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β-Glucosidase Inhibitors Incorporating Pyridinium as a Glucosyl Mimic

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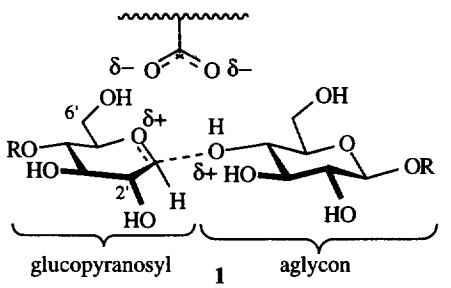
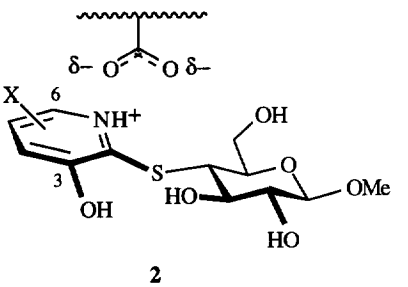
Abstract: *The synthesis of several 2-pyridylthio-based pseudo-disaccharides (5 - 9) is described. One of them, 5a, shows inhibition of* Agrobacterium *β-glucosidase (K_i = 800 μM), possibly because in protonated form it mimics the protonated substrate (or transition state, compare 1) leading to the glucopyranosyl cation.*

A number of naturally-occurring glycosidase inhibitors are thought to function by mimicking the protonated substrate, or the transition state for glycoside hydrolysis as represented loosely by **1**, or the glycopyranosyl cation itself.[1] A few, such as acarbose,[2] adiposin-1,[3] and trehazolin,[4] are multi-subunit pseudo-oligosaccharides; they possess both a "glucopyranosyl" mimic and an "aglycon" mimic, and both portions are important for efficacy and specificity.[5,6] Several designed, linkage-spanning, pseudo-disaccharides that incorporate this multi-subunit attribute of the natural inhibitors have been prepared, and improvements in efficacy and selectivity over the simpler corresponding "glycopyranosyl" mimics have been recorded.[7] In general, the ability to adjust the "aglycon" portion may provide the best means to tune the specificity of inhibition to a particular enzyme, and the most effective "glycopyranosyl" mimics can likely be improved by attaching the appropriate "aglycon".

Designed glycosidase inhibitors that are not directly based on natural products nevertheless do not stray very far in structure from the parent "glycopyranosyl" framework, whereas "aglycone" mimics have included structures ranging from pyranose rings to substituted aromatics to alkyl substituents.[8] Noting the structural similarities between the linkage-spanning portion of **1** and a pyridinium derivative such as **2**, we propose that heteroaromatic rings might serve as suitable "glycopyranosyl" surrogates for design of effective, selective, stable, and easily synthesized glycosidase inhibitors. In this Letter we describe the synthesis and enzymatic evaluation of several methyl 4-deoxy-S-(3-hydroxy-2-pyridyl)-4-thio-β-D-glucopyranosides as a first test of this proposal.



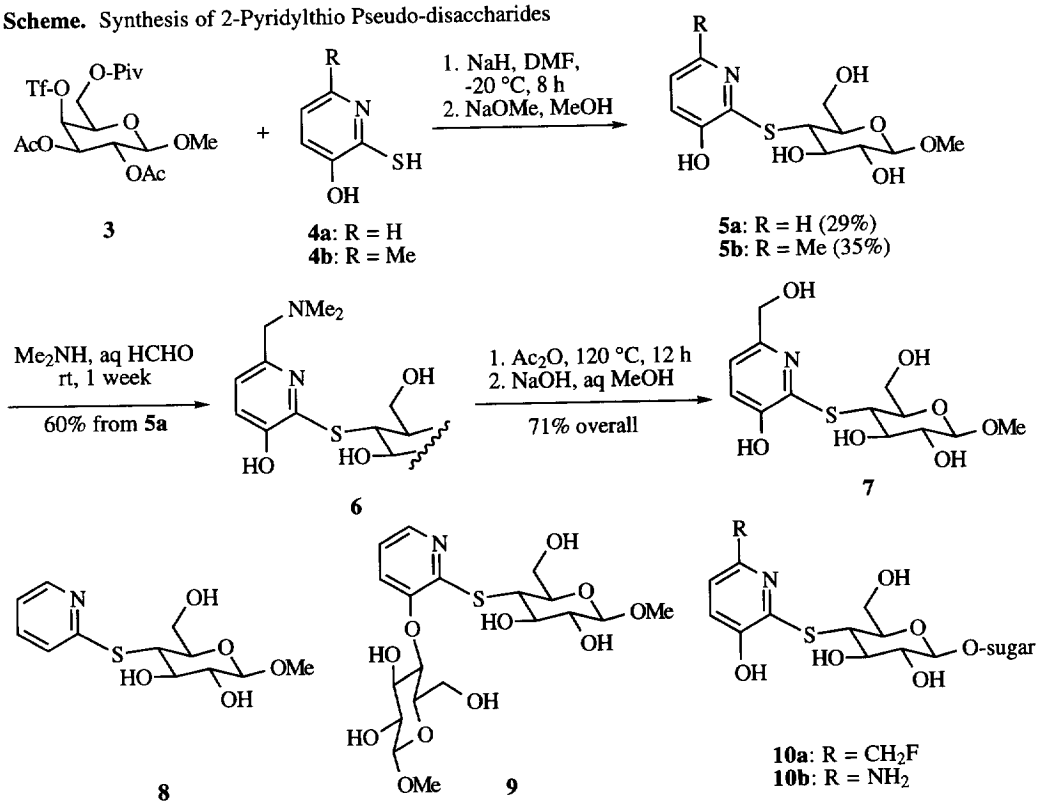
1



2

The Scheme shows the straightforward assembly of the 2-pyridylthio pseudo-disaccharides. The galactose 4-triflate **3** was prepared by selective pivaloylation of methyl 2,3-di-O-acetyl-β-D-galactopyranoside[9] at O-6, followed by triflation[10] at O-4 (91% yield overall). Treatment of **3** with the monosodio derivative of commercially available (Janssen Chimica) 3-hydroxy-2-mercaptopyridine **4a** gave, after deacylation, the product of substitution, thioether **5a** (29%), along with some elimination product and the pseudo-trisaccharide **9** from (O,S) double alkylation (4%). Similarly, the 6-methyl analogue **4b**, prepared in two steps from 5-hydroxy-2-picoline,[11] was coupled with **3** and then deacylated to give **5b**. Mannich substitution[12] of **5a** led to the 6-[(dimethylamino)methyl]-pyridine derivative **6**, which was in turn transformed to the 6-(hydroxymethyl)-pyridine pseudo-disaccharide **7** following replacement of dimethylamino by acetoxy at high temperature.[13] The simpler 2-pyridylthio pseudo-sugar **8** was made analogously from 2-mercaptopyridine and **3**.

Scheme. Synthesis of 2-Pyridylthio Pseudo-disaccharides



The structures of the 2-pyridylthio pseudo-disaccharides were confirmed by ^{1}H and ^{13}C NMR spectroscopic analysis. In particular, the C-6 site selectivity of the Mannich reaction leading to **6**, while expected from literature precedent,[12,13] was authenticated by the disappearance of the (downfield) pyridyl H-6 signal, and by comparison of the observed δ values of the pyridyl carbons of **7** with predicted δ values[14] for the products of both C-6 and C-4 substitution.

Replacement of a glucopyranose ring with the 3-hydroxy-6-(hydroxymethyl)-2-pyridylthio unit of **7** would be expected to alter the overall dimensions of a disaccharide or higher oligomer only slightly.[15] For

example, the O-2 to C-6 distance in a glucopyranoside (≈5.56 Å) should increase to about 5.64 Å for the O-3 to C-7 span across the pyridyl ring of **7**. Because of the longer C-S bond in **7** compared with a normal glucosidic linkage, the C-5 to S distance is about 0.20 Å longer than the corresponding span across the glucopyranose ring (C-4 to O-1). The longer C-S bond would be a favorable attribute in an inhibitor that mimics **1**, since the transition state ought to have a "stretched" glucosidic C-O bond.[7a,16]

The six 2-pyridylthio pseudo-sugars (**5a**, **5b**, **6**, **7**, **8**, and **9**; see Table) were assayed for inhibition of *Agrobacterium* β-glucosidase under the following conditions: 50 mM sodium phosphate buffer at pH 7.00, 0.1% BSA, 37 °C, reaction volumes 0.81 mL or 0.155 mL, and 0.08 mM *p*-nitrophenyl β-D-glucopyranoside as substrate. Inhibitor concentrations ranging from 0.1 - 5 times the K_i were used wherever possible.[17]

Table. β-Glucosidase Inhibitory Activity of 2-Pyridylthio Pseudo-sugars

Compound :	**5a**	**5b**	**6**	**7**	**8**	**9**
K_i :	0.8 mM	4.5 mM	20 mM	4 mM	8 mM	5.5 mM

Although none of the compounds tested showed outstanding activity, **5a** exhibited good enough inhibition to warrant a full kinetic analysis, and was found to be a competitive inhibitor with K_i = 0.8 mM (inhibitor conc. 0.4-4.4 mM, substrate conc. 0.013-0.50 mM). No enzymatic cleavage of **5a** to release methanol was observed. It binds ~8 times better than glucose (K_i = 6.4 mM)[18] and phenyl β-D-glucopyranoside,[19] suggesting that interactions involving the pyridine moiety may be contributing. Some interesting effects involving the pyridyl substituents are also apparent: the 3-hydroxy of **5a** is helpful for activity (which is consistent with the strong interactions known to be present at the glucopyranose 2-hydroxyl[20]), whereas substitution at the pyridine 6-position with methyl, (dimethylamino)methyl, or hydroxymethyl (**5b**, **6**, **7**) is detrimental. The additional glucopyranose ring of the pseudo-trisaccharide **8** also provides no apparent benefit.

The enzyme data could reflect the result of binding **5a** in a manner analogous to the natural substrate, with the pyridine ring occupying the non-reducing site (as in **2**), but they do not require it. Other rotational orientations of the pyridine ring, such as one in which the pyridine nitrogen is positioned close to the substrate 2'-OH site, must also be considered. The failure of **7** to show good binding suggests that there is not a simple correspondence of the pyridyl C-6 substituent and the substrate C-6' (hydroxymethyl), and even the relatively small C-6 methyl of **5b** is not well-tolerated in this subsite.

While more analogues must be examined before a better understanding of the role of pyridine (pyridinium) as a glucopyranosyl surrogate can emerge, this study identifies the first such compound with glycosidase inhibitory activity, and demonstrates the ease with which pyridine-containing pseudo-sugars can be assembled. Future investigations might center on varying the pyridine substitution to produce a potential quinone methide precursor such as **10a**, or an amidine-like (carboxylate-binding) structure **10b**.

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