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Synthesis of a Ribofuranosyl Cation Mimic

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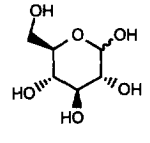
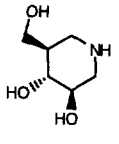
Abstract: (3S,4S)-3,4-dihydroxy-3-hydroxymethylpyrrolidine was prepared and found to be a transitionstate analogue for β-D-ribofuranosyl cleavage.

In a project with the aim of creating glycoside-cleaving catalytic antibodies we were puzzled by the information that a *N*-benzylpiperidine could act as a transitionstate analogue for preparation of catalytic antibodies with the ability to hydrolyse phenolic tetrahydropyranyl ethers[1]. Though the interpretation of those results were not completely unambigeous we decided to elaborate on them and prepare isofagomine, an analogue of glucose having nitrogen instead of the anomeric carbon. We discovered that isofagomine was a powerful inhibitor of glucoside hydrolases, particularly β-glucosidase[2], and thus indeed seemed to be a good transitionstate analogue. Subsequently it was reported by Ichikawa et al. that the galacto-isomer of isofagomine was a potent inhibititor of β-galactosidase[3]. These results indicate a predictable way of creating transitionstate analogues and consequently powerful and specific inhibitors of glycoside hydrolases, by substituting the anomeric carbon and ring-oxygen of the glycon with nitrogen and carbon respectively. We have attempted to investigate this hypothesis in the present work.

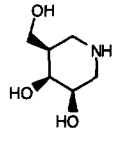


D-glucose



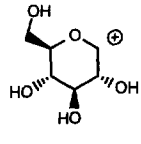
Isofagomine

K_i: 110 nM
(β-Glucosidase)

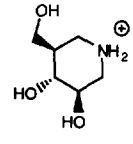


***galacto*-isofagomine**

K_i 4 nM
(β-Galactosidase)



D-glucosyl cation



protonated isofagomin

Fig 1

It was particularly interesting to study whether a pyrrolidine analogue of isofagomine would be a transitionstate analogue for furanoside-cleavage. Such a compound should accordingly inhibit a pentofuranoside cleaving enzyme, but not significantly inhibit other pyranoside cleaving glycosidases. An enzyme of this kind is purin nucleoside phosphorylase (PNP'ase), which catalyses conversion of purin deoxyribo- or ribonucleosides to purin nucleobase and α-D-ribofuranose-1-phosphate (fig 2, R'= purin, X= PO_4^{2-}). The transitionstate for this reaction is the ribofuranosyl cation, and the corresponding transitionstate analogue of isofagomin-like structure is (*3S,4R*)-3-hydroxy-4-hydroxymethylpyrrolidine. We decided however to prepare the analogue with an extra hydroxygroup at the 4-position because the 4-OH was

believed to be less important, and because both it and its enantiomer could more readily be prepared in optical pure form from carbohydrate starting materials.

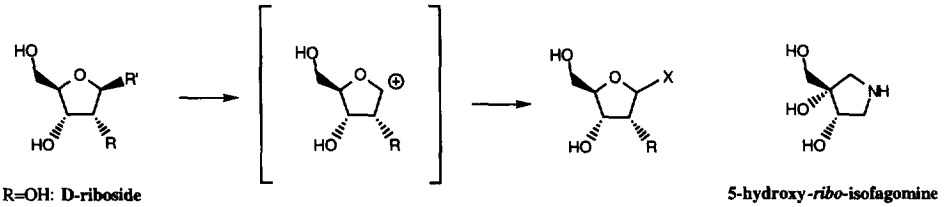
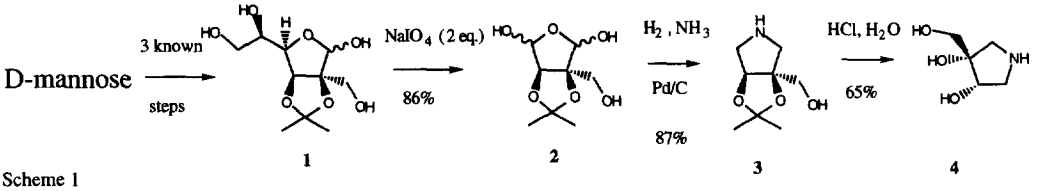


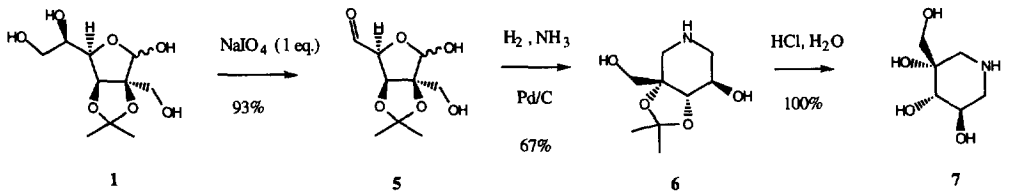
Fig 2

This compound "4-hydroxy-*ribo*-isofagomine" or (3S,4S)-3,4-dihydroxy-3-hydroxymethyl-pyrrolidine (**4**) was prepared as shown in scheme 1. Using the aldolkondensation procedure of Ho[4] D-mannose was converted into its 2,3:5,6-diisopropylidenefuranoside[5], which was hydroxymethylated using formaldehyde and K_2CO_3[4], and the 5,6-acetonide was selectively hydrolysed[4] to give known branched sugar **1**. Treatment of **1** with 4 equivalents of $NaIO_4$ (H_2O, 18 h, 25 °C) led to dialdehydosugar **2** in 86% yield. As expected the NMR spectrum of **2** revealed it to be complex mixture of anomers and aldehyde hydrates. However on high pressure reductive amination (0.25 M NH_4OH, 37 atm. H_2, 5% Pd/C, MeOH) the material transformed into pyrrolidine **3**[6] in 87% yield. High pressure of H_2 and a low concentration



Scheme 1

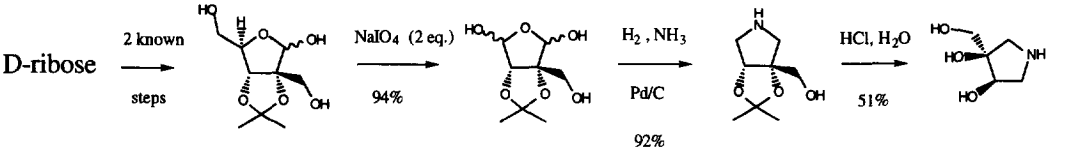
of NH_3 was important in this reaction to avoid formation of sideproducts. Furthermore it was found to be important to remove the formaldehyde formed in the previous periodate cleavage from the starting material **2**, because it led formation of rather large amounts of N-methylated **3**. Flash chromatography of **2** could solve this problem. Finally acidic hydrolysis (HCl, 4 M, 50-55 °C, 2h) of **3** led to cleavage of the isopropylidene to give the hydrochloride of **4**[6] ($[\alpha]_D$ +7.8° (*c* 0.4, H_2O)) in 65 % yield.



Scheme 2

Having **1** available we also prepared the known[7] 5-hydroxy-analogue of isofagomine **7** (scheme 2). Selective cleavage of **1** with 1.1 equivalent of $NaIO_4$ (MeOH-H_2O 1:1, 2 h, 0 °C) gave **5** in 93% yield, which by reductive amination (0.25 M NH_4OH, 37 atm H_2, 5% Pd/C, MeOH) gave the crystalline derivative **6**[6] (mp 97-100 °C, $[\alpha]_D$ +32.2° (*c* 1, EtOH)) in 67% yield. As in the synthesis **4** chromatographic purification of periodate cleavage product **5** was important to remove formaldehyde, that otherwise lead to N-methylated

product in the next step. Acidic hydrolysis of **6** (0.1 M HCl/MeOH, 50-55 °C, 2h) gave **7**[7,8] ($[\alpha]_D$ -22.7° (*c* 1, EtOH)) as the hydrochloride in quantitative yield. This synthesis represent some improvement to the recently published 10 step synthesis[7] of **7**.



Scheme 3 **8** **9** **10** **11**

The enantiomer of **4** was also prepared optically pure with the benefit of Ho's chemistry (scheme 3). D-ribose was by acetonation[9] and aldolcondensation with formaldehyde[4] converted to known **8**. $NaIO_4$-cleavage (2 equiv., H_2O, 18 h, 25 °C) gave **9** in 94% yield, which by reductive amination (0.25 M NH_4OH, 102 atm H_2, 5% Pd/C, EtOH) gave **10**[6] in 92% yield, which finaly by acidic hydrolysis (4 M HCl, 25 °C, 18 h) gave the hydrochloride of enantiomeric pyrrolidine **11**[6] (mp 104-9 °C, $[\alpha]_D$ -10.8° (*c* 1, EtOH)).

Compounds **4**, **6**, **7** and **11** was tested for inhibition of α-glucosidase from bakers yeast (EC 3.2.1.20)[10], β-glucosidase from almonds (EC 3.2.1.21)[11] and purin nucleoside phosphorylase from human blood (EC 2.4.2.1)[12]. The pyrrolidines **4** and **11** showed weak and unspecific inhibition of α- and β-glucosidase. Only 5-hydroxy-isofagomine (**7**) had significant effect on these enzymes confirming previous findings[7].

	α-glucosidase[10]	β-glucosidase[11]	PNP'ase from blood[12]
4	3800	1400	180
6	2000	560	>1000
7	230	12.6	>1000
11	2000	1130	-

Table 1. Inhibition constants (K_i) in μM of **4-11** versus enzymes.

Like isofagomine, **7** inhibit β-glucosidase better than α-glucosidase, but the compound is 100 fold less potent than isofagomine[2] against this enzyme. Also inhibition of α-glucosidase is weaker. So the presence of an "extra" hydroxygroup at the exocyclic C-branch is definitely a feature that decreases this compound's glucosyl cation mimicry. Acetonide **6** is a much weaker inhibitor than **7** of both glucosidases, an information that tells that bulk on the α-side of the piperidine is another undesired feature.

4-hydroxy-*ribo*-isofagomine (**4**) was found to be a moderate inhibitor of human blood PNP'ase[13], while neither **6** nor **7** showed any inhibition of this enzyme. This strongly indicate that **4** is a ribofuranosyl cation mimic. However it is to be expected, in analogy with **7**, that the hydroxygroup at the C-branch makes it less so, and that a better mimic would be obtained by removing this OH-group.

This study showed that it was possible to prepare an inhibitor for a furanoside cleaving enzyme by making the corresponding 1-azasugar. This supports the theory that 1-aza-sugars, like isofagomine, are very good transitionstate-analogues of glycosyl cleavage/formation. The consequence is that rational design of specific inhibitors of glycosyl cleaving enzymes is likely to be possible in the future

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6. ^{13}C-NMR (D_2O, pyrrolidine/piperidine numbering)

	C-2	C-3	C-4	C-5	C-6	exocyclic C	Me_2C
3/10	56.9	95.0	84.9	54.3	-	65.9	114.2 28.6 27.7
4/11	53.5	80.8	72.4	50.8	-	65.1	-
6	50.2	81.6	78.0	67.5	48.3	65.4	111.7 29.7 28.8

7. Ichikawa, M.; Igarashi, Y.; Ichikawa, Y. *Tetrahedron Lett.* **1995** *36* 1767-1770.
8. Spectral data of **7** equivalent to reported values (ref 7).
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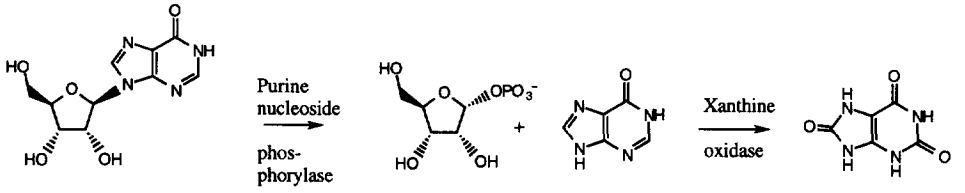
10. α-glucosidase assay was performed according to Halvorson, H.O. *Methods Enzymol.* **1966** *8* 559-562. The type 1 bakers yeast enzyme (Sigma G 5003) was employed to catalyse hydrolysis of 4-nitrophenyl α-D-glucopyranoside to glucose and 4-nitrophenol at pH 6.8 and 22°C with or without inhibitor present. Formation of 4-nitrophenol was followed spectrophotometrically at 400 nm.
11. β-glucosidase assay was performed according to Halvorson, H.O. *Methods Enzymol.* **1966** *8* 559-562. The enzyme from almonds (Sigma G 0395) was employed to catalyse hydrolysis of 4-nitrophenyl β-D-glucopyranoside to glucose and 4-nitrophenol at pH 6.8 and 22°C with or without inhibitor present. Formation of 4-nitrophenol was followed spectrophotometrically at 400 nm.
12. PNP'ase assay was performed according to Stoeckler, J.D.; Agarval, R.P.; Agarwal, K.C.; Parks, R.E. Jr. *Methods Enzymol.* **1978** *51* 530-8. Purin nucleoside phosphorylase from human blood (Sigma N 3514) was employed to catalyse phosphorolysis of inosine to α-D-ribofuranose-1-phosphate and hypoxanthine at pH 7.5 and 22°C in the presence of xanthine oxidase (Sigma X 4875) with or without inhibitor present (see below). Xanthine oxidase catalysed the immidiate oxidation of hypoxanthine to uric acid, formation of which was followed UV-spectrophotometrically at 293 nm.



Inosine hypoxanthin uric acid

13. Since the PNP'ase assay relies on two enzymes it is important to ensure which enzyme is being affected by an inhibitor. Compound **4** did not inhibit xanthine oxidase at the contrations ([**4**] = 1.1 mM) at which the PNP'ase inhibition study was carried out.

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