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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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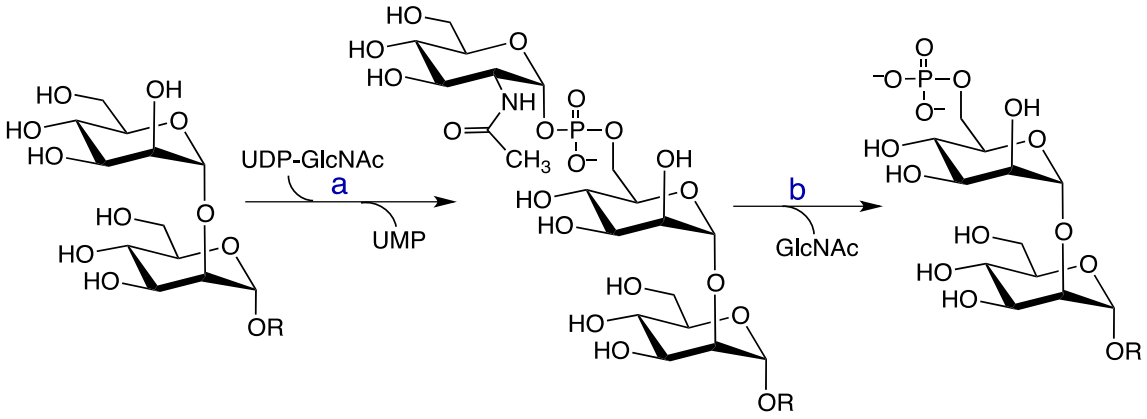


Scheme 1. Enzymatic insertion of phosphate into αMan residues of Golgi glycoproteins destined for transport to lysosomes. Step a requires UDP-GlcNAc:glycoprotein GlcNAc 1P phosphotransferase, which cleaves the phosphoanhydride bond in UDP-GlcNAc and transfers αGlcNAc 1P to O-6 of a terminal αMan residue, 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.

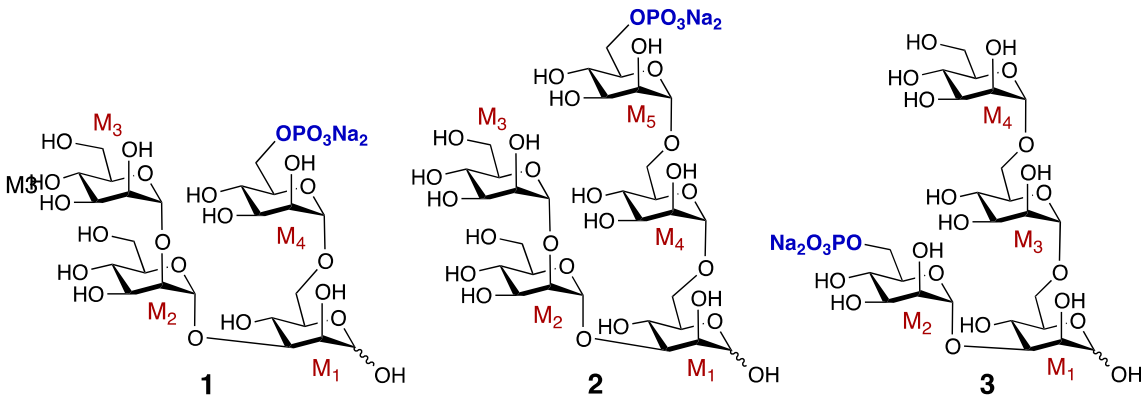
make both their isolation from natural sources and their chemical synthesis challenging [9]. Indeed, the extent of the chemical diversity of the M6P glycoproteome is currently unknown due to difficulties in separating mixtures of these modified proteins and in quantifying their constituents [1,9e]. M6P-containing 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.