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Synthesis and α-Glucosidase II inhibitory activity of valienamine pseudodisaccharides relevant to *N*-glycan biosynthesis

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ABSTRACT

Valienol-derived allylic C-1 bromides have been used as carbaglycosyl donors for α-*xylo* configured valienamine pseudodisaccharide synthesis. We synthesised valienamine analogues of the Glc(α1→3)Glc and Glc(α1→3)Man disaccharides representing the linkages cleaved by α-Glucosidase II in *N*-glycan biosynthesis. These (*N*1→3)-linked pseudodisaccharides were found to have some α-Glucosidase II inhibitory activity, while two other (*N*1→6)-linked valienamine pseudodisaccharides failed to inhibit the enzyme.

The enzyme α-Glucosidase II is found in the Endoplasmic Reticulum (ER), and is involved in *N*-glycan biosynthesis. More specifically, it catalyses the removal of the second and third glucose residues from the $Glc_3Man_9GlcNAc_2$ oligosaccharide after transferral to the protein by Oligosaccharyltransferase, and after α-Glucosidase I has cleaved the outermost of the glucose residues.[1,2] The two oligosaccharide substrates for α-Glucosidase II then are $Glc_2Man_9GlcNAc_2$ and $Glc_1Man_9GlcNAc_2$ (Fig. 1). A difference in the rate of cleavage of glucose from these two oligosaccharides has been observed, the outermore of the two glucose residues being cleaved more quickly than the innermore one.[3] It has been proposed that this rate-difference has consequences for protein-folding mediated by the lectin chaperones Calnexin and Calreticulin, which bind the monoglucosylated oligosaccharide, and for an alternative degradation pathway in a quality control mechanism.[4]

The enzyme consists of two subunits, termed GIIα and GIIβ. The catalytic activity comes from the α-subunit, while the β-subunit appears to contain a mannose-binding lectin domain, to which binding is necessary to ensure efficient trimming of $Glc_{1-2}Man_9GlcNAc_2$-type oligosaccharides.[5] The detailed three-dimensional structure of the enzyme is not known. Based on the irreversible inhibition of enzyme activity by the carbocyclic glucose mimic bromoconduritol, Calvo proposed a model consistent with two active sites, one each for the cleavage of each of the two glucose residues.[6,7] Results from one of our labs show that different *N*-alkylated deoxynojirimycins inhibit each of the two different cleavages to different degrees, which is consistent with a two-site model.[8] Alternative explanations, that there is a single active site,[9] and that the rate differential is presumably due to a difference in the affinity of binding the two linkages, or due to different isoforms of the enzyme,[10] or due to binding of mannose residues in the remainder of the oligosaccharide[5] or in a second oligosaccharide[11] have also been proposed. It has been shown that the disaccharides are substrates for the enzyme, and that also here, the Glc(α1→3)Glc linkage is cleaved more rapidly than the Glc(α1→3)Man linkage.[12] Hence it seems that a substrate of at least disaccharide size is recognised by the enzyme active site(s).

We designed potential inhibitors **2** and **3** mimicking the two disaccharide structures cleaved by α-Glucosidase II, but modified to include the carbocyclic α-glucosidase inhibitor, valienamine **1**, rather than glucose (Fig. 1). Thus, the pseudodisaccharides are stable to hydrolysis by α-Glucosidase II, they contain a bridging nitrogen atom that could be protonated to interact with the catalytic carboxylate residues, and the valienamine ring is unsaturated, so more easily distorted (than a saturated chair) into a conformation whereby the interaction between inhibitor and protein may closely resemble the interaction between substrate and protein at the transition state of the cleavage reaction. We expected that these pseudodisaccharides might be more specific for α-Glucosidase II than monosaccharide-mimicking inhibitors, and that they might be more effective inhibitors than the thioether-linked carbasugar-based pseudodisaccharide mimics of the same two disaccharides, which we had synthesised earlier, and which failed to inhibit α-Glucosidase II at all.[13] Beyond the fundamental importance of

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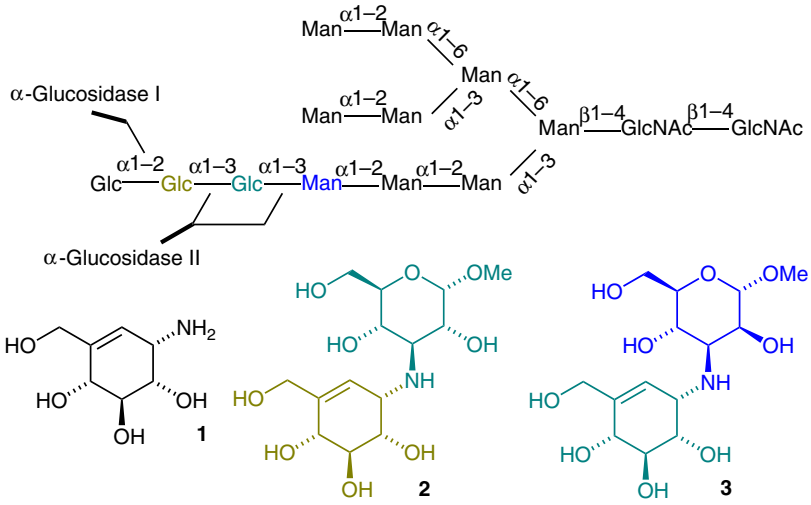


Figure 1. Valienamine **1**, valienamine pseudodisaccharide targets **2** and **3**, and the natural oligosaccharide substrate, indicating the cleavage sites of α-Glucosidase II.

understanding the mechanism of action of this enzyme, specific inhibition of α-Glucosidase II, and thus interfering with *N*-glycan biosynthesis, has possible applications in antiviral medicine, as virally infected cells use the host machinery to manufacture the viral *N*-glycans.[14]

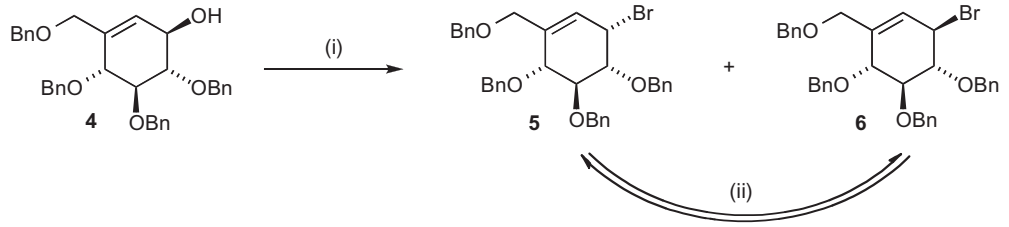
A number of pseudodisaccharides based on *N*-substituted valienamine (or its epimers) have been synthesised over the past thirty years as potential glycosidase inhibitors.[15] Many of the synthetic routes are based on attack of carbohydrate amine nucleophiles on (*epi*-)valienol electrophiles of various types. Valienol 1,2-epoxides result in 1,2-*trans* configured pseudodisaccharides (i.e., 1- or 2-*epi*-valienamines, β-*xylo* or α-*lyxo* configuration).[16,17] Palladium-catalysed[18,19] and Mitsunobu[19,20] coupling methods have also recently been used to access valienamine pseudodisaccharides. Some of the first coupling reactions to be reported involved nucleophilic substitution of C-1 halides with carbohydrate amines.[21–23] A C-2 acetyl protected carbasugar halide gave the 1,2-*trans* product irrespective of the starting C-1 configuration of the bromide (1,2-*cis* or 1,2-*trans*), possibly due to neighbouring group participation.[22] We recently showed that carbasugar C-1 halides with benzyl ether protection coupled with amines to give 1,2-*cis* (α-*xylo*) pseudodisaccharides as the major or exclusive products.[19] In this letter, we describe how we used nucleophilic substitution reactions of valienol C-1 bromides to prepare our target valienamine (*N*1→3)Glc **2** and (*N*1→3)Man **3** pseudodisaccharide structures of relevance to α-Glucosidase II, as well as the results of inhibition assays against the enzyme.

The carbasugar C-1 alcohol **4**, accessible from L-sorbose by a sequence relying on ring-closing metathesis as a key step, was treated with triphenylphosphane and carbon tetrabromide[24] to give the allylic bromides **5** and **6** as a diastereomeric mixture by a low-yielding Appel reaction (Scheme 1). The C-1 epimeric alcohol (1-*epi*-**4**) gave a similar diastereomeric mixture of C-1 bromides under the same reaction conditions. It has been noted before that allylic carbasugar C-1 bromides can sometimes be configurationally unstable.[22,23] The α- **5** and β- **6** bromides were separable by chromatography, and when isolated, did not spontaneously interconvert. However, when we treated each of the diastereomeric bromides (in CD_2Cl_2) with an external bromide anion source, tetrabutylammonium bromide, we were able to follow their interconversion and formation of a *ca* 2:1 (**5**:**6**) thermodynamic mixture by ^{1}H NMR spectroscopy. This result is consistent with the apparent formation of a thermodynamic mixture of diastereomeric C-1 bromides by multiple S_N2 inversions in the Appel bromination. It was possible to run the bromide epimerisation reaction on a preparative scale; treatment of the pure α bromide **5** with tetrabutylammonium bromide gave a mixture (*ca* 2.5:1) of α-**5** and β-**6** bromides that could be easily separated by chromatography. However, leaving the epimerisation reaction for longer periods of time resulted in by-product formation and a more difficult purification of the β diastereomer.

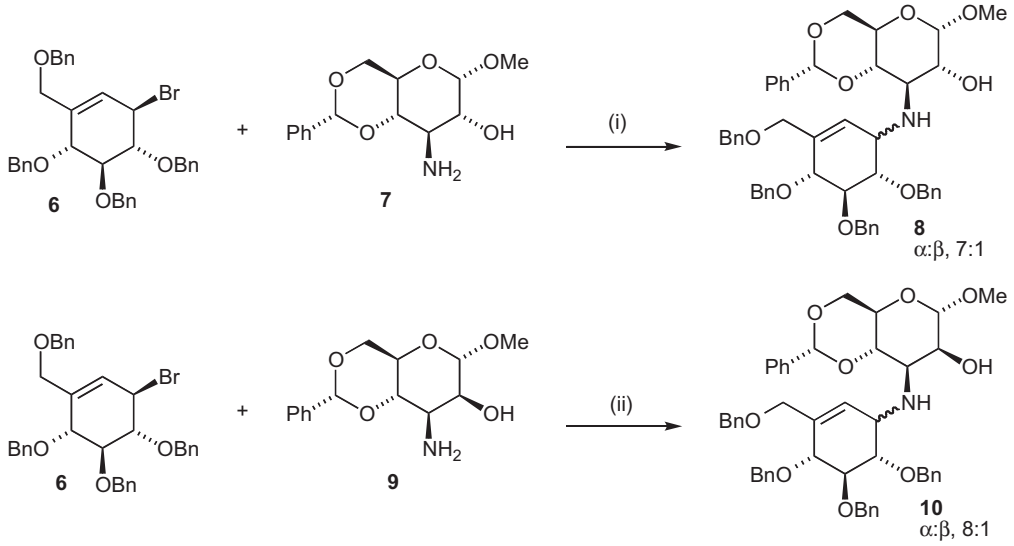
Heating a mixture of the β-bromide **6** and the glucose 3-amine **7**[25] with Hünig's base resulted in the formation of the pseudodisaccharides **8**, with the α-configured compound **8**α predominating (**8**α:**8**β, 7:1) (Scheme 2). Similarly, the β-bromide **6** and mannose 3-amine **9**[26] gave the pseudodisaccharides **10**, again with the α-linked diastereomer as the major component (**10**α:**10**β, 8:1). The stereochemistry of the pseudodisaccharides **8** and **10** was assigned using the $J_{1,2}$ and $J_{1,5a}$ coupling constants from the ^{1}H NMR spectra, in comparison with reported data.[19,27] The α-*xylo* configured had $J_{1,5a}$ values of 4.9 Hz (**8**α) and 3.9 Hz (**10**α), and $J_{1,2}$ values of 4.6 Hz (**8**α) and 4.3 Hz (**10**α). In the β-*xylo* configured by-products, H-5a appeared as a (slightly broadened) singlet. The major α-configured diastereomers were obtained pure by flash column chromatography, but the β-configured by-products were not obtained pure. Hence the method of nucleophilic substitution of valienol C-1 bromides may be used with amines at the secondary carbons of carbohydrates for the synthesis of pseudodisaccharides, with a tendency for the stereoselective formation of α-configured products.

Deprotection of benzyl ethers and benzylidene acetals in **8**α and **10**α and was achieved by treatment of the pseudodisaccharides with sodium in liquid ammonia, to give the two (*N*1→3)-linked target compounds **2** and **3** of relevance to α-Glucosidase II (Scheme 3). We also deprotected our previously synthesised (*N*1→6)-linked pseudodisaccharides **11** and **12** in the same way to give the α and β (*N*1→6)-linked valienamine pseudodisaccharides **13** and **14**.

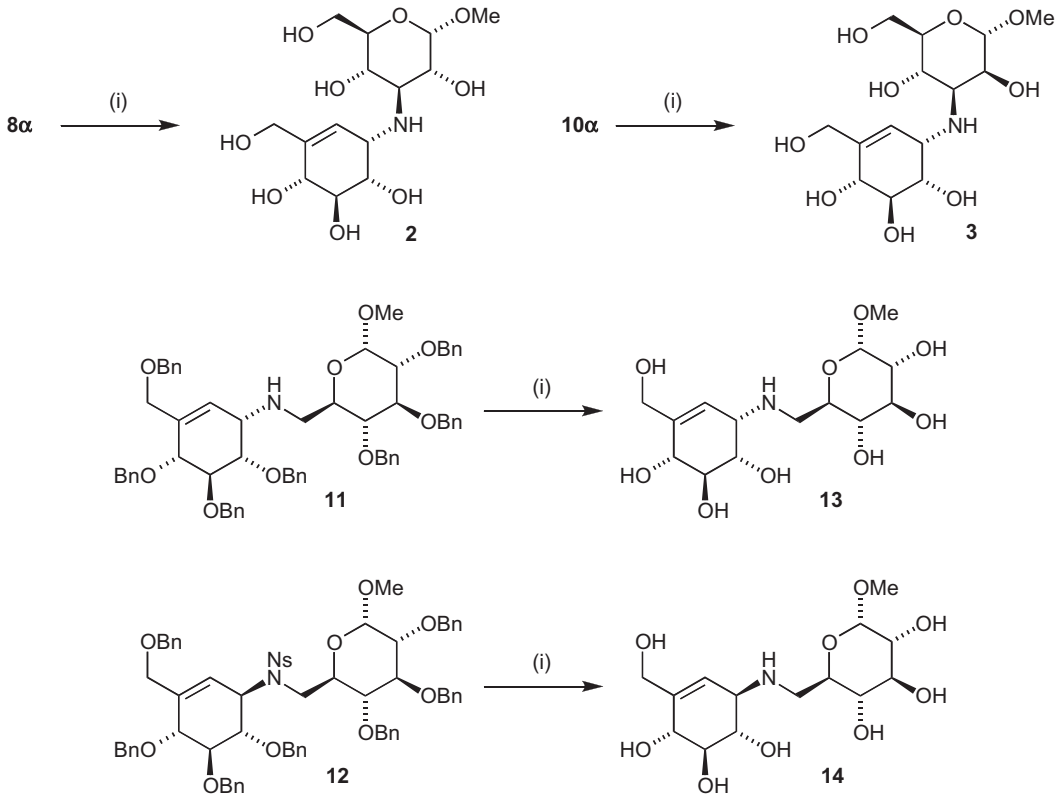
We tested the effects of the pseudodisaccharides **2**, **3**, **13** and **14** on α-Glucosidase II using two assays. Inhibitory activity against isolated rat liver α-Glucosidase II was determined by monitoring the rate of disappearance of substrate for two oligosaccharide substrates, *viz* monoglucosylated $Glc_1Man_5GlcNAc_1$ and diglucosylated $Glc_2Man_7GlcNAc_2$. The results are given in Table 1. The two (*N*1→6)-linked compounds **13** and **14** were non-inhibitory up to 500 μM, while the two (*N*1→3)-linked compounds **2** and **3** inhibited α-Glucosidase II to different extents. We also ran the cell-based free oligosaccharide (FOS) assay in which the formation of free oligosaccharides arising from an ER-associated degradation



Scheme 1. Reagents and conditions: (i) PPh_3, CBr_4, CH_2Cl_2, 0 °C to rt; **5**, 29%; **6**, 16%; (ii) Bu_4NBr, CH_2Cl_2, from **5**: **5**, 58%; **6**, 24%.



Scheme 2. Reagents and conditions: (i) $^{i}Pr_2EtN$, MeCN, 75 °C, 41%; (ii) $^{i}Pr_2EtN$, MeCN, 75 °C, 43%.



Scheme 3. Reagents and conditions: (i) Na, $NH_{3\ (l)}$, MeOH, THF, −78 °C; **2**, 40%; **3**, 38%; **13**, 38%; **14**, 27%.

Table 1
Inhibition of isolated rat liver α-Glucosidase II with compounds **2**, **3**, **13** and **14** as inhibitors and $Glc_2Man_7GlcNAc_2$ and $Glc_1Man_5GlcNAc_1$ as substrates

Entry	Compound	IC_{50}/μM, with substrate: $Glc_2Man_7GlcNAc_2$	$Glc_1Man_5GlcNAc_1$
1	**2**	72 ± 15	143 ± 10
2	**3**	NI	236 ± 15
3	**13**	NI	NI
4	**14**	NI	NI

NI = Non-inhibitory up to 500 μM inhibitor concentration.

pathway is monitored.[28] Also here, the (*N*1→6)-linked compounds **13** and **14** showed no inhibition of α-Glucosidase II compared to the control, but the presence of mannose-terminating species indicated some potentially weak inhibition of α-mannosidase activity (results not shown). The (*N*1→3)-linked compounds **2** and **3** both showed the formation of a significant extra peak not seen in the control (no inhibitor) due to $Glc_1Man_4GlcNAc_1$, a result of α-Glucosidase II inhibition (Fig. 2). Hence, as well as binding to the protein, the pseudodisaccharides are able to cross the cell membrane, and also to enter the ER.

Valienamine **1** itself is a fairly weak inhibitor of α-Glucosidase II (IC_{50} 1 mM),[29] so the (*N*1→3)-linked pseudodisaccharides clearly show an enhanced affinity for the enzyme. Valienamine has also been reported to inhibit the activity of α-Glucosidase I (IC_{50} 780 μM), but from the FOS assay, we did not detect oligosaccharides arising from α-Glucosidase I inhibition (i.e., Glc_3 oligosaccharides) from any of the pseudodisaccharides tested at the concentrations of inhibitors used, so we can say that the pseudodisaccharides **2** and **3** also seem to have an improved specificity for α-Glucosidase II compared to the parent monosaccharide-mimicking valienamine. The enhanced affinity and specificity (over valienamine) of the (*N*1→3)-linked pseudodisaccharides for α-Glucosidase II is consistent with binding of the inhibitors to the active site(s) of the enzyme in a manner that mimics the binding of the substrates.

It is instructive to compare the inhibitory activities of these pseudodisaccharides here with other compounds from the literature. Two amine-linked pseudodisaccharides mimicking linkages from the $Glc_3Man_9GlcNAc_2$ tetradecasaccharide have been tested for activity inhibiting the processing of this oligosaccharide by α-Glucosidases I and II: a 2-amino-2-deoxyglucoside *N*-substituted with valienamine **15** (i.e., a slightly modified salbostatin) was synthesised by Ogawa (Fig. 3);[30] a 3-amino-3-deoxyglucoside *N*-substituted with an inositol **16** was synthesised by Haines and Carvalho.[31] Both of these pseudodisaccharides inhibited Baker's yeast α-Glucosidase to a greater or lesser extent, but neither showed any inhibition of α-Glucosidases I or

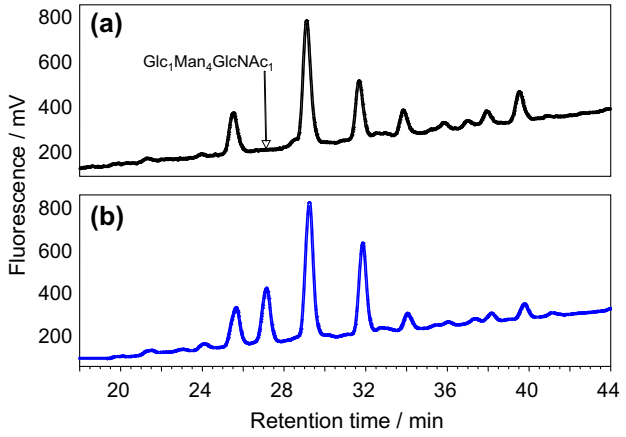


Figure 2. Illustrative HPLC trace of free oligosaccharides from the cell-based FOS assay. (a) Control with HL60 cells only; (b) HL60 cells with 500 μM compound **2** for 24 h.

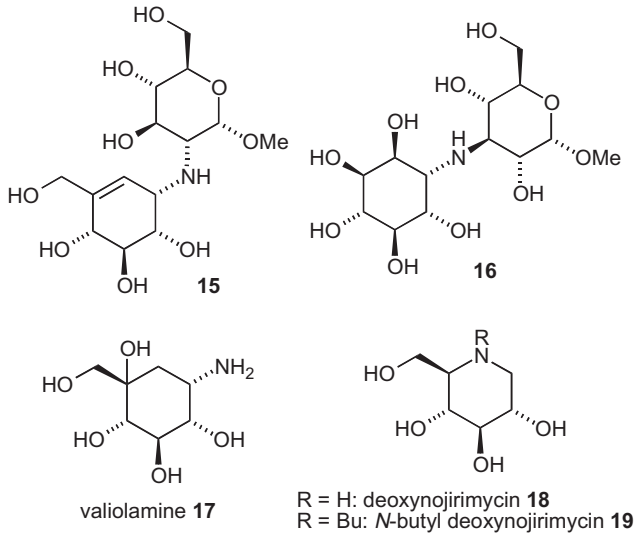


Figure 3. Structures of some previously reported (proposed) inhibitors of α-Glucosidase I and II.

II from rat liver microsomes. Inhibitors of these enzymes with higher potency are known, for example valiolamine **17**,[29] deoxynojirimycin **18** (4.6 μM),[29] and *N*-butyl deoxynojirimycin **19**[8] (IC_{50} values 4.6–53 μM for isolated α-Glucosidase II).

In conclusion, nucleophilic substitution of valienol C-1 bromides as a diastereoselective method for the synthesis of α-*xylo* configured valienamine pseudodisaccharides is extensible to (*N*1→3)-linkages with amine nucleophiles on the secondary position of carbohydrates. The pseudodisaccharides mimicking the natural substrates of α-Glucosidase II inhibit the enzyme (both with the isolated enzyme and in cells), but do not inhibit α-Glucosidase I; (*N*1→6)-linked pseudodisaccharides did not inhibit the enzyme, all of which is consistent with a specific interaction with the active site. The (*N*1→3)Glc compound **2** inhibits the cleavage of both substrates (i.e., Glc(α1→3)Glc and Glc(α1→3)Man), whereas the (*N*1→3)Man compound **3** inhibits only the cleavage of Glc(α1→3)Man. Moreover, also in the FOS assay the *gluco* compound **2** was more effective than the *manno* compound **3**. These data, taken in isolation, could be consistent with a model in which Glc(α1→3)Glc is bound with a higher affinity than Glc(α1→3)Man to a single active site. However, a more detailed kinetic analysis would be needed before definitive conclusions can be drawn.

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Supplementary data

Supplementary data (experimental procedures, characterisation data for new compounds, copies of ^{1}H NMR spectra for new compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.07.046.

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17. In this Letter we have used carbohydrate/carbasugar nomenclature according to IUPAC. Hence, valienamine **1**, which shares C-1–C-4 stereochemistry with α-glucose has α-*xylo* stereochemistry (also compounds **2**, **3**, **5**, **8**α, **10**α, **11** and **13**); 1-*epi*-valienamine has β-*xylo* stereochemistry (also compounds **4**, **6**, **12** and **14**).
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