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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.

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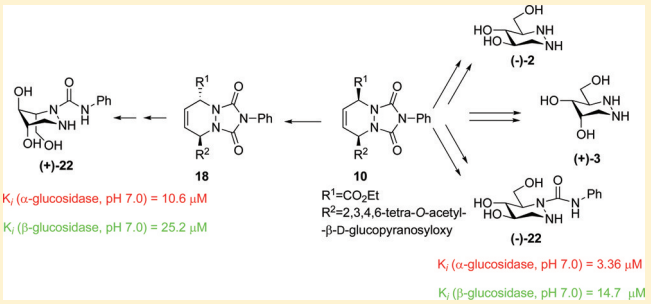
Supporting Information

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's 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 toward 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 modeling. The efficient packing of the aromatic ring of the 1-*N*-phenyl carboxamide moiety into a hydrophobic subsite (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 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 *Escherichia 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.

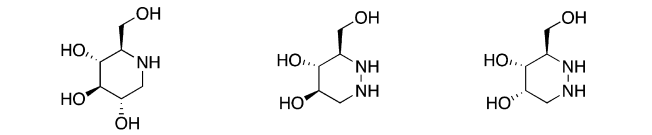


Figure 1. Structure of some known iminosugars.

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**).

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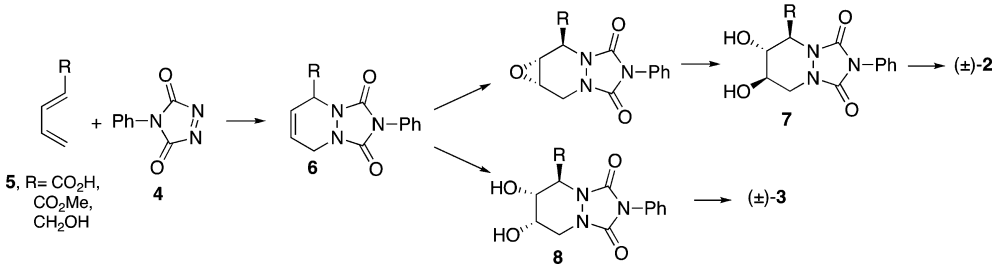
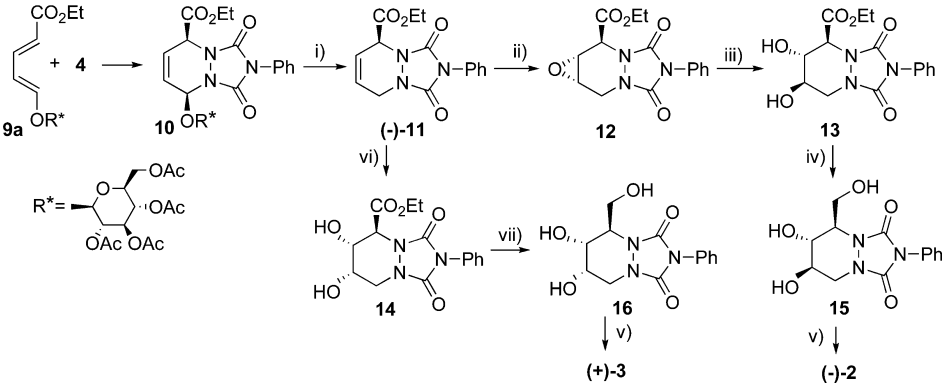
Scheme 1



Scheme 2[a]



[a](i) Et_3SiH, TFA, DCM, 5 h, rt, 61%; (ii) oxone, CF_3COCH_3, $NaHCO_3$, CH_3CN/H_2O (3.5:2), 24 h, 65%; (iii) H_2O, H_2SO_4 (exc.), reflux, 8 h, 52%; (iv) $NaBH_4$ (3 equiv), EtOH, 3 d, rt, 59%; (v) $NH_2NH_2{\cdot}H_2O$, 100 °C, 18 h, 68% (−)-**2**, 64% (+)-**3**; (vi) OsO_4, NMO, acetone:H_2O (2:1), 5 d, rt, 79%; (vii) $NaBH_4$ (3 equiv), EtOH, 3 d, rt, 52%.

Bols[3] also achieved the synthesis of (−)-1-azafagomine (**2**) from relatively expensive L-xylose in six steps. L-2,3,5-Tribenzyl xylofuranose was isolated as an intermediate after three steps with no explicit yield. 1-Azafagomine was then isolated in 37% overall yield from this intermediate.[6,7]

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 1990s, 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 diastereoselectivity (Scheme 2).[8,9] The chemistry of cycloadduct **10** had been pushed forward for the synthesis of dehydropiperazic acid, a nonproteinogenic 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).

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 stereoselectivity by refluxing in aqueous H_2SO_4 giving **13**. Osmilation of compound **11** produced *cis*-diol **14** with total stereoselectivity. 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). Whereas (−)-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 the literature, 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]

(*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, and (iii) triethylamine/*p*-chlorothiophenol in

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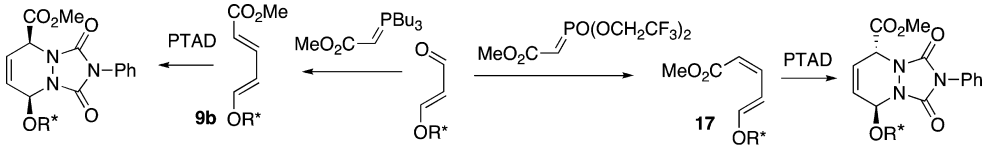
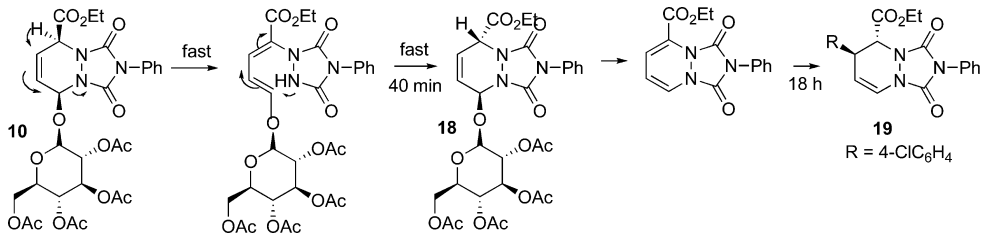
Scheme 3. Stoodley's Synthetic Sequence for Precursors Related to Compounds (−)-2, (−)-22, and (+)-22



Scheme 4. Epimerization of Compound 10 into Compound 18[a]



[a]Reagents: NEt_3, *p*-chlorothiophenol, MeOH, 0 °C → rt.

MeOH (Scheme 4). When triethylamine was the sole reagent, isolation of compound **18** was difficult because of the 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).

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

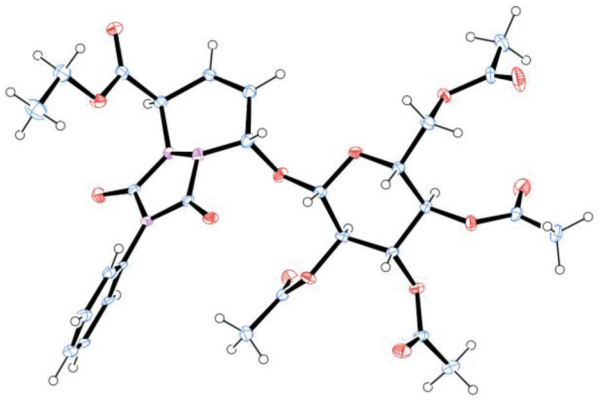


Figure 2. ORTEP view of compound **18**.

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).

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).

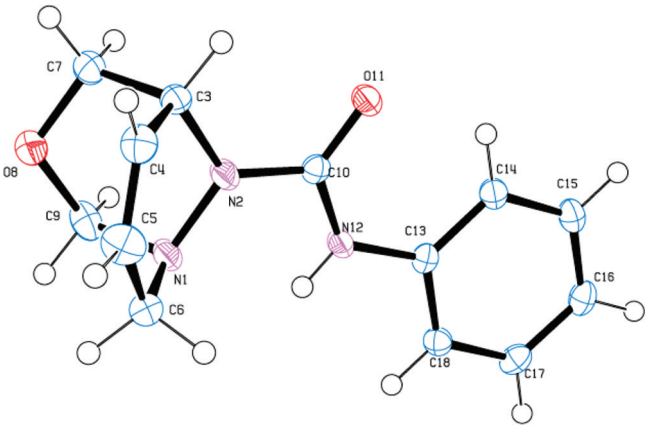


Figure 3. ORTEP view of compound **20**.

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.

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

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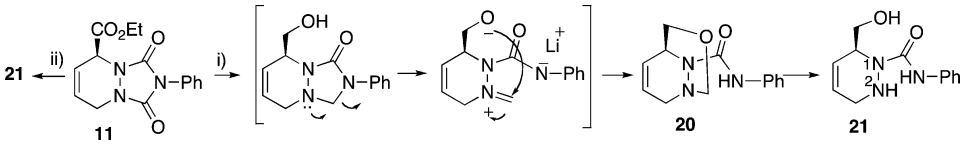
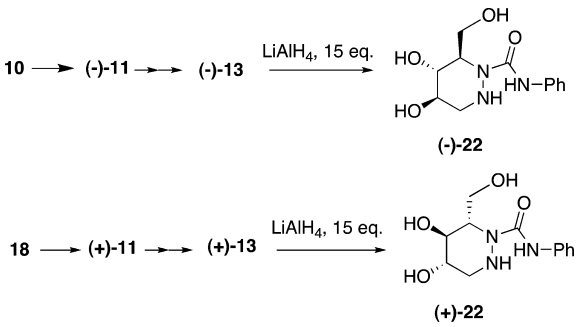
Scheme 5[a]



[a](i) $LiAlH_4$ (7 equiv from a bottle opened for a long time), THF, 4 h, 0 °C → rt, 86%; (ii) $LiAlH_4$ (15 equiv from a recently opened bottle), THF, 4, 0 °C → rt, 48%.

Scheme 6



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).

The *N*-propylphenyl derivative **23** is around an order of magnitude more effective as a 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 values toward 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, whereas its isomer (+)-**22** is slightly less active. Both enantiomers of compound **22** display lower activity toward 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** toward 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, whereas (+)-1-azafagomine is virtually inactive toward the same α-glucosidase that was used in this study.[3]

The yeast α-glucosidase enantioselective discrimination toward (+)-**22** and (−)-**22** was studied using molecular docking methodologies. This enzyme, as well as the *Saccharomyces cerevisiae* enzyme used for the homology modeling,[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 toward hydration of D-glucal and the hydrolysis of *p*-nitrophenyl α-2-deoxyglucosides.[14,15] The active site structure in 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 this, the observation of the binding pose of both enantiomers of **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.

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, and 349, and arginine 212 and 439 and a hydrophobic pocket flaked by phenylalanine 157 and 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, and 349, arginine 212 and 439, and histidine 348 of the α-glucosidase enzyme active site. The highlighted nonbonded 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. On the basis of these observations, the molecular modeling 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 diastereoselective 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** was revealed to be the key step in the synthesis of the dextrorotatory compound **22**. This methodology represents an advantageous alternative to other

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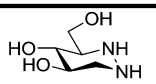
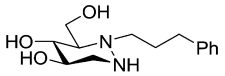
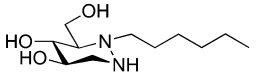
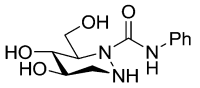
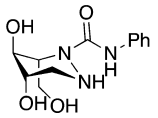
Table 1. K_i Values (μM) for the Inhibition of α- and β-Glucosidases by Compounds 22 and Other Azasugars at Different pH Values

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.

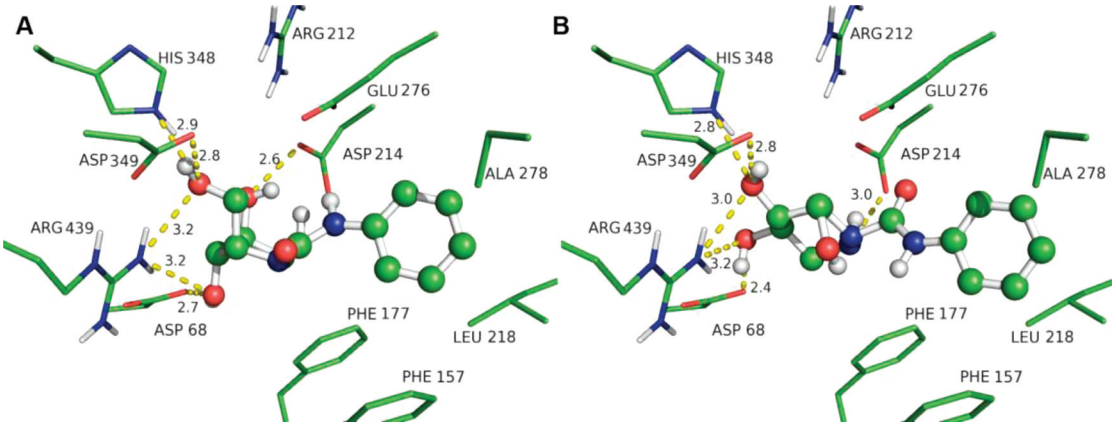


Figure 4. Structure of the lowest binding free energy complexes between yeast α-glucosidase binding site and the enantiomers of compound **22**: (A) (+)-**22** and (B) (−)-**22**. The figure is rendered with Corey−Pauling−Koltun (CPK) coloring scheme. Selected side-chain residues of the yeast α-glucosidase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

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 toward α- and β-glucosidase. The low K_i value determined toward α-glucosidase inhibition is particularly relevant in comparison with 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 modeling 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 Methods. 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 to the literature,[16] and 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 the literature.[4] Functionalization of the double bond was obtained by osmilation with OsO_4 in acetone/water or with

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oxone, trifluoromethylacetone, in the presence of $NaHCO_3$ and aqueous acetonitrile. Reduction of the oxazolidinone was done either with freshly opened $LiAlH_4$ 1 M in THF or with long-term opened bottles (over a month) of $LiAlH_4$ 1 M 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 a 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 24 h. The solvent was evaporated, giving an oil subjected to dry-flash chromatography (petroleum ether/diethyl ether; gradient of polarity) (**9**, 1.17 g; 63%): $[\alpha]_D^{20}$ +27.0° (*c* = 1, CH_2Cl_2); ν_{max} (Nujol, cm^{-1}) 2955, 2934, 1736, 1698, 1635; ^1H 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 colored solution that quickly lost its color. 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%): $[\alpha]_D^{20}$ +23,8° (*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) were 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 redissolved in DCM (30 mL). The solution was washed with an aq saturated solution of $NaHCO_3$ (3 × 50 mL) and water (50 mL). The combined organic layers were dried over magnesium sulfate and filtered, and the solvent was 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%): $[\alpha]_D^{20}$ −311.0° (*c* = 2.15, CH_2Cl_2); mp 140−142 °C; 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) were 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 was redissolved in DCM (25 mL). The solution was washed with an aq saturated solution of $NaHCO_3$ (3 × 25 mL) and water (25 mL). The combined organic layers were dried over magnesium sulfate and filtered, and the solvent was 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%): $[\alpha]_D^{20}$ +370.0° (*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), were added solid $NaHCO_3$ (2.44 g; 29.02 mmol) and oxone (12.00 g; 39.04 mmol) for 20 min at 0 °C. 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, which was extracted with $CHCl_3$ (8 × 40 mL). The organic layers were combined and dried with magnesium sulfate. After removal of the solvent, recrystallization with diethyl ether gave a white solid identified as the title compound ((−)-**12**, 0.329 g; 65%): $[\alpha]_D^{20}$ − 238.0° (*c* = 0.8, CH_2Cl_2); mp 218−220 °C; ν_{max} (Nujol, cm^{-1}) 2950, 2923, 1775, 1750, 1715, 1458, 1094, 1034; ^1H 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) were added solid $NaHCO_3$ (1.86 g; 29.02 mmol) and oxone (9.15 g; 39.04 mmol) for 20 min at 0 °C. 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, which was extracted with DCM (10 × 40 mL). The organic layers were combined and dried with magnesium sulfate. After removal of the solvent, recrystallization with diethyl ether gave a white solid identified as the title compound ((+)-**12**, 0.233 g; 60%): $[\alpha]_D^{20}$ + 232.8° (*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 concentrated 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 was evaporated until 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 sulfate, 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%): $[\alpha]_D^{20}$ −22.8° (*c* = 2, acetone); ν_{max} (Nujol, cm^{-1}) 3596−3540, 2954, 2923, 1729, 1698, 1122, 1088; ^1H 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;

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^{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 concentrated 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 was evaporated until 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 sulfate, 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%): $[\alpha]_D^{20}$ +26.9° (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) were 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 an aq solution of $Na_2S_2O_3$ 5% (25 mL) was added to mixture, which was stirred for 15 min. The solution was extracted with ethyl acetate (4 × 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%): $[\alpha]_D^{20}$ −110.6° (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, so a new amount of $NaBH_4$ (8 mg; 0.22 mmol) was added, and the mixture was 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 was stirred for 10 min and evaporated. The residue was dissolved in water (10 mL) and extracted with ethyl acetate (8 × 15 mL). The organic phases were combined and dried over magnesium sulfate. Evaporation of the solvent gave a white solid identified as the title compound (**15**, 0.037 g; 59%): $[\alpha]_D^{20}$ −70.4° (c = 1.2, acetone). The spectroscopic data of the racemic mixture was 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, and the residue was dissolved in water (20 mL) and saturated aq solution of $NaHCO_3$ (10 mL) and extracted with ethyl acetate (14 × 25 mL). The organic layers were combined, dried over magnesium sulfate, and concentrated. It obtained a white solid identified as the title compound (**16**, 0.112 g; 52%): $[\alpha]_D^{20}$ −8.0° (c = 0.75, acetone). The spectroscopic data of the racemic mixture was 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) were added 4-chorothiophenol (0.10 g; 0.68 mmol) and triethylamine at 0 °C and under magnetic stirring. After 40 min, the solvent was evaporated, and the crude was 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%): mp 154−157 °C; $[\alpha]_D^{20}$ + 219.7° (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, 4 × s, 4 × $\underline{CH_3}CO_2$), 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 ($\underline{CH_3}CO_2$), 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 equiv, 5.2 mL), from a flask containing a white deposit, at 0 °C. The mixture was kept under stirring for 4 h 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 was extracted with ethyl acetate (4 × 25 mL). The combined organic layers were washed with saturated aq $NaHCO_3$ (25 mL) and brine (25 mL) and then dried over $MgSO_4$. After evaporation of the ethyl acetate, a yellowish crude crystallized giving **20** (0.088 g; 0.36 mmol; 48%): $[\alpha]_D^{20}$ −57.5° (c = 0.4, $CHCl_3$); ν_{max} (Nujol, cm^{-1}) 3320, 1670, 1604, 1591, 1530; ^{1}H 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 10.5 Hz, 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 $LiAlH_4$ 1 M in THF (15 equiv; 13.5 mL), freshly open, at 0 °C. The mixture was kept under stirring for 4 h 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 was extracted with ethyl acetate (5 × 40 mL). The combined organic layers were washed with saturated aq $NaHCO_3$ (50 mL) and brine (50 mL) and then dried over $MgSO_4$. After evaporation of the ethyl acetate, a yellowish crude was obtained, from which crystallized a solid (0.10 g; 0.43 mmol; 86%): $[\alpha]_D^{20}$ −150.8° (c = 0.4, $CHCl_3$); ν_{max} (Nujol, cm^{-1}) 3359, 3268, 3058, 1636, 1601, 1592, 1536; ^{1}H 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.

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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 °C a solution of $LiAlH_4$ 1 M in THF (7 equiv; 2.51 mL). The reaction mixture stirred for 3 h at rt, and 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 × 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%): $[\alpha]_D^{20}$ −54.4° (*c* = 0.6, methanol); ν_{max} (neat, cm^{-1}) 3346, 2925, 1656, 1592, 1534; ^1H 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 °C a solution of $LiAlH_4$ 1 M in THF (7 equiv; 5.03 mL). The reaction mixture was stirred for 1.5 h at rt, and 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 × 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%): $[\alpha]_D^{20}$ +51.3° (*c* = 1, methanol).

Measurement of Glycosidase Inhibition. α-Glucosidase from baker's 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 well 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 °C. A range of substrate concentrations from 3.3×10^{-5} M to 2.0×10^{-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**, 5×10^{-6} M and 10×10^{-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 °C, measuring the absorbance (1 reading each minute) at 400 nm. A value of ε_1 = 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 absorbance into product concentration. Initial velocities were calculated from the slopes of the absorbance vs time graphs for each concentration of substrate and used to construct Michaelis−Menten plots. The kinetic parameters K_M and V_{max} 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 two 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}/K_M)-1)), where 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 two independent K_i determinations.

Structural Molecular Modeling Studies. Structural enzyme−compound complexes and theoretical binding free energy of (−)-**2**, (+)-**2**, and **22** toward yeast α-glucosidase structure were done with computational docking methodologies using AUTODOCK 4.[19] The modeling of the enzyme−compound complexes with almond β-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 evidence.[20] The grid for probe-target energy calculations was placed with its center at the enzyme-binding site. The docking grid size was 42 × 40 × 42 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 cutoff of 1 Å. Since no experimental structure exists for the yeast α-glucosidase enzyme, a theoretical structural model of this enzyme was derived using MODELER,[21] employing the crystal structure of isomaltase from *S. cerevisiae* structure (PDB ID: 3A4A)[13] as template. Isomaltase and α-glucosidase from *S. cerevisiae* share 72% sequence similarity. Twenty models were generated using an initial alignment between the isomaltase and α-glucosidase enzyme sequences. The model with the lowest objective function[21] was chosen, and its quality was evaluated on the basis of its stereochemistry given by Procheck.[22] A high quality model of the yeast α-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.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Crystallographic data and ORTEP drawing for compounds **18** and **20** (CIF) and ^1H, ^{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/.

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