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Synthesis of high-mannose oligosaccharides containing mannose-6-phosphate residues using regioselective glycosylation

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ABSTRACT

Molecular recognition of mannose-6-phosphate (M6P)-modified oligosaccharides by transmembrane M6P receptors is a key signaling event in lysosomal protein trafficking *in vivo*. Access to M6P-containing high-mannose *N*-glycans is essential to achieving a thorough understanding of the M6P ligand–receptor recognition process. Herein we report the application of a versatile and reliable chemical strategy to prepare asymmetric di-antennary M6P-tagged high-mannose oligosaccharides in > 20% overall yield and in high purity (> 98%). Regioselective chemical glycosylation coupled with effective phosphorylation and product purification protocols were applied to rapidly assemble these oligosaccharides. The development of this synthetic strategy simplifies the preparation of M6P-tagged high-mannose oligosaccharides, which will improve access to these compounds to study their structures and biological functions.

1. Introduction

α-Mannose 6-phosphate (M6P) residues appended to high-mannose *N*-glycans are key targeting signals for the transport of newly synthesized lysosomal glycoproteins by the M6P pathway [1]. After assembly in the endoplasmic reticulum, newly synthesized lysosomal glycoproteins enter the *cis*-Golgi where their high-mannose oligosaccharide chains are covalently modified by phosphorylation to contain one or more terminal M6P residues. This phosphorylation involves UDP-GlcNAc as a GlcNAc 1-phosphate donor to form a transient phosphodiester linkage with O6 of a terminal αMan residue, followed by phosphodiester hydrolysis to liberate free GlcNAc and the αM6P residue (Scheme 1) [1c]. The presence of M6P residues distinguishes lysosomal glycoproteins from all other types of proteins in the Golgi and enables their vesicular trafficking from the *trans*-Golgi to an acidified prelysosomal compartment. Trafficking involves recognition between the M6P-containing glycoprotein and two kinds of M6P receptors (MPRs): a cation-dependent MPR (CD-MPR) and a cation-independent MPR (CI-MPR) [2]. Selective recognition by these MPRs occurs at pH 6.5–6.7 in the *trans*-Golgi, from which clathrin-coated vesicles containing the lysosomal glycoproteins bud and eventually fuse with the more acidic (pH 6) endosome/lysosome compartments, into which the glycoproteins are released [3].

Defects in the M6P–receptor recognition system cause lysosomal enzyme deficiencies that result in the intra-lysosomal accumulation of non-degraded substrates, a characteristic symptom of lysosomal storage diseases (LSDs). More than fifty inherited metabolic disorders, including Gaucher disease, GM gangliosidoses, lysosomal acid lipase deficiency and metachromatic leukodystrophy, are classified as LSDs, with a relatively high cumulative incidence of ~1 in 8000 [4]. Enzyme replacement therapy (ERT) that exploits the M6P–MPR pathway has been used to treat inherited LSDs [5]. This strategy relies on intravenous injection of exogenous lysosomal enzymes containing M6P modifications to facilitate their proper trafficking. However, treatment of some LSDs with exogenous enzymes has proven ineffective for reasons that remain unclear (e.g., renal impairment in Fabry disease) [3,4,6,7]. Therefore, elucidating the mechanisms of the M6P signal pathway at the molecular level remains imperative.

Efforts to treat LSDs have focused on the biological functions of M6P-tagged oligosaccharides, whose structures display tremendous diversity and informational storage capacity [8]. The location and valency of M6P moieties in high-mannose *N*-glycans, and the higher-order structural properties (e.g., conformation and dynamics) of the latter, influence their binding affinities for MPRs [8c]. A major impediment to these investigations has been the limited availability of structurally defined and structurally diverse sets of phosphorylated high-mannose oligosaccharides. The inherent structural heterogeneity of M6P-containing oligosaccharides on glycoproteins (the M6P glycoproteome [1])

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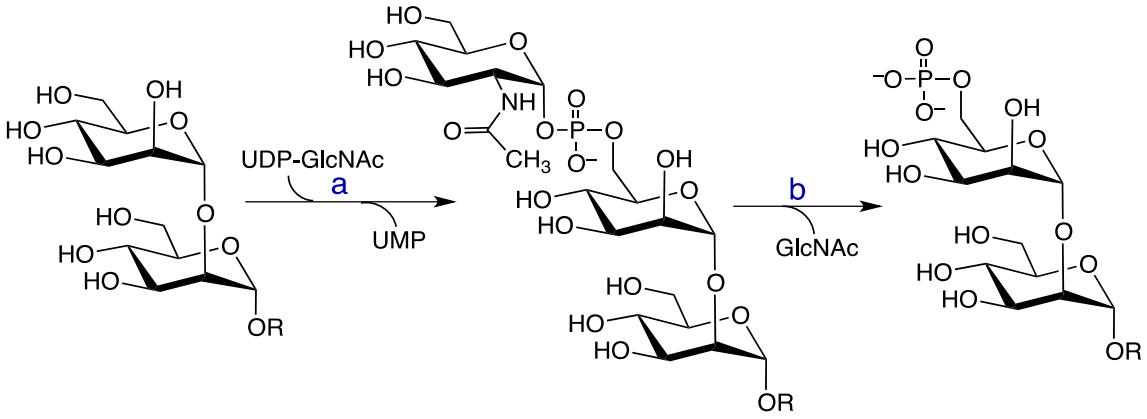


Scheme 1. Enzymatic insertion of phosphate into αMan residues of Golgi glycoproteins destined for transport to lysosomes. Step a requires UDP-GlcNAc:glycoprotein GlcNAc 1P phosphotransferase, which cleaves the phosphoanhydride bond in UDP-GlcNAc and transfers αGlcNAc 1P to O-6 of a terminal αMan residue, releasing UMP. Step b requires an αGlcNAc phosphodiesterase, which liberates GlcNAc and produces the O-6 phosphorylated αMan (αM6P) residue. Man residues in glycoproteins can apparently be mono- or bisphosphorylated *in vivo* and can be attached to the glycan chain by different types of O-glycosidic linkages, and glycoproteins may contain multiple αM6P residues.

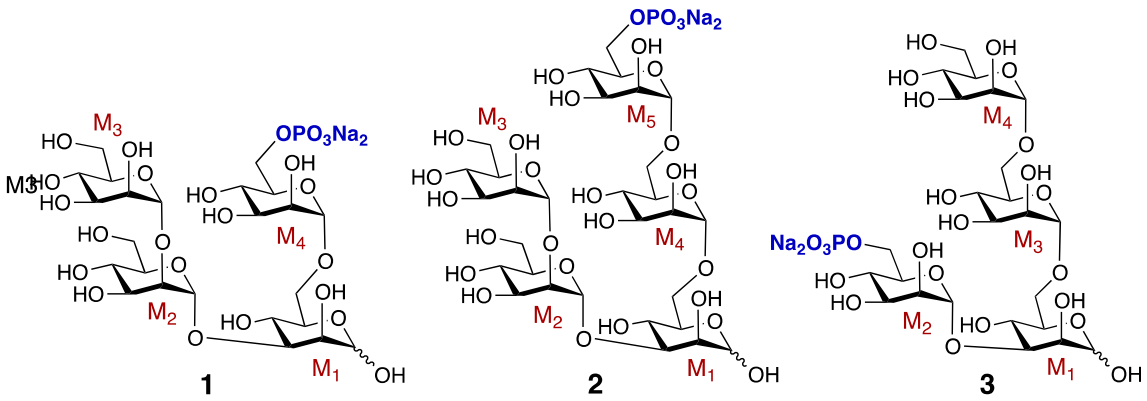
make both their isolation from natural sources and their chemical synthesis challenging [9]. Indeed, the extent of the chemical diversity of the M6P glycoproteome is currently unknown due to difficulties in separating mixtures of these modified proteins and in quantifying their constituents [1,9e]. M6P-containing oligosaccharides have been prepared previously using phosphorylated glycosyl bromides as donors [10a-b], however this approach is hindered by poor reactivity. Recent methods [10c-d] have employed strategically positioned protecting groups during the synthesis and installation of the phosphate esters in the final step of the assembly. In this report, we describe the chemical synthesis of several phosphorylated high-mannose oligosaccharides and their isolation in high purity, with the longer-range goal of expanding the chemical space and availability of these compounds for use as M6P glycoproteome standards and/or biochemical reagents in M6P glycoprotein research. A consecutive regioselective glycosylation strategy [10e] that reduces tedious and time-consuming protection–deprotection steps was applied to enable the rapid assembly of these modified oligosaccharides. Two synthetic routes were developed to incorporate phosphate groups at different locations to demonstrate the scope and general applicability of the strategy to prepare structurally diverse M6P-containing oligosaccharides.

2. Results and discussion

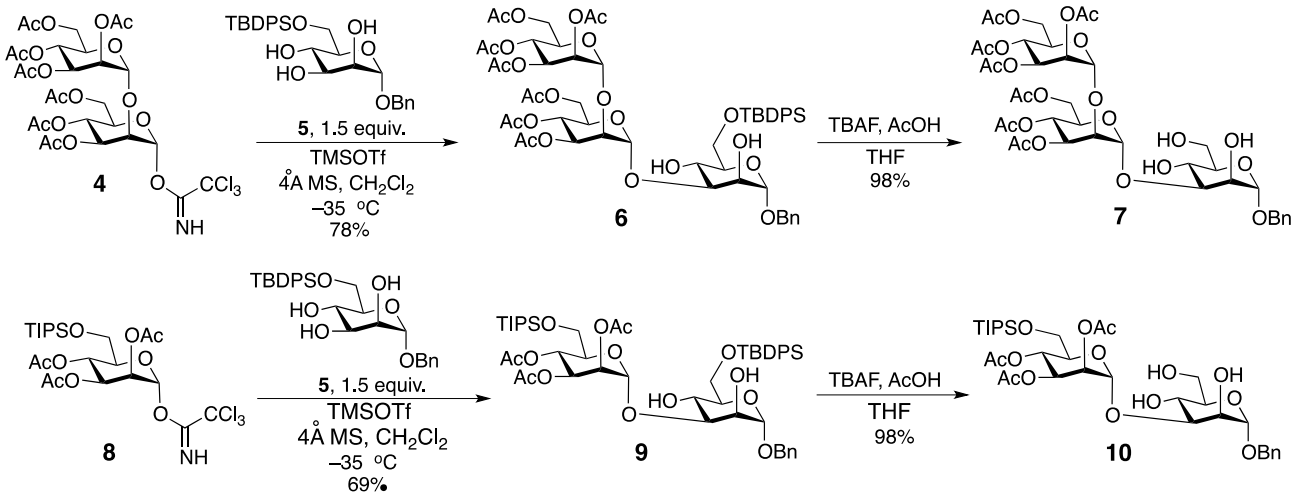
Three phosphorylated oligomannosides **1–3** were prepared (Scheme 2), which represent typical substructures found in naturally occurring M6P-containing high-mannose oligosaccharides (M6P-HMOs). Since **1–3** are asymmetrically monophosphorylated on either the 3- or 6-branches of residue M_1, different synthetic routes incorporating different protection/deprotection strategies were developed to enable their synthesis.

Syntheses of **1–3** initiate from building-block mannosyl donors and acceptors. These reagents were prepared using established chemical methods, including the mannose monosaccharide donors **8** and **11** [11], the α-(1 → 2)-linked disaccharide donor **4** [12], and the partially protected triol acceptor **5** [13]. Both the TIPS-protected donor **8** and the TBDMS-protected donor **11** were prepared to compare their properties with respect to stability during glycosylation and lability towards desilylation.

In our previous assembly of high-mannose oligosaccharides [10e], we demonstrated that chemical glycosylation between Schmidt trichloroacetimidate donors and allyl 6-*O*-TBDPS-α-D-mannopyranoside acceptors in the presence of a TMSOTf catalyst occurs exclusively and regioselectively at O-3 in good yield. A similar strategy was applied in this work, with glycosylations carried out between acceptor **5** and donors **4** and **8** after activation with TMSOTf at −35 °C to afford compounds **6** and **9**, respectively, in good yields (Scheme 3). The identities of the newly formed linkages in **6** and **9** were confirmed from analyses of 1D ^{1}H and 2D ^{1}H-^{1}H COSY spectra. The anomeric configurations of the α-(1 → 3)-glycosidic linkages in **6** and **9** were confirmed using the chemical shifts of H5 in each residue, which is typically observed downfield (> 3.6 ppm) in α-Man residues relative to that in β-Man residues (~3.4 ppm) [14]. For **6,** two cross-peaks observed at 3.19 ppm/3.97 ppm and 2.53 ppm/4.00 ppm in the 2D ^{1}H–^{1}H gCOSY spectrum (see Supplementary Data) indicated that ^{1}H signals associated with two free (non-glycosylated) hydroxyl hydrogens with chemical



Scheme 2. Structures of the target M6P-HMOs **1–3**. Mannose residues in **1–3** are labeled M_1–M_5 as shown.



Scheme 3. Regioselective syntheses of acceptors **7** and **10**.

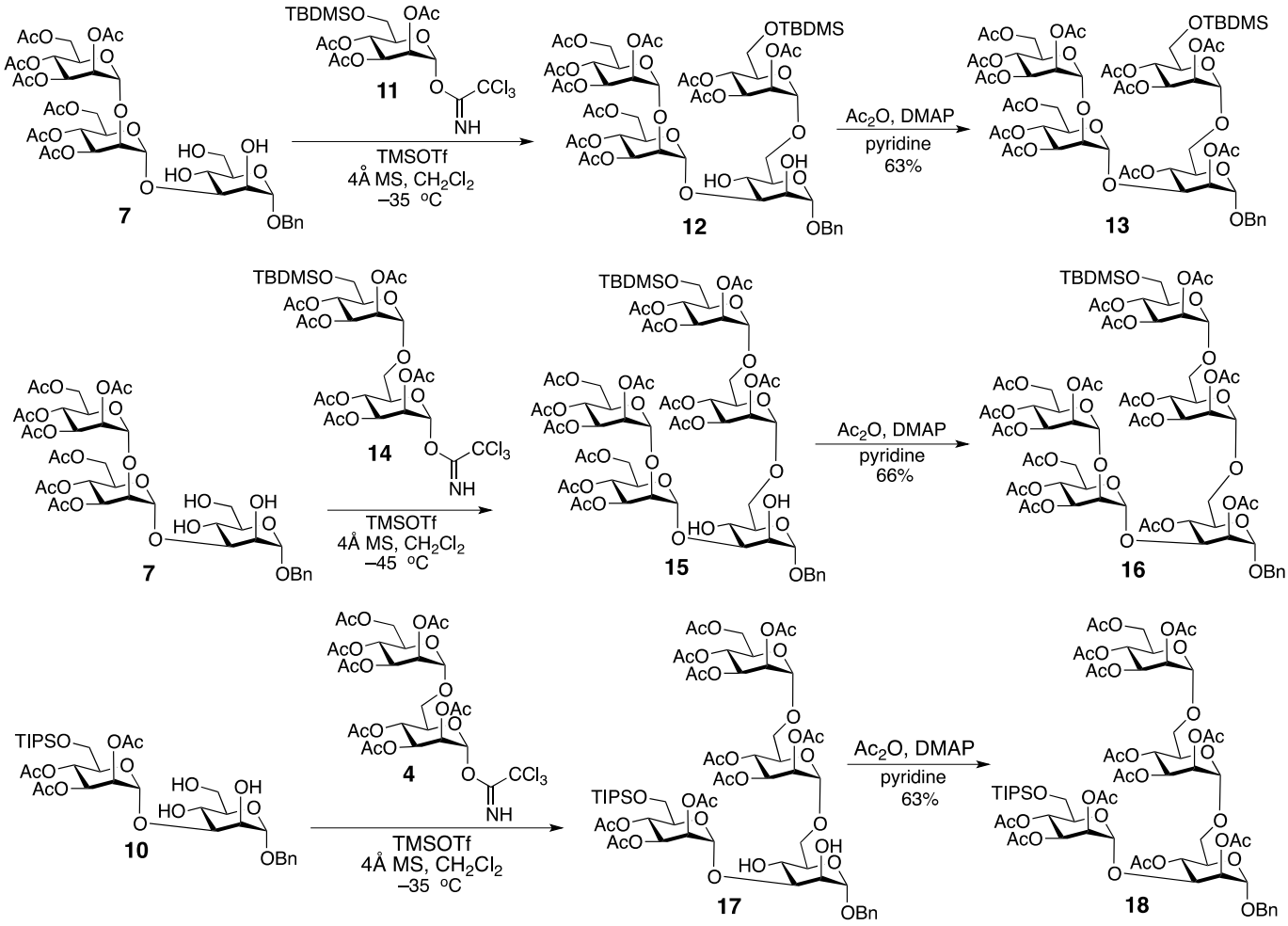
shifts at 3.19 and 2.53 ppm correlated with ^{1}H signals at 3.97 and 4.00 ppm, respectively. Inspection of the *J*-coupling patterns in the latter signals (see Supplementary Data) led to their assignments to H2 and H4, respectively, since the former appears as a broadened signal containing two small splittings (the H2 signal is split by small $^{3}J_{H1,H2}$ and $^{3}J_{H2,H3}$ values) and the latter appears as a pseudo-triplet (the H4 signal is split by large $^{3}J_{H3,H4}$ and $^{3}J_{H4,H5}$ values). These signal multiplicities differ from that expected for H3, which appears as a doublet of doublets containing a small $^{3}J_{H2,H3}$ and a large $^{3}J_{H3,H4}$. These results collectively confirmed that an α-(1 → 3)-glycosidic linkage was formed during the glycosylation. For **9,** the H2 signal is well resolved and coupled to one of the hydroxyl hydrogens, but the H3 and H4 signals overlapped with signals arising from two H6 hydrogens, preventing their analysis. Thus, it was difficult to confirm the site of glycosylation based on the NMR data obtained on **9**. While removal of the 6-*O*-TBDPS group in **9** allowed the H3 and H4 signals of **10** to be cleanly observed, the hydroxyl hydrogen signals were unobservable, presumably due to line-broadening caused by fast exchange with solvent. Consequently, the linkage site in **10** was confirmed using the resolved H3 multiplet (a doublet of doublets containing a small $^{3}J_{H2,H3}$ and a large $^{3}J_{H3,H4}$), which was correlated in the 2D ^{1}H–^{13}C HSQC spectrum with the most deshielded (glycosylated C3) carbon in the non-anomeric region of the ^{13}C{^{1}H} NMR spectrum of **10**.

Compounds **6** and **9** were desilylated with *tert*-butyl ammonium fluoride (TBAF) to give acceptors **7** and **10** in nearly quantitative yields (Scheme 3). Interestingly, for the desilylation of **9**, TBAF removed the TBDPS group completely in ~1 h without affecting the TIPS group [15]; lack of reactivity of the latter might be attributed to its greater steric bulk.

With acceptors **7** and **10** in hand, regioselective glycosylations were performed to construct the frameworks of the target molecules (Scheme 4). Acceptor **7** was coupled to TBDMS-protected monosaccharide donor **11** and disaccharide donor **14**, respectively, with activation afforded by TMSOTf at −35 °C, to give tetrasaccharide **12** and pentasaccharide **15**. Acceptor **10** was coupled to disaccharide donor **4** under the same conditions to obtain tetrasaccharide **17**. The TBDMS protection groups in **12** and **15**, and the TIPS protection group in **17**, were stable under the conditions used for glycosylation. Following chromatographic purification of **12,** its α-(1 → 6) linkage was confirmed by 1D ^{1}H NMR; two free hydroxyl hydrogen signals were observed as doublets split by H2 and H4, and the 2D ^{1}H-^{1}H COSY spectrum confirmed these assignments (see Supplementary Data). Compounds **15** and **17** were purified after peracetylation to give **16** and **18**, respectively; acetylation reduced the polarity of impurities (presumably unreacted acceptors and/or hydrolyzed donors) and greatly facilitated purification by silica-gel column chromatography. The constituent α-(1 → 6) linkages in **13**, **16** and **18** were confirmed by ^{13}C{^{1}H} NMR (see Supplementary Data). For tetrasaccharides **13** and **18**, only three carbon signals at ~62 ppm were observed, indicating that one of the four C6 carbons in the tetrasaccharide was glycosylated (glycosylation shifts the C6 signal downfield to 66–68 ppm). Similarly, only four carbon signals were observed at ~62 ppm for pentasaccharide **16**, indicating that one of the five C6 carbons was glycosylated.

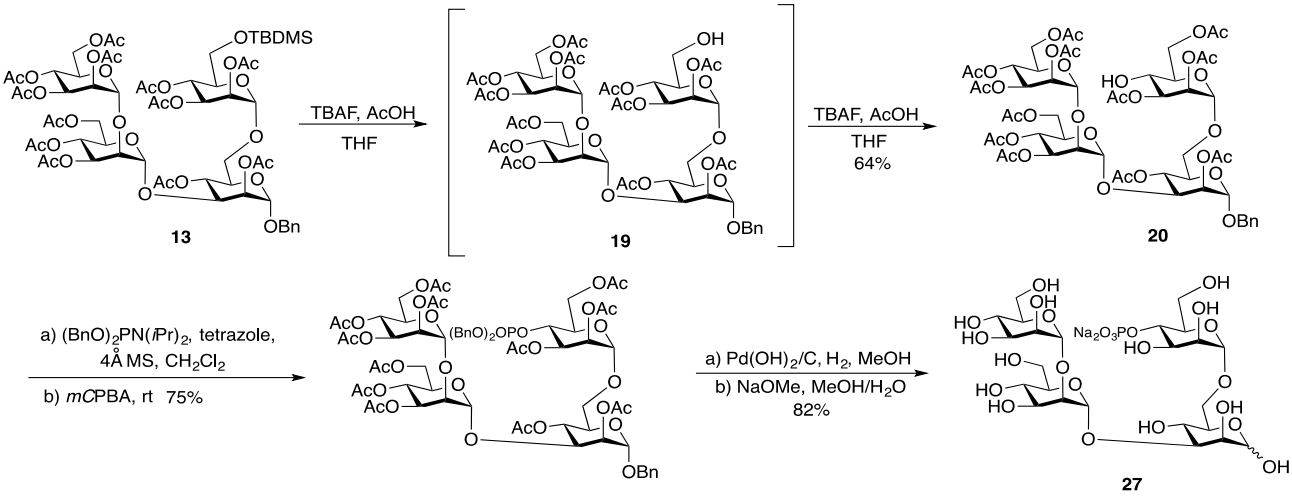
Compounds **13**, **16**, and **18** were then subjected to desilylation. Normally, TBAF in THF and acetic acid solvents effectively removes the silyl groups based on the hard–hard interaction of fluoride anion with silicon [14]. This treatment, however, gave no detectable desilylation product as determined by TLC assay after overnight incubation at room temperature with stirring. After incubation for two days, TLC assay showed that ~60% of the starting material **13** was desilylated. The product was then phosphorylated using dibenzyl *N*,*N*-diisopropylphosphoramidite, followed by oxidation of the intermediate phosphite with *m*CPBA and deprotection to give mannose phosphate **21**, whose structure was characterized by NMR. NMR analysis showed, however, that **21** had the phosphate group attached to O4, giving a M4P derivative instead of the desired M6P derivative (Scheme 5). The ^{13}C{^{1}H} NMR spectrum of **21** contained three C6 signals, all singlets, at ~62 ppm and one doublet at 71.7 ppm. If the M6P derivative had been produced, O6 phosphorylation would have shifted the C6 signal downfield by ~3 ppm and the signal would have appeared as a doublet caused by $^{2}J_{C6,P}$ (~−2.5 Hz) [16]; the remaining two C6 signals would appear as singlets at ~62 ppm. Subsequent NMR analysis of the protected intermediate **20** (Scheme 5) found a broadened H4 signal at 3.83 ppm and a correlation between an hydroxyl hydrogen signal at 2.70 ppm and the H4 signal (see Supplementary Data), indicating that the solution conditions for TBAF-mediated desilylation encouraged acetyl group migration from O4 to O6 (**19** → **20** in Scheme 5), thus explaining the formation of **21**.

In an effort to minimize or eliminate acetyl group transfer during desilylation, $BF_3{\cdot}OEt_2$ was examined as an alternate reagent to remove the TBDMS group in **13** [8a]. Tetrasaccharide **13** was dissolved in CH_2Cl_2 at −10 °C, $BF_3{\cdot}OEt_2$ was added dropwise to the solution, and the solution was stirred for 2.5 h while it was warmed to room temperature (Scheme 6). The solution was then concentrated, and intermediate **19** was purified and subsequently phosphorylated to give tetrasaccharide **22** (Scheme 6). ^{13}C{^{1}H} NMR analysis of **22** showed that phosphorylation had occurred at O6H, since only two C6 signals at ~62 ppm and one doublet at 66.0 ppm were observed in the spectrum (see Supplementary Data). NMR (2D ^{1}H-^{1}H COSY) analysis of the intermediate **19** revealed a pair of broadened H6 signals at 3.68 and

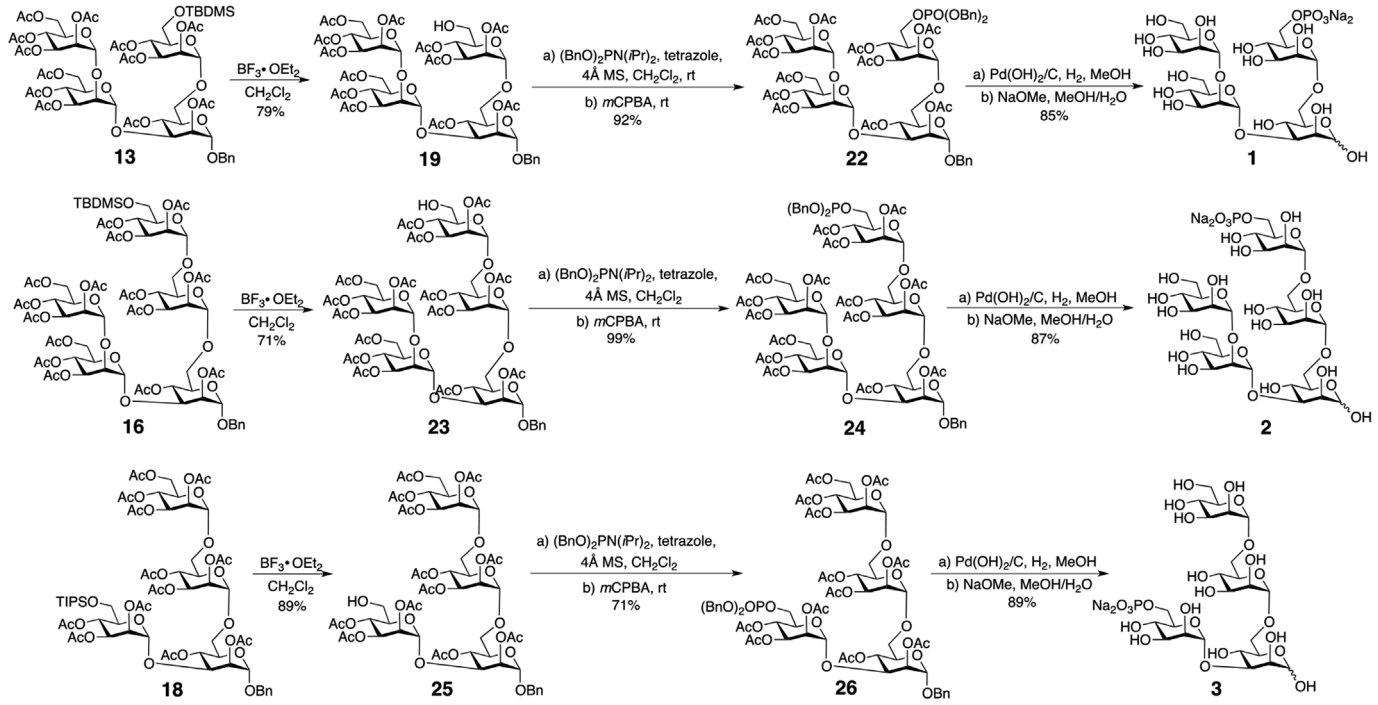


Scheme 4. Synthetic routes to prepare oligosaccharides **13**, **16** and **18**.

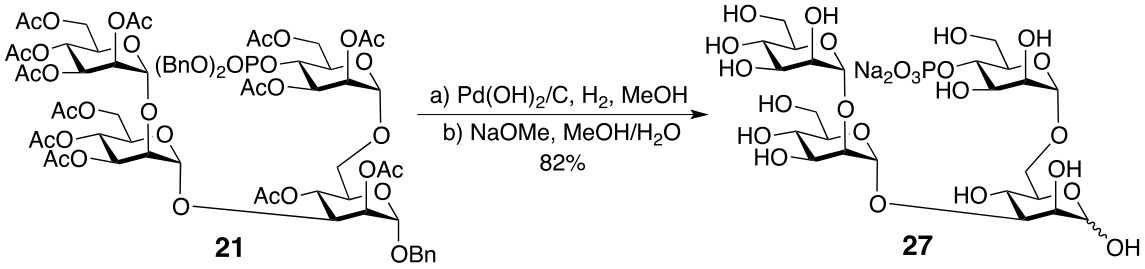
3.61 ppm and correlations between the hydroxyl hydrogen signal at 2.41 ppm and these H6 signals (see Supplementary Data). This desilylation approach proved more efficient than that using TBAF, with both TBDMS and TIPS groups completely removed in 2.5 h. Compounds **16** and **18** were desilylated in the same manner using $BF_3{\cdot}OEt_2$ and phosphorylated to give precursors **24** and **26**, respectively (Scheme 6). Compounds **22**, **24** and **26** were each dissolved in methanol, debenzylated in an H_2 atmosphere using a palladium hydroxide on carbon ($Pd(OH)_2/C$, 20% wt-%) catalyst, and deacetylated with sodium methoxide. Care was taken during deacetylation to add sufficient sodium methoxide to convert protonated phosphoric acid groups into their corresponding disodium phosphate salts. This conversion, however, significantly decreased product solubility in methanol, causing white precipitates to form. To avoid product loss, the precipitates were brought back into solution with the addition of a small volume of water, and the reaction mixture was stirred for another 2 h. The solution was



Scheme 5. Desilylation of **13** with TBAF, and subsequent phosphorylation to give the unexpected phosphorylated tetrasaccharide **27**.



Scheme 6. Desilylation, phosphorylation and deprotection of **13**, **16**, and **18** to give phosphorylated oligosaccharides **1–3**, respectively.



Scheme 7. Deprotection of tetrasaccharide **21** to give phosphorylated tetrasaccharide **27**.

then concentrated, the residue was applied to a DEAE-Sephadex A-25 anion-exchange column (3 × 55 cm), and the column was eluted with aqueous $NaHCO_3$ (linear 0–0.8 M gradient; 3 L total volume; 20 mL fractions; 1.5 mL/min flow rate). Fractions containing organic phosphates, as determined by HPLC (Phenomenex organic acid column in H^+ form), were pooled and concentrated, and the resulting solutions were desalted by passage through Sephadex LH-20 columns (2.5 × 80 cm; water solvent) to give oligosaccharides **1–3** in purities exceeding 98% (Scheme 6). Tetrasaccharide **21** was deprotected in the same manner as **22**, **24** and **26** to afford phosphorylated tetrasaccharide **27** after purification (Scheme 7).

A reliable chemical approach has been developed to prepare asymmetric di-antennary M6P-tagged high-mannose oligosaccharides in > 20% overall yield and with the single M6P residue residing in either a (1 → 3)- or (1 → 6) branch of the oligosaccharide. Introducing more than one M6P residue is feasible by incorporating silylated protecting groups in both arms; an example of this application is shown in the Table of Contents (TOC) graphic. Regioselective chemical glycosylations with partially protected acceptors to introduce (1 → 3)- and (1 → 6)-linkages promoted rapid oligosaccharide assembly by reducing the number of protection–deprotection steps, thereby improving overall efficiency and yield. Purification procedures were developed to provide phosphorylated oligosaccharides in purities exceeding 98%. The overall synthetic strategy developed in this work simplifies the preparation of M6P-tagged high-mannose oligosaccharides and improve access to these compounds to study their structures and biological functions.

3. Experimental section

3.1. General methods

All chemicals were purchased as anhydrous reagent grade and were used without further purification. All reactions were performed under anhydrous conditions unless otherwise noted. Reactions were monitored by thin-layer chromatography (TLC) on silica gel precoated aluminum plates. Zones were detected by heat/charring with a *p*-anisaldehyde–sulfuric acid visualization reagent [10e]. Flash column chromatography on silica gel (preparative scale) was performed on a Reveleris® X2 flash chromatography system. ^1H, $^{13}C\{^1H\}$ and $^{31}P\{^1H\}$ NMR spectra were recorded at 22 °C on Bruker Avance III HD 500-MHz or Varian DirectDrive 600-MHz FT-NMR spectrometers. Chemical shifts are reported in δ-units (ppm) relative to the ^1H signal of residual $CHCl_3$ at δ 7.26 ppm and the ^{13}C signal at δ 77.16 ppm. Chemical shifts for ^{31}P NMR are relative to external 85% H_3PO_4 solution. Abbreviations for NMR signal multiplicities are: s = singlet; dd = doublet of doublets; d = doublet; dt = doublet of triplets; t = triplet; td = triplet of doublets; q = quartet; m = multiplet. Two-dimensional NMR spectra (gCOSY and gHSQC) were recorded on the same instruments using

Bruker or Varian processing software. Mass spectrometric analyses were performed on a Bruker microTOF-Q II quadrupole time-of-flight (QTOF) mass spectrometer with an ESI source.

3.2. Experimental data

3.2.1. Benzyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl-(1 → 3)-6-O-(tert-butyldiphenylsilyl)-α-D-mannopyranoside **6**

Donor **4** (2.02 g, 2.26 mmol) and acceptor **5** (1.72 g, 3.39 mmol) were dissolved in anhydrous CH_2Cl_2 (200 mL), and 4 Å molecular sieves (4 g) were added to the solution. The solution was cooled to −35 °C and was treated with TMSOTf (82 μL, 0.45 mmol) under N_2. The reaction mixture was stirred for 2 h and then quenched with the addition of triethylamine (0.2 mL). The mixture was vacuum-filtered through Celite®, and the filtrate was concentrated to a syrup. The syrup was purified by flash chromatography on an 80-g silica gel column (eluent: hexanes/ethyl acetate = 1:2) to afford compound **6** (1.98 g, 1.76 mmol, 78%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.68 (m, 4H), 7.44–7.35 (m, 7H), 7.33–7.27 (m, 4H), 5.38 (dd, J = 10.0, 3.4 Hz, 1H), 5.31 (m, 2H), 5.25 (m, 3H), 4.94 (d, J = 1.9 Hz, 1H), 4.84 (d, J = 1.8 Hz, 1H), 4.63 (d, J = 11.9 Hz, 1H), 4.42 (d, J = 11.9 Hz, 1H), 4.21 (dd, J = 12.2, 4.5 Hz, 1H), 4.18–4.06 (m, 4H), 4.00 (m, 1H), 3.97 (t, J = 9.4 Hz, 1H), 3.94–3.90 (m, 2H), 3.89 (dd, J = 10.7, 5.1 Hz, 1H), 3.70 (m, 1H), 3.19 (br, 1H), 2.53 (br, 1H), 2.13 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.04 (s, 9H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 170.1, 171.0, 170.8, 170.1, 170.0, 169.8, 169.7, 137.1, 135.8 (× 5), 133.2, 133.1, 130.1(× 2), 128.8 (× 2), 128.6 (× 2), 128.3, 128.0 (× 4), 100.1, 99.0, 98.7, 81.1, 76.5, 72.0, 70.5, 69.9, 69.3, 69.2 (× 3), 68.8, 68.6, 66.8, 66.6, 65.1, 62.8, 62.7, 60.9, 27.1 (× 3), 21.2, 20.9, 20.8 (× 4), 20.6. HRESIMS: (m/z) calcd for $C_{55}H_{70}O_{23}SiNa^+$ $(M + Na)^+$ 1149.3975; found 1149.3984.

3.2.2. Benzyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl-(1 → 3)-α-D-mannopyranoside **7**

Compound **6** (1.53 g, 1.36 mmol) was dissolved in THF (30 mL), and AcOH (0.23 mL, 4.08 mmol) and TBAF (2.72 mL, 2.72 mmol) were added to the solution. The reaction mixture was stirred at rt for 2 h, then concentrated to a syrup. The syrup was purified by flash chromatography on a 40-g silica gel column (eluent: ethyl acetate) to afford compound **7** (1.18 g, 1.33 mmol, 98%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.36–7.25 (m, 5H), 5.38 (dd, J = 10.0, 3.3 Hz, 1H), 5.36 (dd, J = 10.0, 3.2 Hz, 1H), 5.34 (d, J = 2.2 Hz, 1H), 5.28–5.21 (m, 3H), 4.96 (d, J = 1.9 Hz, 1H), 4.90 (d, J = 1.8 Hz, 1H), 4.66 (d, J = 11.9 Hz, 1H), 4.47 (d, J = 11.9 Hz, 1H), 4.24–4.12 (m, 9H), 4.00 (m, 1H), 3.94–3.88 (m, 2H), 3.84–3.78 (m, 2H), 3.74 (d, J = 4.6 Hz, 1H), 3.61 (m, 1H), 3.04 (m, 1H), 2.13 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 171.6, 171.2 (× 2), 170.1, 170.0, 169.8, 169.7, 137.0, 128.7 (× 2), 128.5 (× 2), 128.4, 100.3, 99.3, 99.0, 80.5, 76.8, 72.5, 70.9, 70.6, 70.0, 69.6, 69.3, 69.2, 68.6, 66.8, 66.7, 66.1, 62.9, 61.7, 21.1, 21.0, 20.9 (× 4), 20.8. HRESIMS: (m/z) calcd for $C_{39}H_{52}O_{23}Na^+$ $(M + Na)^+$ 911.2797; found 911.2782.

3.2.3. Benzyl 2,3,4-tri-O-acetyl-6-O-(tri-isopropylsilyl)-α-D-mannopyranosyl-(1 → 3)-α-D-mannopyranoside **10**

Donor **8** (1.0 g, 1.65 mmol) and acceptor **5** (1.26 g, 2.47 mmol) were dissolved in anhydrous CH_2Cl_2 (100 mL), and 4 Å molecular sieves (2.5 g) were added to the solution. The solution was cooled to −35 °C and was treated with TMSOTf (60 μL, 0.33 mmol) under N_2. The reaction mixture was stirred for 2 h and then quenched with the addition of triethylamine (0.2 mL). The mixture was vacuum-filtered through Celite®, and the filtrate was concentrated to a syrup. The syrup was purified by flash chromatography on a 40-g silica gel column (eluent: hexanes/ethyl acetate = 1.5:1) to afford compound **9** (1.08 g, 1.13 mmol, 69%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73 (tt, J = 6.1, 1.6 Hz, 4H), 7.42–7.37 (m, 7H), 7.32–7.27 (m, 4H), 5.40 (dd, J = 9.8, 3.5 Hz, 1H), 5.33 (dd, J = 3.5, 1.8 Hz, 1H), 5.14 (t, J = 10.1 Hz, 1H), 5.11 (d, J = 1.9 Hz, 1H), 4.82 (d, J = 1.7 Hz, 1H), 4.70 (d, J = 11.9 Hz, 1H), 4.47 (d, J = 11.9 Hz, 1H), 4.25 (dd, J = 9.8, 7.4, 2.1 Hz, 1H), 4.18 (dd, J = 6.1, 3.2, 1.7 Hz, 1H), 4.01 (dd, J = 10.9, 3.7 Hz, 1H), 3.96–3.90 (m, 3H), 3.80–3.76 (m, 2H), 3.72 (dd, J = 10.8, 2.0 Hz, 1H), 3.17 (d, J = 6.1 Hz, 1H), 2.89 (d, J = 2.6 Hz, 1H), 2.11 (s, 3H), 2.02 (s, 3H), 1.98 (s, 3H), 1.09 (s, 9H), 1.04 (t, J = 6.5 Hz, 18H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 170.1 (× 2), 170.0, 137.2, 135.8, 135.7, 133.4, 133.3, 129.8 (× 2), 128.4, 128.1, 127.8 (× 3), 98.9, 98.6, 81.7, 72.4, 72.1, 69.9, 69.5, 69.3, 68.6, 67.3, 66.9, 64.7, 63.3, 26.9, 20.9, 20.8 (× 2), 19.3, 17.9, 11.9. Compound **9** (860 mg, 0.9 mmol) was dissolved in THF (50 mL), and AcOH (0.16 mL, 3 mmol) and TBAF (1 mL, 1 mmol) were added to the solution. The reaction mixture was stirred at rt for 2 h, then concentrated to a syrup. The syrup was purified on a 24-g silica gel column (eluent: ethyl acetate) to afford compound **10** (630 mg, 0.88 mmol, 98%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34–7.28 (m, 5H), 5.38 (dd, J = 9.9, 3.4 Hz, 1H), 5.33 (dd, J = 3.4, 1.8 Hz, 1H), 5.17 (t, J = 10.1 Hz, 1H), 5.13 (d, J = 1.8 Hz, 1H), 4.85 (d, J = 1.8 Hz, 1H), 4.70 (d, J = 12.0 Hz, 1H), 4.49 (d, J = 12.0 Hz, 1H), 4.23 (dd, J = 9.8, 7.1, 2.2 Hz, 1H), 4.17 (s, 1H), 4.11 (m, 1H), 3.94 (m, 2H), 3.87 (dt, J = 11.8, 6.4 Hz, 2H), 3.76 (dd, J = 10.9, 7.0 Hz, 1H), 3.72–3.67 (m, 2H), 3.43 (d, J = 3.6 Hz, 1H), 2.78 (s, 1H), 2.11 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.02 (t, J = 6.5 Hz, 18H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 170.6, 170.4, 170.0, 137.2, 128.5, 128.1, 128.0, 99.5, 98.8, 81.4, 72.3, 72.2, 70.0, 69.9, 69.6, 69.2, 66.7, 66.1, 63.3, 62.3, 21.0, 20.9 (× 2), 18.0, 12.0. HRESIMS: (m/z) calcd for $C_{39}H_{52}O_{23}Na^+$ $(M + Na)^+$ 911.2797; found 911.2782.

3.2.4. Benzyl 2,3,4-tri-O-acetyl-6-O-(tert-butyldimethylsilyl)-α-D-mannopyranosyl-(1 → 6)-[2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl-(1 → 3)]-D-mannopyranoside **12**

Donor **11** (180 mg, 0.32 mmol) and acceptor **7** (260 mg, 0.29 mmol) were dissolved in anhydrous CH_2Cl_2 (100 mL), and 4 Å molecular sieves (1 g) were added to the solution. The solution was cooled to −35 °C and treated with TMSOTf (10 μL, 0.058 mmol) under N_2. The reaction mixture was stirred for 2 h and then quenched with the addition of triethylamine (50 μL). The mixture was vacuum-filtered through Celite®, and the filtrate was concentrated to a syrup. The syrup was purified on a 24-g silica gel column (eluent: ethyl acetate) to afford compound **12** (243 mg, 0.18 mmol, 65%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.36–7.28 (m, 5H), 5.39 (dd, J = 9.8, 3.4 Hz, 1H), 5.38 (dd, J = 9.5, 3.4 Hz, 1H), 5.31 (t, J = 10.1 Hz, 1H), 5.29 (t, J = 10.1 Hz, 1H), 5.26–5.20 (m, 5H), 5.00 (d, J = 1.8 Hz, 1H), 4.94 (d, J = 1.8 Hz, 1H), 4.85 (d, J = 1.8 Hz, 1H), 4.68 (d, J = 12.0 Hz, 1H), 4.50 (d, J = 12.0 Hz, 1H), 4.23 (dd, J = 9.7, 5.5, 2.6 Hz, 1H), 4.21–4.10 (m, 7H), 4.07 (m, 1H), 3.95–3.92 (m, 2H), 3.91–3.87 (m, 2H), 3.78–3.70 (m, 4H), 2.88 (d, 1H), 2.54 (d, 1H), 2.13 (s, 3H), 2.11 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 0.90 (s, 9H), 0.06 (s, 3H), 0.04 (s, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 171.3, 171.0, 170.6, 170.5, 170.3, 170.1 (× 2), 169.9, 169.8 (× 2), 136.9, 128.7 (× 2), 128.6 (× 2), 128.3, 100.3, 98.9 (× 2), 97.5, 81.4, 76.3, 72.0, 71.5, 70.5, 70.4, 70.3, 69.9, 69.5, 69.4, 69.3, 69.2, 68.5, 67.0, 66.8, 66.4, 63.2, 62.8, 62.4, 26.1 (× 3), 21.1 (× 3), 21.0 (× 4), 20.9 (× 2), 20.8, 18.5, 1.2, −5.1, −5.2. HRESIMS: (m/z) calcd for $C_{57}H_{82}O_{31}SiNa^+$ $(M + Na)^+$ 1313.4502; found 1313.4511.

3.2.5. Benzyl 2,3,4-tri-O-acetyl-6-O-(tert-butyldimethylsilyl)-α-D-mannopyranosyl-(1 → 6)-[2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl-(1 → 3)]-2,4-di-O-acetyl-α-D-mannopyranoside **13**

Compound **12** was dissolved in dry pyridine (20 mL), and 4-(dimethylamino)pyridine (DMAP, catalytic amt.) and Ac_2O (0.1 mL) were added with stirring for 1 h at rt. After concentrating the solution, the

residue was partitioned between EtOAc and water, and the organic layer was washed with sat. aq. $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash chromatography on a 24-g silica gel column (eluent: hexanes/ethyl acetate = 1:2) to afford compound **13** (250 mg, 0.18 mmol, 98%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37–7.28 (m, 5H), 5.36 (dd, J = 10.0, 3.4 Hz, 1H), 5.34 (dd, J = 10.1, 3.4 Hz, 1H), 5.29 (t, J = 9.9 Hz, 1H), 5.28 (t, J = 9.9 Hz, 1H), 5.24–5.16 (m, 5H), 5.12 (d, J = 2.0 Hz, 1H), 5.11 (dd, J = 10.0, 3.1 Hz, 1H), 4.86 (d, J = 1.7 Hz, 1H), 4.79 (m, 2H), 4.69 (d, J = 11.6 Hz, 1H), 4.52 (d, J = 11.6 Hz, 1H), 4.20–4.10 (m, 5H), 4.07 (dd, J = 12.3, 2.1 Hz, 1H), 3.98 (dd, J = 10.0, 4.8, 2.1 Hz, 1H), 3.89–3.84 (m, 3H), 3.79 (dd, J = 10.6, 6.5 Hz, 1H), 3.70 (dd, J = 11.5, 5.1 Hz, 1H), 3.67 (dd, J = 11.5, 2.6 Hz, 1H), 3.54 (dd, J = 10.6, 2.8 Hz, 1H), 2.16 (s, 3H), 2.13 (s, 3H), 2.11 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H), 0.89 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 171.1, 170.6 (× 2), 170.4, 170.3, 170.0 (× 4), 169.8, 169.7, 169.6, 136.4, 128.8 (× 2), 128.7 (× 2), 128.5, 99.9 (× 2), 97.3, 96.2, 78.5, 74.9, 71.6, 71.0, 69.8 (× 3), 69.7 (× 2), 69.5, 68.9, 68.5, 66.8, 66.6, 66.4, 65.8, 62.8, 62.5, 62.3, 60.6, 26.0 (× 3), 21.1 (× 3), 21.0 (× 2), 20.9 (× 4), 20.8(× 3), −5.1, −5.2. HRESIMS: (*m/z*) calcd for $C_{61}H_{86}O_{33}SiNa^+$ $(M + Na)^+$ 1397.4718; found 1397.4718.

3.2.6. Benzyl 2,3,4-tri-O-acetyl-6-O-(tert-butyldimethylsilyl)-α-D-mannopyranosyl-(1 → 6)-2,3,4-tri-O-acetyl-α-D-mannopyranosyl-(1 → 6)-[2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl-(1 → 3)]-2,4-di-O-acetyl-α-D-mannopyranoside **16**

Donor **14** (190 mg, 0.22 mmol) and acceptor **7** (300 mg, 0.33 mmol) were dissolved in anhydrous CH_2Cl_2 (50 mL), and 4 Å molecular sieves (400 mg) were added to the solution. The solution was cooled to −35 °C and treated with TMSOTf (4 μL, 0.022 mmol) under N_2. The reaction mixture was stirred for 2 h and then quenched with the addition of triethylamine (20 μL). The mixture was vacuum-filtered through Celite®, and the filtrate was concentrated to a syrup. The afforded crude product **15** was dissolved in dry pyridine (20 mL), and 4-(dimethylamino)pyridine (DMAP, catalytic amt.) and Ac_2O (0.1 mL) were added with stirring for 1 h at rt. After concentrating the solution, the residue was partitioned between EtOAc and water, and the organic layer was washed with sat. aq. $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash chromatography on a 40-g silica gel column (eluent: hexanes/ethyl acetate = 1:3) to afford compound **16** (240 mg, 0.14 mmol, 66%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34–7.29 (m, 5H), 5.34–5.21 (m, 11H), 5.12–5.09 (m, 2H), 4.89 (d, J = 1.7 Hz, 1H), 4.81 (d, J = 1.7 Hz, 2H), 4.79 (d, J = 1.9 Hz, 1H), 4.70 (d, J = 11.6 Hz, 1H), 4.53 (d, J = 11.6 Hz, 1H), 4.18–4.05 (m, 7H), 3.99 (ddt, J = 9.4, 4.7, 2.3 Hz, 2H), 3.87 (dt, J = 3.9, 1.9 Hz, 2H), 3.79 (dd, J = 11.0, 5.3 Hz, 3H), 3.68 (m, 2H), 3.50 (dd, J = 10.8, 8.2, 2.7 Hz, 2H), 2.18–1.94 (15s, 15 × 3H), 0.89 (s, 9H), 0.04 (s, 3H), 0.03 (s, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 170.9, 170.6, 170.5, 170.3 (× 2), 170.1, 169.9 (× 2), 169.8 (× 4), 169.7, 169.6, 169.5, 136.4, 128.7, 128.5, 128.4, 99.8, 99.7, 97.6, 97.5, 96.4, 78.4, 74.9, 71.5, 70.9, 69.7 (× 3), 69.6, 69.5 (× 2), 69.4, 69.2, 68.6, 68.4, 66.8, 66.4, 66.3, 66.3, 66.2, 65.7, 62.8, 62.3, 62.2, 25.9, 20.9–20.6, −5.2, −5.3. HRESIMS: (*m/z*) calcd for $C_{73}H_{102}O_{41}SiNa^+$ $(M + Na)^+$ 1685.5564; found 1685.5558.

3.2.7. Benzyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl-(1 → 6)-2,3,4-tri-O-acety-α-D-mannopyranosyl-(1 → 6)-[2,3,4-tri-O-acetyl-6-O-(tri-isopropylsilyl)-α-D-mannopyranosyl-(1 → 3)]-2,4-di-O-acetyl-α-D-mannopyranoside **18**

Donor **4** (240 mg, 0.31 mmol) and acceptor **10** (200 mg, 0.28 mmol) were dissolved in anhydrous CH_2Cl_2 (50 mL), and 4 Å molecular sieves (400 mg) were added to the solution. The solution was cooled to −35 °C and treated with TMSOTf (5 μL, 0.028 mmol) under N_2. The reaction mixture was stirred for 2 h and then quenched with the addition of triethylamine (20 μL). The mixture was vacuum-filtered through Celite®, and the filtrate was concentrated *in vacuo* to a syrup. The afforded crude product **17** was dissolved in dry pyridine (20 mL), and 4-(dimethylamino)pyridine (DMAP, catalytic amt.) and Ac_2O (0.1 mL) were added with stirring for 1 h at rt. After concentrating the solution, the residue was partitioned between EtOAc and water, and the organic layer was washed with sat. aq. $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash chromatography on a 40-g silica gel column (eluent: hexanes/ethyl acetate = 1:2) to afford compound **18** (250 mg, 0.18 mmol, 63%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37–7.28 (m, 5H), 5.40 (t, J = 9.9 Hz, 1H), 5.39 (dd, J = 10.0, 3.6 Hz, 1H), 5.34 (t, J = 9.9 Hz, 1H), 5.30–5.24 (m, 4H), 5.19 (dd, J = 10.1, 3.3 Hz, 1H), 5.16 (t, J = 9.9 Hz, 1H), 4.98–4.94 (m, 3H), 4.90 (d, J = 1.8 Hz, 1H), 4.83 (d, J = 1.5 Hz, 1H), 4.70 (d, J = 11.9 Hz, 1H), 4.52 (d, J = 11.9 Hz, 1H), 4.24–4.10 (m, 6H), 4.08–4.04 (m, 2H), 3.89 (dd, J = 9.8, 6.9, 3.6 Hz, 1H), 3.86 (dd, J = 9.9, 3.2, 2.9 Hz, 1H), 3.78–3.74 (m, 3H), 3.46 (dd, J = 10.6, 2.6 Hz, 1H), 2.17 (s, 3H), 2.14 (s, 3H), 2.13 (s, 3H), 2.11 (s, 3H), 2.07 (2s, 6H), 2.05 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 2.00 (2s, 6H), 1.97 (s, 3H), 1.02 (t, J = 6.5 Hz, 18H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 171.1, 170.7, 170.5, 170.4 (× 3), 170.1 (× 2), 170.0, 169.7 (× 3), 136.6, 128.7 (× 2), 128.3 (× 3), 99.9, 98.9, 98.1, 96.5, 77.3, 74.3, 72.3, 71.1, 71.0, 70.5, 70.4, 70.0, 69.4 (× 3), 68.9 (× 2), 68.8, 68.6, 67.3, 66.5, 66.1, 62.7, 62.3 (× 2), 60.6, 21.3 (× 3), 21.1 (× 2), 21.0 (× 3), 20.9 (× 4), 18.1, 12.2. HRESIMS: (*m/z*) calcd for $C_{64}H_{92}O_{33}SiNa^+$ $(M + Na)^+$ 1439.5188; found 1439.5210.

3.2.8. Benzyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl-(1 → 3)-[2,3,6-tri-O-acetyl-α-D-mannopyranosyl-(1 → 6)]-2,4-di-O-acetyl-α-D-mannopyranoside **20**

Compound **13** (290 mg, 0.21 mmol) was dissolved in THF (30 mL), the solution was cooled to 0 °C, and AcOH (36 μL, 0.63 mmol) and TBAF (0.42 mL, 0.42 mmol) were added. The reaction mixture was stirred at rt for 48 h, and then concentrated to a syrup. The syrup was purified by flash chromatography on a 24-g silica gel column (eluent: ethyl acetate) to afford compound **20** (170 mg, 0.14 mmol, 64%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.18–7.10 (m, 5H), 5.36 (dd, J = 10.0, 3.4 Hz, 1H), 5.32–5.20 (m, 7H), 5.12 (d, J = 1.9 Hz, 1H), 5.11 (dd, J = 9.7, 3.1 Hz, 1H), 4.88 (d, J = 1.9 Hz, 1H), 4.82 (d, J = 1.9 Hz, 1H), 4.80 (d, J = 1.9 Hz, 1H), 4.68 (d, J = 11.5 Hz, 1H), 4.52 (d, J = 11.5 Hz, 1H), 4.51 (dd, J = 12.2, 4.8 Hz, 1H), 4.32 (dd, J = 12.2, 2.2 Hz, 1H), 4.21–4.06 (m, 4H), 4.00 (dd, J = 10.0, 4.8, 2.2 Hz, 1H), 3.92–3.80 (m, 4H), 3.75 (dd, J = 10.8, 5.8 Hz, 1H), 3.48 (dd, J = 10.8, 2.6 Hz, 1H), 2.20–2.00 (12s, 12 × 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 170.9, 170.6, 170.4, 170.4, 170.2, 170.0, 169.9, 169.8 (× 2), 169.5, 169.4, 136.1, 128.8 (× 2), 128.7 (× 2), 128.7, 99.8 (× 2), 97.7, 96.6, 78.4, 74.9, 71.7, 71.2, 70.9, 70.0, 69.8 (× 2), 69.7, 68.7, 68.6, 66.6, 66.4, 65.8, 63.5, 62.8, 62.3, 21.1–20.9. HRESIMS: (*m/z*) calcd for $C_{55}H_{72}O_{33}Na^+$ $(M + Na)^+$ 1283.3854; found 1283.3837.

3.2.9. Benzyl 2,3,4-tri-O-acetyl-α-D-mannopyranosyl-(1 → 6)-[2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl-(1 → 3)]-2,4-di-O-acetyl-α-D-mannopyranoside **19**

Compound **13** (200 mg, 0.15 mmol) was dissolved in CH_2Cl_2 (50 mL), and the solution was cooled to −10 °C. BF_3•Et_2O (45 μL, 0.36 mmol) was then added dropwise, and the reaction mixture was stirred for 2.5 h while it warmed to rt. The reaction mixture was then concentrated *in vacuo*, and the residue was purified on a 24 g silica gel column (eluent: hexanes/ethyl acetate = 1:5) to afford compound **19** (150 mg, 0.12 mmol, 79%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38–7.30 (m, 5H), 5.40 (dd, J = 10.2, 3.4 Hz, 1H), 5.35 (dd, J = 10.0, 3.4 Hz, 1H), 5.30–5.20 (m, 7H), 5.12–5.10 (m, 2H), 4.87 (d, J = 1.7 Hz, 1H), 4.84 (d, J = 1.8 Hz, 1H), 4.80 (d, J = 1.9 Hz, 1H), 4.68 (d, J = 11.6 Hz, 1H), 4.53 (d, J = 11.6 Hz, 1H), 4.51 (dd, J = 12.2, 4.8 Hz, 1H), 4.20–4.06 (m, 5H), 4.07 (dd, J = 12.3, 2.1 Hz, 1H), 3.98 (dd, J = 10.1, 4.7,

2.1 Hz, 1H), 3.88–3.84 (m, 3H), 3.76 (dd, J = 10.8, 6.1 Hz, 1H), 3.68 (br, 1H), 3.61 (br, 1H), 3.48 (dd, J = 10.8, 2.8 Hz, 1H), 2.41 (br, 1H), 2.18–1.97 (12s, 12 × 3H). $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 171.1 (× 2), 170.7 (× 2), 170.5, 170.3, 170.1 (× 2), 170.0 (× 2), 169.9, 169.7, 169.6, 136.4, 128.9 (× 2), 128.7 (× 2), 128.6, 99.9, 99.8, 97.9, 96.4, 78.2, 74.7, 70.8, 70.7, 69.6 (× 3), 69.4, 69.3 (× 3), 68.7, 68.6, 68.3, 66.9, 66.3, 66.2, 65.2, 62.6, 62.1, 61.3, 20.9–20.5. HRESIMS: (m/z) calcd for $C_{55}H_{72}O_{33}Na^+$ $(M + Na)^+$ 1283.3854; found 1283.3823.

3.2.10. 6-O-phosphate-α-D-mannopyranosyl-(1 → 6)-[α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranosyl-(1 → 3)]-α/β-D-mannopyranose **1**

To a solution of compound **19** (130 mg, 0.1 mmol) in anhydrous CH_2Cl_2 (30 mL) was added 4 Å molecular sieves (300 mg) and 1*H*-tetrazole (0.7 mL, 0.45 M in CH_3CN, 0.31 mmol). After stirring for 2 h at rt, dibenzyl *N*,*N*-diisopropyl-phosphoramidite (70 μL, 0.21 mmol) was added to the solution under N_2, and the reaction mixture was stirred overnight. *m*CPBA (55 mg, 0.31 mmol) was then added, and the mixture was stirred at rt for 1 h. The reaction was quenched with the addition of triethylamine (0.5 mL), vacuum-filtered through Celite®, and the filtrate was concentrated *in vacuo* to a syrup. The syrup was purified by flash chromatography on a 24-g silica gel column (eluent: hexanes/ethyl acetate = 1:5) to afford compound **22** (140 mg, 0.092 mmol, 92%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.34–7.30 (m, 15H), 5.37–5.21 (m, 9H), 5.11 (m, 2H) 5.05–5.03 (m, 4H), 4.85 (d, J = 1.7 Hz, 1H), 4.79 (d, J = 2.0 Hz, 1H), 4.78 (d, J = 1.8 Hz, 1H), 4.65 (dd, J = 81.9, 11.6 Hz, 2H), 4.19–4.04 (m, 9H), 3.98 (dd, J = 10.1, 4.8, 2.2 Hz, 1H), 3.88 (dd, J = 3.1, 2.0 Hz, 1H), 3.82 (dd, J = 10.2, 5.8, 2.7 Hz, 1H), 3.75 (dd, J = 10.7, 5.8 Hz, 1H), 3.45 (dd, J = 10.7, 2.8 Hz, 1H), 2.13–1.95 (12s, 12 × 3H). $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 170.9, 170.5 (× 2), 170.3, 170.0, 169.9, 169.8 (× 2), 169.7 (× 2), 169.5, 169.4, 136.3, 135.9, 135.8, 128.7, 128.6 (× 2), 128.5, 128.4, 128.0, 127.9, 99.8, 99.7, 97.5, 96.3, 78.3, 74.8, 70.8, 69.7, 69.7 (× 2), 69.6, 69.5 (× 2), 69.4(× 4), 69.3, 69.1, 68.5, 68.4, 66.8, 66.3, 66.0, 65.9, 65.8, 65.6, 62.7, 62.2, 20.9–20.6. Compound **22** (140 mg, 0.092 mmol) was dissolved in anhydrous methanol (20 mL) and $Pd(OH)_2/C$ (140 mg, 20% wt-%) was added under H_2. After stirring at rt for 2 d, the $Pd(OH)_2/C$ catalyst was removed by filtration, and the solution was treated with NaOMe (0.5 M, 3 mL). After stirring for 10 min, a white precipitate formed. After additional stirring for 2 h, H_2O was added until the precipitate dissolved. The reaction mixture was stirred for another 2 h, and the solution was concentrated *in vacuo* to a syrup. The syrup was purified on a DEAE Sephadex A-25 column (3 × 55 cm) using a 3-L linear gradient of aqueous $NaHCO_3$ solution (0–0.8 M). Fractions (20 mL) were collected at a flow-rate of 1.5 mL/min, and fractions containing organic phosphate were identified using HPLC. Fractions containing **1** were pooled and concentrated *in vacuo* to ~5 mL, and the resulting solution was applied to a Sephadex LH-20 gel-filtration column (2.5 × 80 cm), which was eluted with distilled water at a flow rate of 1.5 mL/min. Fractions containing **1** were pooled and concentrated *in vacuo* to give **1** (62 mg, 0.078 mmol, 85%) as a white solid. ^1H NMR (600 MHz, D_2O) δ 5.35 (d, J = 1.8 Hz, 1H), 5.13 (d, J = 1.8 Hz, 0.67H (α-H)), 5.05 (d, J = 2.0 Hz, 1H), 4.90 (d, J = 1.8 Hz, 0.33H (β-H)), 4.89 (d, J = 2.0 Hz, 1H), 4.14–3.53 (m, 24H). $^{13}C\{^1H\}$ NMR (126 MHz, D_2O) δ 102.3, 100.8, 100.7, 99.6, 99.6, 94.2, 93.8, 80.9, 78.6, 78.5, 74.1, 73.3, 73.2, 71.7, 71.6, 70.9, 70.8, 70.5, 70.5, 70.4, 70.1, 70.0, 69.9, 69.9, 67.0, 66.9, 66.7, 66.2, 65.8, 65.6, 65.5, 65.4, 64.0, 64.0, 61.0, 60.9. $^{31}P\{^1H\}$ NMR (202 MHz, D_2O) δ 0.72. HRESIMS: (m/z) calcd for $C_{24}H_{42}Na_2O_{24}P^+$ $(M + H)^+$ 791.1599; found 791.1589.

3.2.11. 6-O-phosphate-α-D-mannopyranosyl-(1 → 6)-α-D-mannopyranosyl-(1 → 6)-[α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranosyl-(1 → 3)]-α/β-D-mannopyranose **2**

Compound **16** (210 mg, 0.13 mmol) was dissolved in CH_2Cl_2 (50 mL), and the solution was cooled to −10 °C $BF_3{\cdot}Et_2O$ (40 μL, 0.32 mmol) was then added dropwise, and the reaction mixture was stirred for 2.5 h as it warmed to room temperature. The mixture was then concentrated *in vacuo* to a syrup, and the syrup was purified by flash chromatography on a 24-g silica gel column (eluent: hexanes/ethyl acetate = 1:5) to afford compound **23** (140 mg, 0.09 mmol, 71%). To a solution of compound **23** (140 mg, 0.09 mmol) in anhydrous CH_2Cl_2 (30 mL) was added 4 Å molecular sieves (200 mg) and 1*H*-tetrazole (0.6 mL, 0.45 M in CH_3CN, 0.27 mmol). After stirring for 2 h at rt, dibenzyl *N*,*N*-diisopropyl-phosphoramidite (60 μL, 0.18 mmol) was added to the solution under N_2, and the reaction mixture was stirred overnight. *m*CPBA (50 mg, 0.27 mmol) was then added, and the mixture was stirred at rt for 1 h. The reaction was quenched with the addition of triethylamine (0.5 mL), vacuum-filtered through Celite®, and the solution was concentrated *in vacuo* to a syrup. The syrup was purified by flash chromatography on a 24-g silica gel column (eluent: hexanes/ethyl acetate = 1:5) to afford compound **24** (160 mg, 0.089 mmol, 99%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.34–7.29 (m, 15H), 5.36–5.19 (m, 11H), 5.12 (d, J = 2.0 Hz, 1H), 5.10 (dd, J = 10.0, 3.0 Hz, 1H), 5.06–5.01 (m, 4H), 4.88 (d, J = 1.8 Hz, 1H), 4.79 (3 d, J = 1.9 Hz, 3H), 4.70 (d, J = 11.5 Hz, 1H), 4.52 (d, J = 11.6 Hz, 1H), 4.19–4.04 (m, 9H), 4.00–3.97 (m, 2H), 3.93 (ddt, J = 6.8, 4.3, 2.4 Hz, 1H), 3.86 (m, 2H), 3.74 (m, 2H), 3.47 (dd, J = 2.5, 1.2 Hz, 1H), 3.46 (d, J = 2.6 Hz, 1H), 2.15–1.93 (15s, 15 × 3H). $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 170.9, 170.5 (× 2), 170.4, 170.2, 169.9(× 2), 169.8 (× 3), 169.74(× 2), 169.6, 169.5, 169.4, 136.3, 135.9(× 2), 135.8(× 2), 128.6(× 5), 128.5, 128.3, 128.0, 127.9, 99.7 (× 2), 97.8, 97.5, 96.3, 78.4, 74.8, 70.8, 69.6 (× 3), 69.5, 69.4, (× 3), 69.3 (× 2), 69.2, 69.1, 68.6, 68.4, 66.8, 66.3 (× 2), 66.0, 66.0, 65.8, 65.6, 62.7, 62.1, 20.9–20.6. Compound **24** (160 mg, 0.089 mmol) was dissolved in anhydrous methanol (20 mL) and $Pd(OH)_2/C$ (160 mg, 20% wt%) was added under H_2. After stirring at rt for 2 d, the $Pd(OH)_2/C$ catalyst was removed by filtration, and the filtrate was treated with NaOMe (0.5 M, 3 mL). After stirring for 10 min, a white precipitate formed. After stirring for an additional 2 h, H_2O was added until the precipitate dissolved. The reaction mixture was stirred for another 2 h, and the solution was concentrated *in vacuo* to a syrup. The syrup was purified on a DEAE Sephadex A-25 column (3 × 55 cm) using a 3-L linear gradient of aqueous $NaHCO_3$ solution (0–0.8 M). Fractions (20 mL) were collected at a flow-rate of 1.5 mL/min, and fractions containing organic phosphate were identified using HPLC. Fractions containing **2** were pooled and concentrated *in vacuo* to ~5 mL, and the resulting solution was applied to a Sephadex LH-20 gel-filtration column (2.5 × 80 cm), which was eluted with distilled water at a flow rate of 1.5 mL/min. Fractions containing **2** were pooled and concentrated *in vacuo* to give **2** (74 mg, 0.078 mmol, 87%) as a white solid. ^1H NMR (600 MHz, D_2O) δ 5.35 (d, J = 1.8 Hz, 1H), 5.13 (d, J = 1.8 Hz, 0.67H (α-H)), 5.05 (d, J = 1.9 Hz, 1H), 4.90 (d, J = 1.8 Hz, 1H), 4.89 (d, J = 1.0 Hz, 0.33H (β-H)), 4.88 (d, J = 1.7 Hz, 0.33H (β-H)), 4.87 (d, J = 1.7 Hz, 0.67H (α-H)), 4.14–3.53 (m, 30H). $^{13}C\{^1H\}$ NMR (126 MHz, D_2O) δ 102.3, 100.9, 100.7, 99.5 (× 2), 94.2, 93.8, 80.9, 78.6, 78.5, 74.0, 73.4, 73.3, 71.7, 71.7, 70.9, 70.9, 70.8, 70.7, 70.7, 70.5, 70.4, 70.3, 70.1, 70.0, 69.9 (× 2), 67.0, 66.9, 66.7, 66.6, 66.2, 65.7, 65.7, 65.6, 65.4, 65.3, 64.1(× 2), 61.0 (× 2). $^{31}P\{^1H\}$ NMR (202 MHz, D_2O) δ 0.70. HRESIMS: (m/z) calcd for $C_{30}H_{52}Na_2O_{29}P^+$ $(M + H)^+$ 953.2127; found 953.2129.

3.2.12. Benzyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl-(1 → 6)-2,3,4-tri-O-acetyl-α-D-mannopyranosyl-(1 → 6)-[2,3,4-tri-O-acetyl-6-O-dibenzylphosphate-α-D-mannopyranosyl-(1 → 3)]-2,4-di-O-acetyl-α-D-mannopyranoside **26**

Compound **18** (250 mg, 0.16 mmol) was dissolved in CH_2Cl_2 (50 mL), and the solution was cooled to −10 °C $BF_3{\cdot}Et_2O$ (60 μL, 0.49 mmol) was added dropwise, the reaction mixture was stirred for 2.5 h as it warmed to room temperature, and then the reaction solution was concentrated *in vacuo* to a syrup. The syrup was purified by flash

chromatography on a 24-g silica gel column (eluent: hexanes/ethyl acetate = 1:5) to afford compound **25** (180 mg, 0.14 mmol, 89%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35–7.30 (m, 5H), 5.39–5.18 (m, 10H), 5.04 (dd, J = 3.0, 1.8 Hz, 1H), 4.98 (d, J = 1.8 Hz, 1H), 4.86 (d, J = 1.6 Hz, 1H), 4.85 (d, J = 1.7 Hz, 1H), 4.81 (d, J = 1.8 Hz, 1H), 4.74 (d, J = 11.8 Hz, 1H), 4.55 (d, J = 11.9 Hz, 1H), 4.27 (dd, J = 12.3, 5.2 Hz, 1H), 4.21 (dd, J = 9.8, 3.4 Hz, 1H), 4.12–4.08 (m, 2H), 4.06–4.00 (m, 2H), 3.91–3.82 (m, 2H), 3.78 (m, 2H), 3.58 (m, 2H), 3.48 (dd, J = 10.8, 2.7 Hz, 1H), 2.38 (t, J = 7.1 Hz, 1H), 2.18–1.95 (12s, 12 × 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 171.1, 171.0, 170.7, 170.3, 170.1 (× 2), 170.0, 169.9, 169.8 (× 2), 169.7, 169.6 (× 2), 128.6, 128.2 (× 2), 99.2, 97.9, 97.6, 96.3, 75.2, 71.6, 70.9, 70.0, 69.9, 69.5, 69.4 (× 3), 69.2 (× 2), 68.7, 68.3, 68.2, 66.9, 66.5, 66.4, 66.3, 66.0, 62.5, 61.2, 21.0–20.7. To a solution of compound **25** (180 mg, 0.14 mmol) in anhydrous CH_2Cl_2 (30 mL) was added 4 Å molecular sieves (200 mg) and 1*H*-tetrazole (0.96 mL, 0.45 M in CH_3CN, 0.43 mmol). After stirring for 2 h at rt, dibenzyl *N*,*N*-diisopropylphosphoramidite (100 μL, 0.28 mmol) was added to the solution under N_2, and the reaction mixture was stirred overnight. Then *m*CPBA (100 mg, 0.43 mmol) was added, and the mixture was stirred at rt for 1 h. The reaction was quenched with the addition of triethylamine (0.5 mL), vacuum-filtered through Celite®, and the filtrate was concentrated to a syrup. The syrup was purified by flash chromatography on a silica gel column (24 g) (eluent: hexanes/ethyl acetate = 1:5) to afford compound **26** (150 mg, 0.1 mmol, 71%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34–7.24 (m, 15H), 5.37–5.16 (m, 10H), 5.04 (d, J = 1.1 Hz, 1H), 5.02 (d, J = 1.2 Hz, 1H), 5.01 (d, J = 1.1 Hz, 1H), 5.00 (d, J = 1.0 Hz, 1H), 4.94 (dd, J = 3.3, 1.8 Hz, 1H), 4.87 (d, J = 1.8 Hz, 1H), 4.85 (d, J = 1.4 Hz, 1H), 4.79 (d, J = 1.7 Hz, 2H), 4.64 (dd, J = 96.0, 11.8 Hz, 2H), 4.26 (dd, J = 12.3, 5.2 Hz, 1H), 4.15 (dd, J = 9.8, 3.4 Hz, 1H), 4.10–3.98 (m, 6H), 3.84 (dd, J = 10.2, 6.0, 2.8 Hz, 1H), 3.77–3.74 (m, 2H), 3.58 (dd, J = 11.2, 2.6 Hz, 1H), 3.45 (dd, J = 10.7, 2.8 Hz, 1H), 2.17–1.94 (12s, 12 × 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 170.7, 170.6, 170.3, 170.1, 170.0, 169.9, 169.8 (× 2), 169.7 (× 2), 169.6 (× 2), 136.4, 135.98, 136.0, 135.9 (× 2), 128.6 (× 2), 128.5 (× 2), 128.4, 128.3, 128.1, 127.9 (× 2), 98.8, 97.8, 97.5, 96.2, 74.7, 70.8, 70.0, 69.8 (× 3), 69.4 (× 2), 69.3 (× 5), 69.1(× 2), 68.7, 68.4, 68.3, 66.9, 66.5, 66.3, 65.9, 65.7 (× 2), 65.6, 62.4, 20.9–20.7. HRESIMS: (*m*/*z*) calcd for $C_{69}H_{85}O_{36}PNa^+$ $(M + Na)^+$ 1543.4456; found 1543.4457.

3.2.13. α-D-mannopyranosyl-(1 → 6)-α-D-mannopyranosyl-(1 → 6)-[6-O-phosphate-α-D-mannopyranosyl-(1 → 3)]-α/β-D-mannopyranose **3**

Compound **26** (150 mg, 0.1 mmol) was dissolved in anhydrous methanol (20 mL) and $Pd(OH)_2$/C (150 mg, 20% wt%) was added under H_2. After stirring at rt for 2 d, the $Pd(OH)_2$/C catalyst was removed by filtration, and the filtrate was treated with NaOMe (0.5 M, 3 mL). After stirring for 10 min, a white precipitate formed. After stirring for 2 h, H_2O was added until the precipitate dissolved. The reaction mixture was then stirred for another 2 h, and the solution was concentrated *in vacuo* to a syrup. The syrup was purified by chromatography on a DEAE Sephadex A-25 column (3 × 55 cm) using a 3-L linear gradient of aqueous $NaHCO_3$ solution (0–0.8 M). Fractions (20 mL) were collected at a flow-rate of 1.5 mL/min, and fractions containing organic phosphate were identified using HPLC. Fractions containing **3** were pooled and concentrated *in vacuo* to ~5 mL, and the solution was applied to a Sephadex LH-20 gel-filtration column (2.5 × 80 cm), which was eluted with distilled water at a flow rate of 1.5 mL/min. Fractions containing **3** were pooled and concentrated *in vacuo* to give **3** (70 mg, 0.089 mmol, 89%) as a white solid. ^{1}H NMR (600 MHz, D_2O) δ 5.13 (d, J = 1.9 Hz, 0.67H (α-H)), 5.10 (d, J = 1.8 Hz, 0.33H (β-H)), 5.09 (d, J = 1.8 Hz, 0.67H (α-H)), 4.92 (d, J = 1.0 Hz, 0.33H (β-H)), 4.90 (d, J = 1.7 Hz, 1H), 4.88 (d, J = 1.8 Hz, 0.33H (β-H)), 4.87 (d, J = 1.8 Hz, 0.67H (α-H)). 4.17–3.55 (m, 24H). ^{13}C{^{1}H} NMR (126 MHz, D_2O) δ 102.8, 102.5, 99.5, 99.4, 99.3, 94.4, 93.8, 81.1, 79.3, 74.0, 72.7, 72.4, 72.3, 70.8, 70.7, 70.6, 70.3 (× 2), 70.1, 70.0, 69.9 (× 2), 66.8, 66.6, 66.5, 65.7, 65.5, 65.5, 65.3, 65.2, 64.3, 64.3, 61.0. ^{31}P{^{1}H} NMR (202 MHz, D_2O) δ 0.90. HRESIMS: (*m*/*z*) calcd for $C_{24}H_{42}Na_2O_{24}P^+$ $(M + H)^+$ 791.1599; found 791.1591.

3.2.14. 4-O-phosphate-α-D-mannopyranosyl-(1 → 6)-[α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranosyl-(1 → 3)]-α/β-D-mannopyranose **27**

Compound **20** was dissolved in anhydrous CH_2Cl_2 (30 mL), and 4 Å molecular sieves (200 mg) and 1*H*-tetrazole (0.9 mL, 0.45 M in CH_3CN, 0.41 mmol) were added to the solution. After stirring for 2 h at rt, dibenzyl *N*,*N*-diisopropyl-phosphoramidite (100 μL, 0.27 mmol) was added to the reaction mixture under N_2, and the mixture was stirred overnight. *m*CPBA (70 mg, 0.41 mmol) was then added, and the mixture was stirred at rt for 1 h. The mixture was quenched with the addition of triethylamine (0.5 mL), vacuum-filtered through Celite®, and the filtrate was concentrated *in vacuo* to a syrup. The syrup was purified by flash chromatography on a 24-g silica gel column (eluent: hexanes/ethyl acetate = 1:5) to afford compound **21** (150 mg, 0.1 mmol, 75%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30–7.17 (m, 15H), 5.42 (dd, J = 9.8, 3.5 Hz, 1H), 5.35 (dd, J = 10.0, 3.3 Hz, 1H), 5.29–5.11 (m, 7H), 5.09 (dd, J = 10.0, 2.9 Hz, 1H), 4.99 (d, J = 7.9 Hz, 2H), 4.88–4.84 (m, 3H), 4.80 (m, 2H), 4.74 (q, J = 9.5 Hz, 1H), 4.67 (d, J = 11.5 Hz, 1H), 4.47 (m, 2H), 4.25 (dd, J = 12.4, 5.3 Hz, 1H), 4.18–4.06 (m, 7H), 3.98 (dd, J = 10.1, 4.7, 2.1 Hz, 1H), 3.86 (m, 2H), 3.74 (dd, J = 10.6, 6.8 Hz, 1H), 3.49 (dd, J = 10.7, 2.4 Hz, 1H), 2.15–1.78 (12s, 12 × 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 170.9, 170.6, 170.4, 170.4, 170.2, 170.0, 169.9, 169.8 (× 2), 169.5, 169.4, 136.1, 135.5, 135.4 (× 3), 128.7, 128.6 (× 2), 128.5, 128.4, 128.0, 127.8, 99.7 (× 2), 97.0, 96.3, 78.3, 77.4, 77.2, 76.9, 74.6, 70.8, 69.7, 69.6 (× 4), 69.5 (× 2), 69.2, 69.1, 68.6, 68.4, 66.8, 66.2, 65.6, 62.7, 62.5, 62.1, 20.9–20.6. Compound **21** (140 mg, 0.092 mmol) was dissolved in anhydrous methanol (20 mL) and $Pd(OH)_2$/C (140 mg, 20% wt-%) was added under H_2. After stirring at rt for 2 d, the $Pd(OH)_2$/C catalyst was removed by filtration, and the filtrate was treated with NaOMe (0.5 M, 3 mL). After stirring for 10 min, a white precipitate formed, and after stirring for 2 h, H_2O was added until the precipitate dissolved. The reaction mixture was stirred for another 2 h, and the solution was concentrated *in vacuo* to a syrup. The syrup was purified on a DEAE Sephadex A-25 column (3 × 55 cm) using a 3-L linear gradient of aqueous $NaHCO_3$ solution (0–0.8 M). Fractions (20 mL) were collected at a flow-rate of 1.5 mL/min, and fractions containing organic phosphate were identified using HPLC. Fractions containing **27** were pooled and concentrated *in vacuo* to ~5 mL, and the resulting solution was applied to a Sephadex LH-20 gel-filtration column (2.5 × 80 cm), which was eluted with distilled water at a flow rate of 1.5 mL/min. Fractions containing **27** were pooled and concentrated *in vacuo* to give **27** (60 mg, 0.075 mmol, 82%) as a white solid. ^{1}H NMR (600 MHz, D_2O) δ 5.36 (d, J = 1.8 Hz, 1H), 5.13 (d, J = 1.9 Hz, 0.67H (α-H)), 5.05 (d, J = 2.0 Hz, 1H), 4.91 (d, J = 1.7 Hz, 0.33H (β-H)), 4.90 (d, J = 1.7 Hz, 0.67H (α-H)), 4.89 (d, J = 1.0 Hz, 0.33H (β-H)), 4.18–3.52 (m, 24H). ^{13}C{^{1}H} NMR (126 MHz, D_2O) δ 103.7, 102.3, 102.1, 100.8, 100.7, 95.6, 95.2, 82.3, 80.0 (× 2), 75.5, 74.8, 74.7, 73.2, 73.1, 72.8 (× 2), 72.3, 72.2, 71.8, 71.5, 71.4, 71.2, 71.2, 68.4, 68.4, 68.2, 67.2, 67.1, (× 2), 67.0, 62.4 (× 2), 62.2. ^{31}P{^{1}H} NMR (202 MHz, D_2O) δ 1.06. HRESIMS: (*m*/*z*) calcd for $C_{24}H_{42}Na_2O_{24}P^+$ $(M + H)^+$ 791.1599; found 791.1616.

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Appendix ASupplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.carres.2018.07.005.

References

[1] (a) A. Varki, S. Kornfeld, J. Biol. Chem. 255 (1980) 10847–10858;
(b) P.Y. Tong, W. Gregory, S. Komfeld, J. Biol. Chem. 264 (1989) 7962–7969;
(c) M.F. Coutunho, M.J. Prata, S. Alves, Mol. Genet. Metabol. 105 (4) (2012) 542–550;
(d) B. Winchester, A. Vellodi, E. Young, Biochem. Soc. Trans. 28 (2000) 150–154;
(e) A.P. Lieberman, R. Puertollano, N. Raben, S. Slaugenhaupt, S.U. Walkley, A. Ballabio, Autophagy 8 (2012) 719–730;
(f) M.L. Schultz, L. Tecedor, M. Chang, B.L. Davidson, Trends Neurosci. 34 (2011) 401–410.

[2] P. Ghosh, N.M. Dahms, S. Kornfeld, Nat. Rev. Mol. Cell Biol. 4 (2003) 202–212.

[3] M.F. Coutinho, M.J. Prata, S. Alves, Mol. Genet. Metabol. 105 (2012) 542–550.

[4] G. Parenti, G. Andria, A. Ballabio, Annu. Rev. Med. 66 (2015) 471–486.

[5] W.S. Sly, H.D. Fischer, J. Cell. Biochem. 18 (1982) 67–85.

[6] (a) R.O. Brady, Annu. Rev. Med. 57 (2006) 283–296;
(b) R.H. Lachmann, Curr. Opin. Pediatr. 23 (2011) 588–593.

[7] R. Lachmann, Biochem. Soc. Trans. 38 (2010) 1465–1468.

[8] (a) P. Priyanka, T.B. Parsons, A. Miller, F.M. Platt, A.J. Fairbanks, Angew. Chem. Int. Ed. 55 (2016) 5058–5061;
(b) T. Yamaguchi, M.N. Amin, C. Toonstra, L.-X. Wang, J. Am. Chem. Soc. 138 (2016) 12472–12485;
(c) J.J. Distler, J.F. Guo, G.W. Jourdian, O.P. Srivastava, O. Hindsgaul, J. Biol. Chem. 266 (1991) 21687–21692.

[9] (a) X. Song, Y. Lasanajak, L.J. Olson, M. Boonen, N.M. Dahms, S. Kornfeld, R.D. Cummings, D.F. Smith, J. Biol. Chem. 284 (2009) 35201–35214;
(b) R.N. Bohnsack, X. Song, L.J. Olson, M. Kudo, R.R. Gotschall, W.M. Canfield, R.D. Cummings, D.F. Smith, N.M. Dahms, J. Biol. Chem. 284 (2009) 35215–35226;
(c) H. Franzyk, M.K. Christensen, R.M. Jorgensen, M. Meldal, H. Cordes, S. Mouritsen, K. Bock, Bioorg. Med. Chem. 5 (1997) 21–40;
(d) D.B. Berkowitz, G. Maiti, B.D. Charette, C.D. Dreis, R.G. MacDonald, Org. Lett. 6 (2004) 4921–4924;
(e) D.E. Sleat, M.C.D. Valle, H. Zheng, D.F. Moore, P. Lobel, J. Proteome Res. 7 (7) (2008) 3010–3021.

[10] (a) O. Srivastava, O. Hindsgaul, J. Org. Chem. 52 (1987) 2869–2875;
(b) O. Srivastava, O. Hindsgaul, Carbohydr. Res. 161 (1987) 324–329;
(c) Y. Liu, Y.M. Chan, J. Wu, C. Chen, A. Benesi, J. Hu, Y. Wang, G. Chen, Chembiochem 12 (2011) 685–690;
(d) Y. Liu, G. Chen, J. Org. Chem. 76 (2011) 8682–8689;
(e) B. Meng, J. Wang, Q. Wang, A.S. Serianni, Q. Pan, Tetrahedron 73 (2017) 3932–3938.

[11] (a) E.A.B. Kantchev, J.R. Parquette, Synlett 10 (2005) 1567–1570;
(b) J.K. Fairweather, T. Karoli, V. Ferro, Bioorg. Med. Chem. 12 (2004) 6063–6075.

[12] (a) K. Gorska, K.-T. Huang, O. Chaloin, N. Winssinger, Angew. Chem. Int. Ed. 48 (2009) 7695–7700;
(b) A.M. Daines, B.W. Greatrex, C.M. Hayman, S.M. Hook, W.T. Mcburney, T. Rades, P.M. Rendle, I.M. Sims, Bioorg. Med. Chem. 17 (2009) 5207–5218;
(c) M. Upreti, D. Ruhela, R.A. Vishwakarma, Tetrahedron 56 (2000) 6577–6584.

[13] (a) T. Li, L. Wen, A. Williams, B. Wu, L. Li, J. Qu, J. Meisner, Z. Xiao, J. Fang, P.G. Wang, Bioorg. Med. Chem. 22 (2014) 1139–1147;
(b) P. Cmoch, Z. Pakulski, Tetrahedron: Asymmetry 19 (2008) 1494–1503.

[14] (a) C. Rucker, Chem. Rev. 95 (1995) 1009–1064;
(b) S. Yan, N. Ding, W. Zhang, P. Wang, X. Li, M. Li, Carbohydr. Res. 354 (2012) 6–20.

[15] T.W.W. Greene, P. G. M., Protective Groups in Organic Synthesis, John Wiley & Sons, Inc., 1999.

[16] K. Kamien ska-Trela, J. Wo jcik, Nucl. Magn. Reson. 41 (2012) 148–195.