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Synthesis of novel bivalent mimetic ligands for mannose-6-phosphate receptors

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ABSTRACT

Mannose-6-phosphate (M6P)-containing N-linked glycans are essential signaling molecules for sorting hydrolases in eukaryotic cells. Their receptors, especially the cation-independent M6P receptors (CI-MPRs), have emerged as promising protein targets for targeted drug delivery for the treatment of lysosomal storage disease and liver fibrosis. In this Letter, we describe the design and synthesis of novel bivalent mimetic ligands for CI-MPRs. We report that for the first time, a newly-discovered binding motif, GlcNAc-M6P, has been incorporated in mimetic ligands. M6P- and GlcNAc-M6P-containing building blocks, equipped with NH_2 and CO_2H handles, have been prepared and assembled with an ornithine linker through amide coupling reactions. Efficient global deprotection protocols have also been developed which have been showcased in the synthesis of our novel bivalent mimetic ligands.

Mannose-6-phosphate (M6P)-containing N-glycans are a class of oligosaccharide-based signaling molecules that plays an essential role in intracellular transport of hydrolases into the endosomal/lysosomal compartments in eukaryotic cells (Fig. 1).[1] Two major types of M6P receptors (MPRs), cation-dependent (CD-MPRs) and cation-independent (CI-MPRs), are responsible for recognizing M6P moieties of hydrolases, and facilitating their transport to the lysosome.[2] Additionally, CI-MPR, also known as insulin-like growth factor 2 receptor (IGF2R), functions as a membrane-associated receptor that is responsible for the internalization of a number of exogenous proteins in the extracellular matrix. Defects in the M6P and MPR mediated sorting system are known to cause lysosomal storage diseases (LSD). Currently, enzyme replacement therapy (ERT), via the infusion of enzymes produced in vitro, is the only viable method for treating certain LSDs.[3] However, this method suffers from low efficiency and high cost due to the poor uptake of the infused enzymes that lack the critical targeting elements. To improve ERT and develop other MPR-targeted drug delivery strategies, it is crucial that we better understand the M6P-coded sorting system.[4]

The ability to elucidate this M6P/MPR mediated recognition/transport system has been hampered by the heterogeneity of M6P N-glycans and the complex multivalency of their binding towards MPRs.[5] Recently, we successfully developed the first chemical synthesis of N-glycans carrying well-defined M6P motifs for in vitro uptake studies.[6] The total synthesis of the naturally occurring M6P carrying N-glycans provides valuable insight in the MPR system in their native format and these complex N-glycans are well-suited for biomedical applications. There is, however, a formidable cost associated with the total synthesis of these compounds. An alternative strategy is to synthesize simpler model scaffolds containing the key binding motifs from these N-glycans. These scaffolds could exhibit comparable or even enhanced binding ability, when compared to the naturally occurring N-glycans. Herein, we report our efforts to design and synthesize novel bivalent mimetic ligands for MPR-targeted biomedical applications. For the first time, a newly discovered binding motif, GlcNAc-M6P, has been incorporated in mimetic ligands.

The concept of developing simplified mimetic ligands based on the M6P motif has been pioneered by the groups of Hindsgaul, Bock, Berkowitz and MacDonald.[7] Although some promising results were obtained, truly unimolecular multivalent ligands that can span the long distance between the two known M6P binding domains 3 and 9 in CI-MPRs have not be well-established. Recently, the Dahms laboratory made a key discovery by identifying *N*-acetylglucosamine M6P (GlcNAc-M6P), a biosynthesis precursor of M6P, as a new binding element to CI-MPRs, at a new binding domain 5 located between domains 3 and 9 (Fig. 1).[8] Although this structural motif has been long found in many N-glycoproteins, their binding interaction with CI-MPRs was not studied for several decades. We speculate that this was due to its relatively weaker binding to CI-MPRs and difficulty in obtaining homogenous N-glycan carrying this motif. Different from the previously-reported mimetic ligands, we sought to incorporate the newly-discovered binding element, GlcNAc-M6P, in our ligand

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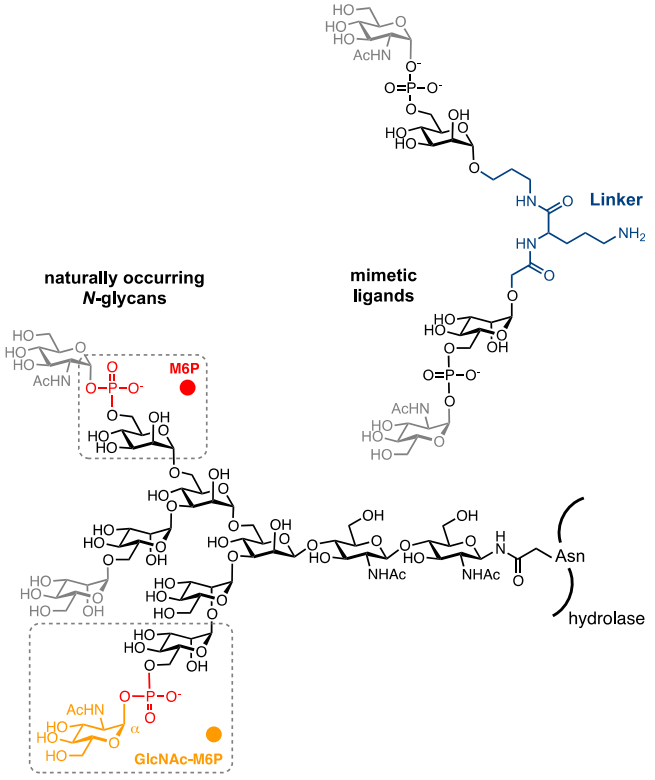
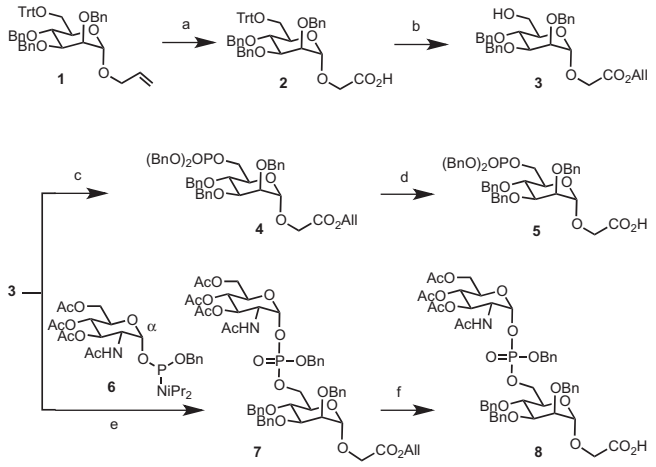


Figure 1. Representative structures of naturally occurring M6P-containing N-glycans and mimetic ligands for CI-MPRs.

design. We expect that unimolecular multivalent ligands carrying suitable M6P and/or GlcNAc-M6P elements are uniquely suited to achieve the elusive high-affinity binding toward the monomeric unit of CI-MPR. To pursue suitable multivalent ligands, we need to prepare and test a series of multivalent ligands that M6P and GlcNAc-M6P moieties are presented on linker molecules with varying lengths and spatial arrangements. Due to the challenging physical and chemical properties of these phosphorylated sugar compounds, their synthesis routes need to be carefully orchestrated to accommodate the labile phosphoester moieties, so as to avoid any difficult purification steps and to ensure that these synthetic routes are amenable to library synthesis.[9]

In contrast to the late-stage phosphorylation strategy used in our previous total synthesis routes, we adopted a more modular synthetic strategy for these mimetic ligands. Our strategy is outlined as follows. First, as shown in Schemes 1 and 2, protected M6P and GlcNAc-M6P building blocks bearing NH_2 or CO_2H handles, were prepared as standard cassette-type building blocks. Second, these building blocks were then coupled with the linker molecules through amide bonds to provide the desired ligands. For the first-generation bivalent ligands, amino acid ornithine was used as a linker to tether two building blocks on its α-NH_2 and CO_2H ends; the remaining δ-NH_2 group on side chain can be potentially used for conjugation with biophysical tags.

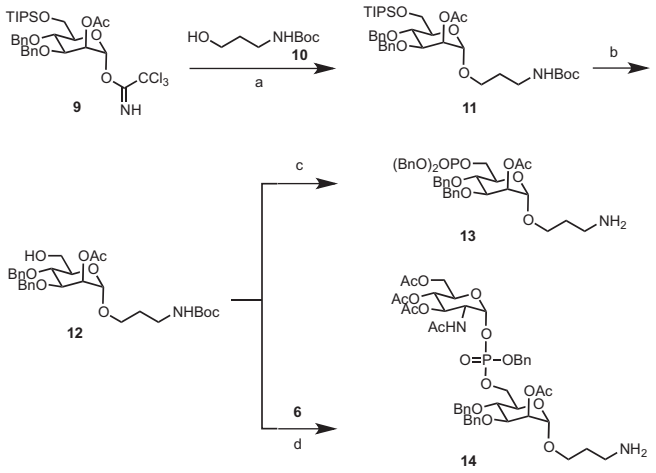
As shown in Scheme 1, building blocks **5** and **8** bearing a CO_2H handle were first prepared. *O*Bn-protected phosphoester linkages are labile under nucleophilic conditions but reasonably stable under acidic conditions. Accordingly, the allyl ester group, was chosen as the protecting group for the intermediates **4** and **7**. The allyl group of the α-*O*-allyl mannoside **1**[10] was first cleaved using $RuCl_3$/$NaIO_4$ to furnish mannosyl glycolic acid **2**. The CO_2H group of **2** was then protected with allyl bromide to give an allyl ester intermediate, whose trityl group was subsequently removed by $FeCl_3{\cdot}6H_2O$ to give **3**. Installation of the *O*Bn-protected phosphoester on the 6-OH



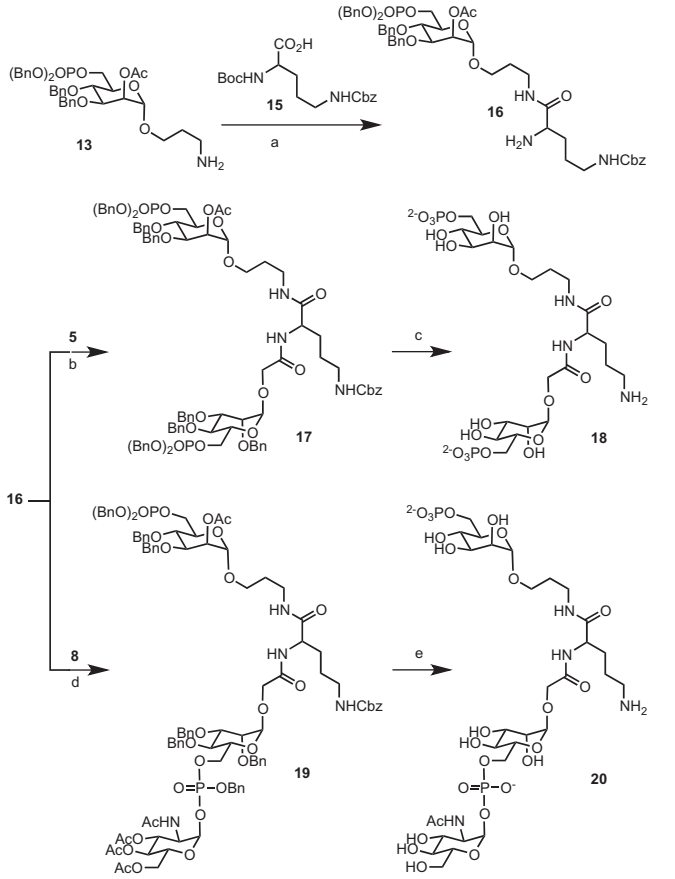
Scheme 1. Synthesis of building blocks **5** and **8** equipped with CO_2H handle. Reagents and conditions: (a) $RuCl_3$, $NaIO_4$, $CH_3CN/CH_2Cl_2/H_2O$ (2:2:3), rt; (b) (i) allyl bromide, K_2CO_3, DMF, rt, 72% over two steps, (ii) $FeCl_3{\cdot}6H_2O$, CH_2Cl_2, 0 °C, 75%; (c) (i) $(BnO)_2PN(iPr_2)$, 1*H*-tetrazole, 4 Å MS, CH_2Cl_2, rt, (ii) *t*-BuO_2H, rt, 91% over two steps; (d) $Pd(PPh_3)_4$, morpholine, CH_3CN, 0 °C; (e) (i) **6**, 1*H*-tetrazole, 4 Å MS, CH_2Cl_2, rt, (ii) *t*-BuO_2H, −40 °C, 90% over two steps; (f) $Pd(PPh_3)_4$, morpholine, CH_3CN, 0 °C.

position of **3** was effected by dibenzyl *N*,*N*-diisopropyl phosphoramidite $(BnO)_2PN(iPr_2)$ and the subsequent oxidation by *t*BuO_2H gave intermediate **4** in 91% yield. The allyl group of **4** was removed efficiently using $Pd(Ph_3P)_4$/morpholine to provide the M6P-OH building block **5.** Installation of the GlcNAc phosphoester on the 6-OH position of **3** was effected using the GlcNAc-derived phosphoramidite **6** and the subsequent oxidation with *t*BuO_2H. Reagent **6** was originally developed by Boons and co-workers for the synthesis of GlcNAc-P-serine motifs containing a similar α phosphodiester linkage.[11] Removal of the allyl group of **7** using $Pd(Ph_3P)_4$/morpholine gave the GlcNAc-M6P-OH building block **8** in good yield.

We then prepared M6P and GlcNAc-M6P building blocks **13** and **14**, equipped with NH_2 handles (Scheme 3). Stereoselective glycosylation of the mannose trichloroacetimidate **9**[6a] with Boc-protected 3-aminopropanol **10**, under the promotion of TMSOTf, yielded compound **11**. The TIPS group of compound **11** was then removed by the treatment of TBAF to give compound **12**. *O*Bn-pro-



Scheme 2. Synthesis of building blocks **13** and **14** equipped with NH_2 handle. Reagents and conditions: (a) **10**, TMSOTf, 4 Å MS, CH_2Cl_2, −20 °C, 70%; (b) TBAF, THF, rt, 78%; (c) (i) $(BnO)_2PN(iPr_2)$, 1*H*-tetrazole, 4 Å MS, CH_2Cl_2, rt, (ii) *t*-BuO_2H, rt, 89% over two steps, (iii) TFA, CH_2Cl_2, rt; (d) (i) **6**, 1*H*-tetrazole, 4 Å MS, CH_2Cl_2, rt, (ii) *t*-BuO_2H, −40 °C, 90% over two steps, (iii) TFA, CH_2Cl_2, rt.

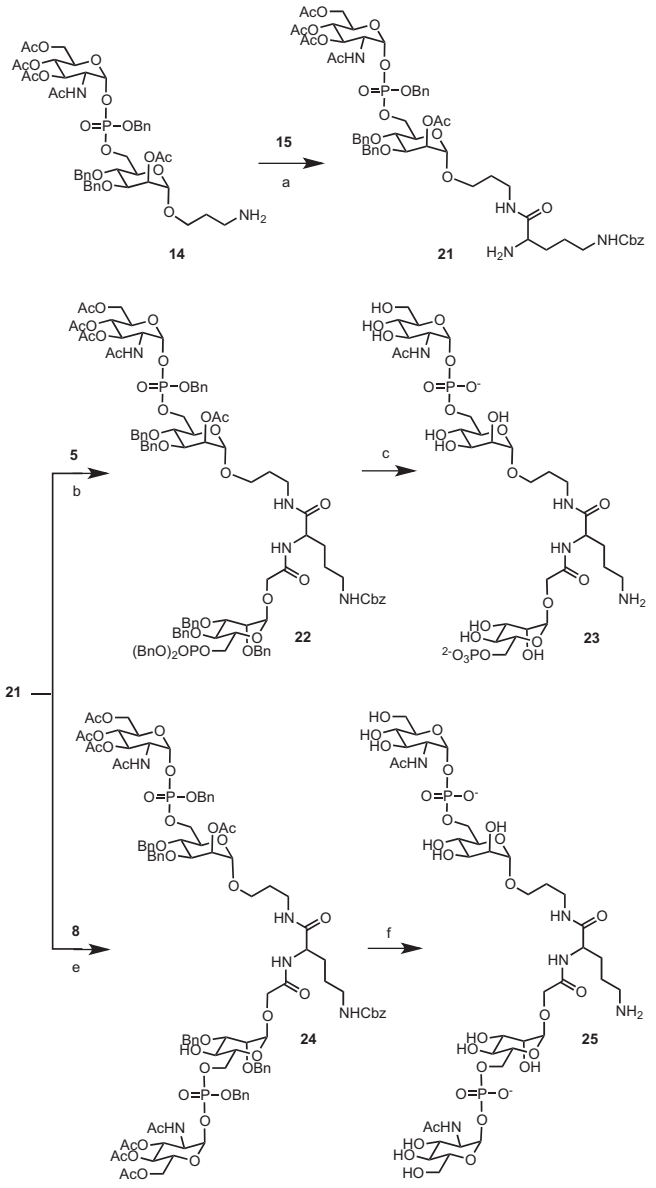


Scheme 3. Synthesis of bivalent ligands **18** and **20**. Reagents and conditions: (a) (i) **15**, HOBt, EDCI, DIPEA, CH_2Cl_2, rt, 86%, (ii) TFA, CH_2Cl_2, rt; (b) **5**, HOBt, EDCI, DIPEA, CH_2Cl_2, rt, 63%; (c) (i) H_2, $Pd(OH)_2$/C, MeOH, rt, (ii) $NH_2NH_2{\cdot}H_2O$, MeOH, rt, 80% over two steps; (d) **8**, HOBt, EDCI, DIPEA, CH_2Cl_2, rt, 68%; (e) (i) H_2, $Pd(OH)_2$/C, MeOH, rt, (ii) $NH_2NH_2{\cdot}H_2O$, MeOH, rt, 78% over two steps.

tected phosphoester group was installed on compound **12** using the standard $(BnO)_2PN(iPr_2)/tBuO_2H$ reagents in 89% yield. The Boc group was removed with TFA/CH_2Cl_2 at room temperature to give the desired M6P-NH_2 building block **13** as a TFA salt. The GlcNAc-M6P-NH_2 building block **14** was prepared from **12** and **6** in analogous fashion to compound **13**.

With the building blocks **5**, **8**, **13** and **14** in hand, we next assembled them with the Boc-N^{δ}-Cbz-L-ornithine linker **15** to provide the bivalent ligands **18**, **20**, **23** and **25** (Schemes 3 and 4). EDCI-mediated amide coupling of M6P-NH_2 **13** with ornithine **15**, and the subsequent Boc deprotection with TFA gave the M6P-Orn-NH_2 **16** in good yield. EDCI-mediated amide coupling of **16** with M6P-OH **5** then gave compound **17**. The protection of all the polar functional groups in compound **17** allowed us to easily purify it using normal silica gel chromatography. The final global deprotection of **17** was first effected by the removal of the Bn and Cbz groups using $Pd(OH)_2$/C-catalyzed hydrogenolysis conditions. The remaining OAc group of **17** was then removed with $NH_2NH_2{\cdot}H_2O$ in MeOH at room temperature to give the fully deprotected bivalent M6P-Orn-M6P ligand **18**. A filtration through a sephadex G-25 column (eluted with H_2O) provided **18** in high yield and purity. The bivalent ligand M6P-Orn-GlcNAc-M6P **20** was prepared in analogous fashion to compound **18**.

It should be noted that the order of the debenzylation and deacetylation steps of the deprotection sequence of **17** is important because the *O*Bn-protected phosphoester is not stable under nucleophilic deacetylation conditions.[11] Upon removal of the Bn group,



Scheme 4. Synthesis of bivalent ligands **23** and **25**. Reagents and conditions: (a) (i) **15**, HOBt, EDCI, DIPEA, CH_2Cl_2, rt, 84%, (ii) TFA, CH_2Cl_2, rt; (b) **5**, HOBt, EDCI, DIPEA, CH_2Cl_2, rt, 65%; (c) (i) H_2, $Pd(OH)_2$/C, MeOH, (ii) $NH_2NH_2{\cdot}H_2O$, MeOH, rt, 85% over two steps; (e) **8**, HOBt, EDCI, DIPEA, CH_2Cl_2, rt, 58%; (f) (i) H_2, $Pd(OH)_2$/C, MeOH, rt, (ii) $NH_2NH_2{\cdot}H_2O$, MeOH, rt, 75% over two steps.

the free phosphate group was stable to nucleophilic conditions. This order is particularly important for the successful deprotection of the intermediate **19**, which carries a more labile GlcNAc-M6P moiety. Following the same synthetic routes for **18** and **20**, another pair of bivalent ligands **23** and **25**, carrying a GlcNAc-M6P moiety on the upper arm, were prepared in good yield from the building block **5**, **8** and **14** (Scheme 4).

In summary, we designed and successfully synthesized novel bivalent mimetic ligands for cation-independent mannose-6-phosphate receptors (CI-MPRs). For the first time, a newly discovered MPR-binding element GlcNAc-M6P has been incorporated in mimetic ligands. A convergent and efficient synthesis strategy based on modular assembly of cassette-type M6P- and GlcNAc-M6P-containing building blocks via amide coupling was developed. Synthesis of other bi- and higher valent mimetic ligands with different linker molecules and in vitro binding analysis with CI-MPRs are under current investigation and will be reported in due course.

Acknowledgments

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Supplementary data

Supplementary data (synthetic procedures and structural characterization data for all new compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.02.068.

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