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Pyridine–sugar conjugates as potent inhibitors of enzyme-catalysed glycoside hydrolysis

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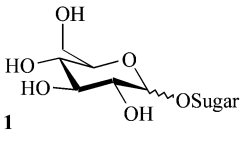
A series of *O*- and *N*-linked pseudo-disaccharides incorporating simple functionalised pyridines were synthesized and demonstrated potent inhibition of the glucoamylase-catalysed reaction.

It is generally recognized[1] that glycosides hydrolyse chemically *via* a cyclic oxocarbenium ion and that a similar transition state is almost certainly involved in the enzyme-catalysed process. Potent known glycosidase inhibitors mimic features of either the enzyme's substrate (*e.g.* **1**) and/or the transition state involved in the enzyme-catalysed process **2**.[2] Unfortunately, the complexity of these molecules often makes their syntheses and that of similar compounds a time consuming and difficult task. Moreover, this synthetic problem is magnified considerably in the development of glycosidase-specific inhibitors by the level of structural and chemical complexity in heterogeneous carbohydrate chains.



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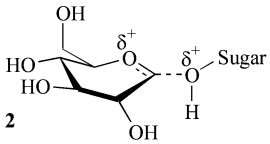

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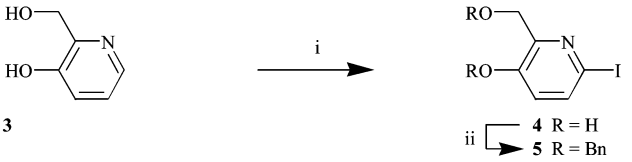
To circumvent these problems some research has recently been directed towards identifying new glycomimetic glycosidase inhibitors which are based on very simple molecular designs. For example, it is known that acyclic molecules such as trihydroxymethylaminomethane (tris)[3] and simple heterocyclic compounds like the cyclic guanidinium ion[4] are competitive inhibitors of glycosidases. Cyclic guanidinium ions have also been employed in the formation of pseudo-disaccharides in an attempt to improve inhibitor efficacy and selectivity.[5] Interestingly, these disaccharide analogues were shown to bind less effectively than simple derivatives of the cyclic guanidinium moiety.

A key, seminal observation by Withers[6] was that carbohydrate–pyridine conjugates also possessed useful inhibitory properties towards glycosidases but, unfortunately, in this case no comparison was made to the effectiveness of the isolated pyridine moiety as an inhibitor of the enzyme. This suggested that a detailed examination of suitably substituted pyridines might be fruitful in the development of a simple yet effective class of new glycosidase inhibitors. An investigation that seemed especially relevant considering the benzyl ring has been successfully mobilised as an effective mimetic of the flat pyranosyl donor sugar proposed for the transition state in a glycosyltransferase reaction.[7]

Work recently performed in this laboratory[8] identified 3-hydroxy-2-hydroxymethylpyridine (Scheme 1; **3**) as a moderate inhibitor of the β-glucosidase [EC 3.2.1.21] from sweet almonds (K_i = 1.9 × 10^{-3} M). This observation compares favourably with the study by Withers and coworkers[6] where kinetic data was obtained using a β-glucosidase and a range of



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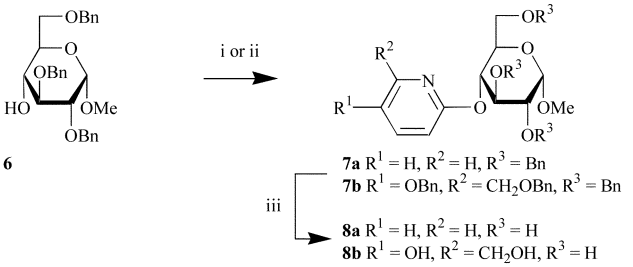
Scheme 1 *Reagents and conditions*: i, I_2, Na_2CO_3, 26%; ii, BnBr, NaH, DMF, 80%.

2-pyridylthio pseudo-sugars and suggests that their effectiveness may largely be due to the pyridine moiety alone.

In the study of 2-pyridylthio pseudo-sugars,[6] as with that where cyclic guanidinium ions were utilised in the formation of pseudo-disaccharides,[5] the enzymes investigated were glycosidases in which it is likely that the majority of bonding interactions occurred solely with the terminal sugar unit. A more pragmatic approach would be to incorporate these simple inhibitor units into pseudo-saccharides as inhibitors of glycosidase reactions where the substrate recognition event is much more dependent on co-operative bonding interactions between the enzyme and more than one sugar unit of the substrate oligosaccharide. Towards these ends we synthesized a series of *O*- and *N*-linked pseudo-disaccharides incorporating simple functionalised pyridines such as **3** as potential inhibitors of well characterized amylase reactions.

Preparation of amino and hydroxy pyridines can be achieved through nucleophilic substitution of a pyridine substrate. For the manufacture of more complex conjugates incorporating pyridine with a spatial arrangement of peripheral hydroxyl groups similar to that observed for **3**, an appropriately substituted pyridine first had to manufactured. In this case an adaptation of the procedure described for the synthesis of 3-hydroxy-2-iodopyridine[9] was utilised to afford 3-hydroxy-2-hydroxymethyl-6-iodopyridine (**4**).‡ Subsequent *O*-benzylation then afforded a substrate (**5**) that could be used in S_NAr reactions.

Nucleophilic substitution worked tolerably well for the preparation of the desired *O*-linked conjugate methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2′-pyridyl)-α-D-glucopyranoside (**7a**) from methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (**6**) and 2-chloropyridine (Scheme 2). The methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (**6**) used in this reaction was prepared from methyl 4,6-*O*-benzylidene-α-D-glucopyranoside[10] *via* benzyl



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Scheme 2 *Reagents and conditions*: i, 2-Chloropyridine, NaH, NMP, 140 °C for 12 h, **7a** = 50%; ii, **5**, NaH, NMP, Cu(I)Br, 140 °C for 18 h, **7b** = 30%; iii, EtOH–cyclohexene (2 : 1), Pd black, reflux, 24 h, **8a** = 82%, **8b** = 46%.

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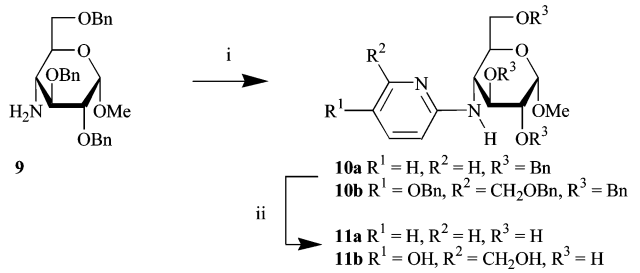
Table 1 Glucoamylase inhibitory activity of 2-pyridyl pseudo-sugars

Compound	**3**	**8a**	**8b**	**11a**	**11b**
K_i (M)	No inhibition at 1×10^{-2}	No inhibition at 1×10^{-3}	1.1×10^{-3}	2.2×10^{-4}	1.8×10^{-6}

protection[11] and regioselective ring opening[12] of the 4,6-*O*-benzylidene acetal moiety.

Unfortunately, when 3-benzyloxy-2-benzyloxymethyl-6-iodopyridine (**5**) was used as substrate for S_NAr reactions, even under harsh reaction conditions (NaH, NMP, 80 °C), formation of the desired pyridine conjugate was not observed. Despite this unreactivity, synthesis of methyl 2,3,6-tri-*O*-benzyl-4-*O*-(5′-benzyloxy-6′-benzyloxymethyl-2′-pyridyl)-α-D-glucopyranoside (**7b**) was achieved *via* copper(I) halide-catalysed alkoxylation[13] of **5**. The synthesis of the equivalent *N*-linked conjugates through straightforward S_NAr reactions of methyl 2,3,6-tri-*O*-benzyl-4-amino-4-deoxy-α-D-glucopyranoside[11] (**9**; Scheme 3) with 2-chloropyridine, 2-chloropyridine *N*-oxide, 2-bromopyridine or **5** also proved unsuccessful, even in the presence of copper(I) halide.



Scheme 3 *Reagents and conditions*: i, 2-Bromopyridine or **5**, $Pd(OAc)_2$, (±)-BINAP, NaO*t*-Bu, toluene, 100 °C under argon for 20 h, **10a** = 22%, **10b** = 29%; iii, EtOH–cyclohexene (2:1), Pd black, reflux, 48 h, **11a** = 80%, **11b** = 25%.

However, the successful palladium-catalysed cross-coupling of pyridyl bromides and primary amines employing chelating bis-(phosphine) ligands has been reported.[14] Utilising similar reaction conditions[15] we found it was possible to synthesize both methyl 2,3,6-tri-*O*-benzyl-4-amino-4-deoxy-4-*N*-(2′-pyridyl)-α-D-glucopyranoside (**10a**) and methyl 2,3,6-tri-*O*-benzyl-4-amino-4-deoxy-4-*N*-(5′-benzyloxy-6′-benzyloxymethyl-2′-pyridyl)-α-D-glucopyranoside (**10b**) from methyl 2,3,6-tri-*O*-benzyl-4-amino-4-deoxy-α-D-glucopyranoside (**9**) and the appropriate pyridyl halide (Scheme 3). Finally, de-*O*-benzylation for all benzyl protected pseudo-disaccharides synthesized (**7a**, **7b**, **10a** and **10b**) by catalytic transfer hydrogenation (cyclohexene–Pd black–EtOH)[16] at reflux temperature for 24–48 h afforded the desired *O*- and *N*-linked pseudo-disaccharides.§

The biological activity of the prepared pyridylglucoconjugates **8a**, **8b**, **11a** and **11b** was then examined using a glucoamylase (GA)-dependent reaction.[17] The observed results (Table 1) clearly define for the first time the potential of these non-carbohydrate carbohydrate mimetics as potent moderators of glycoprocessing events. Indeed, the best inhibitor (**11b**) is comparable in biological activity to the acarvosine unit of the potent GA inhibitor acarbose ($K_i = 1 \times 10^{-6}$ M).[18]

What is most strikingly evident with these simple pseudo-disaccharides is that the pendant polyvalent carbohydrate ligand (*e.g.* in **11a**) clearly overcomes the bonding limitations of the simple transition state mimetic (*e.g.* **3**) and confers additional bonding interactions in the enzyme active site that dramatically increases their inhibitory effectiveness. It should be noted that the X-ray crystal structures of GA with inhibitor bound in the active site clearly show strong bonding interactions between key residues at the catalytic subsite and the *C*-4 and *C*-6 hydroxyls of substrate analogues.[19] The associated improvement in the biological activity of those pseudo-disaccharide inhibitors with peripheral hydroxylation on the flat aromatic structure of the mimetic may therefore indicate an appropriate match for the topological and spatial arrangement of the all-equatorial functionalities at *C*-4 and *C*-5 of the non-reducing glucose unit of the substrate to enable these important hydrogen bond formations. Furthermore, these results, in conjunction with previous studies,[20] also enforce the importance of having a basic exocyclic amino group adjacent to *C*-1 of a sugar for effective binding in the amylase active site.

In conclusion, the preparation of *O*- and *N*-linked pseudo-disaccharides incorporating simple functionalized pyridines has been achieved. Moreover, their associated biological activity demonstrates that this approach represents a viable new alternative for the development of simple non-carbohydrate carbohydrate mimetics for the modification or interception of specific key events in carbohydrate metabolism.

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Notes and references

‡ This procedure also led to the formation of 3-hydroxy-2-hydroxymethyl-4,6-diiodopyridine which was removed from the cooled reaction mix by precipitation and filtration following acidification (pH 6.0) through the dropwise addition of HCl (2 M). The resultant crude 3-hydroxy-2-hydroxymethyl-6-iodopyridine was then purified by successive recrystallization from ethanol. Although the spectroscopic and analytical data for 3-hydroxy-2-hydroxymethyl-6-iodopyridine was in full agreement with the proposed structure, the structure was confirmed by X-ray analysis of its crystal structure.

§ All pseudo-disaccharides were purified by silica-gel chromatography and gave satisfactory elemental analysis or HRMS data. NMR spectra were in full accord with the proposed structures.

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