.2 equiv) and acceptor **4** (36 mg, 0.08 mmol) in dry dichloromethane (2.5 mL), and the reaction mixture was cooled to −40 °C. NIS (21 mg, 0.09 mmol, 1.2 equiv) and TMSOTf (1.1 μL, 0.006 mmol, 0.06 equiv) were added to the solution, and the resulting mixture was stirred for 1.5 h. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$ solution, brought to RT, diluted with dichloromethane (30 mL), and washed with saturated aqueous $NaHCO_3$ solution (20 mL). The organic phase was separated, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography (hexane/EtOAc 3:2), thus providing **14** as a white foam (73 mg, 77 %). R_f = 0.39 (hexane/EtOAc 3:2); $[\alpha]_D$ = −11.0 (c = 0.1 in $CHCl_3$); ^{1}H NMR (600.13 MHz, $CDCl_3$): δ = 7.47–7.13 (m, 35 H; arom. *H*), 5.54 (s, 1 H; C*H*″Ph), 5.06 (dd, $J_{3',4'}$ = 9.3, $J_{3',2'}$ = 9.7 Hz, 1 H; H3′), 4.86 (dd, $J_{2',1'}$ = 8.1 Hz, 1 H; H2′), 4.77 and 4.63 (each d, J = −12.1 Hz, each 1 H; 2″-CH_2Ph), 4.77 and 4.51 (each d, J = −11.9 Hz, each 1 H; 6-CH_2Ph), 4.76 and 4.57 (each d, J = −12.5 Hz, each 1 H; 3-CH_2Ph), 4.73 (d, $J_{1,2}$ = 1.9 Hz, 1 H; H1), 4.72 and 4.67 (each d, J = −12.4 Hz, each 1 H; 2-CH_2Ph), 4.65 and 4.51 (each d, J = −12.4 Hz, each 1 H; 3″-CH_2Ph), 4.65 (d, 1 H; H1′), 4.41 and 4.20 (each d, J = −12.0 Hz, each 1 H; 6′-CH_2Ph), 4.33 (s, 1 H; H1″), 4.25 (dd, $J_{6''a,5''}$ = 4.9, $J_{6''a,6''b}$ = −10.3 Hz, 1 H; H6″a), 4.24 (dd, $J_{4,3}$ = 9.0, $J_{4,5}$ = 9.7 Hz, 1 H; H4), 4.05 (dd, $J_{4'',5''}$ = 9.3, $J_{4'',3''}$ = 9.8 Hz, 1 H; H4″), 3.87 (dd, $J_{4',5'}$ = 9.8 Hz, 1 H; H4′), 3.83 (dd, $J_{3,2}$ = 3.2 Hz, 1 H; H3), 3.79 (dd, $J_{6a,5}$ = 4.6, $J_{6a,6b}$ = −11.0 Hz, 1 H; H6a), 3.77 (dd, $J_{6''b,5''}$ = 10.0 Hz, 1 H; H6″b), 3.74 (dd, 1 H; H2), 3.69 (dd, $J_{6b,5}$ = 1.9 Hz, 1 H; H6b), 3.66 (ddd, 1 H; H5), 3.59 (d, $J_{2'',3''}$ = 3.1 Hz, 1 H; H2″), 3.40 (dd, $J_{6'a,5'}$ = 2.1, $J_{6'a,6'b}$ = −11.3 Hz, 1 H; H6′a), 3.33 (dd, 1 H; H3″), 3.31 (s, 3 H, OCH_3), 3.28 (dd, $J_{6'b,5'}$ = 3.2 Hz, 1 H; H6′b), 3.11 (ddd, 1 H; H5′), 3.09 (dd, 1 H; H5″), 1.99 (s, 3 H; 3′-OCOCH_3), 1.93 ppm (s, 3 H; 2′-OCOCH_3); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 170.0 (3′-O*C*OCH$_3$), 169.7 (2′-O*C*OCH$_3$), 139.3–126.0 (arom. *C*), 102.1 (C1″), 101.3 (*C*″HPh), 100.6 (C1′), 99.2 (C1), 78.5 (C4″), 78.4 (C3), 77.9 (C3″), 76.3 (C2″), 75.8 (C4′), 75.3 (C4), 75.2 (C2), 74.6 (2″-*C*H$_2$Ph), 74.4 (C5′), 73.6 (6-*C*H$_2$Ph), 73.4 (6′-*C*H$_2$Ph), 73.4 (C3′), 73.0 (2-*C*H$_2$Ph), 72.5 (C2′), 72.3 (3″-*C*H$_2$Ph, 3-*C*H$_2$Ph), 71.4 (C5), 68.6 (C6″, C6), 68.0 (C6′), 67.4 (C5″), 54.8 (O*C*H$_3$), 21.0 (3′-OCO*C*H$_3$), 20.7 ppm (2′-OCO*C*H$_3$); HRMS: *m*/*z* calcd for $C_{72}H_{78}O_{18}Na$: 1253.5086 $[M+Na]^+$; found: 1253.5080.

Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-6-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside: NaOMe (2 mg, 0.03 mmol,1 equiv) was added to a solution of **14** (33 mg, 0.027 mmol) in MeOH/THF (1:1, 3 mL), and the resulting mixture was stirred at RT for 19 h. The reaction mixture was neutralized with DOWEX 50 H^+-form, filtered, and concentrated. The crude product was purified by column chromatography (hexane/EtOAc 1:1) to give a white foam (26 mg, 84 %). R_f = 0.40 (hexane/EtOAc 1:1); $[\alpha]_D$ = +6.8 (c = 1.0 in $CHCl_3$); ^{1}H NMR (600.13 MHz, $CDCl_3$): δ = 7.48–7.12 (m, 35 H; arom. *H*), 5.58 (s, 1 H; CH″Ph), 4.76 and 4.56 (each d, J = −12.2 Hz, each 1 H; 3″-CH_2Ph), 4.75 and 4.70 (each d, J = −12.2 Hz, each 1 H; 2″-CH_2Ph), 4.75 and 4.61 (each d, J = −12.3 Hz, each 1 H; 3-CH_2Ph), 4.73 and 4.63 (each d, J = −12.3 Hz, each 1 H; 6-CH_2Ph), 4.72 (d, $J_{1,2}$ = 1.9 Hz, 1 H; H1), 4.71 and 4.65 (each d, J = −12.4 Hz, each 1 H; 2-CH_2Ph), 4.59 (d, $J_{1'2'}$ = 7.9 Hz, 1 H; H1′), 4.44 and 4.09 (each d, J = −12.1 Hz, each 1 H; 6′-CH_2Ph), 4.36 (dd, $J_{4,3}$ = 9.2, $J_{4,5}$ = 9.6 Hz, 1 H; H4), 4.28 (dd, $J_{6''a,5''}$ = 5.0, $J_{6''a,6''b}$ = −10.4 Hz, 1 H; H6″a), 4.26 (s, 1 H; H1″), 4.15 (dd, $J_{4'',5''}$ = 9.0, $J_{4'',3''}$ = 9.9 Hz, 1 H; H4″), 3.96 (dd, $J_{6a,5}$ = 4.2, $J_{6a,6b}$ = −11.5 Hz, 1 H; H6a), 3.90 (dd, $J_{3,2}$ = 3.1 Hz, 1 H; H3), 3.88 (dd, $J_{6''b,5''}$ = 10.1 Hz, 1 H; H6″b), 3.78 (dd, $J_{6b,5}$ = 2.5 Hz, 1 H; H6b), 3.77 (ddd, 1 H; H5), 3.73 (dd, 1 H; H2), 3.54 (dd, $J_{4',3'}$ = 8.7, $J_{4',5'}$ = 9.6 Hz, 1 H; H4′), 3.52 (dd, $J_{3',2'}$ = 9.3 Hz, 1 H; H3′), 3.48 (d, $J_{2'',3''}$ = 3.0 Hz, 1 H; H2″), 3.39 (dd, 1 H; H3″), 3.38 (dd, 1 H; H2′), 3.29 (s, 3 H, OCH_3), 3.29 (ddd, 1 H; H5″), 3.19 (dd, $J_{6'a,5'}$ = 2.0, $J_{6'a,6'b}$ = −10.8 Hz, 1 H; H6′a), 3.11 (dd, $J_{6'b,5'}$ = 3.4 Hz, 1 H; H6′b), 3.11 ppm (dd, 1 H; H5′); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 139.0–126.0 (arom. *C*), 103.4 (C1′), 102.7 (C1″), 101.4 (C″HPh), 99.1 (C1), 81.3 (C4′), 79.1 (C3), 78.4 (C4″), 78.3 (C3″), 75.7 (C2″), 75.4 (C4), 74.9 (C2), 74.8 (2″-O*C*H$_2$Ph), 74.4 (C3′), 74.1 (C2′), 73.8 (C5′), 73.4 (6-O*C*H$_2$Ph), 73.3 (6′-O*C*H$_2$Ph), 73.0 (3″-O*C*H$_2$Ph), 72.8(2-O*C*H$_2$Ph), 71.9 (3-O*C*H$_2$Ph), 70.9 (C5), 69.2 (C6), 68.0 (C6″), 67.9 (C6′), 67.4 (C5″), 54.8 ppm (O*C*H$_3$); HRMS: *m*/*z* calcd for $C_{68}H_{74}O_{16}Na$: 1169.4875 $[M+Na]^+$; found: 1169.4862.

Methyl β-D-mannopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-α-D-mannopyranoside (1): Pd/C (10 % Pd, 59 mg, 2.5 weight equiv) was added to a solution of methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-6-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (26 mg, 0.023 mmol) in dry MeOH (3 mL). The reac-

tion mixture was placed inside a reactor and the H_2 pressure was set to 2.8 bar (40 psi). After 19 h, the reaction mixture was diluted with MeOH (3 mL), filtered through celite, and concentrated to give **1** as a white solid (11 mg, quant.). $[\alpha]_D = +11.1$ ($c = 1.0$ in MeOH); ^{1}H NMR (600.13 MHz, D_2O, 35 °C): $\delta = 4.76$ (d, $J_{1,2} = 1.8$ Hz, 1H; H1), 4.74 (d, $J_{1'',2''} = 1.0$ Hz, 1H; H1″), 4.52 (d, $J_{1',2'} = 8.0$ Hz, 1H; H1′), 4.05 (dd, $J_{2'',3''} = 3.3$ Hz, 1H; H2″), 3.99 (dd, $J_{2,3} = 3.3$ Hz, 1H; H2), 3.95 (dd, $J_{6a,5} = 2.3$, $J_{6a,6b} = -12.3$ Hz, 1H; H6a), 3.93 (dd, $J_{6a,5''} = 2.3$, $J_{6''a,6''b} = -12.3$ Hz, 1H; H6″a)), 3.90 (dd, $J_{6'a,5'} = 2.2$, $J_{6'a,6'b} = -12.4$ Hz, 1H; H6′a), 3.86 (dd, $J_{3,4} = 9.2$ Hz, 1H; H3), 3.85 (dd, $J_{4,5} = 9.7$ Hz, 1H; H4), 3.85 (dd, $J_{6b,5} = 5.5$ Hz, 1H; H6b), 3.73 (dd, $J_{6'b,5'} = 5.2$ Hz, 1H; H6′b), 3.73 (dd, $J_{6''b,5''} = 6.4$ Hz, 1H; H6″b), 3.73 (ddd, 1H; H5), 3.69 (dd, $J_{3',4'} = 8.8$, $J_{3',2'} = 9.7$ Hz, 1H; H3′), 3.68 (dd, $J_{4',5'} = 9.7$ Hz, 1H; H4′), 3.64 (dd, $J_{3'',4''} = 9.7$ Hz, 1H; H3″), 3.61 (ddd, 1H; H5′), 3.58 (dd, $J_{4'',5''} = 9.8$ Hz, 1H; H4″), 3.41 (ddd, 1H; H5″), 3.40 (s, 3H, OCH_3), 3.35 ppm (dd, 1H; H2′); ^{13}C NMR (150.9 MHz, D_2O, 35 °C): $\delta = 103.0$ ($^1J_{C1',H1'} = 163.5$ Hz; C1′), 101.2 ($^1J_{C1,H1} = 173.4$ Hz; C1), 100.6 ($^1J_{C1'',H1''} = 160.8$ Hz; C1″), 79.2 (C4′), 77.2 (C4), 77.0 (C5″), 75.3 (C5′), 74.6 (C3′), 73.5 (C2′), 73.4 (C3″), 71.8 (C5), 71.1 (C2″), 70.1 (C2), 69.8 (C3), 67.3 (C4″), 61.5 (C6″), 60.9 (C6), 60.8 (C6′), 55.4 ppm (OCH_3); HRMS: m/z calcd for $C_{19}H_{34}O_{16}Na$: 541.1745 $[M+Na]^+$; found: 541.1744.

Methyl 2,3,4-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3-di-*O*-acetyl-6-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (16): Preactivated molecular sieves (4 Å) and borane/THF complex in THF (0.28 mL, 0.28 mmol, 5 equiv) were added to a solution of **14** (70 mg, 0.06 mmol) in dry dichloromethane (3 mL). The resulting mixture was stirred for 10 min, and $Cu(OTf)_2$ (1.4 mg, 0.004 mmol, 0.07 equiv) was added with further stirring for 3 h. The reaction mixture was cooled to 0 °C, and the reaction was quenched by the addition of Et_3N and MeOH. The resulting mixture was concentrated to give the crude product, which was purified by column chromatography (hexane/EtOAc 3:2→1:1) to give the product as a colorless oil (60 mg, 85 %). $R_f = 0.52$ (hexane/EtOAc 1:1); $[\alpha]_D = -11.5$ ($c = 0.1$ in $CHCl_3$); ^{1}H NMR (600.13 MHz, $CDCl_3$): $\delta = 7.37$–7.15 (m, 35H; arom. *H*), 5.08 (dd, $J_{3',4'} = 9.3$, $J_{3',2'} = 9.7$ Hz, 1H; H3′), 4.88 (dd, $J_{2',1'} = 8.0$ Hz, 1H; H2′), 4.88 and 4.57 (each d, $J = -11.6$ Hz, each 1H; 3-CH_2Ph), 4.77 and 4.51 (each d, $J = -11.9$ Hz, each 1H; 6-CH_2Ph), 4.76 and 4.62 (each d, $J = -12.5$ Hz, each 1H; 2″-CH_2Ph), 4.76 and 4.56 (each d, $J = -11.1$ Hz, each 1H; 4″-CH_2Ph), 4.72 (d, $J_{1,2} = 1.9$ Hz, 1H; H1), 4.70 and 4.66 (each d, $J = -12.4$ Hz, each 1H; 2-CH_2Ph), 4.64 (d, 1H; H1′), 4.42 and 4.38 (each d, $J = -11.8$ Hz, each 1H; 3″-CH_2Ph), 4.40 and 4.29 (each d, $J = -12.1$ Hz, each 1H; 6′-CH_2Ph), 4.38 (s, 1H; H1″), 4.24 (dd, $J_{4,5} = 9.0$, $J_{4,3} = 10.0$ Hz, 1H; H4), 3.86 (dd, $J_{4',5'} = 9.8$ Hz, 1H; H4′), 3.82 (dd, $J_{6a,5} = 4.3$, $J_{6a,6b} = -11.0$ Hz, 1H; H6a), 3.82 (dd, $J_{6''a,5''} = 2.9$, $J_{6''a,6''b} = -9.9$ Hz, 1H; H6″a), 3.79 (dd, $J_{3,2} = 3.6$ Hz, 1H; H3), 3.74 (dd, $J_{4'',3''} = 9.3$, $J_{4'',5''} = 9.5$ Hz, 1H; H4″), 3.72 (dd, 1H; H2), 3.68 (dd, $J_{6b,5} = 1.8$ Hz, 1H; H6b), 3.66 (ddd, 1H; H5), 3.64 (dd, $J_{6''b,5''} = 5.8$ Hz, 1H; H6″b), 3.61 (d, $J_{2'',3''} = 2.9$ Hz, 1H; H2″), 3.51 (dd, $J_{6'a,5'} = 2.1$, $J_{6'a,6'b} = -11.4$ Hz, 1H; H6′a), 3.34 (dd, $J_{6'b,5'} = 3.5$ Hz, 1H; H6′b), 3.31 (s, 3H, OCH_3), 3.31 (dd, 1H; H3″), 3.23 (ddd, 1H; H5′), 3.19 (ddd, 1H; H5″), 2.00 (s, 3H; 3′-OCOCH_3), 1.93 ppm (s, 3H; 2′-OCOCH_3); ^{13}C NMR (150.9 MHz, $CDCl_3$): $\delta = 170.4$ (3′-O*C*OCH$_3$), 169.7 (2′-O*C*OCH$_3$), 139.3–127.1 (arom. *C*), 100.8 (C1″), 100.7 (C1′), 99.4 (C1), 82.3 (C3″), 78.5 (C3), 76.0 (C5″), 75.4 (C4, C2), 75.3 (3-OCH_2Ph), 75.2 (C4′), 74.9 (C4″), 74.8 (C5′), 74.4 (C2″), 74.0 (2″-OCH_2Ph), 73.8 (6-OCH_2Ph), 73.6 (6′-OCH_2Ph), 73.5 (C3′), 72.9 (2-OCH_2Ph), 72.6 (C2′, 4″-OCH_2Ph), 71.6 (C5), 71.5 (3″-OCH_2Ph), 68.7 (C6), 68.5 (C6′), 62.8 (C6″), 55.0 (OCH_3), 21.0 (3′-OCO*C*H$_3$), 20.9 ppm (2′-OCO*C*H$_3$); HRMS: m/z calcd for $C_{72}H_{80}O_{18}Na$: 1255.5242 $[M+Na]^+$; found: 1255.5249.

Methyl 2,3-di-*O*-benzoyl-4,6-*O*-di-tertbutylsilylene-α-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3-di-*O*-acetyl-6-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (19): Preactivated molecular sieves (4 Å) were added to a mixture containing acceptor **16** (21 mg, 0.017 mmol) and donor **18** (13 mg, 0.020 mmol, 1.2 equiv) in dry dichloromethane (2.5 mL), and the resulting mixture was cooled to 0 °C. NIS (4.5 mg, 0.020 mmol, 1.2 equiv) and TMSOTf (0.2 μL, 0.001 mmol, 0.06 equiv) were added after 10 minutes to the reaction mixture, which was stirred for 1.5 h, brought to RT, quenched with saturated aqueous $NaHCO_3$ solution, diluted with dichloromethane (30 mL), and washed with saturated aqueous $NaHCO_3$ solution (20 mL). The organic phase was separated, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography (hexane/EtOAc 3:2), thus providing the product as a colorless oil (20 mg, 69 %). $R_f = 0.52$ (hexane/EtOAc 3:2); $[\alpha]_D = +24.9$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (600.13 MHz, $CDCl_3$): $\delta = 8.00$–7.10 (m, 45H; arom. *H*), 5.73 (dd, $J_{2''',1'''} = 3.7$, $J_{2''',3'''} = 10.6$ Hz, 1H; H2‴), 5.54 (dd, $J_{3''',4'''} = 3.3$ Hz, 1H; H3‴), 5.28 (d, 1H; H1‴), 5.06 (dd, $J_{3',4'} = 9.3$, $J_{3',2'} = 9.7$ Hz, 1H; H3′), 4.96 and 4.66 (each d, $J = -11.9$ Hz, each 1H; 2″-CH_2Ph), 4.85 (dd, $J_{2',1'} = 8.0$ Hz, 1H; H2′), 4.77 and 4.56 (each d, $J = -12.4$ Hz, each 1H; 3-CH_2Ph), 4.74 and 4.48 (each d, $J = -11.9$ Hz, each 1H; 6-CH_2Ph), 4.72 (d, $J_{1,2} = 2.0$ Hz, 1H; H1), 4.71 and 4.65 (each d, $J = -12.1$ Hz, each 1H; 2-CH_2Ph), 4.67 and 4.50 (each d, $J = -11.8$ Hz, each 1H; 4″-CH_2Ph), 4.67 (dd, $J_{4''',5'''} = 1.0$ Hz, 1H; H4‴), 4.62 (d, 1H; H1′), 4.37 and 4.25 (each d, $J = -12.1$ Hz, each 1H; 6′-CH_2Ph), 4.35 and 4.30 (each d, $J = -11.8$ Hz, each 1H; 3″-CH_2Ph), 4.25 (d, $J_{1'',2''} = 0.5$ Hz, 1H; H1″), 4.22 (dd, $J_{4,3} = 8.9$, $J_{4,5} = 9.6$ Hz, 1H; H4), 4.09 (dd, $J_{6'''a,5'''} = 1.6$, $J_{6'''a,6'''b} = -12.9$ Hz, 1H; H6‴a), 3.89 (dd, $J_{6a,5''} = 1.9$, $J_{6''a,6''b} = -11.7$ Hz, 1H; H6″a), 3.86 (dd, $J_{4'',3''} = 9.1$, $J_{4'',5''} = 9.5$ Hz, 1H; H4″), 3.85 (dd, $J_{6'''b,5'''} = 1.4$ Hz, 1H; H6‴b), 3.83 (ddd, 1H; H5‴), 3.82 (dd, $J_{3,2} = 3.1$ Hz, 1H; H3), 3.81 (dd, $J_{6''b,5''} = 4.3$ Hz, 1H; H6″b), 3.78 (dd, $J_{6a,5} = 4.6$, $J_{6a,6b} = -10.9$ Hz, 1H; H6a), 3.74 (dd, $J_{4',5'} = 9.7$ Hz, 1H; H4′), 3.72 (dd, 1H; H2), 3.69 (dd, $J_{6b,5} = 1.9$ Hz, 1H; H6b), 3.66 (ddd, 1H; H5), 3.57 (dd, $J_{2'',3''} = 3.0$ Hz, 1H; H2″), 3.42 (dd, $J_{6'a,5'} = 2.0$, $J_{6'a,6'b} = -11.7$ Hz, 1H; H6′a), 3.31 (s, 3H, OCH_3), 3.30 (dd, $J_{6'b,5'} = 4.2$ Hz, 1H; H6′b), 3.23 (dd, 1H; H3″), 3.18 (ddd, 1H; H5″), 3.14 (ddd, 1H; H5′), 2.10 (s, 3H; 3′-OCOCH_3), 1.93 (s, 3H; 2′-OCOCH_3), 1.10 and 0.92 ppm (each s, each 9H; C(CH_3)$_3$); ^{13}C NMR (150.9 MHz, $CDCl_3$): $\delta = 170.4$ (3′-O*C*OCH$_3$), 169.5 (2′-O*C*OCH$_3$), 166.1 (2‴-O*C*OPh), 165.8 (3‴-O*C*OPh), 139.3–126.9 (arom. *C*), 101.6 (C1″), 100.7 (C1′), 99.2 (C1), 98.0 (C1‴), 82.2 (C3″), 78.4 (C3), 75.8 (C4′), 75.4 (C2), 75.3 (C4, C5″), 74.6 (2″-OCH_2Ph, C5′), 74.2 (C4″), 73.9 (C2″), 73.6 (4″-OCH_2Ph), 73.5 (6-OCH_2Ph), 73.4 (6′-OCH_2Ph), 73.1 (C3‴), 72.8 (2-OCH_2Ph), 72.4 (C2′, 3-OCH_2Ph), 71.4 (C5), 71.1 (C4‴), 71.0 (3″-OCH_2Ph, C3‴), 68.9 (C6″), 68.8 (C2‴), 68.6 (C6), 68.3 (C6′), 67.0 (C5‴), 66.8 (C6‴), 54.8 (OCH_3), 27.5 and 27.3 (C(*C*H$_3$)$_3$), 23.1 and 20.8 (*C*(CH$_3$)$_3$), 20.8 (3′-OCO*C*H$_3$), 20.7 ppm (2′-OCO*C*H$_3$); HRMS: m/z calcd for $C_{100}H_{114}O_{25}SiNa$: 1765.7316 $[M+Na]^+$; found: 1765.7266.

Methyl 2,3-di-*O*-benzoyl-α-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3-di-*O*-acetyl-6-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (20): The HF/pyridine complex (18 μL) was added to a solution of **19** (50 mg, 0.03 mmol) in dry THF (3 mL) at 0 °C, and the resulting mixture was brought to RT and stirred for 19 h. The reaction mixture was diluted with dichloromethane (30 mL) and quenched by the addition of saturated aqueous $NaHCO_3$ solution. The organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography (hexane/EtOAc 1:1) to give the product as a colorless oil (31 mg, 68 %). $R_f = 0.26$ (hexane/EtOAc 1:2); $[\alpha]_D = +20.8$ ($c = 0.8$ in $CHCl_3$); ^{1}H NMR (600.13 MHz, $CDCl_3$): $\delta = 8.02$–7.18 (m, 45H; arom. *H*), 5.72 (dd, $J_{2''',1'''} = 3.8$, $J_{2''',3'''} = 10.5$ Hz, 1H; H2‴), 5.71 (d, 1H; H1‴), 5.65 (dd, $J_{3''',4'''} = 3.2$ Hz, 1H; H3‴), 5.12 (dd, $J_{3',4'} = 9.5$, $J_{3',2'} = 9.8$ Hz, 1H; H3′), 5.09 (dd, $J_{2',1'} = 8.2$ Hz, 1H; H2′), 4.81 and 4.51 (each d, $J = -11.1$ Hz, each 1H; 4″-CH_2Ph), 4.75 and 4.52 (each d, $J = -11.9$ Hz, each 1H; 6-CH_2Ph), 4.73 (d, $J_{1,2} = 2.0$ Hz, 1H; H1), 4.73 and 4.56 (each d, $J = -12.5$ Hz, each 1H; 3-CH_2Ph), 4.71 (d, 1H; H1′), 4.70 and 4.47 (each d, $J = -12.1$ Hz, each 1H; 2″-CH_2Ph), 4.69 and 4.65 (each d, $J = -12.4$ Hz, each 1H; 2-CH_2Ph), 4.38 and 4.22 (each d, $J = -12.1$ Hz, each 1H; 6′-CH_2Ph), 4.34 and 4.30 (each d, $J = -11.8$ Hz, each 1H; 3″-CH_2Ph), 4.29 (dd, $J_{4''',5'''} = 1.2$ Hz, 1H; H4‴), 4.25 (s, 1H; H1″), 4.21 (dd, $J_{4,3} = 8.8$, $J_{4,5} = 9.8$ Hz, 1H; H4), 4.19 (dd, $J_{4',5'} = 9.7$ Hz, 1H; H4′), 4.10 (ddd, $J_{5''',6'''b} = 3.1$, $J_{5''',6'''a} = 6.3$ Hz, 1H; H5‴), 4.05 (dd, $J_{6'a,5''} = 1.7$, $J_{6''a,6''b} = -12.5$ Hz, 1H; H6″a), 3.89 (dd, $J_{6'''a,6'''b} = -12.4$ Hz, 1H; H6‴a), 3.83 (dd, $J_{3,2} = 3.2$ Hz, 1H; H3), 3.80 (dd, $J_{6a,5} = 4.6$, $J_{6a,6b} = -11.1$ Hz, 1H; H6a), 3.71 (dd, 1H; H2), 3.70 (dd, 1H; H6‴b), 3.69 (dd, $J_{6b,5} = 1.8$ Hz, 1H; H6b), 3.66 (ddd, 1H; H5), 3.64 (dd, $J_{4'',3''} = 9.1$, $J_{4'',5''} = 9.5$ Hz, 1H; H4″), 3.62 (dd, $J_{6''b,5''} = 6.1$ Hz, 1H; H6″b), 3.55 (d, $J_{2'',3''} = 3.0$ Hz, 1H; H2″), 3.38 (dd, $J_{6'a,5'} = 2.4$, $J_{6'a,6'b} = -11.0$ Hz, 1H; H6′a), 3.33 (dd, $J_{6'b,5'} = 2.9$ Hz, 1H; H6′b), 3.32 (s, 3H, OCH_3), 3.31 (ddd, 1H; H5″), 3.23 (dd, 1H; H3″), 3.19 (ddd, 1H; H5′), 2.08 (s, 3H; 3′-OCOCH_3), 2.00 ppm (s, 3H; 2′-OCOCH_3); ^{13}C NMR

(150.9 MHz, $CDCl_3$): δ = 170.9 (3′-O*C*OCH$_3$), 170.0 (2′-O*C*OCH$_3$), 166.0 (3‴-O*C*OPh), 165.8 (2‴-O*C*OPh), 139.3–126.7 (arom. *C*), 101.0 ($^1J_{C1'',H1''}$ = 155.8 Hz; C1″; $^1J_{C1',H1'}$ = 162.1 Hz; C1′), 99.1 ($^1J_{C1,H1}$ = 168.0 Hz; C1), 97.6 ($^1J_{C1''',H1'''}$ = 173.5 Hz; C1‴), 82.1 (C3″), 78.4 (C3), 76.5 (C5″), 75.7 (C4), 74.8 (4″-O*C*H$_2$Ph), 74.3 (C4″, C5′), 73.9 (C4), 73.6 (C2″, 2″-O*C*H$_2$Ph, 6-O*C*H$_2$Ph), 73.4 (6′-O*C*H$_2$Ph), 72.8 (C3′), 72.7 (2-O*C*H$_2$Ph), 72.3 (C2′), 72.0 (3-O*C*H$_2$Ph), 71.3 (C5, C3‴), 71.1 (C5‴), 71.0 (3″-O*C*H$_2$Ph), 69.8 (C4‴), 69.3 (C2‴), 69.0 (C6″), 68.7 (C6), 67.7 (C6′), 63.2 (C6‴), 54.8 (O*C*H$_3$), 20.9 (3′-OCO*C*H$_3$), 20.8 ppm (2′-OCO*C*H$_3$); HRMS: *m*/*z* calcd for $C_{92}H_{98}O_{25}Na$: $[M+Na]^+$ 1625.6295; found: 1625.6266.

Methyl α-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-6-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside: NaOMe (3 mg, 2.4 equiv) was added to a solution of **20** (33 mg, 0.02 mmol) in MeOH/THF (1:1, 4 mL), and the resulting mixture was stirred at RT for 19 h. The reaction mixture was neutralized with DOWEX 50 H^+-form, filtered, and concentrated. The crude product was purified by column chromatography (MeOH/dichloromethane 1:10) to give a white foam (26 mg, quant.). R_f = 0.38 (MeOH/dichloromethane 1:10); ^{1}H NMR (600.13 MHz, $CD_3OD+CDCl_3$): δ = 7.38–7.18 (m, 35H; arom. *H*), 4.89 (d, $J_{1''',2'''}$ = 4.4 Hz, 1H; H1‴), 4.88 and 4.60 (each d, *J* = −11.9 Hz, each 1H; 2-C*H*$_2$Ph), 4.78 and 4.59 (each d, *J* = −11.7 Hz, each 1H; 3-C*H*$_2$Ph), 4.76 and 4.69 (each d, *J* = −12.2 Hz, each 1H; 2″-C*H*$_2$Ph), 4.75 (d, $J_{1,2}$ = 2.0 Hz, 1H; H1), 4.73 and 4.60 (each d, *J* = −12.0 Hz, each 1H; 6-C*H*$_2$Ph), 4.69 and 4.66 (each d, *J* = −12.2 Hz, each 1H; 4″-C*H*$_2$Ph), 4.50 (d, $J_{1',2'}$ = 7.8 Hz, 1H; H1′), 4.46 (s, 2H; 3″-C*H*$_2$Ph), 4.44 and 4.23 (each d, *J* = −12.1 Hz, each 1H; 6′-C*H*$_2$Ph), 4.34 (d, $J_{1'',2''}$ = 0.7 Hz, 1H; H1″), 4.28 (dd, $J_{4,3}$ = 8.8, $J_{4,5}$ = 9.6 Hz, 1H; H4), 4.01 (dd, $J_{6a,5}$ = 4.9, $J_{6a,6b}$ = −11.4 Hz, 1H; H6a), 3.95 (dd, $J_{4''',5'''}$ = 1.2, $J_{4''',3'''}$ = 3.3 Hz, 1H; H4‴), 3.91 (dd, $J_{6b,5}$ = 2.1 Hz, 1H; H6b), 3.89 (dd, $J_{3,2}$ = 3.3 Hz, 1H; H3), 3.86 (dd, $J_{3''',2'''}$ = 9.9 Hz, 1H; H3‴), 3.81 (ddd, 1H; H5), 3.80 (dd, $J_{6''a,5''}$ = 2.7, $J_{6''a,6''b}$ = −11.6 Hz, 1H; H6″a), 3.80 (ddd, $J_{5''',6'''b}$ = 5.5, $J_{5''',6'''a}$ = 6.3 Hz, 1H; H5‴), 3.77 (dd, 1H; H2), 3.75 (dd, $J_{6'''a,6'''b}$ = −11.1 Hz, 1H; H6‴a), 3.74 (dd, 1H; H6‴b), 3.74 (dd, $J_{6b,5''}$ = 8.2 Hz, 1H; H6″b), 3.74 (dd, 1H; H2‴), 3.68 (dd, $J_{4'',3''}$ = 9.2, $J_{4'',5''}$ = 9.8 Hz, 1H; H4″), 3.64 (dd, $J_{2'',3''}$ = 3.0 Hz, 1H; H2″), 3.52 (ddd, 1H; H5″), 3.51 (dd, $J_{4',3'}$ = 8.5, $J_{4',5'}$ = 9.9 Hz, 1H; H4′), 3.48 (dd, $J_{3',2'}$ = 9.4 Hz, 1H; H3′), 3.41 (dd, 1H; H3″), 3.37 (dd, $J_{6'a,5'}$ = 2.2, $J_{6'a,6'b}$ = −11.1 Hz, 1H; H6′a), 3.35 (s, 3H, OC*H*$_3$), 3.29 (dd, 1H; H2′), 3.29 (dd, $J_{6'b,5'}$ = 3.7 Hz, 1H; H6′b), 3.18 ppm (ddd, 1H; H5′); ^{13}C NMR (150.9 MHz, $CD_3OD+CDCl_3$): δ = 139.5–127.5 (arom. *C*), 103.6 (C1′), 102.4 (C1″), 99.6 (C1‴), 99.6 (C1), 82.7 (C3″), 82.0 (C4′), 78.8 (C3), 76.0 (C4), 75.7 (C3′, C2), 75.5 (2-O*C*H$_2$Ph), 75.1 (C4″), 74.7(2″-O*C*H$_2$Ph), 74.3 (C2″, C5″), 74.2 (C5′), 74.1 (C2′), 73.9 (6′-O*C*H$_2$Ph), 73.7 (6-O*C*H$_2$Ph), 73.2 (4″-O*C*H$_2$Ph), 72.6 (3-O*C*H$_2$Ph), 72.4 (3″-O*C*H$_2$Ph), 71.8 (C5), 71.1 (C5‴), 70.3 (C4‴, C3‴), 69.8 (C2‴), 69.7 (C6), 68.7 (C6′), 67.2 (C6″), 62.1 (C6‴), 55.1 ppm (O*C*H$_3$); HRMS: *m*/*z* calcd for $C_{74}H_{86}O_{21}Na$: 1333.5559 $[M+Na]^+$; found: 1333.5548.

Methyl α-D-galactopyranosyl-(1→6)-β-D-mannopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-α-D-mannopyranoside (2): Pd/C (10% Pd, 59 mg, 2.5 weight equiv) was added to a solution of methyl α-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-6-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (24 mg, 0.018 mmol) in dry MeOH (2.3 mL). The reaction mixture was placed inside a reactor and the H_2 pressure was set to 2.8 bar (40 psi). After 19 h, the reaction mixture was diluted with MeOH (3 mL), filtered through celite, and concentrated to give **2** as a colorless oil (12 mg, quant.). $[\alpha]_D$ = +31.8 (*c* = 1.0 in MeOH); ^{1}H NMR (600.13 MHz, D_2O, 35 °C): δ = 5.00 (d, $J_{1''',2'''}$ = 3.9 Hz, 1H; H1‴), 4.76 (d, $J_{1,2}$ = 1.8 Hz, 1H; H1), 4.75 (d, $J_{1'',2''}$ = 0.9 Hz, 1H; H1″), 4.52 (d, $J_{1',2'}$ = 8.0 Hz, 1H; H1′), 4.07 (dd, $J_{2'',3''}$ = 3.3 Hz, 1H; H2″), 3.99 (dd, $J_{4''',3'''}$ = 1.3, $J_{4''',5'''}$ = 3.8 Hz, 1H; H4‴), 3.98 (dd, $J_{2,3}$ = 3.4 Hz, 1H; H2), 3.96 (dd, $J_{6''a,5''}$ = 6.7, $J_{6''a,6''b}$ = −10.6 Hz, 1H; H6″a), 3.95 (dd, $J_{6a,5}$ = 2.7, $J_{6a,6b}$ = −12.6 Hz, 1H; H6a), 3.95 (dd, $J_{5''',6'a}$ = 3.0, $J_{5''',6'''b}$ = 8.8 Hz, 1H; H5‴), 3.95 (dd, $J_{3''',2'''}$ = 10.2 Hz, 1H; H3‴), 3.89 (dd, $J_{6'a,5'}$ = 2.2, $J_{6'a,6'b}$ = −12.5 Hz, 1H; H6′a), 3.86 (dd, $J_{3,4}$ = 9.6 Hz, 1H; H3), 3.85 (dd, $J_{6b,5}$ = 5.3 Hz, 1H; H6b), 3.85 (dd, $J_{4,5}$ = 9.8 Hz, 1H; H4), 3.81 (dd, 1H; H2‴), 3.78 (dd, $J_{6''b,5''}$ = 2.1 Hz, 1H; H6″b), 3.74 (dd, $J_{6'''a,6'''b}$ = −11.1 Hz, 1H; H6‴a), 3.74 (dd, 1H; H6‴b), 3.73 (dd, $J_{6'b,5'}$ = 5.1 Hz, 1H; H6′b), 3.73 (ddd, 1H; H5), 3.68 (dd, $J_{4',3'}$ = 9.5, $J_{4',5'}$ = 9.8 Hz, 1H; H4′), 3.68 (dd, $J_{4'',3''}$ = 9.7, $J_{4'',5''}$ = 9.9 Hz, 1H; H4″), 3.67 (dd, $J_{3',2'}$ = 10.0 Hz, 1H; H3′), 3.65 (dd, 1H; H3″), 3.62 (ddd, 1H; H5′), 3.61 (ddd, 1H; H5″), 3.40 (s, 3H, OC*H*$_3$), 3.34 ppm (dd, 1H; H2′); ^{13}C NMR (150.9 MHz, D_2O, 35 °C): δ = 103.0 ($^1J_{C1',H1'}$ = 163.3 Hz; C1′), 101.2 ($^1J_{C1,H1}$ = 172.5 Hz; C1), 100.8 ($^1J_{C1'',H1''}$ = 159.4 Hz; C1″), 98.9 ($^1J_{C1''',H1'''}$ = 170.6 Hz; C1‴), 79.6 (C4′), 77.3 (C4), 75.1 (C5′, C5″), 74.7 (C3′), 73.5 (C2′, C3″), 71.8 (C5), 71.6 (C3‴), 71.1 (C2″), 70.1 (C2), 70.0 (C5‴), 69.9 (C4‴, C3), 69.1 (C2‴), 67.2 (C4″), 66.8 (C6″), 61.8 (C6‴), 60.9 (C6), 60.8 (C6′), 55.4 ppm (O*C*H$_3$); HRMS: *m*/*z* calcd for $C_{25}H_{44}O_{21}Na$: 703.2273 $[M+Na]^+$; found: 703.2273.

(2′*S*,3′*S*)-Methyl 3,4-*O*-[2′,3′-dimethoxybutan-2′,3′-diyl]-α-D-mannopyranoside: The reaction was performed under previously described experimental conditions[43] starting from commercially available methyl α-D-mannopyranoside (0.46 g, 2.4 mmol), butan-2,3-dione (0.34 mL, 1.6 equiv), trimethylorthoformate (1.15 mL, 4.2 equiv) and CSA (50 mg, 0.1 equiv) to give, after purification by column chromatography with diethyl ether as the eluent, the product as a white foam (0.59 g, 79%). R_f = 0.10 (diethyl ether); ^{1}H NMR (600.13 MHz, $CDCl_3$): δ = 4.75 (d, $J_{1,2}$ = 1.5 Hz, 1H; H1), 4.08 (dd, $J_{4,5}$ = 10.1, $J_{4,3}$ = 10.3 Hz, 1H; H4), 4.01 (dd, $J_{3,2}$ = 3.2 Hz, 1H; H3), 3.92 (ddd, $J_{2,2\text{-OH}}$ = 2.0 Hz, 1H; H2), 3.84 (ddd, $J_{6a,5}$ = 3.0, $J_{6a,6\text{-OH}}$ = 4.9, $J_{6a,6b}$ = −11.9 Hz, 1H; H6a), 3.78 (ddd, $J_{6b,5}$ = 4.7, $J_{6,6\text{-OH}}$ = 7.7 Hz, 1H; H6b), 3.76 (ddd, 1H; H5), 3.37 (s, 3H; 1-OC*H*$_3$), 3.28 and 3.26 (each s, 2′-OC*H*$_3$, each 3H; 3′-OC*H*$_3$), 2.37 (d, 1H; 2-O*H*), 1.98 (dd, 1H; 6-O*H*), 1.32 and 1.29 ppm (each s, each 3H, each C*H*$_3$ (2′, 3′)); HRMS: *m*/*z* calcd for $C_{13}H_{24}O_8Na$: 331.1363 $[M+Na]^+$; found: 331.1373.

(2′*S*,3′*S*)-Methyl 3,4-*O*-[2′,3′-dimethoxybutan-2′,3′-diyl]-6-*O*-*tert*-butyldimethylsilyl-α-D-mannopyranoside: The reaction was performed under previously described experimental conditions[44] starting from 0.14 g (0.4 mmol) of (2′*S*,3′*S*)-methyl 3,4-O[2′,3′-dimethoxybutan-2′,3′-diyl]-α-D-mannopyranoside, 60 mg (2.0 equiv) imidazole and 74 mg (1.1 equiv) *tert*-butyldimethylsilyl chloride to give, after column purification with hexane/EtOAc 3:1 as an eluent, the title compound as a colorless syrup (0.17 g, 90%). R_f = 0.63 (hexane/EtOAc 1:1); $[\alpha]_D$ = +174.4 (*c* = 0.5 in $CHCl_3$); ^{1}H NMR (600.13 MHz, $CDCl_3$): δ = 4.70 (d, $J_{1,2}$ = 1.5 Hz, 1H; H1), 3.99 (dd, $J_{3,2}$ = 2.9, $J_{3,4}$ = 9.2 Hz, 1H; H3), 3.98 (dd, $J_{4,5}$ = 9.9 Hz, 1H; H4), 3.88 (ddd, $J_{2,2\text{-OH}}$ = 3.3 Hz, 1H; H2), 3.85 (dd, $J_{6a,5}$ = 1.8, $J_{6a,6b}$ = −11.3 Hz, 1H; H6a), 3.80 (dd, $J_{6b,5}$ = 5.4 Hz, 1H; H6b), 3.69 (ddd, 1H; H5), 3.35 (s, 3H; 1-OC*H*$_3$), 3.27 and 3.24 (each s, each 3H; 2′-OC*H*$_3$, 3′-OC*H*$_3$), 2.21 (d, 1H; 2-O*H*), 1.31 and 1.28 (each s, each 3H; 2′-C*H*$_3$, 3′-C*H*$_3$), 0.89 (s, 9H; SiC(C*H*$_3$)$_3$), 0.07 and 0.06 ppm (each s, each 3H, each SiC*H*$_3$); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 100.9 (C1), 100.4 and 99.9 (C2′,C3′), 71.6 (C5), 69.9 (C2), 68.5 (C3), 63.0 (C4), 61.8 (C6), 54.7 (1-O*C*H$_3$), 48.2 and 48.0 (2′-O*C*H$_3$, 3′-O*C*H$_3$), 26.0 (SiC(*C*H$_3$)$_3$), 18.5 (Si*C*(CH$_3$)$_3$), 17.9 (2′-*C*H$_3$, 3′-*C*H$_3$), −5.0 and −5.9 ppm (Si(*C*H$_3$)$_2$); HRMS: *m*/*z* calcd for $C_{19}H_{38}O_8SiNa$: 445.2228 $[M+Na]^+$; found: 445.2237.

(2′*S*,3′*S*)-Methyl 2-*O*-acetyl-3,4-*O*-[2′,3′-dimethoxybutan-2′,3′-diyl]-6-*O*-*tert*-butyldimethylsilyl-α-D-mannopyranoside: The reaction was performed under previously described experimental conditions[45] starting from (2′*S*,3′*S*)-methyl 3,4-*O*-[2′,3′-dimethoxybutan-2′,3′-diyl]-6-*O*-*tert*-butyldimethylsilyl-α-D-mannopyranoside (0.13 g, 0.3 mmol), Ac_2O (1.2 mL), and pyridine (1.1 mL) to give, after purification by column chromatography with hexane/EtOAc 4:1 as the eluent, the product as a colorless oil (0.13 g, 92%). R_f = 0.54 (hexane/EtOAc 1:1); $[\alpha]_D$ = +125.8 (*c* = 1.2 in $CHCl_3$); ^{1}H NMR (600.13 MHz, $CDCl_3$): δ = 4.99 (dd, $J_{2,1}$ = 1.7, $J_{2,3}$ = 3.2 Hz, 1H; H2), 4.67 (d, 1H; H1), 4.13 (dd, $J_{3,4}$ = 10.3 Hz, 1H; H3), 4.07 (dd, $J_{4,5}$ = 9.8 Hz, 1H; H4), 3.85 (dd, $J_{6b,5}$ = 4.5, $J_{6b,6a}$ = −11.8 Hz, 1H; H6b), 3.81 (dd, $J_{6a,5}$ = 1.8 Hz, 1H; H6a), 3.66 (ddd, 1H; H5), 3.33 (s, 3H; 1-OC*H*$_3$), 3.27 and 3.25 (each s, each 3H; 2′-OC*H*$_3$, 3′-OC*H*$_3$), 2.11 (s, 3H; 2-OCOC*H*$_3$), 1.28 and 1.27 (each s, each 3H; 2′-C*H*$_3$, 3′-C*H*$_3$), 0.89 (s, 9H; SiC(C*H*$_3$)$_3$), 0.08 and 0.05 ppm (each s, each 3H; each SiC*H*$_3$); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 170.9 (2-O*C*OCH$_3$), 100.3 and 99.8 (C2′,C3′), 98.9 (C1), 71.5 (C5), 70.9 (C2), 66.2 (C3), 63.0 (C4), 61.5 (C6), 54.8 (1-O*C*H$_3$), 48.2 and 48.0 (2′-O*C*H$_3$, 3′-O*C*H$_3$), 26.0 (SiC(*C*H$_3$)$_3$), 21.4 (2-OCO*C*H$_3$), 18.4 (Si*C*(CH$_3$)$_3$), 18.0 and 17.9 (2′-*C*H$_3$, 3′-*C*H$_3$), −5.0 and −5.2 ppm (Si(*C*H$_3$)$_2$); HRMS: *m*/*z* calcd for $C_{21}H_{40}O_9SiNa$: 487.2334 $[M+Na]^+$; found: 487.2317.

(2′*S*,3′*S*)-Methyl 2-*O*-acetyl-3,4-*O*-[2′,3′-dimethoxybutan-2′,3′-diyl]-α-D-mannopyranoside (21): The reaction was performed under previously de-

scribed experimental conditions[46] starting from (2′*S*,3′*S*)-methyl 2-*O*-acetyl-3,4-*O*-[2′,3′-dimethoxybutan-2′,3′-diyl]-6-*O*-*tert*-butyldimethylsilyl-α-D-mannopyranoside (90 mg, 0.2 mmol) and HF/pyridine (80 μL) to give, after purification by column chromatography with hexane/EtOAc (1:2) as the eluent, the product as a white syrup (63 mg, 93%). R_f=0.21 (hexane/EtOAc 1:1); $[\alpha]_D$=+159.7 (c=1.2, $CHCl_3$); ^{1}H NMR (600.13 MHz, $CDCl_3$): δ=5.04 (dd, $J_{2,1}$=1.6, $J_{2,3}$=3.3 Hz, 1H; H2), 4.69 (d, 1H; H1), 4.13 (dd, $J_{3,4}$=10.3 Hz, 1H; H3), 4.03 (dd, $J_{4,5}$=10.0 Hz, 1H; H4), 3.85 (ddd, $J_{6a,5}$=3.0, $J_{6a,6\text{-}OH}$=5.2, $J_{6a,6b}$=−11.8 Hz, 1H; H6a), 3.79 (ddd, $J_{6b,5}$=4.8, $J_{6b,6\text{-}OH}$=7.7 Hz, 1H; H6b), 3.77 (ddd, 1H; H5), 3.36 (s, 3H; 1-OCH_3), 3.264 and 3.260 (each s, each 3H; 2′-OCH_3, 3′-OCH_3), 2.14 (s, 3H; 2-OCOCH_3), 1.88 (dd, 1H; 6-O*H*), 1.28 and 1.27 ppm (each s, each 3H; 2′-CH_3, 3′-CH_3); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ=170.6 (2-O*C*OCH$_3$), 100.2 and 99.7 (C2′,C3′), 99.2 (C1), 70.5 (C2, C5), 65.8 (C3), 63.6 (C4), 61.6 (C6), 55.0 (1-OCH_3), 48.1 and 47.9 (2′-OCH_3, 3′-OCH_3), 21.2 (2-OCOCH_3), 17.8 and 17.6 ppm (2′-CH_3, 3′-CH_3); HRMS: m/z calcd for $C_{15}H_{26}O_9Na$: 373.1469 $[M+Na]^+$; found: 373.1461.

Phenyl 2-*O*-acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside:[47] The reaction was performed according to previously described procedures[45] starting from phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (0.44 g, 0.98 mmol), Ac_2O (3.6 mL), and pyridine (3.2 mL) to give, after purification by column chromatography with hexane/EtOAc (2:1) as the eluent, the product as a yellowish oil (0.44 g, 92%). R_f=0.57 (hexane/EtOAc 2:1); Selected analytical data: ^{1}H NMR (600.13 MHz, $CDCl_3$): δ=7.53–7.27 (m, 15H; arom. *H*), 5.65 (s, 1H; C*H*Ph), 5.62 (dd, $J_{2,1}$=1.4, $J_{2,3}$=3.4 Hz, 1H; H2), 5.46 (d, 1H; H1), 4.73 and 4.70 (each d, J=−12.3 Hz, each 1H; 3-OCH_2Ph), 4.36 (ddd, $J_{5,6a}$=4.8, $J_{5,6b}$=10.2, $J_{5,4}$=10.3 Hz, 1H; H5), 4.24 (dd, $J_{6a,6b}$=−10.1 Hz, 1H; H6a), 4.14 (dd, $J_{4,3}$=9.9 Hz, 1H; H4), 4.01 (dd, 1H; H3), 3.86 (dd, 1H; H6b), 2.16 ppm (s, 3H; 2-OCOCH_3); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ=170.1 (2-O*C*OCH$_3$), 137.8–125.8 (arom. *C*), 101.6 (*C*HPh), 87.2 (C1), 78.5 (C4), 74.1 (C3), 72.4 (3-OCH_2Ph), 71.4 (C2), 68.4 (C6), 65.2 (C5), 21.0 ppm (2-OCO*C*H$_3$).

Phenyl 2-*O*-acetyl-3-*O*-benzyl-1-thio-α-D-mannopyranoside (22): Phenyl 2-*O*-acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (58 mg, 0.11 mmol) was dissolved in AcOH (80%, 3 mL), and the resulting mixture was stirred for 3 h at 60°C and concentrated. The crude product was purified by column chromatography with hexane/EtOAc (1:1) as the eluent to give the product as a colorless oil (43 mg, 89%). R_f=0.18 (hexane/EtOAc 1:1). Selected analytical data: ^{1}H NMR (600.13 MHz, $CDCl_3$): δ=7.50–7.20 (m, 10H; arom. *H*), 5.60 (dd, $J_{2,1}$=1.6, $J_{2,3}$=3.2 Hz, 1H; H2), 5.47 (d, 1H; H1), 4.74 and 4.48 (each d, J=−11.2 Hz, each 1H; 3-OCH_2Ph), 4.18 (ddd, $J_{5,6a}$=3.4, $J_{5,6b}$=4.8, $J_{5,4}$=9.8 Hz, 1H; H5), 3.97 (dd, $J_{4,3}$=9.5 Hz, 1H; H4), 3.86 (dd, $J_{6a,6b}$=−11.7 Hz, 1H; H6a), 3.83 (dd, 1H; H6b), 3.76 (dd, 1H; H3), 2.11 ppm (s, 3H; 2-OCOCH_3).

STD NMR spectroscopic experiments: STD experiments were performed with a lectin concentration of 59 μM on a Bruker Avance 500 MHz NMR spectrometer equipped with a 5 mm TXI inverse probe head. Protein saturation was achieved by using a cascade of 40 selective 70 dB Gaussian pulses of 50 ms in total duration and a total number of 360 scans[39] at a ligand/lectin ratio of 25:1.[38b]

Molecular modeling: Potential-energy maps were performed by using the Coordinate Scan tool implemented in MAESTRO,[34] as described. The generalized Born/surface area (GB/SA solvation model for water was used. The calculations was performed with the AMBER force field.[35] The same force field was employed for the MD simulations performed on two models of tetrasaccharide **2**, built from the global-minimum conformers found for every Φ/Ψ glycosidic linkage. The two models were generated with either the *gg* or *gt* rotamers around the α-Gal*p*-(1→6)-β-Man*p* linkage of **2**. MD simulations of 6 ns were carried out with equilibration of 100 ps, and an integration time step of 2 fs.

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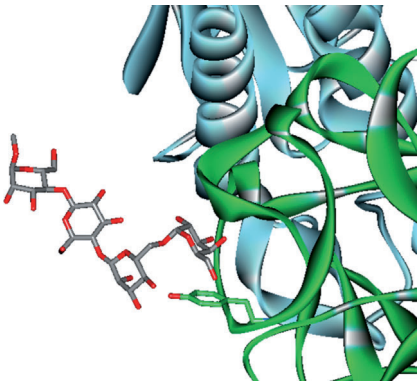
Modeling behavior: Detailed studies on the chemical synthesis, NMR spectroscopic characterization, conformation analysis, and molecular recognition of molecular models of oligosaccharide fragments present in the backbone of galactoglucomannan polysaccharides found in natural Norway spruce are reported (see figure). A tetrasaccharide fragment was further utilized in a binding study with the galactose-binding protein viscumin to test bioaffinity.



Polysaccharides

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Studies Related to Norway Spruce Galactoglucomannans: Chemical Synthesis, Conformation Analysis, NMR Spectroscopic Characterization, and Molecular Recognition of Model Compounds