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Synthesis and Bioactivity of β-(1→4)-Linked Oligomannoses and Partially Acetylated Derivatives

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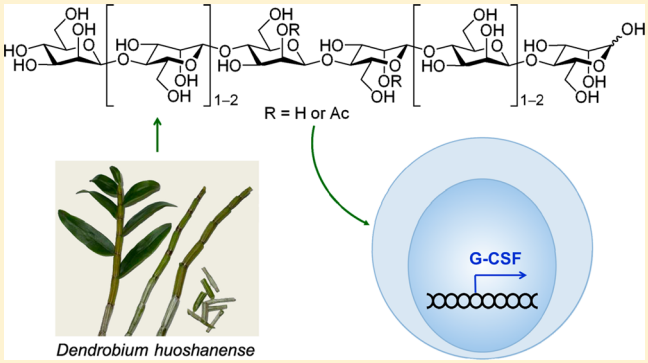
Supporting Information

ABSTRACT: The synthesis of β-(1→4)-linked hexa- to octamannoses and their partially acetylated derivatives was efficiently carried out by assembly of appropriate oligomeric fragments using β-selective glucosylation followed by gluco to manno epimerization at a late stage of the synthetic pathway. In the course of this study, we also observed that 2-*O*-acetylated oligomannoses coexisted in equilibrium with the 3-*O*-acetylated isomers due to intramolecular migration of the acetyl group. Bioactivity of the synthetic oligomannoses and partially acetylated derivatives was investigated in order to identify the possible smallest oligomer for induction of cytokines as that shown in the polysaccharides extracted from *Dendrobium huoshanense*.



INTRODUCTION

The Chinese herb plant *Dendrobium huoshanense* is used as traditional medicine in China for nourishment of the stomach, improvement in circulation of body fluids, and enhancement of the immune system.[1] It was found that *D. huoshanense* exerts important bioactivity such as antiaging, anticancer, and antidiabetes effects.[2] For this reason, many research efforts have been devoted to find bioactive compounds from *D. huoshanense*.[3] We previously reported a bioactive polysaccharide extracted from the aerial parts of *D. huoshanense*.[4] The polysaccharide is composed of β-(1→4)-glucomannan having partial acetylation (∼35%) at the 2- and 3-positions of mannosides in an approximate ratio of 1:10 glucoside to mannoside. Our study revealed that the polysaccharide exhibits specific functions in stimulating murine splenocytes to produce several cytokines including IFN-γ, IL-10, IL-6, IL-1α, GM-CSF, and G-CSF. Among the cytokines examined, a relatively high increase of G-CSF was observed at transcriptional and translational levels.

G-CSF is a hematopoietic growth factor of myeloid lineage.[5] Because of its many important roles in granulopoiesis,[6] G-CSF has currently been used for overcoming granulocytopenia, which is one of the most serious side effects for cancer patients treated with chemotherapies or radiotherapies. In addition, G-CSF exerts profound immunoregulatory effects in adaptive immunity[6b] and has neuroprotective activities in cerebral ischemia and neurodegeneration.[7] Therefore, agents inducing G-CSF production have potential therapeutic applications in autoimmune disease and neurological disorders, which prompted us to investigate a minimal structural requirement responsible for the effect of the polysaccharide on induction of G-CSF.

In our preliminary study,[8] it was found that a mixture of oligomers generated by digestion of the natural polysaccharides using hemicellulase could induce expression of G-CSF. Our study also showed that a mixture of β-(1→4)-linked oligomannoses with the number of mannose unit greater than 6 had G-CSF induction activity. However further study was hampered due to the difficulties in separating and isolating these oligosaccharides. For this reason, we chose to synthesize hexa-, hepta-, and octamannoses **1**, **4**, and **7** (Figure 1) for detailed structure−activity relationship studies. We also envisioned a synthesis of partially acetylated oligomannosides **2**, **3**, **5**, **6**, and **8** (Figure 1) to assess the effect of the acetyl group on the bioactivity. In the natural polysaccharides of *D. huoshanense*, the C-2 hydroxyl groups in the mannose residues are acetylated more abundantly than the C-3 hydroxyl groups. Thus, diacetylated oligomannoses **3** and **6** were designed to mimic the natural oligosaccharides. Monoacetylated oligomannoses **2**, **5**, and **8** were synthesized to evaluate the influence of the acetyl groups on the bioactivity.

RESULTS AND DISCUSSION

Synthesis of Hexasaccharides 1−3. Although synthesis of the related nonacetylated β-(1→4)-linked hexamannoside has been reported,[9] the method involving a procedure to remove the ester-type protecting groups at the final stage

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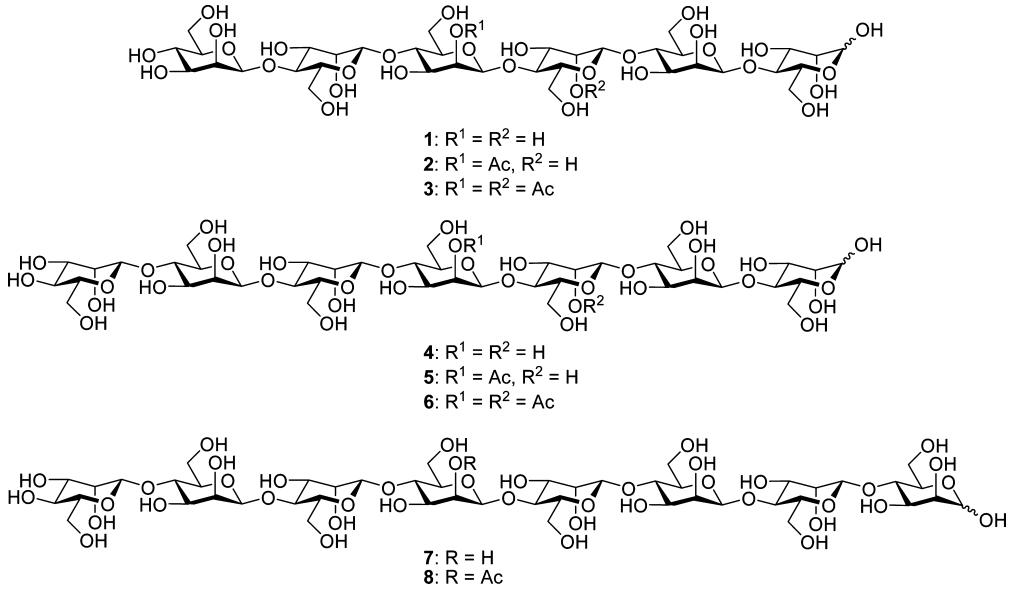
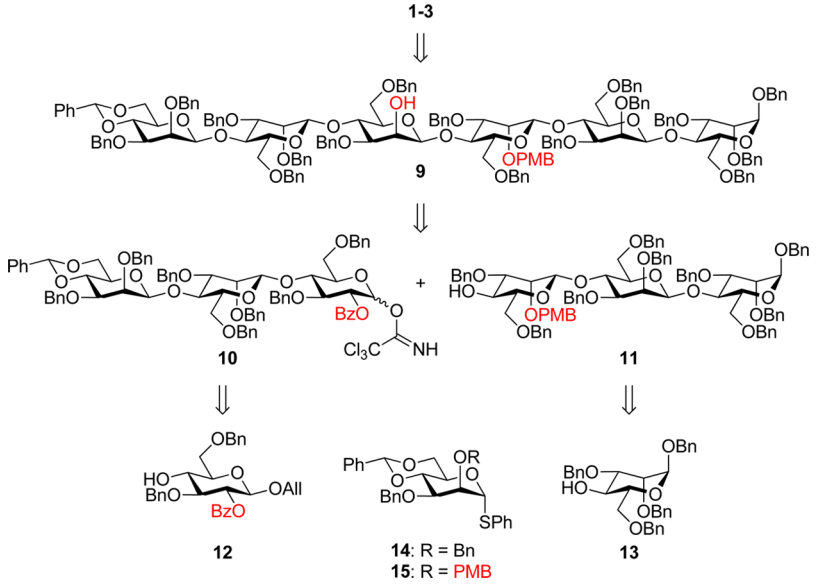


Figure 1. Oligomannoses **1**−**8** as the target molecules for inducing the expression of granulocyte colony-stimulating factor (G-CSF).

Scheme 1. Retrosynthetic Analysis of Hexasaccharides 1−3



cannot be applied to the synthesis of acetylated hexasaccharides **2** and **3**. We thus developed an alternative synthetic route for hexamannoses **1**−**3**. The retrosynthetic analysis (Scheme 1) shows that hexasaccharides **1**−**3** can be derived from a common intermediate **9**. Considering the overall efficiency, it is desirable to synthesize the key intermediate **9** by a [3 + 3] glycosylation. Even though several methods for direct β-selective mannosylation have been developed,[10] direct formation of β-oligomannosides[11] is still difficult because properly protected mannosyl donors are often required for the coupling reactions with oligosaccharide fragments.[12] We chose to synthesize the hexamannoside intermediate **9** by an indirect method involving β-selective glucosylation of trimannoside acceptor **11** with a trisaccharide donor **10**. The subsequent epimerization of the C-2 hydroxyl group in the glucoside moiety would be performed via an oxidation−reduction sequence to afford the desired configuration of β-mannoside. Furthermore, this methodology allows installation of an acetyl group at the designated position. The trisaccharide fragments **10** and **11** can be respectively obtained from monosaccharides **12** and **13**[13] by repeating the β-selective mannosylations using **14**[14] or **15**[14] as the donors. To facilitate the [3 + 3] glycosylation for the assembly of oligosaccharide fragments, the trichloroacetimidate method is preferable to attain high efficiency.[15] Although gluco to manno epimerization in large oligosaccharides seems to be difficult, some successful examples for synthesis of complex oligosaccharides incorporating such epimerization at a late stage have been demonstrated.[16]

We first undertook the synthesis of trisaccharide acceptor **11** (Scheme 2), which proceeded with a β-selective mannosylation of **13** with donor **14** using 1-benzenesulfinyl piperidine (BSP) and trifluoromethanesulfonic anhydride (Tf_2O) as the combined promoters[10c] in the presence of a bulky base 2,4,6-tri-*tert*-butylpyrimidine (TTBP)[17] to afford dimannoside **16** in 73% yield. The reductive ring-opening of benzylidene acetal occurred regioselectively by using Et_3SiH and $BF_3{\cdot}OEt_2$[18] to

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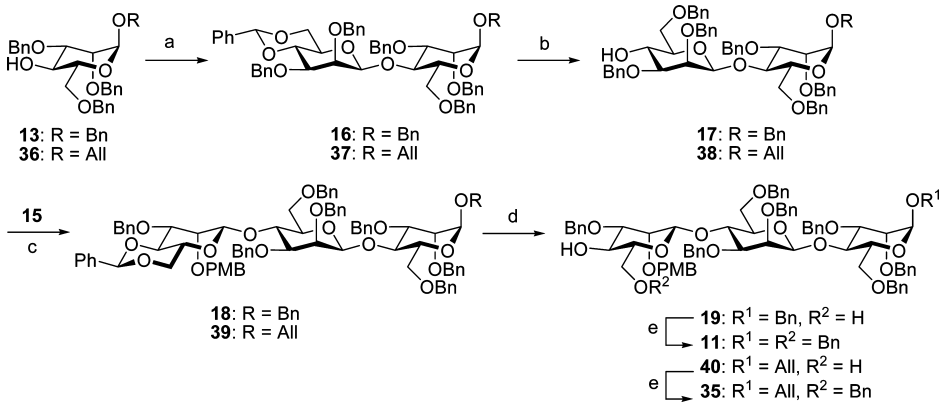
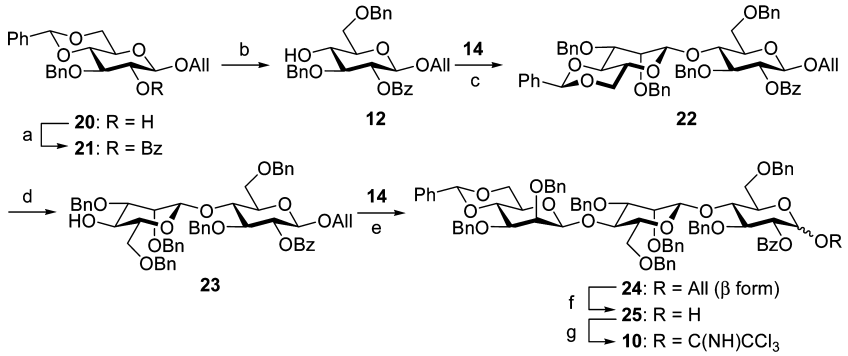
Scheme 2. Synthesis of Trisaccharide Acceptors 11 and 35[a]



[a]Reagents and conditions: (a) **14**, BSP, Tf_2O, TTBP, CH_2Cl_2, MS3A, −78 °C to rt, 5−6 h; 73% for **16**, 79% for **37**; (b) Et_3SiH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0 °C, 7 h; 62% for **17**, 60% for **38**; (c) **15**, BSP, Tf_2O, TTBP, CH_2Cl_2, MS3A, −78 to −60 °C, 2−5 h; Et_3N, $(EtO)_3P$, −60 °C to rt, 15−20 min; 65% for **18**, 53% for **39**; (d) CSA, neopentyl glycol, CH_2Cl_2, rt, 24−35 h; 74% for **19** from **18**, 72% for **40** from **39**; (e) Bu_2SnO, toluene, reflux, 3 h; BnBr, CsF, toluene, reflux, 6 h; 67% for **11**, 80% for **35**.

Scheme 3. Synthesis of Trisaccharide Donor 10[a]



[a]Reagents and conditions: (a) BzCl, pyr, 0 °C to rt, 10 h; 99%; (b) Et_3SiH, TFA, CH_2Cl_2, 0 °C, 1 h; 88%; (c) **14**, BSP, Tf_2O, TTBP, CH_2Cl_2, MS3A, −78 to −60 °C, 5 h; Et_3N, $(EtO)_3P$, −60 °C to rt, 15 min; 71%; (d) Et_3SiH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0 °C, 1.5 h; 74%; (e) **14**, BSP, Tf_2O, TTBP, CH_2Cl_2, MS3A, −78 to −60 °C, 2 h; Et_3N, $(EtO)_3P$, −60 °C to rt, 20 min; 53%; (f) $PdCl_2$, MeOH/CH_2Cl_2 (1:1, v/v), rt, 6 h; 77%; (g) CCl_3CN, DBU, CH_2Cl_2, 0 °C, 1 h; 72%.

give the desired alcohol **17** in 62% yield. Mannosylation of **17** with donor **15** by activation of BSP/Tf_2O furnished trimannoside **18**. It was noted that the addition of triethylamine and triethyl phosphite[19] for quenching the transiently formed electrophilic species, (piperidino)phenyl(*S*-thiophenyl)sulfide triflate, was required to avoid the decomposition of compound **18**. Due to the instability of the PMB ether against $BF_3{\cdot}OEt_2$,[20] conversion of **18** to **11** could not be realized by using Et_3SiH/$BF_3{\cdot}OEt_2$ to effect a direct reductive opening of the benzylidene acetal. Alternatively, we carried out this conversion through two steps: transacetalation[9,21] using neopentyl glycol in the presence of camphorsulfonic acid (CSA) and selective monobenzylation of the resulting diol **19** via formation of a stannylene acetal.[22]

The synthesis of trisaccharide donor **10** began with benzoylation of allyl glucoside **20**[23] to give compound **21** (Scheme 3). Opening of the benzylidene acetal using Et_3SiH and TFA[24] provided the desired 4-hydroxy derivative **12**. Preparation of trisaccharide **24** from monosaccharide **12** was achieved in a three-step sequence similar to that for conversion of **13** to **18**. After removal of the allyl group with $PdCl_2$,[25] hemiacetal **25** was subject to trichloroacetimidation to afford the trisaccharide donor **10**.

Having trisaccharide donor **10** and trisaccharide acceptor **11** in hand, assembly of these fragments was accomplished by treatment with a catalytic amount of TMSOTf to afford hexasaccharide **26** (Scheme 4). The benzoyl group was removed using $LiAlH_4$[16a,b] to obtain hexasaccharide **27** containing a free hydroxyl group at C-2 of the central glucoside residue. To our delight, Albright−Goldman oxidation[26] of alcohol **27** and the subsequent reduction of the resulting ketone using $NaBH_4$[27] afforded the key intermediate **9** having an all-manno configuration. As shown by TLC analysis, the reduction product also contained a small amount of **27** having a gluco configuration at the central saccharide moiety. To verify the all-*β*-manno pyranoside structure of **9**, we determined the $^1J_{CH}$ values between the anomeric carbons and protons. The anomeric C−H coupling constants of the mannoside residues, except for that at the reducing end, are around 160 Hz, in agreement with the *β*-manno pyranoside backbone of hexasaccharide **9**.[28] Our original attempt to prepare non-acetylated hexamer **1** by hydrogenation of **9** encountered difficulties in workup and purification. Alternatively, we considered preparation of **1** by saponification of its acetylated derivatives **2** and **3**. Thus, compound **9** was acetylated to afford hexamannoside **28**, which was subjected to hydrogenation over

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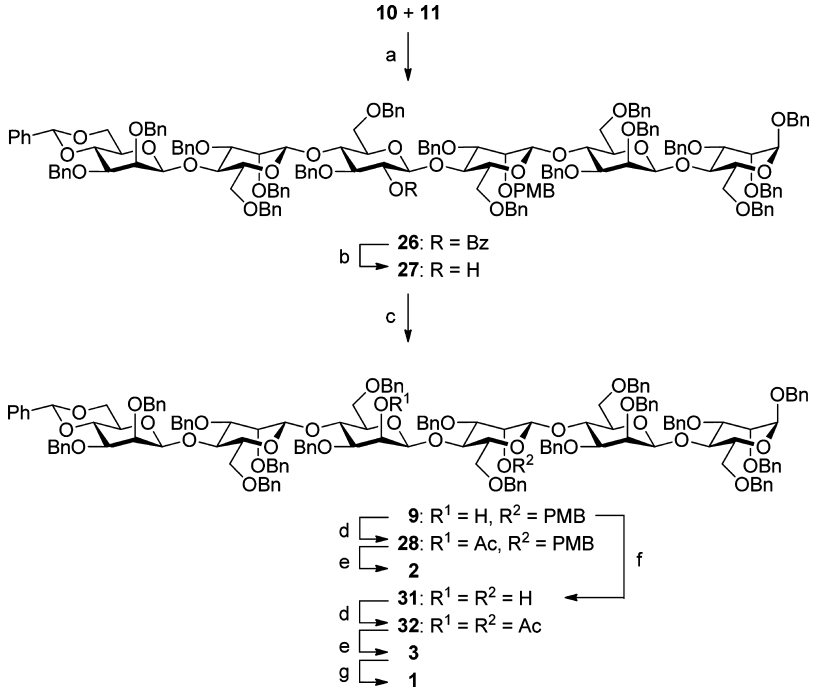
Scheme 4. Synthesis of Hexamannoses 1−3[a]



[a]Reagents and conditions: (a) TMSOTf, CH_2Cl_2, MS4A, −75 to 0 °C, 6.5 h; 80%; (b) $LiAlH_4$, Et_2O, −40 to 0 °C, 30 min; 71%; (c) Ac_2O, DMSO, rt, 16 h; $NaBH_4$, MeOH/CH_2Cl_2 (1:1, v/v), 0 °C to rt, 3 h; 64%; (d) Ac_2O, DMAP, pyr, rt, 3.5−4.0 h; 88% for **28**, 66% for **32**; (e) H_2, Pd/C, MeOH/THF/AcOH (10:20:3, v/v/v), rt, 23 h; 65% for **2**, 74% for **3**; (f) CAN, CH_3CN/toluene/H_2O (18:1:1, v/v/v), 0 °C to rt, 1.5 h; 58%; (g) NaOH, H_2O, rt, 2 h; 84%.

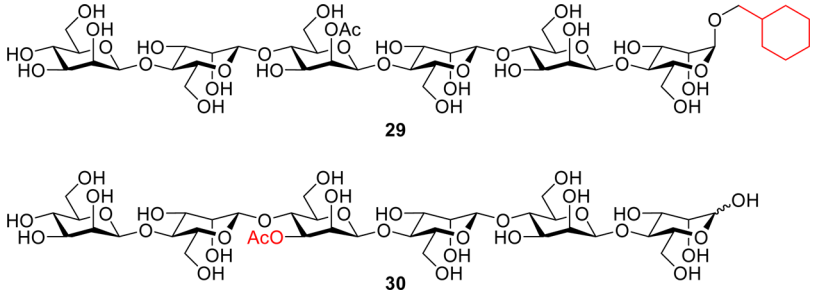


Figure 2. Structures of side products **29** and **30**, which were formed by saturation of the phenyl group and migration of the acetyl group, respectively.

Pd/C in a mixed solvent of MeOH/THF/AcOH. Addition of ∼10% acetic acid in the media was found to suppress migration of the acetyl group during the hydrogenation process. The MALDI-TOF MS analysis of the reaction after 23 h showed the desired product of hexasaccharide **2** (*m*/*z* 1055.3482 for the $[M + Na]^+$ ion) that was formed by global deprotection of all benzyl, benzylidene and *p*-methoxybenzyl groups. However, the MALDI-TOF MS spectrum also showed a small signal at *m*/*z* 1151.4009, which might be from the sodiated molecular ion of mannoside **29** (calcd for $C_{45}H_{76}NaO_{32}$ 1151.4217) having a cyclohexylmethyl substituent at the anomeric position (Figure 2). The formation of cyclohexylmethyl glycoside on hydrogenation, instead of cleaving the anomeric benzyl group, has been previously reported.[29] The side product **29** could be separated from compound **2** by chromatography on a C18 column.

The ^1H NMR analysis (Figure 3) of the crude compound **2**, obtained by removal of the catalyst followed by concentration in vacuo, further revealed the possible migration of the 2-*O*-acetyl group to the neighboring C-3 position, giving a 3-*O*-acetylated isomer **30** (Figure 2). Figure 3a shows the selected areas of ^1H NMR spectrum of the crude compound **2** recorded immediately after dissolving the crude sample in D_2O. The presence of 2-*O*-acetylated mannose residue was confirmed by showing the H-2 signal at δ 5.50 as a broad doublet (J = 3.5 Hz). The singlet signal at δ 2.17 was attributable to the acetyl group. A trace amount of **30** was observed from a weak signal at δ 2.18 (s) corresponding to its acetyl group. After incubation of the mixture in D_2O at 25 °C for 2 h, new signals appearing at δ 4.18 (d, J = 3.1 Hz) and δ 5.10 (dd, J = 9.8, 3.3 Hz) were ascribed to H-2 and H-3, respectively, in compound **30**. The protons in **2** and **30** were assigned according to the reported chemical shifts of an *O*-acetylated galactoglucomannan,[29] and further confirmed by the HMBC and COSY experiments. To our knowledge, this case represents the first detailed NMR study of intramolecular acetyl migration on an oligomannan, though transesterifications of naturally occurring polymeric mannan and synthetic mannopyranose derivatives have been

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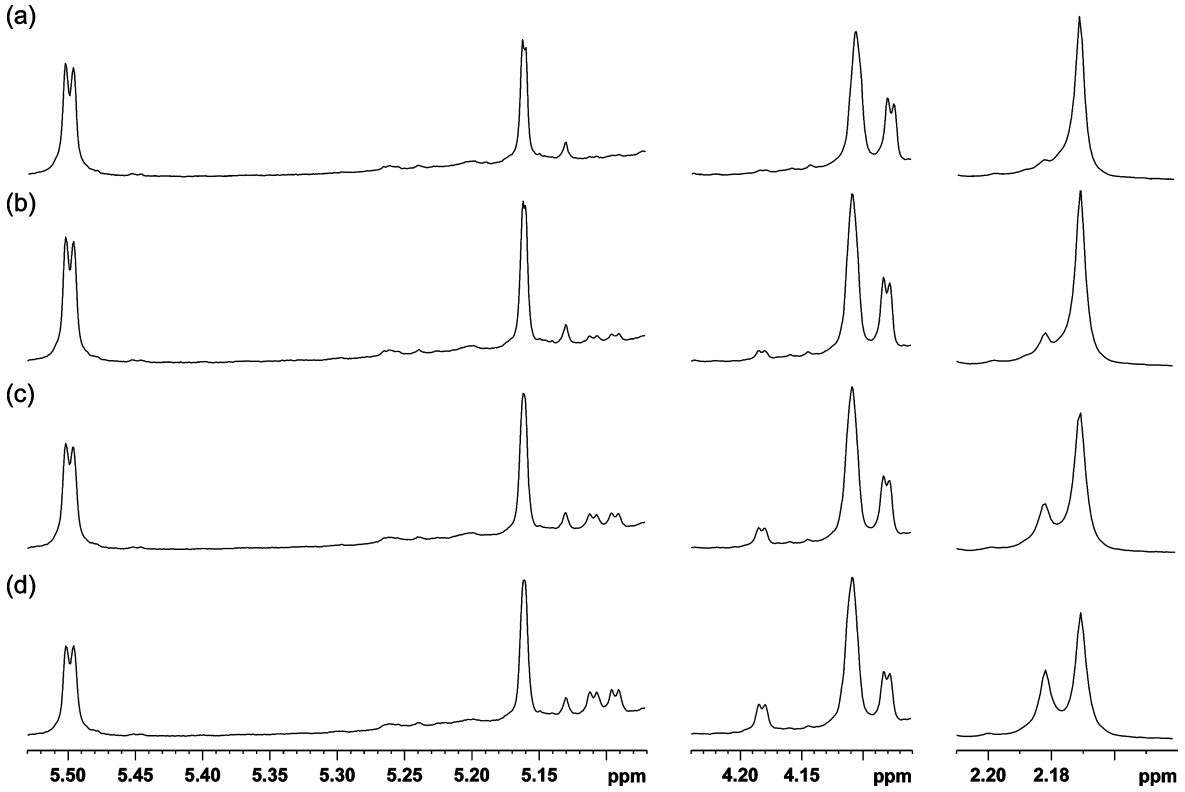
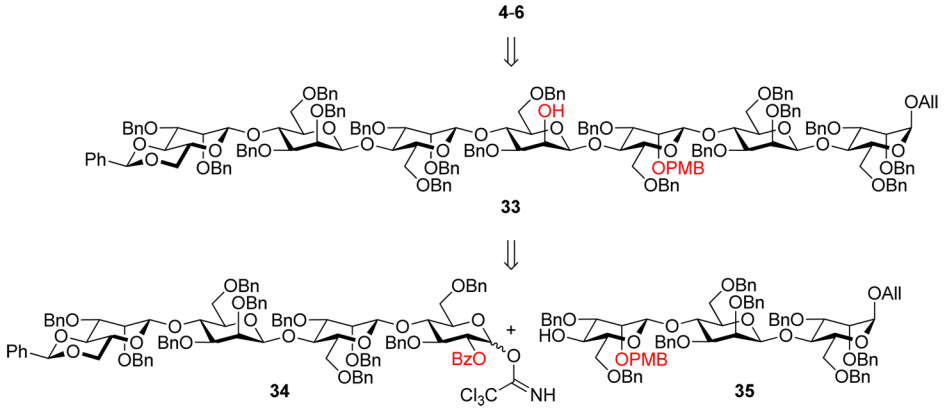


Figure 3. Selected areas of the ^{1}H NMR spectra of the crude product **2** recorded in D_2O: immediately after dissolving the crude sample in D_2O (a) and after incubation at 25 °C for 2 h (b), 8 h (c), and 24 h (d). Compound **30** generated by acetyl migration showed signals at δ 2.18 (s), 4.18 (d), and 5.10 (dd).

Scheme 5. Retrosynthetic Analysis of Heptasaccharides 4−6



reported.[30] Isomerization between **2** and **30** reached an equilibrium (**2**/**30** ≈ 5:3) after incubation at room temperature for 24 h (Figure 3d). We attempted to suppress the acetyl migration by reversed-phase chromatography under mild acidic conditions (1% AcOH in H_2O); however, the isolated hexamannose **2** was still contaminated with its isomer **30**. In contrast to the previous report,[30a] there was no acetyl migration from O2 to O6 position in compound **2**.

The synthesis of diacetylated hexasaccharide **3** commenced with removal of the PMB ether group in **9** using CAN in a solution of CH_3CN/PhMe/H_2O (18:1:1, v/v/v)[31] to give alcohol **31**. After acetylation, the diacetylated compound **32** was subjected to hydrogenation to furnish diacetylated hexamannose **3**, which also existed as a mixture of regioisomers due to migration of the acetyl groups. Saponification of **3** followed by neutralization allowed us to obtain nonacetylated hexamannose **1** in high purity without further purification.

Synthesis of Heptasaccharides 4−6. By a strategy similar to the synthesis of hexasaccharides **1**−**3**, a [4 + 3] glycosylation of **34** with **35** was applied (Scheme 5). The subsequent epimerization would provide heptasaccharide **33** as the pivotal intermediate leading to heptamannoses **4**−**6**. The reducing end of trisaccharide acceptor **35** was protected with an allyl group instead of using benzyl group that might form a cyclohexylmethyl glycoside as the side product on hydrogenation. Trisaccharide **35** was prepared by sequential β-selective mannosylations of allyl mannoside **36**[13] (Scheme 2) in a way similar to that for **11**.

Trisaccharide **41** having a free 4-OH group was designed to synthesize the tetrasaccharide donor **34** via a route incorporat-

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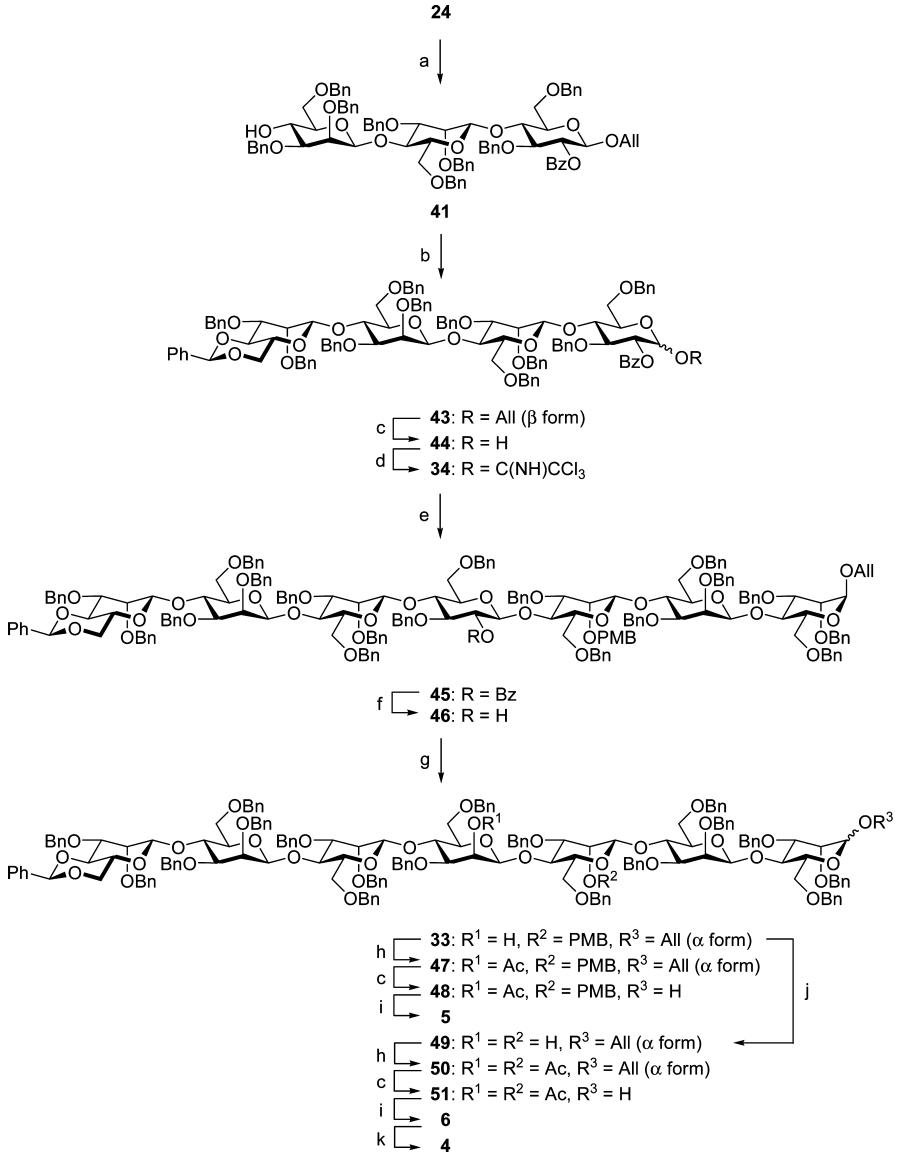
Scheme 6. Synthesis of Heptamannoses 4–6[a]



[a]Reagents and conditions: (a) Et_3SiH, TfOH, CH_2Cl_2, MS4A, −78 °C, 15 h; TBAF, CH_2Cl_2, −78 °C to rt, 2 h; 58%; (b) **14**, BSP, Tf_2O, TTBP, CH_2Cl_2, MS3A, −78 to −60 °C, 1.5 h; Et_3N, $(EtO)_3P$, −60 to −10 °C, 15 min; 68%; (c) $PdCl_2$, MeOH/CH_2Cl_2 (1:1, v/v), rt, 3 h; 79% for **44**, 60% for **48**, 61% for **51**; (d) CCl_3CN, DBU, CH_2Cl_2, 0 °C, 1 h; 56%. (e) **35**, TMSOTf, CH_2Cl_2, MS4A, −71 to 0 °C, 3 h; 65%; (f) $LiAlH_4$, −40 to 0 °C, 30 min; 80%; (g) (i) Ac_2O, DMSO, rt, 16 h; (ii) $NaBH_4$, MeOH/CH_2Cl_2 (1:1, v/v), 0 °C to rt, 3 h; 60%; (h) Ac_2O, DMAP, pyr, rt, 3 h; 92% for **47**, 91% for **50**; (i) H_2, Pd/C, THF/MeOH/AcOH (2.0:1.0:0.3, v/v/v), rt, 23 h; H_2, Pd/C, H_2O/MeOH/AcOH (3:6:1, v/v/v), rt, 17 h; 69% for **5**, 60% for **6**; (j) CAN, CH_3CN/toluene/H_2O (18:1:1, v/v/v), 0 °C to rt, 2.5 h; 75%; (k) NaOH, H_2O, rt, 2 h; 95%.

ing the coupling reaction with mannoside donor **14** under BSP/Tf_2O conditions[10c] (Scheme 6). Though reductive opening of the benzylidene acetal in trisaccharide **24** using $Et_3SiH/BF_3{\cdot}OEt_2$ gave the desired alcohol **41** in 42% crude yield, a considerable amount (26%) of hydrolysis product was also obtained. This problem was not circumvented by addition of molecular sieves. We thus treated **24** with Et_3SiH/TfOH[32] at −78 °C in the presence of molecular sieves to yield a major product **41** according to the TLC analysis. Upon workup by addition of Et_3N, this product disappeared along with occurrence of a less polar compound as shown by TLC analysis. This new compound was tentatively assigned to structure **42** (Figure 4) according to the NMR and MALDI-TOF MS analyses (found m/z 1505.6879 for $[M + Na]^+$, calcd for $C_{90}H_{102}NaO_{17}Si$ 1505.6784). We speculated that alcohol **41** might be silylated with the in situ generated TESOTf, a process conceivably promoted by addition of Et_3N base. To avoid forming the silylated compound **42**, Et_3N was replaced by a THF solution of TBAF on workup to afford the desired product **41** in 58% yield. Trisaccharide **41** was then coupled with monomer **14** to afford tetrasaccharide **43**, which was converted to **34** by deallylation and trichloroacetimidation.

The [4 + 3] coupling reaction of **34** and **35** gave heptasaccharide **45**, which was converted to heptamannoside **33** by epimerization of the central glucoside moiety via a sequence comprising removal of the benzoyl group, oxidation, and reduction (Scheme 6). After acetylation, the anomeric allyl group in **47** was removed using $PdCl_2$, and global reduction of the benzyl and PMB groups by hydrogenation provided monoacetylated heptamannoside **5** (m/z 1217.3980 for [M +

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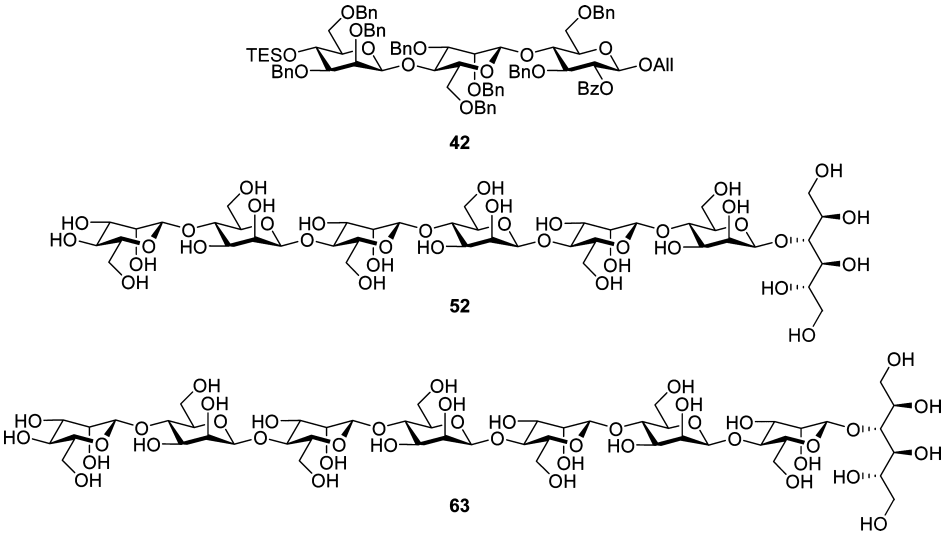
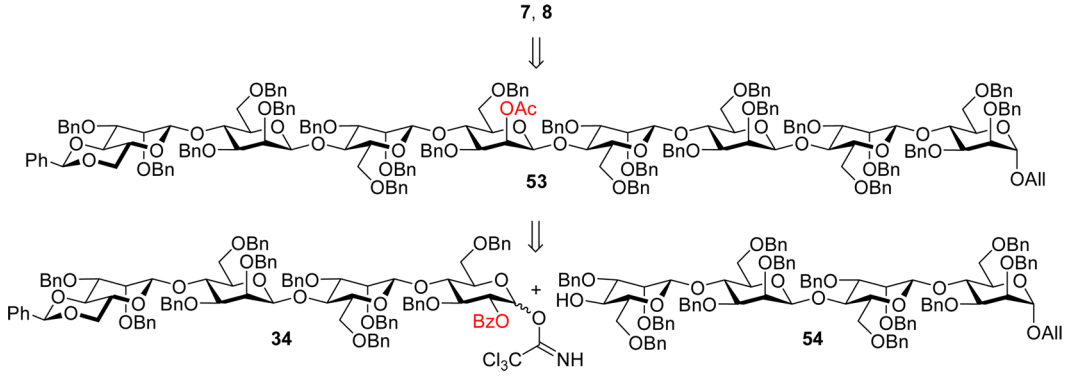


Figure 4. Structures of side products **42**, **52**, and **63**, which were formed by silylation of the hydroxyl group or by over-reduction of the terminal mannose.

Scheme 7. Retrosynthetic Analysis of Octasaccharides 7 and 8



Na]$^+$ ion) in 60% yield along with its 3-*O*-acetylated isomer. Heptasaccharide **4** and the diacetylated derivative **6** were similarly prepared from the common intermediate **33**. By meticulous examination of the ^{13}C NMR and DEPT-135 spectra, we found the product **4** was contaminated with a small amount of **52** bearing a mannitol moiety (Figure 4). Besides the signal at δ_C 69.4 for the C4 at nonreducing end of compound **4**, the corresponding signal for the side product **52** occurred at δ_C 65.1 with ~8% intensity. More accurate percentage of the side product was not determined, partly due to overlapping of the characteristic proton signals in the ^{1}H NMR spectrum. The mannitol moiety in **52** was presumably formed by reduction of the terminal mannose during the process of hydrogenation. The structure of **52** was confirmed by an independent synthesis comprising reduction of the terminal mannose in **51** with $NaBH_4$, debenzylation by catalytic hydrogenation, and removal of the acetyl groups by saponification.

Synthesis of Octasaccharides 7 and 8. Scheme 7 shows our retrosynthetic analysis for octamannoses **7** and **8**. Starting from glycosylation of disaccharide **38** with mannoside donor **14**, the coupling product **55** was then subject to reductive opening of the benzylidene acetal and a second glycosylation with **14** to give tetrasaccharide **57** (Scheme 8). Reduction of the benzylidene acetal in **57** was found inefficient using the combined reagents of Et_3SiH/TfOH/TBAF. We thus converted **57** to alcohol **54** in two steps comprising transacetalization with neopentyl glycol[9,21] and monobenzylation via the stannylene acetal.[22] Octasaccharide **60** was obtained by assembly of oligosaccharide fragments **34** and **54**, followed by removal of the benzoyl group. However, epimerization of the central glucoside moiety in **60** turned out to be problematic. Though oxidation of **60** proceeded smoothly to afford the corresponding ketone as judged by the NMR analysis, the subsequent reduction with $NaBH_4$ did not give the desired axial alcohol in a reproducible manner. This problem was finally overcome by using L-Selectride[33] as the reducing agent to obtain compound **61** in all-manno configuration. After acetylation, the allyl, benzyl, and benzylidene groups were removed to give 2-*O*-acetylated octamannose **8**, which also coexisted with its 3-*O*-acetylated isomer. Saponification of **8** yielded octamannose **7**. A small amount (~8%) of the heptamannose−mannitol compound **63** (Figure 4) was also detected in the ^{13}C NMR and DEPT-135 spectra, similar to that observed in **52**. The structure of **63** was confirmed by an independent synthesis in a five-step sequence: (i) palladium-catalyzed deallylation, (ii) reduction of the terminal mannose by $NaBH_4$, (iii) acetylation, (iv) debenzylation by catalytic hydrogenation, and (v) removal of the acetyl group by saponification. Interestingly, the monoacetylated derivative **8** is more soluble in water than its parental octamannose **7**; even these compounds differ only in one acetyl group. This

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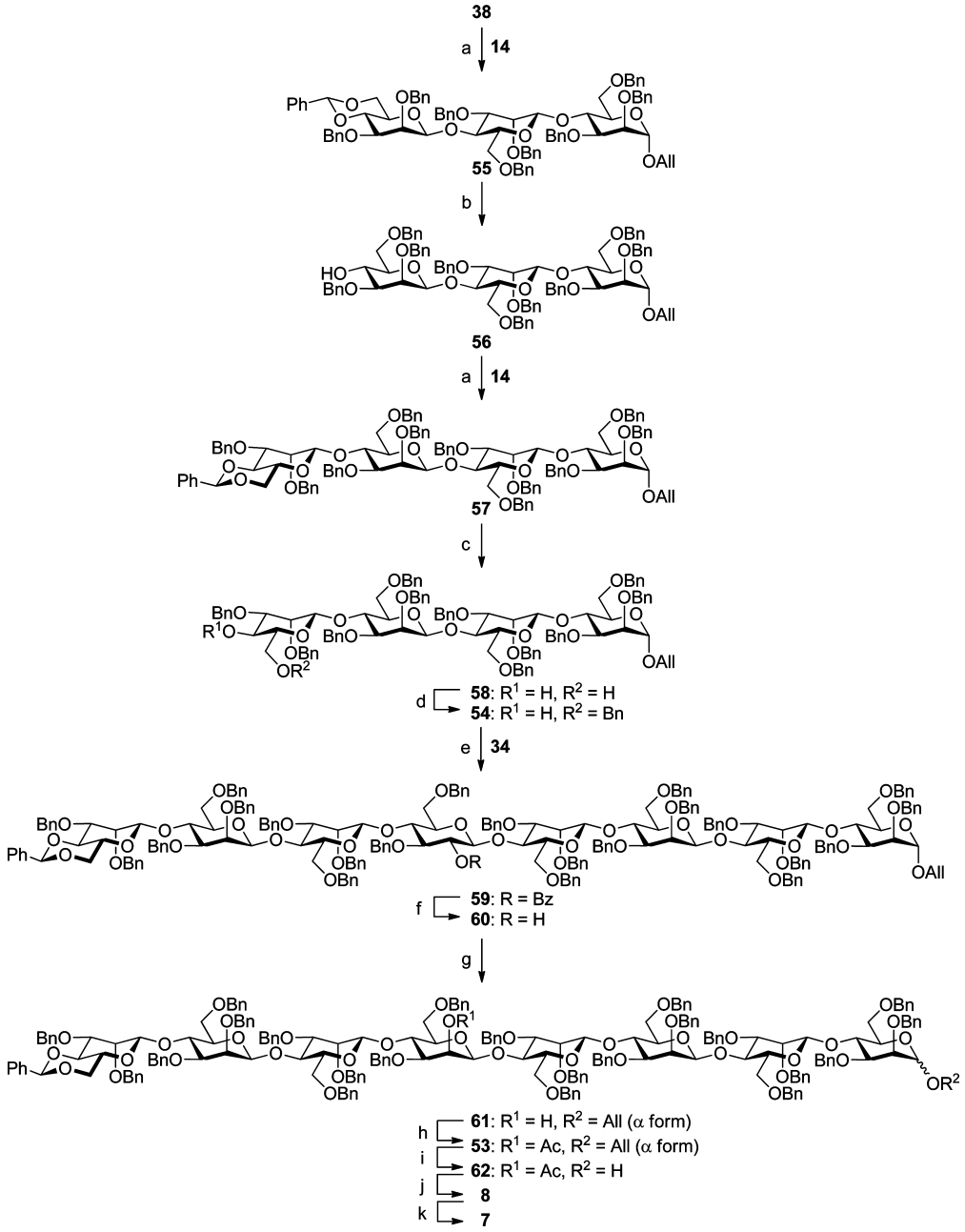
Scheme 8. Synthesis of Octasaccharides 7 and 8[a]



[a]Reagents and conditions: (a) **14**, BSP, Tf_2O, TTBP, CH_2Cl_2, MS3A, −78 to −60 °C, 1.5 h; Et_3N, $(EtO)_3P$, −60 °C to rt, 20 min; 68% for **55**, 65% for **57**; (b) Et_3SiH, TfOH, CH_2Cl_2, MS4A, −78 °C, 15 h; TBAF −78 °C to rt, 2 h; 44%; (c) CSA, neopentyl glycol, CH_2Cl_2, rt, 24 h; 78%; (d) Bu_2SnO, toluene, reflux, 3 h; BnBr, CsF, toluene, reflux, 6 h; 81%; (e) **34**, TMSOTf, CH_2Cl_2, MS4A, −70 to 0 °C, 4 h; 78%; (f) $LiAlH_4$, −40 to 0 °C, 30 min; 67%; (g) Ac_2O, DMSO, rt, 16 h; L-Selectride, THF, −78 °C to rt, 1.5 h; 58%; (h) Ac_2O, DMAP, pyr, rt, 3 h; 91%; (i) $PdCl_2$, MeOH/CH_2Cl_2 (1:1, v/v), rt, 3 h; 59%; (j) H_2, Pd/C, THF/MeOH/AcOH (2:1:0.3, v/v/v), rt, 23 h; H_2, Pd/C, H_2O/MeOH/AcOH (3:6:1, v/v/v), rt, 17 h; 63%; (k) NaOH, H_2O, rt, 1.5 h; 85%.

phenomenon implies that acetylation of β-(1→4)-linked oligomannosides in varied degrees may cause the disturbance of intra- and intermolecular hydrogen bond interactions.

In summary, we have synthesized β-(1→4)-linked hexa-, hepta-, and octamannoses as well as their mono- and diacetylated derivatives. Case by case, various reaction reagents and conditions were tuned to optimize the yields of the desired products and to minimize the side reactions. The efficiency of our strategy relied on the combined use of direct and indirect mannosylation methods to construct β-mannoside backbone and to install acetyl groups at late stage of the synthetic pathways. The acetyl group migrated reversibly from O2 to O3 positions in the central mannose residues, but no acetyl migration from O2 to O6 was observed. The oligomannose compounds **1**−**8** existed as anomers of hemiacetal forms, of which ratio could not be ambiguously determined because the α/β anomeric proton/carbon resonances either overlapped or interfered with HOD in solvent.

Biological Activity. The synthesized β-(1→4)-linked hexa-, hepta-, and octamannoses as well as their mono- and

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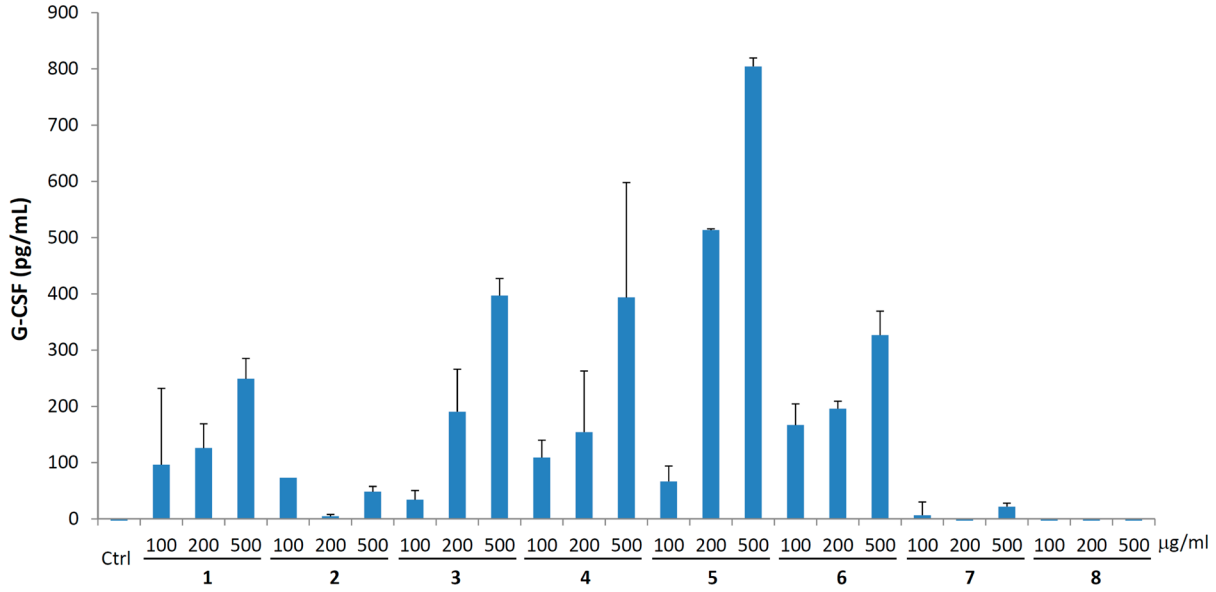

Figure 5. Induced expression of G-CSF by oligomannoses. RAW264.7 cells were treated with oligomannoses **1**−**8** at indicated concentrations for 6 h, and the media were collected for ELISA analysis. Ctrl: nontreated cells.

diacetylated derivatives were evaluated for their activities in inducing G-CSF expression. The oligosaccharide was dissolved in a solution buffered at neutral pH and the dissolved material was directly used for activity evaluation with an incubation time of 6 h. The possible acetyl isomerization of samples during incubation, presumably less than 25% in the case of compound **2** according to the results shown in Figure 3b (for 2 h incubation in D_2O) and Figure 3c (for 8 h incubation), was not rigorously determined. It was shown that both hexa- and heptamannoses (**1** and **4**, respectively) can significantly induce G-CSF expression (Figure 5). Surprisingly, octamannose (**7**) did not significantly induce G-CSF expression. It was also unexpected to observe that the expression of G-CSF induced by oligomannose is similar to the level induced by their acetylated derivatives. We also tested the bioactivity of oligomannose−mannitol compounds **52** and **63**, but no G-CSF induction was found (data not shown). More systematic studies on the location as well as the degree of acetylation may be needed for further investigation.

EXPERIMENTAL SECTION

General Methods. All chemicals were of commercial grade and were used without further purification. Anhydrous solvents were purchased and used without additional purification. Thin-layer chromatography (TLC) was performed on silica gel 60 F254 glass plate. Detection was effected by exposure to UV light (254 nm) and/or by charring with sulfuric acid (5% in ethanol solution). Column chromatography was performed on silica gel 60 (40−60 μm). Melting points were determined with a melting point apparatus and were uncorrected. All reactions were carried out under an argon atmosphere. ^{1}H and ^{13}C NMR spectra were recorded on a 600 MHz spectrometer at 25 °C. In ^{1}H NMR spectra, chemical shifts were referenced to internal tetramethylsilane (δ 0.00 ppm in $CDCl_3$), C_6D_5H (δ 7.16 ppm in C_6D_6), or HOD (δ 4.79 ppm in D_2O or in CD_3CO_2D/D_2O (1:99, v/v)). In ^{13}C NMR spectra, chemical shifts were referenced to the carbon signals in $CDCl_3$ (δ 77.00 ppm) and C_6D_6 (δ 128.39 ppm). In ^{13}C NMR spectra acquired in D_2O and in CD_3CO_2D/D_2O (1:99, v/v), chemical shifts were corrected to 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) as a reference. Coupling constants are given in hertz. Signal splitting patterns are described as singlet (s), doublet (d), triplet (t), double of doublet (dd), double of triplet (dt), triple of doublet (td), double of doublet doublet (ddd), broad signal (br), and multiplet (m). Mass spectra (MS) were recorded on an ESI-TOF mass spectrometer or a MALDI-TOF/TOF mass spectrometer using 2,5-dihydroxybenzoic acid (DHB) or 6-aza-2-thiothymine (ATT) as matrices. High-resolution mass spectra (HRMS) were recorded on an ESI-FTICR mass spectrometer.

Cell Treatment and ELISA Analysis. The mouse macrophage cell line RAW264.7 (ATCC TIB71) was maintained at 37 °C with 5% CO_2 in supplemented RPMI 1640 (10% fetal bovine serum, 0.2 mM L-glutamine, 100 U of penicillin/mL, and 100 μg of streptomycin/mL). RAW cells were plated at 6×10^5 cells in wells of 24-well plates (Greiner Bio-One) in supplemented RPMI 1640 one day prior to treatment. The cells were treated with synthetic oligomannoses at indicated concentrations for 6 h. Following the treatment, the medium were collected for G-CSF quantitation using the Quantikine mouse ELISA kits (R&D Systems) according to the manufacturer's protocol. Shown data are a representative data set from three independent experiments.

Benzyl 2,3,6-Tri-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-α-D-mannopyranoside (16). *Representative Procedure for β-Selective Mannosylation.* To a stirred solution containing compound **14** (948 mg, 1.75 mmol, 1.25 equiv), 1-benzenesulfinylpiperidine (BSP) (404 mg, 1.93 mmol, 1.4 equiv), tri-*tert*-butylpyrimidine (TTBP) (871 mg, 3.51 mmol, 2.5 equiv) and activated 3 Å powdered molecular sieves (5 g) in CH_2Cl_2 (70 mL) at −60 °C under an argon atmosphere was added Tf_2O (350 μL, 2.08 mmol, 1.5 equiv). After 5 min, a solution of benzyl 2,3,6-tri-*O*-benzyl-α-D-mannopyranoside **13** (0.758 g, 1.40 mmol, 1.0 equiv) in CH_2Cl_2 (8 mL) was added. The mixture was cooled to −78 °C, stirred for 6 h, warmed to room temperature, and filtered. The filtrate washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was subjected to column chromatography on silica gel (hexane/EtOAc, 88:12) to give compound **16** (994 mg, 1.02 mmol, 73%): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.06 (td, J = 9.6, 4.7 Hz, 1H), 3.40 (dd, J = 10.0, 3.0 Hz, 1H), 3.54−3.65 (m, 3H), 3.69 (d, J = 2.8 Hz, 1H), 3.75 (dt, J = 9.5, 3.2 Hz, 1H), 3.79 (t, J = 2.7 Hz, 1H), 3.93 (dd, J = 8.4, 3.1 Hz, 1H), 3.99 (dd, J = 10.4, 4.8 Hz, 1H), 4.06 (t, J = 9.6 Hz, 1H), 4.22 (t, J = 9.0 Hz, 1H), 4.40 (d, J = 12.1, Hz, 1H), 4.46−4.59 (d overlapped s, 2H), 4.53−4.59 (2 overlapping d, 2H), 4.62−4.86 (m, 8H), 4.96 (d, J = 2.1 Hz, 1H), 5.50 (s, 1H), 7.15−7.41 (m, integration not determined), 7.44−7.48 (m, 2H); ^{13}C NMR ($CDCl_3$, 151 MHz) δ 67.3, 68.5, 69.1, 71.4, 72.45, 72.49, 72.8, 73.4, 74.9, 75.6, 76.2, 77.0, 78.0, 78.4, 78.7, 97.6, 101.3, 102.0, 126.1, 127.2, 127.3, 127.4, 127.5,

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127.7, 127.75, 127.80, 128.0, 128.1, 128.17, 128.20, 128.22, 128.3, 128.37, 128.44, 128.8, 137.4, 137.7, 138.2, 138.3, 138.6, 138.7, 139.1; HRMS (ESI) found m/z 993.4164 $[M + Na]^+$, calcd for $C_{61}H_{62}NaO_{11}$ 993.4190.

Benzyl 2,3,6-Tri-*O*-benzyl-4-*O*-(2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl)-α-D- mannopyranoside (17). *Representative Procedure for Reductive Ring-Opening of Benzylidene Acetal.* To a stirred solution of compound **16** (837 mg, 0.86 mmol, 1.0 equiv) in CH_2Cl_2 (8.7 mL) at 0 °C were added Et_3SiH (1.7 mL, 10.5 mmol, 12.2 equiv) and $BF_3{\cdot}OEt_2$ (0.17 mL, 1.34 mmol, 1.6 equiv). After the solution was stirred for 9 h at 0 °C, saturated aqueous $NaHCO_3$ was added. The organic layer was separated, dried over Na_2SO_4, and concentrated in vacuo. Chromatography of the residue on a silica gel column (hexane/EtOAc, 85:15) gave compound **17** (523 mg, 0.54 mmol, 62%): colorless oil; ^1H NMR ($CDCl_3$, 600 MHz) δ 2.69 (br s, 1H), 3.14 (dd, J = 9.3, 2.7 Hz, 1H), 3.21 (dt, J = 9.6, 4.9 Hz, 1H), 3.56 (dd, J = 10.4, 5.4 Hz, 1H), 3.59−3.66 (2 overlapping dd, 2H), 3.67−3.73 (dd overlapped with d, 2H), 3.78 (t, J = 2.8 Hz, 1H), 3.83 (ddd, J = 9.5, 4.3, 2.5 Hz, 1H), 3.93 (t, J = 9.5 Hz, 1H), 3.97 (dd, J = 8.3, 2.9 Hz, 1H), 4.26 (t, J = 8.9 Hz, 1H), 4.37−4.50 (m, 6H), 4.51 (s, 1H), 4.56 (d, J = 12.0 Hz, 1H), 4.61−4.74 (m, 5H), 4.78 (d, J = 12.1 Hz, 1H), 4.83 (d, J = 12.0 Hz, 1H), 4.95 (d, J = 2.3 Hz, 1H), 7.17−7.46 (m, integration not determined); ^{13}C NMR ($CDCl_3$, 151 MHz) δ 68.8, 69.1, 69.4, 71.1, 71.46, 71.51, 72.5, 72.7, 73.4, 73.7, 74.2, 74.6, 75.6, 75.7, 78.1, 81.8, 97.7, 101.4, 127.2, 127.3, 127.39, 127.44, 127.5, 127.57, 127.60, 127.70, 127.73, 127.8, 128.05, 128.13, 128.2, 128.3, 128.37, 128.41, 128.42, 137.5, 138.0, 138.1, 138.3, 138.4, 138.9, 139.2; MS (ESI) found m/z 995.4 $[M + Na]^+$, calcd for $C_{61}H_{64}NaO_{11}$ 995.4.

Benzyl 3-*O*-Benzyl-4,6-*O*-benzylidene-2-*O*-*p*-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (18). According to the representative procedure for β-selective mannosylation, the reaction compound of **17** (254 mg, 0.26 mmol) and phenyl 2-*O*-*p*-methoxybenzyl-3-*O*-benzyl-4,6-*O*-benzylidene-1-deoxy-1-thio-α-D-mannopyranoside (**15**, 182 mg, 0.32 mmol) was performed, followed by quench with Et_3N (220 μL, 1.58 mmol) and $(EtO)_3P$ (109 μL, 0.64 mmol), to give compound **18** (244 mg, 0.17 mmol, 65%) after chromatography on silica gel (hexane/EtOAc, 80:20): colorless oil; ^1H NMR ($CDCl_3$, 600 MHz) δ 3.04 (td, J = 9.7, 4.8 Hz, 1H), 3.15−3.25 (m, 1H), 3.31 (dd, J = 9.2, 2.8 Hz, 1H), 3.38 (dd, J = 9.8, 2.9 Hz, 1H), 3.64−3.74 (m, 4H), 3.75−3.84 (m, 5H), 3.92−3.99 (m, 2H), 4.02 (t, J = 9.6 Hz, 1H), 4.14 (t, J = 9.4 Hz, 1H), 4.21−4.29 (t overlapped with d, 2H), 4.40−4.51 (m, 5H), 4.53 (2 overlapping s, 2H), 4.58 (d, J = 12.4 Hz, 1H), 4.61−4.79 (m, 10H), 4.87 (d, J = 12.4 Hz, 1H), 4.95 (d, J = 2.1 Hz, 1H), 5.47 (s, 1H), 6.78−6.84 (m, 2H), 7.07−7.40 (m, integration not determined), 7.41−7.49 (m, 2H); ^{13}C NMR ($CDCl_3$, 151 MHz, selected signals) δ 55.2, 67.2, 68.5, 69.0, 69.29, 69.34, 71.5, 72.2, 72.3, 72.6, 72.7, 73.4, 73.5, 74.2, 74.6, 75.5, 75.7, 75.9, 76.1, 76.7, 78.35, 78.38, 78.6, 80.7, 97.6, 101.3, 101.5, 102.2, 113.5, 126.1, 126.9, 127.1, 127.2, 127.2, 127.35, 127.42, 127.5, 127.59, 127.64, 127.8, 128.0, 128.11, 128.14, 128.2, 128.3, 128.4, 128.7, 137.4, 137.7, 138.3, 138.36, 138.37, 138.6, 139.05, 139.10, 139.3, 159.1; MS (MALDI, DHB) found m/z 1455.6 $[M + Na]^+$, calcd for $C_{89}H_{92}NaO_{17}$: 1455.6.

Benzyl 3-*O*-Benzyl-2-*O*-*p*-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (19). *Representative Procedure for Transacetalation.* To a stirred solution of compound **18** (562 mg, 0.39 mmol, 1.0 equiv) and neopentyl glycol (122 mg, 1.17 mmol, 3.0 equiv) in CH_2Cl_2 (3.9 mL) was added camphorsulfonic acid (18 mg, 0.08 mmol, 0.2 equiv). After being stirred for 1 day at room temperature, saturated aqueous $NaHCO_3$ was added. The organic layer was separated, washed with water, dried over Na_2SO_4, and concentrated in vacuo. Chromatography of the residue on a silica gel column (hexane/EtOAc, 55:45) gave compound **19** (388 mg, 0.29 mmol, 74%): colorless oil; ^1H NMR ($CDCl_3$, 600 MHz) δ 3.01−3.14 (m, 2H), 3.18−3.29 (m, 2H), 3.40 (dd, J = 11.8, 6.7 Hz, 1H), 3.53−3.58 (2 overlapping dd, 2H), 3.62−3.70 (m, 3H), 3.71−3.84 (m, 8H), 3.96 (dd, J = 8.7, 3.0 Hz, 1H), 4.11−4.17 (m, 2H), 4.25−4.34 (m, 3H), 4.41 (d, J = 12.1 Hz, 1H), 4.44−4.53 (m, 5H), 4.59 (d, J = 12.3 Hz, 1H), 4.61−4.80 (m, 9H), 4.90 (d, J = 12.5 Hz, 1H), 4.95 (d, J = 2.0 Hz, 1H), 6.75−6.84 (m, 2H), 7.14−7.41 (m, integration not determined); ^{13}C NMR ($CDCl_3$, 151 MHz, selected signals) δ 55.3, 62.8, 67.3, 69.1, 69.3, 69.5, 70.9, 71.6, 72.6, 72.68, 72.73, 73.2, 73.4, 73.6, 73.7, 74.4, 75.46, 75.51, 75.56, 75.62, 75.8, 76.5, 78.3, 80.3, 82.0, 97.7, 101.37, 101.39, 113.6, 127.1, 127.2, 127.26, 127.28, 127.4, 127.47, 127.52, 127.6, 127.72, 127.74, 127.77, 127.83, 127.88, 127.89, 128.06, 128.14, 128.2, 128.3, 128.35, 128.43, 128.5, 129.6, 130.7, 137.4, 137.8, 138.32, 138.33, 138.4, 138.8, 138.9, 139.3, 159.13; MS (MALDI, DHB) found m/z 1367.5951 $[M + Na]^+$, calcd for $C_{82}H_{88}NaO_{17}$ 1367.5919.

Benzyl 3,6-Di-*O*-benzyl −2-*O*-*p*-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (11). *Representative Procedure for Regioselective Benzylation.* To a stirred solution of diol **19** (367 mg, 0.27 mmol, 1.0 equiv) in toluene (8.0 mL) was added Bu_2SnO (136 mg, 0.55 mmol, 2.0 equiv) at room temperature. The mixture was stirred under reflux for 3 h and cooled to room temperature. CsF (191 mg, 1.26 mmol, 4.7 equiv) and BnBr (130 μL, 1.09 mmol, 4.0 equiv) were added to the above-prepared stannylene acetal. The mixture was stirred under reflux for another 6 h. After the mixture was cooled to 0 °C, saturated aqueous $NaHCO_3$ was added, and the mixture was diluted with EtOAc. The organic phase was separated, washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel (hexane/EtOAc, 80:20) to afford compound **11** (263 mg, 0.18 mmol, 67%): colorless oil; ^1H NMR ($CDCl_3$, 600 MHz) δ 2.67 (br s, 1H), 3.12 (dd, J = 9.4, 2.9 Hz, 1H), 3.19 (dt, J = 9.6, 4.8 Hz, 1H), 3.23−3.28 (m, 1H), 3.34 (dd, J = 9.3, 2.8 Hz, 1H), 3.50−3.56 (m, 2H), 3.56−3.66 (m, 4H), 3.69 (d, J = 2.7 Hz, 1H), 3.73 (d, J = 2.7 Hz, 1H), 3.75 (s, 3H), 3.77−3.83 (m, 2H), 3.89 (t, J = 9.5 Hz, 1H), 3.96 (dd, J = 8.4, 3.1 Hz, 1H), 4.17 (t, J = 9.3 Hz, 1H), 4.22 (t, J = 9.1 Hz, 1H), 4.27−4.33 (2 overlapping d, 2H), 4.35−4.54 (m, 9H), 4.57 (d, J = 12.2 Hz, 1H), 4.59−4.75 (m, 8H), 4.77 (d, J = 12.3 Hz, 1H), 4.87 (d, J = 12.4 Hz, 1H), 4.94 (d, J = 2.3 Hz, 1H), 6.73−6.80 (m, 2H), 7.08−7.40 (m, integration not determined); ^{13}C NMR ($CDCl_3$, 151 MHz, selected signals) δ 55.2, 68.7, 69.0, 69.4, 69.5, 71.1, 71.3, 71.5, 72.3, 72.6, 72.7, 73.3, 73.5, 73.7, 73.8, 74.0, 74.1, 74.6, 75.3, 75.5, 75.7, 75.8, 76.0, 78.3, 80.7, 81.8, 97.7, 101.5, 101.6, 113.5, 127.1, 127.2, 127.3, 127.4, 127.46, 127.51, 127.56, 127.64, 127.71, 127.74, 127.8, 127.9, 128.1, 128.2, 128.25, 128.33, 128.37, 128.41, 129.5, 131.1, 137.5, 138.12, 138.14, 138.3, 138.4, 138.5, 139.1, 139.2, 139.3, 159.0; MS (MALDI, DHB) found m/z 1457.6 $[M + Na]^+$, calcd for $C_{89}H_{94}NaO_{17}$ 1457.6.

Allyl 2-*O*-Benzoyl-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (21). Allyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (**20**, 21.3 g, 53.5 mmol, 1.0 equiv) was dissolved in pyridine (200 mL), and benzoyl chloride (9.4 mL, 80.9 mmol, 1.5 equiv) was added at 0 °C. The mixture was stirred at room temperature for 10 h and quenched with water at 0 °C. After being stirred for another 2 h, the mixture was diluted with EtOAc and washed successively with saturated aqueous $NaHCO_3$, water and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc, 70:30) to give compound **21** (26.7 g, 53.1 mmol, 99%): white solid; mp 118−119 °C; ^1H NMR (C_6D_6, 600 MHz) δ 3.21 (td, J = 9.7, 4.9 Hz, 1H), 3.49 (t, J = 10.2 Hz, 1H), 3.57 (t, J = 9.4 Hz, 1H), 3.78−3.89 (m, 2H), 4.08−4.23 (m, 2H), 4.43 (d, J = 8.0 Hz, 1H), 4.77 (d, J = 12.4 Hz, 1H), 4.82−4.93 (m, 2H), 5.10−5.17 (m, 1H), 5.22 (s, 1H), 5.56−5.65 (m, 1H), 5.77 (t, J = 8.6 Hz, 1H), 6.96−7.02 (m, 3H), 7.07 (t, J = 7.5 Hz, 2H), 7.12−7.18 (m, integration not determined), 7.20−7.27 (m, 4H), 7.63 (d, J = 7.8 Hz, 2H), 8.16 (d, J = 7.8 Hz, 2H); ^{13}C NMR (C_6D_6, 151 MHz) δ 66.9, 69.1, 70.1, 74.1, 74.2, 78.6, 82.3, 101.4, 101.7, 117.1, 126.9, 128.0, 128.7, 128.8, 128.8, 128.9, 129.4, 130.5, 131.2, 133.4, 134.5, 138.7, 139.2, 165.4; HRMS (ESI) found m/z 525.1869 $[M + Na]^+$, calcd for $C_{30}H_{30}NaO_7$ 525.1889.

Allyl 2-*O*-Benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside (12). According to the representative procedure for reductive ring-opening of benzylidene acetal, compound **21** (2.69 g, 5.35 mmol) was treated with Et_3SiH (4.3 mL, 26.6 mmol) and CF_3CO_2H (2.0 mL, 26.9 mmol) at 0 °C for 1 h to give compound **12** (2.38 g, 4.72 mmol, 88%) after

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chromatography on a silica gel column (hexane/EtOAc, 70:30): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.84 (d, J = 2.5 Hz, 1H), 3.54 (dt, J = 9.5, 4.8 Hz, 1H), 3.67 (t, J = 9.1 Hz, 1H), 3.74−3.83 (m, 3H), 4.00−4.10 (m, 1H), 4.25−4.33 (m, 1H), 4.56−4.60 (2 overlapping d, 2H), 4.63 (d, J = 12.1 Hz, 1H), 4.68 (d, J = 11.6 Hz, 1H), 4.72 (d, J = 11.6 Hz, 1H), 5.02−5.10 (m, 1H), 5.16−5.22 (m, 1H), 5.28 (t, J = 8.7 Hz, 1H), 5.70−5.80 (m, 1H), 7.12−7.22 (m, 5H), 7.27−7.32 (m, 1H), 7.33−7.38 (m, 4H), 7.44 (t, J = 7.7 Hz, 2H), 7.53−7.60 (m, 1H), 8.00−8.06 (m, 2H); ^{13}C NMR ($CDCl_3$, 151 MHz) δ 69.6, 70.3, 72.1, 73.3, 73.7, 74.1, 74.3, 82.1, 99.9, 117.2, 127.7, 127.8 128.0, 128.3, 128.4, 129.7, 129.9, 133.0, 133.6, 137.7, 137.9, 165.2; MS (ESI) found m/z 527.2 $[M + Na]^+$, calcd for $C_{30}H_{32}NaO_7$ 527.2; HRMS found m/z 527.2046 $[M + Na]^+$, calcd calcd for $C_{30}H_{32}NaO_7$ 527.2052.

Allyl 2-*O*-Benzoyl-3,6-di-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-*β*-D- mannopyranosyl)-*β*-D-glucopyranoside (22). According to the representative procedure for β-selective mannosylation, the reaction of compounds **12** (3.01 g, 5.97 mmol) and **14** (4.06 g, 7.51 mmol) was performed, followed by quench with Et_3N and $(EtO)_3P$, to give compound **22** (3.98 g, 4.26 mmol, 71%) after chromatography on silica gel (hexane/EtOAc, 90:10): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.10 (td, J = 9.7, 4.8 Hz, 1H), 3.41 (dd, J = 9.9, 3.0 Hz, 1H), 3.43−3.48 (m, 1H), 3.52 (t, J = 10.3 Hz, 1H), 3.59 (dd, J = 11.0, 3.8 Hz, 1H), 3.68 (dd, J = 2.3, 11.0 Hz, 1H), 3.72−3.78 (m, 2H), 4.00−4.12 (m, 4H), 4.28−4.35 (m, 1H), 4.43 (d, J = 12.1 Hz, 1H), 4.54 (s, 1H), 4.55−4.62 (m, 3H), 4.65 (d, J = 12.1 Hz, 1H), 4.73 (d, J = 12.4 Hz, 1H), 4.84 (d, J = 12.0 Hz, 1H), 4.88 (d, J = 12.0 Hz, 1H), 4.92 (d, J = 11.5 Hz, 1H), 5.07−5.11 (m, 1H), 5.16−5.23 (m, 1H), 5.26 (dd, J = 8.1, 9.0 Hz, 1H), 5.50 (s, 1H), 5.69−5.83 (m 1H), 7.03−7.13 (m, 5H), 7.20−7.49 (m, integration not determined), 7.51−7.58 (m, 1H), 7.96−8.02 (m, 2H); ^{13}C NMR ($CDCl_3$, 151 MHz) δ 67.3, 68.5, 68.6, 69.5, 72.6, 73.2, 73.6, 74.4, 74.98, 75.02, 78.1, 78.3, 78.7, 80.6, 99.9, 101.3, 101.8, 117.2, 126.1, 127.2, 127.4, 127.51, 127.53, 127.7, 127.8, 128.0, 128.1, 128.2, 128.26, 128.32, 128.5, 128.8, 129.8, 130.1, 132.9, 133.8, 137.6, 137.8, 138.5, 138.6, 165.1; MS (ESI) found m/z 957.5 $[M + Na]^+$, calcd for $C_{57}H_{58}NaO_{12}$ 957.4.

Allyl 2-*O*-Benzoyl-3,6-di-*O*-benzyl-4-*O*-(2,3,6-tri-*O*-benzyl-*β*-D-mannopyranosyl)-*β*-D-glucopyranoside (23). According to the representative procedure for reductive ring-opening of benzylidene acetal, compound **22** (862 mg, 0.92 mmol) was treated with Et_3SiH (1.8 mL, 11.1 mmol) and $BF_3{\cdot}OEt_2$ (187 μL, 1.48 mmol) at 0 °C for 1.5 h to give compound **23** (637 mg, 0.68 mmol, 74%) after chromatography on a silica gel column (hexane/EtOAc, 70:30): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.78 (br s, 1H), 3.16 (dd, J = 9.5, 3.0 Hz, 1H), 3.26 (dt, J = 9.7, 4.9 Hz, 1H), 3.51−3.57 (m, 2H), 3.61 (dd, J = 10.2, 4.9 Hz, 1H), 3.66 (dd, J = 11.0, 4.1 Hz, 1H), 3.72 (dd, J = 10.9, 2.6 Hz, 1H), 3.75 (d, J = 2.9 Hz, 1H), 3.80 (t, J = 8.9 Hz, 1H), 3.95 (t, J = 9.5 Hz, 1H), 4.02−4.10 (m, 2H), 4.31 (ddt, J = 13.4, 4.7, 1.6 Hz, 1H), 4.35−4.51 (m, 5H), 4.54 (s, 1H), 4.59 (d, J = 7.9 Hz, 1H), 4.60−4.68 (m, 2H), 4.77 (d, J = 11.9 Hz, 1H), 4.85 (d, J = 11.9 Hz, 1H), 4.93 (d, J = 11.7 Hz, 1H), 5.06−5.10 (m, 1H), 5.17−5.23 (m, 1H), 5.28 (dd, J = 9.0, 8.0 Hz, 1H), 5.76 (dddd, J = 17.1, 10.7, 6.2, 4.7 Hz, 1H), 6.96−7.00 (m, 2H), 7.01−7.08 (m, 3H), 7.20−7.35 (m, integration not determined), 7.37−7.43 (m, 4H), 7.51−7.58 (m, 1H), 7.91−8.04 (m, 2H); ^{13}C NMR ($CDCl_3$, 151 MHz) δ 68.8, 68.9, 69.5, 71.1, 71.6, 73.1, 73.6, 73.6, 74.0, 74.2, 74.5, 74.6, 75.0, 77.4, 80.4, 81.7, 99.8, 101.1, 117.2, 127.0, 127.4, 127.5, 127.6, 127.60, 127.67, 127.73, 127.75, 127.82, 127.9, 128.1, 128.2, 128.3, 128.4, 128.5, 129.8, 130.0, 132.9, 133.8, 137.86, 137.93, 138.1, 138.6, 138.7, 165.6; MS (ESI) found m/z 959.4 $[M + Na]^+$, calcd for $C_{57}H_{60}NaO_{12}$ 959.4.

Allyl 2,3-Di-*O*-benzyl-4,6-benzylidene-*β*-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-*β*-D-mannopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-*β*-D-glucopyranoside (24). According to the representative procedure for β-selective mannosylation, the reaction of compounds **23** (2.83 g, 3.02 mmol) and **14** (2.04 g, 3.77 mmol) was performed, followed by quench with Et_3N and $(EtO)_3P$, to give compound **24** (2.19 g, 1.60 mmol, 53%) after chromatography on silica gel (toluene/EtOAc, 95:5): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.06 (td, J = 9.7, 4.8 Hz, 1H), 3.18−3.25 (m, 1H), 3.30 (dd, J = 9.2, 2.9 Hz, 1H), 3.40 (dd, J = 9.8, 3.0 Hz, 1H), 3.45−3.52 (m, 2H), 3.52−3.59 (m, 2H), 3.63 (dd, J = 11.1, 3.9 Hz, 1H), 3.70−3.75 (m, 3H), 3.77 (t, J = 9.0 Hz, 1H), 3.98 (dd, J = 4.8, 10.4 Hz, 1H), 4.03−4.10 (m, 3H), 4.17 (t, J = 9.3 Hz, 1H), 4.23 (d, J = 11.8 Hz, 1H), 4.27−4.34 (m, 1H), 4.41−4.49 (m, 3H), 4.50−4.59 (m, 4H), 4.61−4.69 (m, 3H), 4.74−4.83 (m, 4H), 4.85 (d, J = 12.0, 1H), 4.98 (d, J = 12.1 Hz, 1H), 5.05−5.09 (m, 1H), 5.16−5.22 (m, 1H), 5.29 (t, J = 8.6 Hz, 1H), 5.72−5.82 (m, 1H), 6.92−6.96 (m, 1H), 6.98−7.02 (m, 1H), 7.04 (d, J = 7.5 Hz, 1H), 7.13−7.42 (m), 7.43−7.47 (m, 2H), 7.51−7.57 (m, 1H), 7.96 (d, J = 8.1 Hz, 1H); ^{13}C NMR ($CDCl_3$, 151 MHz) δ 67.2, 68.5, 68.7, 69.3, 69.5, 72.4, 73.1, 73.4, 73.5, 74.1, 74.2, 74.9, 75.0, 75.4, 76.0, 76.1, 77.1, 77.8, 78.4, 78.6, 80.55, 80.61, 99.9, 101.2, 101.3, 102.2, 117.2, 126.1, 126.9, 127.0, 127.2, 127.3, 127.35, 127.43, 127.46, 127.53, 127.66, 127.67, 127.8, 127.9, 128.0, 128.08, 128.10, 128.13, 128.2, 128.26, 128.29, 128.5, 128.8, 129.8, 130.1, 132.9, 133.8, 137.7, 137.9, 138.2, 138.5, 138.7, 138.9, 139.1, 165.2; MS (ESI) found m/z 1389.7 $[M + Na]^+$, calcd for $C_{84}H_{86}NaO_{17}$ 1389.6.

2,3-Di-*O*-benzyl-4,6-benzylidene-*β*-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-*β*-D-mannopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-*β*-D-glucopyranose (25). *Representative Procedure for Removal of Allyl Protecting Group.* To a solution of compound **24** (220 mg, 0.16 mmol, 1.0 equiv) in $MeOH/CH_2Cl_2$ (1:1, v/v, 2.6 mL) was added a suspension of $PdCl_2$ (6 mg, 0.03 mmol, 0.2 equiv) in CH_2Cl_2. After being stirred at room temperature for 6 h, additional $PdCl_2$ (18 mg, 0.10 mmol, 0.6 equiv) was added. The mixture was stirred for another 2 h and then diluted with EtOAc. The mixture was filtered through a small pad of silica gel. The filtrate was washed with saturated aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane/EtOAc, 70:30) to give compound **25** (164 mg, 0.12 mmol, 77%): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.07 (td, J = 4.9, 9.7 Hz, integration not determined), 3.20−3.26 (m, integration not determined), 3.26−3.31 (m, integration not determined), 3.40 (dd, J = 3.0, 9.8 Hz, integration not determined), 3.44−3.52 (m, integration not determined), 3.52−3.66 (m, integration not determined), 3.81 (t, J = 9.1 Hz, integration not determined), 3.95−4.21 (m, integration not determined), 4.23 (d, J = 12.0 Hz, integration not determined), 4.39−4.57 (m, integration not determined), 4.59−4.73 (m, integration not determined), 4.75−4.87 (m, integration not determined), 5.03−5.15 (m, integration not determined), 5.49 (s, integration not determined), 5.55 (d, J = 3.6 Hz, integration not determined), 6.93−7.49 (m, integration not determined), 7.51−7.58 (m, integration not determined), 7.90−8.01 (m, integration not determined); ^{13}C NMR ($CDCl_3$, 151 MHz, selected signals) δ 67.3, 68.49, 68.54, 68.7, 69.3, 69.4, 70.1, 72.4, 72.5, 73.4, 73.5, 73.6, 73.7, 74.2, 74.3, 74.5, 74.8, 74.9, 75.36, 75.40, 75.9, 76.06, 76.10, 76.13, 76.2, 77.5, 77.6, 77.8, 78.4, 78.6, 80.2, 80.50, 80.52, 90.5, 96.1, 101.1, 101.2, 101.3, 102.2, 126.1, 127.0, 127.1, 127.2, 127.3, 127.36, 127.43, 127.47, 127.51, 127.6, 127.65, 127.68, 127.8, 127.90, 127.94, 128.0, 128.05, 128.09, 128.12, 128.2, 128.28, 128.30, 128.4, 128.51, 128.53, 128.8, 129.3, 129.6, 129.8, 129.9, 133.2, 133.4, 137.66, 137.69, 138.2, 138.3, 138.5, 138.66, 138.72, 138.9, 139.1, 165.9, 167.1; MS (MALDI, DHB) found m/z 1349.5 $[M + Na]^+$, calcd for $C_{81}H_{82}NaO_{17}$ 1349.5.

2,3-Di-*O*-benzyl-4,6-benzylidene-*β*-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-*β*-D-mannopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-*α*/*β*-D-glucopyranosyl Trichloroacetimidate (10). *Representative Procedure for Trichloroacetimidation.* To a solution of compound **25** (517 mg, 0.39 mmol, 1.0 equiv) and trichloroacetonitrile (391 μL, 3.90 mmol, 10 equiv) in CH_2Cl_2 (6.7 mL) was added a solution of DBU (5.8 μL, 0.04 mmol, 0.1 equiv) in CH_2Cl_2 (0.1 mL) at 0 °C. After being stirred for 1 h, the mixture was concentrated under reduced pressure. Chromatography of the residue on a silica gel column (hexane/EtOAc/Et_3N, 70:30:1) gave compound **10** (411 mg, 0.28 mmol, 72%): colorless oil; ^{1}H NMR (C_6D_6, 600 MHz) δ 3.03−3.14 (m, integration not determined), 3.23−3.42 (m, integration not determined), 3.45−3.52 (m, integration not determined), 3.53−3.71 (m, integration not determined), 3.73−3.82 (m, integration not determined), 3.87 (d, J = 2.8 Hz, integration not determined), 3.96 (t, J = 8.7 Hz, integration not determined), 4.00−

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4.07 (m, integration not determined), 4.16−4.35 (m, integration not determined), 4.36−4.43 (m, integration not determined), 4.43−4.65 (m, integration not determined), 4.71 (s, integration not determined), 4.75−4.82 (m, integration not determined), 4.90−5.15 (m, integration not determined), 5.21 (s, integration not determined), 5.22 (s, integration not determined), 5.33 (d, J = 12.3 Hz, integration not determined), 5.44 (d, J = 12.4 Hz, integration not determined), 5.66−5.75 (m), 6.02 (t, J = 8.4 Hz), 6.27 (d, J = 7.9 Hz), 6.92−7.30 (m), 7.32−7.40 (m), 7.44−7.52 (m, integration not determined), 7.54−7.62 (m, integration not determined), 8.08−8.18 (m, integration not determined), 8.37 (s, integration not determined), 8.58 (s, integration not determined); ^{13}C NMR (C_6D_6, 151 MHz, selected signals) δ 68.1, 69.0, 69.1, 69.2, 69.86, 69.92, 72.7, 72.9, 73.3, 73.5, 73.6, 74.0, 74.1, 74.18, 74.22, 74.4, 74.7, 75.3, 75.39, 75.43, 76.1, 76.5, 76.7, 76.77, 76.83, 77.0, 77.6, 77.8, 78.3, 78.9, 79.6, 79.7, 81.0, 81.3, 81.6, 91.5, 92.0, 95.0, 97.3, 101.7, 101.8, 102.0, 102.7, 127.0, 127.59, 127.64, 127.8, 127.9, 129.2, 130.4, 130.5, 130.6, 133.4, 133.5, 138.70, 138.73, 139.1, 139.17, 139.24, 139.8, 139.90, 139.94, 140.05, 140.09, 140.5, 161.0, 162.2, 165.5, 166.0; MS (MALDI, ATT) found m/z 1492.5 [M + Na]$^+$, calcd for $C_{83}H_{82}Cl_3NNaO_{17}$ 1492.5.

Benzyl 2,3-Di-*O*-benzyl-4,6-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-*O*-*p*-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (26). *Representative Procedure for Glycosylation.* Acceptor **11** (72 mg, 50.2 μmol, 1.0 equiv) and trichloroacetimidate **10** (111 mg, 75.4 μmol, 1.5 equiv) were combined and coevaporated three times with toluene. The mixture was dissolved in CH_2Cl_2 (1.5 mL), and molecular sieves 4 Å (160 mg) were added. The mixture was stirred for 30 min and cooled to −75 °C, and a solution of TMSOTf (1.35 μL, 7.5 μmol, 0.15 equiv) in CH_2Cl_2 (10 μL) was added. The mixture was stirred for 3.5 h, during which the temperature increased to 0 °C. After being stirred at 0 °C for another 3 h, the reaction was quenched by addition of Et_3N (5.2 μL, 37.4 μmol, 0.75 equiv). Saturated aqueous $NaHCO_3$ was added. The mixture was diluted with CH_2Cl_2 and washed with water. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane/EtOAc, 80:20) to give compound **26** (111 mg, 40.4 μmol, 80%): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.86−2.93 (m, 1H), 3.04 (td, J = 9.6, 4.9 Hz, 1H), 3.14−3.22 (m, 4H), 3.24−3.31 (m, 3H), 3.37−3.51 (m, 7H), 3.52−3.63 (m, 5H), 3.63−3.67 (2 overlapping d, 2H), 3.68−3.74 (m, 5H), 3.74−3.81 (m, 2H), 3.91−4.10 (m, 6H), 4.11−4.23 (m, 5H), 4.28 (d, J = 12.2 Hz, 1H), 4.33 (s, 1H), 4.36−4.85 (m, 33H), 4.92 (d, J = 2.3 Hz, 1H), 4.99 (d, J = 12.2 Hz, 1H), 5.19 (dd, J = 9.4, 8.3 Hz, 1H), 5.48 (s, 1H), 6.61−6.72 (m, 2H), 6.84−6.92 (m, 2H), 6.93−7.00 (m, 3H), 7.02−7.42 (m), 7.42−7.48 (m, 2H), 7.51−7.59 (m, 1H), 7.78−7.97 (m, 2H); ^{13}C NMR ($CDCl_3$, 151 MHz) δ 55.2, 67.2, 68.3, 68.5, 69.0, 69.26, 69.32, 69.34, 71.5, 72.1, 72.29, 72.31, 72.4, 72.6, 72.7, 73.27, 73.29, 73.33, 73.4, 73.5, 73.7, 74.0, 74.16, 74.23, 74.3, 74.9, 75.1, 75.3, 75.4, 75.5, 75.66, 75.69, 75.8, 76.0, 76.1, 77.1, 78.1, 78.2, 78.5, 78.6, 80.46, 80.52, 80.6, 80.9, 97.6, 100.5, 101.27, 101.33, 101.40, 102.2, 113.4, 126.1, 126.8, 126.9, 126.97, 126.99, 127.02, 127.06, 127.09, 127.2, 127.27, 127.30, 127.35, 127.41, 127.44, 127.47, 127.52, 127.60, 127.63, 127.64, 127.7, 127.8, 127.9, 128.0, 128.05, 128.07, 128.11, 128.17, 128.20, 128.26, 128.29, 128.34, 128.4, 128.8, 129.2, 129.8, 129.9, 131.3, 132.9, 137.4, 137.7, 138.1, 138.3, 138.4, 138.5, 138.7, 138.8, 138.9, 139.0, 139.09, 139.11, 139.2, 139.4, 158.8, 165.0; MS (MALDI, DHB) found m/z 2766.0 [M + Na]$^+$, calcd for $C_{170}H_{174}NaO_{33}$ 2766.2.

Benzyl 2,3-Di-*O*-benzyl-4,6-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-*O*-*p*-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (27). *Representative Procedure for Removal of the Benzoyl Group.* To a solution of compound **26** (110 mg, 40.1 μmol, 1.0 equiv) in Et_2O (4.2 mL) was added a suspension of $LiAlH_4$ (6.1 mg, 160.1 μmol, 4.0 equiv) in Et_2O (320 μL) at −40 °C. After being stirred at −40 °C for 30 min, the mixture was warmed to 0 °C. Saturated aqueous $NaHCO_3$ was added. The mixture was diluted with EtOAc and washed with water. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. Chromatography of the residue on a silica gel column (hexane/EtOAc, 85:15) gave compound **27** (75 mg, 28.4 μmol, 71%): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.03 (td, J = 9.6. 4.9 Hz, 1H), 3.08−3.16 (m, 2H), 3.18−3.24 (m, 4H), 3.27 (dd, J = 11.1, 3.2 Hz, 1H), 3.30−3.43 (m, 5H), 3.44−3.76 (m, 17H), 3.77−3.83 (m, 3H), 3.91−3.98 (m, 2H), 4.02 (t, J = 9.6 Hz, 1H), 4.12 (t, J = 9.3 Hz, 1H), 4.14−4.26 (m, 5H), 4.28 (d, J = 11.9 Hz, 1H), 4.32−4.42 (m, 5H), 4.43−4.54 (m, 12H), 4.57 (d, J = 12.3 Hz, 1H), 4.60−4.80 (m, 16H), 4.86 (d, J = 12.3 Hz, 1H), 4.94 (d, J = 2.1 Hz, 1H), 4.99 (d, J = 11.9 Hz, 1H), 5.47 (s, 1H), 6.69−6.74 (m, 2H), 7.11−7.41 (m, integration not determined), 7.42−7.45 (m, 2H); ^{13}C NMR ($CDCl_3$, 151 MHz) δ 55.2, 67.2, 68.5, 68.6, 69.0, 69.3, 69.4, 69.6, 71.2, 71.5, 72.2, 72.3, 72.4, 72.6, 72.7, 73.25, 73.34, 73.39, 73.41, 73.6, 73.8, 74.1, 74.2, 74.6, 74.7, 74.8, 74.9, 75.0, 75.19, 75.23, 75.45, 75.48, 75.7, 75.8, 76.0, 76.1, 77.07, 77.10, 78.2, 78.4, 78.6, 80.6, 80.7, 81.8, 82.5, 97.6, 101.1, 101.3, 101.4, 101.9, 102.2, 103.7, 113.5, 126.1, 126.6, 126.8, 126.9, 127.0, 127.1, 127.15, 127.19, 127.24, 127.3, 127.38, 127.41, 127.43, 127.46, 127.50, 127.56, 127.60, 127.63, 127.7, 128.0, 128.07, 128.10, 128.13, 128.16, 128.21, 128.25, 128.29, 128.32, 128.4, 128.8, 129.3, 131.0, 137.4, 137.7, 137.9, 138.0, 138.29, 138.31, 138.35, 138.42, 138.5, 138.7, 138.97, 139.03, 139.1, 139.2, 139.6, 158.9; MS (MALDI, DHB) found m/z 2662.2 [M + Na]$^+$, calcd for $C_{163}H_{170}NaO_{32}$ 2662.2.

Benzyl 2,3-Di-*O*-benzyl-4,6-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-*O*-*p*-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (9). *Representative Procedure for Gluco to Manno Epimerization.* A solution of compound **27** (66 mg, 25 μmol, 1 equiv) in DMSO (0.82 mL) and acetic anhydride (0.42 mL) was stirred for 16 h at room temperature. After the solution was cooled to 0 °C, saturated aqueous $NaHCO_3$ was added. The mixture was diluted with Et_2O, washed with saturated aqueous $NaHCO_3$, water, and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was coevaporated with toluene three times and dried in vacuo. The residue was dissolved in CH_2Cl_2 (1.0 mL) and MeOH (1.0 mL) and cooled to 0 °C. $NaBH_4$ (12 mg, 317 μmol, 13 equiv) was added in one portion. The mixture was stirred for 3 h, during which time the solution was allowed to warm to room temperature. Water was added. The mixture was diluted with CH_2Cl_2 and washed with water. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane/EtOAc, 65:35) to give compound **9** (42 mg, 15.9 μmol, 64%): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.70 (br s, 1H), 3.03 (td, J = 9.6, 4.8 Hz, 1H), 3.12−3.29 (m, 5H), 3.29−3.41 (m, 4H), 3.43−3.58 (m, 8H), 3.59−3.76 (m, 10H), 3.76−3.82 (m, 2H), 3.90 (br d, 1H), 3.91−3.98 (m, 2H), 4.00−4.09 (2 overlapping d, 2H), 4.11−4.19 (3 overlapping t, 3H), 4.20−4.42 (m, 8H), 4.44−4.54 (m, 13H), 4.57 (d, J = 12.3 Hz, 1H), 4.59−4.80 (m, 17H), 4.87 (d, J = 12.3 Hz, 1H), 4.94 (d, J = 2.1 Hz, 1H), 5.48 (s, 1H), 6.71−6.76 (m, 2H), 7.09−7.39 (m, integration not determined), 7.41−7.47 (m, 2H); ^{13}C NMR ($CDCl_3$, 151 MHz) δ 55.2, 67.2, 68.1, 68.5, 69.0, 69.1, 69.31, 69.34, 69.4, 69.5, 71.5, 71.6, 71.8, 72.2, 72.3, 72.4, 72.6, 72.7, 73.2, 73.3, 73.37, 73.43, 73.7, 74.0, 74.1, 74.7, 74.87, 74.90, 75.0, 75.16, 75.23, 75.51, 75.53, 75.7, 75.8, 75.86, 75.93, 76.1, 77.1, 78.2, 78.5, 78.6, 79.5, 80.7, 81.4, 97.6 ($^1J_{CH}$ = 168.5 Hz), 100.2 ($^1J_{CH}$ = 158.8 Hz), 101.3, 101.4 ($^1J_{CH}$ = 159.2 Hz), 101.6 ($^1J_{CH}$ = 155.7 Hz), 101.8 ($^1J_{CH}$ = 155.1 Hz), 102.1 ($^1J_{CH}$ = 156.4 Hz), 113.5, 126.1, 126.9, 127.1, 127.15, 127.24, 127.25, 127.30, 127.35, 127.39, 127.41, 127.44, 127.46, 127.50, 127.56, 127.62, 127.68, 127.73, 127.8, 127.96, 127.98, 128.06, 128.10, 128.13, 128.16, 128.18, 128.21, 128.25, 128.32, 128.4, 128.8, 129.3, 131.1, 137.4, 137.7, 138.30, 138.32, 138.36, 138.42, 138.5, 138.71, 138.74, 139.03, 139.05, 139.07, 139.14, 139.3, 158.9; MS (MALDI, DHB) found m/z 2662.0 [M + Na]$^+$, calcd for $C_{163}H_{170}NaO_{32}$ 2662.2.

Benzyl 2,3-Di-*O*-benzyl-4,6-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2-*O*-acetyl-3,6-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-*O*-*p*-methoxybenzyl-β-D-mannopyranosyl-(1→4)-

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2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (28). *Representative Procedure for Acetylation.* To a solution of compound **9** (42 mg, 15.9 μmol, 1 equiv) and DMAP (5 mg, 40.9 μmol, 3 equiv) in pyridine (1.8 mL) was added acetic anhydride (0.1 mL). The mixture was stirred for 3.5 h at room temperature and then quenched by addition of MeOH at 0 °C. After the mixture was stirred for 10 min, saturated $NaHCO_3$ was added. The mixture was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$ and water, dried over $MgSO_4$, filtrated, and concentrated in vacuo. Chromatography of the residue on a silica gel column (hexane/EtOAc, 70:30) gave compound **28** (38 mg, 14.2 μmol, 88%): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.01 (s, 3H), 3.03 (td, *J* = 9.7, 4.9 Hz, 1H), 3.12−3.19 (m, 3H), 3.19−3.35 (m, 5H), 3.37−3.43 (m, 3H), 3.43−3.74 (m, 16H), 3.76−3.83 (m, 2H), 3.90−3.99 (m, 3H), 4.02 (t, *J* = 9.6 Hz, 1H), 4.07 (t, *J* = 9.4 Hz, 1H), 4.12−4.24 (m,4H), 4.25−4.32 (2 overlapping d, 2H), 4.32−4.42 (m, 4H), 4.43−4.82 (m, 31H), 4.86 (d, *J* = 12.3 Hz, 1H), 4.94 (d, *J* = 1.8 Hz, 1H), 5.39 (d, *J* = 2.9 Hz, 1H), 5.47 (s, 1H), 6.65−6.72 (m, 2H), 7.09−7.41 (m, integration not determined), 7.41−7.48 (m, 2H); ^{13}C NMR ($CDCl_3$, 600 MHz) δ 21.0, 55.2, 67.2, 68.5, 68.8, 68.95, 69.0, 69.1, 69.36, 69.42, 71.4, 71.5, 71.8, 72.1, 72.3, 72.4, 72.6, 72.7, 73.1, 73.3, 73.39, 73.42, 73.7, 74.1, 74.2, 74.6, 74.90, 74.94, 75.0, 75.2, 75.30, 75.34, 75.5, 75.7, 75.8, 75.9, 76.1, 78.20, 78.23, 78.4, 78.6, 80.6, 80.7, 81.3, 97.7, 99.8, 101.2, 101.3, 101.4, 101.7, 102.2, 113.4, 126.1, 126.77, 126.84, 127.0, 127.10, 127.14, 127.2, 127.26, 127.32, 127.4, 127.45, 127.50, 127.6, 127.66, 127.73, 127.8, 127.96, 128.00, 128.03, 128.08, 128.12, 128.14, 128.18, 128.20, 128.25, 128.32, 128.4, 128.8, 129.3, 131.2, 137.4, 137.7, 138.3, 138.36, 138.42, 138.46, 138.52, 138.76, 138.79, 139.05, 139.14, 139.2, 139.3, 158.8, 170.3; MS (MALDI, DHB) found *m*/*z* 2704.2 $[M + Na]^+$, calcd for $C_{165}H_{172}NaO_{33}$ 2704.2.

β-D-Mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-2-*O*-acetyl-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-D-mannopyranose (2). *Representative Procedure for Global Deprotection of Benzyl, Benzylidene, and p-Methoxybenzyl Groups.* A mixture of compound **28** (8 mg, 2.98 μmol) and 10% Pd/C (8 mg) in THF/MeOH/AcOH (2:1:0.3, v/v/v, 1.0 mL) was stirred under an atmosphere of hydrogen at room temperature for 23 h. The mixture was passed through a pad of Celite to remove the catalyst and then concentrated under reduced pressure. The residue was subject to reversed-phase chromatography on a C18 column (1% AcOH in H_2O), followed by freezing drying, and gave compound **2** (2.0 mg, 1.94 μmol, 65%): white hydroscopic solid; ^{1}H NMR (1% CD_3CO_2D in D_2O, 600 MHz) δ 2.19 (s, integration not determined), 2.20 (s, integration not determined), 3.41−3.60 (m, integration not determined), 3.61−3.68 (m, integration not determined), 3.70−4.08 (m, integration not determined), 4.09−4.15 (m, integration not determined), 4.20 (d, *J* = 3.3 Hz, integration not determined), 4.72 (s, integration not determined), 4.73 (s, integration not determined), 4.76 (s, integration not determined), 4.83 (s, integration not determined), 4.91 (s, integration not determined), 4.95 (s, integration not determined), 5.12 (dd, *J* = 9.8, 3.1 Hz, integration not determined), 5.18 (s, integration not determined), 5.52 (d, *J* = 3.5 Hz, integration not determined); ^{13}C NMR (1% CD_3CO_2D in D_2O, 151 MHz, selected signals) δ 63.2, 63.4, 63.7, 69.4, 71.4, 71.7, 72.67, 72.70, 72.8, 72.85, 72.94, 73.20, 73.23, 73.4, 73.6, 74.1, 74.17, 74.21, 74.27, 74.29, 74.4, 75.48, 75.49, 76.0, 76.1, 77.51, 77.54, 77.71, 77.74, 77.9, 78.0, 79.12, 79.14, 79.19, 79.22, 79.29, 79.31, 79.5, 80.1, 96.4, 96.6, 101.8, 102.3, 102.8, 102.87, 102.92, 102.93, 103.0, 175.7, 176.1; MS found *m*/*z* 1055.3, calcd for $C_{38}H_{64}NaO_{32}$ 1055.3; HRMS found *m*/*z* 1033.3425 $[M + H]^+$, calcd for $C_{38}H_{65}O_{32}$ 1033.3459.

Benzyl 2,3-Di-*O*-benzyl-4,6-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (31). *Representative Procedure for Selective Deprotection of p-Methoxybenzyl Group.* Compound **9** (12 mg, 4.54 μmol, 1.0 equiv) was dissolved in a mixture of CH_3CN/toluene/H_2O (90:5:5, v/v/v, 1.0 mL) and cooled to 0 °C. CAN (12 mg, 21.9 mmol, 4.8 equiv) was added in one portion. The mixture was stirred at 0 °C for 30 min and at room temperature for another 1 h. The mixture was diluted with EtOAc and quenched with saturated aqueous $NaHCO_3$. The organic layer was washed with saturated aqueous $NaHCO_3$ and water, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane/EtOAc, 62:38) to give compound **31** (7 mg, 2.78 μmol, 58%): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.60 (br s, 1H), 2.78 (br s, 1H), 3.03 (td, *J* = 9.7, 4.9 Hz, 1H), 3.15−3.21 (m, 3H), 3.21−3.28 (m, 2H), 3.30 (dd, *J* = 9.1, 3.2 Hz, 1H), 3.35−3.41 (m, 3H), 3.47−3.59 (m, 7H), 3.61−3.69 (m, 5H), 3.72 (d, *J* = 2.9 Hz, 1H), 3.73 (d, *J* = 2.8 Hz, 1H), 3.78 (t, *J* = 2.7 Hz, 1H), 3.79−3.83 (m, 1H), 3.89 (d, *J* = 2.7 Hz, 1H), 3.92−3.98 (m, 3H), 4.03 (t, *J* = 9.6 Hz, 1H), 4.05−4.10 (2 overlapping t, 2H), 4.12−4.20 (2 overlapping t, 2H), 4.21−4.30 (m, 3H), 4.32−4.37 (2 overlapping d, 2H), 4.39 (d, *J* = 11.8 Hz, 1H), 4.41−4.54 (m, 15H), 4.57 (d, *J* = 12.3 Hz, 1H), 4.60−4.80 (m, 15H), 4.83 (d, *J* = 12.3 Hz, 1H), 4.94 (d, *J* = 2.2 Hz, 1H), 5.48 (s, 1H), 7.11−7.39 (m, integration not determined), 7.42−7.46 (m, 2H); ^{13}C NMR ($CDCl_3$, 151 MHz, selected signals) δ 67.2, 67.4, 68.1, 68.5, 69.0, 69.06, 69.14, 69.3, 69.40, 69.42, 71.2, 71.5, 71.8, 71.9, 72.2, 72.4, 72.5, 72.65, 72.70, 72.9, 73.36, 73.38, 73.42, 73.5, 74.1, 74.2, 74.7, 74.8, 74.9, 75.1, 75.17, 75.23, 75.3, 75.7, 75.8, 75.9, 76.1, 77.1, 78.1, 78.5, 78.6, 79.5, 78.0, 80.7, 81.5, 97.6, 100.32, 100.34, 101.3, 101.6, 102.1, 126.1, 126.9, 127.16, 127.19, 127.3, 127.40, 127.43, 127.60, 127.63, 127.72, 127.74, 128.0, 128.05, 128.07, 129.1, 128.2, 128.26, 128.28, 128.30, 128.36, 128.41, 128.8, 137.4, 137.7, 138.09, 138.14, 138.2, 138.26, 138.29, 138.5, 138.67, 138.74, 138.9, 139.0, 139.05, 139.09; MS (MALDI, DHB) found *m*/*z* 2542.1 $[M + Na]^+$, calcd for $C_{155}H_{162}NaO_{31}$ 2542.1.

Benzyl 2,3-Di-*O*-benzyl-4,6-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2-*O*-acetyl-3,6-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2-*O*-acetyl-3,6-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (32). According to the representative procedure for acetylation, alcohol **31** (76 mg, 30 μmol) was treated with acetic anhydride (0.55 mL) in pyridine (5.5 mL) and DMAP (22 mg, 180 μmol) for 4 h at room temperature to give acetate **32** (52 mg, 20.0 μmol, 66%) after chromatography on a silica gel column (hexane/EtOAc, 65:35): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.00−2.05 (2 overlapping s, 6H) 3.03 (td, *J* = 9.7, 4.9 Hz, 1H), 3.10−3.20 (m, 4H), 3.27 (dd, *J* = 9.1, 2.8 Hz, 1H), 3.30 (dd, *J* = 9.3, 2.8 Hz, 1H), 3.33−3.42 (m, 6H), 3.44−3.67 (m, 10H), 3.71 (d, *J* = 3.0 Hz, 1H), 3.76 (t, *J* = 2.7 Hz, 1H), 3.77−3.87 (m, 1H), 3.90−3.98 (m, 3H), 3.98−4.05 (2 overlapping t, 2H), 4.07 (t, *J* = 9.2 Hz, 1H), 4.16−4.23 (m, 2H), 4.25−4.23 (2 overlapping d, 2H), 4.36−4.77 (m, 34H), 4.83 (d, *J* = 12.2 Hz, 1H), 4.93 (d, *J* = 2.3 Hz, 1H), 5.35 (d, *J* = 3.1 Hz, 1H), 5.38 (d, *J* = 3.4 Hz, 1H), 5.47 (s, 1H), 7.09−7.40 (m), 7.42−7.46 (m); ^{13}C NMR ($CDCl_3$, 151 MHz, selected signals) δ 21.0, 67.2, 68.5, 68.6, 68.7, 68.8, 68.9, 69.0, 69.1, 69.4, 71.3, 71.38, 71.44, 72.10, 72.11, 72.4, 72.5, 72.7, 73.0, 73.3, 73.39, 73.40, 74.1, 74.2, 74.57, 74.58, 74.7, 74.9, 74.95, 75.00, 75.3, 75.66, 75.71, 75.73, 75.9, 76.1, 78.10, 78.13, 78.4, 78.6, 78.9, 80.5, 81.3, 97.7, 99.5, 99.7, 101.2, 101.3, 101.4, 102.2, 126.1, 126.8, 126.9, 127.06, 127.11, 127.15, 127.20, 127.3, 127.4, 127.5, 127.62, 127.64, 127.67, 127.70, 127.72, 127.95, 128.02, 128.07, 128.10, 128.12, 128.17, 128.20, 128.23, 128.29, 128.33, 128.4, 128.8, 137.4, 137.6, 138.2, 138.29, 138.31, 138.33, 138.38, 138.39, 138.5, 138.66, 138.71, 138.74, 138.98, 139.01, 139.1, 170.25, 170.26; MS (MALDI, DHB) found *m*/*z* 2625.9 $[M + Na]^+$, calcd for $C_{159}H_{166}NaO_{33}$ 2626.1.

β-D-Mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-2-*O*-acetyl-β-D-mannopyranosyl-(1→4)-2-*O*-acetyl-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-D-mannopyranose (3). According to the representative procedure for global deprotection of benzyl, benzylidene, and *p*-methoxybenzyl groups, compound **32** (21 mg, 8.06 μmol) was treated with 10% Pd/C (21 mg) in THF/MeOH/AcOH (2.0:1.0:0.3, v/v/v) (3.52 mL) under an atmosphere of H_2 for 23 h at room temperature to give compound **3** (6.4 mg, 5.95 μmol, 74%) after reversed-phase chromatography on a C18 column (1% AcOH in H_2O). Hexasaccharide **3** existed as a mixture of regioisomers resulting from migration of the acetyl groups: white hydroscopic solid; ^{1}H NMR (1% CD_3CO_2D in D_2O, 600 MHz) δ 2.17 (s, integration not determined), 2.187 (s, integration not determined), 2.190 (s, integration not determined), 2.202 (s,

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integration not determined), 2.211 (s, integration not determined), 3.40−3.68 (m, integration not determined), 3.71−4.08 (m, integration not determined), 4.08−4.15 (m, integration not determined), 4.17−4.21 (m, integration not determined), 4.73 (s, integration not determined), 4.75 (s, integration not determined), 4.83 (s, integration not determined), 4.90 (s, integration not determined), 4.91 (s, integration not determined), 4.92 (s, integration not determined), 4.93 (s, integration not determined), 4.94 (s, integration not determined), 5.05 (dd, J = 9.7, 3.2 Hz, integration not determined), 5.07−5.14 (m, integration not determined), 5.16−5.19 (m, integration not determined), 5.41 (d, J = 3.6 Hz, integration not determined), 5.48 (d, J = 3.5 Hz, integration not determined), 5.50−5.53 (m, integration not determined); ^{13}C NMR (1% CD_3CO_2D in D_2O, 125 MHz, selected signals) δ 63.08, 63.11, 63.2, 63.7, 69.4, 71.3, 71.4, 71.6, 72.66, 72.69, 72.74, 72.76, 72.81, 72.89, 72.94, 73.20, 73.22, 73.4, 73.6, 74.1, 74.16, 74.21, 74.30, 74.32, 74.34, 74.4, 74.5, 75.5, 75.7, 75.9, 76.09, 76.12, 77.5, 77.66, 77.70, 77.8, 77.87, 77.92, 78.0, 79.1, 79.19, 79.24, 79.3, 79.5, 79.9, 80.1, 96.4, 96.6, 101.3, 101.8, 101.9, 102.3, 102.8, 102.85, 102.93, 103.1, 175.5, 175.7, 176.10, 176.14, 176.2; HRMS found m/z 1097.3382 $[M + Na]^+$, calcd for $C_{40}H_{66}NaO_{33}$ 1097.3384.

β-D-Mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-D-mannopyranose (1). *Representative Procedure for Saponification of Acetate.* NaOH (1.0 M, 80 μL) was added to a solution of hexasaccharide **3** (5.5 mg, 5.12 μmol) in water (720 μL), and the mixture was stirred at room temperature for 2 h. The solution was passed through a short cation-exchange column (Dowex 50W-X8 H^+ form) and washed with water. The eluent was concentrated under reduced pressure and lyophilized to give compound **1** (4.6 mg, 4.34 μmol, 84%): white hydroscopic solid; ^{1}H NMR (D_2O, 600 MHz) δ 3.45 (ddd, J = 9.6, 7.1, 2.4 Hz, integration not determined), 3.51 (ddd, J = 8.6, 5.8, 2.4 Hz, integration not determined), 3.54−3.61 (m, integration not determined), 3.67 (dd, J = 9.6, 3.2 Hz, integration not determined), 3.71−4.03 (m, integration not determined), 4.08 (d, J = 3.4 Hz, integration not determined), 4.12−4.17 (m, integration not determined), 4.74 (s, integration not determined), 4.76 (s, integration not determined), 4.78 (s, integration not determined), 4.92 (s, integration not determined), 5.19 (d, J = 1.5 Hz, integration not determined); ^{13}C NMR (D_2O, 151 MHz, selected signals) δ 63.2, 63.7, 69.4, 71.7, 72.6, 72.67, 72.71, 73.0, 73.2, 73.4, 73.6, 74.15, 74.18, 74.4, 75.5, 77.5, 77.8, 79.15, 79.21, 79.27, 79.30, 79.4, 79.5, 96.4, 96.6, 102.85, 102.88, 102.93; HRMS found m/z 991.3341 $[M + H]^+$, calcd for $C_{36}H_{63}O_{31}$ 991.3353.

Allyl 2,3,6-Tri-O-benzyl-4-O-(2,3-di-O-benzyl-4,6-benzylidene-β-D-mannopyranosyl)-α-D-mannopyranoside (37). According to the representative procedure for selective β-mannosylation, the reaction of allyl 2,3,6-tri-*O*-benzyl-α-$_D$-mannopyranoside (**36**, 3.62 g, 7.38 mmol) and **14** (5.03 g, 9.30 mmol) was performed, followed by quench with Et_3N and $(EtO)_3P$, to give compound **37** (5.39 g, 5.85 mmol, 79%) after chromatography on silica gel (hexane/EtOAc, 83:17): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.05 (td, J = 9.7, 4.8 Hz, 1H), 3.40 (dd, J = 9.8, 3.1 Hz, 1H), 3.57−3.65 (m, 3H), 3.69 (d, J = 2.9 Hz, 1H), 3.72 (dt, J = 9.6, 3.3 Hz, 1H), 3.78 (t, J = 2.8 Hz, 1H), 3.91 (dd, J = 8.6, 3.1 Hz, 1H), 3.94−3.99 (m, 1H), 4.02 (dd, J = 10.4, 5.0 Hz, 1H), 4.06 (t, J = 9.6 Hz, 1H), 4.15−4.24 (m, 2H), 4.39 (d, J = 12.2 Hz, 1H), 4.50 (s, 1H), 4.54−4.59 (2 overlapping d, 2H), 4.63−4.84 (m, 7H), 4.90 (d, J = 2.3 Hz, 1H), 5.16−5.21 (m, 1H), 5.23−5.29 (m, 1H), 5.51 (s, 1H), 5.84−5.93 (m, 1H), 7.18−7.41 (m, integration not determined), 7.44−7.48 (m, 2H); ^{13}C NMR ($CDCl_3$, 151 MHz) δ 67.2, 68.1, 68.6, 69.1, 71.3, 72.4, 72.5, 72.8, 73.4, 74.9, 75.6, 76.1, 77.1, 77.9, 78.4, 78.7, 97.5, 101.3, 101.9, 117.2, 127.2, 127.3, 127.4, 127.5, 127.69, 127.74, 127.8, 128.0, 128.1, 128.17, 128.20, 128.23, 128.3, 128.4, 128.8, 133.8, 137.7, 138.2, 138.4, 138.6, 138.7, 139.1; MS (MALDI, DHB) found m/z 943.5 $[M + Na]^+$, calcd for $C_{57}H_{60}NaO_{11}$ 943.4.

Allyl 2,3,6-Tri-O-benzyl-4-O-(2,3,6-tri-O-benzyl-β-D-mannopyranosyl)-α-D-mannopyranoside (38). According to the representative procedure for reductive ring-opening of benzylidene acetal, compound **37** (187 mg, 0.20 mmol, 1.0 equiv) was treated with Et_3SiH (395 μL, 2.45 mmol) and $BF_3{\cdot}OEt_2$ (41 μL, 0.32 mmol) in CH_2Cl_2 for 7 h at 0 °C to give compound **38** (112 mg, 0.12 mmol, 60%) after chromatography on silica gel (hexane/EtOAc, 80:20): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.71 (br s, 1H), 3.14 (dd, J = 9.4, 2.9 Hz, 1H), 3.22 (dt, J = 9.6, 4.8 Hz, 1H), 3.58 (dd, J = 10.8, 5.4 Hz, 1H), 3.61−3.70 (m, 2H), 3.67−3.73 (m, 2H), 3.76−3.81 (m, 2H), 3.90−3.99 (m, 3H), 4.14−4.19 (m, 1H), 4.24 (t, J = 9.0 Hz, 1H), 4.37−4.49 (m, 5H), 4.51 (s, 1H), 4.56 (d, J = 12.2 Hz, 1H), 4.63−4.72 (m, 4H), 4.78 (d, J = 12.0 Hz, 1H), 4.84 (d, J = 12.1 Hz, 1H), 4.89 (d, J = 2.2 Hz, 1H), 5.15−5.19 (m, 1H), 5.21−5.27 (m, 1H), 5.82−5.92 (m), 7.16−7.37 (m, integration not determined); ^{13}C NMR ($CDCl_3$, 151 MHz) δ 68.0, 68.8, 69.4, 71.1, 71.4, 71.5, 72.5, 72.7, 73.4, 73.7, 74.2, 74.65, 74.67, 75.5, 75.7, 78.2, 81.8, 97.6, 101.4, 117.2, 127.2, 127.3, 127.4, 127.45, 127.50, 127.58, 127.60, 127.71, 127.73, 127.8, 128.06, 128.13, 128.2, 128.3, 128.37, 128.43, 133.9, 138.05, 138.10, 138.3, 138.4, 138.9, 139.2; MS (MALDI, DHB) found m/z 945.4 $[M + Na]^+$, calcd for $C_{57}H_{62}NaO_{11}$ 945.4.

Allyl 3-O-Benzyl-4,6-benzylidene-2-O-p-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-mannopyranoside (39). According to the representative procedure for selective β-mannosylation, the reaction of compounds **38** (250 mg, 0.27 mmol) and **15** (193 mg, 0.34 mmol) was performed, followed by quench with Et_3N and $(EtO)_3P$, to give compound **39** (200 mg, 0.14 mmol, 53%) after chromatography on silica gel (toluene/EtOAc, 95:5): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.04 (td, J = 9.7, 4.9 Hz, 1H), 3.17−3.24 (m, 1H), 3.31 (dd, J = 9.2, 3.0 Hz, 1H), 3.38 (dd, J = 9.9, 3.0 Hz, 1H), 3.48−3.57 (m, 3H), 3.62−3.71 (m, 3H), 3.73 (d, J = 3.1 Hz, 1H), 3.75−3.80 (m, 5H), 3.92−3.98 (m, 3H), 4.02 (t, J = 9.6 Hz, 1H), 4.11−4.19 (m, 2H), 4.23 (t, J = 9.1 Hz, 1H), 4.29 (d, J = 11.9 Hz, 1H), 4.41 (d, J = 12.2 Hz, 1H), 4.43−4.52 (m, 3H), 4.52−4.54 (2 overlapping s, 2H), 4.59 (d, J = 12.4 Hz, 1H), 4.61−4.79 (m, 9H), 4.86−4.91 (2 overlapping d, 2H), 5.15−5.19 (m, 1H), 5.21−5.27 (m, 1H), 5.47 (s, 1H), 5.81−5.94 (m, 1H), 6.78−6.82 (m, 2H), 7.10−7.39 (m, integration not determined), 7.41−7.47 (m, 2H); ^{13}C NMR ($CDCl_3$, 151 MHz) δ 55.2, 67.2, 67.9, 68.5, 69.29, 69.33, 71.3, 72.2, 72.3, 72.6, 72.7, 73.4, 73.5, 74.2, 74.5, 75.4, 75.6, 75.7, 75.9, 76.1, 76.7, 78.4, 78.6, 80.7, 97.5, 101.2, 101.5, 102.2, 113.5, 117.2, 126.1, 126.9, 127.1, 127.16, 127.19, 127.3, 127.4, 127.5, 127.59, 127.64, 127.7, 127.75, 127.76, 128.0, 128.10, 128.13, 128.2, 128.3, 128.7, 129.7, 130.9, 133.8, 137.7, 138.3, 138.37, 138.41, 138.6, 139.06, 139.10, 139.3, 159.1; MS (MALDI, DHB) found m/z 1405.6 $[M + Na]^+$, calcd for $C_{85}H_{90}NaO_{17}$ 1405.6.

Allyl 3-O-Benzyl-2-O-p-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-mannopyranoside (40). According to the representative procedure for transacetalation, compound **39** (969 mg, 0.70 mmol) was treated with neopentyl glycol in the presence of camphorsulfonic acid to give compound **40** (657 mg, 0.51 mmol, 72%) after chromatography on silica gel (hexane/EtOAc, 60:40): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.04−3.10 (m, 2H), 3.23 (dt, J = 9.7, 3.4 Hz, 1H), 3.26 (dd, J = 9.4, 2.9 Hz, 1H), 3.41 (dd, J = 11.6, 6.5 Hz, 1H), 3.55−3.60 (2 overlapping dd, 2H), 3.64−3.71 (m, 3H), 3.72−3.74 (2 overlapping d, 2H), 3.75−3.80 (m, 6H), 3.92−3.99 (m, 2H), 4.11−4.19 (m, 3H), 4.27 (t, J = 9.2 Hz, 1H), 4.30−4.35 (2 overlapping d, 2H), 4.41 (d, J = 12.0 Hz, 1H), 4.46−4.54 (m, 4H), 4.60 (d, J = 12.4 Hz, 1H), 4.62−4.79 (m, 8H), 4.88−4.93 (2 overlapping d, 2H), 5.15−5.21 (m, 1H), 5.21−5.28 (m, 1H), 5.83−5.92 (m, 1H), 6.77−6.83 (m, 2H), 7.14−7.34 (m, integration not determined), 7.36−7.39 (m, 2H); ^{13}C NMR ($CDCl_3$, 151 MHz, selected signals) δ 55.2, 62.7, 67.2, 67.9, 69.3, 69.5, 70.9, 71.4, 72.6, 72.65, 72.74, 73.3, 73.4, 73.6, 73.7, 74.4, 75.46, 75.51, 75.54, 75.6, 75.8, 76.5, 78.3, 80.2, 81.9, 97.5, 101.33, 101.34, 113.6, 117.2, 127.1, 127.16, 127.18, 127.3, 127.4, 127.45, 127.49, 127.6, 127.7, 127.8, 127.9, 128.0, 128.1, 128.2, 128.26, 128.32, 128.4, 129.6, 130.7, 133.8, 137.8, 138.27, 138.30, 138.4, 138.7, 138.9, 139.3, 159.1; MS (MALDI, DHB) found m/z 1317.6 $[M + Na]^+$, calcd for $C_{78}H_{86}NaO_{17}$ 1317.6.

Allyl 3,6-Di-O-benzyl-2-O-p-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-α-D-mannopyranoside (35). According to the representative procedure for selective monobenzylation, diol **40** (765

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mg, 0.59 mmol) was heated with Bu_2SnO (294 mg, 1.18 mmol), followed by treatment with BnBr (280 μL, 2.34 mmol) in the presence of CsF (404 mg, 2.66 mmol), to give compound **35** (652 mg, 0.47 mmol, 80%) after chromatography on silica gel (hexane/EtOAc, 70:30): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.67 (br s, 1H), 3.12 (dd, *J* = 9.4, 2.9 Hz, 1H), 3.19 (dt, *J* = 9.7, 4.8 Hz, 1H), 3.26 (ddd, *J* = 9.5, 4.1, 2.4 Hz, 1H), 3.34 (d, *J* = 9.2, 2.9 Hz, 1H), 3.53 (dd, *J* = 10.5, 5.4 Hz, 1H), 3.55−3.66 (m, 5H), 3.69 (d, *J* = 2.9 Hz, 1H), 3.73 (d, *J* = 2.9 Hz, 1H), 3.74−3.78 (m, 5H), 3.89 (t, *J* = 9.4 Hz, 1H), 3.92−3.98 (m, 2H), 4.14−4.20 (m, 2H), 4.22 (t, *J* = 9.1 Hz, 1H), 4.29−4.34 (2 overlapping d, 2H), 4.36−4.43 (m, 4H), 4.47 (d, *J* = 12.2 Hz, 1H), 4.50−4.54 (d overlapped with 2 s, 3H), 4.56−4.67 (m, 4H), 4.68−4.80 (m, 5H), 4.87−4.91 (2 overlapping d, 2H), 5.14−5.19 (m, 1H), 5.21−5.27 (m, 1H), 5.83−5.91 (m, 1H), 6.75−6.80 (m, 2H), 7.11−7.39 (m, integration not determined); ^{13}C NMR ($CDCl_3$, 151 MHz, selected signals) δ 55.2, 67.9, 68.7, 69.4, 69.5, 71.1, 71.3, 71.4, 72.3, 72.6, 72.7, 73.3, 73.5, 73.6, 73.8, 74.07, 74.12, 74.6, 75.3, 75.5, 75.66, 75.73, 76.1, 78.3, 80.7, 81.8, 97.5, 101.4, 101.6, 113.5, 117.2, 127.1, 127.15, 127.21, 127.4, 127.46, 127.51, 127.56, 127.61, 127.64, 127.7, 127.8, 127.9, 128.13, 128.14, 128.2, 128.25, 128.32, 128.4, 129.5, 131.0, 133.9, 138.11, 138.14, 138.3, 138.4, 138.5, 139.1, 139.2, 139.3, 159.0; MS (MALDI, DHB) found 1407.6256 $[M + Na]^+$, calcd for $C_{85}H_{92}NaO_{17}$ 1407.6232.

Allyl 2,3,6-Tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside (41). According to the representative procedure for reductive ring-opening of benzylidene acetal, trisaccharide **24** (1.031 g, 0.75 mmol) was treated with Et_3SiH (365 μL, 2.26 mmol) and TfOH (225 μL, 2.54 mmol) at −78 °C for 15 h, followed by stirring with TBAF (7.0 mL, 1 M solution in THF) for 2 h at room temperature, to give compound **41** (597 mg, 0.44 mmol, 58%) after chromatography on silica gel (toluene/EtOAc, 92:8): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.69 (br s, 1H), 3.14 (dd, *J* = 9.4, 2.9 Hz, 1H), 3.22 (dt, *J* = 9.6, 4.8 Hz, 1H), 3.29 (ddd, *J* = 9.4, 4.3, 2.3 Hz, 1H), 3.33 (dd, *J* = 9.3, 2.9 Hz, 1H), 3.48 (ddd, *J* = 9.6, 3.9, 2.4 Hz, 1H), 3.53−3.57 (m, 2H), 3.58−3.64 (m, 3H), 3.68−3.78 (m, 4H), 3.92 (t, *J* = 9.4 Hz, 1H), 4.02−4.10 (m, 2H), 4.21 (t, *J* = 9.3 Hz, 1H), 4.26−4.32 (m, 2H), 4.34 (d, *J* = 11.8 Hz, 1H), 4.40−4.49 (m, 6H), 4.53−4.57 (s overlapped with s and d, 3H), 4.60 (d, *J* = 12.1 Hz, 1H), 4.66 (d, *J* = 12.2 Hz, 1H), 4.71 (d, *J* = 12.0 Hz, 1H), 4.78−4.85 (m, 4H), 4.99 (d, *J* = 12.2 Hz, 1H), 5.05−5.09 (m, 1H), 5.16−5.22 (m, 1H), 5.28 (dd, *J* = 9.3, 7.7 Hz, 1H), 5.70−5.81 (m, 1H), 6.91−6.96 (m, 2H), 6.97−7.02 (m, 1H), 7.03−7.07 (m, 2H), 7.14−7.32 (m, integration not determined), 7.33−7.42 (m, 6H), 7.51−7.55 (m, 1H), 7.94−7.97 (m, 2H); ^{13}C NMR ($CDCl_3$, 151 MHz) δ 68.7, 68.8, 69.5, 71.0, 71.4, 72.6, 73.1, 73.4, 73.5, 73.6, 74.06, 74.10, 74.2, 74.6, 74.7, 75.0, 75.4, 76.1, 77.8, 80.5, 80.6, 81.8, 99.9, 101.2, 101.5, 117.1, 126.9, 127.1, 127.18, 127.23, 127.3, 127.4, 127.5, 127.55, 127.56, 127.59, 127.66, 127.67, 127.77, 127.82, 127.9, 128.0, 128.09, 128.12, 128.19, 128.24, 128.3, 128.38, 128.42, 129.8, 130.1, 132.9, 133.8, 137.9, 138.06, 138.10, 138.3, 138.7, 138.86, 138.91; MS (MALDI, DHB) found *m*/*z* 1391.6 $[M + Na]^+$, calcd for $C_{84}H_{88}NaO_{17}$ 1391.6.

Allyl 2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside (43). According to the representative procedure for β-selective mannosylation, the reaction of compounds **41** (112 mg, 82 μmol) and **14** (58 mg, 107 μmol) was performed, followed by quench with Et_3N and $(EtO)_3P$, to give compound **43** (100 mg, 56 μmol, 68%) after chromatography on silica gel (toluene/EtOAc, 92: 8): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.04 (td, *J* = 9.7, 4.9 Hz, 1H), 3.16−3.21 (m, 1H), 3.25 (ddd, *J* = 9.5, 4.2, 2.3 Hz, 1H), 3.28−3.35 (2 overlapping dd, 2H), 3.39 (dd, *J* = 9.9, 3.0 Hz, 1H), 3.45−3.64 (m, 7H), 3.68−3.74 (m, 4H), 3.77 (t, *J* = 9.0 Hz, 1H), 3.94 (dd, *J* = 10.4, 4.9 Hz, 1H), 4.01−4.09 (m, 3H), 4.14 (t, *J* = 9.4 Hz, 1H), 4.21 (t, *J* = 9.4 Hz, 1H), 4.23−4.28 (m, 2H), 4.28−4.33 (m, 1H), 4.38−4.46 (3 overlapping d, 3H), 4.47−4.57 (m, 7H), 4.60 (d, *J* = 12.2 Hz, 1H), 4.62−4.88 (m, 10H), 4.99 (d, *J* = 12.2 Hz, 1H), 5.02−5.10 (m, 1H), 5.15−5.22 (m, 1H), 5.29 (dd, *J* = 9.3, 7.8 Hz, 1H), 5.48 (s, 1H), 5.75 (dddd, *J* = 4.7, 6.2, 10.7, 17.0 Hz, 1H), 6.91−6.95 (m, 1H), 6.97−7.02 (m, 1H), 7.02−7.06 (m, 1H), 7.11−7.46 (m, integration not determined), 7.51−7.56 (m, 1H), 7.94−7.97 (m, 1H); ^{13}C NMR ($CDCl_3$, 600 MHz, selected signals) δ 67.2, 68.5, 68.7, 69.2, 69.4, 69.5, 72.1, 72.3, 72.5, 73.1, 73.3, 73.4, 73.5, 74.0, 74.1, 74.2, 74.9, 75.0, 75.3, 75.4, 75.5, 75.8, 76.0, 76.1, 77.1, 77.7, 78.4, 78.6, 80.5, 80.7, 99.8, 101.15, 101.22, 101.6, 102.1, 117.1, 126.0, 126.9, 127.0, 127.1, 127.16, 127.18, 127.24, 127.3, 127.4, 127.5, 127.6, 127.66, 127.69, 127.75, 127.78, 127.83, 127.99, 128.02, 128.03, 128.05, 128.07, 128.09, 128.13, 128.18, 128.22, 128.3, 128.4, 128.7, 129.7, 130.0, 132.8, 133.7, 137.6, 137.9, 138.3, 138.4, 138.5, 138.65, 138.72, 138.9, 139.0, 139.2, 165.2; MS (MALDI, DHB) found *m*/*z* 1821.8 $[M + Na]^+$, calcd for $C_{111}H_{114}NaO_{22}$ 1821.8.

2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranose (44). According to the representative procedure for deallylation, compound **43** (109 mg, 60.6 μmol) was treated with a catalytic amount of $PdCl_2$ in $MeOH/CH_2Cl_2$ at room temperature for 4 h to give compound **44** (84 mg, 47.7 μmol, 79%) after chromatography on silica gel (hexane/EtOAc, 70:30): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.99 (br s, integration not determined), 3.04 (td, *J* = 9.7, 5.0 Hz, integration not determined), 3.16−3.21 (m, integration not determined), 3.22−3.27 (m, integration not determined), 3.27−3.32 (m, integration not determined), 3.39 (dd, *J* = 9.8, 2.6 Hz, integration not determined), 3.46−3.64 (m, integration not determined), 3.66−3.75 (m, integration not determined), 3.80 (t, *J* = 9.0 Hz, integration not determined), 3.94 (dd, *J* = 10.4, 4.8 Hz, integration not determined), 3.98−4.16 (m, integration not determined), 4.18−4.28 (m, integration not determined), 4.34−4.45 (m, integration not determined), 4.45−4.55 (m, integration not determined), 4.56−4.90 (m, integration not determined), 5.03−5.13 (m, integration not determined), 5.48 (s, integration not determined), 5.54 (d, *J* = 3.5 Hz, integration not determined), 6.95−7.58 (m, integration not determined), 7.91−8.00 (m, integration not determined); ^{13}C NMR ($CDCl_3$, 151 MHz, selected signals) δ 67.2, 68.4, 68.5, 68.6, 69.2, 69.4, 69.5, 70.1, 72.11, 72.14, 72.4, 72.5, 72.6, 73.3, 73.4, 73.46, 73.50, 73.6, 74.17, 74.19, 74.24, 74.5, 74.8, 74.9, 75.35, 75.37, 75.41, 75.58, 75.60, 75.8, 75.9, 76.0, 76.06, 76.12, 76.2, 77.1, 77.5, 77.6, 77.8, 78.4, 78.6, 80.1, 80.6, 80.7, 90.4, 96.1, 101.05, 101.14, 101.2, 101.62, 101.64, 102.2, 126.1, 126.9, 126.95, 127.05, 127.11, 127.13, 127.17, 127.20, 127.27, 127.31, 127.34, 127.36, 127.38, 127.43, 127.48, 127.54, 127.6, 127.67, 127.71, 127.81, 127.83, 127.88, 127.93, 127.95, 128.01, 128.05, 128.08, 128.10, 128.16, 128.21, 128.24, 128.25, 128.28, 128.33, 128.47, 128.49, 128.7, 129.3, 129.6, 129.8, 129.9, 133.1, 133.4, 137.57, 137.64, 138.25, 138.33, 138.4, 138.5, 138.6, 138.7, 138.9, 139.0, 139.25, 139.27, 165.8, 167.1; MS (MALDI, DHB) found *m*/*z* 1781.7 $[M + Na]^+$, calcd for $C_{108}H_{110}NaO_{22}$ 1781.7.

2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-α/β-D-glucopyranosyl trichloroacetimidate (34). According to the representative procedure for trichloroacetimidation, compound **44** (113 mg, 64.2 μmol) was treated with trichloroacetonitrile (65 μL, 648.2 μmol) in the presence of DBU at 0 °C for 1 h to give compound **34** (68 mg, 35.7 μmol, 56%) after chromatography (hexane/EtOAc/Et_3N, 75:25:1): colorless oil; ^{1}H NMR (C_6D_6, 600 MHz) δ 3.04 (td, *J* = 9.6, 4.9 Hz, integration not determined), 3.23−3.32 (m, integration not determined), 3.33−3.39 (m, integration not determined), 3.41−3.48 (m, integration not determined), 3.55−3.73 (m, integration not determined), 3.76 (d, *J* = 3.1 Hz, integration not determined), 3.80−3.86 (m, integration not determined), 3.92 (d, *J* = 2.9 Hz, integration not determined), 3.98 (dd, *J* = 10.2, 4.8 Hz, integration not determined), 4.16−4.24 (m, integration not determined), 4.25−4.33 (m, integration not determined), 4.34−4.39 (m, integration not determined), 4.44−4.59 (m, integration not determined), 4.59−4.77 (m, integration not determined), 4.87−5.09 (m, integration not determined), 5.17−5.21 (m, integration not determined), 5.22−5.28 (m, integration not determined), 5.32 (d, *J* = 12.6 Hz, integration not determined), 5.43 (d, *J* = 12.3 Hz, integration

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not determined), 5.67−5.75 (m, integration not determined), 6.01 (t, J = 8.5 Hz, integration not determined), 6.26 (d, J = 7.9 Hz, integration not determined), 6.91−7.39 (m, integration not determined), 7.45−7.50 (m, integration not determined), 7.52−7.60 (m, integration not determined), 8.06−8.17 (m, integration not determined), 8.37 (s, integration not determined), 8.58 (s, integration not determined); ^{13}C NMR (C_6D_6, 151 MHz, selected signals) δ 68.0, 69.0, 69.2, 70.0, 70.1, 72.9, 73.2, 73.3, 73.9, 74.15, 74.20, 74.22, 74.4, 75.2, 75.3, 75.4, 76.0, 76.46, 76.53, 76.8, 77.7, 77.76, 77.83, 78.2, 78.9, 79.6, 79.7, 81.4, 81.8, 92.0, 95.0, 101.8, 102.0, 102.3, 102.8, 127.0, 127.6, 127.75, 127.80, 127.88, 127.93, 127.95, 128.00, 128.1, 128.79, 128.81, 128.88, 128.94, 128.97, 129.03, 129.2, 130.5, 130.6, 133.5, 138.7, 139.1, 139.35, 139.41, 139.9, 139.97, 140.03, 140.1, 140.5, 140.6, 161.0, 166.0; MS (MALDI, ATT) found m/z 1924.6 [M + Na]$^+$, calcd for $C_{110}H_{110}Cl_3NNaO_{22}$ 1924.6.

Allyl 2,3-Di-*O*-benzyl −4,6-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-*O*-*p*-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (45). According to the representative procedure for glycosylation, the reaction of acceptor **35** (63 mg, 45.5 μmol) and trichloroacetimidate **34** (130 mg, 68.3 μmol) was carried out by the promotion of TMSOTf to give compound **45** (92 mg, 29.4 μmol, 65%) after column chromatography on silica gel (toluene/EtOAc, 90:10): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.86−2.92 (m, 1H), 3.03 (td, J = 9.7, 4.9 Hz, 1H), 3.13−3.23 (m, 5H), 3.23−3.30 (m, 4H), 3.35−3.40 (m, 2H), 3.42−3.66 (m, 14H), 3.68−3.76 (m, 8H), 3.89−3.96 (m, 3H), 3.97−4.09 (m, 4H), 4.10−4.28 (m, 9H), 4.32 (s, 1H), 4.35−4.59 (m, 18H), 4.61−4.86 (m, 19H), 4.87 (d, J = 2.0 Hz, 1H), 4.99 (d, J = 12.2 Hz, 1H), 5.14−5.24 (m, 3H), 5.47 (s, 1H), 5.82−5.89 (m, 1H), 6.65−6.69 (m, 2H), 6.85−6.89 (m, 2H), 6.94−6.98 (m, 3H), 7.00−7.40 (m, integration not determined), 7.42−7.45 (m, 2H), 7.51−7.57 (m, 1H), 7.84−7.89 (m, 2H); ^{13}C NMR ($CDCl_3$, 151 MHz, selected signals) δ 55.2, 67.2, 67.9, 68.3, 68.49, 68.52, 69.2, 69.28, 69.34, 69.4, 71.3, 72.1, 72.3, 72.37, 72.42, 72.6, 72.7, 73.28, 73.30, 73.3, 73.4, 73.7, 74.0, 74.1, 74.2, 74.3, 74.9, 75.1, 75.36, 75.40, 75.41, 75.45, 75.54, 75.6, 75.71, 75.74, 75.9, 76.0, 76.1, 77.1, 78.0, 78.3, 78.4, 78.6, 80.4, 80.5, 80.76, 80.79, 80.9, 97.5, 100.5, 101.26, 101.32, 101.6, 102.2, 113.4, 117.1, 126.1, 126.8, 126.87, 126.91, 126.97, 127.02, 127.1, 127.16, 127.18, 127.22, 127.3, 127.35, 127.42, 127.49, 127.54, 127.56, 127.60, 127.65, 127.70, 127.73, 127.8, 127.9, 128.00, 128.02, 128.06, 128.08, 128.11, 128.17, 128.20, 128.24, 128.28, 128.31, 128.7, 129.3, 129.8, 129.9, 131.3, 132.9, 133.9, 137.7, 138.1, 138.28, 138.31, 138.37, 138.42, 138.5, 138.7, 138.77, 138.82, 139.0, 139.06, 139.09, 139.3, 139.4, 158.8, 165.0; MS (MALDI, DHB) found m/z 3148.4 [M + Na]$^+$, calcd for $C_{193}H_{200}NaO_{38}$ 3148.4.

Allyl 2,3-Di-*O*-benzyl-4,6-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-*O*-*p*-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (46). According to the representative procedure for debenzoylation, compound **45** (56 mg, 17.9 μmol) was treated with $LiAlH_4$ (2.7 mg, 71.1 μmol) in Et_2O to give compound **46** (43 mg, 14.2 μmol, 80%) after chromatography on silica gel (hexane/EtOAc, 70:30): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.02 (td, J = 9.6, 4.8 Hz, 1H), 3.08−3.17 (m, 3H), 3.18−3.28 (m, 6H), 3.29−3.42 (m, 5H), 3.42−3.82 (m, 23H), 3.90−3.98 (m, 3H), 4.02 (t, J = 9.6 Hz, 1H), 4.09−4.25 (m, 9H), 4.29 (d, J = 12.0 Hz, 1H), 4.31−4.43 (m, 7H), 4.44−4.54 (m, 12H), 4.58 (d, J = 12.5 Hz, 1H), 4.59−4.80 (m, 18H), 4.85−4.90 (2 overlapping d, 2H), 5.00 (d, J = 11.9 Hz, 1H), 5.14−5.19 (m, 1H), 5.21−5.26 (m, 1H), 5.47 (s, 1H), 5.82−5.92 (m, 1H), 6.68−6.73 (m, 2H), 7.09−7.39 (m, integration not determined), 7.41−7.46 (m, 2H); ^{13}C NMR ($CDCl_3$, 151 MHz, selected signals) δ 55.2, 67.2, 67.9, 68.5, 68.6, 69.2, 69.3, 69.4, 69.6, 71.3, 71.4, 72.1, 72.26, 72.31, 72.4, 72.6, 72.7, 73.2, 73.3, 73.4, 73.6, 73.8, 74.09, 74.14, 74.2, 74.66, 74.69, 74.8, 74.9, 75.0, 75.2, 75.28, 75.31, 75.46, 75.48, 75.53, 75.6, 75.7, 75.9, 76.06, 76.13, 77.0, 77.1, 78.3, 78.4, 78.6, 80.7, 80.8, 81.8, 82.4, 97.5, 101.1, 101.2, 101.4, 101.7, 101.9, 102.2, 103.7, 113.5, 117.2, 126.1, 126.7, 126.8, 126.9, 127.00, 127.01, 127.11, 127.13, 127.16, 127.18, 127.3, 127.37, 127.41, 127.5, 127.55, 127.61, 127.62, 127.67, 127.71, 127.73, 127.8, 127.97, 127.98, 128.05, 128.08, 128.13, 128.15, 128.19, 128.21, 128.23, 128.27, 128.31, 128.7, 129.3, 131.0, 133.8, 137.7, 137.9, 138.0, 138.3, 138.4, 138.5, 138.7, 138.8, 139.00, 139.04, 139.08, 139.09, 139.12, 139.27, 139.30, 139.5, 158.9; MS (MALDI, DHB) found m/z 3044.3 [M + Na]$^+$, calcd for $C_{186}H_{196}NaO_{37}$ 3044.3.

Allyl 2,3-Di-*O*-benzyl-4,6-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-*O*-*p*-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (33). According to the representative procedure for *gluco* to *manno* epimerization, alcohol **46** (43 mg, 14.2 μmol) was subject to oxidation with DMSO (0.48 mL) and acetic anhydride (0.24 mL) for 16 h at room temperature, and the resulting carbonyl compound was reduced by $NaBH_4$ (7 mg, 185.0 μmol) to give compound **33** (26 mg, 8.6 μmol, 60%) after column chromatography on silica gel (hexane/EtOAc, 65:35): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.69 (br s, 1H), 3.03 (td, J = 9.7, 4.9 Hz, 1H), 3.12−3.25 (m, 5H), 3.26−3.40 (m, 6H), 3.42−3.60 (m, 10H), 3.60−3.80 (m, 13H), 3.90 (br s, 1H), 3.91−3.98 (m, 3H), 4.00−4.07 (2 overlapping t, 2H), 4.10−4.33 (m, 11H), 4.34−4.54 (m, 18H), 4.56−4.80 (m, 20H), 4.85−4.91 (2 overlapping d, 2H), 5.14−5.19 (m, 1H), 5.21−5.26 (m, 1H), 5.47 (s, 1H), 5.80−5.93 (m, 1H), 6.70−6.76 (m, 2H), 7.08−7.38 (m, integration not determined), 7.41−7.47 (m, 2H); ^{13}C NMR ($CDCl_3$, 151 MHz, selected signals) δ 55.2, 67.2, 67.9, 68.1, 68.5, 69.1, 69.2, 69.35, 69.36, 69.43, 69.5, 71.4, 71.7, 71.8, 72.1, 72.26, 72.34, 72.4, 72.6, 72.7, 73.2, 73.3, 73.35, 73.37, 73.41, 73.44, 73.7, 74.0, 74.12, 74.14, 74.7, 74.89, 74.92, 75.1, 75.2, 75.29, 75.33, 75.4, 75.52, 75.54, 75.6, 75.7, 75.9, 76.0, 76.2, 77.1, 78.3, 78.4, 78.6, 79.4, 80.7, 80.76, 80.84, 81.4, 97.5 ($^1J_{CH}$ = 169.3 Hz), 100.2 ($^1J_{CH}$ = 159.3 Hz), 101.3, 101.4 ($^1J_{CH}$ = 155.7 Hz), 101.56 ($^1J_{CH}$ = 157.9 Hz), 101.61 ($^1J_{CH}$ = 156.4 Hz), 101.8 ($^1J_{CH}$ = 155.7 Hz), 102.2 ($^1J_{CH}$ = 157.1 Hz), 113.5, 117.2, 126.1, 126.90, 126.94, 127.0, 127.1, 127.2, 127.25, 127.29, 127.35, 127.37, 127.41, 127.44, 127.5, 127.55, 127.62, 127.64, 127.67, 127.70, 127.72, 127.73, 127.8, 127.96, 127.98, 128.05, 128.09, 128.11, 128.13, 128.15, 128.19, 128.22, 128.23, 128.3, 128.7, 129.3, 131.1, 133.9, 137.7, 138.29, 138.30, 138.33, 138.4, 138.6, 138.7, 138.8, 139.05, 139.11, 139.15, 139.21, 139.3, 158.9; MS (MALDI, DHB) found m/z 3044.3 [M + Na]$^+$, calcd for $C_{186}H_{196}NaO_{37}$ 3044.3.

Allyl 2,3-Di-*O*-benzyl-4,6-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2-*O*-acetyl-3,6-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-*O*-*p*-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (47). According to the representative procedure for acetylation, alcohol **33** (94 mg, 31.1 μmol) was treated with acetic anhydride (0.4 mL) in pyridine in the presence of DMAP for 3 h at room temperature to give acetate **47** (87 mg, 28.4 μmol, 92%) after column chromatography on silica gel (hexane/EtOAc, 70:30): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.01 (s, 3H), 3.02 (td, J = 9.7, 4.9 Hz, 1H), 3.11−3.20 (m, 4H), 3.22 (dt, J = 9.4, 3.2 Hz, 1H), 3.26−3.35 (m, 5H), 3.35−3.60 (m, 12H), 3.60−3.73 (m, 10H), 3.74−3.79 (m, 2H), 3.89−3.99 (m, 4H), 4.02 (t, J = 9.5 Hz, 1H), 4.07−4.24 (m, 8H), 4.27−4.32 (2 overlapping d, 2H), 4.34 (d, J = 12.2 Hz, 1H), 4.36−4.81 (m, 38H), 4.85−4.90 (2 overlapping d, 2H), 5.13−5.19 (m, 1H), 5.20−5.26 (m, 1H), 5.39 (d, J = 3.4 Hz, 1H), 5.47 (s, 1H), 5.78−5.96 (m, 1H), 6.65−6.71 (m, 2H), 7.09−7.39 (m, integration not determined), 7.41−7.46 (m, 2H); ^{13}C NMR ($CDCl_3$, 151 MHz, selected signals) δ 21.0, 55.2, 67.2, 67.9, 68.5, 68.8, 69.0, 69.1, 69.2, 69.36, 69.44, 71.36, 71.41, 71.9, 72.1, 72.2, 72.3, 72.4, 72.6, 72.7, 73.1, 73.3, 73.36, 73.38, 73.40, 73.7, 74.1, 74.2, 74.5, 74.9, 75.1, 75.25, 75.29, 75.40, 75.42, 75.5, 75.6, 75.7, 75.8, 76.09, 76.13, 76.2, 77.1, 78.1, 78.3, 78.4, 78.6, 80.7, 80.8, 81.3, 97.5, 99.7, 101.1, 101.3, 101.4, 101.68, 101.70, 102.2, 113.4, 117.1, 126.1, 126.8, 126.85, 126.90, 127.01, 127.04, 127.09, 127.13, 127.15, 127.21, 127.27, 127.34, 127.37, 127.41, 127.44, 127.5, 127.59, 127.64, 127.65, 127.70, 127.72, 127.8, 127.96,

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127.99, 128.05, 128.07, 128.09, 128.12, 128.16, 128.21, 128.3, 128.7, 129.2, 131.2, 133.9, 137.7, 138.3, 138.35, 138.38, 138.43, 138.45, 138.48, 138.54, 138.77, 138.80, 139.07, 139.10, 139.15, 139.20, 139.23, 139.3, 158.8, 170.3; MS (MALDI, DHB) found m/z 3086.1 [M + Na]$^+$, calcd for $C_{188}H_{198}NaO_{38}$ 3086.3.

2,3-Di-*O*-benzyl-4,6-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2-*O*-acetyl-3,6-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-*O*-*p*-methoxybenzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranose (48). According to the representative procedure for deallylation, compound **47** (83 mg, 27.1 μmol) was treated with a catalytic amount of $PdCl_2$in MeOH/CH_2Cl_2 (1:1, v/v, 4.0 mL) at room temperature for 3 h to give compound **48** (49 mg, 16.2 μmol, 60%) after column chromatography on silica gel (hexane/EtOAc, 60:40): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.01 (s, integration not determined), 2.70 (br s, integration not determined), 3.02 (td, J = 9.7, 4.9 Hz, integration not determined), 3.11−3.20 (m, integration not determined), 3.22−3.74 (m, integration not determined), 3.75 (t, J = 2.8 Hz, integration not determined), 3.77−3.79 (m, integration not determined), 3.90−4.26 (m, integration not determined), 4.28−4.84 (m, integration not determined), 4.92−4.97 (m, integration not determined), 5.22 (d, J = 2.1 Hz, integration not determined), 5.39 (d, J = 3.6 Hz, 1H, integration not determined), 5.47 (s, integration not determined), 6.66−6.71 (m, integration not determined), 7.09−7.39 (m, integration not determined), 7.41−7.46 (m, integration not determined); ^{13}C NMR ($CDCl_3$, 151 MHz, selected signals) δ 21.0, 55.2, 67.2, 68.5, 68.8, 69.0, 69.1, 69.2, 69.45, 69.52, 69.6, 71.4, 71.5, 71.91, 71.94, 72.1, 72.2, 72.28, 72.33, 72.4, 72.5, 72.7, 73.1, 73.4, 73.5, 73.6, 73.71, 73.74, 74.0, 74.1, 74.2, 74.3, 74.5, 74.8, 74.9, 75.1, 75.3, 75.40, 75.42, 75.5, 75.7, 75.8, 75.9, 76.0, 76.07, 76.11, 76.14, 76.2, 77.15, 77.20, 78.0, 78.4, 78.6, 80.4, 80.5, 80.69, 80.73, 81.3, 93.0, 93.4, 99.7, 101.09, 101.14, 101.3, 101.58, 101.61, 101.7, 102.2, 113.4, 126.1, 126.8, 126.86, 126.90, 127.1, 127.16, 127.19, 127.23, 127.35, 127.37, 127.43, 127.45, 127.52, 127.56, 127.60, 127.65, 127.70, 127.74, 127.77, 127.81, 127.9, 128.0, 128.06, 128.08, 128.10, 128.12, 128.15, 128.19, 128.24, 128.29, 128.34, 128.4, 128.5, 128.7, 129.3, 131.12, 137.7, 138.1, 138.3, 138.37, 138.43, 138.5, 138.75, 138.77, 138.9, 139.0, 139.06, 139.09, 139.12, 139.2, 158.8, 170.3; MS (MALDI, DHB) found m/z 3046.4 [M + Na]$^+$, calcd for $C_{185}H_{194}NaO_{38}$ 3046.3.

β-D-Mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-2-*O*-acetyl-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-D-mannopyranose (5). According to the representative procedure for global deprotection of benzyl, benzylidene, and *p*-methoxybenzyl groups, compound **48** (10 mg, 3.31 μmol) was treated with 10% Pd/C (10 mg) in THF/MeOH/AcOH (2.0:1.0:0.3, v/v/v, 1.2 mL) under an atmosphere of hydrogen at room temperature to give compound **5** (2.7 mg, 2.26 μmol, 69%) after reversed-phase chromatography on a C18 column (1% AcOH in H_2O). Heptasaccharide **5** was isolated as a mixture of regioisomers resulting from the migration of the acetyl group: white hydroscopic solid; ^{1}H NMR (1% CD_3CO_2D in D_2O, 600 MHz) δ 2.18 (s, integration not determined), 2.19 (s, integration not determined), 3.40−3.60 (m, integration not determined), 3.60−3.67 (m, integration not determined), 3.69−4.08 (m, integration not determined), 4.08−4.15 (m, integration not determined), 4.19 (d, J = 3.3 Hz), 4.73 (s, integration not determined), 4.75 (s, integration not determined), 4.83 (s, integration not determined), 4.90 (s, integration not determined), 4.94 (s, integration not determined), 5.11 (dd, J = 9.8, 3.1 Hz, integration not determined), 5.17 (s, integration not determined), 5.51 (d, J = 3.6 Hz, integration not determined); ^{13}C NMR (1% CD_3CO_2D in D_2O, 151 MHz, selected signals) δ 63.19, 63.23, 63.3, 63.7, 69.4, 71.4, 71.6, 72.6, 72.65, 72.69, 72.78, 72.84, 72.9, 73.2, 73.4, 73.6, 74.2, 74.26, 74.28, 74.4, 75.5, 76.0, 76.1, 77.50, 77.53, 77.7, 77.9, 78.0, 79.1, 79.19, 79.22, 79.28, 79.33, 79.5, 80.1, 96.4, 96.5, 101.8, 102.3, 102.8, 102.86, 102.91, 103.0, 175.7, 176.1; ESI-HRMS found m/z 1217.3722 [M + Na]$^+$, calcd for $C_{44}H_{74}NaO_{37}$ 1217.3807.

Allyl 2,3-Di-*O*-benzyl-4,6-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (49). According to the representative procedure for selective deprotection of *p*-methoxybenzyl group, compound **33** (123 mg, 40.7 μmol) was treated with CAN (112 mg, 204.3 μmol) in CH_3CN/toluene/H_2O (90:5:5, v/v/v, 8.8 mL) at room temperature for 2 h to give compound **49** (88 mg, 30.3 μmol, 75%) after column chromatography on silica gel (hexane/EtOAc, 60:40): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.58 (d, J = 2.7 Hz, 1H), 2.76 (d, J = 2.3 Hz, 1H), 3.03 (td, J = 9.7, 4.9 Hz, 1H), 3.13−3.21 (m, 4H), 3.24 (ddd, J = 9.5, 3.7, 2.5 Hz, 1H), 3.26−3.32 (m, 3H), 3.35−3.40 (m, 3H), 3.42−3.60 (m, 9H), 3.61−3.75 (m, 8H), 3.75−3.80 (m, 2H), 3.88 (br t, 1H), 3.91−3.98 (m, 4H), 4.00−4.25 (m, 9H), 4.25−4.29 (2 overlapping d, 2H), 4.33−4.55 (m, 20H), 4.57 (d, J = 12.4 Hz, 1H), 4.60−4.80 (m, 17H), 4.84 (d, J = 12.3 Hz, 1H), 4.89 (d, J = 2.3 Hz, 1H), 5.15−5.19 (m, 1H), 5.21−5.26 (m, 1H), 5.47 (s, 1H), 5.82−5.91 (m, 1H), 7.10−7.39 (m, integration not determined), 7.42−7.46 (m, 2H); ^{13}C NMR ($CDCl_3$, 151 MHz, selected signals) δ 67.2, 67.5, 68.0, 68.1, 68.5, 69.0, 69.16, 69.24, 69.4, 69.5, 71.2, 71.4, 71.8, 71.9, 72.1, 72.3, 72.4, 72.5, 72.68, 72.73, 73.0, 73.35, 73.37, 73.42, 73.5, 74.0, 74.1, 74.7, 74.86, 74.89, 75.0, 75.2, 75.29, 75.34, 75.4, 75.7, 75.9, 76.05, 76.06, 77.12, 78.2, 78.4, 78.6, 79.4, 78.0, 80.8, 80.9, 81.5, 97.5, 100.3, 101.2, 101.5, 101.57, 101.62, 102.2, 117.2, 126.1, 126.9, 127.0, 127.06, 127.11, 127.15, 127.17, 127.3, 127.35, 127.43, 127.46, 127.49, 127.53, 127.55, 127.59, 127.62, 127.64, 127.66, 127.69, 127.73, 128.0, 128.05, 128.09, 128.13, 128.20, 128.24, 128.3, 128.4, 128.7, 133.8, 137.7, 138.1, 138.15, 138.21, 138.22, 138.3, 138.37, 138.40, 138.41, 138.6, 138.7, 138.8, 138.9, 139.07, 139.09, 139.13, 139.2; MS (MALDI, DHB) found m/z 2924.4 [M + Na]$^+$, calcd for $C_{178}H_{188}NaO_{36}$ 2924.3.

Allyl 2,3-Di-*O*-benzyl-4,6-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2-*O*-acetyl-3,6-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2-*O*-acetyl-3,6-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (50). According to the representative procedure for acetylation, alcohol **49** (89 mg, 30.7 μmol) was treated with acetic anhydride (0.4 mL) in pyridine (1.5 mL) in the presence of DMAP for 3 h at room temperature to give acetate **50** (84 mg, 28.1 μmol, 91%) after column chromatography on silica gel (hexane/EtOAc, 65:35): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.94−2.10 (2 overlapping s, 6H), 3.02 (td, J = 9.7, 4.9 Hz, 1H), 3.10−3.22 (m, 5H), 3.27−3.32 (m, 3H), 3.34−3.56 (m, 13H), 3.58−3.67 (m, 5H), 3.68−3.72 (2 overlapping d, 2H), 3.74−3.79 (m, 2H), 3.90−3.97 (m, 4H), 3.97−4.05 (2 overlapping t, 2H), 4.07−4.16 (m, 3H), 4.16−4.23 (m, 3H), 4.25−4.31 (2 overlapping d, 2H), 4.36−4.61 (m, 23H), 4.61−4.77 (m, 15H), 4.83 (d, J = 12.2 Hz, 1H), 4.88 (d, J = 2.4 Hz, 1H), 5.13−5.19 (m, 1H), 5.19−5.26 (m, 1H), 5.35 (d, J = 3.6 Hz, 1H), 5.38 (d, J = 3.6 Hz, 1H), 5.47 (s, 1H), 5.81−5.91 (m, 1H), 7.09−7.39 (m, integration not determined), 7.41−7.46 (m, 2H); ^{13}C NMR ($CDCl_3$, 151 MHz, selected signals) δ 21.0, 67.2, 67.9, 68.5, 68.6, 68.7, 68.8, 68.9, 69.15, 69.22, 69.4, 69.5, 71.3, 71.4, 72.11, 72.13, 72.2, 72.4, 72.5, 72.7, 73.1, 73.3, 73.35, 73.38, 73.40, 74.1, 74.2, 74.5, 74.6, 74.7, 74.9, 75.1, 75.29, 75.34, 75.4, 75.6, 75.7, 75.80, 75.83, 76.1, 76.2, 77.2, 78.0, 78.1, 78.4, 78.6, 78.8, 80.7, 81.3, 97.5, 99.5, 99.7, 101.2, 101.3, 101.4, 101.7, 102.1, 117.1, 126.1, 126.86, 126.90, 127.07, 127.10, 127.16, 127.19, 127.23, 127.27, 127.30, 127.32, 127.35, 127.42, 127.45, 127.52, 127.54, 127.59, 127.62, 127.63, 127.66, 127.72, 127.73, 127.96, 128.00, 128.02, 128.05, 128.07, 128.10, 128.13, 128.16, 128.21, 128.24, 128.27, 128.32, 128.7, 133.9, 137.7, 138.26, 138.30, 138.37, 138.41, 138.5, 138.66, 138.73, 138.8, 138.99, 139.04, 139.06, 139.08, 139.16, 139.20, 170.3; MS (MALDI, DHB) found m/z 3008.4 [M + Na]$^+$, calcd for $C_{182}H_{192}NaO_{38}$ 3008.3.

2,3-Di-*O*-benzyl-4,6-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2-*O*-acetyl-3,6-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2-*O*-acetyl-3,6-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-D-mannopyranose (51). According to the representative procedure for deallylation, compound **50** (71

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mg, 23.8 μmol) was treated with a catalytic amount of $PdCl_2$ in $MeOH/CH_2Cl_2$ (1:1, v/v, 3.6 mL) at room temperature for 4 h to give compound **51** (43 mg, 14.6 μmol, 61%) after column chromatography on silica gel (hexane/EtOAc, 60:40): colorless oil; ^1H NMR ($CDCl_3$, 600 MHz) δ 2.01 (s, integration not determined), 2.02 (s, integration not determined), 2.03 (s, integration not determined), 2.65 (br s, integration not determined), 3.02 (td, *J* = 9.6, 4.8 H, integration not determined z), 3.10−3.22 (m, integration not determined), 3.22−3.58 (m, integration not determined), 3.58−3.72 (m, integration not determined), 3.74 (t, *J* = 2.9 Hz, integration not determined), 3.76−3.78 (m, integration not determined), 3.88−3.95 (m, integration not determined), 3.96−4.05 (m, integration not determined), 4.07−4.23 (m, integration not determined), 4.24−4.32 (2 overlapping d, integration not determined), 4.36−4.82 (m, integration not determined), 4.90−4.96 (m, integration not determined), 5.22 (d, *J* = 2.8 Hz, integration not determined), 5.34−5.37 (m, integration not determined), 5.39 (d, *J* = 3.5 Hz, integration not determined), 5.41 (d, *J* = 3.5 Hz, integration not determined), 5.47 (s, integration not determined), 7.09−7.38 (m, integration not determined), 7.41−7.45 (m, integration not determined); ^{13}C NMR ($CDCl_3$, 151 MHz, selected signals) δ 21.0, 67.2, 68.5, 68.6, 68.7, 68.8, 68.9, 69.20, 69.22, 69.3, 69.48, 69.51, 69.6, 71.3, 71.4, 71.5, 72.06, 72.13, 72.2, 72.4, 72.5, 72.7, 73.05, 73.08, 73.13, 73.35, 73.39, 73.40, 73.5, 73.6, 73.9, 74.12, 74.14, 74.19, 74.22, 74.5, 74.6, 74.66, 74.70, 74.89, 74.91, 75.0, 75.07, 75.11, 75.3, 75.4, 75.67, 75.74, 75.8, 75.9, 76.0, 76.1, 76.15, 77.16, 78.0, 78.4, 78.6, 78.8, 80.3, 80.7, 81.2, 93.0, 93.4, 99.5, 99.7, 101.16, 101.22, 101.26, 101.29, 101.7, 102.1, 126.1, 126.86, 126.90, 126.93, 127.07, 127.11, 127.16, 127.19, 127.24, 127.26, 127.32, 127.34, 127.43, 127.45, 127.50, 127.52, 127.54, 127.59, 127.61, 127.63, 127.67, 127.70, 127.73, 127.76, 127.77, 127.80, 127.9, 128.00, 128.02, 128.06, 128.07, 128.10, 128.13, 128.18, 128.20, 128.21, 128.25, 128.27, 128.30, 128.35, 128.40, 128.5, 128.8, 137.7, 138.07, 138.11, 138.25, 138.34, 138.36, 138.42, 138.5, 138.63, 138.64, 138.7, 138.8, 138.89, 138.92, 138.98, 139.00, 139.03, 139.06, 139.09, 139.2, 170.3; MS (MALDI, DHB) found *m*/*z* 2968.3 $[M + Na]^+$, calcd for $C_{179}H_{188}NaO_{38}$ 2968.3.

β-D-Mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-2-*O*-acetyl-β-D-mannopyranosyl-(1→4)-2-*O*-acetyl-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-D-mannopyranose (6). According to the representative procedure for global deprotection of benzyl, benzylidene, and *p*-methoxybenzyl groups, compound **51** (12 mg, 4.07 μmol) was subjected to catalytic hydrogenation with 10% Pd/C (12 mg) in THF/MeOH/AcOH (2.0:1.0:0.3, v/v/v, 1.65 mL) at room temperature for 23 h to give compound **6** (3.0 mg, 2.43 μmol, 60%) after reversed-phase chromatography on a C18 column (1% AcOH in H_2O). Heptasaccharide **6** was isolated as a mixture of regioisomers resulting from the migration of the acetyl groups: white hygroscopic solid; ^1H NMR (1% CD_3CO_2D in D_2O, 600 MHz) δ 2.16 (s, integration not determined), 2.181 (s, integration not determined), 2.183 (s, integration not determined), 2.194 (s, integration not determined), 2.198 (s, integration not determined), 2.204 (s, integration not determined), 3.40−3.68 (m, integration not determined), 3.69−4.07 (m, integration not determined), 4.08−4.14 (m, integration not determined), 4.16−4.21 (m, integration not determined), 4.72 (s, integration not determined), 4.75 (s, integration not determined), 4.82 (s, integration not determined), 4.83 (s, integration not determined), 4.893 (s, integration not determined), 4.899 (s, integration not determined), 4.903 (s, integration not determined), 4.909 (s, integration not determined), 4.93 (s, integration not determined), 4.94 (s, integration not determined), 5.04 (dd, *J* = 9.7, 3.0 Hz, integration not determined), 5.06−5.13 (m, integration not determined), 5.17 (s, integration not determined), 5.41 (d, *J* = 3.2 Hz, integration not determined), 5.48 (d, *J* = 3.5 Hz, integration not determined), 5.49−5.52 (m, integration not determined); ^{13}C NMR (1% CD_3CO_2D in D_2O, 150 MHz, selected signals) δ 63.1, 63.19, 63.24, 63.3, 63.7, 69.4, 71.3, 71.36, 71.40, 71.5, 71.6, 72.62, 72.64, 72.7, 72.75, 72.80, 72.88, 72.93, 73.2, 73.4, 73.6, 74.1, 74.2, 74.3, 74.4, 74.5, 75.5, 75.7, 75.8, 76.0, 76.08, 76.11, 77.5, 77.66, 77.69, 77.74, 77.86, 77.91, 78.0, 79.1, 79.18, 79.23, 79.3, 79.5, 79.9, 80.1, 96.4, 96.5, 101.3, 101.80, 101.84, 102.3, 102.86, 102.91, 103.0, 103.1, 175.5, 175.67, 175.70, 175.74, 176.1, 176.2; HRMS found *m*/*z* 1259.3822 $[M + Na]^+$, calcd for $C_{46}H_{76}NaO_{38}$ 1259.3912.

β-D-Mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-D-mannopyranose (4). According to the representative procedure for saponification, heptasaccharide **6** (7 mg, 5.66 μmol) was treated with NaOH (1.0 M, 100 μL) at room temperature for 2 h to give compound **4** (6.2 mg, 5.38 μmol, 95%) after lyophilization: white hygroscopic solid; ^1H NMR (D_2O, 600 MHz) δ 3.42−3.47 (m, integration not determined), 3.49−3.61 (m, integration not determined), 3.66 (dd, *J* = 9.6, 3.3 Hz, integration not determined), 3.71−4.02 (m), 4.07 (d, *J* = 3.4 Hz, integration not determined), 4.12−4.16 (m, integration not determined), 4.74 (s, integration not determined), 4.76 (s, integration not determined), 4.92 (s, integration not determined), 5.19 (s, integration not determined); ^{13}C NMR (D_2O, 151 MHz, selected signals) δ 63.2, 63.7, 69.4, 71.7, 72.7, 72.9, 73.2, 73.4, 73.6, 74.1, 74.2, 74.4, 75.5, 77.5, 77.8, 79.1, 79.2, 79.27, 79.34, 79.5, 96.4, 96.6, 102.87, 102.93; HRMS found *m*/*z* 1175.3623 $[M + Na]^+$, calcd for $C_{42}H_{72}NaO_{36}$ 1175.3701.

Allyl 2,3-Di-*O*-Benzyl-4,6-benzylidene -β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (55). According to the representative procedure for β-selective mannosylation, the reaction of compounds **38** (883 mg, 0.96 mmol) and **14** (663 mg, 1.23 mmol) was performed, followed by quench with Et_3N and $(EtO)_3P$, to give compound **55** (784 mg, 0.58 mmol, 61%) after chromatography on silica gel (toluene/EtOAc, 95.5:4.5): colorless oil; ^1H NMR ($CDCl_3$, 600 MHz) δ 3.05 (td, *J* = 9.7, 4.9, 1H), 3.19 (dt, *J* = 9.5, 3.1 Hz, 1H), 3.30 (dd, *J* = 9.2, 3.0 Hz, 1H), 3.40 (dd, *J* = 9.9, 3.0 Hz, 1H), 3.47−3.58 (m, 3H), 3.62−3.72 (m, 3H), 3.73 (d, *J* = 3.0 Hz, 1H), 3.76−3.81 (m, 2H), 3.91−3.99 (m, 3H), 4.04 (t, *J* = 9.6 Hz, 1H), 4.12−4.19 (m, 2H), 4.21−4.30 (t overlapped with d, 2H), 4.41 (d, *J* = 12.1 Hz, 1H), 4.45 (d, *J* = 11.9 Hz, 1H), 4.48−4.55 (m, 4H), 4.59 (d, *J* = 12.3 Hz, 1H), 4.62−4.81 (m, 9H), 4.85−4.92 (2 overlapping d, 2H), 5.13−5.21 (m, 1H), 5.21−5.28 (m, 1H), 5.48 (s, 1H), 5.79−5.94 (m, 1H), 7.09−7.41 (m, integration not determined), 7.42−7.47 (m, 2H); ^{13}C NMR ($CDCl_3$, 151 MHz) δ 67.2, 67.9, 68.5, 69.25, 69.32, 71.3, 72.2, 72.4, 72.5, 72.7, 73.3, 73.5, 74.2, 74.9, 75.4, 75.6, 75.7, 76.0, 76.1, 77.1, 78.37, 78.42, 78.6, 80.7, 97.5, 101.3, 101.5, 102.2, 117.8, 126.1, 126.9, 127.10, 127.13, 127.2, 127.35, 127.37, 127.42, 127.5, 127.57, 127.63, 127.70, 127.73, 127.8, 128.0, 128.07, 128.09, 128.12, 128.21, 128.24, 128.3, 128.7, 133.8, 137.7, 138.3, 138.37, 138.42, 138.5, 138.8, 139.07, 139.10, 139.3; MS (MALDI, DHB) found *m*/*z* 1375.6 $[M + Na]^+$; calcd for $C_{84}H_{88}NaO_{16}$: 1375.6.

Allyl 2,3,6-Tri-*O*-Benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (56). According to the representative procedure for reductive ring-opening of benzylidene acetal, trisaccharide **55** (317 mg, 234 μmol) was reduced with Et_3SiH (115 μL, 712 μmol) in the presence of TfOH (70 μL, 791 μmol) at −78 °C for 15 h, followed by treatment with TBAF (2.2 mL, 1 M solution in THF) at room temperature for 2 h, to give compound **56** (138 mg, 102 μmol, 44%) after column chromatography on silica gel (toluene/EtOAc, 92:8): colorless oil; ^1H NMR ($CDCl_3$, 600 MHz) δ 2.68 (d, *J* = 1.0 Hz, 1H), 3.14 (dd, *J* = 9.4, 2.8 Hz, 1H), 3.20 (dt, *J* = 9.6, 4.8, 1H), 3.25 (ddd, *J* = 9.5, 4.0, 2.4, 1H), 3.33 (dd, *J* = 9.2, 2.8, 1H), 3.50−3.61 (m, 4H), 3.61−3.67 (2 overlapping dd, 2H), 3.69 (d, *J* = 2.9 Hz, 1H), 3.72−3.79 (m, 3H), 3.89−3.99 (m, 3H), 4.13−4.24 (m, 3H), 4.30 (d, *J* = 12.0, 1H), 4.34 (d, *J* = 11.7, 1H), 4.37−4.48 (m, 5H), 4.49−4.55 (d overlapped with 2 s, 3H), 4.56−4.80 (m, 9H), 4.86−4.91 (m, 2H), 5.13−5.20 (m, 1H), 5.20−5.28 (m, 1H), 5.80−5.93 (m, 1H), 7.12−7.39 (m, integration not determined); ^{13}C NMR ($CDCl_3$, 151 MHz) δ 67.9, 68.8, 69.3, 69.5, 71.1, 71.35, 71.38, 72.3, 72.6, 72.7, 73.3, 73.5, 73.7, 74.1, 74.2, 74.6, 74.7, 75.3, 75.5, 75.6, 75.7, 76.1, 78.3, 80.7, 81.8, 97.5, 101.4, 101.6, 117.2, 127.10, 127.12, 127.2, 127.3, 127.38, 127.44, 127.5, 127.55, 127.62, 127.7, 127.75, 127.77, 127.9, 128.08, 128.12, 128.13, 128.21, 128.22, 128.25, 128.31, 128.4, 133.9, 138.10, 138.11, 138.3, 138.4, 138.5, 139.0, 139.1, 139.2, 139.3; MS (MALDI, DHB) found *m*/*z* 1377.6 $[M + Na]^+$, calcd for $C_{84}H_{90}NaO_{16}$ 1377.6.

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Allyl 2,3,-Di-*O*-Benzyl-4,6-benzylidene- -β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (57). According to the representative procedure for β-selective mannosylation, the reaction of compounds **56** (308 mg, 227 μmol) and **14** (160 mg, 296 μmol) was performed, followed by quench with Et_3N and $(EtO)_3P$, to give compound **57** (262 mg, 147 μmol, 65%) after chromatography on silica gel (toluene/EtOAc, 94:6): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.03 (td, *J* = 9.7, 4.9 Hz, 1H), 3.13−3.19 (m, 1H), 3.21 (dt, *J* = 9.4, 3.0 Hz, 1H), 3.27−3.35 (2 overlapping dd, 2H), 3.38 (dd, *J* = 9.9, 2.9 Hz, 1H), 3.43−3.51 (2 overlapping dd, 2H), 3.51−3.58 (m, 3H), 3.61−3.68 (2 overlapping dd, 2H), 3.69−3.74 (m, 3H), 3.75−3.80 (m, 2H), 3.91−3.98 (m, 3H), 4.03 (t, *J* = 9.6 Hz, 1H), 4.10−4.26 (m, 5H), 4.28 (d, *J* = 12.1 Hz, 1H), 4.37−4.42 (2 overlapping d, 2H), 4.46−4.54 (m, 7H), 4.56−4.83 (m, 13H), 4.86−4.91 (2 overlapping d, 2H), 5.14−5.19 (m, 1H), 5.20−5.26 (m, 1H), 5.47 (s, 1H), 5.80−5.92 (m, 1H), 7.09−7.39 (m, integration not determined), 7.42−7.46 (m, 2H); ^{13}C NMR ($CDCl_3$, 151 Hz) δ 67.2, 67.9, 68.5, 69.2, 69.3, 69.4, 71.4, 72.1, 72.3, 72.4, 72.6, 72.7, 73.3, 73.4, 74.1, 74.2, 74.9, 75.4, 75.5, 75.6, 75.7, 75.9, 76.1, 76.2, 77.1, 78.3, 78.4, 78.6, 80.8, 80.9, 97.5, 101.3, 101.4, 101.7, 102.2, 117.2, 126.1, 126.90, 126.93, 127.07, 127.12, 127.15, 127.18, 127.35, 127.37, 127.42, 127.45, 127.54, 127.6, 127.66, 127.70, 127.73, 127.96, 127.99, 128.05, 128.09, 128.14, 128.20, 128.21, 128.24, 128.3, 128.7, 133.9, 137.7, 138.3, 138.4, 138.47, 138.54, 138.8, 139.09, 139.11, 139.14, 139.3; MS (MALDI, DHB) found *m*/*z* 1807.8 [M + Na]$^+$, calcd for $C_{111}H_{116}NaO_{21}$ 1807.8.

Allyl 2,3,-Di-*O*-Benzyl -β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (58). According to the representative procedure for transacetalation, compound **57** (1.502 g, 0.84 mmol) was treated with neopentyl glycol (438 mg, 4.21 mmol) in the presence of camphorsulfonic acid (88 mg, 0.38 mmol) for 24 h at room temperature to give compound **58** (1.119 g, 0.66 mmol, 78%) after chromatography on silica gel (toluene/EtOAc, 74:26): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.25 (d, *J* = 2.0 Hz, 1H), 2.36 (br s, 1H), 3.03−3.09 (m, 2H), 3.19−3.23 (m, 2H), 3.26 (dd, *J* = 9.5, 2.8 Hz, 1H), 3.32 (dd, *J* = 9.2, 2.8 Hz, 1H), 3.40 (dd, *J* = 11.5, 6.5 Hz, 1H), 3.51−3.59 (m, 4H), 3.61−3.69 (m, 3H), 3.69−3.73 (m, 2H), 3.73−3.80 (m, 4H), 3.91−3.98 (m, 2H), 4.10−4.25 (m, 5H), 4.26−4.30 (2 overlapping d, 2H), 4.35 (d, *J* = 11.8 Hz, 1H), 4.37−4.43 (2 overlapping d, 2H), 4.46 (s, 1H), 4.48−4.54 (m, 5H), 4.56−4.81 (m, 11H), 4.84 (d, *J* = 12.4 Hz, 1H), 4.86−4.91 (2 overlapping d, 2H), 5.14−5.19 (m, 1H), 5.21−5.28 (m, 1H), 5.80−5.92 (m, 1H), 7.09−7.41 (m, integration not determined); ^{13}C NMR ($CDCl_3$, 151 MHz) δ 62.7, 67.2, 67.9, 69.3, 69.4, 71.0, 71.3, 72.4, 72.45, 72.52, 72.7, 73.3, 73.4, 73.5, 73.8, 74.08, 74.10, 74.4, 75.39, 75.41, 75.43, 75.5, 75.6, 75.7, 76.3, 76.5, 78.2, 80.3, 80.7, 82.0, 97.5, 101.2, 101.4, 101.5, 117.1, 126.9, 127.0, 127.08, 127.11, 127.14, 127.27, 127.33, 127.37, 127.43, 127.52, 127.54, 127.6, 127.67, 127.71, 127.76, 127.80, 127.82, 127.88, 127.92, 128.07, 128.11, 128.13, 128.18, 128.19, 128.23, 128.3, 128.4, 133.8, 137.7, 138.25, 138.32, 138.38, 138.40, 138.65, 138.68, 138.9, 139.1, 139.2, 139.3; MS (MALDI, DHB) found *m*/*z* 1719.7 [M + Na]$^+$, calcd for $C_{104}H_{112}NaO_{21}$ 1719.8.

Allyl 2,3,6-Tri-*O*-benzyl -β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (54). According to the representative procedure for selective monobenzylation, diol **58** (1.047 g, 0.62 mmol) was heated with Bu_2SnO (307 mg, 1.23 mmol) under reflux in toluene, followed by treatment with BnBr (295 μL, 2.47 mmol, 4.0 equiv) and CsF (422 mg, 2.78 mmol) under reflux for 6 h, to give compound **54** (896 mg, 0.50 mmol, 81%) after column chromatography on silica gel (toluene/EtOAc, 88:12): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.69 (d, *J* = 1.4 Hz, 1H), 3.13 (dd, *J* = 9.5, 2.8 Hz, 1H), 3.16−3.25 (m, 3H), 3.28−3.35 (2 overlapping dd, 2H), 3.49−3.59 (m, 6H), 3.61−3.67 (2 overlapping dd, 2H), 3.68−3.74 (3 overlapping d, 3H), 3.74−3.79 (m, 2H), 3.89−3.97 (m, 3H), 4.13−4.19 (m, 3H), 4.22 (t, *J* = 9.1 Hz, 1H), 4.25−4.29 (2 overlapping d, 2H), 4.33 (d, *J* = 11.9 Hz, 1H), 4.37−4.43 (m, 5H), 4.46−4.54 (m, 6H), 4.56−4.63 (2 overlapping d, 2H), 4.63−4.80 (m, 9H), 4.82 (d, *J* = 12.4 Hz, 1H), 4.85−4.91 (2 overlapping d, 2H), 5.13−5.19 (m, 1H), 5.21−5.27 (m, 1H), 5.82−5.91 (m, 1H), 7.10−7.38 (m, integration not determined); ^{13}C NMR ($CDCl_3$, 151 MHz) δ 67.9, 68.7, 69.3, 69.35, 69.40, 71.1, 71.3, 72.2, 72.4, 72.6, 72.7, 73.3, 73.37, 73.38, 73.6, 74.05, 74.10, 74.12, 74.5, 74.6, 75.3, 75.38, 75.44, 75.5, 75.6, 75.7, 76.1, 76.2, 78.2, 80.75, 80.77, 81.8, 97.5, 101.4, 101.5, 101.6, 117.1, 127.0, 127.05, 127.09, 127.18, 127.22, 127.3, 127.4, 127.5, 127.6, 127.65, 127.68, 127.72, 127.8, 127.9, 127.96, 128.04, 128.06, 128.11, 128.17, 128.19, 128.21, 128.3, 128.4, 133.8, 138.06, 138.10, 138.3, 138.40, 138.44, 138.5, 138.9, 139.08, 139.10, 139.13, 139.3; MS (MALDI, DHB) found *m*/*z* 1809.7 [M + Na]$^+$, calcd for $C_{111}H_{118}NaO_{21}$ 1809.8.

Allyl 2,3-Di-*O*-benzyl-4,6-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (59). According to the representative procedure for glycosylation, acceptor **54** (454 mg, 254 μmol) was treated with trichloroacetimidate **34** (725 mg, 381 μmol) in the presence of TMSOTf (6.8 μL, 38 μmol) to give compound **59** (696 mg, 197 μmol, 78%) after column chromatography on silica gel (hexane/EtOAc, 70:30): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.85−2.91 (m, 1H), 303 (td, *J* = 9.6, 5.0 Hz, 2H), 3.11−3.23 (m, 6H), 3.23−3.32 (m, 5H), 3.36−3.41 (m, 2H), 3.41−3.68 (m, 17H), 3.68−3.73 (3 overlapping d, 3H), 3.73−3.78 (m, 2H), 3.90−3.97 (m, 3H), 3.97−4.09 (m, 4H), 4.09−4.27 (m, 11H), 4.32−4.36 (d overlapped with s, 2H), 4.36−4.54 (m, 17H), 4.54−4.61 (3 overlapping d, 3H), 4.61−4.84 (m, 21H), 4.84−4.89 (2 overlapping d, 2H), 5.0 (d, *J* = 12.1 Hz, 1H), 5.14−5.25 (m, 3H), 5.47 (s, 1H), 5.81−5.91 (m, 1H), 6.85−6.90 (m, 2H), 6.93−7.40 (m, integration not determined), 7.42−7.47 (m, 2H), 7.50−7.58 (m, 1H), 7.82−7.89 (m, 2H); ^{13}C NMR ($CDCl_3$, 151 MHz, selected signals) δ 67.2, 67.9, 68.2, 68.49, 68.51, 69.18, 69.24, 69.3, 69.35, 69.42, 71.3, 72.1, 72.2, 72.35, 72.40, 72.6, 72.7, 73.3, 73.35, 73.42, 73.7, 74.0, 74.05, 74.07, 74.2, 74.3, 74.9, 75.0, 75.36, 75.39, 75.43, 75.46, 75.51, 75.6, 75.7, 75.9, 76.0, 76.08, 76.14, 76.2, 76.3, 77.1, 78.0, 78.2, 78.4, 78.6, 80.56, 80.63, 80.7, 80.76, 80.80, 80.9, 97.5, 100.4, 101.2, 101.30, 101.34, 101.5, 101.6, 102.2, 117.1, 126.1, 126.8, 126.9, 126.95, 126.97, 127.05, 127.08, 127.16, 127.20, 127.22, 127.30, 127.34, 127.40, 127.42, 127.48, 127.52, 127.58, 127.60, 127.63, 127.7, 127.8, 127.91, 127.93, 127.96, 127.98, 128.04, 128.1, 128.2, 128.3, 128.7, 129.8, 129.9, 132.9, 133.9, 137.7, 138.1, 138.3, 138.36, 138.38, 138.42, 138.44, 138.5, 138.7, 138.76, 138.82, 139.0, 139.06, 139.09, 139.13, 139.2, 139.26, 139.29, 139.32, 164.9; MS (MALDI, DHB) found *m*/*z* 3551.1 [M + Na]$^+$, calcd for $C_{219}H_{226}NaO_{24}$ 3550.5.

Allyl 2,3-Di-*O*-benzyl-4,6-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (60). According to the representative procedure for debenzoylation, compound **59** (93 mg, 26.3 μmol) was treated with $LiAlH_4$ (8 mg, 210.8 μmol) in Et_2O to give compound **60** (60 mg, 17.5 μmol, 67%) after chromatography on silica gel (hexane/EtOAc, 70:30): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.02 (td, *J* = 9.6, 5.0 Hz, 1H), 3.08−3.42 (m, 16H), 3.42−3.58 (m, 10H), 3.58−3.67 (m, 3H), 3.67−3.82 (m, 10H), 3.90−3.98 (m, 3H), 4.02 (t, *J* = 9.5 Hz, 1H), 4.09−4.19 (m, 6H), 4.19−4.28 (m, 6H), 4.31−4.43 (m, 8H), 4.44−4.54 (m, 14H), 4.57 (d, *J* = 12.3 Hz, 1H), 4.59−4.82 (m, 21H), 4.85−4.91 (2 overlapping d, 2H), 4.99 (d, *J* = 12.0 Hz, 1H), 5.15−5.18 (m, 1H), 5.20−5.26 (m, 1H), 5.47 (s, 1H), 5.83−5.91 (m, 1H), 7.08−7.39 (m, integration not determined), 7.42−7.45 (m, 2H); ^{13}C NMR ($CDCl_3$, 151 MHz, selected signals) δ 67.2, 67.9, 68.5, 68.6, 69.2, 69.3, 69.4, 69.45, 69.52, 71.36, 71.38, 72.1, 72.2, 72.3, 72.4, 72.6, 72.7, 73.2, 73.3, 73.37, 73.40, 73.41, 73.6, 74.08, 74.12, 74.16, 74.19, 74.7, 74.8, 74.9, 75.0, 75.2, 75.29, 75.33, 75.39, 75.41, 75.48, 75.52, 75.6, 75.7, 75.9, 76.06, 76.12, 76.13, 76.2, 77.0, 77.1, 78.3, 78.4, 78.6, 80.75, 80.80, 80.84, 81.9, 82.4, 97.5, 101.0, 101.3, 101.4, 101.6, 101.7, 101.8, 102.2, 103.7, 117.2, 126.1, 126.7, 126.8, 126.9, 126.96, 126.99,

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127.01, 127.06, 127.11, 127.16, 127.19, 127.3, 127.4, 127.46, 127.54, 127.6, 127.66, 127.70, 127.96, 127.98, 128.01, 128.03, 128.05, 128.08, 128.13, 128.19, 128.21, 128.23, 128.26, 128.30, 128.7, 133.9, 137.7, 137.8, 138.0, 138.3, 138.42, 138.44, 138.48, 138.54, 138.7, 138.8, 138.99, 139.01, 139.09, 139.10, 139.2, 139.27, 139.31, 139.5; MS (MALDI, DHB) found m/z 3446.9 $[M + Na]^+$, calcd for $C_{212}H_{222}NaO_{41}$ 3446.5.

Allyl 2,3-Di-*O*-benzyl-4,6-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (61). According to the representative procedure for gluco to manno epimerization, compound **60** (88 mg, 25.7 μmol, 1.0 equiv) was subjected to oxidation with DMSO (1.0 mL) and acetic anhydride (0.5 mL) at room temperature for 16 h, and subsequent reduction with L-Selectride (1 M in THF, 77 μL, 77 μmol) at −78 °C for 30 min, to give compound **61** (51 mg, 14.9 μmol, 58%) after column chromatography on silica gel (hexane/EtOAc, 65:35): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.68 (br s, 1H), 303 (td, J = 9.6, 4.9 Hz, 1H), 3.10−3.23 (m, 6H), 3.26−3.40 (m, 7H), 3.41−3.59 (m, 12H), 3.60−3.78 (m, 11H), 3.89 (br s, 1H), 3.91−3.97 (m, 3H), 4.00−4.07 (2 overlapping t, 2H), 4.10−4.32 (m, 13H), 4.35−4.54 (m, 21H), 4.55−4.82 (m, 23H), 4.85−4.90 (2 overlapping d, 2H), 5.14−5.19 (m, 1H), 5.19−5.28 (m, 1H), 5.47 (s, 1H), 5.75−5.95 (m, 1H), 7.03−7.39 (m, integration not determined), 7.41−7.47 (m, 2H); ^{13}C NMR ($CDCl_3$, 151 MHz, selected signals) δ 67.2, 67.9, 68.0, 68.5, 69.1, 69.2, 69.3, 69.36, 69.42, 71.4, 71.8, 72.1, 72.2, 72.3, 72.36, 72.38, 72.6, 72.7, 73.2, 73.3, 73.35, 73.38, 73.39, 73.41, 74.0, 74.12, 74.13, 74.7, 74.9, 75.1, 75.2, 75.28, 75.33, 75.36, 75.41, 75.43, 75.5, 75.6, 75.7, 75.9, 76.0, 76.1, 76.2, 77.1, 78.3, 78.4, 78.6, 79.3, 80.75, 80.77, 80.84, 81.4, 97.5, 100.2, 101.3, 101.4, 101.57, 101.61, 101.7, 102.2, 117.2, 126.1, 126.90, 126.94, 126.98, 127.0, 127.07, 127.11, 127.16, 127.20, 127.3, 127.35, 127.41, 127.44, 127.45, 127.54, 127.60, 127.62, 127.70, 127.73, 127.95, 127.98, 128.01, 128.05, 128.09, 128.11, 128.18, 128.20, 128.23, 128.3, 128.7, 133.9, 137.7, 138.3, 138.4, 138.45, 138.48, 138.51, 138.6, 138.7, 138.8, 139.05, 139.11, 139.2, 139.25, 139.27; MS (MALDI, DHB) found m/z 3446.1 $[M + Na]^+$, calcd for $C_{212}H_{222}NaO_{41}$ 3446.5.

Allyl 2,3-Di-*O*-benzyl-4,6-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2-*O*-acetyl-3,6-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (53). According to the representative procedure for acetylation, alcohol **61** (92 mg, 26.9 μmol) was treated with acetic anhydride (0.35 mL) in pyridine in the presence of DMAP (catalytic amount) for 3 h at room temperature to give acetate **53** (85 mg, 24.5 μmol, 91%) after column chromatography on silica gel (hexane/EtOAc, 67:33): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.00 (s, 3H), 3.02 (td, J = 9.6, 4.9 Hz, 1H), 3.10−3.23 (m, 6H), 3.25−3.33 (m, 6H), 3.35−3.59 (m, 14H), 3.60−3.73 (m, 8H), 3.74−3.79 (m, 2H), 3.90−3.98 (m, 4H), 4.02 (t, J = 9.5 Hz, 1H), 4.07−4.31 (m, 12H), 4.34−4.54 (m, 22H), 4.55−4.82 (m, 23H), 4.84−4.91 (2 overlapping d, 2H), 5.13−5.20 (m, 1H), 5.20−5.26 (m, 1H), 5.38 (d, J = 3.3 Hz, 1H), 5.47 (s, 1H), 5.80−5.92 (m, 1H), 7.07−7.38 (m), 7.41−7.46 (m, 2H); ^{13}C NMR ($CDCl_3$, 151 MHz, selected signals) δ 21.0, 67.2, 67.9, 68.5, 68.8, 69.0, 69.1, 69.2, 69.4, 69.5, 71.36, 71.40, 72.0, 72.1, 72.15, 72.24, 72.4, 72.6, 72.7, 73.0, 73.3, 73.35, 73.36, 73.38, 73.39, 74.05, 74.10, 74.14, 74.2, 74.5, 74.9, 75.0, 75.3, 75.4, 75.5, 75.6, 75.7, 75.82, 75.84, 76.08, 76.12, 76.17, 76.21, 77.1, 78.1, 78.3, 78.4, 78.6, 80.7, 80.76, 80.82, 81.4, 97.5 ($^1J_{CH}$ = 167.5 Hz), 99.8 ($^1J_{CH}$ = 159.5 Hz), 101.1 ($^1J_{CH}$ = 155.4 Hz), 101.3, 101.4 ($^1J_{CH}$ = 157.3 Hz), 101.5 ($^1J_{CH}$ = 158.2 Hz), 101.6 ($^1J_{CH}$ = 157.0 Hz), 101.7 ($^1J_{CH}$ = 157.6 Hz), 102.2 ($^1J_{CH}$ = 157.0 Hz), 117.1, 126.1, 126.8, 126.85, 126.89, 126.96, 126.98, 127.04, 127.06, 127.10, 127.14, 127.2, 127.26, 127.30, 127.34, 127.37, 127.42, 127.44, 127.50, 127.54, 127.57, 127.58, 127.65, 127.68, 127.70, 127.73, 127.74, 127.95, 127.97, 127.99, 128.05, 128.08, 128.09, 128.13, 128.18, 128.21, 128.24, 128.3, 128.7, 133.9, 137.7, 138.3, 138.35, 138.39, 138.43, 138.45, 138.53, 138.54, 138.78, 138.81, 139.08, 139.11, 139.14, 139.17, 139.23, 139.25, 139.27, 170.3; MS (MALDI, DHB) found m/z 3448.1 $[M + Na]^+$, calcd for $C_{214}H_{224}NaO_{42}$ 3488.5.

2,3-Di-*O*-benzyl-4,6-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2-*O*-acetyl-3,6-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranose (62). According to the representative procedure for deallylation, compound **53** (104 mg, 30.0 μmol) was treated with a catalytic amount of $PdCl_2$ in $MeOH/CH_2Cl_2$ (1:1, v/v, 4.6 mL) at room temperature for 3 h to give compound **62** (61 mg, 17.8 μmol, 59%) after column chromatography on silica gel (hexane/EtOAc, 62:38): colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.00 (s, integration not determined), 2.64 (d, J = 3.5 Hz, integration not determined), 3.02 (td, J = 9.7, 4.9 Hz, integration not determined), 3.10−3.25 (m, integration not determined), 3.25−3.60 (m, integration not determined), 3.60−3.73 (m, integration not determined), 3.75 (t, J = 2.8 Hz, integration not determined), 3.77−3.79 (m, integration not determined), 3.90−4.05 (m, integration not determined), 4.06−4.32 (m, integration not determined), 4.34−4.84 (m, integration not determined), 4.92−4.98 (2 overlapping d, integration not determined), 5.23 (t, J = 2.9 Hz, integration not determined), 5.39 (d, J = 3.4 Hz, integration not determined), 5.47 (s, integration not determined), 7.09−7.40 (m, integration not determined), 7.41−7.46 (m, integration not determined); ^{13}C NMR ($CDCl_3$, 151 Hz, selected signals) δ 21.0, 67.2, 68.5, 68.8, 68.9, 69.1, 69.2, 69.36, 69.42, 69.5, 69.6, 71.4, 71.5, 72.0, 72.1, 72.2, 72.26, 72.30, 72.4, 72.5, 72.7, 73.0, 73.4, 73.5, 73.6, 74.0, 74.06, 74.11, 74.14, 74.16, 74.23, 74.5, 74.8, 74.9, 75.0, 75.30, 75.32, 75.39, 75.41, 75.5, 75.7, 75.8, 75.9, 76.08, 76.11, 76.2, 77.1, 77.2, 78.1, 78.4, 78.6, 80.4, 80.55, 80.59, 80.7, 80.8, 81.4, 93.0, 93.4, 99.7, 101.1, 101.2, 101.3, 101.5, 101.6, 101.7, 102.2, 126.1, 126.8, 126.85, 126.89, 126.98, 127.01, 127.07, 127.11, 127.15, 127.22, 127.27, 127.33, 127.34, 127.37, 127.42, 127.5, 127.58, 127.63, 127.69, 127.73, 127.8, 128.0, 128.05, 128.09, 128.15, 128.18, 128.21, 128.3, 128.4, 128.5, 128.7, 128.8, 130.9, 137.7, 138.1, 138.35, 138.39, 138.42, 138.46, 138.51, 138.54, 138.78, 139.0, 139.1, 139.17, 139.23, 170.3; MS (MALDI, DHB) found m/z 3448.5 $[M + Na]^+$, calcd for $C_{211}H_{220}NaO_{42}$ 3448.5.

β-D-Mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-2-*O*-acetyl-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannopyranose (8). According to the representative procedure for global deprotection of benzyl, benzylidene and *p*-methoxybenzyl groups, compound **62** (25 mg, 7.29 μmol) was subject to hydrogenation by catalysis of 10% Pd/C in THF/MeOH/AcOH (2.0:1.0:0.3, v/v/v, 2.13 mL) at room temperature to give compound **8** (6.2 mg, 1.81 μmol, 63%) after reversed-phase chromatography (CH_3CN/H_2O/AcOH, 2.5/97.5/0.1). Octasaccharide **8** was isolated as a mixture of regioisomers resulting from the migration of the acetyl group: white hygroscopic solid; ^{1}H NMR (1% CD_3CO_2D in D_2O, 600 MHz) δ 2.19 (s, integration not determined), 2.20 (s, integration not determined), 3.41−3.60 (m, integration not determined), 3.60−3.68 (m, integration not determined), 3.71−4.08 (m, integration not determined), 4.09−4.15 (m, integration not determined), 4.20 (d, J = 3.6 Hz, integration not determined), 4.72 (s, integration not determined), 4.73 (s, integration not determined), 4.75 (s, integration not determined), 4.83 (s, integration not determined), 4.91 (s, integration not determined), 4.95 (s, integration not determined), 5.12 (dd, J = 9.8, 3.1 Hz, integration not determined), 5.18 (d, J = 1.5 Hz, integration not determined), 5.51 (d, J = 3.8 Hz, integration not determined); ^{13}C NMR (1% CD_3CO_2D in D_2O, 151 MHz, selected signals) δ 23.0, 23.3, 63.2, 63.3, 63.7, 69.4, 71.4, 71.6, 72.6, 72.66, 72.70, 72.79, 72.84, 72.9, 73.2, 73.4, 73.6, 74.1, 74.2, 74.26, 74.28, 74.4, 75.5, 76.0, 76.1, 77.50, 77.53, 77.7, 77.9, 78.0, 79.10, 79.13, 79.22, 79.23, 79.27, 79.32, 79.5, 80.1, 96.4, 96.6, 101.8, 102.3, 102.8, 102.86, 102.91, 103.0, 175.7, 176.1; HRMS found m/z 1357.4569 $[M + H]^+$, calcd for $C_{50}H_{85}O_{42}$ 1357.4515.

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β-D-Mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-D-mannopyranose (7). According to the representative procedure for saponification, octasaccharide **8** (6.1 mg, 4.49 μmol) was treated with 1.0 M NaOH in water at room temperature for 1.5 h to give compound **7** (5.0 mg, 3.80 μmol, 85%) after lyophilization: white hygroscopic solid; ^{1}H NMR (D_2O, 600 MHz) δ 3.45 (ddd, *J* = 9.6, 7.1, 2.4 Hz, integration not determined), 3.49−3.53 (m, integration not determined), 3.54−3.62 (m, integration not determined), 3.67 (dd, *J* = 9.7, 3.2 Hz, integration not determined), 3.71−4.03 (m, integration not determined), 4.08 (d, *J* = 3.3 Hz, integration not determined), 4.12−4.16 (m, integration not determined), 4.75 (s, integration not determined), 4.77 (s, integration not determined), 4.93 (s, integration not determined), 5.19 (d, *J* = 1.2 Hz, integration not determined); ^{13}C NMR (D_2O, 125 MHz) δ 63.2, 63.7, 69.4, 71.7, 72.6, 72.67, 72.71, 72.9, 73.2, 73.4, 73.6, 74.1, 74.2, 74.4, 75.5, 77.5, 77.8, 79.15, 79.21, 79.27, 79.34, 79.5, 96.4, 96.6, 102.87. 102.93; HRMS found *m*/*z* 1315.4443 [M + H]$^+$, calcd for $C_{48}H_{83}O_{41}$ 1315.4410.

Independent Synthesis of β-D-Mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-D-mannitol (52). To a solution of compound **51** (36 mg, 12.2 μmol) in THF/EtOH (1:2, v/v, 1.5 mL) was added $NaBH_4$ (10 mg) in one portion. The mixture was stirred at room temperature for 17 h, and water was added at 0 °C. The mixture was diluted with CH_2Cl_2, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (toluene/EtOAc, 75:25) to give a mannitol compound **64** (22 mg, 7.5 μmol, 61%).

The above-prepared mannitol compound was treated with 10% Pd/C (22 mg) in THF/MeOH/AcOH (2.0:1.0:0.3, v/v/v, 2.97 mL) under an atmosphere of hydrogen at room temperature for 23 h. The mixture was passed through a pad of Celite to remove the catalyst, and the filtrate was concentrated under reduced pressure. The residue was dissolved in H_2O/MeOH/AcOH (3:6:1, v/v/v, 3.00 mL) and treated with 10% Pd/C (22 mg), and the suspension was stirred under an atmosphere of hydrogen at room temperature for 17 h. The suspension was filtered through a pad of Celite and concentrated under reduced pressure. Reversed-phase chromatography (CH_3CN/H_2O/AcOH, 2:98:1) of the residue gave a debenzylation compound (5.3 mg).

The above-prepared debenzylated compound (5.3 mg) was treated in 0.1 M NaOH (0.7 mL) at room temperature for 1.0 h. The solution was passed through a short cation-exchange column (Dowex 50W-X8 H^+ form) and washed with water. The eluent was concentrated under reduced pressure and lyophilized to give compound **52** (4.7 mg, 3.46 μmol, 47%): white hydroscopic solid; ^{1}H NMR (D_2O, 600 MHz) δ 3.46 (ddd, *J* = 9.7, 7.1, 2.5 Hz, 1H), 3.55−3.62 (m, 6H), 3.66−3.72 (m, 3H), 3.73−3.88 (m, 18H), 3.90−3.99 (m, 9H), 4.06−4.10 (m, 2H), 4.12−4.19 (m, 5H), 4.75 (s, integration not determined), 4.76 (s, integration not determined), 4.78 (s, integration not determined), 4.83 (s, integration not determined); ^{13}C NMR (D_2O, 151 MHz) δ 63.2, 63.5, 63.7, 65.1, 65.8, 69.4, 72.2, 72.6, 72.7, 72.9, 73.2, 73.3, 74.1, 74.2, 74.3, 75.5, 77.5, 77.7, 79.1, 79.25, 79.33, 79.7, 102.7, 102.86, 102.92; HRMS found *m*/*z* 1155.4041 [M + H]$^+$, calcd for $C_{42}H_{75}O_{36}$ 1155.4038.

Independent Synthesis of β-D-Mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-D-mannitol (63). To a solution of compound **61** (93 mg, 27.1 μmol, 1.0 equiv) in MeOH/CH_2Cl_2 (1:1, v/v, 4.0 mL) was added a catalytic amount of $PdCl_2$. The mixture was stirred at room temperature for 3 h, diluted with EtOAc, and filtered through a small pad of silica gel. The filtrate was washed with saturated aqueous $NaHCO_3$, water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane/EtOAc, 55:45) to give a deallylation product (54 mg).

The above-prepared deallylated product (54 mg) was dissolved in THF/EtOH (1:2, v/v, 2.1 mL), and $NaBH_4$ (10 mg) was added in one portion. The mixture was stirred at room temperature for 15 h, and water was added at 0 °C. The mixture was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (toluene/EtOAc, 70:30) to give a mannitol product **65** (40 mg, 11.8 μmol, 43%).

The above-prepared mannitol product (31 mg, 9.15 μmol, 1.0 equiv) was treated with acetic anhydride (0.25 mL) and DMAP (catalytic amount) in pyridine (1.0 mL) for 3.0 h at room temperature and then quenched by addition of MeOH at 0 °C. After removal of the solvent by evaporation in vacuo, the residue was dissolved in Et_2O. The mixture was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane/EtOAc, 65:35) to give the acetylation product **66** (26 mg, 7.40 μmol, 81%).

The above-prepared product (11 mg, 3.13 μmol) was treated with 10% Pd/C (11 mg) in THF/MeOH/AcOH (2.0:1.0:0.3, v/v/v, 1.32 mL) under an atmosphere of hydrogen at room temperature for 23 h. The mixture was passed through a pad of Celite to remove the catalyst and then concentrated in vacuo. The residue was dissolved in H_2O/MeOH/AcOH (3:6:1, v/v/v, 1.73 mL) and treated with 10% Pd/C (11 mg) under an atmosphere of hydrogen at room temperature for 17 h. The suspension was filtered through a pad of Celite and concentrated under reduced pressure. Reversed-phase chromatography (CH_3CN/H_2O/AcOH, 5:95:1) of the residue gave the debenzylation product (1.9 mg).

The above-prepared product was treated in 0.1 M NaOH (0.4 mL) at room temperature for 1.5 h. The solution was passed through a short cation-exchange column (Dowex 50W-X8 H^+ form) and washed with water. The eluent was concentrated under reduced pressure and lyophilized to give compound **63** (1.6 mg, 1.21 μmol, 39%): white hydroscopic solid; ^{1}H NMR (D_2O, 600 MHz) δ 3.45 (ddd, *J* = 9.6, 7.1, 2.4 Hz, 1H), 3.54−3.62 (m, 7H), 3.65−3.71 (m, 3H), 3.72−3.88 (m, 21H), 3.89−3.99 (m, 10H), 4.05−4.10 (m, 2H), 4.11−4.19 (m, 6H), 4.75 (s, integration not determined), 4.76 (s, integration not determined), 4.77 (s, integration not determined), 4.82 (s, integration not determined); ^{13}C NMR (D_2O, 151 MHz) δ 63.2, 63.5, 63.7, 65.1, 65.8, 69.4, 72.2, 72.6, 72.7, 72.9, 73.2, 73.3, 74.15, 74.17, 74.3, 75.5, 77.5, 77.8, 79.2, 79.3, 79.4, 79.72, 79.73, 102.7, 102.87, 102.93; HRMS found *m*/*z* 1317.4609 [M + H]$^+$, calcd for $C_{48}H_{85}O_{41}$ 1317.4566.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR spectra of synthetic compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) Tang, Z.-Z.; Cheng, S.-J. *Bull. Bot. Res.* **1984**, *4*, 141−146. (b) Bao, X.-S.; Shun, Q.-S.; Chen, L.-Z. *Chinese Medicinal Dendrobium*; Fudan University Press and Shanghai Medical University Press: Shanghai, 2001.

(2) Lu, S.-F.; Guo, G.-J.; Cai, Y.-P. *Chin. Trad. Herbal Drugs* **2005**, *5*, 790−793.
(3) (a) Yang, L.; Wang, Z.; Xu, L. *J. Chromatogr. A* **2006**, *1104*, 230−237. (b) Lee, Y. H.; Park, J. D.; Baek, N. I.; Kim, S. I.; Ahn, B. Z. *Planta Med.* **1995**, *61*, 178−180. (c) Honda, C.; Yamaki, M. *Phytochemistry* **2000**, *53*, 987−990. (d) Yang, H.; Chou, G. X.; Wang, Z. T.; Hu, Z. B.; Xu, L. S. *Asian Nat. Prod. Res.* **2004**, *53*, 35−38. (e) Wrigley, T. C. *Nature* **1960**, *188*, 1108. (f) Morita, H.; Fujiwara, M.; Yoshida, N.; Kobayashi, J. *Tetrahedron* **2000**, *56*, 5801−5805. (g) Zhao, W. M.; Ye, Q. H.; Tan, X. J.; Jiang, H. L.; Li, X. Y.; Chen, K. X.; Kinghorn, A. D. *J. Nat. Prod.* **2001**, *64*, 1196−1200. (h) Ye, Q. H.; Qin, G. W.; Zhao, W. M. *Phytochemistry* **2002**, *61*, 885−890. (i) Ye, Q. H.; Zhao, W. M. *Planta Med.* **2002**, *68*, 723−729.
(4) Hsieh, Y. S.-Y.; Chien, C.; Liao, S. K.-S.; Liao, S.-F.; Hung, W.-T.; Yang, W.-B.; Lin, C.-C.; Cheng, T.-J. R.; Chang, C.-C.; Fang, J.-M.; Wong, C.-H. *Bioorg. Med. Chem.* **2008**, *16*, 6054−6068.
(5) (a) Nicola, N. A.; Metcalf, D.; Matsumoto, M.; Johnson, G. R. *J. Biol. Chem.* **1983**, *258*, 9017−9023. (b) Metcalf, D. *Science* **1985**, *229*, 16−22. (c) Welte, K.; Platzer, E.; Lu, L.; Gabrilove, J. L.; Levi, E.; Mertelsmann, R.; Moore, M. A. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 1526−1530. (d) Nomura, H.; Imazeki, I.; Oheda, M.; Kubota, N.; Tamura, M.; Ono, M.; Ueyama, Y.; Asano, S. *EMBO J.* **1986**, *5*, 871−876. (e) Nagata, S.; Tsuchiya, M.; Asano, S.; Kaziro, Y.; Yamazaki, T.; Yamamoto, O.; Hirata, Y.; Kubota, N.; Oheda, M.; Nomura, H.; Ono, M. *Nature* **1986**, *319*, 415−418. (f) Souza, L. M.; Boone, T. C.; Gabrilove, J.; Lai, P. H.; Zsebo, K. M.; Murdock, D. C.; Chazin, V. R.; Bruszewski, J.; Lu, H.; Chen, K. K.; Barendt, J.; Platzer, E.; Moore, M. A. S.; Mertelsmann, R.; Welte, K. *Science* **1986**, *232*, 61−65. (g) Welte, K.; Gabrilove, J.; Bronchud, M. H.; Platzer, E.; Morstyn, G. *Blood* **1996**, *88*, 1907−1929.
(6) (a) Viret, F.; Goncalves, A.; Tarpin, C.; Chabannon, C.; Viens, P. *Bull. Cancer* **2006**, *93*, 463−471. (b) Franzke, A. *Cytokine Growth Factor Rev.* **2006**, *17*, 235−244. (c) Panopoulos, A. D.; Watowich, S. S. *Cytokine* **2008**, *42*, 277−288.
(7) Xiao, B. G.; Lu, C. Z.; Link, H. *J. Cell. Molec. Med.* **2007**, *11*, 1272−1290.
(8) Wong, C.-H.; Yang, W.-B.; Cheng, T.-J.; Hsieh, Y. S.-Y.; Chien, C.; Lin, C.-C.; Wen, H.-Y.; Fang, J.-M. Structure and Bioactivity of the Polysaccharides and Oligomers in Medicinal Plant Dendrobium huoshanense. US Patent 8,354,127 B2, 2013.
(9) Crich, D.; Banerjee, A.; Yao, Q. *J. Am. Chem. Soc.* **2004**, *126*, 14930−14934.
(10) (a) Crich, D.; Sun, S. X. *J. Am. Chem. Soc.* **1998**, *120*, 435−436. (b) Crich, D.; Sun, S. X. *Tetrahedron* **1998**, *54*, 8321−8348. (c) Crich, D.; Smith, M. *J. Am. Chem. Soc.* **2001**, *123*, 9015−9020. (d) Crich, D. *J. Carbohydr. Chem.* **2002**, *21*, 667−690.
(11) Crich, D.; Li, W. J.; Li, H. M. *J. Am. Chem. Soc.* **2004**, *126*, 15081−15086.
(12) Crich, D. *Acc. Chem. Res.* **2010**, *43*, 1144−1153.
(13) Koto, S.; Asami, K.; Hirooka, M.; Nagura, K.; Takizawa, M.; Yamamoto, S.; Okamoto, N.; Sato, M.; Tajima, H.; Yoshida, T.; Nonaka, N.; Sato, T.; Zen, S.; Yago, K.; Tomonaga, F. *Bull. Chem. Soc. Jpn.* **1999**, *72*, 765−777.
(14) Oshitari, T.; Shibasaki, M.; Yoshizawa, T.; Tomita, M.; Takao, K.; Kobayashi, S. *Tetrahedron* **1997**, *53*, 10993−11006.
(15) Schmidt, R. R.; Kinzy, W. *Adv. Carbohyd. Chem. Biochem.* **1994**, *50*, 21−123.
(16) (a) Danishefsky, S. J.; Hu, S.; Cirillo, P. F.; Eckhardt, M.; Seeberger, P. H. *Chem.—Eur. J.* **1997**, *3*, 1617−1628. (b) Wang, Z. G.; Warren, J. D.; Dudkin, V. Y.; Zhang, X. F.; Iserloh, U.; Visser, M.; Eckhardt, M.; Seeberger, P. H.; Danishefsky, S. J. *Tetrahedron* **2006**, *62*, 4954−4978. (c) Ghosh, S.; Misra, A. K. *Tetrahedron: Asymmetry* **2010**, *21*, 725−730. (d) Ghosh, S.; Misra, A. K. *Tetrahedron: Asymmetry* **2010**, *21*, 2755−2761.
(17) Crich, D.; Smith, M.; Yao, Q. J.; Picione, J. *Synthesis* **2001**, 323−326.
(18) Debenham, S. D.; Toone, E. J. *Tetrahedron: Asymmetry* **2000**, *11*, 385−387.
(19) (a) Sliedregt, L. A. J. M.; Vandermarel, G. A.; Vanboom, J. H. *Tetrahedron Lett.* **1994**, *35*, 4015−4018. (b) Codee, J. D. C.; van den Bos, L. J.; Litjens, R. E. J. N.; Overkleeft, H. S.; van Boeckel, C. A. A.; van Boom, J. H.; van der Marel, G. A. *Tetrahedron* **2004**, *60*, 1057−1064.
(20) Jayaprakash, K. N.; Radhakrishnan, K. V.; Fraser-Reid, B. *Tetrahedron Lett.* **2002**, *43*, 6953−6955.
(21) Andrews, M. A.; Gould, G. L. *Carbohydr. Res.* **1992**, *229*, 141−147.
(22) Nagashima, N.; Ohno, M. *Chem. Lett.* **1987**, 141−144.
(23) Csuk, R.; Prell, E.; Korb, C.; Kluge, R.; Strohl, D. *Tetrahedron* **2010**, *66*, 467−472.
(24) Deninno, M. P.; Etienne, J. B.; Duplantier, K. C. *Tetrahedron Lett.* **1995**, *36*, 669−672.
(25) Ogawa, T.; Kaburagi, T. *Carbohydr. Res.* **1982**, *110*, C12−C15.
(26) Albright, J. D.; Goldman, L. *J. Am. Chem. Soc.* **1965**, *87*, 4214−4216.
(27) Liu, K. K. C.; Danishefsky, S. J. *J. Org. Chem.* **1994**, *59*, 1892−1894.
(28) Bock, K.; Pedersen, C. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293−299.
(29) Hannuksela, T.; du Penhoat, C. H. *Carbohydr. Res.* **2004**, *339*, 301−312.
(30) (a) Willför, S.; Sundberg, K.; Tenkanen, M.; Holmbom, B. *Carbohydr. Polym.* **2008**, *72*, 197−210. (b) Ekholm, F. S.; Ardá, A.; Eklund, P.; André, S.; Gabius, H. J.; Jiménez-Barbero, J.; Leino, R. *Chem.—Eur. J.* **2012**, *18*, 14392−14405.
(31) Pekari, K.; Tailler, D.; Weingart, R.; Schmidt, R. R. *J. Org. Chem.* **2001**, *66*, 7432−7442.
(32) Sakagami, M.; Hamana, H. *Tetrahedron Lett.* **2000**, *41*, 5547−5551.
(33) (a) Brown, H. C.; Krishnam, S. *J. Am. Chem. Soc.* **1972**, *94*, 7159−7161. (b) Lichtenthaler, F. W.; Lergenmuller, M.; Peters, S.; Varga, Z. *Tetrahedron: Asymmetry* **2003**, *14*, 727−736.