JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

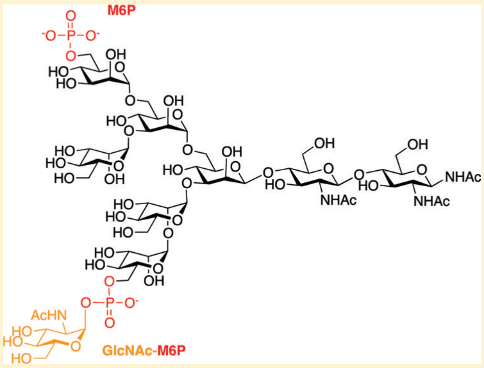
Chemical Synthesis of N-Linked Glycans Carrying Both Mannose-6-phosphate and GlcNAc-Mannose-6-phosphate Motifs

Yunpeng Liu and Gong Chen*

Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802, United States

Supporting Information

ABSTRACT: Mannose-6-phosphate (M6P) containing N-linked glycans are the essential targeting signals for hydrolases sorting in eukaryotic cells. To facilitate their structural and binding analyses, a highly efficient and convergent method has been developed to prepare complex N-linked glycans with well-defined M6P and *N*-acetylglucosamine (GlcNAc)-M6P motifs, a newly identified binding element for M6P receptors. The GlcNAc-M6P motif was stereoselectively installed at the late stage of the synthesis. Sequential deprotection of benzyl and acetate groups provided the fully deprotected *N*-glycans in excellent yield.



INTRODUCTION

Mannose-6-phosphate (M6P)-containing N-linked glycans are the essential targeting signals for the transport of hydrolases and other M6P-containing proteins in eukaryotic cells (Figure 1A).[1] In the ER-Golgi network, hydrolases become distinct from the other *N*-glycoproteins by acquiring M6P residues in a two-step process: First, the GlcNAc-phosphotransferase transfers *N*-acetylglucosamine-1-phosphate to mannose residues on a *N*-glycan to generate a phosphodiester intermediate GlcNAc-M6P. Second, α-*N*-acetyl-glucosaminidase removes the *N*-acetylglucosamine residue to generate the M6P phosphomonoester (Figure 1B).[2] Depending on the M6P-based binding signals embedded in *N*-glycans, M6P receptors (MPRs) can guide the transport of hydrolases to different destinations, mainly lysosomes or extracellular matrix. Because of the complexity caused by the inherent heterogeneity and multivalence of M6P *N*-glycans, the detailed mechanism of this universal M6P-coded protein sorting process is still poorly understood.[3] The conventional binding model relies on a simple binary coding system (M and M6P) where M6P serves as the sole positive binding element for MPRs. However, recent studies from Dahms and co-workers provided critical insights to the functional roles of the GlcNAc-M6P motif.[4] They discovered that M6P receptor homology (MRH) domain 5 of the cation-independent (CI)-MPR provides a previously unnoticed weak binding site for GlcNAc-M6P (Figure 1B). More surprisingly, MRH domain 5 shows a stronger binding affinity for GlcNAc-M6P as compared to M6P.[5] Even though GlcNAc-M6P-containing *N*-glycans were found in secreted hydrolases, they were mostly ignored due to their unknown binding abilities.[6] Inspired by Dahms's discovery, we hypothesized that GlcNAc-M6P could be a key element necessary to control the diversion of hydrolases at the trans Golgi network. Therefore, a much more complex ternary coding system consisting of M, M6P, and Glc-M6P might exist for the hydrolase sorting system. To test this hypothesis via structural and binding analyses, we need to obtain enough homogeneous *N*-glycan materials with various well-defined GlcNAc-M6P and M6P modification patterns. Herein, we report the first chemical synthesis of GlcNAc-M6P-containing *N*-glycans, which proceeds in a highly efficient fashion.

RESULTS AND DISCUSSION

Phosphor-containing *N*-glycans pose a greater synthetic challenge when compared to unmodified *N*-glycans.[7,8] Synthetic routes have to be delicately orchestrated and executed to accommodate the labile phosphor moieties. In our previous exploration of the chemical synthesis of multiphosphorylated *N*-glycans, a late stage phosphorylation strategy was successfully employed to install two benzyl-protected phosphate groups on an octasaccharide substrate using the phosphoramidite chemistry.[9] Accordingly, such a late stage phosphorylation strategy was adopted for the synthesis of GlcNAc-M6P-containing *N*-glycans. To synthesize complex GlcNAc-M6P *N*-glycans, we first need to develop a highly efficient and robust procedure to install GlcNAc to a monomeric mannopyranose substrate through the phosphodiester linkage in a stereoselective manner (Scheme 1).

Received: June 10, 2011
Published: September 28, 2011

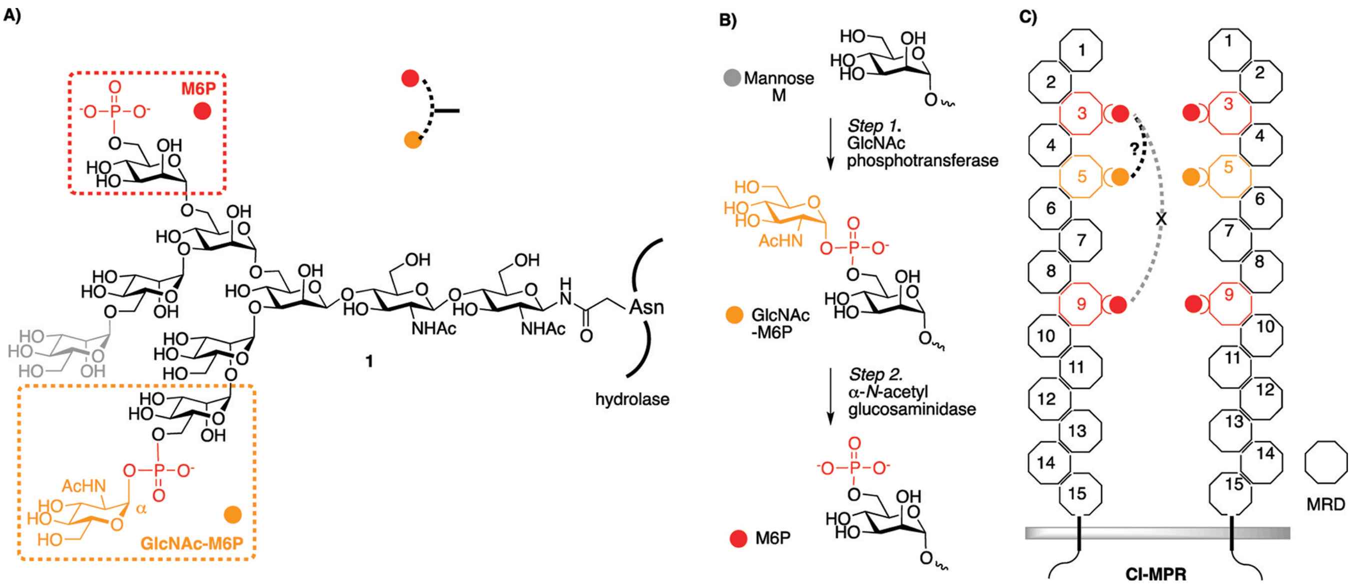
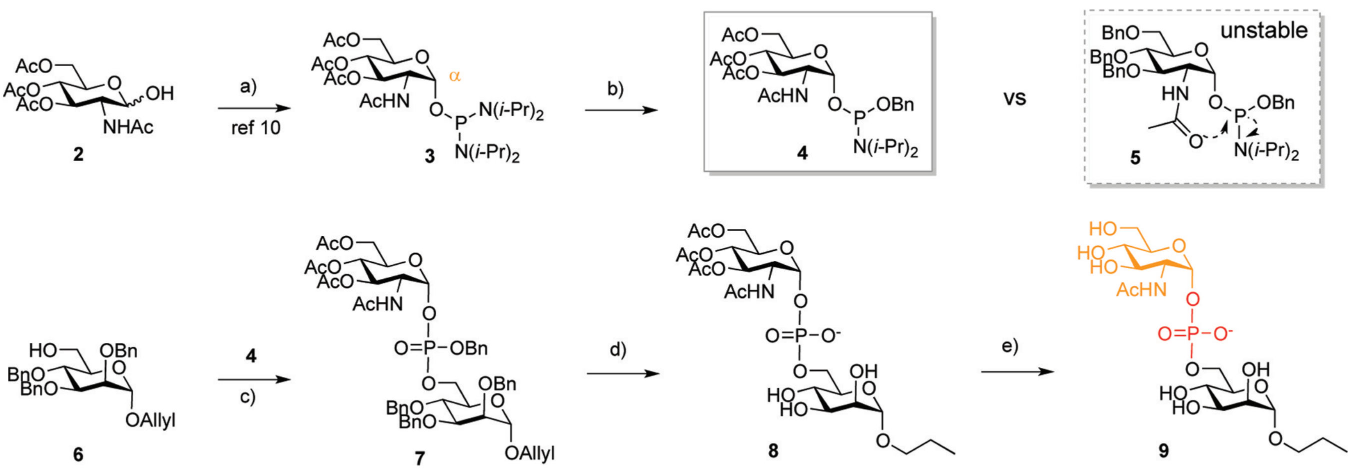


Figure 1. M6P *N*-glycans for hydrolase sorting. (A) A representative structure *N*-glycan carrying M6P and/or GlcNAc-M6P motifs. (B) Biosynthesis of M6P. (C) A schematic structure of a homodimeric cation-independent MPR: Each octahedron is a MPH domain, and domains 3 and 9 are known binding sites for M6P, while domain 5 was recently revealed as a GlcNAc-M6P binding site.

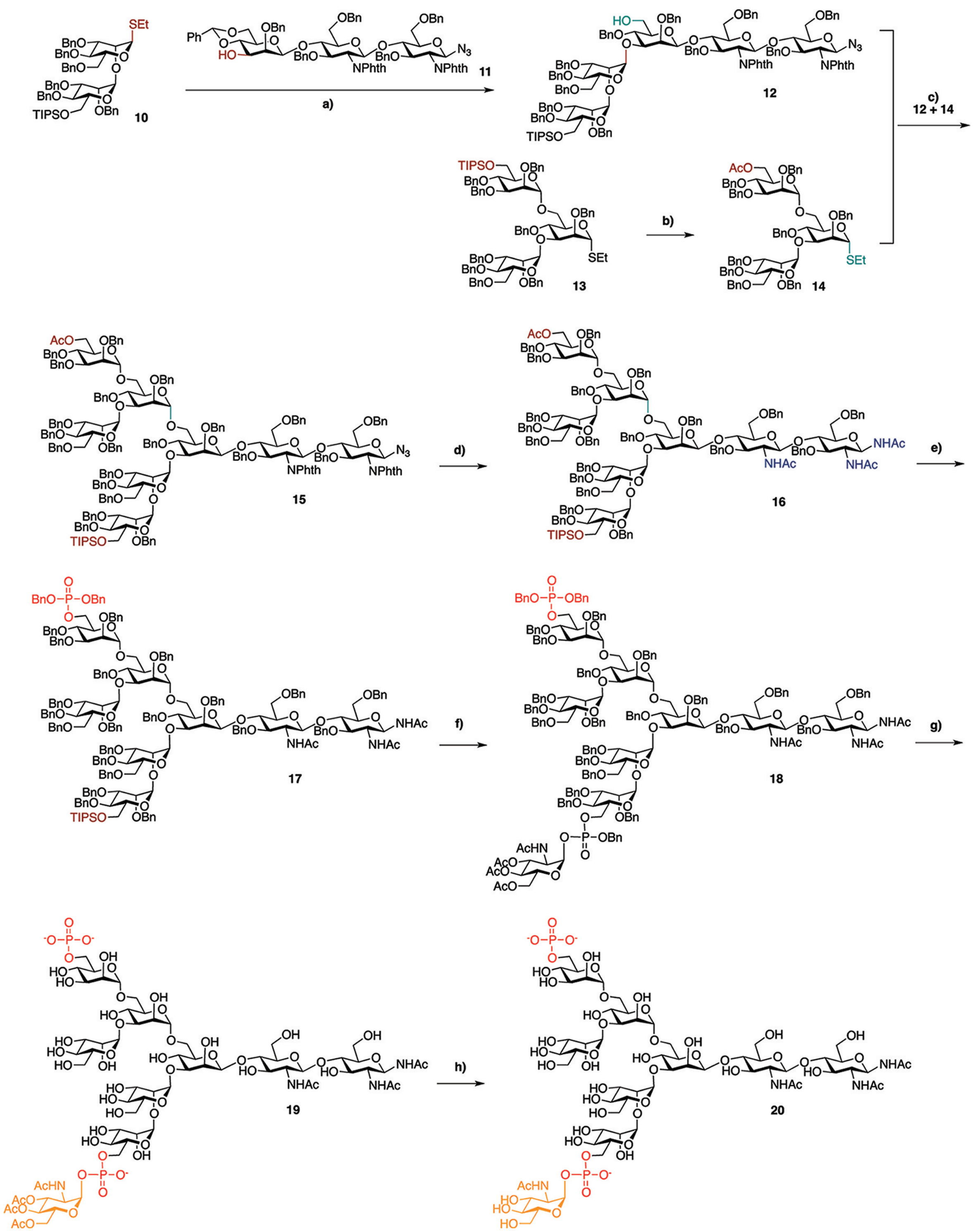
Scheme 1. Synthesis of GlcNAc-M6P Disaccharide[a]



[a]Reagents and conditions: (a) $(i\text{-}Pr_2N)_2PCl$, DIPEA, CH_2Cl_2, MS 4 Å, rt, 32%. (b) BnOH, 1*H*-tetrazole, CH_2Cl_2, rt, 36%. (c) (i) **4** (2 equiv), **6**, 1*H*-tetrazole, CH_2Cl_2, MS 4 Å, rt; (ii) *t*-BuO_2H, 90% over two steps. (d) H_2, Pd/C, MeOH, rt. (e) $NH_2NH_2{\cdot}H_2O$, MeOH; rt, 67% over steps d and e.

Among the various options for the construction of the GlcNAc-M6P phosphodiester linkage, the disconnection between the C_6-OH group on the mannopyranose and the phosphorus (P) group linked to $C_1\alpha$-OH of GlcNAc looked particularly attractive. An O-(P-X) (X as a leaving group) group can be first stereoselectively installed on the C_1-OH of GlcNAc, which then reacts as an electrophilic phosphorylation reagent with the C_6-OH group of a mannopyranose. If successful, the difficult $C_1\alpha$-O-P stereochemistry can be pre-established in a simple intermediate, and the overall sequence to synthesize complex *N*-glycans will also be more convergent. The blueprint of this approach had been elegantly demonstrated by Boons and co-workers in the synthesis of GlcNAc-P-serine motifs bearing a similar α phosphodiester linkage between the C_1 of GlcNAc and the β-OH group of serine.[10] Using this strategy, GlcNAc-P-N$(i\text{-}Pr)_2$ **4** was prepared from GlcNAc **2** in moderate yield and excellent α stereoselectivity, which reacted with the β-OH group of protected serine to form the desired phosphotriester upon oxidation with tBuO$_2$H. Initially, we explored a new phosphoramidite reagent **5**, a Bn-protected version of reagent **4**, for the synthesis of GlcNAc-M6P because of its facile deprotection by catalytic hydrogenolysis. However, this compound failed to undergo the desired phosphorylation with mannopyranose due to intramolecular cyclization presumably caused by the increased nucleophilicity of the NHAc group (Scheme 1). To our delight, compound **4** reacted well with monomeric mannopyranose **6** to give GlcNAc-M6P **7** in excellent yield under the tetrazole-promoted coupling and tBuO$_2$H-mediated oxidation condition. Following Boons's procedure, a clean deprotection of GlcNAc-M6P **7** was achieved to provide the disaccharide **9** in a two-step sequence: first removal of the OBn groups via catalytic hydrogenolysis, followed by removal of the OAc groups with $NH_2NH_2{\cdot}H_2O$ in MeOH. It is noteworthy that, in contrast to the labile Bn-protected phosphotriester linkage, phosphodiester linkage was stable under the above nucleophilic conditions.

Scheme 2. Synthesis of the Hybrid *N*-Glycan 20[a]

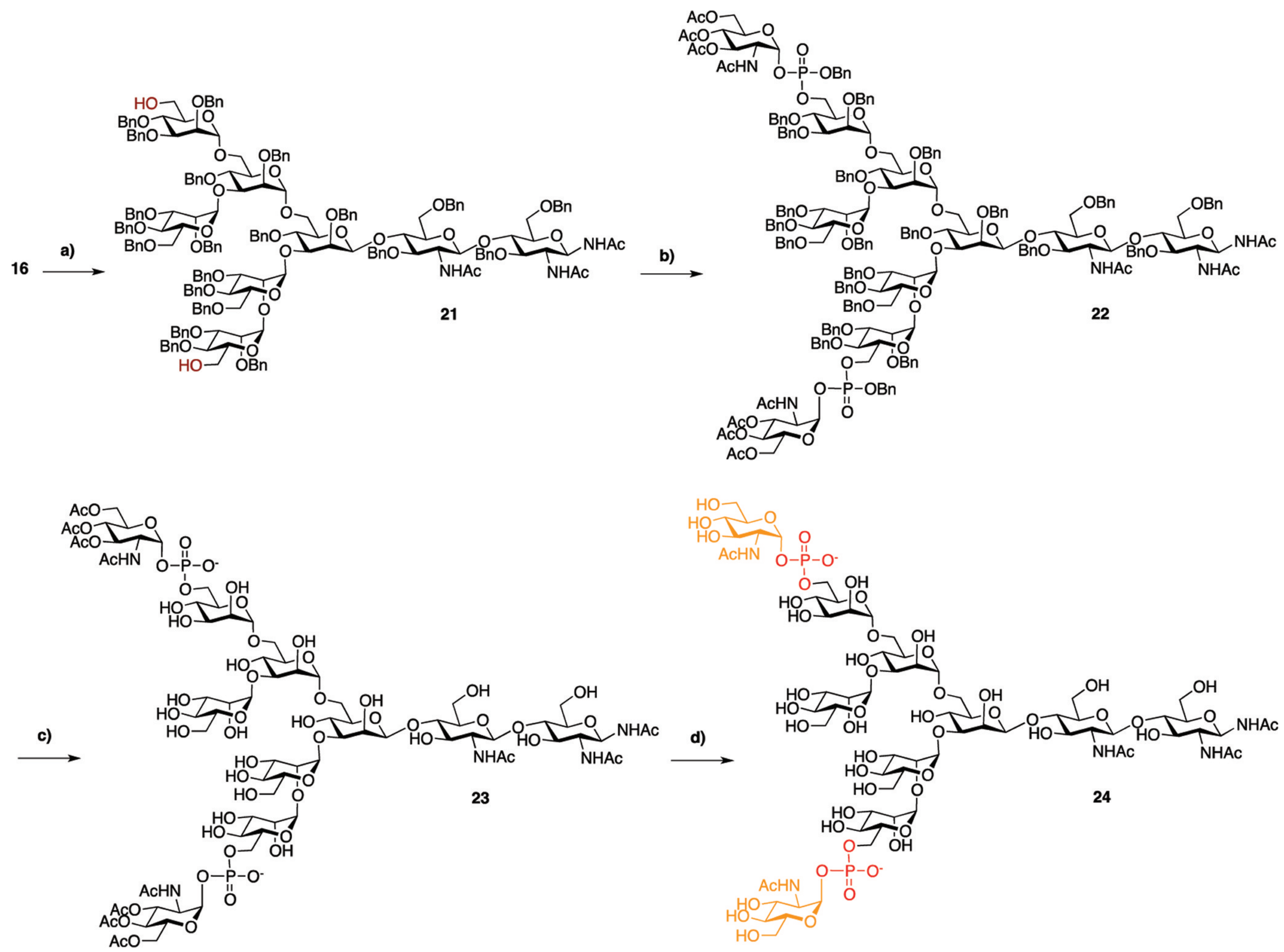


[a]Reagents and conditions: (a) (i) NIS, TfOH, Et_2O, MS 4 Å, −20 °C−rt, 92%; (ii) $PhBCl_2$, Et_3SiH, CH_2Cl_2, −78 °C, 92%. (b) (i) TABF, THF, rt, 90%; (ii) Ac_2O, Py, rt, 95%. (c) **12**, **14** (2 equiv), NIS, AgOTf, Et_2O, MS 4 Å, −20 °C−rt. (d) (i) Ethylenediamine, *n*-BuOH, then Ac_2O, Py, rt, ~50% over steps c and d−i; (ii) CH_3COSH, $CHCl_3$, reflux, 82%. (e) (i) NaOMe, MeOH, rt, >95%; (ii) dibenzyl *N*,*N*-diisopropylphosphoramidite (3 equiv), 1*H*-tetrazole; (iii) *m*CPBA, 95%, over two steps. (f) (i) TABF, THF, rt, >95%; (ii) **4** (2 equiv), 1*H*-tetrazole, CH_2Cl_2, MS 4 Å; then *t*-BuO_2H, >95%, over two steps. (g) H_2, Pd/C, MeOH, rt. (h) $NH_2NH_2 \cdot H_2O$, MeOH, rt, 81%, over two steps g and h.

With this highly efficient GlcNAc-M6P construction method in hand, we set out to synthesize complex *N*-glycans carrying GlcNAc-M6P and/or M6P motifs. An octasaccharide skeleton as shown in structure **1** (Figure 1A) was chosen as our target.[4] To allow for different modes of phosphorylation at the late stage of synthesis, two mannose branches bearing differentially protected C_6-OH groups with OTIPS and OAc needed to be built into the skeleton (compound **16**, Scheme 2).

Following our previous synthetic route for the bis-M6P *N*-glycan, Man_2 compound **10** carrying the O-TIPS group was

Scheme 3. Synthesis of the Bis GlcNAc-M6P *N*-Glycan 24[a]



[a]Reagents and conditions: (a) (i) NaOMe, MeOH, rt, >95%; (ii) TABF, THF, rt, >95%. (b) **4** (5 equiv), 1*H*-tetrazole, CH_2Cl_2, MS 4 Å; then *t*-BuO_2H, >95%, over two steps. (c) H_2, Pd/C, MeOH, rt. (d) $NH_2NH_2 \cdot H_2O$, MeOH, rt, 87%, over steps c and d.

first installed to the central trisaccharide **11** under the promotion of NIS and TfOH, followed by selective benzylidene opening, to give pentasaccharide **12** in good yield.[9] The TIPS group of Man_3 branch **13**, used in our previous synthesis,[9] was converted to the OAc group. The resulting trisaccharide **14** was installed onto **12** under the promotion of NIS and AgOTf. Subsequently, the Phth groups were converted to the NHAc groups; the anomeric azido group was transformed to NHAc by AcSH[11] to provide octasaccharide **16** with two different masked phosphorylation sites. The OAc group of **16** was then removed by the treatment with NaOMe in MeOH; the unmasked OH group was phosphorylated with dibenzyl *N*,*N*-diisopropylphosphoramidite, followed by tBuO$_2$H oxidation, to place the first Bn-protected phosphoester motif in **17**. The TIPS group of **17** was then removed by TBAF. To our delight, installation of the GlcNAc phosphotriester on the octasaccharide substrate proceeded cleanly with 2 equiv of reagent **4** under the standard coupling/oxidation conditions. Following the sequential deprotection of OBn and OAc groups of **18**, analytically pure product **20** carrying both M6P and GlcNAc-M6P motifs was obtained in nearly quantitative yield. The stereochemistry of $C_1\alpha$ of GlcNAc in **20** was confirmed by ^{1}H NMR (see the Supporting Information).

Following the same late stage phosphorylation strategy, *N*-glycan **24** carrying two GlcNAc-M6P motifs, a compound found in secreted hydrolases,[6] was also synthesized. As shown in Scheme 3, both OAc and OTIPS groups of compound **15** were removed to provide intermediate **21**. Installation of two GlcNAc phosphotriester linkages on **21** proceeded cleanly with 5 equiv of phosphoramidite **4** under the standard phosphorylation condition to give compound **22**. Final deprotection of OBn and OAc groups of **22** provided the decasaccharide **24** in excellent yield.

■ CONCLUSION

In summary, we developed a highly efficient and convergent method for the synthesis of GlcNAc-M6P motif. Following a late stage phosphorylation strategy, we have achieved the first syntheses of complex *N*-glycans carrying well-defined GlcNAc-M6P and/or M6P motifs. We expect this strategy would allow us to prepare a series of *N*-glycans with various glycoforms and phosphoforms for detailed structural and binding analyses with CI-MPRs.

■ EXPERIMENTAL SECTION

Allyl 6-[Benzyl-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)]-phosphonato-2,3,4-tri-*O*-benzyl-α-D-manno-

pyranoside (7). To a mixture of compounds **4**[10] (120 mg, 0.2 mmol) and **6**[12] (50 mg, 0.1 mmol) in CH_2Cl_2 (4 mL) under an atmosphere of N_2 was added 1*H*-tetrazole solution (1.0 mL, 0.45 M solution in CH_3CN). The reaction mixture was stirred at room temperature for 12 h and then cooled to −40 °C. *t*-BuO_2H (47 μL, 70% solution in water) was then added and stirred for 2 h at −40 °C. The solvent was then removed under reduced pressure, and the resulting residue was purified by silica gel column chromatography (EtOAc/Hex, 4/1). This product was further purified by size-exclusion column chromatography over Sephadex LH-20 ($MeOH/CH_2Cl_2$, 1/1) to give compound **7** as a yellow oil (75 mg, 90%). R_f = 0.4 (EtOAc/Hex, 4/1). $[\alpha]_D^{20}$ 25 (*c* 0.44, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.25−7.32 (m, 20 H), 6.07 (d, 1 H, *J* = 9.3 Hz, NH), 5.70 (dd, *J* = 3.3 Hz, J_1 = 5.8 Hz), 5.85 (m, 1H), 5.06−5.22 (m, 6 H), 4.86−4.96 (m, 2 H), 4.72 (s, 1 H), 4.56−4.68 (m, 4 H), 4.34−4.43 (m, 2 H), 4.28−4.31 (m, 1 H), 4.09−4.15 (m, 2 H), 3.88−4.03 (m, 5 H), 3.79−3.82 (m, 2 H), 2.00 (s, 3 H, Ac), 1.99 (s, 3 H, Ac), 1.97 (s, 3 H, Ac), 1.82 (s, 3 H, Ac). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.5, 171.0, 170.6, 169.5, 138.5, 133.8, 129.1, 128.8, 128.7, 128.4 (2 C), 128.2, 128.0, 117.8, 97.7, 80.3, 77.7, 75.6, 74.8, 74.3, 73.1, 72.5, 70.7, 69.9, 68.6, 67.7, 67.6, 61.5, 35.6, 23.3, 21.0, 20.9. ^{31}P NMR ($CDCl_3$, 146 MHz): δ −1.82, −1.94 (diastereomers). IR (NaCl, cm^{-1}): 2942, 1755, 1244, 1028, 959. HRMS (ESI$^+$) calcd for $C_{51}H_{64}N_2O_{17}P^+$ [M + NH_4^+], 1007.3943; found, 1007.3923.

Propyl 6-(2-Acetamido-2-deoxy-α-D-glucopyranosyl)-phosphonato-1-α-D-mannopyranoside (9). The phosphoester compound 7 (60 mg, 0.06 mmol) was dissolved in MeOH (2 mL), and Pd/C (100 mg, 10 wt %) was added. The resulting suspension was placed under an atmosphere of H_2. The reaction mixture was stirred for 24 h. The progress of the reaction was monitored by ^{1}H NMR spectroscopy, which showed the disappearance of Ar-*H* peaks at δ_H = 7.40−7.15 ppm. The reaction mixture was filtered through a polytetrafluoroethylene (0.2 μM) membrane filter, which, upon concentration, gave the fully debenzylated phosphodiester intermediate **8**. Compound **8** was then dissolved in MeOH (1 mL), $NH_2NH_2{\cdot}H_2O$ (0.24 mL) was added to the solution at room temperature, and the reaction mixture was stirred for 8 h. The solvent was then removed under reduced pressure, and the crude product was purified via size-exclusion column chromatography (Sephadex G-25, eluent H_2O) to give compound **9** as a white amorphous powder (20 mg, 67%). $[\alpha]_D^{20}$ 20.4 (*c* 0.49, H_2O). ^{1}H NMR (500 MHz; D_2O): δ 5.39 (dd, 1 H, $J_{1,2}$ = 2.9 Hz, $J_{1,P}$ = 6.7 Hz, H-1, α isomer)[1], 4.78 (s, 1 H), 3.98−4.08 (m, 2 H), 3.85−3.89 (m, 2 H), 3.78−3.80 (m, 2 H), 3.70−3.75 (m, 3 H), 3.67−3.68 (m, 2 H), 3.58−3.60 (m, 1 H), 3.41−3.50 (m, 2 H), 1.98 (s, 3 H, Ac), 1.52−1.56 (m, 2 H), 0.82−0.85 (m, 3 H). ^{13}C NMR (D_2O, 75 MHz): δ 174.9, 100.0, 94.4, 73.3, 71.8, 70.9, 70.8, 70.4, 70.0, 69.8, 66.6, 64.9, 60.6, 54.1, 22.4, 22.2, 10.2. ^{31}P NMR (D_2O, 146 MHz): δ −1.99. IR (NaCl, cm^{-1}): 3337, 1654, 1034. HRMS (ESI$^+$) calcd for $C_{17}H_{33}NO_{14}P^+$ [M + H^+], 506.1639; found, 506.1624.

Ethanethiol (6-*O*-Acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-*O*-[2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-*O*]-2,4-di-*O*-benzyl-1-α-D-mannopyranoside (14). To the solution of compound **13**[9] (2.05 g, 1.35 mmol) in THF (30 mL) was added TBAF/THF (1 M, 4.05 mL, 4.05 mmol) at room temperature. After it was stirred at room temperature for 10 h, the reaction mixture was concentrated in vacuo. The resulting residue was purified via silica gel flash chromatography (EtOAc/Hex, 1/3) to give compound **25** as a yellow oil (1.67 g, 90%, see the Supporting Information for its structure). R_f = 0.24 (EtOAc/Hex, 1/2). $[\alpha]_D^{20}$ 18.3 (*c* 0.6, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.15−7.33 (m, 45 H), 5.31 (s, 1 H), 5.29 (s, 1 H), 4.96 (s, 1H), 4.90 (d, 1 H, *J* = 11.0 Hz), 4.83 (d, 1 H, *J* = 10.6 Hz), 4.55−4.68 (m, 9 H), 4.41−4.52 (m, 4 H), 4.26−4.29 (m, 3 H), 3.95−4.06 (m, 3 H), 3.84−3.93 (m, 9 H), 3.68−3.79 (m, 6 H), 2.46−2.58 (m, 2 H), 1.16−1.21 (m, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 138.7 (2 C), 138.6, 138.5, 138.3, 137.8, 137.7, 128.5, 128.4, 128.3 (3 C), 128.2, 128.1, 127.9, 127.8, 127.7 (2 C), 127.6 (2 C), 127.5, 127.3, 127.2, 127.1, 99.9, 97.7, 81.2, 80.3, 80.2, 79.9, 77.2, 75.7, 75.4, 75.2, 75.1, 75.0, 74.9, 74.8, 74.7, 73.4, 73.1, 72.7, 72.2, 72.0, 71.9, 71.8, 71.1, 71.0, 69.3, 66.8, 62.3, 25.3, 15.0. IR (NaCl, cm^{-1}): 2907, 1453, 1098. HRMS (ESI$^+$) calcd for $C_{83}H_{94}NO_{15}S^+$ [M + NH_4^+], 1376.6344; found, 1376.6349. Compound **25** (100 mg, 0.07 mmol) was dissolved in anhydrous pyridine (2 mL) and Ac_2O (2 mL). After it was stirred at room temperature for 12 h, the solution was diluted with EtOAc and washed with aqueous HCl (1 M), saturated $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified via silica gel column chromatography (EtOAc/Hex, 1/4) to give compound **14** as a yellow oil (98 mg, 95%). R_f = 0.67 (EtOAc/Hex, 1/2). $[\alpha]_D^{20}$ 17.9 (*c* 2.6, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.14−7.33 (m, 45 H), 5.32 (s, 1 H), 5.30 (s, 1 H), 4.98 (s, 1 H), 4.91 (d, 1 H, *J* = 10.9 Hz), 4.82 (d, 1 H, *J* = 10.4 Hz), 4.36−4.66 (m, 14 H), 4.20−4.28 (m, 4 H), 3.97−4.06 (m, 3 H), 3.69−3.89 (m, 13 H), 2.41−2.60 (m, 2 H), 1.97 (s, 3 H, Ac), 1.14−1.19 (m, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.4, 139.1 (2 C), 138.9, 138.8 (2 C), 138.7, 138.2, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2 (2 C), 128.1, 128.0 (2 C), 127.9 (2 C), 127.8, 127.6, 100.4, 97.9, 81.4, 80.8, 80.6, 80.3, 77.7, 76.2, 75.8, 75.7, 75.4, 75.1, 74.8, 73.8, 73.5, 72.8, 72.5, 72.3, 72.1, 71.7, 71.5, 70.3, 69.5, 67.1, 63.9, 25.6, 21.4, 15.3. IR (NaCl, cm^{-1}): 2917, 1453, 1104. HRMS (ESI$^+$) calcd for $C_{85}H_{96}NO_{16}S^+$ [M + NH_4^+], 1418.6450; found, 1418.6461.

Acetamido (6-*O*-Acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-*O*-[2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl-(1→3)-*O*]-(2,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-*O*-[(2,3,4-tri-*O*-benzyl-6-*O*-triisopropylsilyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-*O*-tribenzyl-α-D-mannopyranosyl)-(1→3)-*O*](2,4-di-*O*-benzyl-β-D-mannopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-*O*-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (16). A mixture of donor **14** (470 mg, 0.335 mmol), acceptor **12**[9] (525 mg, 0.234 mmol), and MS (4 Å, 500 mg) in anhydrous Et_2O (6 mL) was stirred at room temperature under argon for 2 h. NIS (75.0 mg, 0.335 mmol) and AgOTf (5.0 mg, 0.112 mmol) were added at −20 °C. The reaction mixture was stirred at −20 °C for 0.5 h before it was quenched with a few drops of triethylamine. The resulting mixture was filtered through Celite. The filtrate was diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified via silica gel chromatography (EtOAc/Hex, 1/4) to give the crude intermediate product **15**. To the suspension of compound **15** in *n*-BuOH (5 mL) was added ethylenediamine (2.5 mL) at room temperature. The mixture was stirred at 90 °C under Ar for 12 h and then concentrated in vacuo. The residue was azeotroped with toluene for 3 times and was then dissolved in anhydrous pyridine (5 mL) and Ac_2O (5 mL). After it was stirred at room temperature for 12 h, the solution was diluted with EtOAc and washed with aqueous HCl (1 M), saturated $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified via silica gel column chromatography (EtOAc/Hex, 1/1) to give compound **26** as a yellow oil (410 mg, ~50% over three steps, see the Supporting Information for its structure). R_f = 0.21 (EtOAc/Hex, 1/1). $[\alpha]_D^{20}$ −4.6 (*c* 0.65, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.05−7.42 (m, 105 H), 6.22 (d, 1 H, *J* = 8.3 Hz, NH), 5.31 (s, 1 H), 5.20 (s, 1 H), 5.02−5.11 (m, 3 H), 4.70−4.94 (m, 10 H), 4.18−4.70 (m, 38 H), 3.35−4.14 (m, 43 H), 3.09−3.11 (m, 2 H), 1.94 (s, 3 H, Ac), 1.88 (s, 3 H, Ac), 1.59 (s, 3 H, Ac), 0.99 (brs, 21 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.0, 170.4 (2 C), 139.1, 139.0, 138.8, 138.7, 138.6, 138.5, 138.4, 138.2, 137.8, 128.7, 128.6, 128.4 (3 C), 128.3 (2 C), 128.2 (2 C), 128.1 (3 C), 127.9, 127.7 (2 C), 127.5, 127.4, 127.0, 102.0, 101.7, 100.0, 99.8, 98.7, 98.3, 98.1, 88.4, 82.7, 80.4, 80.2, 79.6, 79.5, 78.8, 78.6, 78.0, 77.3, 76.4, 75.9, 75.2, 75.1, 74.9, 74.8, 74.7, 74.4, 74.3, 74.2 (2 C), 73.9, 73.7, 72.7, 72.6, 72.3, 72.1, 71.9, 71.6, 71.5, 70.4, 70.0, 69.9, 69.1, 68.9, 68.3, 66.6, 66.2, 64.4, 63.6, 62.6, 55.3, 51.7, 23.4, 23.2, 21.0, 18.1, 12.0. IR (NaCl, cm^{-1}): 2864, 2113, 1454, 1054. HRMS (ESI$^+$) calcd. for $C_{210}H_{240}N_6O_{41}Si^{2+}$ [M + NH_4^+ + H^+], 1765.8351; found, 1765.8373. To a stirred solution of compound **26** (560 mg, 0.16 mmol) in $CHCl_3$ (5 mL) was added 2,6-lutidine (1.1 mL, 8.0 mmol) followed by dropwise addition of thioacetic acid (563 μL, 8.0 mmol).[13] The reaction mixture was stirred at reflux and monitored by TLC. After completion of the reaction, the solvent was evaporated, and the residue was dried under vacuum. The residue was purified by a silica gel flash column chromatography (MeOH/CH_2Cl_2, 1/50) to give compound **16** as a yellow oil (450 mg, 82%). R_f = 0.35 (MeOH/CH_2Cl_2, 1/30). $[\alpha]_D^{20}$ −1.8 (*c* 0.55, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.21−7.52 (m, 105 H), 5.38 (s, 1 H), 5.18−5.30 (m, 3 H), 5.09 (s, 1 H), 4.80−5.00 (m, 9 H), 4.30−4.76 (m, 38 H), 3.50−4.19 (m, 42 H), 3.30−3.48 (m, 2 H), 3.10−3.21 (m, 2 H), 2.00 (s, 3 H, Ac), 1.96 (s, 3 H, Ac), 1.80 (s, 3 H, Ac), 1.64 (s, 3 H, Ac), 1.06 (brs, 21 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.1, 171.0, 170.9, 170.1, 139.4, 139.2, 139.0, 138.8, 138.7 (2 C), 138.5, 138.4, 138.3, 138.2, 138.1, 138.0, 137.8, 137.6, 128.8, 128.7, 128.5, 128.4 (2 C), 128.3 (3 C), 128.2 (2 C), 128.1, 128.0, 127.9, 127.7 (2 C), 127.6 (2 C), 127.5, 127.4 (2 C), 127.3 (2 C), 127.2, 127.1, 127.0, 126.9, 126.8, 126.6, 101.9, 101.7, 100.0, 98.6, 98.3, 98.1, 82.9, 80.4, 80.2, 79.7, 79.5, 78.7, 78.4, 77.3, 75.9, 75.2, 75.1, 74.9, 74.6, 74.3, 74.2, 73.9, 73.6, 73.4 (2 C), 73.1, 73.0, 72.5, 72.3, 72.2, 72.1, 71.9, 71.6, 71.4, 70.4, 70.0, 69.8, 69.1, 68.4, 67.8, 66.7, 66.0, 64.4, 63.6, 62.6, 55.4, 52.7, 23.4, 23.3, 23.0, 20.9, 18.1, 12.0. IR (NaCl, cm^{-1}): 2933, 1664, 1053. HRMS (ESI$^+$) calcd for $C_{212}H_{244}N_4O_{42}Si^{2+}$ [M + NH_4^+ + H^+], 1773.8451; found, 1773.8427.

Acetamido (2,3,4-Tri-*O*-benzyl-6-dibenzylphosphonato-α-D-mannopyranosyl)-(1→6)-*O*-[(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-*O*]-(2,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-*O*-[(2,3,4-tri-*O*-benzyl-6-*O*-triisopropylsilyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-*O*]-(2,4-di-*O*-benzyl-β-D-mannopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-*O*-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (17). Compound **16** (440 mg, 0.125 mmol) was dissolved in MeOH/CH_2Cl_2 (2/1, 10 mL) at room temperature. NaOMe in MeOH was added until the pH of the reaction mixture reached 10. After it was stirred at room temperature for 12 h, the solution was neutralized with ion-exchange resin (H^+), filtered, and concentrated in vacuo. The residue was purified via silica gel chromatography (MeOH/CH_2Cl_2, 1/50) to give the desired product **27** as a yellow oil (420 mg, >95%, see the Supporting Information for its structure). R_f = 0.35 (MeOH/CH_2Cl_2, 1/30). $[\alpha]_D^{20}$ 28.0 (*c* 0.5, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.01−7.45 (m, 105 H), 5.32 (s, 1 H), 5.20 (s, 1 H), 5.12 (s, 1 H), 5.03 (s, 1 H), 4.92−4.95 (m, 2 H), 4.76−4.88 (m, 6 H), 4.64−4.71 (m, 7 H), 4.30−4.60 (m, 26 H), 4.10−4.30 (m, 6 H), 3.83−4.10 (m, 15 H), 3.40−3.82 (m, 28 H), 3.31−3.37 (m, 2 H), 3.07−3.15 (m, 2 H), 1.89 (s, 3 H, Ac), 1.74 (s, 3 H, Ac), 1.57 (s, 3 H, Ac), 1.00 (m, 21 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.7, 169.6, 168.8, 137.8 (2 C), 137.6, 137.4, 137.3, 137.2, 137.1 (2 C), 136.9 (2 C), 136.7, 136.6, 136.5, 127.4 (2 C), 127.2, 127.1, 126.9 (2 C), 126.8, 126.7 (2 C), 126.5 (2 C), 126.4, 126.3 (2 C), 126.2, 126.1 (2 C), 125.9, 125.8, 125.7, 125.6, 125.4, 100.6, 100.3, 98.5, 97.2, 96.6, 95.5, 81.3, 79.1, 78.8, 78.7, 78.1, 77.8, 77.5, 77.2, 76.0, 74.6, 74.4, 73.9, 73.7, 73.5, 73.4, 73.3, 73.1, 72.9, 72.5, 72.3, 72.1, 72.0, 71.7, 71.1, 71.0, 70.7, 70.6, 70.4, 70.3, 70.2, 69.0, 68.4, 67.8, 66.9, 66.4, 64.8, 64.6, 61.2, 60.9, 54.2, 51.4, 22.1, 21.7, 16.7, 10.7. IR (NaCl, cm^{-1}): 2917, 1659, 1454, 1051. HRMS (ESI$^+$) calcd for $C_{210}H_{242}N_4O_{41}Si^{2+}$ [M + NH_4^+ + H^+], 1752.8398; found, 1752.8414. A mixture of compound **27** (72 mg, 0.02 mmol), 1*H*-tetrazole (222 μL, 0.45 M in CH_3CN, 0.1 mmol), and 4 Å molecular sieves (50 mg) in anhydrous CH_2Cl_2 (3 mL) was stirred at room temperature under Ar for 2 h. Dibenzyl *N*,*N*-diisopropylphosphoramidite (27 μL, 0.08 mmol) was then added, and the reaction mixture was stirred at room temperature for another 3 h. Subsequently, *m*CPBA (18.0 mg, 0.08 mmol) was added; the solution was stirred at room temperature for another 1 h. The reaction was quenched by the addition of a few drops of triethylamine and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by silica gel flash chromatography (MeOH/CH_2Cl_2, 1/50) to give product **17** as a yellow oil (74 mg, 95%). R_f = 0.33 (MeOH/CH_2Cl_2, 1/30). $[\alpha]_D^{20}$ −1.8 (*c* 0.55, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.00−7.45 (m, 115 H), 5.37 (s, 1 H), 5.32 (s, 1 H), 4.60−5.23 (m, 22 H), 4.15−4.60 (m, 31 H), 3.35−4.15 (m, 45 H), 3.15−3.20 (m, 2 H), 1.93 (s, 3 H, Ac), 1.78 (s, 3 H, Ac), 1.63 (s, 3 H, Ac), 1.01 (brs, 21 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.3, 171.3, 170.6, 139.2, 139.1, 138.9, 138.6, 138.5, 138.3, 138.2, 138.0, 137.9, 137.8, 135.7, 128.7, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.5, 127.2, 126.9, 126.6, 102.0, 101.6, 100.0, 99.7, 98.5, 98.3, 98.2, 82.8, 80.4, 80.2, 79.9, 79.4, 78.8, 78.5, 77.3, 75.8, 75.2, 75.0, 74.8, 74.6, 74.2, 73.9, 73.4, 73.0, 72.5, 72.3, 72.1, 72.0, 71.5, 71.4, 70.7, 70.4, 69.8, 69.5, 69.4, 69.0, 68.3, 67.7, 66.8, 66.5, 66.1, 29.7, 23.4, 23.0, 18.0, 12.0. ^{31}P NMR ($CDCl_3$, 146 MHz): δ −1.06. IR (NaCl, cm^{-1}): 2923, 1659, 1454, 1052. HRMS (ESI$^+$) calcd for $C_{224}H_{255}N_4O_{44}PSi^{2+}$ [M + NH_4^+ + H^+], 1882.8700; found, 1882.8684.

Acetamido (6-Phosphato-α-D-mannopyranosyl)-(1→6)-*O*-[α-D-mannopyranosyl-(1→6)-*O*]-(α-D-mannopyranosyl)-(1→3)-*O*-[6-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-phosphonato-(α-D-mannopyranosyl)-(1→2)-*O*-(α-D-mannopyranosyl)-(1→3)-*O*]-(β-D-mannopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranoside (20). To the solution of compound **17** (68 mg, 0.018 mmol) in THF (2 mL) was added TBAF/THF (1 M, 363 μL, 0.363 mmol) at room temperature. After it was stirred at room temperature for 10 h, the reaction mixture was concentrated in vacuo. The resulting residue was purified via silica gel flash chromatography (MeOH/CH_2Cl_2, 1/50) to give compound **28** as an oil (64 mg, >95%, see the Supporting Information for its structure). R_f = 0.33 (MeOH/CH_2Cl_2, 1/30). $[\alpha]_D^{20}$ −2.3 (*c* 0.43, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.99−7.44 (m, 115 H), 5.24 (s, 1 H), 5.21 (s, 1 H), 4.71−5.10 (m, 17 H), 4.18−4.70 (m, 35 H), 3.40−4.15 (m, 45 H), 3.28−3.33 (m, 2 H), 3.06−3.13 (m, 2 H), 1.89 (s, 3 H, Ac), 1.72 (s, 3 H, Ac), 1.57 (m, 3 H, Ac). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 172.1, 170.9, 170.1, 139.4, 139.1, 138.7, 138.6, 138.5, 138.4, 138.2, 138.0, 137.9, 137.7, 136.0, 135.9, 135.8, 128.8, 128.7, 128.5, 128.4 (2 C), 128.3, 128.2, 128.1, 128.0 (2 C), 127.9 (2 C), 127.8, 127.6, 127.5, 127.4, 127.3, 127.2, 127.1, 127.0, 101.8, 101.1, 100.0, 99.6, 98.2, 98.0, 82.4, 80.2, 80.0, 79.9, 79.8, 79.5, 78.6, 78.3, 77.2, 75.9, 75.8, 75.2, 75.1, 75.0, 74.6, 74.2, 74.1, 73.6, 73.4 (2 C), 73.3, 73.0, 72.9, 72.4, 72.2, 72.1, 71.9, 71.3, 70.3, 69.3, 69.2, 69.1, 69.0, 66.6, 62.1, 29.7, 23.4, 23.0. ^{31}P NMR ($CDCl_3$, 146 MHz): δ −0.77. IR: υ 2925, 1659, 1454, 1027 cm^{-1}. HRMS (ESI$^+$) calcd for $C_{215}H_{235}N_4O_{44}P^{2+}$ [M + NH_4^+ + H^+], 1804.8033; found, 1804.7988. To a mixture of compounds **28** (66 mg, 0.018 mmol) and **4** (21 mg, 0.036 mmol) in CH_2Cl_2 (4 mL) under an atmosphere of nitrogen was added 1*H*-tetrazole solution (176 μL, 0.45 M solution in acetonitrile). The reaction mixture was stirred at room temperature for 12 h and cooled to −40 °C, and *t*-BuO_2H (92 μL, 70% solution in water) was added and stirred for another 2 h. The solvent was removed under reduced pressure, and the resulting residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2, 1/50). This product was further purified by size-exclusion column chromatography over Sephadex LH-20 (MeOH/CH_2Cl_2, 1/1) to give compound **18** as a yellow oil (60 mg, >95%). R_f = 0.37 (MeOH/CH_2Cl_2, 1/30). $[\alpha]_D^{20}$ 3.57 (*c* 1.12, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.03−7.28 (m, 120 H), 5.95 (d, 1 H, *J* = 6.0 Hz, NH), 5.68 (dd, 1 H, $J_{1,2}$ = 3.2 Hz, $J_{1,P}$ = 5.7 Hz, H-1, α isomer), 5.29 (brs, 2 H), 5.10−5.15 (m, 5 H), 4.10−5.09 (m, 11 H), 4.74−4.82 (m, 5 H), 4.57−4.71 (m, 6 H), 4.18−4.55 (m, 31 H), 3.82−4.32 (m, 22 H), 3.35−3.81 (m, 26 H), 3.25−3.33 (m, 1 H), 3.16−3.20 (m, 1 H), 3.11−3.14 (m, 1 H), 1.94 (s, 3 H, Ac), 1.93 (s, 3 H, Ac), 1.89 (s, 3 H, Ac), 1.77 (s, 3 H, Ac), 1.71 (s, 3 H, Ac), 1.70 (s, 3 H, Ac), 1.57 (s, 3 H, Ac). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.0, 170.0, 169.9, 169.4, 169.1, 168.1, 138.2, 138.1, 137.7, 137.6, 137.4, 137.2, 137.1, 136.8, 134.9, 134.8, 134.1, 128.7, 127.8, 127.6, 127.4, 127.3, 127.2, 127.1, 127.0 (2 C), 126.9, 126.8, 126.7, 126.6, 126.5, 126.4, 126.3, 126.2, 126.1, 126.0, 125.8, 100.9, 100.2, 99.0, 98.8, 98.2, 97.4, 97.1, 95.3, 79.2, 78.9, 78.7, 78.4, 77.8, 77.5, 76.3, 75.0, 74.8, 74.1, 73.8, 73.6, 73.3, 72.7, 72.5, 72.3, 72.0, 71.8, 71.5, 71.0, 70.9, 70.8, 70.3, 69.4, 69.2, 68.9, 68.6, 68.3, 68.1, 67.6, 66.2, 65.3, 65.1, 60.0, 54.7, 51.7, 50.8, 28.7, 22.5, 22.4, 22.0, 21.8, 19.6, 19.5. ^{31}P NMR ($CDCl_3$, 146 MHz): δ −0.98, −1.95. IR (NaCl, cm^{-1}): 2923, 1748, 1665, 1454, 1047. HRMS (ESI$^+$) calcd for $C_{236}H_{261}N_5O_{55}P_2^{2+}$ [M + NH_4^+ + H^+], 2054.3655; found, 2054.3630. Phosphoester **18** (36 mg, 0.009 mmol) was dissolved in MeOH (2 mL), and Pd/C (50 mg, 10 wt %) was added. The resulting suspension was placed under an atmosphere of H_2. The reaction mixture was stirred at room temperature under H_2 atmosphere for 24 h. The progress of the reaction was monitored by ^{1}H NMR spectroscopy, which showed the disappearance of Ar-*H* peaks at δ_H = 7.40−7.15 ppm. The reaction mixture was filtered through a polytetrafluoroethylene (0.2 μM) membrane filter to give the fully debenzylated phosphoester intermediate **19**. $NH_2NH_2 \cdot H_2O$ (0.24 mL) was added to a solution of **19** in MeOH (1 mL), and the reaction mixture was stirred for 8 h. The solvent was removed under reduced pressure, and the crude product was purified by size-exclusion column chromatography over Sephadex G-25 (eluent: H_2O) to give compound **20** as a white amorphous powder upon freeze drying (13 mg, 81%). $[\alpha]_D^{20}$ 15 (c 0.4, H_2O). ^1H NMR (600 MHz; D_2O): δ 5.33 (dd, 1 H, $J_{1,2}$ = 3.2 Hz, $J_{1,P}$ = 7.1 Hz, H-1, α isomer), 5.22 (s, 1 H), 5.07 (s, 1 H), 4.89 (d, 1 H, J = 9.7 Hz), 4.86 (s, 1 H), 4.79 (s, 1 H), 4.75 (s, 1 H), 4.65 (s, 1 H), 4.44−4.47 (m, 1 H), 4.05−4.15 (m, 1 H), 3.98−4.04 (m, 1 H), 3.40−3.95 (m, 52 H), 1.92 (s, 3 H, Ac), 1.91 (s, 3 H, Ac), 1.84 (s, 3 H, Ac), 1.75 (s, 3 H, Ac). ^{13}C NMR (D_2O, 125 MHz): δ 181.4, 174.9, 174.7, 174.5, 102.3, 101.1, 100.8, 100.7, 100.0, 99.8, 99.5, 93.9, 93.8, 80.8, 79.2 (2 C), 79.1, 78.5, 78.2, 76.1, 74.4, 74.2, 73.9, 73.5, 73.3, 73.0, 72.6, 72.2, 72.0, 71.9, 71.7, 70.3 (2 C), 70.2 (2 C), 70.1, 70.0, 69.8, 69.4, 69.3, 66.9, 66.5, 66.4, 66.1, 65.9, 65.7, 65.6, 65.1, 62.7, 61.0, 60.7, 60.2, 59.8, 59.7, 55.0, 53.9, 53.8, 53.7, 23.2, 22.1, 22.0, 21.9, 21.8, 21.6. ^{31}P NMR (D_2O, 146 MHz): δ −0.72, 4.76. IR (NaCl, cm^{-1}): 3292, 1061. HRMS (ESI$^-$) calcd for $C_{62}H_{104}N_4O_{52}P^{2-}$ [M − 2H$^+$], 899.2546; found, 899.2545.

Acetamido (2,3,4-Tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-*O*-[2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl-(1→3)-*O*]-(2,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-*O*-[(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-*O*-tribenzyl-α-D-mannopyranosyl)-(1→3)-*O*]-(2,4-di-*O*-benzyl-β-D-mannopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-*O*-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (21). To the solution of compound **27** (68 mg, 0.02 mmol, derived from **16**) in THF (2 mL) was added TBAF/THF (1 M, 100 μL, 0.1 mmol) at room temperature. After it was stirred at room temperature for 10 h, the reaction mixture was concentrated in vacuo and purified via silica gel flash chromatography (MeOH/CH_2Cl_2, 1/50) to give compound **21** as a yellow oil (64 mg, >95%). R_f = 0.35 (MeOH/CH_2Cl_2, 1/30). $[\alpha]_D^{20}$ −4.5 (c 0.44, CH_2Cl_2). ^1H NMR ($CDCl_3$, 400 MHz): δ 7.00−7.50 (m, 105 H), 5.28 (s, 1 H), 5.20 (s, 1 H), 5.11 (s, 1 H), 5.08 (s, 1 H), 4.93−5.05 (m, 4 H), 4.78−4.88 (m, 6 H), 4.20−4.73 (m, 35 H), 4.09−4.17 (m, 3 H), 3.83−4.03 (m, 14 H), 3.42−3.77 (m, 28 H), 3.28−3.34 (m, 2 H), 3.07−3.14 (m, 2 H), 1.89 (s, 3 H, Ac), 1.72 (s, 3 H, Ac), 1.56 (s, 3 H, Ac). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 172.0, 170.9, 170.0, 139.2, 139.1, 138.7, 138.6, 138.5 (2 C), 138.4, 138.3, 138.2, 138.0 (2 C), 137.9, 137.8, 137.7, 128.7, 128.6, 128.5, 128.4, 128.3 (2 C), 128.2, 128.1, 127.8, 127.6, 127.5, 127.4 (2 C), 127.2, 127.1, 127.0, 126.8, 101.8, 101.1, 100.0, 99.8, 97.9, 97.8, 82.3, 80.2, 80.0, 79.8, 79.4, 79.1, 78.7, 78.5, 77.2, 75.9, 75.7, 75.3, 75.0, 74.9, 74.7, 74.6, 74.3, 74.1, 73.6, 73.4, 73.3, 73.0, 72.9, 72.4, 72.2, 72.0, 71.8, 71.7, 71.6, 70.3, 69.5, 69.1, 68.2, 67.7, 66.1, 62.2, 55.4, 52.7, 29.7, 23.4, 23.0. IR (NaCl, cm^{-1}): 2924, 1654, 1051. HRMS (ESI) calcd for $C_{201}H_{222}N_4O_{41}^{2+}$ [M + NH_4^+ + H$^+$], 1674.7732; found, 1674.7770.

Acetamido 6-[Benzyl-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-phosphonato]-2,3,4-tri-*O*-benzyl-(α-D-mannopyranosyl)-(1→6)-*O*-[(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-*O*]-(2,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-*O*-{6-[benzyl-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-phosphonato-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-*O*}-(2,4-di-*O*-benzyl-β-D-mannopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-*O*-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (22). To a mixture of compounds **21** (65 mg, 0.02 mmol) and **4** (57 mg, 0.1 mmol) in CH_2Cl_2 (4 mL) under an atmosphere of nitrogen was added 1*H*-tetrazole solution (260 μL, 0.45 M solution in acetonitrile). The reaction mixture was stirred at room temperature for 12 h and then cooled to −40 °C, and t-BuO_2H (27 μL, 70% solution in water) was added and stirred for another 2 h. The solvents were then removed under reduced pressure, and the residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2, 1/50). The resulting product was further purified by size-exclusion column chromatography over Sephadex LH-20 (MeOH/CH_2Cl_2, 1/1) to give compound **22** (75 mg, >95%) as an oil. R_f = 0.36 (MeOH/CH_2Cl_2, 1/30). $[\alpha]_D^{20}$ 9.6 (c 2.7, CH_2Cl_2). ^1H NMR ($CDCl_3$, 500 MHz): δ 7.78 −8.02 (4 H), 7.03−7.49 (m, 111 H), 6.28 (d, 1 H, J = 6.8 Hz), 6.05 (d, 1 H, J = 8.6 Hz), 5.69 (s, 2 H), 3.31−5.69 (m, 114 H), 1.98 (s, 3 H, Ac), 1.95 (s, 3 H, Ac), 1.94 (s, 3 H, Ac), 1.93 (s, 3 H, Ac), 1.89 (s, 3 H, Ac), 1.85 (s, 3 H, Ac), 1.80 (s, 3 H, Ac), 1.72 (s, 3 H, Ac), 1.67 (s, 3 H, Ac), 1.64 (s, 3 H, Ac), 1.57 (s, 3 H, Ac). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.4, 170.9, 169.7, 169.6, 169.4, 169.3, 169.0, 168.9, 168.8, 168.0, 167.9, 138.1, 137.9, 137.5, 137.4 (2 C), 137.3, 137.1, 137.0, 136.9, 136.8, 136.7, 136.5, 134.2, 134,1 (2 C), 134.0, 127.6, 127.5, 127.3, 127.2, 127.1, 127.0 (2 C), 126.8 (2 C), 126.7, 126.6, 126.5, 126.4, 126.3 (2 C), 126.2, 126.1, 126.0, 125.9, 125.8, 125.6, 125.4, 100.6, 100.0, 98.7, 98.1, 98.0, 97.3, 95.3, 95.2, 95.1, 81.6, 81.0 (2 C), 78.7, 78.6, 78.2, 77.4, 77.3, 76.1, 74.7, 74.6, 74.1, 74.0, 73.9, 73.8, 73.7, 73.7, 73.6, 73.5, 73.4, 73.3, 73.1, 73.0, 72.4, 72.2, 72.1, 71.9, 71.5, 71.3, 71.2, 71.0, 70.8 (2 C), 70.6, 70.1, 69.8, 69.3, 69.2, 69.1, 68.7, 68.6, 68.4, 68.3, 67.8, 67.3, 66.5, 66.2, 66.0, 65.8, 65.6, 60.0, 59.9, 54.3, 51.5, 50.5, 50.3 (2 C), 50.2, 22.2 (2 C), 21.8, 21.6 (2 C), 19.5, 19.4, 19.3 (2 C). ^{31}P NMR ($CDCl_3$, 146 MHz): δ −1.99. IR (NaCl, cm^{-1}): 3286, 2939, 1751, 1666, 1041. HRMS (ESI$^+$) calcd for $C_{243}H_{273}N_6O_{63}P_2^{2+}$ [M + NH_4^+ + H$^+$], 2173.8977; found, 2173.8948.

Acetamido 6-(2-Acetamido-2-deoxy-α-D-glucopyranosyl)-phosphonato-(α-D-mannopyranosyl)-(1→6)-*O*-[α-D-mannopyranosyl-(1→6)]-(α-D-mannopyranosyl)-(1→3)-*O*-[6-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-phosphonato-(α-D-mannopyranosyl)-(1→2)-*O*-(α-D-mannopyranosyl)-(1→3)-*O*]-(β-D-mannopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranoside (24). Compound **22** (50 mg, 0.01 mmol) was dissolved in MeOH (2 mL), and Pd/C (50 mg, 10 wt %) was added. The resulting suspension was placed under an atmosphere of H_2. The reaction mixture was stirred at room temperature for 24 h. The progress of the reaction was monitored by ^1H NMR spectroscopy, which showed the disappearance of Ar-*H* peaks at δ_H = 7.40−7.15 ppm. The reaction mixture was filtered through a polytetrafluoroethylene (0.2 μM) membrane filter to give the fully debenzylated intermediate **23**. $NH_2NH_2 \cdot H_2O$ (0.24 mL) was added to the solution of compound **23** in MeOH (1 mL), and the reaction mixture was stirred at room temperature for 8 h. The solvent was removed under reduced pressure, and the crude product was purified by size-exclusion column chromatography over Sephadex G-25 (eluent: H_2O) to give **24** as a white amorphous powder upon freeze drying (20 mg, 87%). $[\alpha]_D^{20}$ 23 (c 0.65, H_2O). ^1H NMR (500 MHz; D_2O): δ 5.40−5.41 (m, 2 H), 5.30 (s, 1 H), 5.15 (s, 1 H), 4.97 (d, 1 H, J = 9.8 Hz), 4.94 (s, 1 H), 4.86 (s, 1 H), 4.82 (s, 1 H), 4.52−4.55 (m, 1 H), 4.17 (s, 1 H), 3.46−4.20 (m, 60 H), 2.00 (s, 9 H, Ac), 1.93 (s, 6 H, Ac). ^{13}C NMR (D_2O, 75 MHz): δ 175.3, 175.1, 175.0, 174.9, 102.8, 101.5 (2 C), 101.2, 100.4 (2 C), 99.9, 94.5, 94.4 (2 C), 79.6, 79.0, 78.6, 76.5, 76.5, 74.8, 74.4, 74.0, 73.7, 73.4, 73.3, 72.4, 72.1, 71.0, 70.8, 70.7, 69.9, 69.8, 67.3, 66.8, 66.4, 66.1, 65.5, 64.9, 61.5, 61.2, 60.7, 60.6, 60.2, 55.4, 54.0 (2 C), 22.5, 22.4, 22.3 (2 C). ^{31}P NMR (D_2O, 146 MHz): δ −1.23, −1.31. IR (NaCl, cm^{-1}): 3324, 1646, 1031. HRMS (ESI$^-$) calcd for $C_{70}H_{117}N_5O_{57}P_2^{2-}$ [M − 2H$^+$], 1000.7948; found, 1000.7927.

ASSOCIATED CONTENT

Supporting Information

General experimental information, structures of all intermediates, and NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: guc11@psu.edu.

ACKNOWLEDGMENTS

We gratefully acknowledge The Pennsylvania State University for financial support of this work.

REFERENCES

(1) Ghosh, P.; Dahms, N. M.; Kornfeld, S. *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 202−212.

(2) (a) Hasilik, A.; Kelin, U.; Waheed, A.; Strecker, G.; von Figura, K. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 7074−7078. (b) Kornfeld, S.; Bao, M.; Brewer, K.; Noll, C.; Canfield, W. *J. Biol. Chem.* **1999**, *274*, 32778−32785.

(3) (a) Srivastava, O. P.; Hindsgaul, O. *J. Org. Chem.* **1987**, *52*, 2869−2875. (b) Franzyk, H.; Christensen, M. K.; Joergensen, R. M.; Meldal, M.; Cordes, H.; Mouritsen, S.; Bock, K. *Bioorg. Med. Chem.* **1997**, *5*, 21−40. (c) Berkowitz, D. B.; Maiti, G.; Charette, B. D.; Dreis, C. D.; MacDonald, R. G. *Org. Lett.* **2004**, *6*, 4921−4924. (d) Fei, X.; Connelly, C. M.; MacDonald, R. G.; Berkowitz, D. B. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3085−3089.

(4) (a) Song, X.; Lasanajak, Y.; Olson, L. J.; Boonen, M.; Dahms, N. M.; Kornfeld, S.; Cummings, R. D.; Smith, D. F. *J. Biol. Chem.* **2009**, *284*, 35201−35214. (b) Bohnsack, R. N.; Song, X.; Olson, L. J.; Kudo, M.; Gotschall, R. R.; Canfield, W. M.; Cummings, R. D.; Smith, D. F.; Dahms, N. M. *J. Biol. Chem.* **2009**, *284*, 35215−35226.

(5) Chavez, C. A.; Bohnsack, R. N.; Kudo, M.; Gotschall, R. R.; Canfield, W. M.; Dahms, N. M. *Biochemistry* **2007**, *46*, 12604−12617.

(6) Varki, A.; Kornfeld, S. *J. Biol. Chem.* **1980**, *255*, 10847−10858.

(7) (a) Totani, K.; Ihara, Y.; Matsuo, I.; Ito, Y. *J. Am. Chem. Soc.* **2008**, *130*, 2101−2107. (b) Nagorny, P.; Fasching, B.; Li, X.; Chen, G.; Aussedat, B.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2009**, *131*, 5792−5799. (c) Huang, W.; Li, C.; Li, B.; Umekawa, M.; Yamamoto, K.; Zhang, X.; Wang, L.-X. *J. Am. Chem. Soc.* **2009**, *131*, 2214−2223. (d) Piontek, C.; Ring, P.; Harjes, O.; Heinlein, C.; Mezzato, S.; Lombana, N.; Pöhner, C.; Püttner, M.; Silva, D. V.; Martin, A.; Schmid, F. X.; Unverzagt, C. *Angew. Chem., Int. Ed.* **2009**, *48*, 1936−1940.

(8) (a) Xue, J.; Guo, Z. *J. Am. Chem. Soc.* **2003**, *125*, 16334−16339. (b) Jayaprakash, K. N.; Lu, J.; Fraser-Reid, B. *Angew. Chem., Int. Ed.* **2005**, *44*, 5894−5898. (c) Liu, X.; Kwon, Y.-U.; Seeberger, P. H. *J. Am. Chem. Soc.* **2005**, *127*, 5004−5005.

(9) Liu, Y.; Chan, Y. M.; Wu, J.; Chen, C.; Benesi, A.; Hu, J.; Wang, Y.; Chen, G. *ChemBioChem* **2011**, *12*, 685−690.

(10) Majumdar, D.; Elsayed, G. A.; Buskas, T.; Boons, G. J. *J. Org. Chem.* **2005**, *70*, 1691−1697.

(11) Shangguan, N.; Katukojvala, S.; Greenberg, R.; Williams, L. J. *J. Am. Chem. Soc.* **2003**, *125*, 7754−7755.

(12) Ali, A.; Vishwakarma, R. A. *Tetrahedron Lett.* **2000**, *66*, 4357−4369.

(13) Shangguan, N.; Katukojvala, S.; Greenberg, R.; Williams, L. J. *J. Am. Chem. Soc.* **2003**, *125*, 7754−7755.