Synthesis and Glycosidase Inhibitory Activity of 7-Deoxycasuarine

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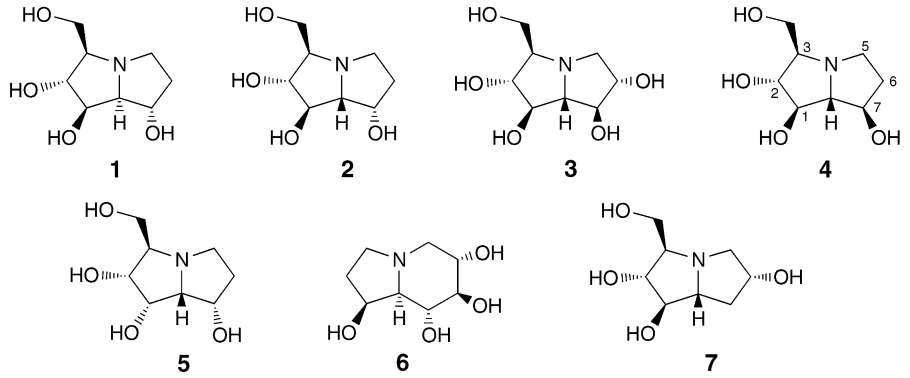
Reaction of 1,4-anhydro-2,3,5-tri-*O*-benzyl-1-deoxy-1-imino-D-arabinitol *N*-oxide (**8**) with allyl alcohol produced a 3.6 : 1 mixture of the two pyrrolo[1,2-*b*]isoxazole derivatives **13** and **14**. The major adduct **13** was converted to 7-deoxycasuarine (**7**), a potent, specific, and competitive inhibitor of amyloglucosidase from *Rhizopus mold* (see *Table*).

Introduction. – Polyhydroxylated pyrrolizidines constitute an important class of glycoprotein-processing glycosidases and consequently display a range of important biological activities and have potential as chemotherapeutic agents [1]. Alexine (**1**), australine (**2**), and casuarine (**3**) are natural pyrrolizidine alkaloids that have as common structural feature a hydroxymethyl group at C(3), that differentiates them from the more-common necines that have substituents at C(1). Pyrrolizidines **1** and **2** were first isolated at about the same time from *Alexa liopetala* [2] and *Castanospermum australe* [3], respectively. Casuarine (**3**), the most-recently isolated member of this class [4], has the most-oxygenated framework bearing a hydroxy group at C(6). Casuarine and its derivatives have generated interest in the study of possible approaches for the treatment of cancer and AIDS [5].



The importance of pyrrolizidine alkaloids as potential drugs and their interesting bicyclic structures have provoked much effort towards their chemical syntheses. Preparation of these natural products and other non-natural structural analogues useful for structure–activity-relationship (SAR) studies have been reported [6].

Enantiomerically pure five-membered cyclic nitrones have a well known importance in organic synthesis [7]. Such nitrones have shown remarkable reactivity as 1,3-dipoles in cycloadditions toward alkenes [8], and this type of reaction has been used in the synthesis of pyrrolizidines[1)][2)][3)][4)][5)]. Recently, *Denmark* and co-workers have reported [10] the synthesis of australine (**2**), 7-epiaustraline (**4**), 1-epiaustraline (**5**), and casuarine (**3**) based on the preparation of a nitrosoacetal created in the key step by asymmetric tandem [4 + 2]/[3 + 2] cycloaddition between a silaketal nitroalkene and a chiral vinyl ether. During the preparation of our manuscript, *Goti* and co-workers [11] published the synthesis of 7-deoxycasuarine (**7**) based on a 1,3-dipolar cycloaddition of nitrone **8** and maleic acid and acrylic derivatives.

In this report, we present our own efforts toward the synthesis of 7-deoxycasuarine (**7**), also using 1,3-dipolar cycloaddition of nitrone **8** but with allylic alcohol. We have also studied the inhibitory activity of **7** toward 25 glycosidases and have found that this pyrrolizidine is a potent, competitive, and specific inhibitor of amyloglucosidase from *Rhizopus* mold ($IC_{50} = 4.2$ μM). It is a much more selective inhibitor than casuarine (**3**) and analogs **1**, **2**, **4**, and **5**. Whereas the latter pyrrolizidine derivatives inhibit also α-glucosidase from rice and amyloglucosidase from *Aspergillus niger* moderately, **7** does not inhibit these enzymes.

Synthesis. – Following the methodology of *Holzapfel et al.* [8b], the reaction of 2,3,5-tri-*O*-benzyl-D-arabinofuranose **9** with hydroxylamine hydrochloride afforded oximes **10** [12] in 91% yield (*Scheme 1*). Selective silylation with (*tert*-butyl)chlorodiphenylsilane in pyridine (92%), followed by iodination [13] with inversion of the configuration at C(4) led to the formation of a mixture of (*E*)- and (*Z*)-iodo derivatives **12** in 66% yield, that were separated by chromatography. Desilylation of the major compound **12a** (*E*) with anhydrous tetrabutylammonium fluoride in boiling toluene and subsequent intramolecular nucleophilic displacement afforded crystalline nitrone **8** in 92% yield.

Heating a mixture of nitrone **8** and allyl alcohol in toluene under reflux led to the formation of cycloadducts **13** and **14** (3.6 : 1) in 93% combined yield (*Scheme 2*). The structures of **13** and **14** were assigned based on ^{1}H-NMR NOE experiments. In the case of the major isomer **13**, the proximities of pairs of protons H_β–C(3)/H–C(3a), H_α–C(3)/H–C(2), and H_α–C(3)/H–C(4) were demonstrated (*Fig.*). For compound **14**, the proximities of pairs of protons H_α–C(3)/H–C(4), H_β–C(3)/H–C(3a), and H_β–C(3)/H–C(2) were observed. The preferred formation of **13** can be interpreted in terms of steric factors. The (benzyloxy)methyl group in **8** makes the nitrone face *anti* to it less sterically hindered than its *syn* face for the cycloaddition. Mesylation of the major alcohol **13** with methanesulfonyl chloride in pyridine/CH_2Cl_2 afforded **15** in 94% yield. Reductive cleavage of the N–O bond was achieved with [$Mo(CO)_6$] in aqueous

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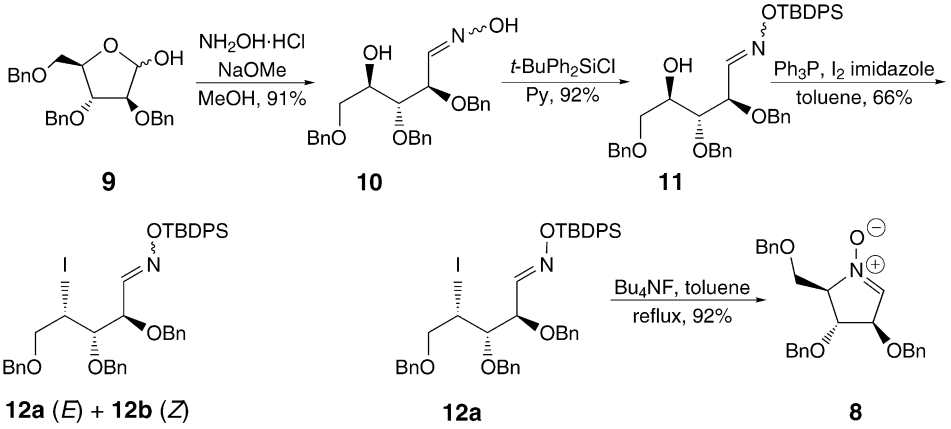
1) For the synthesis of trihydroxypyrrolizidines, see [9a].
2) For the synthesis of pyrrolizidines related to alexine, see [9b].
3) For the synthesis of (−)-rosmarinecine, see [9c].
4) For the synthesis of (−)-hastanecine, croalbinecine, and 7-epicroalbinecine, see [9d].
5) For the synthesis of aspargamine A, see [9e].

Scheme 1



TBDPS = *t*-BuPh$_2$Si

Scheme 2

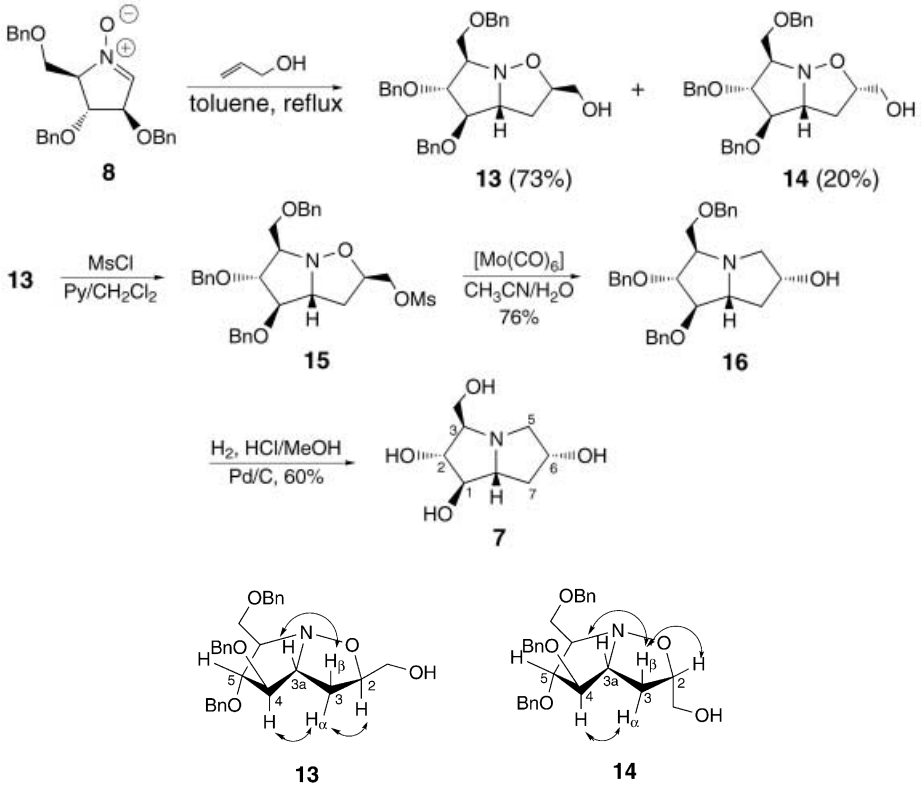


Figure. *NOEs in the* ^{1}*H-NMR spectra of* **13** *and* **14**

MeCN, yielding the pyrrolizidine derivative **16**. Hydrogenolysis of **16** gave the target hydroxylated pyrrolizidine **7** in 60% yield.

Glycosidase Inhibition Assays. – Pyrrolizidine **7** was tested [8c][14] toward 25 commercially available glycosidases and shown to be a potent and highly selective and competitive inhibitor of amyloglucosidase from *Rhizopus* mold, with $K_i = 6$ μM, and $IC_{50} = 4.2$ μM (see *Table*). At 1 mM concentration, no inhibition was observed for two α-L-fucosidases (from bovine epididymis and human placenta), three α-galactosidases (from coffee beans, *Aspergillus niger*, and *E. coli*), five β-galactosidases (from *E. coli*, bovine liver, *Aspergillus niger*, *Aspergillus orizae*, and jack beans), two α-glucosidases (from yeast and rice), one isomaltase (from baker yeast), amyloglucosidase (from *Aspergillus niger*), two β-glucosidases (from almonds and *Caldocellum saccharolyticum*), two α-mannosidases (from jack beans and almonds), one β-mannosidase (from *Helix pomatia*), one β-xylosidase (from *Aspergillus niger*), one α-*N*-acetylgalactosaminidase (from chicken liver), and three β-*N*-acetylglucosaminidases (from jack beans and bovine epididymis A and B).

Table. *Inhibitory Activity* (IC_{50}, μM) *for Compounds* **1**–**5** *and* **7** *toward* α*-Glucosidases and Amyloglucosidases*[a])

	α-Glucosidases		Amyloglucosidases		Ref.
	rice	yeast	*Aspergillus niger*	*Rhizopus* mold	
1	250	n.i.	n.i.	n.d.	[15]
2	21	n.i.	28	n.d.	[15]
3	1.2	n.i.	0.7	n.d.	[15]
4	350	n.i.	92	n.d.	[15]
5	280	n.i.	300	n.d.	[15]
7	n.i.	44[b])	n.i.	4.2	this work

[a]) n.i. = no inhibition at 1 mM concentration; n.d. = not determined. [b]) % Inhibition at 1 mM concentration.

The glycosidase inhibitory activities of related pyrrolizidine alkaloids **1**–**5** have been reported recently [15]. The results toward α-glucosidases and amyloglucosidases are summarized in the *Table*, together with our results for 7-deoxycasuarine (**7**). Alexine (**1**), australine (**2**), 7-epiaustraline (**4**), and 1-epiaustraline (**5**) are reported to be weak inhibitors of α-glucosidases from rice, while casuarine (**3**) is a good inhibitor of this enzyme. Our results for 7-deoxycasuarine (**7**) show no inhibition toward α-glucosidases from rice and very weak inhibition toward α-glucosidases from yeast, indicating that the absence of a hydroxy group at C(7) nearly abolishes the inhibition toward α-glucosidases. Australine (**2**), 7-epiaustraline (**4**), and 1-epiaustraline (**5**) have also proved to be moderate-to-good inhibitors of amyloglucosidases [16].

Conclusions. – The most interesting result is that 7-deoxycasuarine (**7**) is a potent, specific, and competitive inhibitor of amyloglucosidase from *Rhizopus* mold ($IC_{50} = 4.2$ μM, $K_i = 6$ μM). This behavior contrasts with that of casuarine (**3**), which is also a potent inhibitor of amyloglucosidase from *Aspergillus niger* ($IC_{50} = 0.7$ μM) but not specific, as it presents also strong inhibition toward α-glucosidases and β-glucosidases [15].

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Experimental Part

General. Anh. solvents and reagents were freshly distilled under N_2 prior to use. TLC: silica gel HF_{254} (*Merck*); detection by UV light and charring with H_2SO_4 or *Pancaldi* reagent ($(NH_4)_6MoO_4$, $Ce(SO_4)_2$, H_2SO_4, H_2O). Column chromatography (CC): silica gel *60* (*Merck*, 230 mesh). M.p.: *Gallenkamp MFB-595* apparatus; uncorrected. Optical rotations: 1.0-cm tube; *Perkin-Elmer 241-MC* and *Bendix NPL-143D* spectropolarimeters. IR Spectra: *Bomem MB-120* FT-IR instrument; in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *Bruker WH-400*, *Bruker AMX-300*, and *Bruker AMX-500* spectrometers; $CDCl_3$ and CD_3OD solns., J values in Hz, δ in ppm: confirmation of all assignments by two-dimensional NMR experiments. MS: *KRATOS MS-80-RFA* instrument for FAB and CI; *Micromass AutoSpeQ* and *V.G.-ZABE* instruments for HR-FAB and HR-CI; in *m/z* (rel. %).

Enzymatic Inhibition Assays. Appropriate 4-nitrophenyl glycoside substrates buffered to optimum pH of the enzymes were used; for details, see [8c][14]. The inhibition constants (K_i) and the type of inhibition (competitive, noncompetitive, mixed) were determined from *Lineweaver–Burk* plots. For each plot, a blank and two concentrations of inhibitor were used corresponding to IC_{50} and $IC_{50}/2$.

*(1*E*)- and (1*Z*)-2,3,5-Tri*-O-*benzyl*-D-*arabinose* O-*[(*tert-*Butyl)diphenylsilyl]oximes* (**11**). (*tert*-Butyl)chlorodiphenylsilane (2.2 ml, 8.22 mmol) was added to a stirred soln. of oxime **10** [12] (3.58 g, 8.22 mmol) in dry pyridine (20 ml). After stirring overnight at 25°, the solvent was evaporated, the residue dissolved in CH_2Cl_2, and the soln. sequentially washed with 1% HCl soln., sat. aq. $NaHCO_3$ soln., and brine, dried ($MgSO_4$), and evaporated. The residue was purified by CC (silica gel, Et_2O/petroleum ether 1:6): **11** (5.1 g, 92%). Oil. HR-CI-MS: 674.3318 ($[C_{42}H_{47}NO_5Si+H]^+$; calc. 674.3302).

*(1*E*)- and (1*Z*)-2,3,5-Tri*-O-*benzyl*-4-*deoxy*-4-*iodo*-L-*xylose* O-*[(*tert-*Butyl)diphenylsilyl]oximes* (**12a** and **12b**, resp.). A mixture of **11** (2.23 g, 3.31 mmol), Ph_3P (2.60 g, 9.93 mmol), 1*H*-imidazole (0.68 g, 9.93 mmol), and I_2 (1.68 g, 6.62 mmol) in toluene (150 ml) was stirred under reflux for 2 h. The mixture was cooled, an equal volume of sat. aq. $NaHCO_3$ soln. was added, and the mixture was stirred for 5 min. I_2 was added in portions until the toluene phase remained violet. It was then stirred for an additional 10 min, and the excess I_2 was destroyed by the addition of aq. $Na_2S_2O_3$ soln. The mixture was diluted with toluene, and the org. phase was washed with H_2O, dried ($MgSO_4$), and evaporated. PPh_3O was then precipitated in Et_2O, the mixture filtered, and the filtrate evaporated. The residue was purified by CC (silica gel, Et_2O/petroleum ether 1:15): **12a** (1.4 g, 54%) and **12b** (0.325 g, 12%), both as oils.

Data for **12a**: $[\alpha]_D^{25} = -27.6$ ($c = 1.05$, $CHCl_3$). IR (film): 3065, 2930, 2860, 1595 (C=N), 1110 (C–O), 740, 700, 615 (C–I). ^1H-NMR (500 MHz, $CDCl_3$): 7.77–7.73 (*m*, 4 H, Ph); 7.61 (*d*, *J*(1,2) = 8.0, H–C(1)); 7.41–7.18 (*m*, 21 H, Ph); 4.91, 4.71 (2*d*, ^2J = 11.6, 1 H each, PhCH_2); 4.50, 4.33 (2*d*, ^2J = 11.6, 1 H each, PhCH_2); 4.34 (*s*, PhCH_2); 4.29 (*dd*, *J*(2,3) = 7.2, H–C(2)); 4.16 (*ddd*, *J*(4,5a) = 8.7, *J*(4,3) = 3.0, *J*(4,5b) = 5.3, H–C(4)); 3.75 (*dd*, ^2J(5a,5b) = 10.0, H_a–C(5)); 3.62 (*dd*, H_b–C(5)); 3.55 (*dd*, H–C(3)); 1.16 (*s*, *t*-Bu). ^{13}C-NMR (125.7 MHz, $CDCl_3$): 147.1 (C(1)); 132.6, 131.9, 131.8, 127.6, 127.4 (5 C(1) of Ph); 129.8, 124.0, 122.6–121.8 (Ph); 75.2 (C(2)); 71.7 (C(3)); 69.0 (PhCH_2); 67.0 (PhCH_2); 66.8 (C(5)); 65.6 (PhCH_2); 25.5 (C(4)); 21.4 (Me_3C); 13.5 (Me_3C). HR-CI-MS: 784.2316 ($[C_{42}H_{46}INO_4+H]^+$; calc. 784.2319).

Data for **12b**: $[\alpha]_D^{25} = -25.4$ ($c = 1.5$, CH_2Cl_2). IR (film): 3060, 2935, 2860, 1595 (C=N), 1110 (C–O), 740, 700, 615 (C–I). ^1H-NMR (500 MHz, $CDCl_3$): 7.74–7.71 (*m*, 4 H, Ph); 7.44–7.26 (*m*, 21 H, Ph); 7.06 (*d*, *J*(1,2) = 6.6, H–C(1)); 5.37 (*dd*, *J*(2,3) = 5.2, H–C(2)); 4.74, 4.70 (2*d*, ^2J = 11.2, 1 H each, PhCH_2); 4.61, 4.47 (2*d*, ^2J = 11.5, 1 H each, PhCH_2); 4.43, 4.36 (2*d*, ^2J = 12.0, 1 H each, PhCH_2); 4.39 (*ddd*, *J*(4,3) = 5.0, *J*(4,5a) = 6.6, *J*(4,5b) = 5.9, H–C(4)); 3.86 (*t*, H–C(3)); 3.76 (*dd*, ^2J(5a,5b) = 10.6, H_a–C(5)); 3.63 (*dd*, H_b–C(5)); 1.14 (*s*, *t*-Bu). ^{13}C-NMR (75.4 MHz, $CDCl_3$): 154.8 (C(1)); 137.7, 137.6, 137.1, 135.4, 132.8 (5 C(1) of Ph); 135.4, 129.7, 128.3–127.5 (Ph); 79.0 (C(4)); 74.6 (PhCH_2); 73.5 (C(2)); 72.7, 72.4, 72.2 (2 PhCH_2, C(5)); 31.0 (C(3)); 27.0 (Me_3C); 19.2 (Me_3C). FAB-MS: 806 (100, $[M+Na]^+$). CI-MS: 784 (20, $[M+H]^+$). HR-CI-MS: 784.2316 ($[C_{42}H_{46}INO_4+H]^+$; calc. 784.2319).

1,4-Anhydro-2,3,5-tri-O-*benzyl*-1-*deoxy*-1-*imino*-D-*arabinitol* N-*Oxide* (=*(2*R*,3*R*,4*R*)-3,4-Dihydro-3,4-bis(phenylmethoxy)-2-[(phenylmethoxy)methyl]-2*H*-pyrrole 1-Oxide*; **8**). A mixture of **12a** (0.66 g, 0.84 mmol)

and anh. Bu_4NF (0.31 g, 1.19 mmol) in toluene (45 ml) was heated under reflux for 30 min. After evaporation, the residue was purified by CC (silica gel, Et_2O/MeOH 100:1 → 70:1): **8** (0.32 g, 92%). White solid. M.p. 88–90°. $[\alpha]_D^{25} = -41$ ($c = 1$, $CHCl_3$). IR (KBr): 3055, 2875, 1590 (C=N), 1455, 1095 (C−O), 860, 745, 700. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.38–7.25 (*m*, 15 H, Ph); 6.90 (*t*, $J(1,2) = {}^5J(1,4) = 2.2$, H−C(1)); 4.65 (*td*, $J(2,3) = 2.2$, $^4J(2,4) = 0.7$, H−C(2)); 4.60, 4.37 (2*d*, $^2J = 12.0$, 1 H each, PhCH_2); 4.54 (*s*, PhCH_2); 4.53 (*d*, PhCH_2); 4.37 (*ddd*, $J(3,4) = 3.6$, $^4J(3,5b) = 0.4$, H−C(3)); 4.04 (*dd*, $J(5a,4) = 5.1$, $^2J(5a,5b) = 9.9$, H_a−C(5)); 4.00 (*m*, H−C(4)); 3.76 (*ddd*, $J(5b,4) = 2.9$, H_b−C(5)). ^{13}C-NMR (125.7 MHz, $CDCl_3$): 137.6, 137.2, 137.1 (3 C(1) of Ph); 132.7 (C(1)); 128.6–127.7 (Ph); 82.7 (C(2)); 80.3 (C(3)); 77.5 (C(4)); 73.5, 71.9, 71.6 (3 Ph*C*H$_2$); 66.1 (C(5)). HR-CI-MS: 418.2024 ($[C_{23}H_{26}NO_4 + H]^+$; calc. 418.2018). Anal. calc. for $C_{26}H_{27}NO_4$: C 74.80, H 6.52, N 3.36; found: C 74.37, H 6.39, N 3.46.

*(2*R*,3a*R*,4*R*,5*R*,6*R*)- and (2*S*,3a*R*,4*R*,5*R*,6*R*)-Hexahydro-4,5-bis(phenylmethoxy)-6-[(phenylmethoxy)-methyl]pyrrolo[1,2-*b*]isoxazole-2-methanol* (**13** and **14**, resp.). A soln. of nitrone **8** (250 mg, 0.600 mmol) and allyl alcohol (122 µl, 1.8 mmol) in toluene (10 ml) was heated under reflux for 3 h. After evaporation, the residue was purified by CC (silica gel, petroleum ether/AcOEt 1:1): **13** (209 mg, 73%) and **14** (58 mg, 20%), both as oils.

Data for **13**: $[\alpha]_D^{25} = -45$ ($c = 1$, $CHCl_3$). IR (film): 3030, 2925, 2865, 1625, 1105 (C−O), 740, 695. ^{1}H-NMR (400 MHz, $CDCl_3$)[6]: 7.33–7.15 (*m*, 15 H, Ph); 4.60, 4.37 (2*d*, $^2J = 12.01$, 1 H each, PhCH_2); 4.54 (*s*, PhCH_2); 4.53 (*d*, PhCH_2); 4.30 (*m*, H−C(2)); 4.04 (*dd*, $J(5,4) = 4.1$, $J(5,6) = 6.2$, H−C(5)); 3.96 (*t*, $J(4,3a) = 4.0$, H−C(4)); 3.76 (*dd*, $J(2'a,2) = 8.8$, $^2J(2'a,2'b) = 12.2$, H_a−C(2′)); 3.75 (*ddd*, $J(3a,3\beta) = 6.9$, $J(3a,3\alpha) = 7.7$, H−C(3a)); 3.66 (*dd*, $J(6'a,6) = 4.8$, $^2J(6'a,6'b) = 9.9$, H_a−C(6′)); 3.60 (*dd*, $J(6',b,6) = 5.8$, H_b−C(6′)); 3.56 (*dd*, $J(2'b,2) = 4.4$, H_b−C(2′)); 3.33 (*ddd*, H−C(6)); 2.33 (*ddd*, $^3J(3\beta,2) = 9.0$, $^2J(3\beta,3\alpha) = 12.4$, H_β−C(3)), 2.17 (*ddd*, $J(3\alpha,2) = 5.4$, H_α−C(3)); 2.12 (br. *s*, OH). ^{13}C-NMR (125.7 MHz, $CDCl_3$)[6]: 138.2, 137.9, 137.2 (3 C(1) of Ph); 128.5–127.6 (Ph); 87.2 (C(4)); 83.9 (C(5)); 77.2 (C(2)); 73.4, 72.3, 71.8 (3 Ph*C*H$_2$); 69.7 (C(6), C(6′)); 68.6 (C(3a)); 63.2 (C(2′)); 35.4 (C(3)). CI-MS: 476 (30, $[M + H]^+$). HR-CI-MS: 476.2434 ($[C_{29}NO_5 + H]^+$; calc. 476.2437).

Data for **14**: $[\alpha]_D^{25} = -29$ ($c = 1.34$, CH_2Cl_2). IR (film): 3020, 2915, 2870, 1625, 1105 (C−O), 745, 700. ^{1}H-NMR (500 MHz, $CDCl_3$)[6]: 7.36–7.27 (*m*, 15 H, Ph); 4.57, 4.54 (2*d*, $^2J = 12.0$, 1 H each, PhCH_2); 4.56 (*s*, PhCH_2); 4.49 (*s*, PhCH_2); 4.18 (*m*, H−C(2)); 4.14–4.11 (*m*, H−C(4), H_a−C(5)); 3.80 (*dd*, $J(2'a,2) = 2.4$, $^2J(2'a,2'b) = 12.2$, H_a−C(2′)); 3.76–3.71 (*m*, H−C(6), H−C(3a)); 3.63 (*dd*, $J(6'a,6) = 5.7$, $^2J(6'a,6'b) = 9.7$, H_a−C(6′)); 3.52 (*dd*, $J(6'b,6) = 6.5$, H_b−C(6′)); 3.52 (*m*, H_b−C(2′)); 2.49 (br. *s*, OH); 2.48 (*dt*, $J(3\beta,3a) = J(3\beta,2) = 8.0$, $^2J(3\beta,3\alpha) = 12.3$, H_β−C(3)); 2.29 (*ddd*, $J(3\alpha,3a) = 5.8$, $J(3\alpha,2) = 8.2$, H_α−C(3)). ^{13}C-NMR (75.4 MHz, $CDCl_3$)[6]: 137.9, 137.6, 137.5 (3 C(1) of Ph); 128.3–127.4 (Ph); 89.4, 86.0 (C(4), C(5)); 79.0 (C(2)); 73.2, 72.1, 71.9 (3 Ph*C*H$_2$); 70.6, 70.1 (C(3a), C(6)); 69.3 (C(6′)); 61.9 (C(2′)); 35.8 (C(3)). CI-MS: 476 (100, $[M + H]^+$). HR-CI-MS: 476.2436 ($[C_{29}H_{33}NO_5 + H]^+$; calc. 476.2437).

*(2*R*,3a*R*,4*R*,5*R*,6*R*)-Hexahydro-4,5-bis(phenylmethoxy)-6-[(phenylmethoxy)methyl]pyrrolo[1,2-*b*]isoxazole-2-methanol Methanesulfonate* (**15**). To a stirred soln. of **13** (150 mg, 0.316 mmol) in CH_2Cl_2 (4 ml) and pyridine (1.4 ml) cooled to 0°, methanesulfonyl chloride (75 µl, 0.63 mmol) was added dropwise. The mixture was allowed to warm to 25° and after 3 h, H_2O (1–2 ml) was added. The solvent was evaporated and the crude product partitioned between CH_2Cl_2 and H_2O. The org. phase was washed with brine, dried ($MgSO_4$), and evaporated and the residue purified by CC (silica gel, Et_2O/petroleum ether 4:1): **15** (165 mg, 94%). White solid. M.p. 90–92°. $[\alpha]_D^{25} = -42$ ($c = 1$, CH_2Cl_2). IR (KBr): 3025, 2890, 1600, 1350 (SO_2−OR), 1110 (C−O), 965, 735, 690. ^{1}H-NMR (500 MHz, $CDCl_3$)[6]: 7.35–7.26 (*m*, 15 H, Ph); 4.59, 4.58, 4.55 (4*d*, $^2J = 12.0$, 1 H each, PhCH_2); 4.52 (*s*, PhCH_2); 4.45 (*m*, H−C(2)); 4.28 (*dd*, $J(2'a,2) = 3.5$, $^2J(2'a,2'b) = 11.3$, H_a−C(2′)); 4.24 (*dd*, $J(2'b,2) = 5.6$, H_b−C(2′)); 4.04 (*dd*, $J(5,6) = 5.6$, $J(5,4) = 3.8$, H−C(5)); 3.97 (*t*, $J(4,3a) = 3.8$, H−C(4)); 3.76 (*ddd*, $J(3a,3\alpha) = 5.4$, $J(3a,3\beta) = 8.8$, H−C(3a)); 3.67 (*dd*, $J(6'a,6) = 5.0$, $^2J(6'a,6'b) = 9.9$, H_a−C(6′)); 3.58 (*dd*, $J(6'b,6) = 6.2$, H_b−C(6′)); 3.36 (*ddd*, H−C(6)); 2.29 (*ddd*, $J(3\alpha,2) = 7.8$, $^2J(3\alpha,3\beta) = 12.8$, H_α−C(3)); 2.22 (*ddd*, $J(3\beta,2) = 6.8$, H_β−C(3)). ^{13}C-NMR (125.7 MHz, $CDCl_3$)[6]: 132.4, 132.0, 131.8 (3 C(1) of Ph); 122.7–121.8 (Ph); 81.3 (C(4)); 78.4 (C(5)); 68.6 (C(2)); 67.6, 66.5, 66.1 (3 Ph*C*H$_2$); 64.4 (C(6)); 64.2 (C(6′)); 63.6 (C(2′)); 62.5 (C(3a)); 31.9 ($MeSO_3$); 30.0 (C(3)). FAB-MS: 554 (25, $[M + H]^+$), 576 (100, $[M + Na]^+$). CI-MS: 553 (4, $M^{+\cdot}$). HR-CI-MS: 553.2133 ($C_{30}H_{35}NO_7S$; calc. 553.2134). Anal. calc. for $C_{30}H_{35}NO_7S$: C 65.08, H 6.42, N 2.53; found: C 64.84, H 6.42, N 2.59.

*(1*R*,2*R*,3*R*,6*R*,7a*R*)-Hexahydro-1,2-bis(phenylmethoxy)-3-[(phenylmethoxy)methyl]-1*H*-pyrrolizine-6-ol* (**16**). A mixture of **15** (100 mg, 0.18 mmol) and $[Mo(CO)_6]$ (75 mg, 0.27 mmol) in MeCN/H_2O 15:1 (3 ml) was heated at reflux under N_2 for 8 h. Silica gel (1 g) was then added, and the mixture was stirred at 25° for 16 h. The

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[6] For convenience, the exocyclic C-atoms bound to C(2) or C(6) are labelled C(2′) or C(6′), respectively.

mixture was diluted with AcOEt and filtered through *Celite*. After evaporation of the filtrate, the residue was purified by CC (CH_2Cl_2/MeOH 80:1 → 20:1): **16** (63 mg, 76%). Oil. $[\alpha]_D^{25} = +8$ ($c = 1.2$, CH_2Cl_2). IR (film): 3465 (OH), 1595, 1110 (C–O), 740, 695. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.37–7.24 (*m*, 15 H, Ph); 4.66, 4.58 (2*d*, 1 H each, $^2J = 11.7$, CH_2Ph); 4.53 (*s*, 2 PhCH_2); 4.33 (*m*, H–C(6)); 4.12 (*t*, $J(1,2) = (1,7a) = 4.5$, H–C(1)); 4.09 (*t*, $J(2,3) = 4.6$, H–C(2)); 3.60 (*ddd*, $J(7a,7) = 9.1$, $J(7a,7') = 4.5$, H–C(7a)); 3.55 (*d*, $J(CH_2,3) = 6.5$, CH_2–C(3)); 3.47 (*dt*, H–C(3)); 3.22 (*dd*, $J(5a,6) = 4.5$, $^2J(5a,5b) = 12.2$, H_a–C(5)); 2.99 (br. *d*, H_b–C(5)); 2.86 (br. *s*, OH); 2.22 (*ddd*, $J(7,6) = 5.5$, $^2J(7,7') = 13.8$, H–C(7)); 1.84 (*ddd*, $J(7',6) = 3.3$, H′–C(7)). ^{13}C-NMR (75.4 MHz, $CDCl_3$): 138.2, 137.9, 137.6 (3 C(1) of Ph), 128.3–127.3 (Ph); 88.9 (C(1)); 85.3 (C(2)); 73.8 (C(6)); 73.1, 72.2, 71.8 (3 PhCH_2); 71.7 (CH_2–C(3)), 70.0 (C(3)); 67.6 (C(7a)); 63.4 (C(5)); 40.1 (C(7)). FAB-MS: 460 (100, $[M+H]^+$), 482 (50, $[M+Na]^+$). HR-FAB-MS: 482.2334 ($[C_{29}H_{33}NO_4+Na]^+$; calc. 482.2307), 460.2492 ($[C_{29}H_{34}NO_4+H]^+$; calc. 460.2489).

*(1*R*,2*R*,3*R*,6*R*,7a*R*)-Hexahydro-3-(hydroxymethyl-1*H*-pyrrolizine-1,2,6-triol* (= *7-Deoxycasuarine*; **7**). A soln. of **16** (23 mg, 0.052 mmol) in 1% HCl/MeOH (2.5 ml) was hydrogenated at 1 atom over (10% Pd/C 12 mg) for 16 h. The mixture was diluted with MeOH, filtered through *Celite*, and evaporated. The residue was then redissolved in H_2O (2 ml) and stirred with *Dowex 1 × 8-OH* resin (40 mg) for 30 min. Filtration and evaporation gave **7** (5.8 mg, 60%). White solid. $[\alpha]_D^{25} = +23$ ($c = 0.3$, MeOH). ^{1}H-NMR (300 MHz, CD_3OD): 4.35 (*m*, H–C(6)); 4.01 (*t*, $J(1,2) = J(1,7a) = 7.8$, H–C(1)); 3.72 (*dd*, $J(CH_2,3) = 3.3$, $^2J = 11.1$, 1 H, CH_2–C(3)); 3.70 (*dd*, $J(2,3) = 9.3$, H–C(2)); 3.53 (*dd*, $J(CH_2,3) = 6.2$, 1 H, CH_2–C(3)); 3.22 (*td*, $J(7a,7') = 4.4$, $J(7a,7) = 8.4$, H–C(7a)); 3.04 (*ddd*, H–C(3)); 3.03 (*dd*, $J(5a,6) = 4.3$, $^2J(5a,5b) = 11.7$, H_a–C(5)); 2.88 (*ddd*, $J(5b,6) = 3.4$, $^4J(5b',7') = 1.4$, H_b–C(5)); 2.09 (*ddd*, $J(7,6) = 5.1$, $^2J(7,7') = 13.4$, H–C(7)); 1.89 (*dddd*, $J(7',6) = 3.9$, H′–C(7)). ^{13}C-NMR (125.7 MHz, CD_3OD): 82.9 (C(1)); 79.0 (C(2)); 74.5 (C(6)); 72.8 (C(3)); 68.1 (C(7a)); 64.4 (C(8)); 63.3 (C(5)); 39.4 (C(7)): CI-MS: 190 (100, $[M+H]^+$). HR-CI-MS: 190.1078 ($[C_8H_{15}NO_4+H]^+$; calc. 190.1079).

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