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Increasing the inhibitory potency of L-*arabino*-imidazolo-[1,2]-piperidinose towards β-D-glucosidase and β-D-galactosidase

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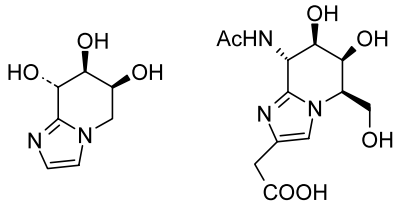
Abstract—The synthesis of some potent inhibitors of two retaining β-glycosidases was achieved by introducing aglycon-mimics into the imidazole moiety of L-*arabino* azasugar **1**. The strongest inhibition was observed with the phenyl-ethyl substituent at C(2) of **1** against β-D-galactosidase and β-D-glucosidase, whereas the hydroxymethyl group at C(2) increased only slightly the inhibitory properties. © 2003 Published by Elsevier Science Ltd.

Several non-natural tetrazole-, triazole-, and imidazole derivatives fused to furanoses or pyranoses have been synthesised as potential transition-state- analogue mimics of glycosidase inhibitors. Indeed, some of them proved to be selective and potent glycosidase inhibitors.[1] Along these lines, L-*arabino*-imidazolo-piperidinose **1** together with its seven stereomers had been prepared in our laboratory, starting from easily available D- and L-erythrose and from D- and L-threose.[2] Tested against six commonly encountered α- and β-glycosidases, the L-*arabino* stereomer **1** inhibited markedly both β-D-glucosidase of almonds ($K_i = 1$ μM) and β-D-galactosidase of *Escherichia coli* ($K_i = 1$ μM).[2] The D-*ribo* and the D-*xylo* stereomers showed a selective but less potent inhibition of β-D-glucosidase of almonds ($K_i = 20$ μM in both cases), whereas the five remaining stereomers were either inactive or of poor activity only.[2] It is worth noticing that nagstatine **2**, which is a powerful inhibitor of *N*-acetyl-β-D-glucosaminidase of bovine kidney ($IC_{50} = 4$ nM), is the only carbohydrate fused to an azole moiety found so far in nature.[3,4] All eight type **1** imidazolo-carbohydrate stereomers are piperidino–pentose derivatives, whereas the six glycosidases with which they have been tested, normally catalyse the hydrolysis of pyrano–aldohexose polysaccharides. Even though the half-chair conformation of these imidazolosugars do mimic an oxocarbenium transition state, they lack the hydroxymethyl group which may be important for optimal docking to most glycosidases' active sites. Therefore, it was unlikely that they would lead to very strong inhibitions. We surmised that a stronger inhibition may result from additional substitution of the imidazole moiety with some aglycone-mimics.[5] Along this line of thought, we describe herein the application of Vasella's methodology to introduce phenylethyl and hydroxymethyl groups to the imidazole moiety of azasugar **1**,[5] as well as the inhibition data of these novel imidazolosugar derivatives.



1

K_i = 1μM
β-D-galactosidase
(*Escherichia coli*)

K_i = 1μM
β-D-glucosidase
(almonds)

2

Nagstatine

In order to obtain the key L-*arabino* intermediate **7**, we developed a new and more straightforward synthetic approach. The dithioethyl-acetal of L-arabinose **3** permitted sequential protection of all four alcohol groups

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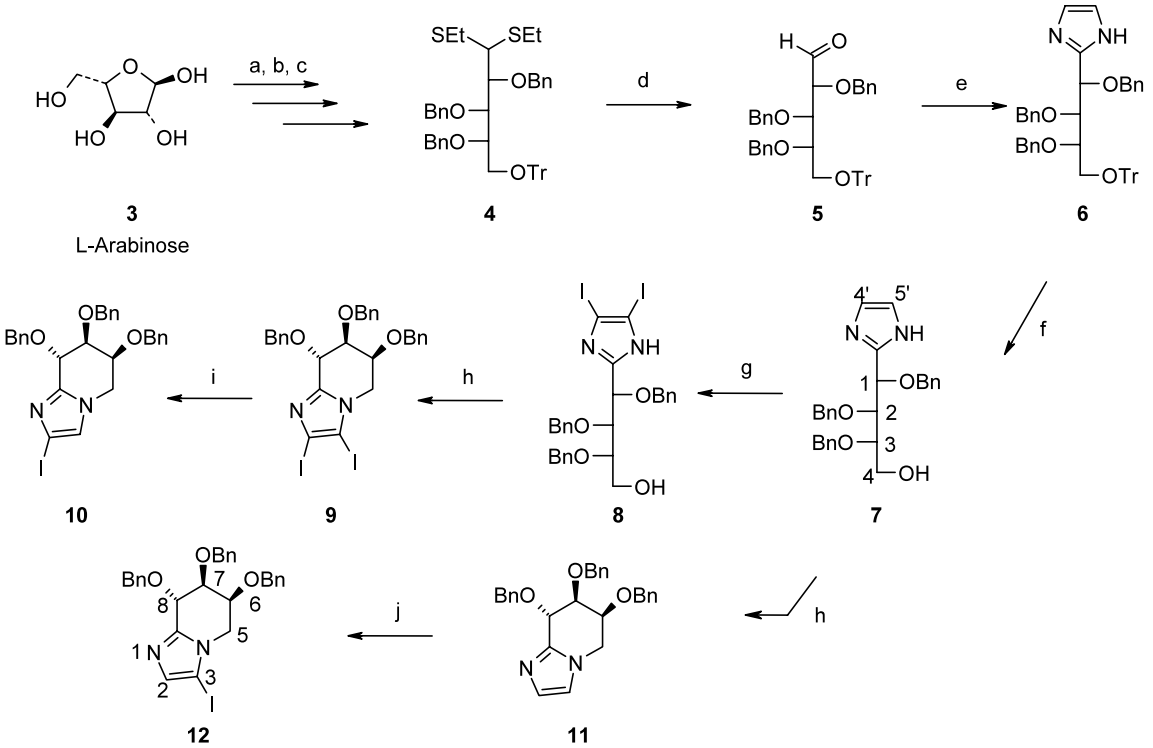
leading thereby to derivative **4** whose selective deprotection gave aldehyde **5**. Condensation of **5** with glyoxal in methanol solution saturated with gaseous ammonia, according to the procedure of Rothenberg, Dauplaise and Panzer,[7,8] gave the linear imidazole **6**. The latter compound was selectively deprotected (HCl 4N) to L-*arabino* derivative **7**.

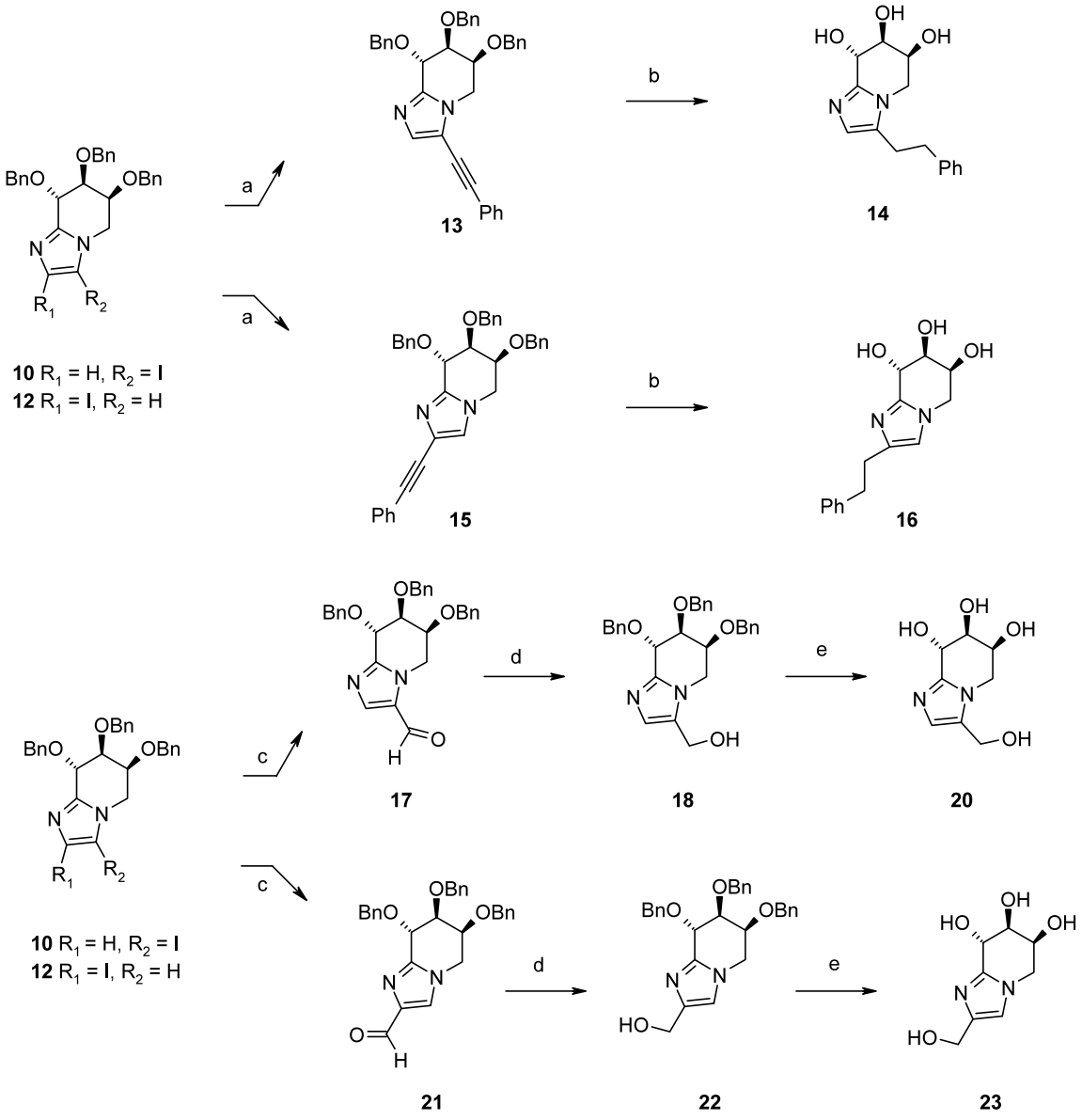
Starting from **7**, two pathways were chosen, in order to introduce an iodine atom, either to C(2) or to C(3), by taking advantage of the known difference of reactivity of these two imidazole carbon atoms.[9] Reaction of **7** with NIS (2.5 equiv.) gave di-iodo compound **8** whose mesylate cyclised at once to **9**, a compound which was selectively reduced to 2-iodo derivative **10**. Alternatively, mesylation of **7** led to **11** via an intramolecular Walden inversion. Iodination of bicyclic compound **11** required NIS in excess (5 equiv.) and gave 3-iodo derivative **12** (Scheme 1).

Introduction of the substituents to carbon atoms C(2) and C(3) was performed as follows. Reaction of the mono-iodo derivatives **10** and **12** with phenylacetylene according to the standard Sonogashira methodology led to **13** and **15**, respectively.[6] Total hydrogenation of the acetylene triple bond of the latter two products over a palladium catalyst was accompanied by hydrogenolysis of the benzyl ether protections, and gave the corresponding phenyl-ethyl imidazolo-sugar target molecules **14** and **16**. A second set of reactions led to the introduction of the hydroxymethyl groups to the same carbon atoms: reaction of the monoiodo derivatives **10** and **12** in THF with ethyl-magnesium bromide, followed by addition of DMF gave in good yield the formyl derivatives **17** and **21**, respectively. Reduction of these aldehyde moieties with $LiAlH_4$, followed by hydrogenolysis over palladium of the three benzyl ether groups, led in good overall yields to the corresponding hydroxymethyl target molecules **20** and **23** (Scheme 2).

The four substituted imidazolo-L-*arabino*-piperidinoses **14**, **16**, **20** and **23** were submitted to inhibition assays which were performed with β-D-galactosidase of *Escherichia coli* and β-D-glucosidase of almonds, according to a methodology described in detail in a previous paper.[2] The inhibition data, as determined via Michaelis–Menten kinetics (Table 1), demonstrate: (i) the very powerful inhibition of β-galactosidase ($K_i = 4$ nM) with imidazolosugar **16**, a compound which also strongly inhibits β-glucosidase ($K_i = 80$ nM); (ii) that all four imidazolo–piperidino derivatives **14**, **16**, **20** and **23** are reversible inhibitors. The inhibition data indicate that substituents at carbon atom C(3) interact unfavourably with retaining β-galactosidase and β-glucosidase, while the substituents at C(2) significantly increase the inhibition. These results are in agreement with a molecular modelling as proposed by Vasella and his collaborators for a β-glucosidase.[10] These authors showed that only substituents at C(2) project into the aglycon binding subsite of such glycosidases, while



Scheme 1. *Reagents and conditions*: (a) HSEt, HCl, 0°C, 15 min, 79%; (b) TrCl, DMAP cat., pyridine, 80°C, 3 h, 97%; (c) BnBr, NaH, Bu_4NI cat., DMF, 0°C to rt, 96%, (d) $Hg(ClO_4)_2 \times H_2O$, $CaCO_3$, THF/H_2O, rt, 2 h, 66%; (e) glyoxal, $MeOH/NH_3$, −20 to 80°C, 1 h, 62%; (f) HCl 4N, dioxane, 80°C, 2 h, 81%; (g) NIS, CH_3CN, rt, 12 h, 91%; (h) MsCl, pyridine, 0–80°C, 87% of **9**, and 92% of **11**; (i) EtMgBr, CH_2Cl_2, 0°C to rt, 30 min, 87%; (j) 5 equiv. NIS, CH_3CN, 80°C, 24 h, 57%.



Scheme 2. *Reagents and conditions*: (a) phenylacetylene, $Pd(PPh_3)_4$, CuI, Et_3N, DMF, 80°C, 3 h, 92% of **13** and 62% of **15**; (b) H_2, $Pd(OH)_2/C$, EtOH/AcOH, rt, 12 h, 68% of **14** and 56% of **16**; (c) EtMgBr, THF, DMF, 0°C to rt, 2 h, 88% of **17** and 84% of **21**; (d) $LiAlH_4$, THF, −78 to −30°C, 1 h 30, 85% of **18** and 83% of **22**; (e) H_2, $Pd(OH)_2/C$, EtOH/AcOH, rt, 12 h, 87% of **20** and 68% of **23**.

Table 1. Inhibition constants of C(2)- and C(3)-substituted imidazolo-L-*arabino*-piperidinoses

	1 (μM)	**14** (μM)	**16** (μM)	**20** (μM)	**23** (μM)
β-D-galactosidase (*Escherichia coli*)	1	2	**0.004**	70	0.6
β-D-glucosidase (Almonds)	1	15	**0.080**	20	**0.1**

substituents at C(3) interact unfavourably with the active site. We assume that the strong inhibition by the phenyl-ethyl derivative **16** reflects hydrophobic interactions of the substituents at C(2) with putative aromatic residues located close to the aglycon binding site of these glycosidases.

Since the D-*ribo* and D-*xylo* stereomers also exhibited inhibition of glycosidases,[2] we shall prepare several imidazolosugars possessing a range of hydrophobic substituents at their imidazole C(2) carbon atom, in order to confirm the structure-activity relationship as determined herein for the L-*arabino* derivative **16**.

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