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INHIBITORS OF GLUCOSIDASES WITH RELATED STRUCTURES AND INVERSE SPECIFICITY

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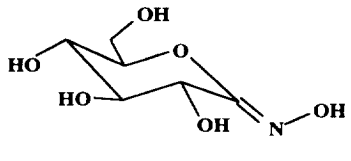
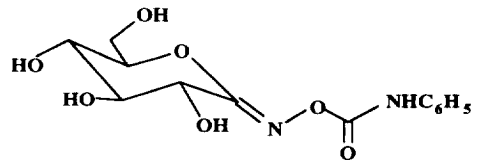
Abstract.
A dinitrophenyl ether (**III**) of gluconolactone oxime **I** was synthesized and tested as an inhibitor of glucosidases. Surprisingly, in contrast to the known compound **II**, **III** was found to be a modest inhibitor of beta-glucosidase, but a good inhibitor of alpha-glucosidase (Ki = 5 μM).

Inhibitors of glycosidases have been considered as compounds of interest for a long time, since some of them could act as antiviral agents by blocking the biosynthesis of viral glycoproteins. Besides this potential role in therapeutics, new inhibitors can give some informations regarding the mechanism of the cleavage of a glycosidic bond by a given enzyme. Inhibitors can either be considered as analogs of substrates in their "ground state" (pseudosugars, deoxynojirimycin, glycosylamines, ...) or as analogs of a reaction intermediate of high energy ("transition-state" analogs: aldonolactones, aldonolactames, amidine-based inhibitors..). In this last case, the inhibitor is supposed to mimic an oxocarbenium intermediate formed by the loss of the aglycone group following its protonation. Thus, it should have a half-chair conformation and, if possible, bear a well placed positive charge [1].

Some years ago, Vasella *et al* reported the synthesis and some inhibitory properties of several aldonolactone oximes derived from **I** [2]. In most cases, these compounds are synthesized as a single geometric isomer with Z configuration, and have been shown by NMR studies to be in a partially half-chair conformation [3]. Gluconohydroxymolactone **I** itself is a rather weak competitive inhibitor of β-glucosidase from almond, with a Ki of 100 μM, whereas the carbamate **II** exhibit a Ki of 2.5 μM. According to Vasella, this improvement could be explained by additional interactions between the aromatic nucleus and an hydrophobic part of the binding site of the enzyme [4].



I



II

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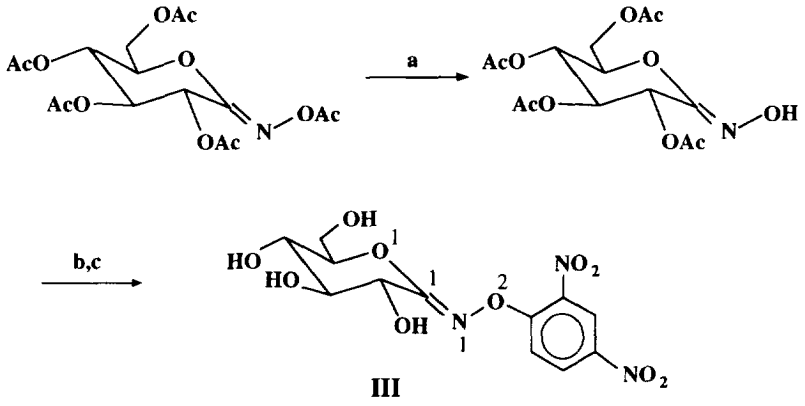
Relying on these findings, we reasoned that a strong electron withdrawing group bound to the oxygen atom of the hydroxymolactone should improve the inhibitory properties by increasing the partial positive charge on the endocyclic oxygen atom and by changing the conformation to a complete half-chair. Compound **III** was synthezised from the known peracetyl hydroxymolactone according to scheme I. It is an amorphous, yellow, very hygroscopic compound, which was fully characterized as its peracetylated derivative (F = 123-125° C, methanol).

Scheme I



a: Hydrazine acetate, 1eq., DMF, 0°--> RT, (98%). **b**: DNFB, 2eq., NaH, 18-C-6, DMF, RT.
c: NEt_3 / MeOH / H_2O 2:8:1, -20°C, 20h (60 % 2 steps)

Then **III** was tested as an inhibitor of β-glucosidase from almond and was found a competitive inhibitor with a Ki around 80 µM (versus 2.5 - 5 µM for **II**, as we measured it in a comparative experiment). **III** is also a weak competitive inhibitor of β–galactosidase from *E.coli* (Ki = 120 µM) but has no significant effect on α-mannosidase from Jack Bean. Since **II** had apparently not been tested on α–glucosidases, we decided to assay both compounds **II** and **III** on α-glucosidase from yeast. To our surprise, **III** was found to be a very good competitive inhibitor, with a Ki value of 2.5 to 5 µM, while **II** showed only moderate competitive-inhibition properties (Ki = 75 µM). Kinetics measurements gave identical results when the experiment was launched either by adding the enzyme to the mixture of substrate plus inhibitor, or by adding the substrate to the enzyme preincubated with the inhibitor, showing that compounds **II** and **III** do not behave as slow-binding inhibitors [5]. Moreover, preincubation of the enzyme for several hours with millimolar concentrations of **III**, followed by elimination of the inhibitor by dilution showed no evidence of an irreversible inactivation.

Thus, although **II** and **III** differ only in their aglycone part, they exhibit inverse specificity towards α- and β-glucosidases. Although there are many examples of improvement of the properties of known inhibitors by changing substituents (for example, by alkylating the nitrogen atom of azasugars [1b, 6]), this is however a rare, if not unique case where a small change in the structure of an inhibitor brings a total reversal

of selectivity (for example, derivatives of 1-deoxynojirimycin bearing various n-alkyl subtituents are all specific of α-glucosidases [1b, 6]).

Due to the strong electron withdrawing character of the dinitrophenyl group, we looked for some subtle differences in the structures of the two transition-state analogs **II** and **III**. However, analysis of the ^1H NMR spectra of the two compounds indicated that the polyhydroxylated cycles have the same conformation (see Table I).

chemical shift, ppm

	C_2-H	C_3-H	C_4-H	C_5-H	C_6-H	C_6-H'
II	4.2	3.78	3.65	4.25	3.95	3.78
III	4.35	3.92	3.82	4.4	4.07	3.92

coupling constant, Hz

	J (2-3)	J (3-4)	J (4-5)	J (5-6)	J (5-6')	J (6-6')
II	5.5	6	10	2	4.5	12.5
III	5.5	6	9.5	2	4.5	12.5

Table I: ^1H NMR data of compounds **II** and **III** (CD_3OD, 250 MHz).

Moreover, the measured coupling constants indicate that both compounds adopt a half chair conformation (sugars in chair conformation have more commonly J (2-3) and J (3-4) around 10 Hz).
^1H NMR of **III** in D_2O gave similarly J values very close to that reported for **II** [2].

That compounds **II** and **III** have the same conformation within the active sites of glucosidases is far from being obvious. It has been demonstrated that **II** has mainly the same conformation in solution and bound to phosphorylase b, for wich it is an inhibitor [7]. On the other hand, a parallel experiment done with **I** indicated that while it is in the same conformation as **II** and **III** in solution, it can adopt a chair conformation when bound to the enzyme. This clearly indicates that this class of compounds has some conformational flexibility [8].

We then looked for a difference in charge distribution on O-1, C-1, N-1, O-2. In fact, ^{13}C NMR spectra showed no difference for C-2 to C-6 of the sugar ring, while the signal attributed to C-1 was shifted 3 ppm downfield in **III** as compared to **II**. This represents however a negligible difference between the two compounds. ^{15}N NMR gave also some information about the charge distribution on N-1 between the two compounds: Chemical shifts are respectively 177,7 ppm and 189,3 ppm for N-1 of **II** and **III** (DMF as an external reference in methanol). Again, this represents a small difference.
This was also independently confirmed by a semi-empirical AM1 calculation [9], which gave very close values for the charges borne by C-1, O-1, N-1 on the two compounds (respectively + 0.09, - 0.24, - 0.166 and + 0.11, - 0.23, - 0.18 on **II** and **III**)[10]. In fact, only C-1 was found to be slightly positive, while O-1 and N-1 were negative. Thus, compounds **II** and **III** (and probably other related products) are good inhibitors mainly because of their half-chair conformation, since they do not bear positive charges on strategic atoms (see introduction).

Aldonolactones are strong inhibitors of β-glycosidases and much less good inhibitors of α-glycosidases. Aldonolactone-oxymes derivatives such as **II** or **III** should behave similarly. Indeed this is the case for **II**. It was postulated that α-glycosides should react in their ground state conformation (chair) in opposition to β-glycosides. In contradiction to this theory, some known inhbitors of α-glycosidases are considered as "transition state " analogs similar to inhibitors of β-glycosidases (Swainsomine, D.I.M., mannolactame amidrazone against α-mannosidases, acarbose against sucrase, isomaltase.....). That similar "transition state" analog structures can act on both classes of enzymes is then not so surprising.

Although the reasons of the change in specificity we observed remain unclear, this work demonstrates that any modification of a substituant on an inhibitor can not only improve or alter its performance on a given enzyme, but also can more deeply change its properties.

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