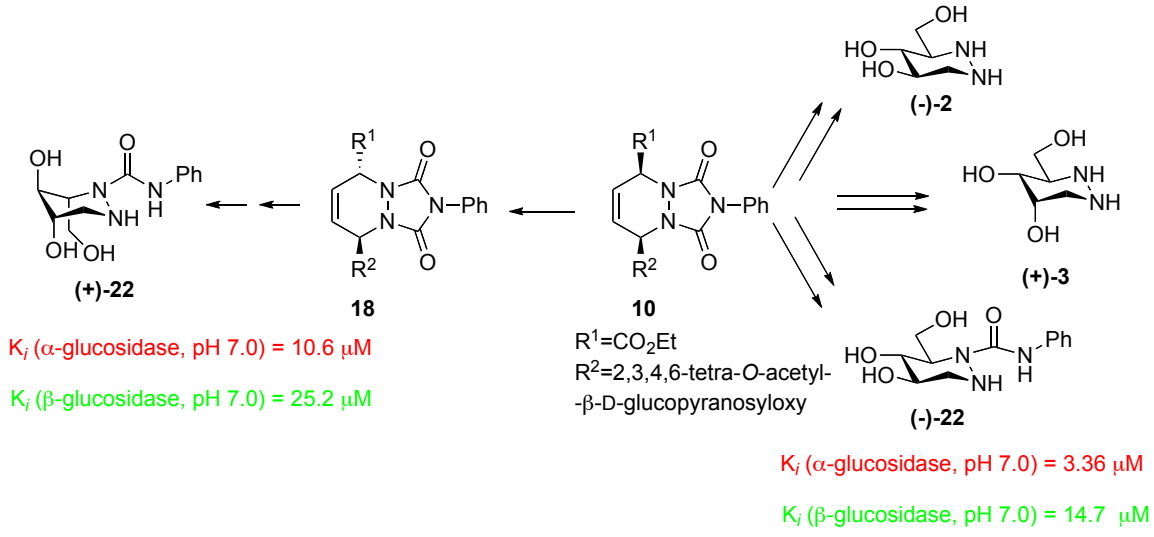
Advances in the Synthesis of Homochiral (-)-1-Azafagomine and (+)-5-*epi*-1-Azafagomine. 1-*N*-Phenyl Carboxamide Derivatives of both Enantiomers of 1-Azafagomine: Leads for the Synthesis of Active α-Glycosidase Inhibitors.

M. J. Alves;[a]* F. T. Costa;[b] Vera C. M. Duarte;[a] A. Gil Fortes;[a] J. A. Martins;[a] N. M. Micaelo[a]

[a] *Departamento de Química, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal.*

[b] *Faculdade de Ciências da Saúde, Universidade Fernando Pessoa, R. Carlos da Maia, 296, 4100-150 Porto, Portugal.*

*Corresponding Author E-mail Address: mja@quimica.uminho.pt

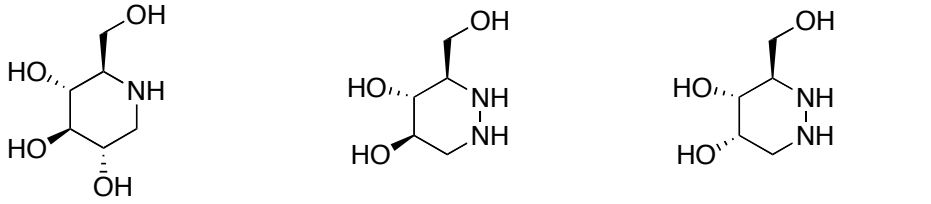


Abstract- A new expeditious preparation of homochiral (-)-1-azafagomine, and (+)-5-epi-1-azafagomine has been devised. Stoodley´s diastereoselective cycloaddition of dienes bearing a 2,3,4,6-tetraacetyl glucosyl chiral auxiliary to 4-phenyl-1,2,4-triazole-3,5-dione, was merged with Bols protocol for functionalizing alkenes into molecules bearing a glucosyl framework. Homochiral (+)-5-epi-1-azafagomine was synthetized for the first

time. Partial reductive cleavage of the phenyltriazolidinone moiety afforded new homochiral 1-N-phenyl carboxamide derivatives of 1-azafagomine. Both enantiomers of these derivatives were synthetized and tested, displaying a very good enzymatic inhibition towards baker´s yeast α-glucosidase. The molecular recognition mechanism of the 1-N-phenyl carboxamide derivative of 1-azafagomine by α-glucosidase from baker´s yeast was studied by molecular modelling. The efficient packing of the aromatic ring of the 1-N-phenyl carboxamide moiety into a hydrophobic sub-site (pocket) in the enzyme´s active site, seems to be responsible for the improved binding affinity in relation to underivatized (-)1-azafagomine and (+)1-azafagomine.

Introduction

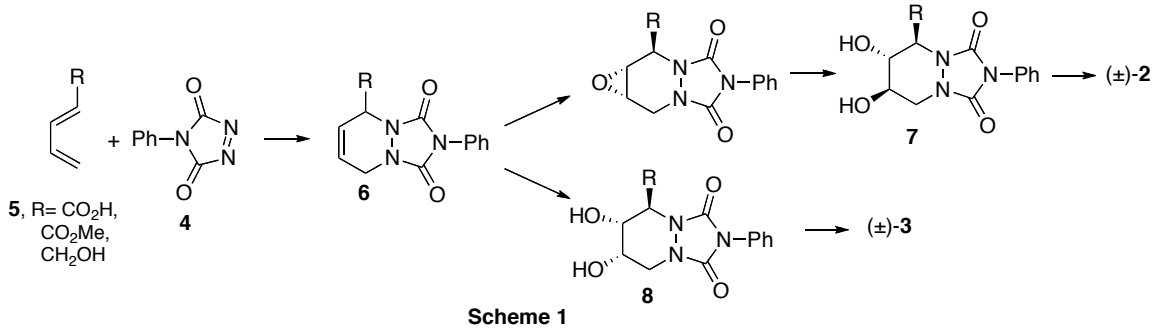
The synthesis of iminosugars is receiving an increasing interest because many of these structures are biological tools and potential therapeutics. The first iminosugar medicine registered was miglitol, (Glyset, PHARMACIA and UPJOHN).[1] The biological properties of iminosugars arise from their interference with glycosidases, the natural carbohydrate degrading enzymes, and with carbohydrate recognizing receptors spread in all living organisms. 1-Deoxynojirimycine (**1**) is a natural iminosugar resembling the structure of glucose. The biological activity of this compound seems to be dependent on its conjugated ammonium form mimicking the transition state for glycoside cleavage.[2] Bols and co-workers have demonstrated that (-)-1-azafagomine (**2**) is a potent competitive inhibitor of almond β-glucosidase (K_i= 0.32 μM), yeast α-glucosidase (K_i= 6.9 μM) and isomaltase (K_i= 0.27 μM).[3] On the other hand, racemic (±)-5-*epi*-1-azafagomine (**3**), was found to be a much weaker glycosidase inhibitor of almond β-glucosidase (K_i= 137 μM) and *E. coli* β-galactosidase (K_i= 149 μM) (Figure 1).[4] 5-*epi*-1-Azafagomine (**3**), as far as we could find, was previously unknown in any of the enantiomeric pure forms.



(+)-1-Deoxynojirimycine (**1**) (-)-1-Azafagomine (**2**) (±)-5-*epi*-1-Azafagomine (**3**)

Figure 1 Structure of some known iminosugars

The synthesis of homochiral (-)-1-azafagomine (**2**) was accomplished by Bols and co-workers through a synthetic sequence based on the Diels-Alder cycloaddition to 4-phenyl-1,2,4-triazole-3,5-dione (PTAD) **4** to achiral dienes: 2,4-pentadienoic acid, methyl 2,4-pentadienoate and 2,4-pentadienol (**5**).[4] The racemic cycloadduct **6** obtained from 2,4-pentadienol and PTAD was resolved by lipase-mediated transesterification. The olefin portion of each enantiomer´s precursor of **2** was oxidized to oxirane and further opened under highly acidic conditions to yield the glucosyl framework of 1-azafagomine, compound **7**. After hydrazinolysis, both enantiomers of 1-azafagomine were obtained in 9% total yield[5] (Scheme 1). Osmilation of the double bond on the racemic cycloadduct **6**, led to racemic diol **8**, which after hydrazinolysis gave 5-*epi*-1-azafagomine (**3**). Bols[3] also achieved the synthesis of (-)-1-azafagomine (**2**) from relatively expensive L-xylose in 6 steps. L-2,3,5-Tribenzyl xylofuranose was isolated as an intermediate after 3 steps with no explicit yield. 1-Azafagomine was then isolated in 37% overall yield from this intermediate.[6,7]



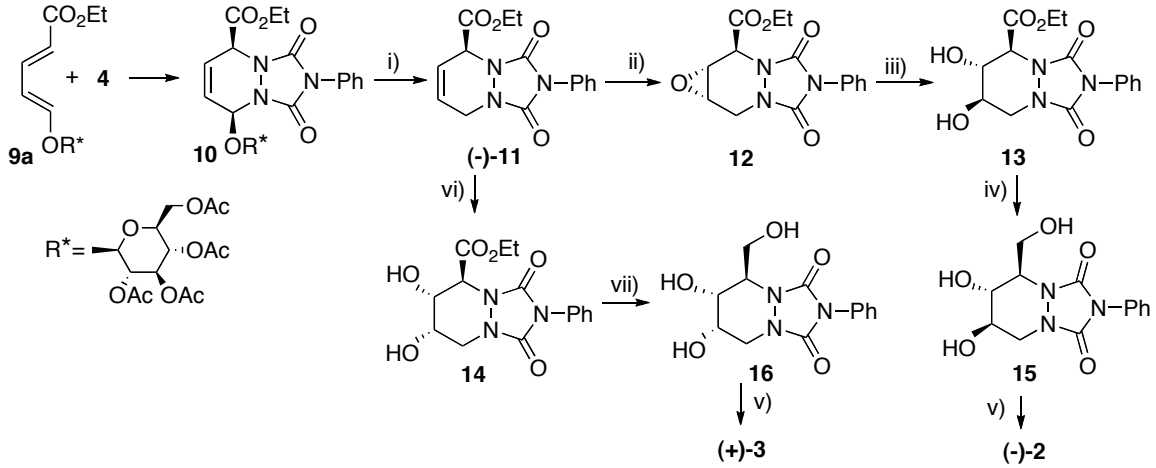
Scheme 1

Alternatives to the enzymatic resolution of racemic adducts of type **6** are desirable for the production of chiral synthons for further elaboration into homochiral compounds. Stoodley in the nineties combined 2,4-pentadienoates, bearing a tetraacetyl glucosyl chiral auxiliary in the position 1, compound **9a**, with PTAD to obtain cycloadduct **10** in 70 % yield and in a high degree of diastereo-selectivity (Scheme 2).[8,9] The chemistry of cycloadduct **10** had been pushed forward for the synthesis of dehydropiperazic acid, a non-proteinogenic amino acid constituent of antrimycins-linear heptapeptides with antitubercular activity.[10] Lately, dienes bearing oxazolidinone chiral auxiliary were combined with PTAD to generate (*S*)-piperazinic acid.[11] To the best of our knowledge, nobody has merged the Stoodley cycloaddition entry into chiral alkenes of type **11** with Bols´s olefin functionalization methodology for synthetizing enantiopure iminosugars.

Results and Discussion

New synthetic sequence for preparing homochiral (-)-1-azafagomine, and (+)-5-*epi*-1-azafagomine.

In this paper we report a new synthetic route for obtaining homochiral (-)-1-azafagomine **(-)-2**, and (+)-5-*epi*-1-azafagomine **(+)-3** from chiral alkene **(-)-11**. *(*Scheme 2*)*

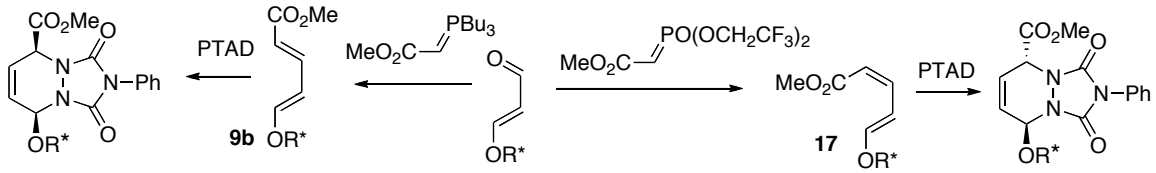


Scheme 2 - i) Et_3SiH, TFA, DCM, 5h, rt, 61 %; ii) oxone, CF_3COCH_3, $NaHCO_3$, CH_3CN/H_2O (3.5:2), 24h, 65% iii) H_2O, H_2SO_4 (exc.), reflux, 8h, 52%; iv) $NaBH_4$ (3 eq.), EtOH, 3d, rt, 59 %; v) $NH_2NH_2.H_2O$, 100 ºC, 18h, 68 % (-)-**2**; 64 % (+)-**3**; vi) OsO_4, NMO, Acetone:H_2O (2:1), 5d, rt, 79 %; vii) $NaBH_4$ (3 eq.), EtOH, 3d, rt, 52%.

Cycloadduct **10** was submitted to reductive cleavage with triethylsilane, according to Stoodley´s protocol, to generate known compound **(-)-11**.[9] Treatment of **11** with oxone/trifluoroacetone in the presence of $NaHCO_3$ at room temperature for 1 day, generated a 3:1 mixture of oxiranes as reported previously by Bols for his racemic compounds.[4] Selective crystallization afforded the major isomer **12** in 65 % yield. Opening of oxirane **12** was achieved with total *regio*- and *stereo*-selectivity by refluxing in aqueous H_2SO_4 giving **13**. Osmilation of compound **11** produced *cis*-diol **14** with total *stereo*-selectivity. Selective reduction of *trans*-diol **13** and *cis*-diol **14** with $NaBH_4$, gave respectively triols **15** and **16**. These compounds were further treated with hydrazine under reflux to produce the target compounds: (-)-1-azafagomine (**2)** in 14 % overall yield and (+)-5-*epi*-1-azafagomine (**3)** in 26 % overall yield, from alkene **(-)-11** (Scheme 2). While (-)-1-azafagomine **2** is a known compound, (+)-5-*epi*-1-azafagomine **3** was obtained enantiomerically pure for the first time. Pure (+)-5-*epi*-1-azafagomine displays NMR spectra compatible with the data published for the racemic compound **3**. The specific optical rotation obtained for (+)-5-*epi*-1-azafagomine is [α_D = + 65 (H_2O, c = 0.70)]. The specific optical rotation value measured for (-)-1-azafagomine [α_D = - 20 (H_2O, c = 0.85)] differs from the one reported in lit [α_D = -9.8 (H_2O, c = 0.85)].[3]

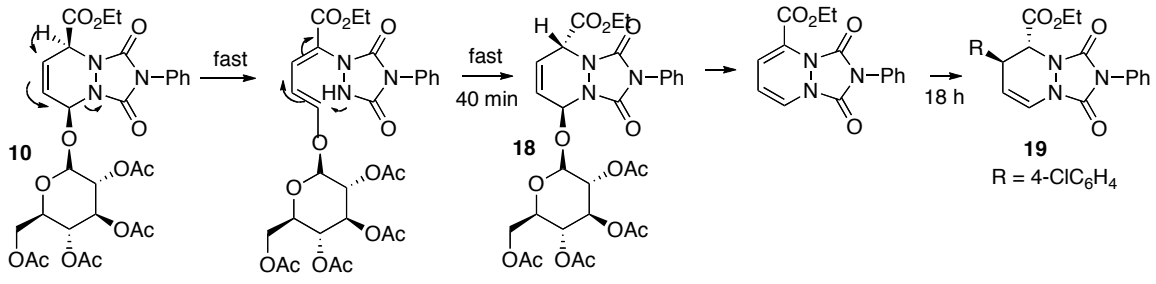
New synthetic sequence for preparing homochiral 1-*N*-phenyl carboxamide derivatives of 1-azafagomine (+)-22 and (-)-22

Stoodley was able to prepare the precursors of **(-)-22** and **(+)-22** from (*E,E)*-diene **9b** and (*E,Z)*-diene **17** respectively by the method described in scheme 3.[9]



Scheme 3 - Stoodley´s synthetic sequence for precursors related to compounds **(-)-2**, **(-)-22** and **(+)-22**

(*E,E*)-Diene **9b** was isolated in 70 % yield and *(E,Z)*-diene **17** in 14 % yield. Applying Still´s olefination the yield of the *(E,Z)*-diene could be improved to 36 %.[9] (Scheme 3) Having in mind the shortcomings in the synthesis of compound **17**, epimerization of compound **10** obtained by Stoodley´s method was tried in various conditions: i) triethylamine in MeOH, ii) NaN_3 in MeOH, iii) triethylamine / *p*-chlorothiophenol in MeOH. (Scheme 4) When triethylamine was the sole reagent, isolation of compound **18** was difficult, due to competing elimination of glucosyl moiety giving the 1,3-diene compound. The same applied for the attempt with NaN_3. The mixture of triethylamine / *p*-chlorothiophenol afforded after 40 min of reaction 88 % yield of compound **18**. This represents an important achievement concerning the synthesis of compound **18**. Extending the reaction time, a Michael addition of *p*-chlorothiophenol occurs leading to compound (±)-**19**. (Scheme 4)



Scheme 4 - Epimerization of compound **10** into compound **18.** Reagents: NEt_3, *p*-chlorothiophenol, MeOH, 0ºC -> rt.

The structure of compound **18** was unambiguously confirmed by X-ray crystallography. (Figure 2)

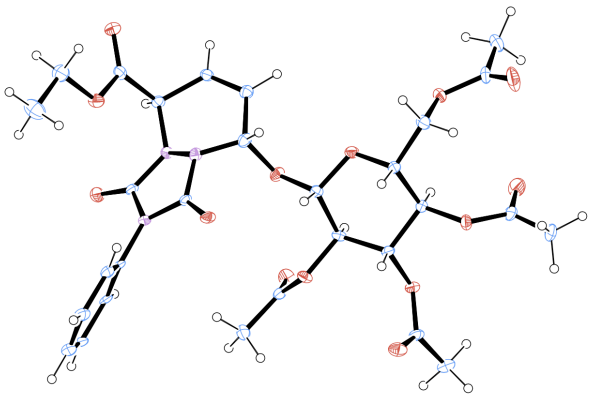


Figure 2 ORTEP view of compound **18**

Reduction of the ester and urea groups in the triazolidinone moiety

i) in the synthesis of compound (-)-21

When compound **(-)-11** was treated with 7 equivalents of freshly opened $LiAlH_4$, a new compound formed, according to ^1H NMR spectroscopy. If the $LiAlH_4$ was not strictly fresh a mixture of two compounds was observed on the ^1H NMR spectrum. Further treatment with $LiAlH_4$ converted the mixture into the same compound observed before. The structure of the intermediate in the reduction process was determined by X-ray crystallography and identified as compound **20** (Figure 3).

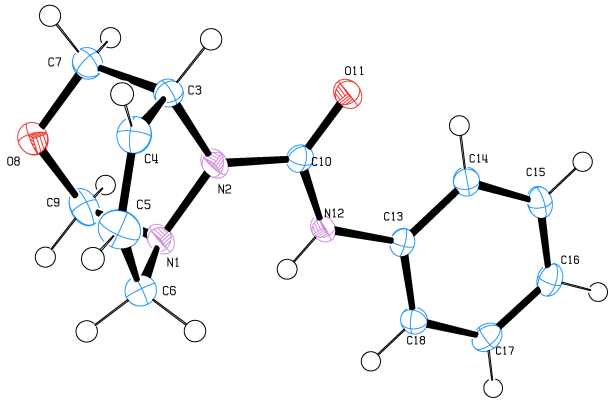
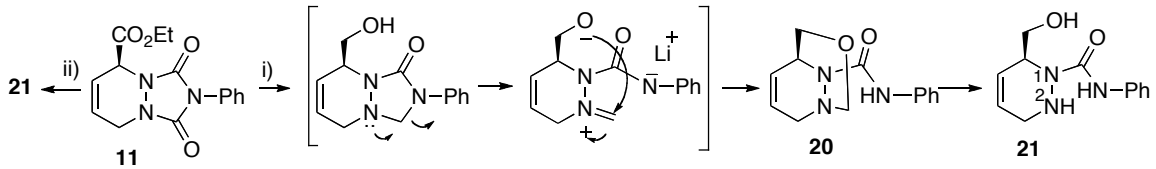


Figure 3 ORTEP view of compound **20**

Knowledge of the bridged structure of compound **20**, allowed us to propose a plausible mechanism for its formation and the formation of compound **21**. (Scheme 5)



Scheme 5 - i) $LiAlH_4$ (7 eq.)[a)], THF, 4h, 0 ºC -> rt, 86 %; ii) $LiAlH_4$ (15 eq.)[b)], THF, 4, 0 ºC -> rt, 48 %

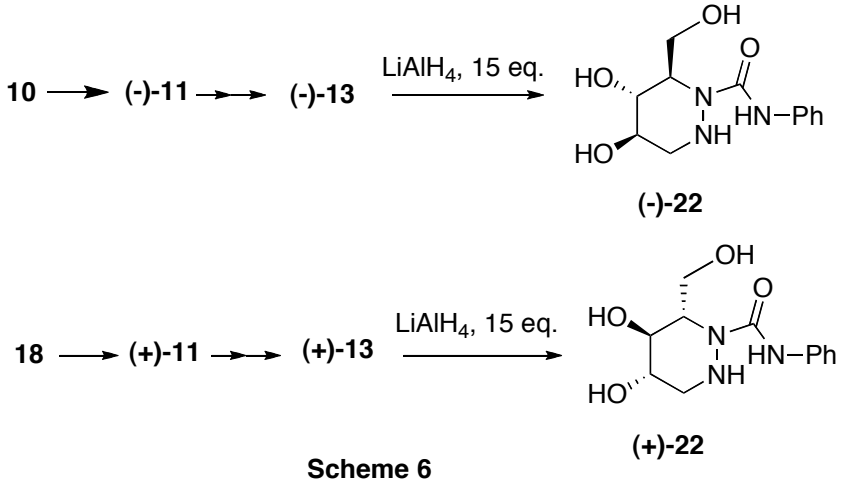
[a)] from a long-time opened bottle
[b)] from a recently opened bottle

The Schiff salt initially formed by reduction of one of the carbonyl groups is trapped by internal nucleophilic attack of the alcohol function. A large excess of hydride was necessary to cleave intermediate **20** to final product **21**. Compounds **20** and **21** display a major difference in their ^{13}C NMR spectra: a peak at δ_C = 86.3 ppm, assigned to the methylene attached to the oxygen and nitrogen atoms in compound **20** is not apparent in the ^{13}C NMR spectrum of compound **21**.

ii) in the synthesis of compounds (-)-22 and (+)-22

Attempted epoxidation of compound **21** was unsuccessful leading to a complex mixture. As an alternative, compound **(-)-13** was subjected to treatment with $LiAlH_4$ in THF to give compound **(-)-22**. The synthesis of compound **(+)-22** was obtained from **18**, (Scheme 4) by reductive cleavage of the glucosyl moiety to give **(+)-11** followed by the functional group transformation described in scheme 6.

The enantiopure compounds (-)-1-*N*-phenyl carboxamide 1-azafagomine **(-)-22** and (+)-1-*N*-phenyl carboxamide 1-azafagomine **(+)-22** were obtained following the same sequence of reactions in 29 % and 10 % overall yield starting from compounds **10** and **18** respectively.

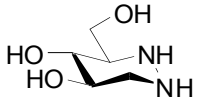
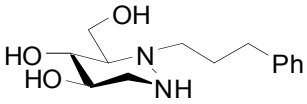
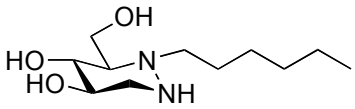
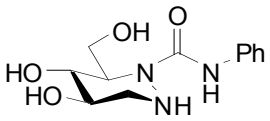
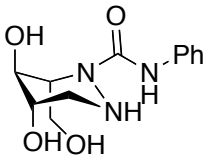


Scheme 6

1-*N*-Phenyl carboxamide derivatives of 1-azafagomine: new leads for the synthesis of potent α-glycosidase inhibitors

A structure-activity relationship study of a series 2-*N*-alkylated 1-azafagomines as glycosidase inhibitors, revealed that these compounds are better β-glycosidase inhibitors than α-glycosidase inhibitors. Moreover, the inhibition constant (K_i) was found to be dependent on the chain length. The best results have been obtained with the *N*-propylphenyl derivative **23** (K_i=0.032 μM) and the *N*-hexyl derivative **24** (K_i=0.055 μM).[12] (Table 1)

Table 1. K_i values (μM) for the inhibition of α- and β-glucosidases by compounds **22** and other azasugars at different pH values.

Compound		α-glucosidase (bakers' yeast)	β-glucosidase (almonds)	α/β-selectivity
	**(-)-2**[3]	6.90[a]	0.32[a]	22
	**23**[12]	158[a]	0.032[a]	4938
	**24**[12]	278[a]	0.55[a]	5054
	**(-)-22**	3.36[b] ____[c,d]	14.7[b] 67.4[c]	0.23
	**(+)-22**	10.6[b] ____[c,d]	25.2[b] 90.0[c]	0.42

[a] pH 6.8; [b] pH 7.0; [c] pH 5.0; [d] enzyme inactive

The *N*-propylphenyl derivative **23** is around an order of magnitude more effective as β-glucosidase inhibitor than (-)-1-azafagomine **2**. On the other hand, derivative **23** is a

much weaker inhibitor of α-glucosidase than its parent compound (**2**), making compound **23** a potent inhibitor selective for β-glucosidase.[12]

The most striking results of the inhibition studies with the 1-*N*-phenyl carboxamide derivatives of 1-azafagomines **22** are K_i towards α-glucosidase substantially lower than the *N*-propylphenyl derivative **23.** Compound **(-)-22** displaying the same stereochemistry as (-)-1-azafagomine **2** is around two times more active, while its isomer **(+)-22** is slightly less active. Both enantiomers of compound **22** display lower activity towards β-glucosidase than their parent compound **2** and the *N*-propylphenyl derivative. A moderate α/β selectivity was observed for compounds **22**. The low K_i values obtained for compounds **22** towards α-glucosidase suggests an efficient recognition mechanism between both enantiomers and the enzyme. The levorotatory isomer was slightly more active than the dextrorotatory isomer. (Table 1) This observation is in contrast with the results reported by Bols who demonstrated that (-)-1-azafagomine is the active enantiomer while (+)-1-azafagomine is virtually inactive towards the same α-glucosidase that was used in this study.[3]

The yeast α-glucosidase enantioselective discrimination towards **(+)-22** and **(-)-22** was studied using molecular docking methodologies. This enzyme, as well as the *S. cerevisiae* enzyme used for the homology modelling[13], belongs to the glycoside hydrolase family 13 (GH 13). This family of retaining glucosidases is characterized by strong recognition of the a glucoside moiety of synthetic *p*-nitrophenyl glucosides and heterogeneous substrates such as sucrose, while being inactive towards hydration of D-glucal and the hydrolysis of *p*-nitrophenyl α-2-deoxyglucosides.[14,15] The active site structure on Figure 4 illustrates that this enzyme retains the nucleophile aspartate 214 and the catalytic residues glutamate 276 and aspartate 349. Our theoretical binding affinity estimate for **(+)-22** and **(-)-22** against yeast α-glucosidase are of -7.8 and -7.5 kcal/mol, respectively. The binding affinity free energy difference between the two enantiomers is within the docking standard error (~2 kcal/mol), and consequently, it suggests, low enantiomeric discrimination of **(+)-22** and **(-)-22**. However, our experimental K_i data (Table 1) indicates a preferential binding of **(-)-22** when compared to **(+)-22.** This discrepancy can be explained by the lack of mobility of enzyme to reorganize during the docking experiments, and consequently, to properly recognize the most potent enantiomeric compound, **(-)-22**. Despite of this, the observation of the binding pose of both enantiomers **22** provides a structural explanation of their binding

mechanism (Figure 4) and a rational approach for their future improvement. These complexes correspond to lowest binding energy pose. They belong to the most populated cluster of docking solutions, 7 and 8 docking poses out of 20, respectively for each enantiomer.

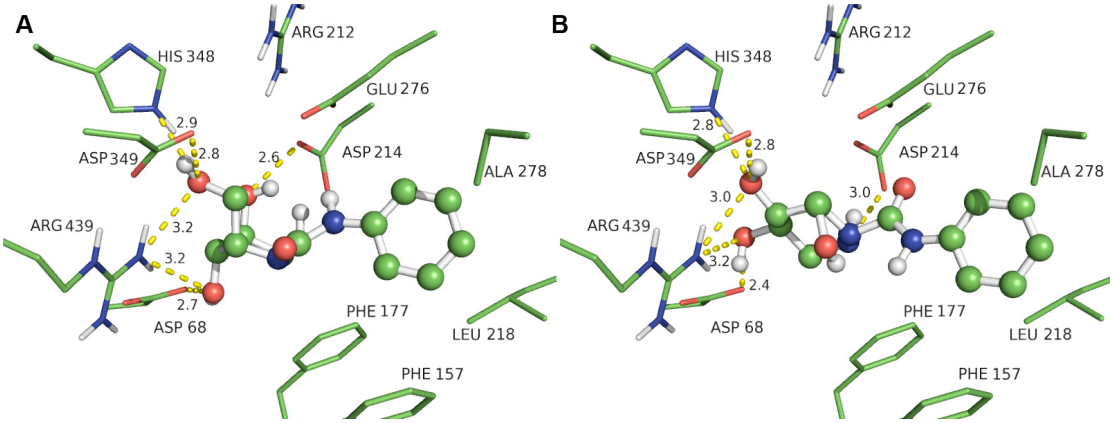


Figure 4. Structure of the lowest binding free energy complexes between yeast α-glucosidase binding site and the enantiomers of compound **22**: A) **(+)-22** and B) **(-)-22**. Figure is rendered with Corey-Pauling-Koltun (CPK) colouring scheme. Selected side-chain residues of the yeast α-glucosidase binding pocket are rendered in sticks and labeled with the 3-letter aminoacid code name and sequence residue number. Compounds are rendered in ball-and-stick style.

The α-glucosidase enzyme active site is characterized by two distinct regions, a highly polar region due to the presence of histidine 348, glutamate 276, aspartate 68, 214, 349, arginine 212, 439; and a hydrophobic pocket flaked by phenylalanine 157, 177, leucine 218 and alanine 278 (Figure 4). The binding affinity between the two **22** enantiomers and yeast α-glucosidase is the result of several strong hydrogen bonding interactions between two hydroxyl and amine groups of **(+)-22** and **(-)-22** with aspartate 68, 214, 349, arginine 212, 439 and histidine 348 of the α-glucosidase enzyme active site. The highlighted non-bonded interactions of **(-)-22** with histidine 348, arginine 439 and aspartate 68 are shorter for this enantiomer when compared to **(+)-22**, suggesting a stronger interaction of **(-)-22** with the enzyme. Based on these observations, the molecular modelling study suggests that the binding affinity can be improved either by: a) increasing the length of the *N*-phenyl-1-carboxamide moiety or, b) introducing

donor/acceptor moieties on the *p*- position of the aromatic ring. These avenues are being currently pursued in order to improve the binding affinity, and ideally selectivity.

Conclusions

In this paper we report the preparation of homochiral 1-azafagomine **(-)-2**, and (+)-5-*epi*-1-azafagomine **(+)-3**. The synthetic route devised merges Stoodley diastereo-selective Diels-Alder cycloaddition methodology with Bols protocol for functionalizing alkenes into molecules bearing sugar-like frameworks. Novel 1-*N*-phenyl carboxamide derivatives of 1-azafagomine **22** were obtained in enantiomeric pure forms. The epimerization of cycloadduct **10** revealed to be the key step on the synthesis of the dextrorotatory compound **22.** This methodology represents an advantageous alternative to other more conventional approaches for obtaining enantiopure **(+)-2** (not isolated in this work) and its derivatives (*e.g*. *N*-phenyl carboxamides)**.** Compounds **22** were tested as inhibitors against α- and β-glucosidases. Both enantiomeric forms of **22** are potent inhibitors of α-glucosidase in contrast to the current wisdom that only **(-)-2** enantiomer of 1-azafagomine is active towards α- and β-glucosidase. The low K_i value determined towards α-glucosidase inhibition is particularly relevant comparing to its analogue *N*-propylphenyl azafagomine, the compound in table 1 with a closer side chain length. The molecular recognition mechanism between the enatiomeric compounds **22** and the α-glucosidase studied by molecular modelling has shown that the aromatic group is accommodated in a hydrophobic pocket of the enzyme binding site with polar characteristics at its end. This evidence has provided further clues for improving the binding affinity and, possibly, the α/β selectivity, by increasing the length of the *N*-carboxamide moiety and the introduction of donor/acceptor hydrogen bond groups on the aromatic ring.

The results of this study suggest that 1-*N*-phenyl carboxamide derivatives of 1-azafagomine are potential new leads for the synthesis of potent α-glycosidase inhibitors.

Experimental Section

General: Solvents were distilled under anhydrous conditions. The (*S*)-ethyl 2-phenyl-2,4,9-triazabicyclo[4.3.0]non-6-ene-1,3-dione-5-carboxylate **(-)-11** was obtained according to Stoodley´s protocol for the *(S)*-methyl carboxylate derivative; 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (ABG) was prepared according lit.[16], potassium 3-hydroxypropenal[17] was combined to ABG[18] followed by addition of tributylphosphorane.[9]

The diene obtained was subjected to 4-phenyl-1,2,4-triazole-3,5-dione (PTAD) to obtain the adduct **10**. The glucose moiety was removed by reduction with triethylsilane.[10] Compound **7** (R=CH_2OH) was obtained according to lit.[4] Functionalization of the double bond was obtained by osmilation with OsO_4 in acetone/water or with oxone, trifluoromethylacetone, in the presence of $NaHCO_3$ and aqueous acetonitrile. Reduction of the oxazolidinone was done either with freshly opened $LiAlH_4$ 1M in THF, or with long term open bottles (over a month) of $LiAlH_4$ 1M in THF. All reagents were purchased and used without further purification. Glassware was dried prior to use. Compounds were purified by dry flash chromatography, using silica 60, <0,063 mm and water pump vacuum or by flash-chromatography, using silica 60A 230-400 Mesh as stationary phases. TLC plates (Silica Gel 60 F_{254}) were visualized either at UV lamp or in I_2.

Synthesis of ethyl 5-(2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-2,4-pentadienoate 9

To a solution of ethyl tributyl phosphorane[9] (3.37 g; 11.70 mmol) in DCM (15 mL) was added 3-(2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-1-propenal[17] (1.32 g; 3.42 mmol), the orange solution formed was stirred at rt for 24h. The solvent was evaporated giving an oil subjected to dry-flash chromatography (petroleum ether: diethyl ether; gradient of polarity) (**9**, 1.17 g; 63 %). $[\alpha]_D^{20}$ +27.0º (c = 1, CH_2Cl_2). ν_{max}(Nujol, cm^{-1}) 2955, 2934, 1736, 1698, 1635. ^{1}H NMR (δ_H, 300 MHz, $CDCl_3$) 1.27 (3H, t, *J* 7.2 Hz, CH_3), 2.01, 2.03, 2.04, 2.08 (12H, 4×s, 4× $\underline{CH_3}CO_2$), 3.81 (1H, ddd, *J* 9.0, 6.0, 3.0 Hz, H-5'), 4.13 (1H, dd, *J* 9.0, 3.0 Hz, H-6'), 4.18 (2H, q, *J* 6.0 Hz, CH_2), 4.26 (1H, dd, *J* 12.0, 3.0 Hz, H-6'), 4.87 (1H, d, *J* 9.0 Hz, H-1'), 5.08-5.29 (3H, m, H-2' + H-3' + H-4'), 5.78 (1H, d, *J* 15.0 Hz, H-2), 5.90 (1H, t, *J* 12.0 Hz, H-4), 6.81 (1H, d, *J* 12.0 Hz, H-5), 7.20 (1H, dd, *J* 15.0, 12.0 Hz, H-3) ppm. ^{13}C NMR (δ_C, 75.5 MHz, $CDCl_3$) 14.2 (CH_3), 20.5, 20.5, 20.7 ($\underline{C}H_3CO_2$), 60.1 (CH_2), 61.6 (C-6'), 67.8, 70.6, 72.3 (C-2', C-3', C-4'), 72.4 (C-5'), 99.7 (C-1'), 110.1 (C-4), 118.7 (C-2), 140.9 (C-3), 152.6 (C-5), 167.0 (C=O ester), 169.1, 169.2, 170.1, 170.5 ($CH_3\underline{C}O_2$) ppm.

Synthesis of (5*S*,8*S*)-ethyl 8-(2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-2-phenyl-2,4,9-triazabicyclo[4.3.0]non-6-eno-1,3-dione-5-carboxylate 10

To a solution of diene **9** (1.31 g; 2.86 mmol) in DCM (15 mL) was added 4-phenyl-1,2,4-triazole-3,5-dione (0.50 g; 2.86 mmol), giving a red coloured solution that quickly lost its

colour. The reaction mixture was stirred for a further 30 min and then evaporated. The residue was tritured with ethyl ether. A white solid was formed and filtered giving the title compound (**10**, 1.302 g; 70 %). $[\alpha]_D^{20}$ +23,8º (c = 1, CH_2Cl_2). ν_{max}(Nujol, cm^{-1}) 2954, 2853, 1743, 1620, 1635, 1218. ^1H NMR (δ_H, 300 MHz, $CDCl_3$) 1.35 (3H, t, *J* 6.0 Hz, CH_3), 2.00, 2.01, 2.02, 2.04 (12H, 4×s, 4×$\underline{CH_3}CO_2$), 3.87 (1H, ddd, *J* 12.0, 6.0, 3.0 Hz, H-5'), 4.05 (1H, dd, *J* 12.0, 3.0 Hz, H-6'), 4.27 (1H, dd, *J* 15.0, 9.0 Hz, H-6'), 4.34 (2H, dq, *J* 7.2, 1.2 Hz, CH_2), 5.01 (2H, m, H-5 + H-2'), 5.09 (1H, t, *J* 9.9 Hz, H-4'), 5.19-5.26 (2H, m, H-3' + H-1'), 6.06-6.12 (2H, m, H-8 + H-7), 6.19-6.26 (1H, m, H-6), 7.39-7.56 (5H, m, Ph) ppm. ^{13}C NMR (δ_C, 75.5 MHz, $CDCl_3$) 14.0 (CH_3), 20.5, 20.6, 20.6 ($\underline{C}H_3CO_2$), 56.7 (C-5), 61.5 (C-6'), 62.9 (CH_2), 67.8 (C-4'), 71.0 (C-2'), 72.1 (C-5'), 72.8 (C-3'), 74.6 (C-8), 97.2 (C-1'), 123.3 (C-7), 124.9 (C-6), 125.4 (C-H, Ph), 128.5 (C-H, Ph), 129.2 (C-H, Ph), 130.7 (Cq, Ph), 150.2 (C=O), 151.4 (C=O), 165.6 (C=O ester), 169.4, 169.4, 170.2, 170.7 ($CH_3\underline{C}O_2$) ppm. HRMS (FAB): Calcd for $C_{29}H_{33}N_3O_{14}$, 648.2041; Found, 648.2041.

Synthesis of (*S*)-ethyl 2-phenyl-2,4,9-triazabicyclo[4.3.0]non-6-ene-1,3-dione-5-carboxylate (-)-11

To a solution of the cycloadduct **10** (1.31 g; 2.03 mmol) in DCM (20 mL) was added triethylsilane (12.7 mL; 0.78 mol) and trifluoroacetic acid (12.7 mL; 0.17 mol). The resulting yellow suspension was kept under stirring at rt for 5 h. The solvent was removed under vacuum and the residue re-dissolved in DCM (30 mL). The solution was washed with aq. sat. sol. of $NaHCO_3$ (3×50 mL) and water (50 mL). The combined organic layers were dried over magnesium sulphate, filtered and the solvent evaporated. From the residual oil crystallized a white solid that was washed with diethyl ether and proved to be the title compound (**(-)-11**, 0.313 g; 51 %). $[\alpha]_D^{20}$ -311.0º (c = 2.15, CH_2Cl_2); m.p. 140-142 ºC. ν_{max}(Nujol, cm^{-1}) 2954, 2923, 1742, 1714. ^1H NMR (δ_H, 300 MHz, $CDCl_3$) 1.29 (3H, t, *J* 7.2 Hz, CH_3), 3.99-4.06 (1H, dm, H-8), 4.25 (2H, q, *J* 7.2 Hz, CH_2), 4.38-4.45 (1H, dm, H-8), 5.09-5.12 (1H, m, H-5), 6.05-6.16 (2H, m, H-6 + H-7), 7.38-7.57 (5H, m, Ph) ppm. ^{13}C NMR (δ_C, 75.5 MHz, $CDCl_3$) 14.1 (CH_3), 43.1 (C-8), 55.9 (C-5), 62.4 (CH_2), 119.7 (C-6 or C-7), 123.3 (C-6 or C-7), 125.6 (C-H, Ph), 128.2 (C-H, Ph), 129.1 (C-H, Ph), 131.1 (Cq, Ph), 152.3 (C=O), 153.3 (C=O), 166.7 (C=O ester) ppm. EA Calcd for $C_{15}H_{15}N_3O_4$, C, 59.79 %; H, 5.02 %; N, 13.95 %; Found, C, 59.90 %; H, 4.93 %; N, 13.86 %.

Synthesis of (*R*)-ethyl 2-phenyl-2,4,9-triazabicyclo[4.3.0]non-6-ene-1,3-dione-5-carboxylate (+)-11

To a solution of the cycloadduct **18** (1.03 g; 1.59 mmol) in DCM (25 mL) was added triethylsilane (9.85 mL; 0.78 mol) and trifluoroacetic acid (9.85 mL; 0.17 mol). The resulting yellow solution was kept under stirring at rt for 5 h. The solvent was removed under vacuum and the residue re-dissolved in DCM (25 mL). The solution was washed with aq. sat. sol. of $NaHCO_3$ (3×25 mL) and water (25 mL). The combined organic layers were dried over magnesium sulphate, filtered and the solvent evaporated. From the residual oil crystallized a white solid that was washed with diethyl ether and proved to be the title compound (**(+)-11**, 0.292 g; 61 %). $[\alpha]_D^{20}$ +370.0º (c = 1, CH_2Cl_2).

Synthesis of (5*S*,6*R*,7*S*)-ethyl 6,7-epoxy-2-phenyl-2,4,9-triazabicyclo[4.3.0]nonano-1,3-dione-5-carboxylate (-)-12

To a solution of compound **(-)-11** (0.48 g; 1.59 mmol) in acetonitrile (28.0 mL), water (16.0 mL) and 1,1,1-trifluoracetone (3.21 mL), was added solid $NaHCO_3$ (2.44 g; 29.02 mmol), oxone (12.00 g; 39.04 mmol) for 20 min. at 0ºC. The mixture was stirred for 18 h. A new portion of solid $NaHCO_3$ (2.44 g; 29.02 mmol) and oxone (12.00 g; 39.04 mmol) was added and stirred for another 4 h. Then water (100 mL) was added to the reaction mixture, extracted with $CHCl_3$ (8×40 mL). The organic layers were combined and dried with magnesium sulphate. After removal of the solvent and recrystallization with diethyl ether gave a white solid identified as the title compound (**(-)-12**, 0.329 g; 65 %). $[\alpha]_D^{20}$ – 238.0º (c = 0.8, CH_2Cl_2); m.p. 218-220 ºC. ν_{max}(Nujol, cm^{-1}) 2950, 2923, 1775, 1750, 1715, 1458 , 1094, 1034. ^{1}H NMR (δ_H, 300 MHz, $CDCl_3$) 1.32 (3H, t, *J* 7.1 Hz, CH_3), 3.53-3.65 (2H, m, H-7 + H-8), 3.89 (1H, dd, *J* 5.6, 3.8 Hz, H-6), 4.19-4.40 (2H, m, CH_2), 4.47 (1H, dd, *J* 13.6, 1.4 Hz, H-8), 5.01 (1H, d, *J* 5.7 Hz, H-5), 7.33-7.51 (5H, m, Ph) ppm. ^{13}C NMR (δ_C, 75.5 MHz, $CDCl_3$) 14.1 (CH_3), 43.0 (C-8), 49.0 (C-6), 50.1 (C-7), 54.8 (C-5), 62.7 (CH_2), 125.6 (C-H, Ph), 128.4 (C-H, Ph), 129.1 (C-H, Ph), 130.9 (Cq, Ph), 153.3 (C=O), 153.56 (C=O), 165.6 (C=O ester) ppm. HRMS (FAB): Calcd for $C_{15}H_{16}N_3O_5$, 318.1089; Found, 318.1087.

Synthesis of (5*R*,6*S*,7*R*)-ethyl 6,7-epoxy-2-phenyl-2,4,9-triazabicyclo[4.3.0]nonano-1,3-dione-5-carboxylate (+)-12

To a solution of compound **(+)-11** (0.37 g; 1.21 mmol) in acetonitrile (21.1 mL), water (12.3 mL) and 1,1,1-trifluoracetone (2.40 mL), was added solid $NaHCO_3$ (1.86 g; 29.02

mmol), oxone (9.15 g; 39.04 mmol) for 20 min. at 0ºC. The mixture was stirred for 18 h. A new portion of solid $NaHCO_3$ (1.86 g; 29.02 mmol) and oxone (9.15 g; 39.04 mmol) was added and stirred for another 4 h. Then water (60 mL) was added to the reaction mixture, extracted with DCM (10×40 mL). The organic layers were combined and dried with magnesium sulphate. After removal of the solvent and recrystallization with diethyl ether gave a white solid identified as the title compound (**(+)-12**, 0.233 g; 60 %). $[\alpha]_D^{20}$ + 232.8º (c = 0.8, CH_2Cl_2).

Synthesis of (5*S*,6*R*,7*R*)-ethyl 6,7-dihydroxy-2-phenyl-2,4,9-triazabicyclo[4.3.0] nonane-1,3-dione-5-carboxylate (-)-13

To a solution of epoxide **(-)-12** (0.20 g; 0.63 mmol) in water (30 mL) was added conc. H_2SO_4 (0,5 mL) and the mixture was refluxed for 8 h. After this time solid $NaHCO_3$ (0.86 g; 10.24 mmol) was added and the water evaporated till dryness. The residue was dissolved in ethyl acetate (100 mL) and washed with NaCl (50 mL). The organic phase was separated and the aqueous phase was extracted with ethyl acetate (100 mL). The organic phases were combined and dried with magnesium sulphate, filtered and concentrated in the rotary evaporator. The yellowish solid obtained was washed with diethyl ether and found to be the title compound (**(-)-13**, 0.110 g; 52 %). $[\alpha]_D^{20}$ -22.8º (c = 2, acetone). ν_{max}(Nujol, cm^{-1}) 3596-3540, 2954, 2923, 1729, 1698, 1122, 1088. ^{1}H NMR (δ_H, 400 MHz, $CDCl_3$) 1.24 (3H, m, CH_3), 3.64 (1H, d, *J* 12.0 Hz, H-8), 3.95 (1H, bs, H-7), 3.99 (1H, d, *J* 12.8 Hz, H-8), 4.13-4.28 (2H, m, CH_2), 4.46 (1H, t, *J* 2.8 Hz, H-6), 4.74 (1H, d, *J* 2.8 Hz, H-5), 7.38-7.50 (5H, m, Ph) ppm. ^{13}C NMR (δ_C, 100 MHz, $CDCl_3$) 13.9 (CH_3), 44.3 (C-8), 59.4 (C-5), 62.6 (CH_2), 65.9 (C-7), 67.4 (C-6), 125.9 (C-H, Ph), 128.6 (C-H, Ph), 129.3 (C-H, Ph), 131.0 (Cq, Ph), 152.1 (C=O), 154.1 (C=O), 167.3 (C=O ester) ppm. HRMS (FAB): Calcd for $C_{15}H_{18}N_3O_6$, 336.1196; Found, 336.1207.

Synthesis of (5*R*,6*S*,7*S*)-ethyl 6,7-dihydroxy-2-phenyl-2,4,9-triazabicyclo[4.3.0] nonane-1,3-dione-5-carboxylate (+)-13

To a solution of epoxide **(+)-12** (0.23 g; 0.73 mmol) in water (35 mL) was added conc. H_2SO_4 (0.7 mL) and the mixture was refluxed for 10 h. After this time solid $NaHCO_3$ (1.42 g; 16.90 mmol) was added and the water evaporated till dryness. The residue was dissolved in ethyl acetate (100 mL) and washed with NaCl (50 mL). The organic phase was separated and the aqueous phase was extracted with ethyl acetate (3×100 mL). The organic phases were combined and dried with magnesium sulphate, filtered and

concentrated in the rotary evaporator. The yellowish solid obtained was washed with diethyl ether and found to be the title compound (**(+)-13**, 0.121 g; 49 %). $[\alpha]_D^{20}$ +26.9º (c = 0.5, acetone).

Synthesis of (5*S*,6*R*,7*S*)-ethyl 6,7-dihydroxy-2-phenyl-2,4,9-triazabicyclo[4.3.0] nonane-1,3-dione-5-carboxylate 14

To a solution of **(-)-11** (0.30 g; 1.00 mmol) in acetone (1 mL) and water (0.5 mL) was added 4-methylmorpholine *N*-oxide (0.18 g; 1.49 mmol) and a solution of OsO_4 in water 4 % (108 mL). The mixture of stirred for 5 days. Then a aq. sol. of $Na_2S_2O_3$ 5 % (25 mL) was added to mixture and stirred for 15 min. After the solution was extracted with ethyl acetate (4 x 30 mL) and the organic phases were washed with water (10 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated to give a white solid (**14**, 0.26 g; 79 %). $[\alpha]_D^{20}$ -110.6º (c = 2.05, acetone). ν_{max}(Nujol, cm^{-1}) 3425, 1768, 1749, 1736, 1287, 1204. ^{1}H NMR (δ_H, 400 MHz, $CDCl_3$) 1.27 (3H, t, *J* 7.2 Hz, CH_3), 3.35 (1H, d, *J* 10.8 Hz, H-8), 3.83 (1H, ddd, *J* 10.0, 5.2, 2.8 Hz, H-7), 4.03 (1H, dd, *J* 11.6, 5.2 Hz, H-8), 4.24 (2H, q, *J* 7.2 Hz, CH_2), 4.52 (1H, t, *J* 2.8 Hz, H-6), 4.90 (1H, d, *J* 3.6 Hz, H-5), 7.40-7.49 (5H, m, Ph) ppm. ^{13}C NMR (δ_C, 100 MHz, $CDCl_3$) 14.0 (CH_3), 43.2 (C-8), 60.6 (C-5), 62.8 (CH_2), 65.1 (C-7), 67.2 (C-6), 125.8 (C-H, Ph), 128.6 (C-H, Ph), 129.3 (C-H, Ph), 130.9 (Cq, Ph), 151.4 (C=O), 153.8 (C=O), 166.4 (C=O ester) ppm. HRMS (FAB): Calcd for $C_{15}H_{18}N_3O_6$, 336.1196; Found, 336.1195.

Synthesis of (5*S*,6*R*,7*R*)-6,7-dihydroxy-5-hydroxymethyl-2-phenyl-2,4,9-triazabicyclo[4.3.0] nonane-1,3-dione 15

To a solution of the diol **(-)-13** (0.07 g; 0.22 mmol) in ethanol (3 mL) was added $NaBH_4$ (8 mg; 0.22 mmol), under magnetic stirring at room temperature. After 1 h an aliquot was quenched with HCl 0.4 M, extracted with ethyl acetate, dried over magnesium sulfate and concentrated. ^{1}H NMR spectrum showed that the reaction was not completed and a new amount of $NaBH_4$ (8 mg; 0.22 mmol) was added and the mixture stirred for another 4 h. The procedure was repeated with addition of $NaBH_4$ (8 mg; 0.22 mmol). The reaction was quenched with aq. HCl 0.4 M (4.4 mL), the mixture stirred for 10 min and evaporated. The residue is dissolved in water (10 ml) and extracted with ethyl acetate (8 x 15 mL). The organic phases were combined and dried over magnesium sulphate. Evaporation of the solvent gave a white solid identified as the title compound (**15**, 0.037

g; 59 %). $[\alpha]_D^{20}$ -70.4º (c = 1.2, acetone). The spectroscopic data of the racemic mixture is reported before.[4]

Synthesis of (5*S*,6*R*,7*S*)-6,7-dihydroxy-5-hydroxymethyl-2-phenyl-2,4,9-triazabicyclo[4.3.0] nonane-1,3-dione 16

To a solution of the diol **14** (0.224 g; 0.73 mmol) in ethanol (7 mL) was added $NaBH_4$ (0.083 g). The mixture was stirred at room temperature overnight. After addition of aq. HCl 0.4 M (15.3 mL) the mixture was stirred for 15 min. Then the solvent was removed under vacuum, the residue was dissolved in water (20 mL) and sat. aq. sol. of $NaHCO_3$ (10 mL), and extracted with ethyl acetate (14 x 25 mL). The organic layers were combined, dried over magnesium sulphate and concentrated. It was obtained a white solid identified as the title compound (**16**, 0.112 g; 52 %). $[\alpha]_D^{20}$ - 8.0º (c = 0.75, acetone). The spectroscopic data of the racemic mixture is reported before.[4]

Synthesis of ethyl (5*R*,8*S*)-8-(2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-2-phenyl-2,4,9-triazabiciclo[4.3.0]non-6-ene-1,3-dione-5-carboxylate 18

To a suspension of compound **10** (0.22 g; 0.33 mmol) in methanol (5 mL) was added 4-chorothiophenol (0.10 g; 0.68 mmol) and triethylamine at 0ºC and under magnetic stirring. After 40 min. the solvent was evaporated and the crude subjected to dry-flash chromatography (petroleum ether / ether 1:3). The product was obtained as a white solid (**18**; 0.187 g; 0.30 mmol; 88 %), m.p. 154-157 ºC. $[\alpha]_D^{20}$ + 219.7º (c = 1, acetone). ν_{max}(Nujol, cm^{-1}) 2955, 2924, 1744, 1723, 1226. ^{1}H NMR (δ_H, 400 MHz, $CDCl_3$) 1.28 (3H, t, *J* 7.2 Hz, CH_3), 1.88, 1.98, 2.02, 2.07 (12H, 4xs, 4x$\underline{CH_3}CO_2$), 3.74-3.79 (1H, m, H-5'), 4.15 (1H, dd, *J* 12.4, 2.4 Hz, H-6'), 4.23 (2H, q, *J* 7.2 Hz, CH_2), 4.25 (1H, dd, *J* 12.0, 4.4 Hz, H-6'), 4.95 (1H, dd, *J* 9.6, 8.0 Hz, H-2'), 5.07 (1H, t, *J* 10.0 Hz, H-4'), 5.15 (1H, dd, *J* 5.2, 2.0 Hz, H-5), 5.18-5.23 (2H, m, H-1'+ H-3'), 5.96 (1H, d, *J* 4.8 Hz, H-8), 6.07 (1H, ddd, *J* 10.0, 4.4, 2.0 Hz, H-6), 6.31 (1H, ddd, *J* 10.0, 5.2, 0.8 Hz, H-7), 7.27-7.56 (5H, m, Ph) ppm. ^{13}C NMR (δ_C 100 MHz, $CDCl_3$) 14.0 (CH_3), 20.5, 20.5, 20.7 ($\underline{C}H_3CO_2$), 56.0 (C-5), 61.6 (C-6'), 62.7 (CH_2), 68.0 (C-4'), 71.1 (C-2'), 72.2 (C-5'), 72.6 (C-3'), 76.0 (C-8), 99.6 (C-1'), 123.9 (C-7), 124.1 (C-6), 125.6 (C-H, Ph), 128.6 (C-H, Ph), 129.2 (C-H, Ph), 130.8 (Cq, Ph), 151.5 (C=O), 153.4 (C=O), 166.0 (C=O ester), 169.2, 169.4, 170.1, 170.6 ($CH_3\underline{C}O_2$) ppm. EA Calcd for $C_{29}H_{33}N_3O_{14}$, C, 53.79 %; H, 5.14 %; N, 6.49 %; Found, C, 53.58 %; H, 5.23 %; N, 6.38 %.

Synthesis of (*S*)-*N*-phenyl-3-oxa-1,9-diazabicyclo[3.3.1]non-6-ene-9-carboxylate 20

To a solution of ester **(-)-11** (0.205 g; 0.68 mmol) solubilized in dry THF (13 mL) was added $LiAlH_4$ 1 M in THF (7 eq., 5.2 mL), from a flask containing a white deposit, at 0ºC. The mixture was kept under stirring for 4h at rt. The reaction was quenched with a sequence addition of water (1 drop), aq. NaOH 15 % (2 drops) and water (1 drop) during which time a large amount of H_2 was released. Then a portion of water (15 mL) was added and the mixture extracted with ethyl acetate (4 x 25 mL). The combined organic layers were washed with sat. aq. $NaHCO_3$ (25 mL) and brine (25 mL), then dried over $MgSO_4$. After evaporation of the ethyl acetate a yellowish crude crystalized giving **20** (0.088 g; 0.36 mmol; 48 %). $[\alpha]_D^{20}$ -57.5º (c = 0.4, $CHCl_3$); ν_{max}(Nujol, cm^{-1}) 3320, 1670, 1604, 1591, 1530. ^1H NMR (δ_H, 300 MHz, $CDCl_3$) 3.45 (1H, dd, *J* 18.3, 1.2 Hz, H-8), 3.71 (1H, d, *J* 10.5 Hz, H-4), 3.90 (dd, *J* 2.7, 11.1 Hz, H-4), 4.00 (1H, dd, *J* 18.3, 1.2 Hz, H-8), 4.56 (1H, d, *J* 10.5Hz, H-2), 4.66(1H, bs, H-5), 4.69 (d, *J* 10.5 Hz, H-2) , 6.07 (2H, bs, H-6+ H-7), 7.04 (t, *J* 7.2 Hz, CH, Ph), 7.31 (2H, t, *J* 7.2 Hz, CH, Ph), 7.49 (2H, d, *J* 7.2 Hz, CH, Ph), 8.00 (1H, bs, NH) ppm. ^{13}C NMR (δ_C, 100 MHz, $CDCl_3$) 44.4 (C-5), 50.7 (C-8), 66.7 (C-4), 86.3 (C-2), 118.7 (CH, Ph), 122.9 (CH, Ph), 127.8 (C-7 or C-6), 128.9 (C-6 or C-7), 128.9 (C-H, Ph), 138.5 (Cq, Ph), 153.1 (C=O) ppm. HRMS (FAB): Calcd for $C_{13}H_{16}N_3O_2$, 246.124252; Found, 246.124172.

Synthesis of (*S*)-6-(hydroxymethyl)-*N*-phenyl-2,3-dihydropyridazine-1(6*H*)-carboxamide 21

To the ester **(-)-11** (0.15 g; 0.5 mmol) solubilized in dry THF (10 mL) was added $LiAlH_4$ 1M in THF (15 eq.; 13.5 mL), freshly open, at 0 ºC. The mixture was kept under stirring for 4h at rt. The reaction was quenched by a drop of water followed by 2 drops of aq. NaOH 15 % and another drop of water during which time a large amount of H_2 was released. Then a portion of water (40 mL) was added and the mixture extracted with ethyl acetate (5 x 40 mL). The combined organic layers were washed with sat. aq. $NaHCO_3$ (50 mL) and brine (50 mL), then dried over $MgSO_4$. After evaporation of the ethyl acetate a yellowish crude was obtained from which crystalized a solid (0.10 g; 0.43 mmol; 86 %). $[\alpha]_D^{20}$ -150.8º (c = 0.4, $CHCl_3$); ν_{max}(Nujol, cm^{-1}) 3359, 3268, 3058, 1636, 1601, 1592, 1536. ^1H NMR (δ_H, 300 MHz, $CDCl_3$) 3.30 (1H, bd, *J* 17.2 Hz, H-3), 3.48-3.55 (1H, m, H-3), 3.75 (1H, dd, *J* 10.8, 5.2 Hz, $\underline{CH_2}OH$), 3.95 (1H, dd, *J* 11.2, 3.2 Hz, $\underline{CH_2}OH$), 4.20 (1H, dd, *J* 11.2, 2.4 Hz, OH), 4.78 (1H, bs, H-6), 5.83 (1H, dm, *J* 8.4 Hz, H-4 or H-5), 6.13 (1H, dm, *J* 8.4 Hz, H-4 or H-5), 7.02 (1H, t, *J* 7.6 Hz, CH, Ph), 7.30

(2H, t, *J* 7.6 Hz, CH, Ph), 7.47 (2H, d, *J* 7.6 Hz, CH, Ph), 8.60 (1H, bs, NH) ppm. ^{13}C NMR (δ_C, 100 MHz, $CDCl_3$) 45.3 (C-6), 50.7 (C-3), 65.1 (CH_2OH), 122.7 (CH, Ph), 124.5 (C-4 or C-5), 128.3 (C-5 or C-4), 128.9 (CH, Ph), 138.7 (Cq, Ph), 155.0 (C=O) ppm. HRMS (FAB): Calcd for $C_{12}H_{16}N_3O_2$, 234.124432; Found, 244.124252.

Synthesis of (4*R*,5*R*,6*R*)-4,5-dihydroxy-6-(hydroxymethyl)-*N*-phenylhexahydropyridazine-1-carboxamide (-)-22

To a solution of **(-)-13** (0.06 g; 0.18 mmol) in dry THF (8 mL) was added at 0 ºC a solution of $LiAlH_4$ 1M in THF (7 eq.; 2.51 mL). The reaction mixture stirred for 3 h at rt, then the quenching was followed by sequential addition of 1 drop of water, one drop of aq. NaOH 15% and water (20 mL). The aqueous solution was extracted with ethyl acetate (6 x 60 mL). The organic layers were combined, dried and evaporated giving an oil that was submitted to PLC (DCM/ methanol 10%) giving the title compound **(-)-22** (0.014 g; 0.05 mmol, 29 %). $[\alpha]_D^{20}$ -54.4º (c = 0.6, methanol). ν_{max}(neat, cm^{-1}) 3346, 2925, 1656, 1592, 1534. ^{1}H NMR (δ_H, 400 MHz, $CDCl_3$) 2.92 (1H, dt, *J* 1.2, 14.8 Hz, H-3), 3.32 (1H, dd, *J* 14.8, 2.0 Hz, H-3), 3.69-3.73 (1H, m, H-6), 3.82 (1H, dd, *J* 12.0, 4.8 Hz, CH_2OH), 3.90-3.92 (1H, m, H-4), 4.11(1H, dd, *J* 12.2, 9.0 Hz, CH_2OH), 4.44-450 (1H, m, H-5), 7.19-7.43 (5H, m, Ph); ^{13}C NMR (δ_C, 100 MHz, $CDCl_3$) 46.4 (C-3), 56.1 (C-5), 59.0 (CH_2OH), 64.8 (C-6), 66.2 (C-4), 121.5 (CH, Ph), 125.0 (CH, Ph), 129.1 (CH, Ph), 138.0 (Cq, Ph), 153.6 (C=O) ppm. HRMS (FAB): Calcd for $C_{12}H_{18}N_3O_4$, 268.1219; Found, 268.1222.

Synthesis of (4*S*,5*S*,6*S*)-4,5-dihydroxy-6-(hydroxymethyl)-*N*-phenylhexahydropyridazine-1-carboxamide (+)-22

To a solution of **(+)-13** (0.12 g; 0.36 mmol) in dry THF (10 mL) was added at 0 ºC a solution of $LiAlH_4$ 1M in THF (7 eq.; 5.03 mL). The reaction mixture stirred for 1.5 h at rt, then the quenching was followed by sequential addition of 1 drop of water, one drop of aq. NaOH 15% and water (50 mL). The aqueous solution was extracted with ethyl acetate (10 x 40 mL). The organic layers were combined, dried and evaporated giving an oil that was submitted to PLC (DCM/ methanol 10%) giving the title compound **(+)-22** (0.010 g; 0.04 mmol, 10 %). $[\alpha]_D^{20}$ +51.3º (c = 1, methanol).

Measurement of glycosidase inhibition

α-Glucosidase from bakers' yeast (EC 3.2.1.20, Sigma G-5003) and β-glucosidase from

almonds (EC 3.2.1.21, Sigma G-0395) were used as model glycosidases. Enzyme assays were conducted in 96 wells *Nunc* plates using 4-nitrophenyl α-D-glucopyranoside or 4-nitrophenyl β-D-glucopyranoside as substrates, in phosphate buffer 100 mM, pH 7.0 or citrate buffer 100 mM, pH 5.0 at 25 ºC. A range of substrate concentrations from 3.3 x 10^{-5} M to 2.0 x 10^{-3} M (11 different concentrations), in a final volume of 300 µL, was tested using 0.2 units/mL of β-glucosidase or 0.15 units/mL of α-glucosidase, in the absence and in the presence of inhibitor (**(+)-** and **(-)-22**, 5 x 10^{-6} M and 10 x 10^{-6} M). Blanks were set containing all reaction components but enzyme. All assays were performed in triplicate.

The formation of 4-nitrophenol was monitored for 20 min at 25 ºC, measuring the absorbance (1 reading each minute) at 400 nm. A value of εI = 787.73 M^{-1} (pH 7.0) or 28.29 M^{-1} (pH 5.0), determined in the same conditions as used for the enzyme assays, was used to convert absorvance into product concentration. Initial velocities were calculated from the slopes of the Abs *vs* time graphs for each concentration of substrate and used to construct Michaelis-Menten plots. The kinetic parameters KM and Vmax were determined by fitting the experimental results to a rectangular hyperbole using the Origin 8 Graph Pad and by Lineweaver-Burk analysis. The inhibition type was established as competitive for all enzymes and inhibitors tested, using 2 different concentrations of inhibitors (in duplicate) and by examining the Lineweaver-Burk plot. For each inhibitor concentration, individual K_i values were obtained using the expression for competitive inhibition (K_i= [I]/((K_{Mapp}/KM)-1)) were K_M and K_{Mapp} represent the Michaelis-Menten constant in the absence and in the presence of inhibitor, respectively. Reported K_i values are expressed as average of 2 independent K_i determinations.

Structural molecular modelling studies

Structural enzyme-compound complexes and theoretical binding free energy of **(-)-2**, **(+)-2**, and **22** towards yeast a-glucosidase structure were done with computational docking methodologies using AUTODOCK 4.[19] The modelling of the enzyme-compound complexes with almond b-glucosidase was not calculated, because to the best of our knowledge, no structure or protein sequence is available. In the docking calculations, all possible torsions of the compounds were set flexible except the amide bonds in both enantiomers of compound **22**. The protonation state of the amine N-1 and N-2 of the compounds was set neutral, in agreement with previous NMR evidences.[20] The grid for probe-target energy calculations was placed with its centre at the enzyme-binding site.

The docking grid size was 42x40x42 grid points with 0.375 Å spacing. For each ligand, 20 runs using the Lamarckian genetic algorithm with 150 individuals in each population were carried out. The maximum number of generations was set to 27×10^3 and the maximum number of energy evaluations to 5×10^6. The resulting docking solutions were clustered using AUTODOCK with a structural root mean square deviation cut-off of 1 Å. Since no experimental structure exists for the yeast α-glucosidase enzyme, a theoretical structural model of this enzyme was derived using MODELLER,[21] employing the crystal structure of isomaltase from *Saccharomyces cerevisiae* structure (PDB ID:3A4A)[13] as template. Isomaltase and a-glucosidase from *Saccharomyces cerevisiae* share 72% sequence similarity. 20 models were generated using an initial alignment between the isomaltase and a-glucosidase enzyme sequences. The model with the lowest objective function[21] was chosen and its quality was evaluated based on its stereochemistry given by Procheck.[22] A high quality model of the yeast a-glucosidase enzyme was obtained with no residues in disallowed regions in the Ramachandran plot. The protonation states of the acidic and basic residues were set to their standard state found in aqueous solution at pH 7.

Acknowledgments

We thank FCT for project funding PTDC/QUI/67407/2006; FCT and FEDER for funding NMR spectrometer Bruker Avance III 400 as part of the National NMR Network. Micaelo, N.M. acknowledges the contract research program "Compromisso com a Ciência" reference: C2008-UMINHO-CQ-03 and access to the Minho University GRIUM cluster.

Supporting Information. Crystallographic data and ORTEP drawing for compounds **18** and **20** (CIF), copies of ^{1}H, ^{13}C, HMBC and HMQC NMR spectra of all compounds. This material is available free of charge via the Internet at http://pubs.acs.org/.

References

1- Scott, L.J.; Spencer, C.M. *Drugs*, **2000**, *59*, 521-549.

2- Bols, M. *Acc. Chem. Res.* **1998**, *31*, 1-8.

3- Ernholt, V. B.; Thomsen, Ib B.; Lohse, A.; Plesner, I.W.; Jensen, K.B.; Liang, X.; Jakobsen, A.; Bols, M *Chem. Eur. J.,* **2000**, 278-287.

4- Bols, M.; Hazell, R.G. Thomsen, Ib B. *Chem. Eur. J.,* **1997**, 940-947.

5- Liang, X.; Bols, M. *J. Org. Chem.* **1999**, *64*, 8485-8488.

6- Baker, B.R.; Schaus, R.E.; Williams, J.H. *J. Am. Chem. Soc.* **1955**, 7-12.

7- Fleet, G.W.J.; Nicholas, S.J.; Smith, P.W.; Evans, S.V.; Fellows, L.E., and Nash, R.J. *Tetrahedron Lett.,* **1985**, *26*, 3127-3130.

8- Aspinall, I.H.; Cowley, P.M.; Mitchell, G.; Stoodley, R.J. *J. Chem. Soc., Chem. Commun.*, **1993**, 1179-1180.

9- Cowley, P.M.; Stoodley, R.J. *Tetrahedron Lett.,* **1994**, *35*, 7853-7856.

10- Aspinall, I.H.; Cowley, P.M.; Mitchell, G.; Raynor, C.M. and Stoodley, R.J. *J. Chem. Soc. Perkin Trans 1*, **1999**, 2591-2599.

11- Makino, K.; Henmi.,Y; Terasawa, M.; Hara, O; Hamada, Y. *Tetrahedron Lett.,* **2005**, 555-558.

12- Lopez, O.L; Bols, M. *ChemBioChem.,* **2007**, *8*, 657-661

13- Yamamoto, K.; Miyake, H.; Kusunoki, M.; Osaki, S. *FEBS J.*, 2010, 277, 4205-4214.

14- Henrissat, B. *Biochem. J.* **1991** 280, *309-316*.

15- Kuriki, T; Imanaka, T. *J. Biosci. Bioeng*. **1999**, 87*, 557–565*.

16- *Vogel's Textbook of Practical Organic Chemistry*, 4ª ed.; Longman: London, 1978.

17- Lubineau, A.; Queneau, Y. *J. Org. Chem.* **1987**, *52*, 1001-1007 and references cited in.

18- Larsen, D. S.; Stoodley, R. J. *J. Chem. Soc., Perkin Trans.,* **1989**, 1841-1852.

19- Huey, R.; Morris, G.M.; Olson, A.J., Goodsell, D.S. *J. Comput. Chem*., **2007**. *28*, 1145-1152.

20- Sivertsen, A.C.; Gasior, M.; Bjerring, M.; Hansen, S.U.; Lopez, O.L.; Nielson, N.C.; Bols, M. *Eur. J. Org. Chem.* **2007**, *11*, 1735-1742.

21- Sali, A.; Blundell, T.L. *J. Mol. Biol*. **1993**, *234*, 779-815.

22- Laskowski, R.A.; MacArthur, M.W.; Moss, D.S.; Thornton, J.M. *J. Appl. Crystallogr*. **1993**, *26*, 283-291.