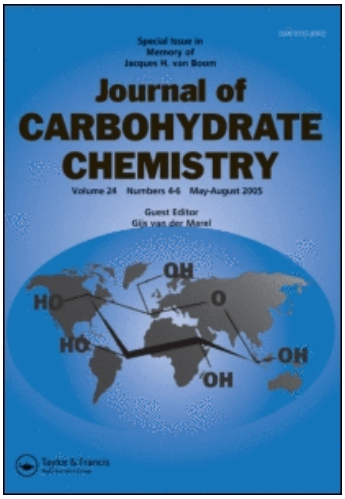

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POLYHYDROXYPYRROLIDINE GLYCOSIDASE INHIBITORS RELATED TO (+)-LENTIGINOSINE[1]

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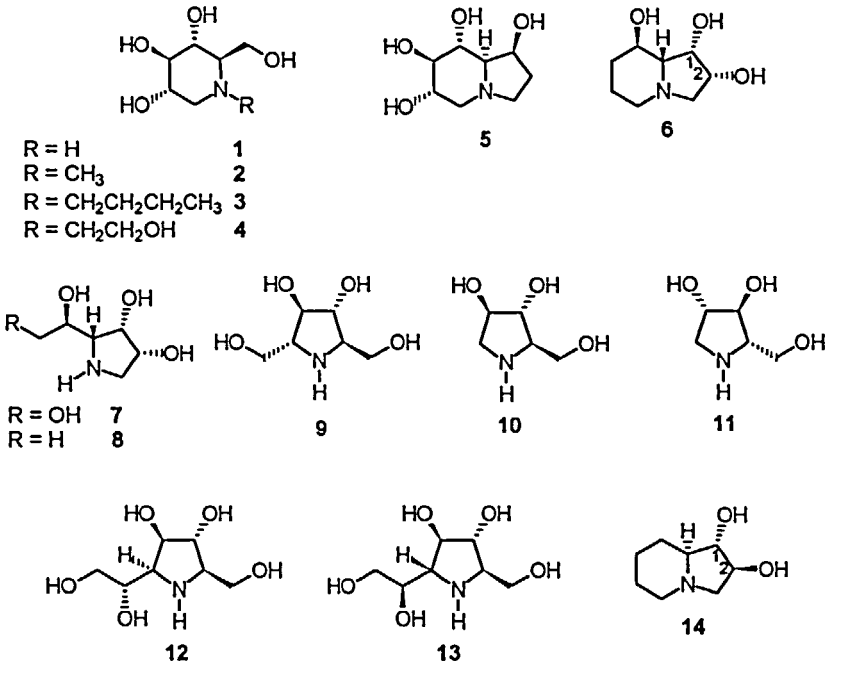
ABSTRACT

(+)-Lentiginosine (**14**) and (7*R*)-7-hydroxylentiginosine (**26**), powerful inhibitors of amyloglucosidases, and their enantiomers were obtained in high overall yields by a multistep synthesis involving 1,3-dipolar cycloaddition of enantiopure tartaric acid derived pyrroline *N*-oxides. Structurally related (*S*,*S*)-3,4-dihydroxypyrrolidines **29-33** were synthesized as simpler models and tested towards 24 glycosidases.

INTRODUCTION

Inhibitors of glycosidases are potential chemotherapeutics towards a range of diseases (diabetes, viral and bacterial infections, cancer).[2] The mechanism of action is related to inhibition of the biosynthesis of glycoproteins, which are responsible for recognition and adhesion of exogenous agents.[3] Effective inhibitors are compounds able

to mimic the terminal unit of oligosaccharides (or the transition state involved in the process),[4] which compete with the natural substrates for occupying the enzyme active site.



Among the best glycosidase inhibitors, imino sugars (sugar analogues with basic nitrogen instead of oxygen in the ring, i.e., polyhydroxypiperidines and pyrrolidines) and related bicyclic *N*-bridgehead heterocycles, i.e., polyhydroxylated indolizidines and pyrrolizidines, occupy a prominent place.[5] For example, deoxynojirimycin, DNJ (**1**), its derivatives **2-4** and castanospermine (**5**) are powerful inhibitors of α-glucosidases[5,6] and swainsonine (**6**) is a very good inhibitor of α-mannosidases.[5,6] Castanospermine and derivatives display also excellent anti-HIV properties.[2f-i] It is apparent that these sugar mimics inhibit the glycosidic enzyme specific for hydrolyzing the saccharide whose terminal carbohydrate has the same configuration at all the stereogenic centres. However, often the structure-activity relationship is not obvious. This is frequently the case for pyrrolidine iminocyclitols.[6a,7] As expected, both iminomannitols **7** and **8** are in fact, like swainsonine, powerful mannosidase inhibitors.[5,6] However, 2,5-dideoxy-2,5-imino-D-mannitol (DMDP, **9**), a fructose mimic in its furanose form, displays opposite inhibition

properties towards invertases from different organisms[7] and the related lower homologue 1,4-dideoxy-1,4-imino-D-arabinitol (DAB1, **10**) is, in contrast, a powerful inhibitor of α-glucosidases.[8] Its synthetic enantiomer LAB1 (**11**) is even more active towards α-glucosidases[8] and its higher homologues **12** and **13** are good inhibitors of both α- and β-glucosidases.[9] It has been reported recently that a much less functionalized indolizidine than castanospermine (**5**) or swainsonine (**6**) such as lentiginosine (**14**) is a very good inhibitor of α-glucosidases. It showed high selectivity towards amyloglucosidases.[10] The first synthesis of this natural compound as a pure enantio- and diastereoisomer was reported by us.[11] We could thus establish its relative and absolute configuration and glycosidase inhibition activity. Importantly, the pure compound obtained by synthesis displayed inhibition of amyloglucosidases better than the natural product. This finding prompted us to study in detail the behavior of (+)-lentiginosine and of some related compounds. Therefore, we pursued a computational study in order to establish the peculiarities and the interactions in the active site region of the complex enzyme-inhibitor.[12] Then we accomplished the synthesis of compounds structurally related to (+)-lentiginosine, e.g., its 7-hydroxy derivatives, and studied their biological properties.[13]

Compared to the other known glycosidase inhibitors possessing the indolizidine structure, the peculiarity of (+)-lentiginosine and their analogues rests in their 1,2-*trans*-dihydroxy substitution. We then expanded our efforts in two opposite directions, aimed at simplification and at sophistication of the structure. On one hand the *trans*-3,4-dihydroxypyrrolidine substructure was linked to a saccharide unit,[14] since recent suppositions and findings suggested that an oligomeric pseudodisaccharide might improve selectivity towards glycosidases.[15] This was confirmed experimentally, albeit it was accompanied by a decrease in activity,[14] presumably due to a more conflicting fitting of the compound into the enzyme's active site. On the other hand, simplification in the structure was expected to bring an easier accommodation into the active site, but possibly a loss in stability of the complex and/or in selectivity towards different enzymes. Our next goal was, then, to synthesize analogues of lentiginosine, still possessing all the important features of the alkaloid for recognition by the enzyme, i.e. the *trans*-configured hydroxy groups and the tertiary amine as suggested by the computational study,[12] but much more readily synthetically available. We thus report the synthesis of a series of *N*-substituted

3,4-*trans*-dihydroxypyrrolidines and their inhibition activity towards glycosidases, as well as the details of the novel and advantageous synthesis of (+)-lentiginosine.[16]

RESULTS AND DISCUSSION

Our previous syntheses of (+)-lentiginosine **(14)**[11] and of (7*R*)-7-hydroxylentiginosine[13] present several critical points which prohibit their preparation in large amounts as required for their *in vivo* testings. The poor regioselectivity of the methylenecyclopropane cycloaddition and the low chemoselectivity of the following rearrangement are two main drawbacks of our first protocol.[11,17] A suitable alternative was envisaged in the use of the *tert*-butyl or *tert*-butyldimethylsilyl protected dihydroxy nitrones **15**[18] and **16**[19] and their cycloaddition to 3-buten-1-ol **(17)** as dipolarophile for the construction of the indolizidine nucleus.[20]

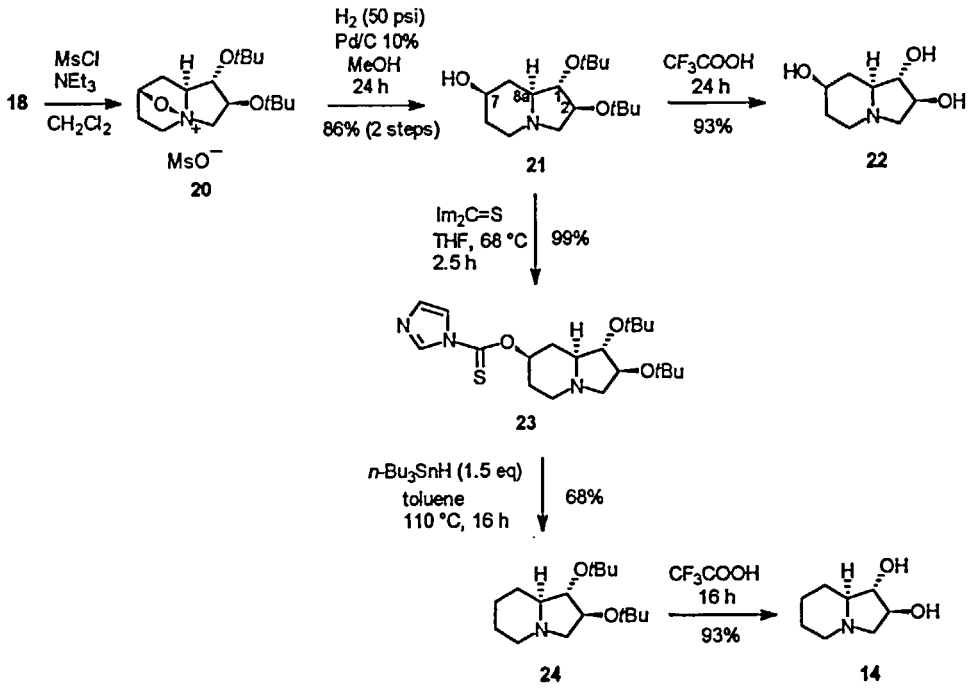
R = *t*Bu **15** 60 °C, 2 d 100% (10:2:1) **18** 74%
R = TBDMS **16** 60 °C, 3 d 74% (6:2:1) **19** 55%

Scheme 1

The cycloaddition of nitrones **15** and **16** to 3-buten-1-ol **(17)** were highly regioselective affording mixtures of the expected 2-(2-hydroxyethyl)isoxazolidines (Scheme 1).[11,14,19,20b] The major isomer was separated in each case and completely characterized on the basis of spectral data and comparison with our previous findings on related reactions.[11,14,19,20b] Their structures were then assigned as **18** and **19**, respectively, derived as expected from the most favored *exo-anti* transition state. Two other diastereoisomers were visible in the ^{1}H NMR spectra of the crude reaction mixtures: the integration of the corresponding signals gave a 10:2:1 diastereomeric ratio for the reaction with nitrone **15** and a 6:2:1 ratio from nitrone **16**. The minor diastereoisomers were only partially separated (see Experimental Part): they presumably derive from the less viable

endo-anti and *exo-syn* transition states.[11,14,19,20b] As the yield to convert L-tartaric acid into **16** was lower than its conversion into **15**, and considering the lower *exo-anti/exo-syn* (3:1) diastereoselectivity for reaction **16** + **17** than for reaction **15** + **17** (5:1), the latter cycloaddition was selected for our synthetic purposes.

Compound **18** could be obtained in 74% isolated yield after column chromatography. It was then transformed into the corresponding mesylate which underwent internal S_N2 type attack by the amino group[20] giving the salt **20** quantitatively (Scheme 2). The crude salt was subjected to reductive ring-opening with H_2 (50 psi, Pd/C) to afford the diprotected indolizidine triol **21** in 86% yield. Deprotection of **21** by trifluoroacetic acid afforded (1*S*,2*S*,7*R*,8a*S*)-trihydroxyoctahydroindolizine (**22**)[13] in excellent yield (93%).



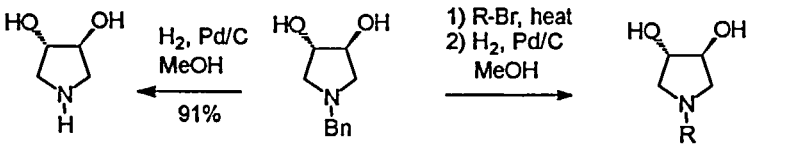
Scheme 2

The synthesis of lentiginosine (**14**) was completed by a Barton-McCombie reaction[21] via the thiocarbonylimidazolide **23**. This derivative underwent clean reduction to the diprotected lentiginosine **24** by reaction with tri(*n*-butyl)tin hydride in refluxing

toluene (Scheme 2). Final deprotection afforded lentiginosine (**14**) that was identical with an authentic sample ($[\alpha]_D^{23} = +2.2$ (*c* 0.28, MeOH)).[11]

Thus, a new synthetic protocol for (+)-lentiginosine (**14**) in 10 steps and 25% overall yield (vs 9 steps, 2.4%)[11] and 7-hydroxylentiginosine **22** in 8 steps and 37% overall yield (vs 9 steps, 3.6%)[13] has been developed starting from inexpensive commercially available L-tartaric acid derivatives. Starting from D-tartaric acid, enantiomeric nitrone (***ent*)-15** was obtained. Its cycloaddition to **17** and further transformations as above provided (1*R*,2*R*,7*S*,8a*R*)-1,2,7-trihydroxyoctahydroindolizine (***ent*)-22** and (−)-lentiginosine (***ent*)-14**, much less active than the enantiomer,[11b] with the same ease as **22** and **14**.

The synthesis of a series of *N*-substituted 3,4-dihydroxypyrrolidines with the same configuration as (+)-lentiginosine (**14**) to mimic the lipophilic ring of this indolizidine was then carried out. The monocyclic *N*-substituted pyrrolidines **26-28** were obtained readily from *N*-benzylpyrrolidine (**25**),[22] an intermediate in the synthesis of nitrone **16**,[19] by nucleophilic substitution with an appropriate bromo derivative followed by catalytic hydrogenation of the quaternary ammonium salt (Scheme 3). Appropriate structural variations for enzymatic testings were considered such as the length of the chain at the nitrogen center and the presence of a further hydroxy functionality at the end of the *N*-chain. Dihydroxypyrrolidine **29**,[23] derived from **25** by hydrogenolysis, was also considered.



R = CH_2CH_3 **26** (46%)
R = CH_2CH_2OH **27** (66%)
R = $CH_2CH_2CH_2OH$ **28** (80%)

Scheme 3

All the dihydroxypyrrolidines **25-29** were then tested for their inhibitory activities towards a large variety of glycosidases (see Experimental). Table 1 reports the results towards the glycosidases which showed some degree of inhibition. The inhibition rate (in %) in the presence of 1 mM concentration of the compound **25-29** is reported. IC_{50} values

Table 1. Inhibition rate (in %) towards glycosidases at 1 mM concentration of inhibitors. IC_{50} (in parentheses) and K_i values are given in μM concentrations.

		25	26	27	28	29
Amyloglucosidase EC 3.2.1.3	from *Rhizopus* mold	30%	52% (950)	0	55% (495)	0
β-Glucosidase EC 3.2.1.21	from almonds	0	91% (124) 534	78% (210) 538	54% (825)	0
	from caldocellum saccharolyticum	0	90% (55) 37	75% (282) 591	40%	0
β-Galactosidase EC 3.2.1.23	from bovine liver	43%	0	0	0	0
	from *Aspergillus orizae*	0	0	40%	0	0
	from jack beans	0	0	71% (451) 723	0	0

were calculated for those compounds which showed ≥50% inhibition and K_i values for those compounds which showed ≥70%.

Our enzymatic assays demonstrated that some activity and selectivity is still retained by the simple dihydroxypyrrolidines, since only a few glycosidases were inhibited among the 24 enzymes tested (see Experimental). Pyrrolidines **26** and **28** and the simple benzylpyrrolidine **25** itself showed some inhibition of amyloglucosidase from *Rhizopus* mold, analogously to (+)-lentiginosine (**14**). However, the rate of inhibition is much decreased, suggesting that subtle factors are involved in the recognition by the enzyme. Indeed, pyrrolidine **27**, having the same number of carbon atoms as **26** and the final hydroxy group as in **28**, was not able to inhibit the enzyme. Moreover, none of the pyrrolidines did inhibit amyloglucosidase from *Aspergillus niger*. The *N*-unsubstituted pyrrolidine **29** showed no inhibition towards any of the enzymes tested, albeit it had been previously demonstrated to be an inhibitor of arabinofuranosidase III.[24] More importantly, the pyrrolidines studied gained activity towards different glycosidases, namely β-

galactosidases and β-glucosidases. Again, subtle structural changes control the inhibitory activity, since only pyrrolidine **27** (which behaves differently from **26** and **28**) inhibited β-galactosidases from *Aspergillus orizae* and jack beans and only pyrrolidine **25** showed a modest inhibition of β-galactosidase from bovine liver. Noteworthy is the observation that all the pyrrolidines **26-28** are fairly good inhibitors of β-glucosidases from almonds and caldocellum saccharolyticum, the extent of the inhibition likely depending on the length of the chain at the nitrogen center. These results suggest that the synthesized dihydroxypyrrolidines **26-28** are better mimics of the transition state for β-glycosidase reactions, where the positive charge generated at the nitrogen atom corresponds to the charged anomeric position of the substrate.[4a,h]

EXPERIMENTAL

General methods. The reactions which required anhydrous conditions were run under a nitrogen or an argon atmosphere using solvents dried according to the usual procedures. Melting points (mp) were measured with an RCH Kofler apparatus and are uncorrected. R_f values are referred to TLC on 0.25 mm silica gel plates (Merck F_{254}) by eluting with the same eluent used for the chromatographic separation of the compound. Optical rotations were measured with a JASCO DIP-370 polarimeter. 200 MHz ^{1}H NMR and 50.3 MHz ^{13}C NMR spectra were recorded on a Varian Gemini spectrometer. 500 MHz ^{1}H NMR spectra were recorded on a Bruker DRX 500 spectrometer. When not specified, spectra were recorded in $CDCl_3$. NMR signal attributions of proton signals was also allowed by bidimensional spectroscopy (COSY, NOESY) experiments. IR spectra were recorded with a Perkin Elmer 881. Mass spectra were measured with a QMD 1000 Carlo Erba Instrument (EI, 70 eV). Elemental analyses were recorded with a Perkin-Elmer 240 C instrument.

Cycloaddition of nitrone 15 to but-3-en-1-ol (17). A solution of (3*S*,4*S*)-3,4-bis(*tert*-butoxy)-1-pyrroline *N*-oxide[18] (**15**, 1.1 g, 4.8 mmol) and but-3-en-1-ol (**17**, 2.05 mL, 23.9 mmol) in benzene (2 mL) was heated to 60 °C in a sealed tube for 2 days. The crude mixture was then concentrated to give a 10:2:1 (by 500 MHz ^{1}H NMR) mixture

(1.444 g, 100%) of **18** with 2 diastereoisomers. Purification by column chromatography on silica gel (EtOAc) afforded pure **18** (R_f 0.32, 1.07 g, 3.55 mmol, 74%) as an oil and one minor diastereoisomer (R_f 0.15, 188 mg, 0.624 mmol, 13%), containing traces of impurities, as an oil.

(2*S*,3a*S*,4*S*,5*S*)-4,5-Bis(*tert*-butoxy)-2-(2-hydroxyethyl)hexahydropyrrolo [1,2-*b*]isoxazole (18). $[\alpha]_D^{21}$ +65.9 (*c* 0.59, $CHCl_3$). ^{1}H NMR (500 MHz): δ 4.41 (dq, $J_{1,3}$ = 4.3, 7.8 Hz, 1 H, H-2), 3.90 (dt, $J_{1,3}$ = 8.1, 5.8 Hz, 1 H, H-5), 3.78-3.74 (m, 1 H, H-4), 3.75 (t, $J_{1,3}$ = 5.9 Hz, 2 H, H-2'), 3.49 (dt, $J_{1,3}$ = 9.3, 4.4 Hz, 1 H, H-3a), 3.46 (dd, $J_{1,2}$ = 10.3 Hz, $J_{1,3}$ = 5.9 Hz, 1 H, Ha-6), 2.85 (dd, $J_{1,2}$ = 10.3 Hz, $J_{1,3}$ = 8.0 Hz, 1 H, Hb-6), 2.37-1.78 (m, 4 H), 1.19 (s, 9 H, *t*Bu), 1.18 (s, 9 H, *t*Bu); ^{13}C NMR (50.3 MHz): δ 81.9 (d), 75.9 (d), 74.6 (d), 73.9 (s, 2 C, *t*Bu), 69.6 (d), 60.6 (t), 59.4 (t), 40.2 (t), 35.9 (t), 28.8 (q, 3 C, *t*Bu), 28.5 (q, 3 C, *t*Bu); IR ($CDCl_3$): 3895, 3752, 3687, 3609, 2979, 2938, 1364, 1187 cm^{-1}; MS: *m/z* (relative intensity) 301 (M^+, 11), 245 (9), 244 (69), 188 (50), 116 (57), 84 (60).

Anal. Calcd for $C_{16}H_{31}NO_4$: C, 63.76; H, 10.37; N, 4.65. Found: C, 63.36; H, 10.43; N, 4.87.

Minor diastereoisomer, presumably (2*R*,3a*R*,4*S*,5*S*)-4,5-Bis(*tert*-butoxy)-2-(2-hydroxyethyl)hexahydropyrrolo [1,2-*b*]isoxazole. ^{1}H NMR (200 MHz): δ 4.22 (dq, $J_{1,3}$ = 4.0, 6.5 Hz, 1 H, H-2), 4.04-3.67 (m, 4 H), 3.26 (dd, $J_{1,2}$ = 13.6 Hz, $J_{1,3}$ = 6.3 Hz, 1 H, Ha-6), 2.92 (dd, $J_{1,2}$ = 13.5 Hz, $J_{1,3}$ = 8.1 Hz, 1 H, Hb-6), 2.62 (ddd, $J_{1,2}$ = 12.4 Hz, $J_{1,3}$ = 6.9, 3.6 Hz, 1 H, Ha-3), 1.96-1.67 (m, 4 H), 1.18 (s, 9 H, *t*Bu), 1.17 (s, 9 H, *t*Bu); ^{13}C NMR (50.3 MHz): δ 77.2 (d), 76.9 (d), 75.4 (d), 73.6 (s, 2 C, *t*Bu), 65.5 (d), 60.0 (t), 59.7 (t), 36.6 (t), 35.8 (t), 28.5 (q, 6 C, *t*Bu); IR ($CDCl_3$): 3622, 3537, 2978, 2934, 1364, 1188 cm^{-1}; MS: *m/z* (relative intensity) 301 (M^+, 6), 245 (3), 244 (31), 188 (21), 116 (33).

Cycloaddition of nitrone 16 to but-3-en-1-ol (17). A solution of (3*S*,4*S*)-3,4-bis[(*tert*-butyl)dimethylsilyloxy]-1-pyrroline *N*-oxide[19] (**16**, 188 mg, 0.54 mmol) and but-3-en-1-ol (**17**, 0.23 mL, 2.7 mmol) in benzene (2 mL) was heated to 60 °C in a sealed tube for 3 days. The crude mixture was then concentrated to give a 6:2:1 (by 500 MHz ^{1}H NMR) mixture (166 mg, 0.398 mmol, 74%) of **19** with 2 diastereoisomers. Purification by column chromatography on silica gel (light petroleum ether-EtOAc 1:1,

afforded pure **19** (R_f 0.33, 124 mg, 0.297 mmol, 55%) as an oil and a mixture of one minor diastereoisomer with traces of the third one (R_f 0.20, 33.7 mg, 0.081 mmol, 15%).

(2*S*,3a*S*,4*S*,5*S*)-4,5-Bis(*tert*-butyldimethylsilyloxy)-2-(2-hydroxyethyl) hexahydropyrrolo[1,2-*b*]isoxazole (19). $[\alpha]_D^{26}$ +34.5 (*c* 0.50, MeOH). ^{1}H NMR (200 MHz): δ 4.37 (tdd, $J_{1,3}$ = 8.0, 6.2, 4.2 Hz, 1 H, H-2), 4.01 (td, $J_{1,3}$ = 5.4, 4.1 Hz, 1 H, H-5), 3.89 (t, $J_{1,3}$ = 3.8 Hz, 1 H, H-4), 3.75 (t, $J_{1,3}$ = 5.6 Hz, 2 H, H-2'), 3.56 (dt, $J_{1,3}$ = 9.0, 3.6 Hz, 1 H, H-3a), 3.54 (dd, $J_{1,2}$ = 12.2 Hz, $J_{1,3}$ = 5.2 Hz, 1 H, Ha-6), 2.98 (dd, $J_{1,2}$ = 12.2 Hz, $J_{1,3}$ = 5.6 Hz, 1 H, Hb-6), 2.28 (ddd, $J_{1,2}$ = 12.2 Hz, 6.2, $J_{1,3}$ = 4.0 Hz, 1 H, H-3), 2.18-2.03 (m, 1 H), 1.91-1.79 (m, 2 H), 0.89 (s, 18 H, *t*Bu TBDMS), 0.08 (s, 12 H, CH_3 TBDMS); ^{13}C NMR (50.3 MHz): δ 83.6 (d), 77.3 (d), 74.4 (d), 71.1 (d), 61.4 (t), 60.0 (t), 40.0 (t), 35.6 (t), 25.6 (q, 6 C, TBDMS), 17.8 (s, 2 C, TBDMS), –4.6 (q, 2 C, TBDMS), –4.9 (q, TBDMS), –5.0 (q, TBDMS); MS: *m/z* (relative intensity) 417 (M^+, 11), 360 (10), 286 (3), 171 (22), 147 (55), 129 (72), 75 (27), 73 (100), 57 (39).

Anal. Calcd for $C_{20}H_{43}NO_4Si_2$: C, 57.51; H, 10.38; N, 3.35. Found: C, 58.01; H, 10.68; N, 2.90.

Minor diastereoisomer, presumably (2*R*,3a*R*,4*S*,5*S*)-4,5-Bis(*tert*-butyldimethylsilyloxy)-2-(2-hydroxyethyl)hexahydropyrrolo[1,2-*b*]isoxazole. ^{13}C NMR (50.3 MHz): δ 78.2 (d), 77.9 (d), 76.7 (d), 66.8 (d), 60.8 (t), 60.2 (t), 35.9 (t), 35.3 (t), 25.7 (q, 6 C, TBDMS), 17.9 (s, TBDMS), 17.9 (s, TBDMS), –4.6 (q, TBDMS), –4.7 (q, TBDMS), –4.8 (q, TBDMS), –5.0 (q, TBDMS); MS: *m/z* (relative intensity) 417 (M^+, 13), 360 (12), 171 (12), 147 (49), 129 (73), 84 (45), 75 (29), 73 (100), 57 (40).

(1*S*,2*S*,7*R*,8a*S*)-1,2-Bis(*tert*-butoxy)-7-hydroxyoctahydroindolizine (21). To a cooled (0 °C) solution of the cycloadduct **18** (170 mg, 0.56 mmol) in dry CH_2Cl_2 (1.5 mL) and TEA (117 μL, 0.84 mmol), MsCl (48 μL, 0.62 mmol) was added dropwise under a nitrogen atmosphere. The mixture was stirred for 30 min at room temperature, then concentrated, dissolved in MeOH (7 mL) and shaken under hydrogen in a Parr apparatus (45 psi) for 24 h in presence of 10% Pd/C (70 mg). The suspension was then filtered through Celite, and the methanolic solution was passed through a short column of strongly basic Amberlyst A-26. Concentration afforded an analytically pure sample of **21** (136 mg, 0.48 mmol, 86%) as a white solid, mp 118-119 °C. R_f 0.40 (CH_2Cl_2-MeOH 8:1). $[\alpha]_D^{20}$ +53.0 (*c* 1.02, $CHCl_3$). ^{1}H NMR (200 MHz): δ 3.84 (ddd, $J_{1,3}$ = 7.0, 4.0, 1.6

Hz, 1 H, H-2), 3.68 (dd, $J_{1,3}$ = 8.6, 3.9 Hz, 1 H, H-1), 3.63-3.58 (m, 1 H, H-7), 2.94 (ddd, $J_{1,2}$ = 11.2 Hz, $J_{1,3}$ = 4.3, 2.6 Hz, 1 H, Ha-5), 2.91 (dd, $J_{1,2}$ = 10.1 Hz, $J_{1,3}$ = 1.4 Hz, 1 H, Ha-3), 2.44 (dd, $J_{1,2}$ = 10.1 Hz, $J_{1,3}$ = 7.1 Hz, 1 H, Hb-3), 2.22-2.18 (m, 1 H), 2.02-1.52 (m, 5 H), 1.21 (s, 9 H, *t*Bu), 1.18 (s, 9 H, *t*Bu); ^{13}C NMR (50.3 MHz): δ 83.2 (d), 78.0 (d), 73.9 (s, *t*Bu), 73.8 (s, *t*Bu), 69.8 (d), 65.4 (d), 61.2 (t, C-3), 50.5 (t, C-5), 37.9 (t), 34.2 (t), 29.2 (q, 3 C, *t*Bu), 28.7 (q, 3 C, *t*Bu); IR (CCl_4): 3623, 2975, 2935, 1388, 1364, 1187, 1064 cm^{-1}; MS: *m/z* (relative intensity) 285 (M^+, 2), 228 (M^+-57, 100), 212 (7), 172 (33), 113 (44), 57 (97).

Anal. Calcd for $C_{16}H_{31}NO_3$: C, 67.33; H, 10.95; N, 4.91. Found: C, 67.70; H, 11.20; N, 4.78.

(1*S*,2*S*,7*R*,8a*S*)-1,2,7-Trihydroxyoctahydroindolizine (22). A solution of the *tert*-butyl ether **21** (29.5 mg, 0.104 mmol) in TFA (0.45 mL) was stirred at room temperature for 24 h. The mixture was then concentrated, dissolved in MeOH and passed through a short column of strongly basic Amberlyst A-26. After solvent evaporation, the residue was purified by flash column chromatography on silica gel (CH_2Cl_2-CH_3OH-30% aq NH_3 35:19:1) to afford pure **22** (R_f 0.35, 16.7 mg, 0.097 mmol, 93%) as a white solid, identical with an authentic sample.[13] $[\alpha]_D^{21}$ +1.8 (*c* 0.81, MeOH) [lit. $[\alpha]_D^{22}$ +2.1 (*c* 0.36, MeOH)[13]].

(1*S*,2*S*,7*R*,8a*S*)-1,2-Bis(*tert*-butoxy)-7-[(imidazolothiocarbonyl)oxy]octahydroindolizine (23). A solution of **21** (41 mg, 0.14 mmol) and *N,N'*-thiocarbonylimidazole (50 mg, 0.28 mmol) in dry THF (1 mL) was heated under reflux for 2.5 h. Evaporation of the solvent and purification over silica gel (CH_2Cl_2-MeOH 12:1) afforded pure **23** (R_f 0.28, 48.8 mg, 0.139 mmol, 99%). ^{1}H NMR (200 MHz): δ 8.34 (br s, 1 H, Im), 7.63 (m, 1 H, Im), 7.04 (m, 1 H, Im), 5.42 (tt, $J_{1,3}$ = 11.0, 4.4 Hz, 1 H, H-7), 3.88 (ddd, $J_{1,3}$ = 6.9, 3.5, 1.3 Hz, 1 H, H-2), 3.72 (dd, $J_{1,3}$ = 8.4, 3.6 Hz, 1 H, H-1), 3.06-3.01 (m, 1 H, H-8)), 2.94 (br d, $J_{1,2}$ = 9.9 Hz, 1 H, Ha-3), 2.50 (dd, $J_{1,2}$ = 9.8 Hz, $J_{1,3}$ = 6.9 Hz, 1 H, Hb-3), 2.48-2.41 (m, 1 H), 2.18-1.77 (m, 4 H), 1.57 (q, $J_{1,2}$ = 11.0 Hz, $J_{1,3}$ = 11.0 Hz, 1 H, Ha-8), 1.20 (s, 9 H, *t*Bu), 1.18 (s, 9 H, *t*Bu); ^{13}C NMR (50.3 MHz): δ 183.1 (s, C=S Im), 136.8 (d, Im), 130.6 (d, Im), 117.8 (d, Im), 83.2 (d), 81.4 (d), 77.8 (d), 73.9 (s, *t*Bu), 73.8 (s, *t*Bu), 65.0 (d, C-8a), 60.8 (t, C-3), 49.8 (t, C-5), 33.2 (t), 29.4 (t), 29.1 (q, 3 C, *t*Bu), 28.6 (q, 3 C, *t*Bu).

(1*S*,2*S*,8a*S*)-1,2-Bis(*tert*-butoxy)octahydroindolizine (24). To a refluxing solution of *n*-Bu_3SnH (46 μL, 0.173 mmol) in dry toluene (4.6 mL) under an argon atmosphere, was added dropwise a solution of the thiocarbonylimidazolide **23** (89.8 mg, 0.256 mmol) in dry toluene (4.6 mL). After 2 h at reflux temperature, another 46 μL of *n*-Bu_3SnH were added, and the mixture was stirred at reflux for 16 h. After evaporation of the solvent, purification on silica gel (CH_2Cl_2-MeOH 13.5:1) afforded pure **24** (R_f 0.21, 46.8 mg, 0.174 mmol, 68%). $[\alpha]_D^{21}$ +42.8 (*c* 0.48, $CHCl_3$). ^1H NMR (200 MHz): δ 3.77 (ddd, $J_{1,3}$ = 7.0, 4.1, 1.5 Hz, 1 H, H-2), 3.62 (dd, $J_{1,3}$ = 8.8, 4.0 Hz, 1 H, H-1), 2.94-2.87 (m, 2 H), 2.40 (dd, $J_{1,2}$ = 10.3 Hz, $J_{1,3}$ = 7.3 Hz, 1 H, Hb-3), 1.94-1.47 (m, 8 H), 1.19 (s, 9 H, *t*Bu), 1.16 (s, 9 H, *t*Bu); ^{13}C NMR (50.3 MHz): δ 83.7 (d), 76.8 (d), 73.7 (s, *t*Bu), 73.5 (s, *t*Bu), 67.0 (d, C-8a), 62.2 (t, C-3), 53.6 (t, C-5), 29.2 (q, 3 C, *t*Bu), 28.7 (q, 3 C, *t*Bu), 28.6 (t), 24.8 (t), 24.1 (t); MS: *m/z* (relative intensity) 269 (M^+, 4), 212 (100), 196 (8), 156 (72), 126 (10), 97 (37), 57 (33).

Anal. Calcd for $C_{16}H_{31}NO_2$: C, 71.33; H, 11.60; N, 5.20. Found: C, 71.48; H, 11.80; N, 4.82.

(1*S*,2*S*,8a*S*)-1,2-Dihydroxyoctahydroindolizine ((+)-lentiginosine, 14). A solution of the *tert*-butyl ether **24** (70 mg, 0.26 mmol) in TFA (0.8 mL) was stirred at room temperature for 16 h. The mixture was then concentrated, dissolved in MeOH and passed through a short column of strongly basic Amberlyst A-26. After solvent evaporation, the residue was purified by flash column chromatography on silica gel (CH_2Cl_2-CH_3OH-30% aq. NH_3 41:8:1) to afford pure **14** (R_f 0.3, 37.9 mg, 0.241 mmol, 93%) as a white solid, identical with an authentic sample.[11] $[\alpha]_D^{23}$ +2.2 (*c* 0.28, MeOH) [lit. $[\alpha]_D^{25}$ +3.2 (*c* 0.27, MeOH)[11]; $[\alpha]_D^{20}$ +2.8 (*c* 0.28, MeOH)[25]; $[\alpha]_D^{25}$ +1.7 (*c* 0.60, MeOH)[26]].

(3*S*,4*S*)-3,4-Dihydroxy-*N*-ethylpyrrolidine (26). A solution of (3*S*,4*S*)-3,4-dihydroxy-*N*-benzylpyrrolidine (**25**)[22] (143 mg, 0.74 mmol) in bromoethane (2 mL) was heated under reflux for 5 h, then concentrated, dissolved in MeOH (3 mL) and stirred under H_2 for 5 days in the presence of 20% $Pd(OH)_2/C$ (90 mg). The mixture was then filtered through Celite, stirred in presence of Amberlyst A-26 for 30 minutes, filtered and purified on silica gel (CH_2Cl_2-MeOH-30% aq NH_3 30:19:1) to afford **26** (R_f 0.24, 44.1 mg, 0.34 mmol, 46%) as a yellow solid, mp 70-72 °C. $[\alpha]_D^{26}$ +36.5 (*c* 0.43, MeOH). ^1H

NMR (200 MHz, D_2O): δ 4.06 (t, $J_{1,3}$ = 4.6 Hz, 2 H, H-3, H-4), 2.91 (dd, $J_{1,2}$ = 9.9 Hz, $J_{1,3}$ = 4.9 Hz, 2 H, Hb-2, Ha-5), 2.50-2.43 (m, 4 H, Ha-2, Hb-5, H-1'), 0.98 (t, $J_{1,3}$ = 7.3 Hz, 3 H, CH_3); ^{13}C NMR (50.3 MHz, D_2O): δ 79.9 (d, 2 C, C-3, C-4), 61.6 (t, 2 C, C-2, C-5), 53.0 (t, C-1'), 15.0 (q, C-2'); MS: *m/z* (relative intensity) 131 (M^+, 14), 116 (46), 112 (4), 71 (36), 58 (45), 43 (100).

Anal. Calcd for $C_6H_{13}NO_2$: C, 54.94; H, 9.99; N, 10.68. Found: C, 54.47; H, 9.78; N, 10.97.

(3*S*,4*S*)-3,4-dihydroxy-*N*-(2-hydroxyethyl)pyrrolidine (27). A solution of (3*S*,4*S*)-3,4-dihydroxy-*N*-benzylpyrrolidine (**25**)[22] (100 mg, 0.52 mmol) and 2-bromoethanol (120 μL, 1.69 mmol) in toluene (1 mL) was heated under reflux for 5 h, then concentrated, dissolved in MeOH (3 mL) and stirred under H_2 for 18 h in the presence of 20% $Pd(OH)_2/C$ (70 mg). The mixture was then filtered through Celite, stirred in the presence of Amberlyst A-26 for 30 min, filtered and purified on silica gel (CH_2Cl_2-MeOH-30% aq NH_3 5:5:0.2) to afford **27** (R_f 0.3, 50.7 mg, 0.345 mmol, 66%) as a waxy solid. $[\alpha]_D^{26}$ +11.0 (*c* 0.72, MeOH). ^1H NMR (200 MHz, D_2O): δ 4.04 (t, $J_{1,3}$ = 4.7 Hz, 2 H, H-3, H-4), 3.60 (t, $J_{1,3}$ = 6.2 Hz, 2 H, H-2'), 2.94 (dd, $J_{1,2}$ = 10.6 Hz, $J_{1,3}$ = 5.5 Hz, 2 H, Hb-2, Ha-5), 2.72-2.58 (m, 2 H, H-1'), 2.54 (dd, $J_{1,2}$ = 10.6 Hz, $J_{1,3}$ = 4.6 Hz, 2 H, Ha-2, Hb-5); ^{13}C NMR (50.3 MHz, D_2O): δ 79.8 (d, 2 C, C-3, C-4), 62.5 (t, 2 C, C-2, C-5), 62.3 (t), 60.3 (t); MS: *m/z* (relative intensity) 147 (M^+, 2), 117 (9), 116 (100), 98 (9), 74 (21), 56 (18), 44 (89).

Anal. Calcd for $C_6H_{13}NO_3$: C, 48.97; H, 8.90; N, 9.52. Found: C, 48.46; H, 9.17; N, 9.88.

(3*S*,4*S*)-3,4-Dihydroxy-*N*-(3-hydroxypropyl)pyrrolidine (28). A solution of (3*S*,4*S*)-3,4-dihydroxy-*N*-benzylpyrrolidine (**25**)[22] (143 mg, 0.74 mmol) and 3-bromopropanol (77 μL, 0.89 mmol) in toluene (1.5 mL) was heated at reflux for 4 h, then concentrated, dissolved in MeOH (3 mL) and stirred under H_2 for 5 days in the presence of 20% $Pd(OH)_2/C$ (90 mg). The mixture was then filtered through Celite, stirred in presence of Amberlyst A-26 for 30 min, filtered and purified on silica gel (CH_2Cl_2-MeOH-30% aq NH_3 5:5:0.2) to afford **28** (R_f 0.25, 95.2 mg, 0.59 mmol, 80%) as a waxy solid. $[\alpha]_D^{26}$ +18.1 (*c* 0.25, MeOH). ^1H NMR (200 MHz, D_2O): δ 4.02 (m, 2 H, H-3, H-4), 3.54 (t, $J_{1,3}$ = 6.4 Hz, 2 H, H-3'), 2.91 (dd, $J_{1,2}$ = 10.6 Hz, $J_{1,3}$ = 5.8 Hz, 2 H, Hb-2, Ha-5), 2.52-

2.42 (m, 4 H, Ha-2, Hb-5, H-1'), 1.64 (q, $J_{1,3}$ = 6.6 Hz, 2 H, H-2'); ^{13}C NMR (50.3 MHz, D_2O): δ 79.8 (d, 2 C, C-3, C-4), 63.0 (t), 62.0 (t, 2 C, C-2, C-5), 55.9 (t), 32.6 (t, C-2'); MS: *m/z* (relative intensity) 161 (M^+, 2), 117 (5), 116 (100), 98 (4), 57 (29).

Anal. Calcd for $C_7H_{15}NO_3$: C, 52.16; H, 9.38; N, 8.69. Found: C, 52.30; H, 9.50; N, 8.00.

(3*S*,4*S*)-3,4-Dihydroxypyrrolidine (29). A solution of (3*S*,4*S*)-3,4-dihydroxy-*N*-benzylpyrrolidine (**25**)[22] (100 mg, 0.52 mmol) in MeOH (2.5 mL) was stirred under hydrogen for 20 h in presence of 20% $Pd(OH)_2/C$ (55 mg). Filtration through Celite and purification on silica gel (eluent CH_2Cl_2-MeOH-30% aq NH_3 8:2:1.5) afforded pyrrolidine **29** (R_f 0.3, 48.8 mg, 0.474 mmol, 91%) as a waxy solid.[23] $[\alpha]_D^{26}$ +20.7 (*c* 0.30, MeOH). ^{1}H NMR (200 MHz, D_2O): δ 4.07-4.04 (m, 2 H, H-3, H-4), 3.07 (dd, $J_{1,2}$ = 12.8 Hz, $J_{1,3}$ = 4.8 Hz, 2 H, Hb-2, Ha-5), 2.72 (dd, $J_{1,3}$ = 12.4 Hz, $J_{1,3}$ = 1.8 Hz, 2 H, Ha-2, Hb-5); ^{13}C NMR (50.3 MHz, D_2O): δ 79.8 (d, 2 C, C-3, C-4), 54.2 (t, 2 C, C-2, C-5); MS: *m/z* (relative intensity) 103 (M^+, 19), 97 (24), 91 (23), 86 (53), 85 (71), 84 (74), 83 (36), 60 (66), 57 (100).

Anal. Calcd for $C_4H_9NO_2$: C, 46.59; H, 8.80; N, 13.58. Found: C, 46.61; H, 8.93; N, 13.52.

Enzymatic Assays. Twenty-four commercially available (Sigma Chemical Co) glycosidases (bovine epididymis α-L-fucosidase (EC 3.2.1.51), *Aspergillus niger* and *Escherichia coli* α and β-galactosidases (EC 3.2.1.22 and 3.2.1.23), coffee beans α-galactosidase (EC 3.2.1.22), bovine liver, jack beans and *Aspergillus orizae* β-galactosidases (EC 3.2.1.23), yeast and rice maltases (EC 3.2.1.20), isomaltase from bakers yeast (EC 3.2.1.10), *Aspergillus niger* and *Rhizopus* mold amyloglucosidases (EC 3.2.1.3), almonds and caldocellum saccharolyticum β-glucosidases (EC 3.2.1.21), jack beans and almonds α-mannosidases (EC 3.2.1.24), *Helix pomatia* β-mannosidase (EC 3.2.1.25), *Aspergillus niger* β-xylosidase (EC 3.2.1.37), chicken liver α-*N*-acetylgalactosaminidase (EC 3.2.1.49) and jack bean, bovine epididymis A and bovine epididymis B β-*N*-acetylglucosaminidases (EC 3.2.1.30)) were assayed with appropriate *p*-nitrophenyl glycoside substrates (Sigma).

A typical enzymatic assay (final volume 0.1 mL), contains 0.01 to 0.5 U/mL of the enzyme (1 U = 1 enzyme unit liberates 1 μmole of glycoside per minute from *p*-

nitrophenyl glycoside), 5 mM aqueous solution of the appropriate *p*-nitrophenyl glycoside substrate and the potential inhibitor buffered to the optimum pH of the enzyme. Enzyme and inhibitor were preincubated for 5 min at 20 °C and the reaction started by addition of the substrate. After 20 min incubation at 37 °C, the reaction was stopped by addition of 0.25 mL 0.2 M sodium borate buffer pH 9.8. The *p*-nitrophenolate released was measured by visible absorption spectroscopy at 405 nm.

In preliminary screenings, enzymatic activity was determined in the presence of high concentrations of the potential inhibitors **25-29** (1 mM). For inhibition rates ≥50% at 1mM concentration, the IC_{50} values (concentration of inhibitor required for 50% inhibition of enzyme activity) was calculated by measuring glycosidase activity in the presence of various concentrations of inhibitor. The inhibition constant K_i was determined when ≥70% inhibition of enzymatic activity was reached at 1 mM concentration of inhibitor.

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