Stereoselective synthesis of (2*S*,3*S*,4*R*,5*S*)-5-methylpyrrolidine-3,4-diol derivatives that are highly selective α-L-fucosidase inhibitors†

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***N*-Phenylaminomethyl benzimidazolyl moieties attached at C-2 of (2*S*,3*S*,4*R*,5*S*)-5-methylpyrrolidine-3,4-diol increase the potency and selectivity of the inhibitory activity of these systems towards α-L-fucosidases.**

Glycosidases are enzymes playing a crucial role in the biosynthesis of glycoproteins.[1] They modulate the cellular processes including cell/cell, cell/invader recognition and inflammation.[2] Inhibitors of glycosidases are potential drugs against diseases that imply cellular dysfunctions or alter cell/invader communication.[3,4]

1,5-Dideoxy-iminoalditols or 1,4-dideoxy-1,4-iminoalditols (aza-sugars) resembling natural sugar substrates and/or mimicking the corresponding oxycarbenium ion intermediate are classical glycosidase inhibitors.[5] Unfortunately these compounds quite often lack enzyme specificity.

We have found that simple *meso*-pyrrolidine-3,4-diol (**1**) is a weak and non-selective glycosidase inhibitor.[6] Enzyme selectivity can be improved if the iminosugar, which mimics the pyranosyl cation, includes some information on the aglycon undergoing the hydrolytic process.[7] We have reported that activity and selectivity can be improved on joining aromatic and heteroaromatic moieties to **1**. Thus, derivatives **2** with (2*R*)-aminomethyl side chains can be highly selective and competitive inhibitors of α-mannosidases.[8] Derivatives with (2*R*)- or (2*S*)-aminoethyl side chains **3** or **4** are less selective. Enantiomers *ent*-**3** and *ent*-**4** are moderate and specific inhibitors of β-glucosidases.[6] We have also found that [(2*S*,3*S*,4*R*)-3,4-dihydroxypyrrolidin-2-yl]furan derivatives **5** are good β-galactosidase inhibitors whereas their C-2 epimers **6** are good and selective α-L-fucosidase inhibitors.[9]

α-L-Fucosidases participate in the last stages of glycoprotein biosynthesis and their inhibitors have been found to inhibit the cytophatic effect of HIV and reduce infection.[4] α-L-Fucosidases also facilitate sperm transport and sperm–egg interactions. Inhibitors of these enzymes could have contraceptive properties.[10] Consequently, a great deal of effort has been made towards the synthesis of new fucosidase inhibitors.

Up to now, the most potent fucosidase inhibitors are derivatives of 1,5-dideoxy-1,5-iminoalditols, such as L-fuconojirimycin (**7**) which inhibits α-L-fucosidases with K_i values in the low nanomolar range. Structural modifications of **7** generated less potent inhibitors.[11,12]

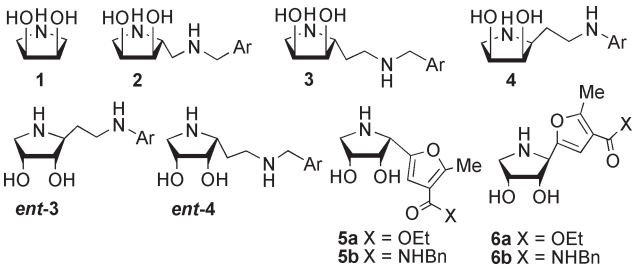

Chart 1

Interestingly, however, Wong and co-workers have found that (1*R*)-aminomethyl-1-deoxy-L-fucononojirimycin (**8**) is a useful template to construct libraries of amides that can be very potent α-L-fucosidase inhibitors. For instance, amide **9** is a picomolar slow, tight binding inhibitor of α-L-fucosidases.[13]

Derivatives of 1,4-dideoxy-1,4-iminoalditol can also be good α-L-fucosidase inhibitors,[11,14–17] whereas 1,6-dideoxy-1,6-iminoalditols have shown up to now only moderate inhibitory activities.[18] Recent work in the search for anti-cancer agents has shown that α-mannosidase inhibitors such as **2** have low cell membrane permeability and must be esterified to generate compounds with anti-cancer activity.[19] This suggests that less polar compounds than fuconojirimycin analogues **9** might be required to construct α-L-fucosidase inhibitors able to penetrate cells. We thus have decided to explore the use of (2*S*,3*S*,4*R*,5*S*)-5-methylpyrrolidine-3,4-diol derivatives **10** as templates for the creation of potential drugs. It is believed that analogs of **10** with fewer hydroxyl groups represent a novel scaffold for α-L-fucosidase inhibitors with potentially improved properties. Imitating the strategy we used to develop selective α-mannosidase inhibitors,[6,8] we are now searching for derivatives of type **10** with high α-L-fucosidase inhibitory activity and high selectivity toward this type of enzymes. Pyrrolidine-diol derivative **10** can be made applying a chemoenzymatic cross-aldol reaction of (2*S*)-azido propanal and dihydroxyacetone monophosphate.[16] Stereoisomers of **10** were obtained *via* an asymmetric Diels–Alder reaction of (*E*,*E*)-sorbaldehyde dimethyl acetal with an α-chloronitroso-D-mannose derivative[17] through a 13-step synthetic route.

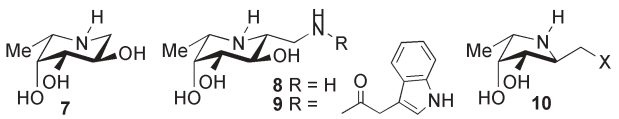

Chart 2

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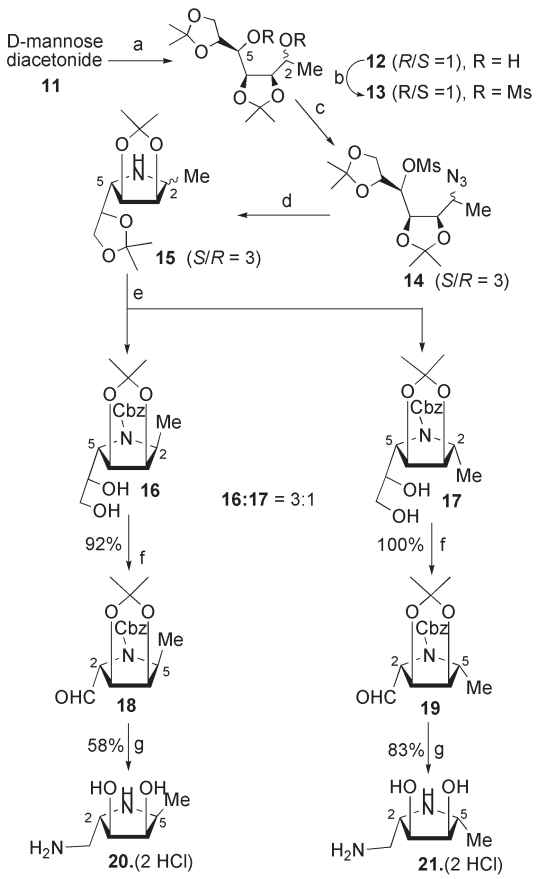
We disclose here an alternative shorter and more efficient route to systems **10** and their conversion into new selective α-L-fucosidase inhibitors.

According to Wightman and co-workers,[20] the reaction of methylmagnesium chloride with commercially available D-mannose diacetonide (**11**) should occur already at −78 °C and give a major diol. In our hands, no reaction occurs below 20 °C. At room temperature, a 1 : 1 mixture of diastereoisomeric diols (2*R*)-**12** and (2*S*)-**12** was formed and isolated in 95% yield. Standard esterification of the diols with an excess of methanesulfonyl chloride and DMAP provided a 1 : 1 mixture of dimesylates (2*R*)-**13** and (2*S*)-**13** in 74% yield (Scheme 1). Using tetrabutylammonium azide (generated *in situ* by reaction of Me_3SiN_3 and Bu_4NF in DMF) as nucleophile, the chemoselective S_N2 displacement of the mesyloxy group at C-2 of **13** is by some means faster with (2*R*)-**13** than with (2*S*)-**13**. Indeed, after 75% conversion (DMF, 90 °C) a 3 : 1 mixture of azides (2*S*)-**14** and (2*R*)-**14**, respectively, was formed and isolated in 55% yield.

The selectivities observed are attributed to steric factors. Mesylate (2*S*)-**13** offers a population of rotamers less suitable for the S_N2 displacement than (2*R*)-**13**.[21]

Catalytic hydrogenation of azides **14** gave a 3 : 1 mixture of primary amines that were not isolated but treated directly with DBU in MeOH. This promoted the intramolecular displacement



Scheme 1 ‡*Reagents and conditions*: a) MeMgCl, THF, r.t., 95%; b) MsCl, pyridine, DMAP, 74%; c) $TMSN_3$ (4 eq.)/TBAF (4 eq.), DMF, 90 °C, 4.5 h, 55%; d) (i) H_2, Pd/C, MeOH; (ii) MeOH, DBU, 99% (i + ii); e) (i) CbzCl, $NaHCO_3$, EtOH/H_2O (1/1); (ii) $Zn(NO_3)_2{\cdot}6H_2O$, MeCN, 50 °C; (iii) Flash chromatography; 58% (i + ii + iii); f) $NaIO_4$, THF–H_2O; g) (i) $BnNH_2$, $NaBH(OAc)_3$, $ClCH_2CH_2Cl$; (ii) H_2, Pd/C, MeOH; (iii) HCl (1 M).

of the mesyloxy group at C-5 giving a 3 : 1 mixture of (2*S*)-**15** and (2*R*)-**15** in quantitative yield. The ratio of pyrrolidines **15** indicates the stereoselectivity of the displacement reaction at C-2 in the open chain compounds **13** that gives the azido derivatives **14**. Amine protection as benzylcarbamate followed by selective hydrolysis of the least sterically hindered acetonides (H_2O/MeCN, $Zn(NO_3)_2$)[22] provided a 3 : 1 mixture of diols **16** and **17** that were readily separated by flash chromatography on silica gel (58% overall on three steps). Standard oxidative cleavage of the diols **16** and **17** with $NaIO_4$ furnished aldehydes **18** (92%) and **19** (100%), respectively.

Attempts to generate *N*-benzylpyrrolidine analogues of **15** by direct reaction of bis-mesylates **13** with benzylamine were not successful, even on using better leaving groups such as iodide or triflates. Similarly, oxidation of diols **12** into the corresponding 1,4-diketone and subsequent reductive amination using ammonium formate and $NaBH_3CN$[23] failed to produce any pyrrolidine derivative, but led to the formation of mixtures of diastereoisomeric tetrahydrofuran derivatives.

Structures of the 2,5-dideoxy-2,5-iminoalditol derivatives **16** and **17** were established by their spectral data. A strong NOE was observed between the signals of **16** assigned to pair of protons H2 (δ = 3.85)/H3 (δ = 4.59), thus confirming the (2*S*)-configuration. In the case of **17**, a NOE was observed between the signals assigned to H2 (δ = 3.96) and H5 (δ = 4.15) and and also between signals assigned to H3 (δ = 4.37) and Me (δ = 1.28), thus confirming the (2*R*,5*S*)-configuration. No NOE between signals assigned to protons H4/H5 and a coupling constant $J_{4,5}$ = 0 in both compounds is observed, thus confirming the *trans* relative configuration of these protons.

In order to obtain 1,2-diamines to be used for the rapid discovery of new glycosidase inhibitors through a combinatorial approach,[8] we have synthesized diamines **20**[14] and **21** by reductive amination of aldehydes **18** and **19** with $BnNH_2$ followed by deprotection (Scheme 1). On the other hand, reductive amination of aldehyde **18** with aniline provided **22** (75%). After deprotection of **22** under standard conditions, diamine **24** was obtained in quantitative yield (Scheme 2).

Oxidation of aldehyde **18** gave the corresponding carboxylic acid **23** (82% yield). It reacted with *o*-phenylenediamine in the presence of PyBOP and DIPEA to give amide **25** in 65% yield. On heating in AcOH at 50 °C,[24] benzimidazole **26** was formed and isolated in high yield. Deprotection of **26** under standard conditions gave benzimidazole **27** (Scheme 2). The same reaction sequence applied to aldehyde **19** provided benzimidazole **28**. The structures of **27** and **28** were based on their spectral data and confirmed by NOE experiments.

Diamines **20**, **21**, **24** and benzimidazoles **27** and **28** have been analyzed for their inhibitory activities towards fifteen commercially available glycosidases.[25] Apart from a weak inhibition (34% at 1 mM concentration) towards β-galactosidases from bovine liver, diamine **20** was a selective and competitive inhibitor of α-L-fucosidases from bovine epididymis (K_i = 1.8 μM)[26] and did not inhibit α-galactosidases from coffee beans and from *E. coli*, β-galactosidases from coffee beans and from *E. coli*, *Aspergillus niger* and *Aspergillus orizae*, α-glucosidases from yeast and from rice, amyloglucosidases from *Aspergillus niger* and from *Aspergillus mold*, β-glucosidases from almonds and from *Saccharomyces cerevisae*, α-mannosidases from Jack beans and from almonds, β-mannosidases from *Helix pomatia*, β-xylosidases from *Aspergillus*

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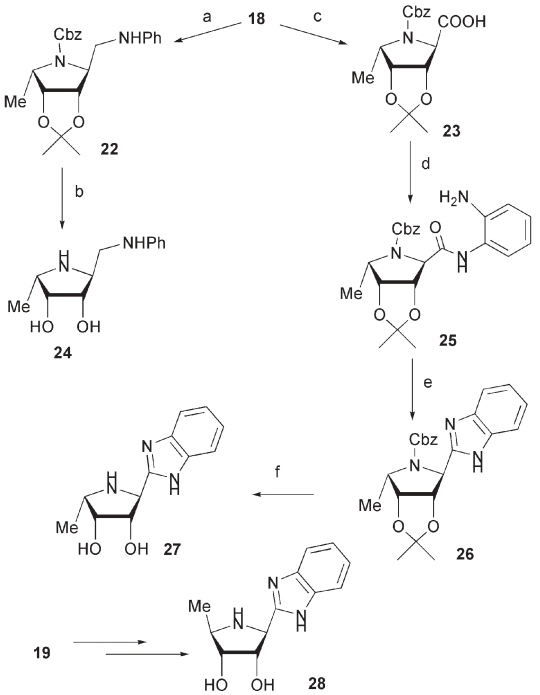

Scheme 2 *Reagents and conditions*: a) $PhNH_2$, $NaBH(OAc)_3$, 1,2-dichloroethane; 75%; b) (i) HCl (1 M); (ii) H_2, Pd/C, MeOH; 100% (i + ii); c) $NaClO_2$, KH_2PO_4, Bu^tOH–H_2O, 2-methyl-2-butene, 82%; d) *o*-phenylenediamine, PyBOP, DIPEA, DMF, 65%; e) AcOH, 50 °C, 100%; f) (i) THF : HCl (1 M) 1 : 1, (ii) H_2, Pd/C, MeOH; 87% (i + ii).

niger, *N*-acetylgalactosaminidase from chicken liver and β-*N*-acetyl glucosaminidases from Jack beans and from bovine kidney. Diastereoisomeric diamine **21** is a much weaker inhibitor of α-L-fucosidases from bovine epididymis (52% at 1 mM concentration) and inhibits α-glucosidases from yeast (37% at 1 mM) and α-mannosidases from Jack beans (36% at 1 mM). The aniline derivative **24** is a selective inhibitor of α-L-fucosidase from bovine kidney and from bovine epididymis (K_i = 0.24 μM, competitive). Thus, as for α-mannosidase inhibitors of type **2**, the aromatic moiety enhanced the inhibitory activity of diamine **20** by a factor of about 10. It contributes also to the high selectivity of the inhibitor toward a single type of glycosidases. This phenomenon appears to be further enhanced with benzimidazole **27** which is a competitive inhibitor of α-L-fucosidase from bovine kidney with K_i = 80 nM. Importantly, this compound did not inhibit any of the other enzymes assayed. As expected, benzimidazole **28** is a much weaker inhibitor of α-L-fucosidase from bovine epididymis (94% inhibition at 1 mM, K_i = 240 μM) than **27** (100% inhibition at 1 mM). Furthermore, **28** is not a selective inhibitor as it inhibits also β-galactosidase from bovine liver (48%), α-glucosidase from yeast (94%, K_i = 46 μM, uncompetitive) and α-mannosidases from Jack beans (80%) and from almonds (60% inhibition at 1 mM concentration, optimal pH).

In conclusion, a new method has been developed for the preparation of (2*S*,3*S*,4*R*,5*S*)-5-methylpyrrolidine-3,4-diols bearing either aminomethyl or heterocyclic moieties at C-2. New types of highly selective and competitive inhibitors of α-L-fucosidases have been discovered. Although they are less active than 1-deoxyfuconojirimycin analogs reported,[13] they are less polar than the latter and thus represent valuable leads as *in vivo* α-L-fucosidase inhibitors.

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‡ For the sake of clarity, the numbering indicated in the scheme was used.

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