Assignment of the Absolute Configuration of Natural Lentiginosine by Synthesis and Enzymatic Assays of Optically Pure (+) and (−)-Enantiomers

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Received April 12, 1995®

The structure and absolute configuration of natural lentiginosine isolated from plant sources was determined to be (1*S*,2*S*,8a*S*)-1,2-dihydroxyindolizidine ((+)-**4**) on the basis of the synthesis of both enantiomers (+)-**4** and (−)-**4** and their inhibition of amyloglucosidases. Alkaloid (+)-**4** was derived from (L)-(+)-tartaric acid via a highly stereo- and regioselective 1,3-dipolar cycloaddition of (3*S*,4*S*)-3,4-bis[(*tert*-butyldiphenylsilyl)oxy]-1-pyrroline *N*-oxide to methylenecyclopropane, followed by thermal rearrangement of the adduct into (1*S*,2*S*,8a*S*)-1,2-[(*tert*-butyldiphenylsilyl)oxy]octahydroindolizin-7-one. The enantiomer (−)-**4** was derived in the same way from (D)-(−)-tartaric acid. Both (+)-**4** and (−)-**4** displayed inhibition specificity for amyloglucosidases, being inactive toward 17 other glycosidases. With amyloglucosidase from *Aspergillus niger*, synthetic (+)-**4** displayed inhibition ($K_i = 2\ \mu M$) 5 times stronger than that reported for natural lentiginosine, 35 times that measured for (−)-**4**, and twice that of castanospermine. Alkaloid (+)-**4** is thus the most potent and specific competitive inhibitor of amyloglucosidases among azasugars and their analogues.

Introduction

A large number of polyhydroxylated indolizidines[1] have been extracted from natural sources,[2–4] mainly plants and microorganisms, and display interesting biological activity as inhibitors of glycosidases.[1,5–8] Glycosidases are key enzymes in the biosynthesis and processing of glycoproteins,[1,5] which are macromolecules involved in recognition (cell–cell, host–pathogene interactions) and in control of biological mechanisms and structures.[9] Thus, substances able to inhibit the biosynthetic pathway of glycoproteins have become important as potential antibacterial,[10] antiviral,[11,12] antitumoral,[13] or antidiabetic[14] agents. For example, inhibitors of α-glucosidases

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® Abstract published in *Advance ACS Abstracts,* September 15, 1995.

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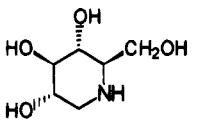
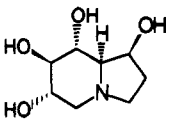
are useful drugs for controlling non-insulin dependent diabetes mellitus since they prevent the rise of blood glucose. These inhibitors have also shown potential in the treatment of obesity and hyperlipoproteinemia.[14d,e] Azasugars (e.g. 1-deoxynojirimycin (**1**) and isomers) and polyhydroxyindolizidines (e.g. castanospermine (**2**) and swainsonine (**3**)) have been the objects of intense synthetic efforts in the last decade[1,5b,15] because of their close structural analogy to natural enzyme substrates. These endeavors have initially targeted natural products,[16-18] generally employing natural sugars as starting materials,[19] and have been extended to all the possible stereoisomers and analogues[20-23] for studies of structure–activity relationships.



1-Deoxynojirimycin (**1**)



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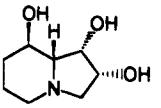
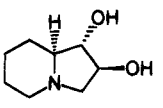


Swainsonine (**3**)



Lentiginosine (**4**)

An examination of the structural features of the known azasugars that act as inhibitors of glycosidases led to the establishment of a general empirical rule stating that a substance should possess at least three hydroxyl groups β to the amino moiety in order to display inhibitory activity.[1a] The first compound that violated this rule was lentiginosine (**4**), an alkaloid isolated in 1990 from a sample of *Astragalus lentiginosus*,[24] and assigned the *trans*-1,2-dihydroxyindolizidine structure. In spite of its low degree of hydroxylation,[24] lentiginosine was found to be a good and selective inhibitor of amyloglucosidase, an enzyme that hydrolyzes 1,4- and 1,6-α-glucosidic linkages.

The absolute configuration of the alkaloid, however, has not been assigned conclusively. Elbein and coworkers, who first isolated the compound, proposed the (1*S*,2*S*,8a*S*) configuration on the basis of a reasonable biosynthetic hypothesis (Scheme 1)[24] which proposes that **4** derives from L-pipecolinic acid (**5**) through intermediate **6**. Compound **6** is common to the biosyntheses of the 2-epimer **7** and swainsonine (**3**), all three alkaloids having been isolated together from the same natural source.

The isolated **4** displayed a low, negative optical rotation ($[\alpha]_D$ −3.3°) and this has created some confusion. In the first synthesis by Yoda et al.,[25] the *all-S* enantiomer was derived from L-tartaric acid. The lentiginosine so-obtained had an optical rotation of $[\alpha]^{25}_D$ +0.19°. This positive value was ascribed by the authors to contamination by the C-8a-epimer.[25] In a preliminary communication,[26] we reported our total synthesis of chemically pure *all-S* lentiginosine ((+)-**4**), for which a rotation of +3.2° was measured. This value is exactly the opposite of that of the isolated natural lentiginosine (**4**). The question was then raised as to whether the natural alkaloid was the *all-R* enantiomer or whether impurities evident in the published NMR spectrum of the natural material were responsible for the difference. In order to solve this puzzle, we decided to synthesize both enantiomeric forms of lentiginosine ((+)-**4** and (−)-**4**).

Our synthetic approach constitutes the first application of our methylenecyclopropane 1,3-dipolar cycloaddition–rearrangement methodology to the preparation of enantiomerically pure compounds. The evaluation of the inhibitory properties of (+)-**4** and (−)-**4** toward a wide range of glycosidases clearly demonstrates that the natural lentiginosine is the (1*S*,2*S*,8a*S*) enantiomer and

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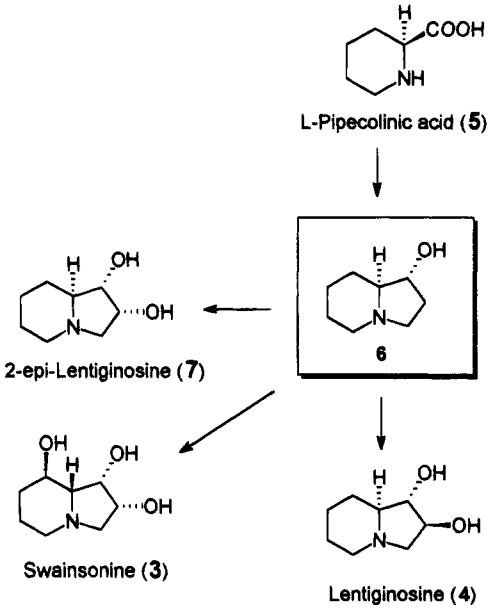
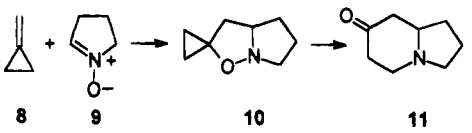
Scheme 1



Scheme 2



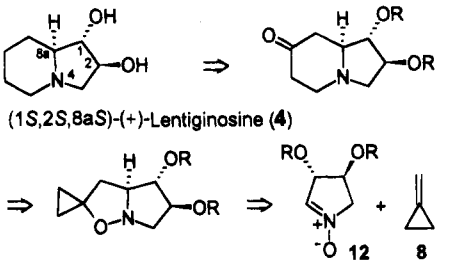
that it is dextrorotatory ((+)-**4**). Our results also show that pure (+)-lentiginosine ((+)-**4**) is the strongest inhibitor of amyloglucosidases among the known azasugars and indolizidine derivatives.

Results and Discussion

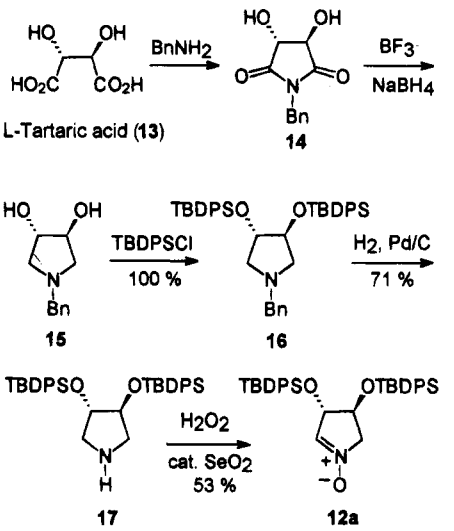
The thermal rearrangement of bicyclic 5-spirocyclopropane isoxazolidines **10** is a versatile and practical means of construction of the indolizidinone skeleton **11** with high regio- and stereocontrol (Scheme 2).[27] Spirocyclopropyl compounds **10**, in turn, can be obtained efficiently by 1,3-dipolar cycloadditions of cyclic nitrones to methylenecyclopropane derivatives.[27,28] This methodology has already been applied to the total or formal syntheses of several alkaloids of different classes in their racemic forms.[27,29]

The application of this methodology to the total synthesis of lentiginosine ((+)-**4**), as illustrated in Scheme 3, requires the initial cycloaddition of methylenecyclopropane (**8**) to a five-membered cyclic dihydroxy nitrone such as **12**. Very recently, such nitrones have been made available in enantiomerically pure form by two related and complementary procedures[30] starting from L-(+)-tartaric acid, an inexpensive, homochiral starting material). Nitrone **12a** was synthesized by using the procedure of Petrini and co-workers (Scheme 4).[30b,31]

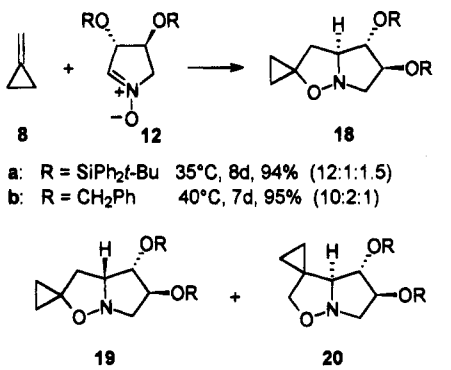
Scheme 3



Scheme 4



Scheme 5



The *N*-benzyl-3,4-dihydroxypyrrolidine **15**,[32] obtained by reaction of L-tartaric acid (**13**) with benzylamine followed by reduction of the resulting pyrrolidine-2,5-dione **14**, was silylated quantitatively with *tert*-butyldimethylsilyl chloride/imidazole and debenzylated by hydrogenolysis in the presence of palladium on charcoal. Oxidation of the resulting pyrrolidine **17** with SeO_2/H_2O_2[33] gave the corresponding nitrone **12a** (18% overall yield, five steps from **13**). The enantiomeric nitrone was obtained in the same way from D-(−)-tartaric acid.

The TBDPS-protected nitrone **12a** was treated with an excess of methylenecyclopropane (**8**) (35 °C in a sealed tube, 8 d) which gave a 12:1 mixture of isoxazolidines **18a** and **19a** in high yield (Scheme 5). Minor amounts

(27) (a) Brandi, A.; Cordero, F. M.; Goti, A.; De Sarlo, F.; Guarna, A. *Synlett* **1993**, 1. (b) Brandi, A.; Garro, S.; Guarna, A.; Goti, A.; Cordero, F. M.; De Sarlo, F. *J. Org. Chem.* **1988**, *53*, 2430. (c) Cordero, F. M.; Brandi, A.; Querci, C.; Goti, A.; De Sarlo, F.; Guarna, A. *J. Org. Chem.* **1990**, *55*, 1672.

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(29) (a) Brandi, A.; Dürüst, Y.; Cordero, F. M.; De Sarlo, F. *J. Org. Chem.* **1992**, *57*, 5666. (b) Brandi, A.; Cordero, F. M.; Goti, A.; Guarna, A. *Tetrahedron Lett.* **1992**, *33*, 6697. (c) Cordero, F. M.; Anichini, B.; Goti, A.; Brandi, A. *Tetrahedron* **1993**, *49*, 9867.

(30) (a) Cicchi, S.; Höld, I.; Brandi, A. *J. Org. Chem.* **1993**, *58*, 5274. (b) Ballini, R.; Marcantoni, E.; Petrini, M. *J. Org. Chem.* **1992**, *57*, 1316.

(31) Brandi, A.; Cicchi, S.; Goti, A.; Koprowski, A.; Pietrusiewicz, K. M. *J. Org. Chem.* **1994**, *59*, 1315.

(32) Nagel, U.; Kinzel, E.; Andrade, J.; Prescher, G. *Chem. Ber.* **1986**, *119*, 3326.

(33) Murahashi, S.-I.; Shiota, T. *Tetrahedron Lett.* **1987**, *28*, 2383.

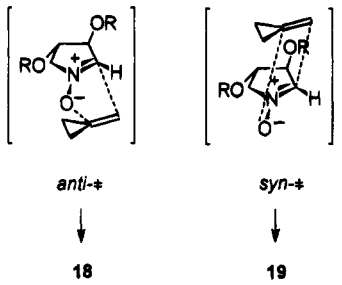
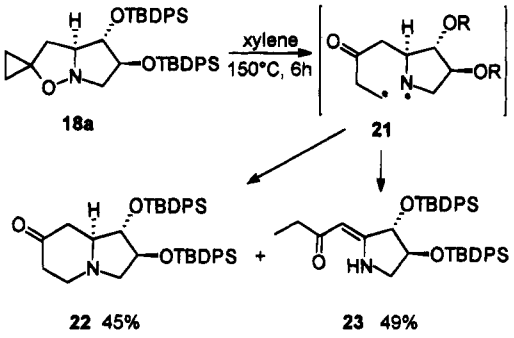


Figure 1. Transition state trajectories for cycloaddition.

Scheme 6



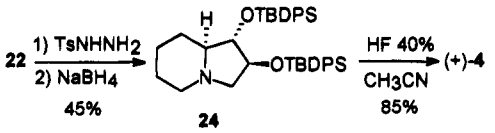
(ca. 10%) of the regioisomeric compound **20a** could also be detected in the crude reaction mixture.

The cycloaddition of known nitrone **12b**[30a] occurred with equal regioselectivity but, predictably, with less stereoselectivity, giving a 5:1 mixture of isoxazolidines **18b** and **19b** (Scheme 5). The role of the hydroxyl protecting group in determining the stereoselectivity of the cycloaddition was thus demonstrated. This observation can be interpreted in terms of a differential steric factor. The preferred mode of cycloaddition results in the attack of the dipolarophile from the face of the nitrone *anti* to the RO group vicinal to the dipole functionality (Figure 1).[31] Therefore, a larger RO group will give a greater *anti* face preference.

The structures of isoxazolidines **18** and **19** were confirmed by their spectral data, including that derived from 2D COSY and NOESY experiments. Relatively small ^3J(H-3a,H-4) coupling constants were observed in **18a** (2.6 Hz) and **18b** (4.4 Hz), consistent with a *trans* relationship between protons H-3a and H-4, while diastereoisomer **19a** displayed a higher value (6.6 Hz). These values are in accord with those reported for diastereomeric cycloadducts of the same nitrone with other dipolarophiles,[31] for which the stereochemistry has been confirmed by X-ray analysis.[34]

The lower stereoselectivity observed with benzyloxy-protected nitrone **12b** as well as the difficulties encountered in separating the major and minor diastereoisomers motivated us to continue the synthesis of (+)-**4** with **18a**. Heating **18a** in xylene at reflux gave a mixture from which indolizidinone **22** was isolated in 45% yield. The isomeric enone **23** was also formed in 49% yield (Scheme 6).[35] Whereas the former product arose from ring closure of intermediate diradical **21**, the latter undesired enone arose from its 1,5-hydrogen atom transfer, followed by double bond migration.[27a,29a]

Scheme 7



These two products were separated by column chromatography. Reduction of the ketone **22** via its tosylhydrazone[36] gave TBDPS-protected lentiginosine **24** in 45% yield (Scheme 7). Deprotection of **24** with 40% aqueous HF in acetonitrile[37] gave (+)-**4** in 85% yield as an analytically pure material which solidified upon standing. Its spectroscopic data were identical with those of natural lentiginosine.

At lower wavelengths, both our sample of (+)-**4** and natural lentiginosine showed counter-clockwise changes in the specific optical rotations. Since our synthesis of (+)-**4** from optically pure L-tartaric acid does not involve conditions under which epimerization of the two alcoholic carbon centers could have occurred, there are two possible interpretations for the opposing optical rotations observed for natural lentiginosine and our sample of (+)-**4**. Either the natural alkaloid has the (1*R*,2*R*,8a*R*) configuration contrary to the proposal of Elbein and co-workers,[24] or the sample isolated by these authors was contaminated by impurities that caused their (1*S*,2*S*,-8a*S*)-*trans*-1,2-dihydroxyindolizidine to display a negative optical rotation. While our work was in progress, Yoda and co-workers[25] reported an alternative synthesis of lentiginosine and observed an optical rotation of +0.19 for (+)-**4**, the deviation from the reported value being attributed to contamination of their synthetic lentiginosine with diastereomeric impurities. Such confusion led us to prepare (−)-**4** from (D)-(−)-tartaric acid following the same procedure as that described above for (+)-**4**. We produced a sample of (−)-**4**, which had an optical rotation of −1.6 (*c* 0.24, MeOH) and evaluated its inhibitory activity in parallel with that of pure (+)-**4** toward 19 different glycosidases (see Experimental Section).

Both synthetic (+)-**4** and (−)-**4** displayed specificity for amyloglucosidases (1,4-α-D-glucanglucohydrolase EC 3.2.1.3) and were inactive toward other glycosidases. The enzymatic activities as a function of concentration are shown in Figures 2A and 2B for *Aspergillus niger* amyloglucosidase and *Rhizopus* mold amyloglucosidase, respectively, together with that of castanospermine (**2**) (from *Castanospermum australe*). The IC_{50} (concentration of inhibitor required for 50% inhibition of enzyme activity) and K_i values are shown in Table 1. For natural lentiginosine, Elbein and co-workers measured an IC_{50} of 5 μg/mL and a K_i of 10 μM (inhibition of *Aspergillus* amyloglucosidase).

Since the inhibitory activities of (−)-**4** are significantly lower than those of both natural lentiginosine isolated by Elbein and co-workers and our synthetic sample of (+)-**4**, we interpret the deviations observed between the specific optical rotations and the enzymatic activities of these compounds as due to impurities present in the natural alkaloid. We thus conclude that the configuration of natural lentiginosine is (+)-**4**, i.e. (1*S*,2*S*,8a*S*). It is interesting to note that (+)-**4** is ca. twice as potent as castanospermine (**2**) in its inhibition of amyloglucosi-

(34) Pietrusiewicz, K. M; Wieczorek, W.; Cicchi, S.; Brandi, A. *Phosphorus, Sulfur, Silicon* **1994**, *97*, 233.

(35) Attempted rearrangement of isoxazolidine **18b** showed that also the benzyloxy groups gave roughly the same indolizidinone/enaminone ratio.

(36) Caglioti, L. *Tetrahedron* **1966**, *22*, 487.

(37) (a) Ogawa, Y.; Nunomoto, M.; Shibasaki, M. *J. Org. Chem.* **1986**, *51*, 1625. (b) Newton, R. F.; Reynolds, D. P.; Finch, M. A. W.; Kelly, D. R.; Roberts, S. M. *Tetrahedron Lett.* **1979**, *41*, 3981.

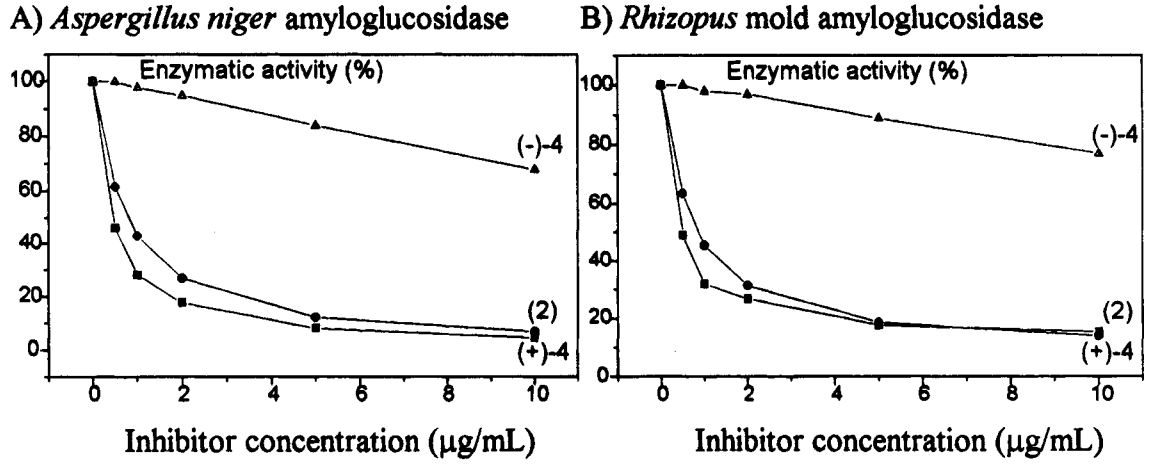


Figure 2. α-Glucosidase activities based on the hydrolysis of *p*-nitrophenyl α-D-glucopyranoside at an optimum pH of 5.4 and 45 °C as a function of the concentration of lentiginosine (+)-**4**, its enantiomer (−)-**4**, and castanospermine (**2**).

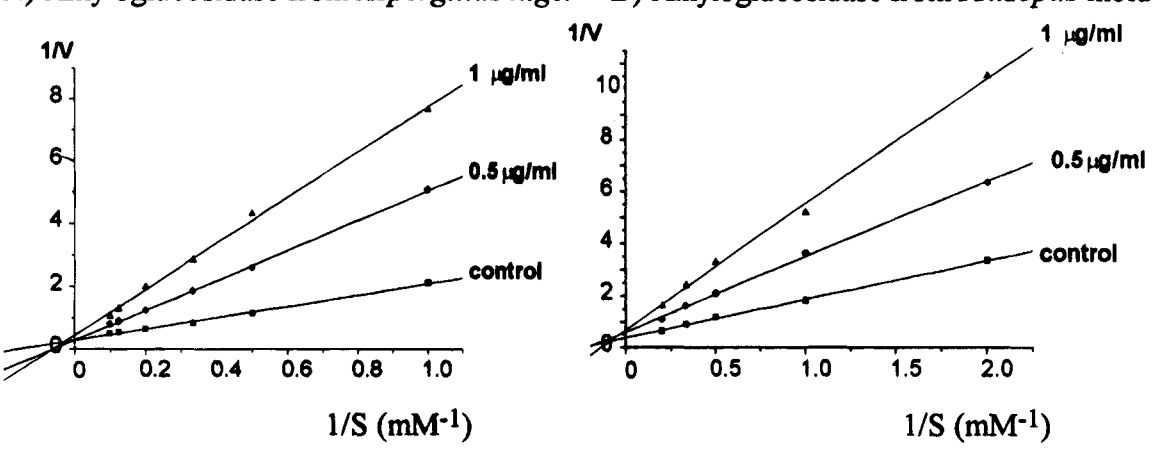


Figure 3. Effect of substrate (*p*-nitrophenyl α-D-glucopyranoside) concentration on amyloglucosidase inhibition by various concentrations of (+)-**4**.

Table 1. IC_{50} and K_i Values with Amyloglucosidases

	from *Aspergillus niger*		from *Rhizopus* mold	
inhibitor	IC_{50} (μg/mL)	K_i (μM)	IC_{50} (μg/mL)	K_i (μM)
(+)-**4**	0.43	2	0.48	3
(−)-**4**	17	70	28	98
2	0.82		0.92	

dases, making this alkaloid the most potent inhibitor of this type of α-glucosidase.[38]

In order to determine the nature of the inhibition by (+)-**4**, we estimated the reaction rate as a function of concentration of substrate for different concentrations of inhibitor. Lineweaver–Burk plots[39] (Figure 3) show that only K_m values and not V_m values are affected by increasing concentrations of inhibitor, suggesting that (+)-**4** is a competitive inhibitor.

Conclusion

Efficient syntheses of natural lentiginosine ((+)-**4**) and its enantiomer (−)-**4** have been realized starting from inexpensive (L)-tartaric acid and (D)-tartaric acid, respectively.[40] Pure alkaloid (+)-**4** is the most potent amyloglucosidase inhibitor found thus far[38] and is ca. 35 times more potent than (−)-**4**. We have demonstrated that natural lentiginosine has the (1*S*,2*S*,8a*S*) configuration, as proposed by Elbein and co-workers, and that it is dextrorotatory at $\lambda = 589$ nm. Our conclusion contradicts that of Gurjar and co-workers[41] who derived (+)-**4** and (−)-**4** from pipecolinic acids. The simplicity of the structure of (+)-**4** and its potency and specificity as an amyloglucosidase inhibitor suggest that other simple analogues might be useful glycosidase inhibitors.

Experimental Section

All reactions that required dry conditions were run under a nitrogen atmosphere using anhydrous solvents. R_f values refer to TLC on 0.25 mm silica gel plates (Merck F_{254}) using the same eluent used for the chromatographic separation of the compound. Melting points (mp) are uncorrected. ^1H and ^{13}C NMR spectra (in $CDCl_3$ solution, unless otherwise stated) were recorded at 200 MHz and 50 MHz, respectively.

(3*S*,4*S*)-3,4-Bis[(*tert*-butyldiphenylsilyl)oxy]-*N*-benzylpyrrolidine (16). *tert*-Butyldiphenylsilyl chloride (6.25 g, 22.8 mmol) was added at 0 °C to a solution of (3*S*,4*S*)-3,4-dihydroxy-*N*-benzylpyrrolidine[32] (**15**, 2 g, 10.4 mmol) and imidazole (3.1 g, 45.5 mmol) in dimethylformamide (10 mL), and the mixture was then heated at 60 °C for 12 h. The

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(39) Lineweaver, H.; Burk, D. *J. Am. Chem. Soc.* **1934**, *56*, 658.
(40) Part 12 in the series: Rearrangement of Isoxazoline 5-Spiro Derivatives. Part 11: ref 22h.
(41) Gurjar, M. K.; Ghosh, L.; Syamala, M.; Jayasree, V. *Tetrahedron Lett.* **1994**, *35*, 8871.

resulting reaction mixture was added to H_2O (20 mL) and extracted with petroleum ether. The organic layer was washed with H_2O and saturated aqueous $NaHCO_3$ solution, dried over Na_2SO_4, and concentrated to give 7.13 g of a crude mixture containing only very minor impurities (^1H NMR monitoring) which was not purified for the following reaction. An analytically pure sample of **16** (oil) was obtained by elution with CH_2Cl_2 from a short pad of silica gel: $[\alpha]^{25}_D$ +47.0° (*c* 1.21, $CHCl_3$). ^1H NMR: δ 7.70–7.20 (m, 25 H), 4.39 (t, *J* = 4.0 Hz, 2 H), 3.50 (AB system, *J* = 13.2 Hz, 2 H), 2.62 (dd, *J* = 10.3, 5.1 Hz, 2 H), 2.41 (dd, *J* = 10.3, 3.7 Hz, 2 H), 1.04 (s, 18 H). Anal. Calcd for $C_{43}H_{51}NO_2Si_2$: C, 77.08; H, 7.67; N, 2.09. Found: C, 77.31; H, 7.93; N, 2.01.

(3*S*,4*S*)-3,4-Bis[(*tert*-butyldiphenylsilyl)oxy]pyrrolidine (17). The crude *N*-benzylpyrrolidine **16** (5 g, 7.5 mmol) was dissolved in MeOH (90 mL), added to 5% $Pd(OH)_2$ on activated carbon (0.5 g), and hydrogenated at 60 psi and 20 °C for 3 d. The solution was filtered over Celite and concentrated. Purification of the crude product by chromatography over a short pad of silica gel, eluent petroleum ether–ethyl acetate–NEt_3 50:50:1, gave the recovered benzylpyrrolidine **16** (R_f = 0.85, 1 g, 20%) and the desired diprotected pyrrolidine **17** (R_f = 0.25, 3.1 g, 5.33 mmol, 71% from **15**) as a colorless oil: $[\alpha]^{25}_D$ +6.9° (*c* 1.67, $CHCl_3$). ^1H NMR: δ 7.60–7.25 (m, 20 H), 4.20 (d, *J* = 2.4 Hz, 2 H), 3.42 (dd, *J* = 12.0, 2.8 Hz, 2 H), 3.15 (d, *J* = 12.0 Hz, 2 H), 2.12 (s, 1 H), 0.97 (s, 18 H). ^{13}C NMR: δ 135.6 (d, 8 C), 133.7 (s, 4 C), 129.6 (d, 4 C), 127.6 (d, 8 C), 79.5 (d, 2 C), 53.8 (t, 2 C), 26.8 (q, 6 C), 19.0 (s, 2 C). Anal. Calcd for $C_{36}H_{45}NO_2Si_2$: C, 74.56; H, 7.82; N, 2.41. Found: C, 74.24; H, 8.01; N, 2.57.

(3*S*,4*S*)-3,4-Bis[(*tert*-butyldiphenylsilyl)oxy]-1-pyrroline *N*-Oxide (12a). To a suspension of SeO_2 (14 mg, 0.13 mmol) and pyrrolidine **17** (1.5 g, 2.6 mmol) in acetone (5 mL) cooled to 0 °C was added dropwise 0.9 mL of a 30% aqueous solution of hydrogen peroxide (7.9 mmol). The resulting mixture was stirred at 20 °C for 2 h and then concentrated, and CH_2Cl_2 (20 mL) was added. The organic layer was washed with H_2O, dried over Na_2SO_4, and filtered and the solvent evaporated. The residue was purified by column chromatography, eluent petroleum ether–Et_2O 1:1, to afford the pure **12a** (R_f = 0.25, 0.8 g, 1.4 mmol, 53%), colorless oil: $[\alpha]^{25}_D$ +66.5° (*c* 1.45, $CHCl_3$). ^1H NMR: δ 7.70–7.25 (m, 20 H), 6.34 (q, *J* = 1.6 Hz, 1 H), 4.90 (m, 1 H), 4.53 (dt, *J* = 5.6, 2.7 Hz, 1 H), 3.76 (m, 1 H), 3.49 (m, 1 H), 1.03 (s, 9 H), 1.01 (s, 9 H). ^{13}C NMR (arom C and C=N signals not reported): δ 80.7 (d), 75.9 (d), 65.8 (t), 26.7 (q, 6 C), 15.2 (s, 2 C). Anal. Calcd for $C_{36}H_{43}NO_3Si_2$: C, 72.80; H, 7.30; N, 2.36. Found: C, 72.68; H, 7.34; N, 2.54.

Cycloaddition of Nitrone 12a to Methylenecyclopropane (8). Methylenecyclopropane (**8**, 308 mg, 5.7 mmol) was added to a solution of **12a** (1.69 g, 2.85 mmol) in benzene (3.5 mL) and the mixture heated in a sealed tube at 35 °C for 8 d. The crude mixture was then concentrated to give a clean 12:1:1.5 (by 500 MHz ^1H NMR) mixture (1.73 g, 2.68 mmol, 94%) of **18a**, **19a**, and **20a**, respectively. Purification of this mixture by column chromatography, eluent petroleum ether–ethyl acetate 5:1, afforded **18a** containing about 5% of diastereomeric **19a** and regioisomeric **20a** (R_f = 0.33, 1.24 g, 1.9 mmol, 67%). Further fractioning of part of this mixture afforded small quantities of analytically pure **18a** as an oil.

(3a′*S*,4′*S*,5′*S*)-4′,5′-Bis[(*tert*-butyldiphenylsilyl)oxy]-hexahydrospiro[cyclopropane-1,2′-pyrrolo[1,2-*b*]isoxazole] (18a): $[\alpha]^{25}_D$ +42.6° (*c* 1.05, $CHCl_3$). ^1H NMR (500 MHz): δ 7.78–7.30 (m, 20 H), 4.45 (dt, *J* = 3.3, 4.5 Hz, C5-H), 4.34 (t, *J* = 2.9 Hz, C4-H), 3.75 (ddd, *J* = 9.4, 6.9, 2.6 Hz, C3a-H), 3.18 (X part of an AXY system, *J* = 12.6, 4.0 Hz, C6-H), 3.08 (Y part of an AXY system, *J* = 12.6, 4.6 Hz, C6-H), 2.06 (dd, *J* = 11.8, 6.9 Hz, C3-H), 1.62 (dd, *J* = 11.8, 9.0 Hz, C3-H), 1.05 (s, 9 H), 1.03 (s, 9 H), 0.90 (m, 1 H), 0.71 (m, 1 H), 0.52 (m, 1 H), 0.33 (m, 1 H). ^{13}C NMR (arom C not reported): δ 83.9 (d), 79.8 (d), 73.0 (d), 61.4 (t), 60.8 (s), 39.1 (t), 26.9 (q, 6 C), 19.1 (s, 2 C), 9.7 (t), 8.3 (t). Anal. Calcd for $C_{40}H_{49}NO_3Si_2$: C, 74.14; H, 7.62; N, 2.16. Found: C, 74.02; H, 7.96; N, 1.88.

(3a′*R*,4′*S*,5′*S*)-4′,5′-Bis[(*tert*-butyldiphenylsilyl)oxy]-hexahydrospiro[cyclopropane-1,2′-pyrrolo[1,2-*b*]isoxazole] (19a): ^1H NMR (500 MHz): δ 7.78–7.30 (m, 20 H), 4.56 (dt, *J* = 8.0, 7.0 Hz, C5-H), 4.44 (t, *J* = 6.6 Hz, C4-H), 3.29 (m, C3a-H), 2.81 (dd, *J* = 13.8, 8.2 Hz, C6-H), 2.61 (dd, *J* = 13.8, 7.0 Hz, C6-H), 2.27 (dd, *J* = 12.3, 4.2 Hz, C3-H), 1.82 (dd, *J* = 12.3, 10.0 Hz, C3-H), 1.15–0.45 (m, 4 H), 1.10 (s, 9 H), 1.08 (s, 9 H).

(3a′*S*,4′*S*,5′*S*)-4′,5′-Bis[(*tert*-butyldiphenylsilyl)oxy]-hexahydrospiro[cyclopropane-1,3′-pyrrolo[1,2-*b*]isoxazole] (20a): ^1H NMR (500 MHz): δ 7.78–7.30 (m, 20 H), 4.28 (d, *J* = 4.6 Hz, C5-H), 4.25 (d, *J* = 7.3 Hz, C2-H), 4.07 (s, C4-H), 3.63 (dd, *J* = 14.3, 4.6 Hz, C6-H), 3.33 (d, *J* = 7.3 Hz, C2-H), 3.27 (d, *J* = 14.3 Hz, C6-H), 3.23 (s, C3a-H), 1.15–0.45 (m, 4 H), 1.03 (s, 9 H), 0.97 (s, 9 H).

Cycloaddition of Nitrone 12b to Methylenecyclopropane (8). Methylenecyclopropane (**8**, 1 g, 18.5 mmol) was added to a solution of **12b** (1.82 g, 3.98 mmol) in benzene (1.2 mL) and the mixture heated in a sealed tube at 40 °C for 7 d. The crude mixture was then concentrated to give a clean 10:2:1 (by ^1H NMR) mixture (1.33 g, 3.78 mmol, 95%) of **18b**, **19b**, and **20b**, respectively. Purification of this mixture by column chromatography, eluent CH_2Cl_2–MeOH 40:1, afforded cycloadduct **18b** containing about 15% of diastereomeric **19b** and minor impurities of **20b** (R_f = 0.31, 0.83 g, 2.3 mmol, 58%). Further fractioning of part of this mixture afforded small quantities of analytically pure **18b** as an oil.

(3a′*S*,4′*S*,5′*S*)-4′,5′-Bis(benzyloxy)hexahydrospiro[cyclopropane-1,2′-pyrrolo[1,2-*b*]isoxazole] (18b): $[\alpha]^{25}_D$ +24.0° (*c* 1.00, $CHCl_3$). ^1H NMR: δ 7.41–7.24 (m, 10 H), 4.67–4.55 (m, 4 H), 4.19–4.03 (m, 2 H), 3.89 (dt, *J* = 8.8, 4.4 Hz, 1 H), 3.60 (dd, *J* = 12.0, 5.6 Hz, 1 H), 3.42 (dd, *J* = 12.0, 6.4 Hz, 1 H), 2.51 (dd, *J* = 12.1, 8.8 Hz, 1 H), 2.24 (dd, *J* = 12.1, 5.2 Hz, 1 H), 1.17–0.50 (m, 4 H). ^{13}C NMR (arom C not reported): δ 88.2 (d), 83.1 (d), 72.1 (t), 72.0 (t), 70.1 (d), 61.9 (s), 58.2 (t), 40.2 (t), 11.0 (t), 8.0 (t). IR ($CDCl_3$): 3005, 2933, 2873, 1602, 1450, 1360, 1205, 1102 cm^{-1}. Anal. Calcd for $C_{22}H_{25}NO_3$: C, 75.19; H, 7.17; N, 3.99. Found: C, 74.83; H, 7.52; N, 3.98.

(3a′*R*,4′*S*,5′*S*)-4′,5′-Bis(benzyloxy)hexahydrospiro[cyclopropane-1,2′-pyrrolo[1,2-*b*]isoxazole] (19b): ^1H NMR (the only discernible signals): δ 4.35 (dt, *J* = 5.2, 7.0 Hz, 1 H), 3.20 (dd, *J* = 13.6, 7.0 Hz, 1 H), 2.59 (dd, *J* = 12.2, 4.2 Hz, 1 H), 2.23 (dd, *J* = 12.2, 8.0 Hz, 1 H).

Thermal Rearrangement of 18a. The cycloadduct **18a** (1.24 g, 1.91 mmol) was dissolved in xylene (38 mL) and heated under reflux for 100 min. After solvent evaporation, the residue was purified by column chromatography, eluent petroleum ether–ethyl acetate 8:1, to give the indolizidinone **22** (R_f = 0.35, 554 mg, 0.86 mmol, 45%) and the enaminone **23** (R_f = 0.26, 607 mg, 0.94 mmol, 49%).

(1*S*,2*S*,8a*S*)-1,2-Bis[(*tert*-butyldiphenylsilyl)oxy]octahydroindolizin-7-one (22). Colorless oil: $[\alpha]^{25}_D$ +8.9° (*c* 0.62, CH_3OH). ^1H NMR: δ 7.68–7.62 (m, 8 H), 7.45–7.33 (m, 12 H), 4.40 (dt, *J* = 6.2, 1.0 Hz, 1 H), 4.08 (dd, *J* = 5.8, 1.8 Hz, 1 H), 2.98–2.89 (m, 1 H), 2.79 (d, *J* = 10.8 Hz, 1 H), 2.46–1.90 (m, 7 H), 1.03 (s, 9 H), 0.96 (s, 9 H). ^{13}C NMR (signals of arom C not reported): δ 208.8 (s), 87.4 (d), 81.1 (d), 69.4 (d), 59.3 (t), 49.5 (t), 44.5 (t), 39.9 (t), 26.9 (q, 3 C), 26.7 (q, 3 C), 19.2 (s), 19.1 (s). MS: *m*/*z* (relative intensity) 647 (M^+, 1), 590 (63), 570 (28), 513 (22), 199 (56), 135 (45), 135 (57), 111 (100), 83 (42), 82 (34), 55 (54). IR ($CDCl_3$): 3073, 3053, 2962, 2933, 2859, 2804, 1709, 1426, 1360, 1222, 1104 cm^{-1}. Anal. Calcd for $C_{40}H_{49}NO_3Si_2$: C, 74.14; H, 7.62; N, 2.16. Found: C, 74.54; H, 7.76; N, 1.91.

(3*S*,4*S*)-2-(2-Oxobutylidene)-3,4-bis[(*tert*-butyldiphenylsilyl)oxy]tetrahydropyrrole (23). Colorless oil: $[\alpha]^{25}_D$ +22.0° (*c* 0.30, CH_3OH). ^1H NMR: δ 9.25 (br, 1 H), 7.80–7.25 (m, 20 H), 4.68 (s, 1 H), 4.48 (d, *J* = 2.0 Hz, 1 H), 4.32 (m, 1 H), 3.57 (dd, *J* = 11.2, 4.1 Hz, 1 H), 3.30 (dd, *J* = 11.2, 1.4 Hz, 1 H), 2.12 (q, *J* = 7.4 Hz, 2 H), 0.96 (s, 9 H), 0.95 (s, 9 H), 0.94 (t, *J* = 7.4 Hz, 3 H). ^{13}C NMR (arom C not reported): δ 200.5 (s), 163.8 (s), 90.9 (d), 81.0 (d), 76.3 (d), 53.3 (t), 34.9 (t), 26.8 (q, 3 C), 26.7 (q, 3 C), 19.2 (s), 19.0 (s), 9.8 (q). IR (CCl_4): 3304 (br), 3073, 3053, 2961, 2931, 2859, 1641, 1560, 1462, 1426, 1103 cm^{-1}. Anal. Calcd for $C_{40}H_{49}NO_3Si_2$: C, 74.14; H, 7.62; N, 2.16. Found: C, 74.00; H, 7.87; N, 1.82.

Reduction of Ketone 22. (*p*-Toluenesulfonyl)hydrazine (210 mg, 1.12 mmol) and activated 3 Å powdered molecular

sieves were added to a solution of indolizidinone **22** (194 mg, 0.30 mmol) in dry MeOH (5 mL), and the mixture was refluxed for 7 h (TLC monitoring). After cooling at 0 °C, a large excess of $NaBH_4$ (279 mg) was added and the mixture heated under reflux for 3 h. The reaction mixture was filtered over Celite and, after removal of MeOH *in vacuo*, extracted with Et_2O, washed with H_2O, and dried over Na_2SO_4. After filtration and concentration, the crude product (149 mg) was purified by column chromatography, eluent petroleum ether–ethyl acetate 10:1, to give **24** (R_f = 0.38, 86 mg, 0.136 mmol, 45%).

(1*S*,2*S*,8a*S*)-1,2-Bis[(*tert*-butyldiphenylsilyl)oxy]octahydroindolizine (24). Colorless oil: $[\alpha]^{25}_D$ +11.8° (*c* 1.40, $CHCl_3$). ^1H NMR: δ 7.77–7.62 (m, 8 H), 7.50–7.25 (m, 12 H), 4.33 (m, 1 H), 4.06 (dd, *J* = 7.6, 2.8 Hz, 1 H), 2.73–2.60 (m, 2 H), 2.03 (dd, *J* = 10.3, 6.6 Hz, 1 H), 1.92–1.25 (m, 8 H), 1.03 (s, 9 H), 0.96 (s, 9 H). ^{13}C NMR (arom C not reported): δ 87.1 (d), 80.0 (d), 69.9 (d), 60.9 (t), 52.7 (t), 28.4 (t), 27.0 (q, 3 C), 26.8 (q, 3 C), 24.7 (t), 24.1 (t), 19.3 (s), 19.1 (s). MS: *m*/*z* (relative intensity) 633 (M^+, 2), 556 (17), 378 (16), 259 (10), 199 (49), 183 (13), 135 (30), 122 (13), 97 (100). Anal. Calcd for $C_{40}H_{51}NO_2Si_2$: C,75.78; H,8.11; N, 2.21. Found: C, 76.09; H, 8.37; N, 2.13.

Deprotection of 24. The silyl ether **24** (86 mg, 0.13 mmol) was stirred at 20 °C in a 7:3 mixture of CH_3CN–aqueous 40% HF (8 mL) in a Teflon flask for 46 h. The mixture was then neutralized by portionwise addition of anhydrous Na_2CO_3 at 0 °C. After filtration and solvent evaporation, the residue was purified by column chromatography, eluent CH_2Cl_2–CH_3OH–30% aqueous NH_3 41:8:1, to afford **4** (R_f = 0.30, 18 mg, 0.11 mmol, 85%), which crystallized as a white solid upon standing.

(+)-Lentiginosine [(1*S*,2*S*,8a*S*)-1,2-Dihydroxyoctahydroindolizine] ((+)-4). Mp 106–107 °C. $[\alpha]^{25}_D$ +3.2°, $[\alpha]^{25}_{546}$ = +2.0°, $[\alpha]^{25}_{435}$ −0.7°, $[\alpha]^{25}_{405}$ −2.5° (*c* 0.27, MeOH). ^1H NMR (D_2O): δ 4.02 (ddd, *J* = 7.3, 4.0, 1.8 Hz, 1 H), 3.62 (dd, *J* = 8.8, 4.0 Hz, 1 H), 2.98 (br d, *J* = 11.2 Hz, 1 H), 2.88 (dd, *J* = 11.3, 1.8 Hz, 1 H), 2.76 (dd, *J* = 11.3, 7.3 Hz, 1 H), 2.22 (dd, *J* = 11.2, 2.9 Hz, 1 H), 2.20–2.10 (m, 1 H), 1.95–1.10 (m, 6 H). ^{13}C NMR (D_2O): δ 84.9 (d), 78.0 (d), 71.5 (d), 62.7 (t), 55.4 (t), 29.8 (t), 26.3 (t), 25.4 (t). MS: *m*/*z* (relative intensity) 157 (M^+, 22), 140 (10), 97 (100), 84 (29), 69 (37). Anal. Calcd for $C_8H_{15}NO_2$: C, 61.12; H, 9.62; N, 8.91. Found: C, 60.99; H, 9.63; N, 8.66.

(1*R*,2*R*,8a*R*)-1,2-Bis[(*tert*-butyldiphenylsilyl)oxy]octahydroindolizine ((−)-24): $[\alpha]^{20}_D$ −12.3° (*c* 4.32, $CHCl_3$).

(−)-Lentiginosine [(1*R*,2*R*,8a*R*)-1,2-Dihydroxyoctahydroindolizine] ((−)-4). Mp 106–107 °C. $[\alpha]^{23}_D$ −1.6° (*c* 0.24, MeOH). Anal. Calcd for $C_8H_{15}NO_2$: C, 61.12; H, 9.62; N, 8.91. Found: C, 60.90; H, 9.41; N, 9.24.

Enzymatic Assays. Nineteen commercially available (Oxford Glycosystem, Sigma Chemical Co) glycosidases (bovine epididymis α-L-fucosidase (EC 3.2.1.51), coffee beans, *Aspergillus niger* and *Escherichia coli* α and β-galactosidases (EC 3.2.1.22 and 3.2.1.23), bovine liver and *Aspergillus orizae* β-galactosidases (EC 3.2.1.23), yeast and rice maltases (EC 3.2.1.20), isomaltase from baker yeasts (EC 3.2.1.10), *Aspergillus niger* and *Rhizopus* mold amyloglucosidases (EC 3.2.1.3), almonds β-glucosidase (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) and *Aspergillus niger* β-xylosidase (EC 3.2.1.37)) were assayed following the method of Saul et al.[7a] with appropriate *p*-nitrophenyl glycoside substrates (Sigma). The reference inhibitor castanospermine (**2**) was purchased from Sigma.

A typical enzymatic assay (final volume 0.1 mL) contains 0.01 to 0.5 units/mL of the enzyme (1 unit = 1 enzyme unit liberates 1 μmol of glycoside per minute from *p*-nitrophenyl glycoside) and 5 mM aqueous solution of the appropriate *p*-nitrophenyl glycoside substrate 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 (45 °C for the amyloglucosidases), the reaction was stopped by addition of 0.25 mL 0.2 M sodium borate buffer pH 9.8. The *p*-nitrophenolate formed was measured by visible absorption spectroscopy at 410 nm. Under these conditions of the assay, the *p*-nitrophenolate released led to optical densities linear with both time of the reaction and concentration of the enzyme.

In preliminary screenings, enzymatic activity was determined in the presence of two different high concentration of the inhibitors (+)-**4** and (−)-**4** (1 mM, 2.5 mM). For the highest inhibition rates, the IC_{50} values (concentration of inhibitor required for 50% inhibition of enzyme activity) were calculated by measuring glycosidase activity in the presence of various concentrations of inhibitor. The inhibition constant K_i was determined when 100% inhibition of enzymatic activity was reached at 1 mM concentration of inhibitor.

Acknowledgment. We thank the Ministry of University and Scientific and Technological Research, Italy (M.U.R.S.T. 60%/40%), the C.N.R. (National Research Council, Italy), the Swiss National Science Foundation, and the Fonds Herbette (Lausanne) for financial support.

JO9506948