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(1*S*,2*S*,7*R*,8a*S*)- and (1*S*,2*S*,7*S*,8a*S*)-Trihydroxyoctahydroindolizine: Two New Glycosidase Inhibitors by Nitrone Cycloaddition Strategy

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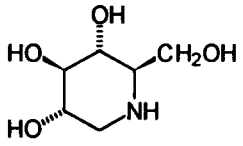
Abstract: The two new epimeric (1*S*,2*S*,7*R*,8a*S*)- and (1*S*,2*S*,7*S*,8a*S*)-1,2,7-trihydroxyoctahydroindolizines **4** and **5** have been synthesized via methylenecyclopropane-nitrone cycloaddition-rearrangement methodology employing an enantiomerically pure L-tartaric acid derived nitrone **7b**. Highly stereoselective reductions of the intermediate indolizidinone **10b** and final deprotection furnished the two title indolizidinetriols **4** and **5**, the inhibiting abilities of which toward 24 commercially available glycosidases were tested. Both **4** and **5** are good competitive inhibitors of amyloglucosidases with K_i values of ca. 6 and 75 μM, respectively. Compared with (+)-lentiginosine **3**, **4** and **5** are less powerful inhibitors but, in contrast to **3**, the (7*R*)-hydroxy analogue **4** possesses a weak inhibiting activity toward α-L-fucosidase from bovine epididymis. A model to rationalize the structure-activity relationship of (+)-lentiginosine and the two new 7-hydroxylentiginosines toward glucoamylases is proposed on the basis of their structural comparison with known inhibitors and with the natural enzyme's substrate amylose.

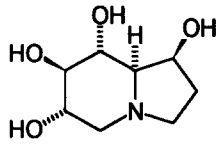
INTRODUCTION

A large number of natural and unnatural polyhydroxylated indolizidines (e.g. castanospermine **2**, lentiginosine **3**),[1] as well as monocyclic azasugars (e.g. 1-deoxynojirimycin **1**), display interesting biological activity as inhibitors of glycosidases.[1,2] Since glycosidases are key enzymes in biosynthesis and processing of glycoproteins, their inhibitors are widely investigated as potential antibacterial, antiviral, antitumoral, antidiabetic, or antiinflammatory agents.[2,3]

The biological activity of azasugars and polyhydroxyindolizidines is related to their stereochemical structural resemblance with the enzyme natural substrates, but in many cases their activity cannot be reliably



1-Deoxynojirimycin **1**



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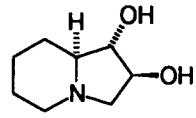
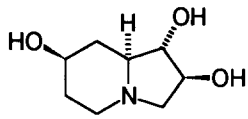


Lentiginosine **3**

predicted on the basis of a superficial correspondence. Thus, the synthetic efforts have been focused on the synthesis of all the possible stereoisomeric polyhydroxyindolizidines with the aim of testing their biological activity.[4] In this paper, we report the synthesis from L-tartaric acid of two new 1,2,7-trihydroxyindolizidines **4** and **5** epimeric at C-7 and their biological activity toward a wide range of commercially available glycosidases. An effort to rationalize the structure-activity relationship is also reported, taking into account the activities toward amyloglucosidases of the new compounds and of known examples from the literature.



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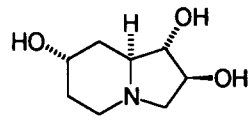


5

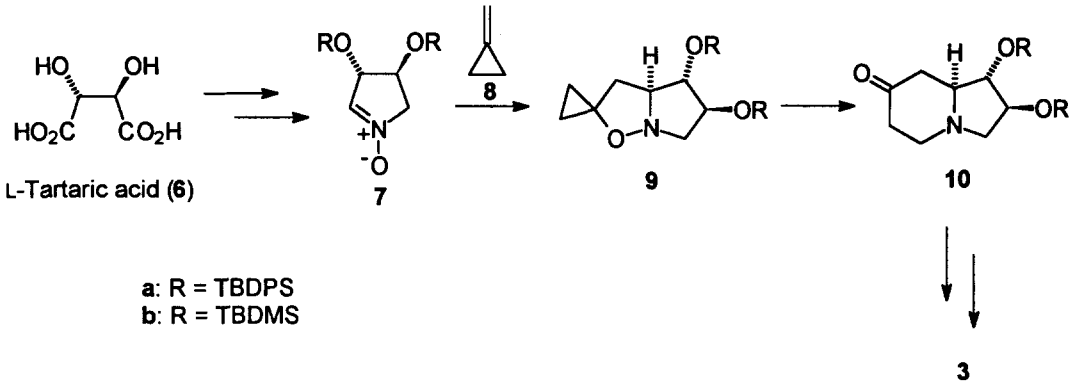
RESULTS

Synthesis of Trihydroxyindolizidines 4 and 5

Recently, we have reported the synthesis of (+)-lentiginosine **3**,[4e,5] a natural occurring dihydroxyindolizidine,[6] which has been shown to be among the most powerful inhibitors of amyloglucosidases (α-1,4-D-glucan glucohydrolase, EC 3.2.1.3) found so far.[4e] The high activity of **3** is surprising, since it is the only compound of this class of inhibitors which possesses less than three -OH groups, and it has no immediate structural relationship to other amyloglucosidase inhibitors, such as **1** and **2**.

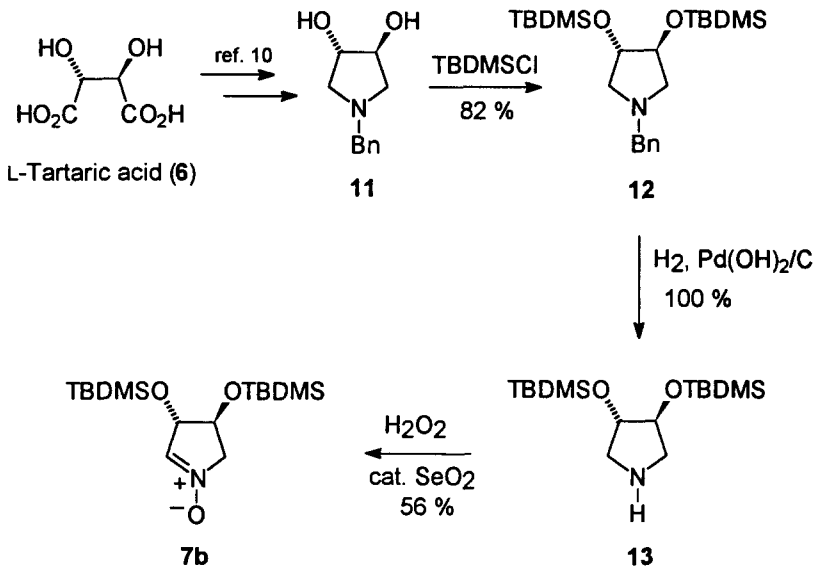
Our synthetic route to lentiginosine **3** employed a 1,3-dipolar cycloaddition of an appropriately protected dihydroxy cyclic nitrone **7a** derived from L-tartaric acid **6** to methylenecyclopropane **8**,[7] followed by thermal rearrangement of the adduct **9a** to the indolizidinone **10a** (Scheme 1),[7a] which gives the desired alkaloid by reduction and deprotection.[4e,5] The intermediate ketone **10** appeared to be a suitable precursor for the production of new polyhydroxyindolizidine derivatives, such as the 7-hydroxylentiginosines. The bioassay of these unknown products[8] would contribute to our information about structure-activity relationships.

Scheme 1



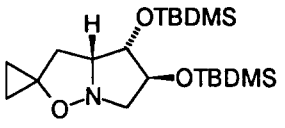
The synthesis of the two trihydroxyindolizidines epimeric at C(7) has been performed by using a *tert*-butyldimethylsilyl (TBDMS) protected nitrone **7b**,[9] obtained in 22% overall yield from L-tartaric acid in five steps via the pyrrolidine **11**[10] (Scheme 2), accordingly to the analogous procedure for the synthesis of **7a**.[4c]

Scheme 2



The TBDMS-protected ketone **10b** was prepared in two steps from nitrone **7b** by the usual cycloaddition-thermal rearrangement procedure[7a] in 26% overall yield (Scheme 1). The features of both the cycloaddition and rearrangement steps were similar to those of the corresponding nitrone **7a**.[4c,5] Indeed, the cycloaddition displayed the same high regioselectivity and a somewhat poorer diastereoselectivity, as expected

on the basis of the minor steric requirements of the TBDMS group compared with the *tert*-butyldiphenylsilyl (TBDPS) group, thus producing a 6:1 ratio of **9b** and **14**, contaminated with minor impurities of **15** (500 MHz ^{1}H NMR spectrum). The thermal rearrangement of **9b** gave an inseparable 1.3:1 mixture of the desired indolizidinone **10b** and the open-chain enaminone **16**.



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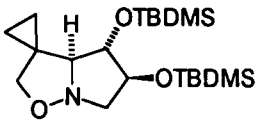

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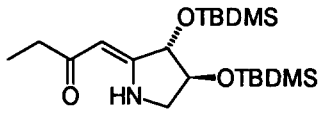
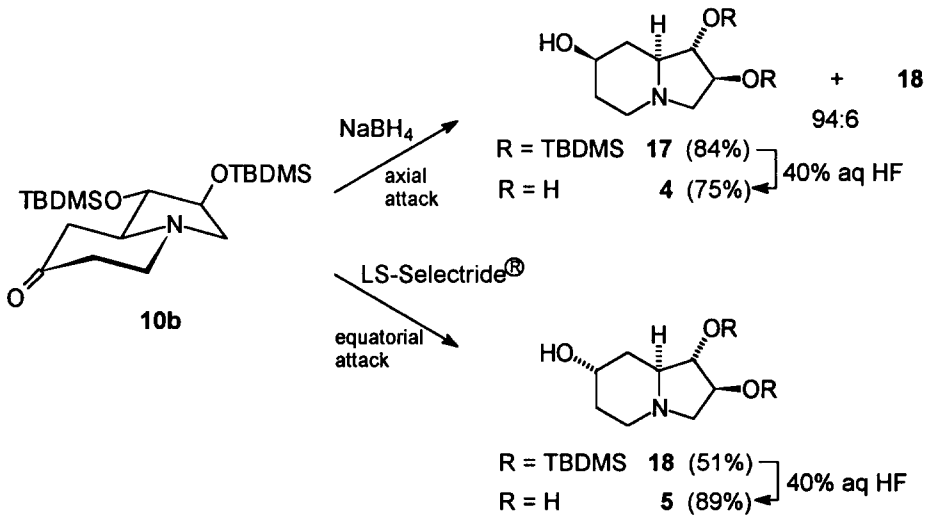


16

The mixture of **10b** and **16** can be used directly in the reduction since the enaminone **16** does not interfere, being unreactive or partially decomposed by metal hydride complexes.[11] With $NaBH_4$ a 94:6 mixture of alcohols **17** and **18** (89%) was obtained, whereas, with the bulkier LS-Selectride® (lithium trisyamylborohydride), exclusive formation of **18** (51%) was observed (Scheme 3). These findings realize very efficient and stereoselective syntheses[12] of the 7-hydroxylentiginosine precursors **17** and **18** that were deprotected with 40% aqueous HF in CH_3CN[13] into **4** (75%) and **5** (89%), respectively (Scheme 3).

Scheme 3



The diastereoselectivities of the above reductions result from the balance of steric and torsional factors. The conformationally biased azacyclohexanone moiety of **10b**[14] implies torsional strain that favors axial attack of the ketone that leads to the corresponding equatorial alcohol **17**.[15] Alternatively, when a bulky hydride is used the steric requirements are such that they lead to a reversal of the face selectivity of the reduction of the ketone and consequently produces the corresponding axial alcohol **18** through an equatorial attack.[15] The

structures of alcohols **17** and **18** (and of **4** and **5**) were confirmed by their ^{1}H NMR spectra. In the case of **17**, the signal of the axial H-C(7) at 3.60 ppm appears as a triplet of a triplet with J = 10.8 and 4.7 Hz (as a tt at 3.63 ppm with J = 11.4 and 4.4 Hz in the corresponding triol **4**), whereas, in the case of **18**, the corresponding equatorial proton resonates at 4.17 ppm as a narrower multiplet with a half-height width of 6.6 Hz (a quintet at 4.12 ppm with J = 2.9 Hz in **5**).

Glycosidase Inhibition

Both indolizidines **4** and **5** displayed the same specificity than (+)-lentiginosine **3** as inhibitors toward amyloglucosidases (1,4-α-D-glucanglucohydrolase EC 3.2.1.3), important industrial enzymes used to produce glucose from starch.[16] The concentrations of inhibitors required for 50% inhibition of enzyme activity (IC_{50}) and the corresponding inhibition constants (K_i) are reported in Table 1 for (+)-lentiginosine **3** and the 7-hydroxy analogues **4** and **5** for the amyloglucosidase from *Aspergillus niger*[17] and from *Rhizopus* mold. Inhibition rate (in %) in the presence of 1 mM inhibitor are given for α-L-fucosidase (EC 3.2.1.51) from bovine epididymis. Lineweaver-Burk plots[18] of the data showed that the three indolizidines **3-5** behave as competitive inhibitors. These compounds did not inhibit the following 21 enzymes: *Aspergillus niger*, *Escherichia coli* and coffee bean α-galactosidases (EC 3.2.1.22), coffee bean, *Aspergillus niger*, *Escherichia coli*, bovine liver and *Aspergillus orizae* β-galactosidases (EC 3.2.1.23), yeast and rice maltases (EC 3.2.1.20), baker yeast isomaltase (EC 3.2.1.10), jack bean and almond 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), jack bean and bovine epididymis A and B β-*N*-acetylglucosaminidases (EC 3.2.1.30) and β-glucosidases (EC 3.2.1.21) from almonds and *Caldocellum saccharolyticum*.

Table 1

IC_{50} and K_i Values with Amyloglucosidases and % of Inhibition at 1 mM with α-L-Fucosidase of **3**, **4**, and **5**

	Amyloglucosidase (from *Aspergillus niger*)		Amyloglucosidase (from *Rhizopus* mold)		α-L-Fucosidase (from bovine epididymis)
Inhibitor	IC_{50} (μM)	K_i (μM)	IC_{50} (μM)	K_i (μM)	% Inhibition at 1 mM
3	2.7	2.0	3.1	3.0	No Inhibition
4	9.8	4.4	12.5	7.2	60%
5	290	69	400	80	No Inhibition

DISCUSSION

Amyloglucosidases are 1,4-α-D-glucanglucosidases tuned by Nature to recognize the non-reductive end of amylose, glycogen and other α-D-glucopyranosides.[16,19] In the crystalline state, amylose adopts a helical structure[20] for which the non-reducing end, *O*-(α-D-glucopyranoyl)-(1→4)-α-D-glucopyranoside unit is represented in Figure 1.

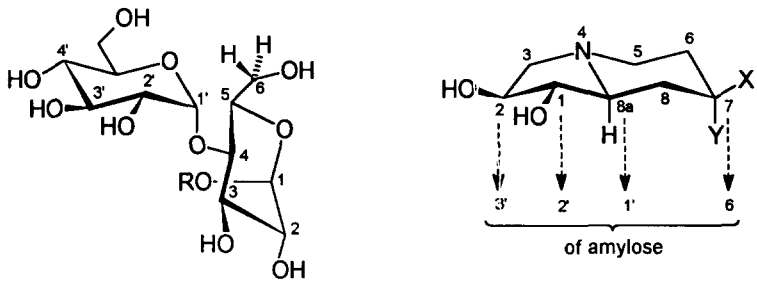
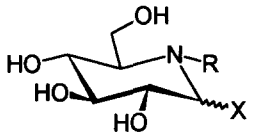


Figure 1

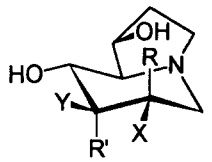
Comparison of the Structure of the Non-Reducing End of Amylose with that of (+)-Lentiginosine **3** and Its 7-Hydroxy Derivatives

As many other glycosidases, glucoamylases are inhibited by sugar analogues possessing a basic nitrogen atom adjacent to C(1),[21] such as nojirimycin **19**,[22] 1-deoxynojirimycin **1**,[23] *N*-substituted 1-deoxynojirimycins (*e.g.* **20-22**),[23a] castanospermine **2**,[6,24] and acarbose,[22,23a25] a pseudotetrasaccharide analogue whose non-reducing end contains a pseudosugar moiety with a carbon-carbon double bond instead of the glycosidic oxygen (see **26**, Figure 2). These inhibitors have structures that resemble that of the transition state of the hydrolysis of α-D-glucopyranosides.[2g-i,26] The enzyme active site enjoys some flexibility since the 6-epimer of castanospermine **23** is only 2.5 times less active than castanospermine **2** and 6,7-di*epi*-castanospermine **24** still displays a quite good inhibiting activity.[24] This phenomenon is also seen on comparing the inhibiting activities of **1**, **2**, **19-22** (Figure 2) with those reported for australine **27**,[27] its 1-epimer **28**,[24] as well as for several other polyhydroxypyrrolizidines possessing the same absolute stereochemistry at C(2) and C(3),[28] and for (+)-lentiginosine **3**.[4e,6] In this case, the *trans*-dihydroxypyrrolidine unit probably takes the space of the enzyme active site reserved for the terminal α-D-glucopyranoside unit of amylose. The *trans* configuration of the dihydroxypyrrolidine moiety is an absolute requirement for activity as it has been reported that swainsonine **31**[29] and 2-*epi*-lentiginosine **32**[6] are inactive as inhibitors toward *Aspergillus niger* amyloglucosidase, while 2-*epi*-swainsonine **30** is still a good inhibitor.[30] The *S,S* absolute configuration of the two carbon atoms bearing these hydroxy groups appears also essential, since *ent*(−)-lentiginosine **29**[4e] is ca. 40 times less active than its (+)-enantiomer **3**. This behavior parallels the decreased activity of **24** with respect to **2**. Furthermore, our

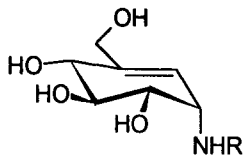
finding that the two 1,7-dihydroxyindolizidines **33** and **34**[31] also lack activity toward amyloglucosidases and other glycohydrolases[32] testifies the need for the two hydroxy groups on C(1) and C(2), in agreement with the 11 times decrease in activity of 7-deoxy-6-*epi*-castanospermine **25** compared with its analogue **23**.[33]



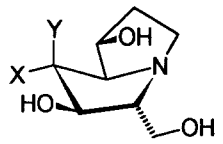
1 X = H, R = H (29 μM; K_i = 2 μM)
19 X = OH, R = H (0.8 μM)[a]
20 X = H, R = Me (K_i = 2 μM)
21 X = H, R = $(CH_2)_2OC_6H_4p$COOEt (K_i = 4 μM)
22 X = H, R = $(CH_2)_2$OH (K_i = 6 μM)



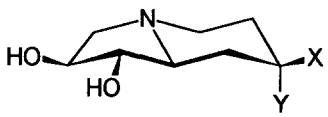
2 X,Y = OH, R,R' = H (8 μM)
23 R,Y = OH, R',X = H (20 μM)
24 R,R' = OH, X,Y = H (84 μM)
25 R = OH, R',X,Y = H (230 μM)



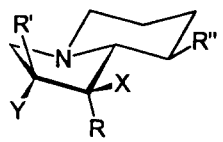
26[b] (0.06 μM, K_i = 10-6 μM)
R = trisaccharide unit



27 X = OH, Y = H (6 μM)
28 X = H, Y = OH (26 μM)



3 X,Y = H (2.7 μM)
4 X = OH, Y = H (10 μM)
5 X = H, Y = OH (290 μM)



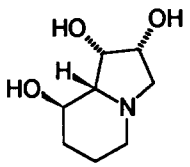
29 X,Y = OH, R,R',R" = H (108 μM)
30 X,Y = H, R,R',R" = OH (K_i = 50 μM)

[a]Value referred to intestinal glucoamylase (cf. IC_{50} = 0.1 μM for 1 toward the same enzyme).
[b]Acarbose.

Figure 2

Examples of *Aspergillus niger* Amyloglucosidase Inhibitors:

Comparison of Their Structures and IC_{50} or K_i Values (in parentheses)



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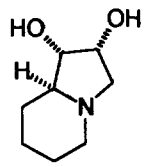

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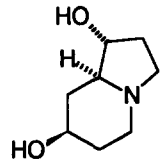

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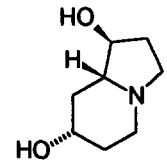

34

The six-membered ring of (+)-lentiginosine **3** is accepted by the amyloglucosidases because centers C(8a) and C(7) take the space of C(1') and C(6) of the terminal *O*-α-D-Glc*p*(1→4)-α-D-Glc*p* unit of amylose (see Figure 1). This hypothesis implies that centers C(5) and C(6) of **3** occupy an empty space in the enzyme, which is consistent with the structures published for amyloglucosidase from *Aspergillus awamori* var. X100.[17,34] Accordingly, the (7*R*) 7-hydroxy group of **4** takes the space of the 6-hydroxymethyl group of amylose inside the enzyme; this is probably also the case for the 2-hydroxyethyl moiety of miglitol (**22**).[23a] It is disappointing that **4** is in fact about 2 to 3 times less active than **3** toward amyloglucosidases (Table 1). Our model, however, explains why **5** is definitely less potent than **3** and **4** since its (7*S*) 7-hydroxy group cannot take the space reserved in the enzyme for the 6-hydroxymethyl group of *O*-α-D-Glc(1→4)-α-D-Glc.

The relatively weak inhibiting activity of **4** toward bovine α-L-fucosidase must be noticed; it cannot be interpreted yet.

CONCLUSION

Efficient and stereoselective syntheses of (7*R*)- and (7*S*)-7-hydroxylentiginosine have been realized starting from L-tartaric acid. The new 1,2,7-trihydroxyindolizidines **4** and **5** are specific inhibitors of amyloglucosidases; they do not inhibit 22 commercially available glucosidases, except for **4** that is a weak inhibitor of bovine α-L-fucosidase. (+)-Lentiginosine **3** is 2 to 3 times more potent than **4** as an inhibitor of amyloglucosidases. The comparison of the structures of known inhibitors and of **3**, **4** and **5** now suggests the synthesis of other analogues as good inhibitors of amyloglucosidases, and studies in this direction are in progress in our laboratories.

EXPERIMENTAL

Enzymatic Assays

The enzymatic inhibition tests were performed under standard conditions with 24 commercially available (Oxford Glycosystem, Sigma Chemical Co.) enzymes (see above).

Enzymes were assayed following the method of Saul *et al.*[35] 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) 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 room temperature 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.

General Procedures

All the reactions which required dry conditions were run under a nitrogen atmosphere using anhydrous solvents. 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. Melting points (mp) were measured with a RCH Kofler apparatus and are uncorrected. Specific optical rotatory values were recorded on a JASCO DIP-370 polarimeter. ^1H and ^{13}C NMR spectra (in $CDCl_3$ solution, unless otherwise stated) were recorded on a Varian Gemini 200 spectrometer (^1H, 200 MHz; ^{13}C, 50 MHz); notations s, d, t, q, m, and br indicate singlet, doublet, triplet, quartet, multiplet, and broad, respectively. IR spectra were recorded with a Perkin-Elmer 881 spectrophotometer. Mass spectra (MS) were recorded on a QMD 1000 Carlo Erba instrument by GC or direct inlet (EI, 70 eV). Microanalyses were measured with a Perkin-Elmer 240 C instrument.

Synthesis of (3S,4S)-3,4-Bis[(tert-butyl)dimethylsilyloxy]-1-pyrroline N-oxide 7b

(3S,4S)-3,4-Bis[(tert-butyl)dimethylsilyloxy]-N-benzylpyrrolidine 12. *Tert*-butyldimethylsilyl chloride (5.16 g, 34.2 mmol) was added portionwise at 0 °C to a solution of (3*S*,4*S*)-3,4-dihydroxy-*N*-benzylpyrrolidine **11**[10] (3.31 g, 17.1 mmol) and imidazole (2.33 g, 34.2 mmol) in dimethylformamide (40 mL) and the mixture was then heated at 60 °C for 1 h. The resulting reaction mixture was added to H_2O (30 mL) and extracted with petroleum ether (2x40 mL). The organic layer was washed with H_2O and brine, dried over Na_2SO_4 and concentrated. The crude mixture was purified by elution on a short pad of silica gel, eluent petroleum ether-ethyl acetate 10:1 to give the protected pyrrolidine **12** (R_f 0.40, 5.88 g, 13.9 mmol, 82%) as a colorless oil. $[\alpha]_D^{19} = -59.7$ (*c* 1.54, $CHCl_3$). ^1H NMR: δ 7.32-7.28 (m, 5 H), 4.13 (m, 2 H), 3.74 (d, *J* = 13.2 Hz, 1 H), 3.50 (d, *J* = 13.2 Hz, 1 H), 2.88 (dd, *J* = 9.6, 6.1 Hz, 2 H), 2.47 (dd, *J* = 9.6, 4.0 Hz, 2 H), 0.89 (s, 18 H), 0.07 (s, 6 H), 0.04 (s, 6 H); ^{13}C NMR: δ 138.6 (s), 128.7 (d, 2 C), 128.2 (d, 2 C), 126.9 (d), 79.8 (d, 2 C), 60.8 (t, 2 C), 60.7 (t), 25.8 (q, 6 C), 18.0 (s, 2 C), –4.6 (q, 2 C), –4.7 (q, 2 C); IR ($CDCl_3$): 3030, 2957, 2930, 1462, 1251 cm^{-1}; MS: m/z (relative intensity) 421 (M^+, 1), 406 (2), 364 (18), 133 (65), 132 (38), 91 (100), 73 (53). Anal. Calcd for $C_{23}H_{43}NO_2Si_2$: C, 65.50; H, 10.28; N, 3.32. Found: C, 65.34; H, 10.40; N, 3.62.

(3S,4S)-3,4-Bis[(tert-butyl)dimethylsilyloxy]pyrrolidine 13. The *N*-benzylpyrrolidine **12** (5.68 g, 13.5 mmol) was dissolved in MeOH (70 mL), added with 20% $Pd(OH)_2$ (1.98 g) and hydrogenated at atmospheric pressure and 20 °C for 20 h. The solution was filtered over Celite and concentrated to give the desired diprotected pyrrolidine **13** (4.47 g, 13.5 mmol, 100%) as a yellowish solid, which was used for the following step without purification (attempted chromatography on silica gel brought to extensive decomposition). Recrystallization from petroleum ether gave an analytically pure sample as a white solid, mp 132-133 °C. $[\alpha]_D^{22}$ = +23.9 (*c* 0.98, $CHCl_3$). ^1H NMR: δ 3.96 (m, 2 H), 3.10 (dd, *J* = 11.8, 4.0 Hz, 2 H), 2.67 (d, *J* = 11.8 Hz, 2 H), 2.04 (br s, 1 H), 0.87 (s, 18 H), 0.07 (s, 6 H), 0.05 (s, 6 H); ^{13}C NMR: δ 79.3 (d, 2 C), 54.2 (t, 2 C), 25.8 (q, 6 C), 18.0 (s, 2 C), –4.8 (q, 4 C); IR ($CDCl_3$): 2957, 2931, 2858, 1462, 1253 cm^{-1}; MS: m/z (relative intensity) 316 ($M\text{-}CH_3^+$, 6), 274 (4), 224 (88), 91 (100). Anal. Calcd for $C_{16}H_{37}NO_2Si_2$: C, 57.95; H, 11.25; N, 4.22. Found: C, 57.80; H, 11.21; N, 4.43.

(3S,4S)-3,4-Bis[(tert-butyl)dimethylsilyloxy]-1-pyrroline N-Oxide 7b. To a suspension of SeO_2 (2 mg, 0.02 mmol) and the diprotected pyrrolidine (111 mg, 0.33 mmol) in acetone (1.5 mL) cooled to 0 °C was added dropwise a 35% aqueous solution of hydrogen peroxide (0.093 mL).[36] The resulting mixture was stirred at 20 °C for 1 h, then concentrated and CH_2Cl_2 and H_2O were added. The separated organic layer was washed with H_2O and brine, dried over Na_2SO_4, filtered and the solvent evaporated. The residue was purified by column chromatography, eluent petroleum ether-ethyl acetate 1:1, to afford the pure nitrone **7b** (R_f 0.35, 64 mg, 0.2 mmol, 56%) as a solid, mp 49-51 °C. $[\alpha]_D^{19}$ = +75.8 (*c* 1.30, $CHCl_3$). ^1H NMR: δ 6.82 (q, *J* = 1.7 Hz, 1 H), 4.68 (br s, 1 H), 4.33-4.21 (m, 2 H), 3.73-3.62 (m, 1 H), 0.89 (s, 18 H), 0.12 (s, 3 H), 0.10 (s, 3 H), 0.09 (s, 3 H), 0.08 (s, 3 H); ^{13}C NMR: δ 134. 4 (d), 80.5 (d), 75.4 (d), 69.0 (t), 25.6 (q, 6 C), 17.9 (s), 17.8 (s), –4.6 (q, 2 C), –4.8 (q, 2 C); IR ($CDCl_3$): 2956, 2932, 2888, 1583, 1462, 1360, 1255 cm^{-1}; MS: m/z (relative intensity) 345 (M^+, 2), 330 (1), 288 (9), 187 (34), 147 (33), 75 (77), 73 (100), 57 (47). Anal. Calcd for $C_{16}H_{35}NO_3Si_2$: C, 55.60; H, 10.21; N, 4.05. Found: C, 55.83; H, 10.28; N, 4.00.

Cycloaddition of Nitrone 7b to Methylenecyclopropane8

Methylenecyclopropane (**8**, 413 mg, 7.6 mmol) was added to a solution of **7b** (754 mg, 2.2 mmol) in benzene (2 mL) and the mixture was heated in a sealed tube at 35 °C for 11 d. The crude mixture was then concentrated to give a 6:1 (by 500 MHz ^1H NMR) mixture (830 mg, 95%) of **9b** and **14** contaminated with impurities of **15**. Purification by column chromatography, eluent petroleum ether-ethyl acetate 6:1, afforded pure **9b** (R_f 0.34, 558 mg, 1.4 mmol, 63%) as an oil and a mixture of **14** with traces of **15** (R_f 0.20, 132 mg, 15%).

(3a'S,4'S,5'S)-4',5'-Bis(tert-butyldimethylsilyloxy)hexahydrospiro[cyclopropane-1,2'-pyrrolo[1,2-b]isoxazole] 9b. $[\alpha]_D^{23}$ = +41.9 (*c* 1.20, $CHCl_3$). ^1H NMR (500 MHz): δ 4.09 (dt, *J* = 6.9, 5.1 Hz, 1 H), 4.03 (t, *J* = 4.6 Hz, 1 H), 3.69 (dt, *J* = 8.8, 4.7 Hz, 1 H), 3.46 (dd, *J* = 11.0, 5.1 Hz, 1 H), 3.19 (dd, *J* = 11.3, 6.6 Hz, 1 H), 2.46 (dd, *J* = 11.7, 8.8 Hz, 1 H), 2.28 (dd, *J* = 11.7, 5.5 Hz, 1 H), 1.09-0.53 (m, 4 H), 0.89 (s, 18 H), 0.09

(s, 3 H), 0.08 (s, 3 H), 0.07 (s, 6 H); ^{13}C NMR: δ 83.2 (d), 78.0 (d), 71.8 (d), 61.7 (s), 60.5 (t), 39.9 (t), 25.7 (q, 6 C), 17.9 (s, 2 C), 10.6 (t), 7.8 (t), –4.4 (q), –4.5 (q), –4.7 (q), –4.8 (q); IR ($CDCl_3$): 2930, 2891, 2858, 1461, 1407, 1360, 1252 cm^{-1}; MS: m/z (relative intensity) 399 (M^+, 11), 384 (2), 342 (28), 315 (75), 313 (46), 147 (100), 73 (73). Anal. Calcd for $C_{20}H_{41}NO_3Si_2$: C, 60.10; H, 10.43; N, 3.50. Found: C, 60.63; H, 10.23; N, 3.64.

(3a'R,4'S,5'S)-4',5'-Bis(tert-butyldimethylsilyloxy)hexahydrospiro[cyclopropane-1,2'-pyrrolo[1,2-b]isoxazole] 14. ^{1}H NMR (500 MHz): δ 4.33 (dt, *J* = 7.7, 6.2 Hz, 1 H), 4.21-3.86 (m, 2 H), 3.36 (dd, *J* = 13.5, 6.5 Hz, 1 H), 3.02 (dd, *J* = 13.6, 8.1 Hz, 1 H), 2.49 (dd, *J* = 11.8, 7.7 Hz, 1 H), 2.14 (dd, *J* = 12.4, 3.6 Hz, 1 H), 1.02-0.60 (m, 4 H), 0.91 (s, 18 H), 0.08 (s, 6 H), 0.07 (s, 6 H); ^{13}C NMR: δ 78.4 (d), 76.4 (d), 67.7 (d), 62.8 (s), 60.6 (t), 35.3 (t), 25.8 (q, 6 C), 18.0 (s, 2 C), 10.5 (t), 9.2 (t), –4.8 (q, 2 C), –5.2 (q, 2 C); MS: m/z (relative intensity) 399 (M^+, 3), 372 (8), 147 (48), 73 (100).

Thermal Rearrangement of 9b

The cycloadduct **9b** (480 mg, 1.2 mmol) was dissolved in *o*-dichlorobenzene (12 mL) and heated at 130 °C for 3 h. The crude mixture was poured on silica gel into a chromatographic column and eluted with petroleum ether in order to remove the reaction solvent. Then the product was purified by flash chromatography, eluent petroleum ether-ethyl acetate 5:1, to give an inseparable 1.3:1 mixture of the indolizidinone **10b** and the enaminone **16** (R_f 0.32, 348 mg, 72%).

(1S,2S,8aS)-1,2-Bis(tert-butyldimethylsilyloxy)octahydroindolizin-7-one 10b. ^{1}H NMR: δ 4.10 (ddd, *J* = 7.4, 3.7, 2.2 Hz, 1 H), 3.85 (dd, *J* = 6.9, 3.8 Hz, 1 H), 3.24-3.12 (m, 1 H), 2.96 (dd, *J* = 9.9, 2.2 Hz, 1 H), 2.72-2.52 (m, 2 H), 2.68 (dd, *J* = 9.9, 7.4 Hz, 1 H), 2.44-2.20 (m, 4 H), 0.92 (s, 9 H), 0.88 (s, 9 H), 0.09 (s, 3 H), 0.08 (s, 3 H), 0.06 (s, 3 H), 0.05 (s, 3 H); ^{13}C NMR (TBDMS signals not reported): δ 208.4 (s), 86.1 (d), 79.3 (d), 68.2 (d), 60.7 (t), 50.6 (t), 45.1 (t), 40.2 (t).

(3S,4S)-2-(2-Oxobutylidene)-3,4-bis(tert-butyldimethylsilyloxy)tetrahydropyrrole 16. ^{1}H NMR: δ 9.23 (br s, 1 H), 5.16 (s, 1 H), 4.46 (d, *J* = 6.2 Hz, 1 H), 4.20 (q, *J* = 6.6 Hz, 1 H), 3.63 (dd, *J* = 9.9, 7.0 Hz, 1 H), 3.23 (dd, *J* = 9.9, 6.6 Hz, 1 H), 2.30 (q, *J* = 7.7 Hz, 2 H), 1.09 (t, *J* = 7.7 Hz, 3 H), 0.93 (s, 9 H), 0.88 (s, 9 H), 0.15 (s, 3 H), 0.14 (s, 3 H), 0.08 (s, 3 H), 0.06 (s, 3 H); ^{13}C NMR (TBDMS signals not reported): δ 200.5 (s), 165.2 (s), 88.7 (d), 79.8 (d), 76.8 (d), 51.0 (t), 35.1 (t), 9.9 (q); IR ($CDCl_3$): 3305, 2957, 2931, 2887, 1632, 1548, 1461, 1252 cm^{-1}.

Reduction of Indolizidinone 10b with Sodium Borohydride

To a solution of indolizidinone **10b** and enaminone **16** (339 mg, 0.46 mmol of **10b**) in EtOH (2 mL), $NaBH_4$ (64 mg) was added at 0 °C and the mixture was stirred at r.t. for 1 d. After removal of EtOH *in vacuo*, H_2O was added, the mixture extracted with CH_2Cl_2 and the organic layer dried over Na_2SO_4. After filtration and concentration, the crude product was purified by column chromatography, eluent petroleum ether-ethyl

acetate 2:1, to give pure **17** (R_f0.15, 155 mg, 0.38 mmol, 84%) as a colorless oil and **18** (R_f0.09, 10 mg, 0.025 mmol, 5%).

(1S,2S,7R,8aS)-1,2-Bis[(tert-butyl)dimethylsilyloxy]-7-hydroxyoctahydroindolizine ***17.*** $[\alpha]_D^{23}$ = +23.5 (*c* 2.40, $CHCl_3$). ^1H NMR: δ 4.03 (ddd, *J* = 7.7, 4.0, 2.1 Hz, 1 H), 3.77 (dd, *J* = 8.4, 4.0 Hz, 1 H), 3.60 (tt, *J* = 10.8, 4.7 Hz, 1 H), 2.94 (ddd, *J* = 11.4, 4.4, 2.6 Hz, 1 H), 2.85 (dd, *J* = 10.2, 2.1 Hz, 1 H), 2.54 (dd, *J* = 10.2, 7.7 Hz, 1 H), 2.16 (dt, *J* = 11.3, 2.2 Hz, 1 H), 2.01-1.84 (m, 2 H), 1.68-1.51 (m, 3 H), 0.90 (s, 18 H), 0.09 (s, 3 H), 0.08 (s, 3 H), 0.06 (s, 3 H), 0.04 (s, 3 H); ^{13}C NMR: δ 85.2 (d), 79.5 (d), 70.0 (d), 67.2 (d), 61.6 (t), 50.9 (t), 38.4 (t), 34.8 (t), 26.4 (q, 3 C), 26.3 (q, 3 C), 18.4 (s), 18.3 (s), –3.6 (q), –3.7 (q), –3.8 (q), –4.2 (q); IR ($CDCl_3$): 3609, 2929, 2858, 2801, 1461, 1384, 1359, 1250 cm^{-1}; MS: m/z (relative intensity) 401 (M^+, 1), 386 (1), 344 (5), 113 (100), 75 (24), 73 (60). Anal. Calcd for $C_{20}H_{43}NO_3Si_2$: C, 59.80; H, 10.79; N, 3.49. Found: C, 60.18; H, 10.77; N, 3.52.

Reduction of Indolizidinone 10b with LS-Selectride®

To a solution of indolizidinone **10b** and enaminone **16** (538 mg, 0.7 mmol of **10b**) in THF (36 mL) was added a 1 M solution of LS-Selectride® in THF (2.2 mL) at –78 °C. After stirring 1 h at –78 °C and 1 h at 0 °C, sat. NH_4Cl (66 mL) was added slowly at 0 °C. Ethyl acetate was added and the organic layer separated, washed with brine and dried over Na_2SO_4. After filtration and concentration, the crude product was purified by column chromatography, eluent petroleum ether-ethyl acetate 1:1, to give diastereochemically pure **18** (R_f0.25, 147 mg, 0.36 mmol, 51%) as a colorless oil.

(1S,2S,7S,8aS)-1,2-Bis[(tert-butyl)dimethylsilyloxy]-7-hydroxyoctahydroindolizine ***18.*** $[\alpha]_D^{25}$ = +11.9 (*c* 0.84, $CHCl_3$). ^1H NMR: δ 4.17 (br s, half height width = 6.6 Hz, 1 H), 4.02 (ddd, *J* = 7.7, 4.1, 2.0 Hz, 1 H), 3.72 (dd, *J* = 8.8, 4.1 Hz, 1 H), 2.86 (dd, *J* = 9.9, 2.0 Hz, 1 H), 2.74 (m, 1 H), 2.62 (dd, *J* = 9.9, 7.7 Hz, 1 H), 2.41-2.20 (m, 2 H), 2.05-1.44 (m, 4 H), 0.90 (s, 9 H), 0.89 (s, 9 H), 0.08 (s, 3 H), 0.06 (s, 6 H), 0.04 (s, 3 H); ^{13}C NMR: δ 85.0 (d), 77.9 (d), 64.2 (d), 61.6 (d), 61.5 (t), 47.0 (t), 35.7 (t), 31.6 (t), 25.6 (q, 6 C), 17.7 (s), 17.6 (s), –4.3 (q), –4.4 (q), –4.5 (q), –4.9 (q); IR ($CDCl_3$): 3615, 2955, 2930, 2858, 1461, 1384, 1359, 1250 cm^{-1}; MS: m/z (relative intensity) 401 (M^+, 4), 386 (4), 344 (11), 113 (100), 73 (38). Anal. Calcd for $C_{20}H_{43}NO_3Si_2$: C, 59.80; H, 10.79; N, 3.49. Found: C, 60.28; H, 10.43; N, 3.96.

Deprotection of Silyl Ethers 17 and 18

A solution of the silylether **17** or **18** was stirred at 20 °C in a 7:3 mixture of CH_3CN-aqueous 40% HF (8.6 mL) in a polyethylene flask for 24 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 to afford **4** or **5**, respectively.

(1S,2S,7R,8aS)-1,2,7-Trihydroxyoctahydroindolizine ***4.*** Obtained from silyl ether **17** (63 mg, 0.16 mmol). Flash column chromatography, eluent CH_2Cl_2-CH_3OH-30% aq. NH_3 41:8:1, gave pure **4** (R_f0.35, 20

mg, 0.12 mmol, 75%) as a white solid, mp 171-172 °C. $[\alpha]_D^{22}$ = +2.1 (*c* 0.36, MeOH). ^{1}H NMR (D_2O): δ 4.02 (ddd, *J* = 7.5, 4.0, 1.8 Hz, 1 H), 3.63 (tt, *J* = 11.4, 4.4 Hz, 1 H), 3.61 (dd, *J* = 8.8, 4.0 Hz, 1 H), 2.86 (ddd, *J* = 11.3, 4.4, 2.5 Hz, 1 H), 2.74 (dd, *J* = 11.4, 1.8 Hz, 1 H), 2.54 (dd, *J* = 11.4, 7.5 Hz, 1 H), 2.16-1.81 (m, 4 H), 1.38 (dq, *J* = 4.7, 12.8 Hz, 1 H), 1.18 (q, *J* = 11.4 Hz, 1 H); ^{13}C NMR (D_2O): δ 85.0 (d), 79.3 (d), 71.0 (d), 69.5 (d), 62.1 (t), 52.3 (t), 38.8 (t), 35.3 (t); MS: m/z (relative intensity) 173 (M^+, 16), 156 (12), 113 (100), 69 (78). Anal. Calcd for $C_8H_{15}NO_3$: C, 55.47; H, 8.73; N, 8.09. Found C, 55.45; H, 8.72; N, 7.78.

(1S,2S,7S,8aS)-1,2,7-Trihydroxyoctahydroindolizine (5). Obtained from silyl ether **18** (74 mg, 0.18 mmol). Flash column chromatography, eluent CH_2Cl_2-CH_3OH-30% aq. NH_3 35:14:1, afforded pure **5** (R_f 0.24, 28 mg, 0.16 mmol, 89%) as a foam, which was crystallized from THF to give a white solid, mp 172-173 °C. $[\alpha]_D^{24}$ = + 6.5 (*c* 0.35, MeOH). ^{1}H NMR (D_2O): δ 4.12 (quint, *J* = 2.9 Hz, 1 H), 4.02 (ddd, *J* = 7.5, 3.8, 2.0 Hz, 1 H), 3.58 (dd, *J* = 8.8, 3.8 Hz, 1 H), 2.78 (dd, *J* = 11.0, 2.0 Hz, 1 H), 2.73 (dt, *J* = 11.4, 3.2 Hz, 1 H), 2.62 (dd, *J* = 11.0, 7.5 Hz, 1 H), 2.38-2.21 (m, 2 H), 1.94 (br d, *J* = 14.3 Hz, 1 H), 1.70-1.62 (m, 2 H), 1.47 (ddd, *J* = 16.8, 12.1, 2.9 Hz, 1 H); ^{13}C NMR (D_2O): δ 85.5 (d), 78.3 (d), 66.4 (d), 65.0 (d), 62.8 (t), 49.6 (t), 36.8 (t), 33.0 (t); MS: m/z (relative intensity) 173 (M^+, 13), 156 (8), 113 (100), 69 (57). Anal. Calcd for $C_8H_{15}NO_3$: C, 55.47; H, 8.73; N, 8.09. Found C, 55.75; H, 8.79; N, 8.06.

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