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Isolation synthesis and glycosidase inhibition profile of 3-*epi*-casuarine

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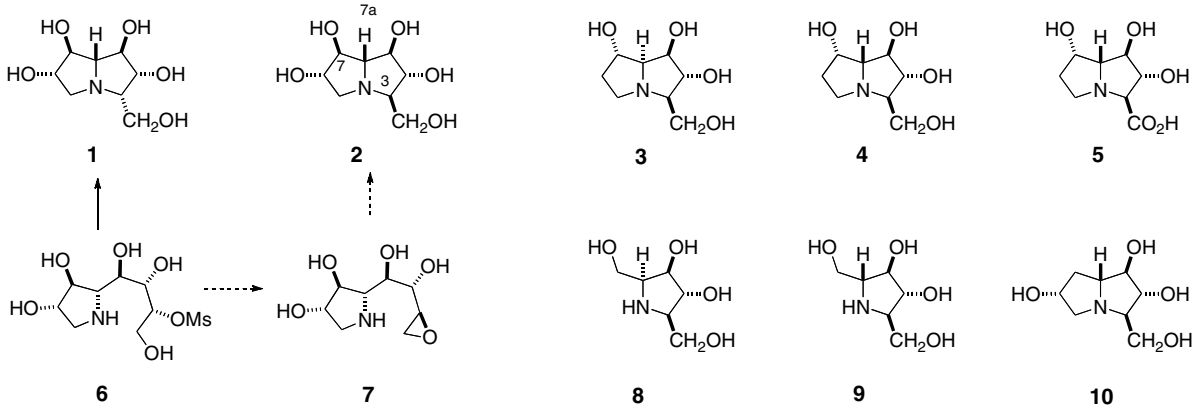
Abstract—3-*epi*-Casuarine, the first naturally occurring stereoisomer of casuarine, was isolated from *Myrtus communis* L. The glycosidase inhibition profile and NMR spectra of 3-*epi*-casuarine are compared with those of casuarine; the change in configuration at one of the six stereogenic centres causes a dramatic change in the conformation of the bicyclic system. The key step in the 6% overall yield synthesis of 3-*epi*-casuarine from D-gluconolactone is the efficient cyclization of a completely unprotected pentahydroxyaminomesylate to the pyrrolizidine nucleus. A low yield synthesis of casuarine is also reported.

1. Introduction

Herein, we report the isolation of 3-*epi*-casuarine **1** from the shrub *Myrtus communis* L. (Myrtle), and comparison of its glycosidase inhibition profiles and its NMR spectra with those of casuarine **2**. The synthesis of **1** from D-gluconolactone **11** depends on the clean cyclization of the completely unprotected aminomesylate **6**; in spite of possible competitive formation of epoxides, the ring closure to the pyrrolizidine ring is very efficient. Less than 5% of casuarine **2** is formed, presumably via epoxide **7**. Casuarine **2**, from *Casuarina equisetifolia*, is the most heavily oxygenated of the naturally occurring pyrrolizidine alkaloids with six adjacent stereogenic centres and is a potent and specific α-glucosidase inhibitor;[1] its 6-α-glucopyranosyl derivative has been isolated from *C. equisetifolia* and *Eugenia jambolana*.[2] None of its 63 stereoisomers have been hitherto identified as a natural product.

Polyhydroxylated monocyclic and bicyclic alkaloids can be viewed as sugar mimics in which the ring oxygen is replaced by nitrogen;[3] the biological properties and uses as chemo-



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therapeutic agents have ensured an escalating interest in synthesizing both naturally occurring and synthetic compounds.[4] Alexine **3**, isolated from *Alexa leiopetala*, was the first pyrrolizidine alkaloid found with a carbon substituent at C-3.[5] The 7a epimer of alexine, australine **4**, was found in *Castanospermum australe*;[6] the corresponding amino acid, 7a-epialexaflorine **5** was isolated from the leaves of *Alexa grandiflora*.[7] Alexine **3** and australine **4** may be viewed as bicyclic analogues of the monocyclic pyrrolidine natural products DGDP **8** and DMDP **9**. Alexines have five chiral centres; seven of the 32 stereoisomers have been isolated as natural products from plants.[8] The natural products and the synthetic analogues have significant glycosidase inhibition[9] and antiviral properties.[10] Alexine **3** and some of its stereoisomers were first made from glucose;[11] the biological potential and the complexity of five adjacent stereogenic centres have led to a large number of subsequent papers on their synthesis.[12] The isomeric tetrahydroxypyrrolizidine 7-deoxycasuarine **10** has also been identified as a specific inhibitor of amyloglucosidase.[13]

In contrast to the synthetic endeavours on the alexines, there are only two syntheses of casuarine **2**[14] and very limited studies on its stereoisomers.[15] The isolation of 3-*epi*-casuarine **1** as a natural product indicates that, similar to the alexines, many stereoisomers may exist in Nature. The X-ray crystal structure of **1** firmly established the relative stereochemistry of 3-*epi*-casuarine;[16] the absolute configuration is shown by the synthesis reported herein from D-gluconolactone **11**. Casuarines possess six contiguous stereogenic centres; the challenge of devising strategies for the efficient synthesis of reasonable amounts for biological evaluation of such compounds remains.

1.1. Isolation and glycosidase inhibition of 3-*epi*-casuarine 1

3-*epi*-Casuarine **1** was isolated as a natural product from the European shrub *M. communis* L. (Myrtle) growing in the grounds of the Institute of Grassland and Environmental Research in Aberystwyth. The isolation was conducted using ion exchange chromatography and fractionation was followed by GC–MS of the pertrimethylsilyl-derivatives.[17] Casuarine **2** was the major alkaloid present in *M. communis* (eluted first with water from the anion exchange resin Dowex 1 in the hydroxyl form) and 3-*epi*-casuarine **1** was the only epimer detected in the plant (eluted after casuarine from Dowex 1). Casuarine **2** and 3-*epi*-casuarine **1** were crystallized from warm 95% aqueous ethanol by layering with acetone. The ^{1}H and ^{13}C NMR of the natural 3-*epi*-casuarine were identical to those of the synthetic material; the natural sample had a specific rotation, $[\alpha]_D^{23} = +5.7$ (*c* 0.5, H_2O) in comparison to $[\alpha]_D^{22} = +5.8$ (*c* 0.69, H_2O) for the synthetic material.

The glycosidase inhibition properties of **1** and **2** were determined by the methods previously described and using commercially available enzymes and 5 mM *para*-nitrophenylsubstrates.[18]

Casuarine **2** is a good inhibitor of α-D-glucosidases (Table 1). In contrast, the epimer **1** only significantly inhibited β-D-glucosidases with an IC_{50} just below 700 μM; by contrast castanospermine (the benchmark for β-D-glucosidase inhibition) in the same assay has an IC_{50} of 2×10^{-5} M. Glucosidase inhibition by 3-*epi*-casuarine **1** is very different than that by casuarine **2**.[19]

Table 1. Results of the glycosidase inhibition study

Enzyme	% Inhibition (3-*epi*-Casuarine **1**, 700 μM)	% Inhibition (Casuarine **2**, 700 μM)
α-D-Glucosidase (yeast)	0	64
α-D-Glucosidase (rice)	13	76
α-D-Glucosidase (*Bacillus*)	12	86
β-D-Glucosidase (almond)	56	0
α-D-Galactosidase (coffee bean)	4	4
β-D-Galactosidase (bovine liver)	6	0
α-D-Mannosidase (Jack bean)	0	5
α-L-Fucosidase (bovine kidney)	0	0
β-*N*-Acetyl-D-glucosaminidase (bovine kidney)	0	14
Naringinase (*Pencillium*)	21	0

1.2. NMR Studies on 3-*epi*-casuarine 1

The ^{1}H NMR spectra of **1** and **2** are shown in Figure 1 and the resonance assignments are given in Table 2. Despite the structural similarities of the two compounds, their NMR spectra are dramatically different. ^{1}H NMR Studies on five alexines and casuarine have shown that changes at one sterocentre can lead to substantial conformational changes of the bicyclic pyrrolizidine ring system and hence significant changes in chemical shifts.[20] Differences in ^{13}C chemical shifts are helpful in fingerprint analysis for identifying known alexines.[21]

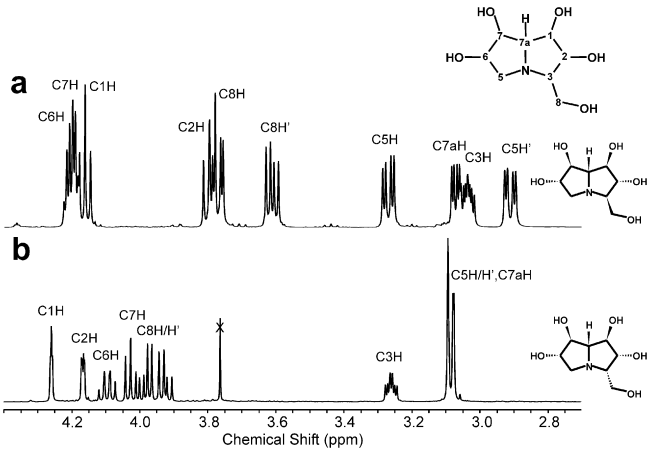


Figure 1. ^{1}H NMR spectra in $^{2}H_2O$ at 30 °C of (a) casuarine **2**, pH 8.3, and (b) 3-*epi*-casuarine **1**, pH 9.3. The ring numbering scheme is shown top right.

For bicyclic pyrrolizidines, the proton coupling constants are a characteristic of the relative orientations of the ring protons.[20] For 3-*epi*-casuarine **1**, the very small C7aH–C1H and C1H–C2H couplings are only consistent with these two proton pairs being trans but not di-axial. The relatively large C6H–C7H and C7H–C7aH couplings suggest that C6H, C7H and C7aH are all trans and all axial. The

Table 2. NMR resonance assignments in ^2H_2O at 30 °C for 3-*epi*-casuarine **1**, pH 9.3, and casuarine **2**, pH 8.3

	3-*epi*-Casuarine **1**			Casuarine **2**		
	^{1}H		^{13}C	^{1}H		^{13}C
	δ (ppm)[a]	$^3J_{HH}$ (Hz)	δ (ppm)[b]	δ (ppm)[a]	$^3J_{HH}$ (Hz)	δ (ppm)[c]
C5	3.09	Overlapping	51.6	3.270	12.2/4.7	59.1
				2.911	12.2/4.0	
C6	4.098	8.5/7.7/7.7	75.9	4.21	4.7/4.0/3.1	78.5
C7	4.029	7.7	79.2	4.19	3.1/3.5	79.9
C7a	3.087	7.7/1.6	75.5	3.071	3.5/8.0	73.2
C1	4.261	1.6/1.4	80.4	4.162	8.0/8.0	78.9
C2	4.169	1.4/3.3	79.7	3.796	8.0/8.0	77.7
C3	3.262	3.3/6.4/7.3	65.0	3.036	8.0/3.8/6.6	71.1
C8	3.984	−11.8/6.4	57.4	3.771	−11.9/3.8	63.3
	3.926	−11.8/7.3		3.611	−11.9/6.6	

[a] Referenced to trimethylsilylpropanesulfonic acid at 0.000 ppm.
[b] Reference to dioxane at 67.2 ppm.
[c] Referenced to acetone at 30.9 ppm.

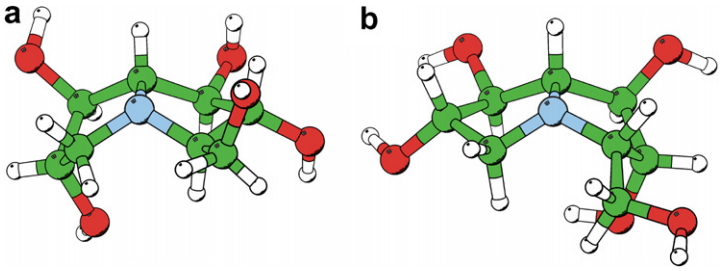


Figure 2. Conformations of (a) casuarine **2** and (b) 3-*epi*-casuarine **1** in water determined by analysis of the proton coupling constants and NOE results.

large C6H–C5H/H′ couplings are also consistent with a cis axial–equatorial and a trans di-axial arrangement for C6H with the two C5 protons, confirming C6H as axial. As C7aH is axial, C1H must be equatorial. The all trans C7aH–C1H–C2H configuration is consistent with the relatively weak C7aH–C1H and C1H–C2H NOEs. A strong C1H–C7H NOE indicates that these two protons are on the same side of the ring, consistent with a trans C7H–C7aH and a trans C7aH–C1H configurations. A strong C2H–C3H NOE indicates that these two protons are on the same side of the ring, the medium C2H–C3H coupling also being consistent with a cis arrangement for these protons. These relative configurations confirm **1** as 3-*epi*-casuarine or its enantiomer.

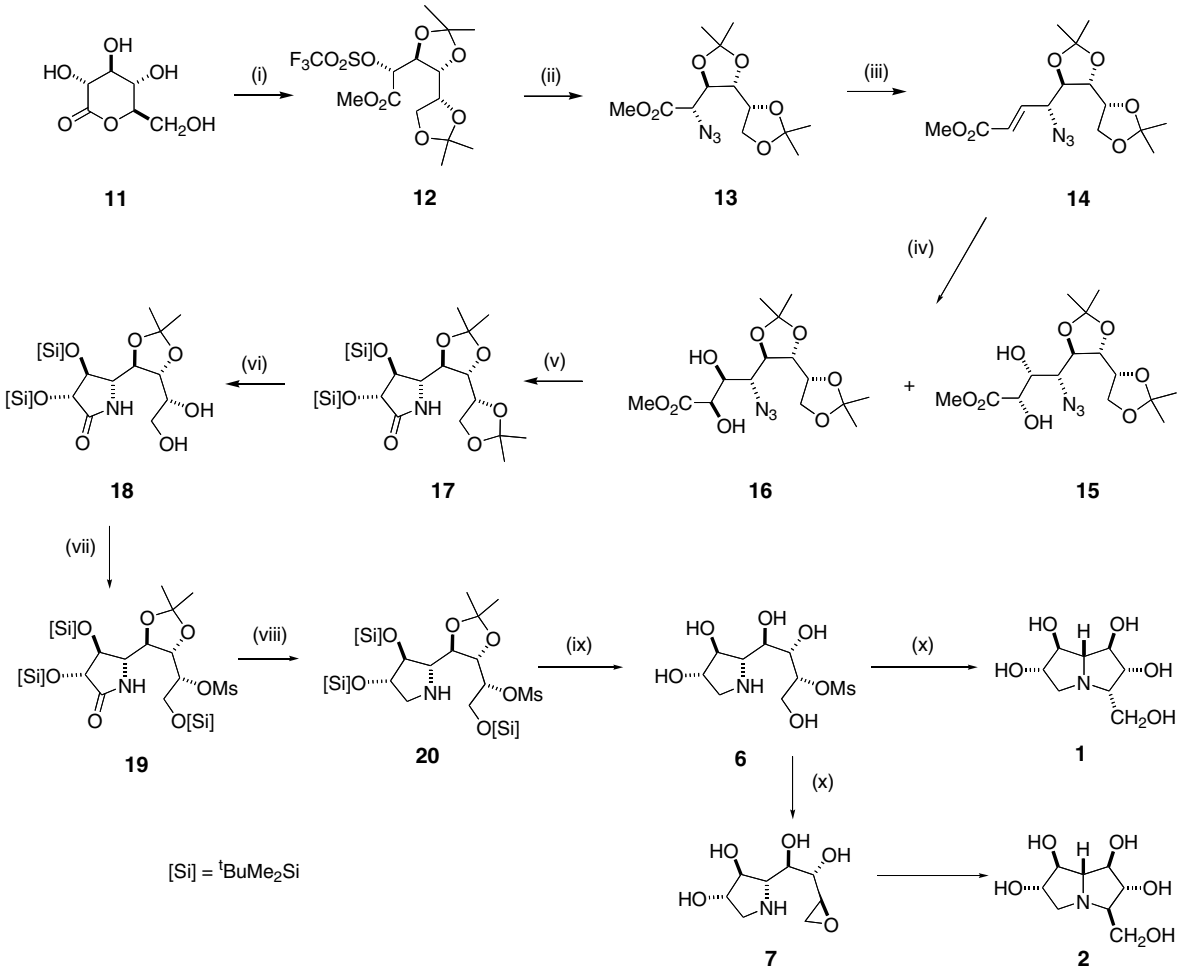
Although **1** and **2** only differ at one stereocentre, the proton coupling constants show the ring conformations to be very different. Casuarine **2** has an *exo*-/*endo*-buckled conformation (C6 *exo* relative to the nitrogen lone pair, C2 *endo*)[1] resulting in C6H and C7H being equatorial and C1H and C2H being axial; 3-*epi*-casuarine has an *endo*-/*exo*-conformation, with C6H and C7H being axial and C1H and C2H being equatorial (Fig. 2).

1.3. Syntheses of 3-*epi*-casuarine 1 and casuarine 2 from D-gluconolactone

The conversion of inexpensive and easily available D-gluconolactone **11**—a rare example of a thermodynamically stable 1,5-lactone—to 3-*epi*-casuarine **1** (Scheme 1) involved the introduction of nitrogen at C-2, a subsequent Wittig extension at C-1 to give, after osmium tetroxide hydroxylation, an 8 carbon sugar derivative with all the correct stereochemistry for the formation of the target; the overall yield was 6.0% over 15 steps (83% per step) (Scheme 1).

The reaction of lactone **11** with dimethoxypropane gave an open chain diacetonide[22] in which the C-2 hydroxyl function was subsequently esterified with trifluoromethanesulfonic (triflic) anhydride, as previously described,[23] to give the stable crystalline triflate **12** in 72% overall yield on a multigram scale. Displacement of the triflate in **12** with sodium azide in dimethyl formamide gave azide **13** (97% yield)[24] in which a nitrogen function has been introduced at C-2 of the gluconolactone.

Reduction of the azidoester **13** with diisobutylaluminium hydride (DIBAL) gave the corresponding azidoaldehyde with no significant over-reduction to the corresponding alcohol. The isolation of the free aldehyde was extremely cumbersome and led to a significant loss of material; however treatment of the crude aldehyde in situ with the stabilized Wittig reagent $Ph_3P{=}CHCOOMe$ **21** afforded the unsaturated eight carbon carbohydrate **14** in 75% yield over the two steps. An *E*:*Z* ratio of 10:1 was typically observed for this reaction, provided that the reaction mixture containing the crude aldehyde was allowed to warm to room temperature before the addition of the ylid; when the Wittig step was carried out at lower temperatures, significant amounts of the *Z*-isomer of **14** were isolated.



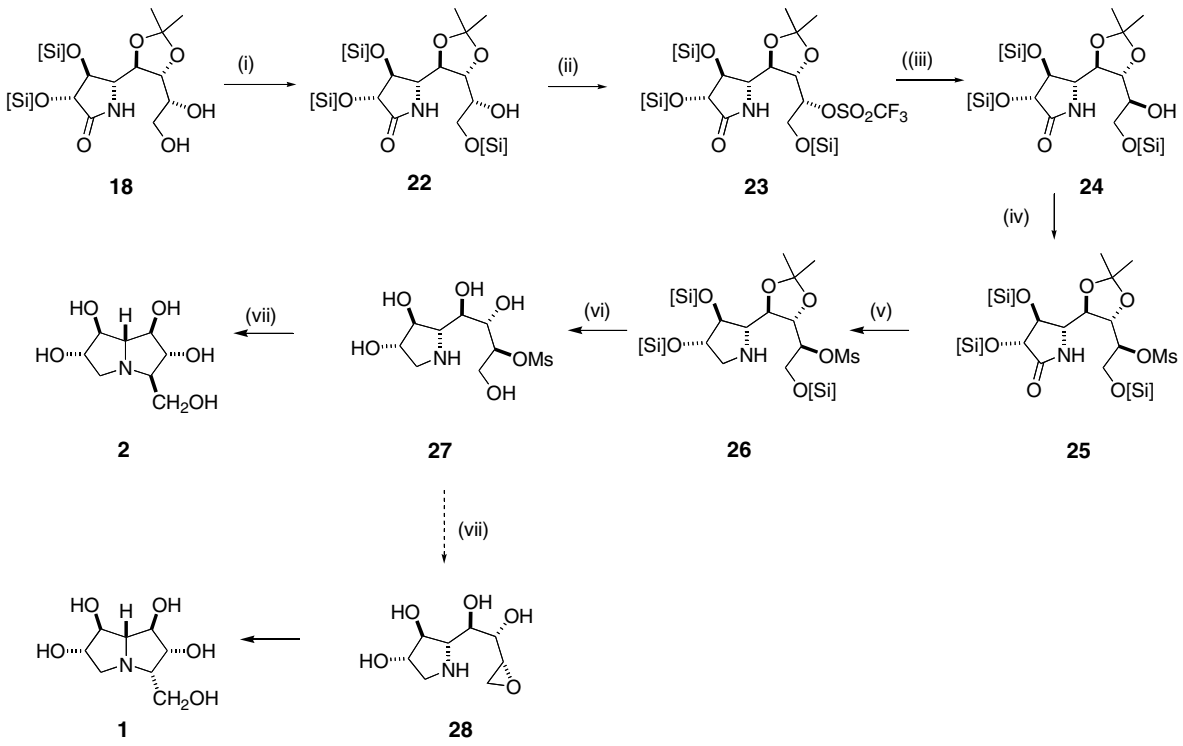
Scheme 1. Reagents and conditions: (i) $Me_2C(OMe)_2$, *p*-TsOH, MeOH; then $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, 72%; (ii) NaN_3, DMF, 97%; (iii) 3 equiv $^tBu_2AlH$, −78 °C; then $Ph_3P{=}CHCO_2Me$ **21**, toluene (75% over two steps); (iv) cat. OsO_4, NMO, *t*BuOH/H_2O, 72%; (v) H_2, Pd/C, THF; then toluene, Δ; then $^tBuMe_2SiCl$, imidazole, THF (70% over three steps); (vi) 60% HOAc, H_2O/MeOH, 69%; (vii) $^tBuMe_2SiCl$, pyridine; then $MeSO_2Cl$, Et_3N, CH_2Cl_2, 66%; (viii) BH_3·THF, THF, 57%; (ix) 90% CF_3CO_2H, H_2O; (x) NaOAc, H_2O (89% over two steps).

The unsaturated ester **14** was subjected to a dihydroxylation using catalytic osmium tetroxide and a stoichiometric amount of *N*-methylmorpholine *N*-oxide (NMO) as reoxidant to afford an inseparable 4:1 mixture of **16**:**15** isomers in a total yield of 72%; the isomer with the required stereochemistry **16** thus greatly predominated. Hydrogenation of the mixture of azides **16** and **15** gave the corresponding amines, which on heating in toluene, resulted in the formation of the first ring of the pyrrolizidine framework. The two diastereomeric polar lactams were difficult to separate, so the mixture was treated with *tert*-butyldimethylsilyl (TBDMS) chloride in the presence of imidazole to allow the isolation of the protected lactam **17** as a single diastereomer in 70% over the three steps (93% calculated from the original isomer content).

The terminal acetonide in **17** was then removed by treatment with aqueous acetic acid to afford **18** in 69% yield. Selective protection of the primary hydroxyl group in **18** with TBDMS chloride in pyridine allowed the esterification of the remaining secondary alcohol with mesyl chloride and triethylamine to afford **19** in 66% overall yield. Reduction of the lactam **19** with THF:borane at elevated temperatures gave the pyrrolidine **20** in 57% yield. No spontaneous cyclization of **20** occurred because the resulting pyrrolizidine would contain a *trans*-diol protected as a strained ketal. Accordingly, all the protecting groups in **20** were removed by heating with 90% aqueous trifluoroacetic acid to give the pentahydroxyaminomesylate **6** as its trifluoroacetate salt. Treatment of the salt **6** with sodium acetate as a base resulted in a clean cyclization step to 3-*epi*-casuarine **1** in 89% yield over the last two steps. A small amount of casuarine **2** (less than 5% yield) was formed, presumably via an initial epoxide. GC–MS analysis of trimethylsilyl-derivatives of polyhydroxylated alkaloids is an exquisitely sensitive method[16] of detecting impurities; no other stereoisomers were formed.

D-Gluconolactone **11** could also be used for a synthesis of casuarine but would require an additional inversion of configuration at C-7 of the diol **18** (Scheme 2).

TBDMS chloride in pyridine reacted with the diol **18** selectively at C-8 to afford the primary silyl ether **22** in 81% yield in which the hydroxyl at C-7 is unprotected. A variety of strategies for inversion of the configuration at C-7 were



Scheme 2. Reagents and conditions: (i) $^{t}BuMe_2SiCl$, pyridine, 81%; (ii) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2; (iii) CF_3CO_2Cs, 2-butanone; then K_2CO_3, MeOH (20% from **22**); (iv) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 90%; (v) BH_3·THF, THF, 57%; (vi) 90% CF_3CO_2H, H_2O; (vi) NaOAc, H_2O (91% over two steps).

attempted, though none proceeded in high yield. A method involving displacement of a triflate by trifluoroacetate was successful though the yield was poor.[25] The free hydroxyl group in **22** was esterified by treatment with triflic anhydride and pyridine to afford the unstable triflate **23**, which was treated with caesium trifluoroacetate in butanone; reaction of the resulting trifluoroacetate ester with potassium carbonate in methanol afforded the inverted alcohol **24** in 20% yield over the three steps. The low yield is due to decomposition of the triflate **23** to uncharacterized baseline material. The free hydroxyl group in **24** was esterified with methanesulfonyl chloride in pyridine (90% yield) to give the lactam mesylate **25**, which was reduced with THF:borane to the protected amine **26** (57% yield). Complete deprotection of **26** by aqueous trifluoroacetic acid gave the unprotected aminomesylate **27**, which with sodium acetate gave essentially pure casuarine **2** (91% yield over two steps) except for a very small amount of 3-*epi*-casuarine **1**. The closure to epoxide **28** followed by a 5-exo-*tet* closure to the pyrrolizidine would account for the tiny amount of 3-*epi*-casuarine **1** that is formed. Again GC–MS analysis of pertrimethylsilyl ethers from the crude reaction mixture showed that only a trace amount of **1** was produced and that no other polyhydroxylated alkaloid was formed. The overall yield of this synthesis of casuarine **2** from D-gluconolactone **11** was 1.4% over 18 steps (78.8% per step); unless a better method can be found for the inversion of the alcohol **22** to **24**, this route would not allow the preparation of significant amounts of casuarine **2**. The efficient high yield closure of another unprotected pentahydroxyamino mesylate **27** to a complex pyrrolizidine, with no significant competition from alternative ring closures, suggest that this may be a general strategy for the synthesis of casuarines.

2. Conclusion

3-*epi*-Casuarine **1** is the first stereoisomer of casuarine **2** to be isolated as a natural product. A practical synthesis of 3-*epi*-casuarine **1** from D-gluconolactone **11** in an overall yield of 6% relies on the efficient cyclization of an unprotected polyhydroxyaminomesylate **6**. The synthesis of casuarine **2** gives an overall yield of 1.4%, in which the only low yielding step is an inversion of configuration of a secondary alcohol; however, the cyclization of another unprotected polyhydroxyaminomesylate **27** to casuarine is also very efficient. It may be that green strategies for the synthesis of pyrrolizidine alkaloids in which none of the hydroxyl groups is protected may allow easy access to alexines and casuarines.

3. Experimental

3.1. General procedures

All solvents were used as supplied (Analytical or HPLC grade), without further purification, except for DMF and pyridine, which were dried on Molecular Sieves prior to use. Reactions performed under a hydrogen atmosphere were maintained by an inflated balloon. Flash chromatography was performed using Sorbsil C60 40/60 silica. Thin layer chromatography (TLC) was carried out on alumin-

ium backed sheets coated with 60 F_{254} silica from Merck. Plates were developed using a spray of 0.2% w/v cerium sulfate and 5% ammonium molybdate in 2 M sulfuric acid with subsequent heating. Melting points were recorded on a Köfler hot block and are corrected. NMR spectra for **1** and **2** were recorded on a Varian UnityINOVA 500 (^{1}H—500 MHz: ^{13}C—125 MHz) spectrometer, in ^2H_2O with a probe temperature of 30 °C. Chemical shifts were measured relative to internal standards (^{1}H—trimethylsilylspronesulfonic acid at 0.000 ppm: ^{13}C—either dioxane at 67.2 ppm or acetone at 30.9 ppm). COSY and HSQC spectra were used to aid assignment of ^{1}H and ^{13}C spectra, respectively. NOESY spectra were recorded with a 400 ms mixing time. NMR spectra for all other samples were recorded on a Bruker DQX 400 (^{1}H—400 MHz: ^{13}C—100.6 MHz) instrument, with residual signals from the solvents used as the internal references. ES mass spectra were measured on a Micromass BioQ II-ZS mass spectrometer. High resolution mass spectra (HRMS) were recorded on a Micromass LCT (ESI) spectrometer. HRMS spectra were correlated relative to PEG with tetraoctylammonium bromide as internal lock mass. Infrared spectra (IR) were recorded on a Bruker Tensor Fourier Transform IR spectrometer using thin film on NaCl plates. Optical rotations were measured on a Perkin–Elmer 241 polarimeter with a path length of 1 dm. Concentrations are quoted in g/100 ml. Microanalyses were carried out by the analytical service of the ICL in Oxford. The protected triflate **12** was prepared from D-gluconolactone **11** in 71% yield, as previously described.[23]

GC–MS was carried out using a Perkin–Elmer Autosystem XL gas chromatograph with a high polarity fused-silica column (Varian 'Factor Four' VF-5 ms column, 25 m × 0.25 mm i.d., 0.25 μm phase thickness). The carrier gas (helium) flow rate was 1 ml min^{-1}. Trimethylsilyl-(TMS) derivatives were separated using a temperature programme that started at 160 °C and was held for 5 min, followed by a linear increase to 300 °C at a rate of 10 °C min^{-1}. The temperature was held at 300 °C for an additional 10 min; the total analysis time was 29 min. Electron impact mass spectrometry of the column eluant was carried out using a Perkin–Elmer TurboMass Gold mass spectrometer, with a quadrupole ion filter system, which was run at 250 °C constantly during analysis. The detector mass range was set to 100–650 amu. The temperature of the transfer line (GC to MS) was held at 250 °C. Samples were injected onto the column via a split vent (split ratio 50:1) through a fused silica narrow bore injection liner packed with deactivated quartz wool; the injection port temperature was maintained at 200 °C. The injection volume was 1 μl. System control, data collection and mass spectral analysis were carried out using Perkin–Elmer TurboMass software (TurboMass v. 4.4).

3.2. Methyl 2-azido-2-deoxy-3,4:5,6-di-*O*-isopropylidene-D-mannonate 13

Sodium azide (2.55 g, 39.2 mmol) was added to a solution of the protected triflate **12** (15.1 g, 35.7 mmol) in DMF (30 ml). The reaction mixture was stirred at room temperature under a nitrogen atmosphere for 12 h, after which the solvent was removed in vacuo. The residue in ethyl acetate (100 ml) was washed with water (100 ml), dried (sodium sulfate) and purified by column chromatography (EtOAc/cyclohexane 1/4 v/v), to give the azide **13**, as a clear, colourless oil (10.96 g, 97%); R_f 0.36 (EtOAc/cyclohexane 1/3 v/v); $[\alpha]_D^{22} = +21.5$ (*c* 0.69, $CHCl_3$) {lit.[15] +21.3 (*c* 1.30, $CHCl_3$)}; IR *v* 2114 (N_3), 1754 (C=O); δ_H (400 MHz, $CDCl_3$) 1.33, 1.37, 1.41, 1.46 (12H, 4 × s, C(CH_3)$_2$), 3.81 (3H, s, OCH_3), 3.97 (1H, dd, H-6′, *J* 4.8, 8.0 Hz), 4.04 (2H, m, H-4, H-5), 4.15 (1H, dd, H-6, *J* 5.2, 8.0 Hz), 4.31 (1H, d, H-2, *J* 3.5 Hz), 4.38 (1H, dd, H-3, *J* 3.5, 6.6 Hz); δ_C (100 MHz, $CDCl_3$) 25.3, 26.4, 26.9, 27.3 (C(*C*H$_3$)$_2$), 52.6 (OCH_3), 63.0 (C-2), 67.6 (C-6), 76.8 (C-5), 77.6 (C-4), 80.7 (C-3), 110.0, 110.4 (*C*(CH$_3$)$_2$), 167.7 (*C*=O); MS *m*/*z* (ESI +ve) 316.21 (M+H$^+$), 288.01 (M−N$_2$+H$^+$); for $C_{13}H_{21}N_3O_6$ calcd C 49.52, H 6.71, N 13.33, found C 49.44, H 6.69, N 13.12.

3.3. Methyl 4-azido-5,6:7,8-di-*O*-isopropylidene-2,3,4-trideoxy-D-manno-oct-2-enoate 14

Diisobutylaluminium hydride in toluene (35 ml, 1.5 M) was added dropwise to a solution of azide **13** (10.96 g, 34.8 mmol) in toluene (200 ml) at −78 °C; the reaction mixture was stirred for 3 h at −78 °C under a nitrogen atmosphere when excess hydride was quenched by dropwise addition of methanol (10 ml) and then allowed to warm to room temperature. Methyl (triphenylphosphoranylidene)-acetate (12.8 g, 38.3 mmol) was added in one portion and the resulting mixture was stirred at room temperature overnight under a nitrogen atmosphere. The solvents were removed in vacuo. The residue was dissolved in diethyl ether (100 ml), and the resulting solution extracted with sodium hydroxide (100 ml, 2 M) and the organic phase dried (sodium sulfate). The solvent was removed and the residue was purified by chromatography (EtOAc/cyclohexane 1/6 v/v) to afford the unsaturated *E*-ester **14** (8.95 g, 75%), R_f 0.89 (EtOAc/cyclohexane 1/1 v/v) as a white crystalline solid; mp 45–46 °C; $[\alpha]_D^{22} = +35.5$ (*c* 0.71, $CHCl_3$); IR *v* 2095 (N_3), 1725 (C=O); δ_H (400 MHz, $CDCl_3$) 1.36, 1.38, 1.51 (12H, 3 × s, C(CH_3)$_2$), 3.73 (1H, m, H-7), 3.78 (3H, s, OCH_3), 3.93 (1H, dd, H-8′, *J* 5.2, 8.4 Hz), 4.05 (1H, m, H-7), 4.14 (1H, dd, H-8, *J* 4.0, 8.4 Hz), 4.17 (1H, dd, H-5, *J* 3.0, 3.6 Hz), 4.33 (1H, ddd, H-4, *J* 1.2, 3.6, 7.2 Hz), 6.11 (1H, dd, H-2, *J* 1.2, 15.6 Hz), 6.96 (1H, dd, H-3, *J* 7.2, 15.2 Hz); δ_C (100 MHz, $CDCl_3$) 25.1, 26.4, 26.9, 27.2 (C(*C*H$_3$)$_2$), 51.8 (OCH_3), 63.1 (C-4), 67.7 (C-8), 76.8 (C-7), 78.3 (C-6), 81.4 (C-5), 109.9, 110.6 (*C*(CH$_3$)$_2$), 125.2 (C-2), 140.3 (C-3), 165.7 (*C*=O); HRMS *m*/*z* (ESI +ve): Found 683.3251 (2M+H$^+$); $C_{30}H_{47}N_6O_{12}$ requires 683.325; for $C_{15}H_{23}N_3O_6$ calcd C 52.78, H 6.79, N 12.31, found C 52.78, H 6.78, N 12.10.

3.4. Methyl 4-azido-4-deoxy-5,6:7,8-di-*O*-isopropylidene-D-*erythro*-L-*altro*-octonate 16 and methyl 4-azido-4-deoxy-5,6:7,8-di-*O*-isopropylidene-D-*erythro*-L-gluco-octonate 15

A solution of azido ester **14** (6.85 g, 20.0 mmol) in a mixture of acetone (50 ml) and water (10 ml) was treated with *N*-methylmorpholine oxide (4.69 g, 40.0 mmol) and osmium tetroxide (50 mg). The reaction mixture was stirred overnight at room temperature under a nitrogen atmo-

sphere and then treated with ethyl acetate (100 ml) and sodium bicarbonate (100 ml, 5% w/v in H_2O). The organic layer was dried (sodium sulfate), the solvent removed and the residue purified by column chromatography (EtOAc/cyclohexane 1/1 v/v) to afford an inseparable mixture of diols **16** and **15** in a ratio of approximately 4:1, respectively (5.37 g, 72%), R_f 0.36 (EtOAc/cyclohexane 1/3 v/v); IR ν 3465 (OH), 2111 (N_3), 1748 (C=O); δ_H (400 MHz, $CDCl_3$) 1.35, 1.38, 1.40, 1.43, 1.47, 1.52 (15.0H, 6 × s, maj/min $C(CH_3)_2$), 2.91 (0.25H, min (C-3)O*H*), 3.03 (1.00H, maj (C-2)O*H*), 3.19 (0.25H, min (C-2)O*H*), 3.73 (1.00H, dd, maj H-3), 3.81 (3.25H, m, maj OCH_3, min (C-3)O*H*), 3.85 (0.75H, s, min OCH_3), 3.95 (0.25H, m, min H-4), 4.05 (4.00H, m, maj H-6, maj H-7, maj H-8′, min H-3, min H-6, min H-7, min H-8′), 4.24 (2.50H, maj H-4, maj-H-8, min H-5, min H-8), 4.48 (1.25H, m, maj H-2, min H-2), 4.55 (1.00H, m, maj H-5); δ_C (100 MHz, $CDCl_3$) 25.0, 25.7, 26.9, 27.2 (maj $C(CH_3)_2$), 25.4, 26.5, 27.3 (min $C(CH_3)_2$), 52.5 (maj OCH_3), 52.8 (min OCH_3), 61.5 (maj C-4), 65.0 (min C-4), 68.0 (min C-8), 68.6 (maj C-8), 70.9, 71.1 (maj C-2, C-3), 71.1, 71.3 (min C-2, C-3), 76.9 (maj C-6), 77.0 (min C-6), 77.5 (maj C-7), 78.6 (min C-7), 80.2 (min C-5), 80.7 (maj C-5), 110.0, 110.1, 110.3 (maj/min $C(CH_3)_2$), 173.1 (min C=O), 173.3 (maj C=O); HRMS m/z (ESI +ve): Found 398.1528 ($M+Na^+$); $C_{15}H_{25}N_3O_8Na$ requires 398.1539; for $C_{15}H_{25}N_3O_8$ calcd C 47.99, H 6.71, N 11.19, found C 48.39, H 6.79, N 10.87.

3.5. 4-Deoxy-2,3-di-*O*-*tert*-butyldimethylsilyl-5,6:7,8-di-*O*-isopropylidene-D-*erythro*-L-*altro*-octono-1,4-lactam 17

A solution of a 4:1 mixture of diols **16** and **15** (5.28 g, 14.07 mmol) in tetrahydrofuran (50 ml) was stirred for 48 h at room temperature under a hydrogen atmosphere in the presence of a palladium catalyst (20% $Pd(OH)_2$ on carbon, 150 mg). The catalyst was removed by filtration and the solvent removed in vacuo. A solution of the residue in toluene (200 ml) was heated under reflux for 3 h under a nitrogen atmosphere. The solvent was then removed in vacuo and a solution of the crude product in tetrahydrofuran (50 ml) was treated with imidazole (4.22 g, 62.04 mmol), and *tert*-butyldimethylsilyl chloride (4.68 g, 31.02 mmol) was added. The reaction mixture was stirred overnight at reflux temperature under a nitrogen atmosphere, the solvent removed in vacuo and the residue redissolved in dichloromethane (50 ml) and washed with aqueous hydrochloric acid (50 ml, 1 M), over sodium bicarbonate (50 ml, 5% w/v in H_2O), and brine (50 ml). The organic layer was dried over sodium sulfate and the solvent removed to give a residue, which was purified by column chromatography (EtOAc/cyclohexane 1/6 v/v) to afford the protected lactam **17** as a single diastereomer (5.41 g, 70%), colourless oil; R_f 0.48 (EtOAc/cyclohexane 1/4 v/v); $[\alpha]_D^{22} = +1.8$ (c 1.59, $CHCl_3$); IR ν 1725 (C=O); δ_H (400 MHz, $CDCl_3$) 0.18, 0.20 (12H, 2 × s, $SiCH_3$), 0.95 (18H, s, $SiC(CH_3)_3$), 1.36, 1.38, 1.40, 1.46 (12H, 4 × s, $C(CH_3)_2$), 3.27 (1H, dd, H-4, J 8.3, 6.8 Hz), 3.62 (1H, app. t, H-6, J 8.1 Hz), 3.76 (1H, app. t, H-5, J 8.2 Hz), 3.99 (2H, m, H-7, H-8′), 4.20 (2H, m, H-3, H-8), 4.28 (1H, d, H-2, J 8.1 Hz); δ_C (100 MHz, $CDCl_3$) −5.1, −4.6 ($SiCH_3$), 18.4 ($SiC(CH_3)_3$), 24.9 ($SiC(CH_3)_3$), 26.6, 26.7 ($C(CH_3)_2$), 27.5 (C-4), 26.8 (C-8), 75.8 (C-2), 76.5 (C-3), 79.5 (C-7), 81.7 (C-6), 83.5 (C-5), 109.8, 110.4 ($C(CH_3)_2$), 172.5 (C=O); HRMS m/z (ESI +ve): Found 546.3265 ($M+H^+$); $C_{26}H_{52}NO_7Si_2$ requires 546.3282; for $C_{26}H_{51}NO_7Si_3$ calcd C 57.21, H 9.42, N 2.57, found C 57.20, H 9.68, N 2.26.

3.6. 4-Deoxy-2,3-di-*O*-*tert*-butyldimethylsilyl-5,6-*O*-isopropylidene-D-*erythro*-L-*altro*-octono-1,4-lactam 18

A solution of the fully protected lactam **17** (3.76 g, 6.89 mmol) in methanol (6 ml), acetic acid (12 ml) and water (2 ml) was stirred at reflux temperature for 5 h under a nitrogen atmosphere. Sodium bicarbonate was then added as a solid until all acetic acid had been neutralized. The reaction was then treated with diethyl ether (100 ml) and water (50 ml); the organic layer was dried over sodium sulfate and the solvent removed. Purification of the residue by chromatography (EtOAc/cyclohexane 1/1 v/v) gave diol **18** (2.39 g, 69%), colourless oil R_f 0.17 (EtOAc/cyclohexane 1/1 v/v); $[\alpha]_D^{23} = +2.9$ (c 0.41, $CHCl_3$); IR ν 3336 (OH), 1701 (C=O); δ_H (400 MHz, $CDCl_3$) 0.14, 0.17, 0.19 (12H, 3 × s, $SiCH_3$), 0.91, 0.92 (18H, 2 × s, $SiC(CH_3)_3$), 1.36, 1.40 (6H, 2 × s, $C(CH_3)_2$), 2.98 (1H, br s, O*H*), 3.37 (1H, dd, H-4, J 2.8, 9.2 Hz), 3.66 (1H, dd, H-6, J 7.2, 15.2 Hz), 3.73 (2H, m, H-7, H-8′), 3.82 (1H, dd, H-5, J 7.2, 9.2 Hz), 3.87 (1H, dd, H-8, J 5.6, 13.2 Hz), 4.04 (1H, d, H-2, J 2.8 Hz), 4.26 (1H, app. t, H-3, J 2.8 Hz), 4.99 (1H, s, O*H*), 7.48 (1H, s, N*H*); δ_C (100 MHz, $CDCl_3$) −4.8, −4.7, −4.4, −4.2 ($SiCH_3$), 17.9, 18.1 ($SiC(CH_3)_3$), 25.8 ($SiC(CH_3)_3$), 26.6, 26.8 ($C(CH_3)_2$), 63.4 (C-4), 64.7 (C-8), 72.9 (C-7), 77.9 (C-7), 80.9 (C-6), 82.0 (C-5), 109.1 ($C(CH_3)_2$), 175.3 (C=O); HRMS m/z (ESI +ve): Found 506.2970 ($M+H^+$); $C_{23}H_{48}NO_7Si_2$ requires 506.2969; for $C_{23}H_{47}NO_7Si_2$ calcd C 54.62, H 9.37, N 2.77, found C 54.85, H 9.64, N 2.54.

3.7. 4-Deoxy-5,6-*O*-isopropylidene-7-*O*-methanesulfonyl-2,3,8-tri-*O*-*tert*-butyldimethylsilyl-D-*erythro*-L-*altro*-octono-1,4-lactam 19

tert-Butyldimethylsilyl chloride (784 mg, 5.20 mmol) was added to a solution of diol **18** (2.39 g, 4.73 mmol) in dry pyridine (10 ml). The reaction mixture was stirred at room temperature for 5 h under a nitrogen atmosphere after which dichloromethane (100 ml) was added; the organic layer was washed with aqueous hydrochloric acid (100 ml, 1 M), sodium bicarbonate (50 ml, 5% w/v in H_2O) and brine (50 ml). The organic layer was dried over sodium sulfate and the solvent reduced in vacuo until a volume of 10 ml remained. Then, triethylamine (1.0 ml, 7.1 mmol) and methanesulfonyl chloride (439 μl, 5.68 mmol) were added to the solution of the crude trisilyl ether **22** (see below for the preparation of pure **22**). The reaction mixture was stirred for 30 min at room temperature under a nitrogen atmosphere, washed with aqueous hydrochloric acid (10 ml, 1 M), sodium bicarbonate (10 ml, 5% w/v in H_2O) and brine (10 ml). The organic layer was dried (sodium sulfate) and the solvent removed to give a residue, which was purified by chromatography (EtOAc/cyclohexane 1/4 v/v) to give the trisilyl mesylate **19** (2.18 g, 66%), colourless oil, R_f 0.23 (EtOAc/cyclohexane 1/4 v/v); $[\alpha]_D^{23} = +28.9$ (c 0.37, $CHCl_3$); IR ν 1720

(C=O); δ_H (400 MHz, $CDCl_3$) 0.11, 0.12, 0.17, 0.18 (18H, 4 × s, $SiCH_3$), 0.87, 0.90, 0.91, 0.92, 0.92 (27H, 5 × s ($SiC(CH_3)_3$), 1.40, 1.46 (6H, 2 × s, $C(CH_3)_2$), 3.09 (3H, s, SO_2CH_3), 3.41 (1H, d, H-4, *J* 8.0 Hz), 3.81 (1H, dd, H-8′, *J* 4.0, 12.0 Hz), 3.85 (1H, s, H-2), 3.93 (1H, dd, H-8, *J* 7.1, 12.0 Hz), 4.07 (1H, app. t, H-5, *J* 8.0 Hz), 4.16 (1H, dd, H-6, *J* 3.7, 8.0 Hz), 4.23 (1H, s, H-3), 4.64 (1H, ddd, H-7, *J* 3.7, 4.0, 7.1 Hz), 6.09 (1H, s, N*H*); δ_C (100 MHz, $CDCl_3$) −5.5, −5.5, −5.2, −4.8, −4.7, −4.4 ($SiCH_3$), 17.8, 18.0, 18.2, 18.3 ($SiC(CH_3)_3$), 25.7, 25.7, 25.8 ($SiC(CH_3)_3$), 26.9, 27.1 ($C(CH_3)_2$), 38.4 (SO_2CH_3), 61.5 (C-8), 65.5 (C-4), 76.0 (C-6), 77.3 (C-3), 78.1 (C-2), 79.2 (C-5), 82.0 (C-7), 110.4, 110.5 ($C(CH_3)_2$), 175.0 (C=O); HRMS *m*/*z* (ESI +ve): Found 698.3615 ($M+H^+$); $C_{30}H_{64}NO_9SSi_3$ requires 698.3210; for $C_{30}H_{63}NO_9SSi_3$ calcd C 51.61 H 9.10 N 2.01, found C 52.00 H 9.05 N 2.33.

3.8. 1,4-Dideoxy-1,4-imino-5,6-*O*-isopropylidene-7-*O*-methanesulfonyl-2,3,8-tri-*O*-*tert*-butyldimethylsilyl-D-*erythro*-L-*altro*-octitol 20

A solution of borane in tetrahydrofuran (21 ml, 1 M) was added to a solution of the protected lactam **19** (2.87 g, 4.11 mmol) in tetrahydrofuran (40 ml) and the resulting mixture stirred at reflux temperature for 1 h under a nitrogen atmosphere. The reaction mixture was cooled to room temperature and treated with methanol (5 ml). Removal of solvents gave a residue, which was purified by chromatography (EtOAc/cyclohexane 1/8 v/v) to afford the protected amino mesylate **20** (1.60 g, 57%), as a colourless oil, $[\alpha]_D^{24} = -67.1$ (*c* 0.38, $CHCl_3$); IR *v* 3394 (NH); δ_H (400 MHz, $CDCl_3$) 0.10, 0.11, 0.11, 0.12, 0.17, 0.18 (18H, 6 × s, $SiCH_3$), 0.90, 0.92, 0.94 (27H, 3 × s, $SiC(CH_3)_3$), 1.39, 1.44 (6H, 2 × s, $C(CH_3)_2$), 3.06 (1H, dd, H-4, *J* 6.4, 8.5), 3.25 (3H, s, SO_2CH_3), 3.63 (1H, app. d, H-1′, *J* 10.4), 3.90 (2H, m, H-1, H-8′), 4.04 (1H, dd, H-8, *J* 2.0, 12.0), 4.16 (1H, br m, H-3), 4.28 (1H, dd, H-6, *J* 6.3, 9.2), 4.43 (1H, dd, H-5, *J* 6.3, 8.5), 4.66 (1H, ddd, H-7, *J* 2.0, 7.6, 9.2), 4.85 (1H, ddd, H-2, *J* 2.8, 8.0, 10.4), 5.06 (1H, br s, N*H*); δ_C (100 MHz, $CDCl_3$) −5.5, −5.4, −4.9, −4.8, −4.8, −4.7 ($SiCH_3$), 17.7, 18.3, 18.4 ($SiC(CH_3)_3$), 25.6, 25.8, 25.9, 26.0 ($SiC(CH_3)_3$), 26.5, 27.1 ($C(CH_3)_2$), 39.2 (SO_2CH_3), 63.8 (C-8), 75.1 (C-4), 76.0 (C-6), 76.2 (C-1), 77.2 (C-5), 77.8 (C-3), 85.0 (C-7), 92.9 (C-2), 110.4 ($C(CH_3)_2$); HRMS *m*/*z* (ESI +ve): Found 684.3804 ($M+H^+$); $C_{30}H_{66}NO_8SSi_3$ requires 684.3817; for $C_{30}H_{65}NO_8SSi_3$ calcd C 52.67 H 9.58 N 2.05, found C 52.54 H 9.41 N 1.99.

3.9. 1,4-Dideoxy-1,4-imino-7-*O*-methanesulfonyl-D-*erythro*-L-*altro*-octitol 6

A solution of the protected amine **20** (1.18 g, 1.72 mmol) in trifluoroacetic acid (4.5 ml) and water (0.5 ml) was stirred for 3 h at reflux temperature under a nitrogen atmosphere. The solvents were evaporated in vacuo and the residue co-evaporated with toluene, to afford the fully deprotected amino mesylate **6** (978 mg), a light brown oil, which was used without further purification. δ_H (400 MHz, D_2O) 3.19 (3H, s, SO_2CH_3), 3.35 (1H, dd, H-1′, *J* 2.1, 12.4 Hz), 3.49 (1H, dd, H-1, *J* 4.0, 12.4 Hz), 3.64 (1H, dd, H-4, *J* 3.6, 6.5 Hz), 3.85 (1H, dd, H-8′, *J* 5.2, 13.2 Hz), 3.98 (2H, m, H-6, H-8), 4.10 (1H, dd, H-5, *J* 1.2, 6.5 Hz), 4.28 (1H, app. p, H-2, *J* 2.1, 4.0, 3.6 Hz), 4.34 (1H, app. t, H-3, *J* 3.6 Hz), 4.80 (1H, m, H-7); δ_C (100 MHz, D_2O) 27.5 (SO_2CH_3), 39.9 (C-1), 49.4 (C-8), 56.0 (C-5), 56.7 (C-4), 57.8 (C-6), 64.0 (C-2), 65.1 (C-3), 71.0 (C-7); MS *m*/*z* (ESI +ve): 301.82 ($M+H^+$).

3.10. 3-*epi*-Casuarine 1

3.10.1. Synthesis. A solution of the amino mesylate **6** in water (20 ml) in the presence of sodium acetate (423 mg, 5.16 mM) was stirred overnight at room temperature under a nitrogen atmosphere. The solvents were removed in vacuo; the residue was subjected to ion-exchange chromatography on Amberlite CG120 (H^+), using 1 M NH_4OH as eluent, to afford 3-*epi*-casuarine **1** (314 mg, 89%), white solid; $[\alpha]_D^{22} = +5.8$ (*c* 0.69, H_2O); NMR data see Table 2; HRMS *m*/*z* (ESI +ve): Found 206.1030 ($M+H^+$); $C_8H_{16}NO_5$ requires 206.1028. GC–MS analysis of the TMS product showed only a trace (<2%) of casuarine **2**. Pure 3-*epi*-casuarine **1** was obtained as its monohydrate by crystallization of the crude product from warm 95% aqueous ethanol by adding a layer of acetone. The minor synthetic epimer, casuarine **2**, was purified by chromatography on Dowex 1 strongly basic anion exchange resin in the OH^- form. Casuarine is eluted with water before the major 3-*epi* product **1**.

3.10.2. Isolation. 3-*epi*-Casuarine was isolated as a natural product from the shrub *M. communis* L. (Myrtle) growing in the grounds of the Institute of Grassland and Environmental Research in Aberystwyth. The aerial parts of the plant were shredded and the alkaloids were extracted into 50% aqueous ethanol. Alkaloids and amino acids were bound on to strongly acidic cation exchange resin (IR-120 H^+ form) and after washing with copious water they were displaced with 2 M ammonium hydroxide. Basic amino acids were removed using weakly acidic cation exchange resin (Amberlite CG50 in the ammonium form) and the unbound material was fractionated in water on strongly basic anion exchange resin (Dowex 1 in the hydroxide form). Fractions were analyzed by GC–MS of trimethylsilyl-derivatives prepared using 200 μl of Pierce TriSil per mg of material. Casuarine was the major alkaloid present in *M. communis* (displaced first from Dowex 1), and 3-*epi*-casuarine was the only epimer detected in the plant (displaced after casuarine from Dowex 1). Casuarine **2** and 3-*epi*-casuarine **1** were crystallized from warm 95% aqueous ethanol by layering with acetone. The ^1H and ^{13}C NMR of the natural 3-epi-casuarine were identical to those of the synthetic material; the natural sample had a specific rotation, $[\alpha]_D^{23} = +5.7$ (*c* 0.5, H_2O).

3.11. 4-Deoxy-5,6-*O*-isopropylidene-2,3,8-tri-*O*-*tert*-butyldimethylsilyl-D-*erythro*-L-*altro*-octono-1,4-lactam 22

A solution of the diacetonide **17** (2.70 g, 4.76 mmol) in methanol (1.5 ml), acetic acid (6 ml) and water (0.5 ml) was stirred at reflux temperature for 5 h under a nitrogen atmosphere. Ethyl acetate (60 ml) and aqueous sodium hydroxide (60 ml, 2 M) were then added and the layers were separated. The organic layer was dried (sodium sul-

fate) and the solvents were evaporated. The resulting crude diol **18** was dissolved in pyridine (10 ml) and treated with *tert*-butyldimethylsilyl chloride (860 mg, 5.71 mmol) overnight at room temperature under a nitrogen atmosphere. Then, dichloromethane (60 ml) was added and the resulting mixture washed with aqueous hydrochloric acid (60 ml, 2 M), sodium bicarbonate (60 ml, 5% w/v in H_2O) and brine (60 ml). The organic layer was dried over sodium sulfate and the solvent removed to give a residue, which was purified by chromatography (EtOAc/cyclohexane 1/6 v/v) to afford the trisilyl ether **22** (2.38 g, 81%), a colourless oil, R_f 0.35 (EtOAc/cyclohexane 1/4 v/v); $[\alpha]_D^{23} = +13.5$ (*c* 0.65, $CHCl_3$); IR *v* 3306 (OH), 1716 (C=O); δ_H (400 MHz, $CDCl_3$) 0.10, 0.10, 0.12, 0.17, 0.19 (18H, 5 × s, SiCH_3), 0.90, 0.91, 0.92 (27H, 3 × s, SiC(CH_3)$_3$), 1.34, 1.37 (6H, 2 × s, C(CH_3)$_2$), 3.07 (1H, d, O*H*, *J* 2.8 Hz), 3.35 (1H, dd, H-4, *J* 9.0, 2.0 Hz), 3.50 (1H, app. t, H-6, *J* 3.8 Hz), 3.60 (2H, br m, H-7, H-8), 3.80 (1H, dd, H-5, *J* 9.0, 7.6 Hz), 3.85 (1H, dd, H-8′, *J* 2.8, 9.6 Hz), 3.97 (1H, d, H-2, *J* 2.8 Hz), 4.27 (1H, app. t, *J* 2.8 Hz); δ_C (100 MHz, $CDCl_3$) −5.5, −4.9, −4.7, −4.5, −4.2 (SiCH_3), 17.9, 18.2, 18.2 (SiC(CH$_3$)$_3$), 25.6, 25.8, 25.8 (SiC(CH_3)$_3$), 26.7 (C(CH_3)$_2$), 63.1 (C-4), 64.6 (C-8), 71.9 (C-7), 77.5 (C-3), 77.7 (C-2), 80.7 (C-6), 82.4 (C-5), 108.8 (C(CH$_3$)$_2$), 174.4 (*C*=O); HRMS *m*/*z* (ESI +ve): Found 620.3857 ($M+H^+$); $C_{29}H_{62}NO_7Si_3$ requires 620.3834; for $C_{29}H_{61}NO_7Si_3$ calcd C 56.17, H 9.92, N 2.26, found C 56.09, H 10.05, N 2.25.

3.12. 4-Deoxy-5,6-*O*-isopropylidene-2,3,8-tri-*O*-*tert*-butyldimethylsilyl-L-*threo*-L-*altro*-octono-1,4-lactam 24

Trifluoromethanesulfonic anhydride (1.29 ml, 7.66 mmol) was added dropwise to a solution of the trisilyl ether **22** (2.38 g, 3.83 mmol) and pyridine (1.87 ml, 23.0 mmol) in dry dichloromethane (10 ml) at −50 °C, after which time the reaction mixture was stirred for 5 h under a nitrogen atmosphere while slowly warming to 0 °C. The reaction mixture was treated with dichloromethane (40 ml) and aqueous hydrochloric acid (60 ml, 1 M); the organic layer was then washed with water (50 ml) and dried over sodium sulfate. The solvent was removed; the crude triflate was dissolved in dry butanone (10 ml), treated with caesium trifluoroacetate (922 mg, 3.75 mmol) and stirred overnight at 50 °C under a nitrogen atmosphere. Then, potassium carbonate (500 mg) was added and the mixture stirred for 15 min. The solvent was removed and the residue dissolved in ethyl acetate (50 ml); the organic layer was washed with water (50 ml), dried over sodium sulfate and the solvent removed. The residue was purified by chromatography (EtOAc/cyclohexane 1/4 v/v), to give the inverted alcohol **24** (469 mg, 20%), a light yellow oil, R_f 0.25 (EtOAc/cyclohexane 1/4 v/v); $[\alpha]_D^{23} = +11.1$ (*c* 0.55, $CHCl_3$); IR *v* 3269 (OH), 1715 (C=O); δ_H (400 MHz, $CDCl_3$) 0.09, 0.10, 0.12, 0.12, 0.17, 0.17 (18H, 6 × s, SiCH_3), 0.90, 0.90, 0.91 (27H, 3 × s, SiC(CH_3)$_3$), 1.38, 1.40 (6H, 2 × s, C(CH_3)$_2$), 2.66 (1H, d, O*H*, *J* 3.6 Hz), 3.42 (1H, dd, H-4, *J* 1.6, 8.0 Hz), 3.64 (1H, dd, H-8, *J* 6.2, 9.8 Hz), 3.76 (2H, m, H-7, H-8′), 3.92 (1H, d, H-2, *J* 2.4 Hz), 3.94 (1H, app. t, H-6, *J* 3.8 Hz), 4.04 (1H, app. t, H-5, *J* 8.0 Hz), 4.17 (1H, app. s, H-3), 6.36 (1H, br s, N*H*); δ_C (100 MHz, $CDCl_3$) −5.4, −5.3, −4.9, −4.7, −4.5, −4.3 (SiCH_3), 17.9, 18.1, 18.3 (SiC(CH$_3$)$_3$), 25.7, 25.7, 25.9 (SiC(CH_3)$_3$), 26.9, 27.0 (C(CH_3)$_2$), 63.7 (C-4), 63.8 (C-8), 71.3 (C-7), 76.8 (C-3), 77.1 (C-5), 77.5 (C-2), 79.3 (C-6), 109.0 (C(CH$_3$)$_2$), 174.5 (*C*=O); HRMS *m*/*z* (ESI +ve): Found 642.3685 ($M+Na^+$); $C_{29}H_{61}NO_7Si_3Na$ requires 642.3654; for $C_{29}H_{61}NO_7Si_3$ calcd C 56.17, H 9.92, N 2.26, found C 56.22, H 9.59, N 2.31.

3.13. 4-Deoxy-5,6-*O*-isopropylidene-7-*O*-methanesulfonyl-2,3,8-tri-*O*-*tert*-butyldimethylsilyl-L-*threo*-L-*altro*-octono-1,4-lactam 25

Triethylamine (308 μl, 2.19 mmol) and methanesulfonyl chloride (69 μl, 0.88 mmol) were added to a solution of the inverted alcohol **24** (452 mg, 0.73 mmol) in dichloromethane (10 ml) and the reaction mixture was stirred for 2 h at room temperature under a nitrogen atmosphere. More dichloromethane (40 ml) was added and the solution was successively washed with aqueous hydrochloric acid (50 ml, 2 M), sodium bicarbonate (50 ml, 5% w/v in H_2O) and brine (60 ml). The organic layer was dried over sodium sulfate and the solvent was removed to give a residue, which was purified by chromatography (EtOAc/cyclohexane 1/4 v/v) to give the mesylate **25** (461 mg, 90%), as an oil, R_f 0.37 (EtOAc/cyclohexane 1/4 v/v); $[\alpha]_D^{22} = +6.0$ (*c* 0.57, $CHCl_3$); IR *v* 1715 (C=O), 1177 (SO_3); δ_H (400 MHz, $CDCl_3$) 0.11, 0.11, 0.12, 0.14, 0.18, 0.18 (18H, 6 × s, SiCH_3), 0.87, 0.89, 0.91 (27H, 3 × s, SiC(CH_3)$_3$), 1.38, 1.40 (6H, 2 × s, C(CH_3)$_2$), 3.50 (1H, app. d, H-4, *J* 6.4 Hz), 3.88 (3H, m, H-5, H-8, H-8′), 4.03 (2H, m, H-3, H-6), 4.24 (1H, app. s, H-2), 4.66 (1H, m, H-7), 6.61 (1H, br s, N*H*); δ_C (100 MHz, $CDCl_3$) −5.4, −5.4, −5.1, −4.8, −4.7, −4.4 (SiCH_3), 17.8, 18.2, 18.3 (SiC(CH$_3$)$_3$), 25.7, 25.8, 25.9 (SiC(CH_3)$_3$), 27.1 (C(CH_3)$_2$), 38.8 (SO_2CH_3), 62.9 (C-8), 64.5 (C-4), 75.7 (C-2), 77.4, 77.6 (C-3, C-5, C-6), 82.9 (C-7), 109.5 (C(CH$_3$)$_2$), 174.9 (*C*=O); HRMS *m*/*z* (ESI +ve): Found 720.3432 ($M+Na^+$); $C_{30}H_{63}NO_9SSi_3Na$ requires 720.3429; for $C_{30}H_{63}NO_9SSi_3$ calcd C 51.61 H 9.10 N 2.01, found C 51.60 H 9.04 N 2.28.

3.14. 1,4-Dideoxy-1,4-imino-5,6-*O*-isopropylidene-7-*O*-methanesulfonyl-2,3,8-tri-*O*-*tert*-butyldimethylsilyl-L-*threo*-L-*altro*-octitol 26

A solution of borane in tetrahydrofuran (5.9 ml, 1 M) was added to lactam **25** (411 mg, 0.59 mmol) dissolved in tetrahydrofuran (5 ml) and the reaction mixture was stirred at reflux temperature for 1 h under a nitrogen atmosphere. Methanol (5 ml) was then added and the combined solvents evaporated. The residue was purified by chromatography (EtOAc/cyclohexane 1/8 v/v) to afford the amine **26** (231 mg, 57%), as a colourless oil, R_f 0.60 (EtOAc/cyclohexane 1/4 v/v); $[\alpha]_D^{23} = -8.5$ (*c* 1.32, $CHCl_3$); IR *v* 3487 (NH), 1771 (SO_3); δ_H (400 MHz, $CDCl_3$) 0.07, 0.08, 0.10, 0.11, 0.17, 0.18 (18H, 6 × s, SiCH_3), 0.88, 0.90, 0.93 (27H, 3 × s, SiC(CH_3)$_3$), 1.39, 1.43 (6H, 2 × s, C(CH_3)$_2$), 3.08 (1H, app. dd, H-4, *J* 6.8, 9.6 Hz), 3.15 (3H, s, SO_2CH_3), 3.44 (1H, app. d, H-1, *J* 8.8 Hz), 3.86 (2H, m, H-3, H-8), 4.03 (1H, dd, H-8′, *J* 8.8, 11.6 Hz), 4.07 (1H, dd, H-6, *J* 7.4, 8.2 Hz), 4.24 (1H, dd, H-5, *J* 8.2, 9.6 Hz), 4.27 (1H, app. s, H-2), 4.71 (1H, br s, N*H*), 4.85 (1H, app. dt, H-

1′, *J* 2.4, 8.4 Hz), 5.09 (1H, app. d, H-7, *J* 7.6 Hz); δ_C (100 MHz, $CDCl_3$) −5.4, −5.0, −4.9, −4.8, −4.8, −4.7 (SiCH_3), 17.9, 18.1, 18.5 (Si$C(CH_3)_3$), 25.6, 25.7, 25.8, 25.9, 26.1, 26.9 (SiC(CH_3)$_3$), 26.9 (C(CH_3)$_2$), 38.8 (SO_2CH_3), 64.1 (C-8), 74.8 (C-5), 75.5 (C-4), 76.4 (C-2), 77.2 (C-3), 80.1 (C-6), 82.2 (C-7), 93.0 (C-1), 109.0 ($C(CH_3)_2$); HRMS *m*/*z* (ESI +ve): Found 684.3810 ($M+H^+$); $C_{30}H_{66}NO_8SSi_3$ requires 684.3817; for $C_{30}H_{65}NO_8SSi_3$ calcd C 52.67 H 9.58 N 2.05, found C 52.49 H 9.65 N 1.84.

3.15. 1,4-Dideoxy-1,4-imino-7-*O*-methanesulfonyl-L-*threo*-L-*altro*-octitol 27

The protected mesylate **26** (138 mg, 0.20 mmol) was stirred in a mixture of trifluoroacetic acid (1.8 ml) and water (0.2 ml) for 5 h at reflux temperature under a nitrogen atmosphere. The solvents were removed; the residue was dissolved in water (10 ml) and the resulting solution washed with ethyl acetate (10 ml). The water layer was subsequently evaporated to dryness to give crude amine **27** (105 mg), oil, which was used without further purification, δ_H (400 MHz, D_2O) 3.18 (3H, s, SO_2CH_3), 3.29 (1H, dd, H-1, *J* 2.8, 12.4 Hz), 3.47 (1H, dd, H-1′, *J* 3.2, 12.4 Hz), 3.66 (1H, app. t, H-4, *J* 5.2 Hz), 3.78 (1H, dd, H-8, *J* 5.8, 13.3 Hz), 3.85 (1H, dd, H-8′, *J* 3.2, 13.3 Hz), 3.92 (1H, dd, H-6, *J* 4.2, 5.8 Hz), 4.02 (1H, dd, H-5, *J* 4.2, 5.4 Hz), 4.25 (2H, m, H-2, H-3), 4.76 (1H, m, H-7); δ_C (100 MHz, D_2O) 38.2 (SO_2CH_3), 50.4 (C-1), 60.8 (C-8), 66.4 (C-4), 67.5 (C-5), 69.2 (C-6), 74.7, 75.5 (C-2, C-3), 84.3 (C-7); MS *m*/*z* (ESI +ve): 301.76 ($M+H^+$).

3.16. Casuarine 2

A solution of the crude amino mesylate **27** (105 mg) and sodium acetate (49 mg, 0.6 mmol) in water (5 ml) was stirred at room temperature for 3 h under a nitrogen atmosphere. The solvents were removed in vacuo; the residue was subjected to ion-exchange chromatography on Amberlite CG120 (H^+) using 1 M aqueous ammonium hydroxide as eluent to yield casuarine (37 mg, quantitative). The synthetic product run as the trimethylsilyl-derivative, have the same GC–MS retention time (10.27 min) and mass spectrum as authentic natural casuarine. $[\alpha]_D^{23} = +16.8$ (*c* 0.33, H_2O) {lit.[1] $[\alpha]_D^{24} = +16.9$ (*c* 0.80, H_2O)}; NMR data see Table 2; MS *m*/*z* (ESI +ve) 205.95 ($M+H^+$).

3.17. Glycosidase inhibition assay

All enzymes and *para*-nitrophenyl substrates were purchased from Sigma. Enzymes were assayed at 20 °C in 0.1 M citric acid/0.2 M disodium hydrogen phosphate buffers at the optimum pH for the enzyme. The incubation mixture consisted of 10 μl enzyme solution, 10 μl inhibitor solution in water and 50 μl of the appropriate 5 mM *para*-nitrophenyl substrate made up in buffer at the optimum pH for the enzyme. The reactions were stopped by addition of 0.4 M glycine (pH 10.4) during the exponential phase of the reaction, which was determined at the beginning of the assay using blanks with a 5 mM substrate solution. Absorbances were read at 405 nm using a Biorad microtitre plate reader (Benchmark). Water was substituted for the inhibitors in controls.

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