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Synthesis of the enantiomers of 6-epicastanospermine and 1,6-diepicastanospermine from D- and L-gulonolactone*

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ABSTRACT

The synthesis of the enantiomers of 6-epicastanospermine and of 1,6-diepicastanospermine from the enantiomeric gulonolactones is reported and the structure of the former is established as (1*S*,6*R*,7*R*,8*R*,8a*R*)-1,6,7,8-tetrahydroxyoctahydroindolizine. The inhibitory activities of the diastereomers against the amyloglucosidase-catalysed hydrolysis of *p*-nitrophenyl α-D-glucopyranoside were investigated, and the effects of 6-epicastanospermine and of 1,6-diepicastanospermine on 14 human liver glycosidases are reported.

INTRODUCTION

A remarkable range of monocyclic[1] and bicyclic[2] polyhydroxylated alkaloids that are specific and potent glycosidase inhibitors has been isolated from plants[3], but only three polyhydroxylated octahydroindolizines have been identified. Swainsonine (**1**), isolated from *Swainsona*[4] and *Astragalus*[5] species, is a potent and specific inhibitor of lysosomal and one of the processing forms of α-D-mannosidase. The pyrrolidine ring in **1** is heavily oxygenated and is structurally similar to mannofuranose. The first total synthesis[6,7] of **1** started from D-glucose. The potential chemotherapeutic value of **1** as an antimetastatic[8] or anti-tumour[9] agent has caused much interest in the stereoisomers and other analogues[10,11].

* Dedicated to Professor Leslie Hough in the year of his 65th birthday.
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Castanospermine (**2**), which has a highly oxygenated piperidine ring that is structurally similar to glucopyranose, was isolated from the seeds of the Australian legume *Castanospermum australe*[12] and is a potent inhibitor of several glucosidases[13], including that involved in the processing of glycoproteins[14]. Castanospermine inhibits experimental metastasis in mice[15], the formation of human immunodeficiency virus syncytium, and viral replication in $CD4^+$ lymphocyte cultures[16]. 6-*O*-Butyrylcastanospermine[17] shows considerable promise as a chemotherapeutic agent[18].

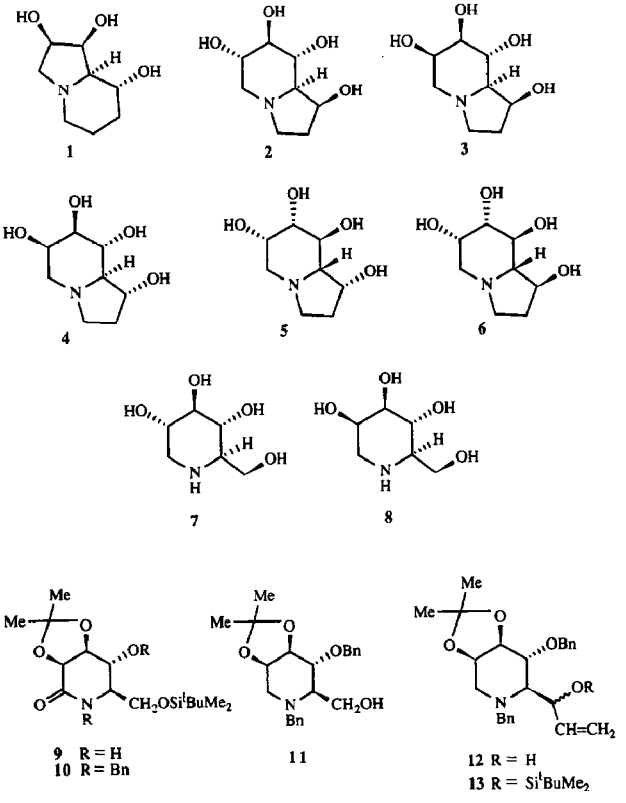
The third octahydroindolizine, first isolated as an oil from the mother liquors of the crystallisation of castanospermine, was assigned the structure **3**, *i.e.*, the 6-epimer of **2**, principally on the basis of ^{1}H- and ^{13}C-n.m.r. data[19]. Recently, 6-epicastanospermine (**3**) has been crystallised as the hydrochloride salt, and the relative stereochemistry was shown by X-ray crystallography to be that proposed[20]. Castanospermine (**2**) is related structurally to the specific glucosidase inhibitor deoxynojirimycin (**7**) in the same way that 6-epicastanospermine (**3**) is related to the mannosidase inhibitor deoxymannojirimycin (**8**). However, early studies of the enzyme inhibitory effects indicated **3** to be a glucosidase, rather than a mannosidase, inhibitor and it is also a feeding deterrent to the aphid *Schizapis graminum*[21]. The natural product is an oil with a low specific optical rotation (+2°, methanol) and its synthesis[22] from a sugar indicated that the correct structure was the enantiomer **5**. However, the specific optical rotation of 1,6-diepicastanospermine (**4**) is −72° (methanol), so that the presence of only small amounts of **4** in 6-epicastanospermine (**3**) may give rise to an incorrect assignment of absolute configuration.

The synthesis is now described of **3** and **4** from L-gulonolactone, and of their respective enantiomers **5** and **6** from D-gulonolactone. Comparison of the effects of **3**–**6** on the activity of amyloglucosidase, combined with the X-ray crystal structure of the hydrochloride of **3**, show that the correct structure of **3** is that originally proposed. The effect of **3** and **4** on 14 human liver glycosidases is also reported (a preliminary account of this work has been published[23]); **5** and **6** did not inhibit the formation of HIV-induced syncytia formation[24].

RESULTS AND DISCUSSION

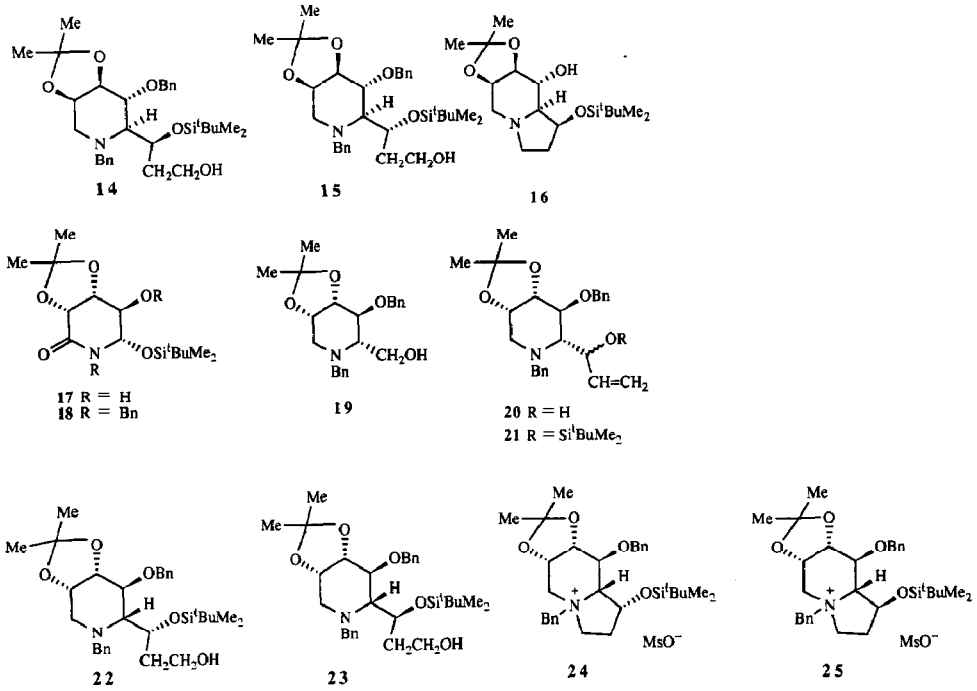
Castanospermine (**2**) and its diastereomers have five adjacent chiral centres. Thus, in a synthesis from a hexose, the introduction of an extra chiral centre is required. Although a recent synthesis of homomannojirimycin began with a protected heptonolactone having five contiguous chiral centres[25], the majority of syntheses of castanospermine[26,27] and of other hydroxylated alkaloids, such as the alexines[28] or homonojirimycins[29] with five contiguous chiral centres, have involved the late introduction of the additional chiral carbon. An advantage of this strategy is that intermediates developed in the synthesis of simpler alkaloids may be used in the synthesis of the required alkaloid. This is the strategy used here.

The lactam **9**, readily available from L-gulonolactone in an overall yield of



~30%, has been used as an intermediate in the synthesis of D-deoxymannojirimycin (**8**) and of D-mannonolactam[30,31]. 6-Epi- (**3**) and 1,6-diepi-castanospermine (**4**) may be derived from **9** by a C_2-extension from the primary hydroxyl group followed by a subsequent ring closure. Benzylation of **9** with sodium hydride, benzyl bromide, and tetrabutylammonium iodide in tetrahydrofuran gave **10** (92%) which, with lithium aluminium hydride and aluminium trichloride in tetrahydrofuran, afforded the tertiary amine **11**; the *tert*-butyldimethylsilyl group was removed during the reduction. Swern oxidation of the primary alcohol group in **11** with methyl sulphoxide and oxalyl chloride, followed by triethylamine, gave the corresponding aldehyde which, with vinylmagnesium bromide in tetrahydrofuran, gave 80% of a 1:1 mixture of the diastereomers **12**. A lack of stereoselectivity was required in the addition of the vinyl group to the aldehyde in order to provide both epimers of **12** and give access to both 6-epi- and 1,6-diepi-castanospermine. The diastereomers **12** were reacted with *tert*-butyldimethylsilyl chloride in the presence of imidazole to give 73% of **13**, which was hydroborated and then oxidised with alkaline hydrogen peroxide to give the separable alcohols **14** (25%) and **15** (19%).

The more polar alcohol **14** was treated with triethylamine and methanesulphonyl chloride, and the product was hydrogenated in methanol in the presence of palladium black to give the 6-epicastanospermine derivative **16**. Treatment of **16** with aqueous



trifluoroacetic acid removed the protecting groups to give 6-epicastanospermine (**3**, 42% from **14**). The stereochemistry of the additional chiral centre in **14** was thus demonstrated. Although **3** is an oil, it gave a crystalline hydrochloride. Likewise, the less polar alcohol **15** was converted into 1,6-diepicastanospermine (**4**, 70%).

The enantiomeric L-castanospermines **5** and **6** were prepared from the lactam **17**, an intermediate that has been used for the preparation of L-deoxymannojirimycin[31], by procedures essentially identical to those described for the above preparations of the D-castanospermines **3** and **4**. Benzylation of **17** afforded **18** (94%) which, on reduction with lithium aluminium hydride in the presence of aluminium trichloride, gave **19** (82%). Subsequent Swern oxidation and reaction of the resulting aldehyde with vinylmagnesium bromide gave a 1:1 mixture of the alcohols **20** (73%). Protection of the hydroxyl group in **20** by *tert*-butyldimethylsilylation gave **21** (85%), and hydroboration and oxidation with alkaline hydrogen peroxide then gave the diastereomeric alcohols **22** (24%) and **23** (22%). The more polar alcohol **22** was esterified with methanesulphonyl chloride and triethylamine in dichloromethane to give the corresponding mesylate which cyclised spontaneously to the methanesulphonate salt **24**. Removal of the protecting groups by hydrogenolysis followed by acid hydrolysis gave L-6-epicastanospermine (**5**, 79% overall yield). Similar treatment of the alcohol **23** gave the epimeric indolizinium mesylate **25** which, on deprotection, afforded L-1,6-diepicastanospermine (**6**, 85%).

Castanospermine is a potent inhibitor of α- and β-D-glucosidases because the four substituents on the piperidine ring have the same structure and chirality as C-2/4 in the

glucopyranosides[32,33]. Although 6-epicastanospermine (**3**) appears to be the corresponding mannose analogue, the naturally occurring 6-epicastanospermine (**3**) was originally reported as a potent inhibitor of amyloglucosidase and a weak inhibitor of bovine liver β-D-galactosidase, and showed only very weak inhibition of jack bean α-D-mannosidase at high concentrations; no inhibitory effect was found even at 200 μg/mL on coffee bean α-D-galactosidase, *Aspergillus fumigatus* β-D-mannosidase, or α-L-fucosidase. 6-Epicastanospermine was also reported[19] as a poor inhibitor of the mung bean-processing enzymes, glucosidases I and II.

6-D-Epicastanospermine (**3**) is a strong inhibitor of fungal amyloglucosidase (EC 3.2.1.3), giving 50% inhibition at 1.4 μg/mL. In contrast, none of the other stereoisomers **4–6** showed > 10% inhibition at 20 μg/mL. These results clearly indicate the natural product to have the 6-epicastanospermine structure (**3**) originally proposed.

6-Epicastanospermine (**3**) is a moderate inhibitor (I_{50} 1mM) of α-D-glucosidase from human liver. Lysosomal α-D-mannosidase is inhibited more effectively by amino sugars that mimic mannofuranose [such as swainsonine (**1**) and 1,4-dideoxy-1,4-imino-D-mannitol] than by those that mimic mannopyranose [such as deoxymannojirimycin (**8**)] (ref. 10). Since **3** is structurally closely related to **8**, this may explain its mode of action. No other glycosidases from human liver, including α- and β-D-mannosidase, β-D-glucosidase, and α-L-fucosidase were affected significantly by **3**. Similarly, 1,6-diepicastanospermine (**4**) did not inhibit any of the glycosidases tested by more than 15% at 1mM concentration. Thus, alteration of the configurations of the sole hydroxyl group in the pyrrolidine ring destroys the inhibition of α-D-glucosidase shown by 6-epicastanospermine (**3**).

Each compound has the minimum structural requirements for the inhibition of α-L-fucosidase by piperidine derivatives, namely, the configurations of the hydroxyl groups on the piperidine ring which correspond to the configurations of the secondary hydroxyl groups in fucopyranosides[34]. Whereas α-L-fucosidase is 94% inhibited by 1mM deoxymannojirimycin (**8**), < 15% inhibition was caused by **3** and **4** at this concentration. These results suggest that either the active site of α-L-fucosidase cannot accommodate the additional pyrrolidine ring, or that the pK_a of 6.1 of the ring nitrogen in **3** is too low. The inhibition of α-L-fucosidase by deoxyfuconojirimycin and its analogues is due to the formation of an ion-pair between the protonated amino sugar and an ionised carboxyl group in the active site of the enzyme.

EXPERIMENTAL

General. — Melting points were recorded on a Kofler hot block. ^{1}H-N.m.r. spectra were recorded with a Varian Gemini 200 (at 200 MHz), Bruker WH 300 (300 MHz), or Bruker AM 500 (500 MHz) spectrometer, and ^{13}C-n.m.r. spectra were recorded with a Varian Gemini 200 (50 MHz) or Bruker 250 (62.9 MHz) spectrometer. Multiplicities were assigned using the DEPT sequence on the Gemini spectrometer and by off-resonance decoupling on the Bruker instrument. Spectra for solutions in D_2O

were referenced to internal methanol. I.r. spectra were recorded with a Perkin–Elmer 297 or 781 spectrophotometer. Mass spectra were recorded with a VG Micromass 30F, ZAB 1F, Masslab 20-250, or Trio-1 GCMS (DB-5 column) spectrometer, using desorption c.i. (NH_3), e.i., c.i. (NH_3), and f.a.b. techniques, as stated. Optical rotations were measured with a Perkin–Elmer 241 polarimeter (1-dm path length). Hydrogenations involved an inflated balloon. Microanalyses were performed at the Dyson Perrins Laboratory. T.l.c. was performed on silica gel $60F_{254}$ with detection by 5% conc. sulphuric acid in methanol, 0.2% cerium(IV) sulphate and 5% ammonium molybdate in 2M sulphuric acid, and 0.5% ninhydrin in methanol. Flash chromatography was carried out using Sorbsil C60 40/60 silica. Ion-exchange chromatography was performed using Dowex 50W-X8 (H^+) and Sigma CG 400 (Cl^-) resins. Dichloromethane was distilled from calcium hydride, methanol from magnesium methoxide, pyridine from (and stored over) potassium hydroxide, and tetrahydrofuran (oxolane) from a purple solution of sodium benzophenone ketyl immediately before use. Hexane was distilled at 68° before use in order to remove involatile fractions. 6-*O*-*tert*-Butyldimethylsilyl-2,3-*O*-isopropylidene-D- (**9**) and -L-mannono-δ-lactam (**17**) were prepared from L- and D-gulonolactone, respectively, as described[30,31].

N-*Benzyl*-4-O-*benzyl*-6-O-tert-*butyldimethylsilyl*-2,3-O-*isopropylidene*-D-*mannono*-δ-*lactam* (**10**). — Sodium hydride (509 mg) was washed with hexane (2 × 10 mL) and to a suspension in dry oxolane (100 mL) was added a solution of **9** (1.69 g, 5.1 mmol) in oxolane (50 mL) with stirring under dry nitrogen for 15 min. Benzyl bromide (1.27 mL, 10.69 mmol) and tetrabutylammonium iodide (100 mg) were added, and the mixture was stirred under dry nitrogen for 15 h when t.l.c. (ethyl acetate) revealed no **9** (R_F 0.4) and one major product, R_F 0.8. Methanol (5 mL) was added, the mixture was diluted with ether (200 mL) and filtered through Celite, the Celite was washed with ether (2 × 100 mL), and the solvent was removed from the combined filtrate and washings under reduced pressure. Flash-column chromatography (ethyl acetate–hexane, 1:5) of the residue gave **10** (2.40 g, 92%), m.p. 72–74°, $[\alpha]_D^{20}$ +50° (*c* 1, chloroform); ν_{max} ($CHCl_3$) 1670 cm^{-1} (C=O). D.c.i.-mass spectrum: *m/z* 512 ($M^+ + H$), 454 ($M^+ + H - Me_2CO$), 91 (100%, $C_7H_7^+$). N.m.r. data ($CDCl_3$): ^1H, δ 7.29 and 7.07 (2 m, 10 H, 2 Ph), 5.53 (d, 1 H), 4.69 (d, 1 H), 4.49 (m, 1 H), 4.15 (m, 3 H), 3.88 (m, 3 H), 3.71 (m, 1 H), 1.52 and 1.37 (2 s, each 3 H, CMe_2), 0.90 (s, 9 H, ^{t}Bu), 0.06 (s, 6 H, $SiMe_2$); ^{13}C, δ 166.61 (s, C-1), 137.92 and 136.90 (2 s, Ar), 128.63, 128.49, 128.32, 127.94, 127.81, and 127.56 (6 d, Ar), 109.89 (s, CMe_2), 75.53, 73.45, and 71.86 (3 d, C-2,3,4), 71.01 (t, C-6), 63.42 (t, $ArCH_2O$), 59.52 (d, C-5), 48.95 (t, $ArCH_2N$), 25.74 [q, $SiC(CH_3)_3$], 25.55 and 23.34 [2 q, $C(CH_3)_2$], 17.98 [s, $SiC(CH_3)_3$], −5.57 (q, $SiCH_3$).

N-*Benzyl*-4-O-*benzyl*-1,5-*dideoxy*-1,5-*imino*-2,3-O-*isopropylidene*-D-*mannitol* (**11**). — To a solution of **10** (2.40 g, 4.3 mmol) in dry oxolane (50 mL) was added lithium aluminium hydride (331 mg, 8.6 mmol). The mixture was stirred under dry nitrogen for 15 min when t.l.c. (ethyl acetate–hexane, 1:5) showed no **10** (R_F 0.2) and one major product, R_F 0.3. Aluminium trichloride (579 mg, 4.3 mmol) was added, and the mixture was stirred for a further 15 min when t.l.c. (ethyl acetate–hexane, 1:5) showed one major product, R_F 0.4. The reaction was quenched with saturated aqueous sodium sulphate

(25 mL), the aqueous phase was extracted with dichloromethane (3 × 25 mL), the organic phases were combined and dried ($MgSO_4$), and the solvents were removed under reduced pressure. Flash-column chromatography (ethyl acetate–hexane, 1:3) of the residue gave **11** as a colourless oil (1.37 g, 82%), $[\alpha]_D^{20}$ +8° (*c* 0.5, chloroform); ν_{max} (film) 3300 cm^{-1} (br, OH). D.c.i.-mass spectrum: *m/z* 384 ($M^+ + H$), 352 ($M^+ + H - MeOH$). N.m.r. data ($CDCl_3$): ^1H, δ 7.32 (m, 10 H, Ar), 4.85 and 4.66 (2 d, 2 H, $ArCH_2O$), 4.32 (m, 2 H), 3.98 and 3.60 (2 d, 2 H, $ArCH_2N$), 3.80 (m, 3 H), 2.93 (dd, 1 H), 2.71 (m, 3 H), 1.55 and 1.37 (2 s, each 3 H, CMe_2); ^{13}C, δ 138.64 and 138.52 (2 s, Ar), 129.11, 128.64, 128.13, 127.98, and 127.49 (5 d, Ar), 109.27 (s, CMe_2), 77.99 (d), 76.73 (d), 72.76 (t), 72.28 (d), 62.83 (d), 60.46 (t), 57.16 (t), 49.22 (t), 27.46 and 25.39 [2 q, $C(CH_3)_2$].

Anal. Calc. for $C_{23}H_{29}NO_4$: C, 72.06; H, 7.57; N, 3.65. Found: C, 72.36; H 7.57; N, 3.67.

N-*Benzyl-4*-O-*benzyl-1,5,7,8-tetradeoxy-1,5-imino-2,3*-O-*isopropylidene*-DL-glycero-D-manno-*oct-7-enitol* (**12**). — To methyl sulphoxide (1.09 mL, 15.4 mmol) in dry dichloromethane (25 mL) under dry nitrogen at −40° was added oxalyl chloride (686 μL, 7.8 mmol) dropwise with stirring. After 15 min, a solution of **11** (1.37 g, 2.6 mmol) in dry dichloromethane (25 mL) was added dropwise, and the mixture was stirred for a further 15 min. Triethylamine (2.5 mL, 17.9 mmol) was added, the mixture was stirred for 30 min, then washed with 2M hydrochloric acid, saturated aqueous copper sulphate, and saturated aqueous sodium hydrogencarbonate, and dried ($MgSO_4$), and the solvent was removed under reduced pressure.

To a solution of the residue in dry oxolane (50 mL) was added M vinylmagnesium bromide in oxolane (7.8 mL, 7.8 mmol). The mixture was stirred under dry nitrogen for 2 h when t.l.c. (ethyl acetate–hexane, 1:3) showed one product, R_F 0.7. Saturated aqueous ammonium chloride (25 mL) was added, the aqueous phase was extracted with dichloromethane (3 × 25 mL), the combined extracts were dried ($MgSO_4$), and the solvent was removed under reduced pressure. Flash-column chromatography of the residue gave **12** as a 1:1 mixture of diastereomers (880 mg, 80%). D.c.i.-mass spectrum: *m/z* 410 (100%, $M^+ + H$), 352 ($M^+ + H - Me_2CO$).

N-*Benzyl-4*-O-*benzyl-6*-O-tert-*butyldimethylsilyl-1,5,7,8-tetradeoxy-1,5-imino-2,3*-O-*isopropylidene*-DL-glycero-D-manno-oct-*7-enitol* (**13**). — A solution of **12** (880 mg, 2.2 mmol) in dry *N,N*-dimethylformamide (50 mL) was stirred under dry nitrogen with imidazole (293 mg, 4.3 mmol) and *tert*-butyldimethylsilyl chloride (488 mg, 3.2 mmol) for 15 h when t.l.c. (ethyl acetate–hexane, 1:5) revealed no **12** (R_F 0.3) and one major product, R_F 0.8. Methanol (5 mL) was added to the mixture, the solvents were removed under reduced pressure, a solution of the residue in brine (100 mL) was extracted with dichloromethane (3 × 100 mL), the combined extracts were dried ($MgSO_4$), and the solvent was removed under reduced pressure. Flash-column chromatography (ether–hexane; 1:10) of the residue gave **13** as a mixture of diastereomers (823 mg, 73%). D.c.i.-mass spectrum: *m/z* 524 ($M^+ + H$).

Anal. Calc. for $C_{31}H_{45}NO_4Si$: C, 71.51; H, 8.75; N, 2.61. Found: C, 71.32; H, 8.96; N, 2.32.

N-*Benzyl-4-O-benzyl-6-O*-tert-*butyldimethylsilyl-1,5,7-trideoxy-1,5-imino-2,3-O-isopropylidene*-D-(**14**) *and*-L-glycero-D-manno-*octitol* (**15**). — To a stirred solution of **13** (823 mg, 1.58 mmol) in dry oxolane (15 mL) under dry nitrogen was added 10M borane–dimethyl sulphide complex, and the mixture was stirred for 30 min when t.l.c. (ethyl acetate–hexane, 1:7) revealed no **13** (R_F 0.8) and two close-running products, R_F 0.6. Water was added dropwise until all effervescence had ceased, followed by 3M sodium hydroxide (1.56 mL) and aqueous 30% hydrogen peroxide (0.5 mL). The mixture was stirred for 15 h when t.l.c. (ethyl acetate–hexane, 1:7) revealed only two close-running products, R_F 0.2. The mixture was treated with brine (20 mL) and extracted with dichloromethane (3 × 20 mL), the combined extracts were dried ($MgSO_4$), and the solvent was removed under reduced pressure. Flash-column chromatography (ethyl acetate–hexane, 1:10) of the residue yielded **14** (207 mg, 25%) and **15** (155 mg, 19%).

Compound **15** had $[\alpha]_D^{20}$ +3° (*c* 0.35, choroform); ν_{max} 3300 cm^{-1} (br, OH). D.c.i.-mass spectrum: *m/z* 542 ($M^+ + H$). N.m.r. data ($CDCl_3$): ^{13}C, δ 138.86 and 138.53 (2 s, Ar), 129.21, 128.56, 128.23, 127.91, and 127.41 (5 d, Ar), 109.06 (s), 78.97 (d), 76.84 (d), 72.54 (t), 71.81 (d), 69.93 (d), 65.85 (d), 60.17 (t), 58.68 (t), 47.69 (t), 34.99 (t), 27.62 and 25.43 (2 q), 25.81 (q), 17.84 (s), −4.51 (q).

Anal. Calc. for $C_{31}H_{47}NO_5Si$: C, 68.76; H, 8.69; N, 2.59. Found: C, 69.02; H, 8.43; N, 2.99.

Compound **14** had $[\alpha]_D^{20}$ +20° (*c* 0.5, chloroform). D.c.i.-mass spectrum: *m/z* 542 ($M^+ + H$). N.m.r. data ($CDCl_3$): ^{13}C, δ 138.87 and 138.46 (2 s, Ar), 129.73, 128.62, 128.42, 127.51, and 127.38 (5 d, Ar), 109.02 (s), 79.69 (d), 74.49 (d), 73.50 (t), 71.67 (d), 70.52 (d), 64.55 (d), 58.46 (t), 55.68 (t), 47.81 (t), 37.94 (t), 27.76 and 25.34 (2 q), 25.89 (q), 17.99 (s), −4.06 (q).

(*1*R,*6*R,*7*R,*8*R,*8a*R)-*1,6,7,8-Tetrahydroxyoctahydroindolizine (6-epicastanospermine,* **3**). — To a solution of **14** (155 mg, 0.29 mmol) in dry dichloromethane (5 mL) were added triethylamine (60 μL, 0.43 mmol) and methanesulphonyl chloride (32 μL, 0.43 mmol). The mixture was stirred under dry nitrogen for 15 h when t.l.c. (ethyl acetate–hexane, 1:3) revealed baseline material only. The solvents were removed under reduced pressure and the residue was purified by flash-column chromatography (methanol–dichloromethane, 1:9).

A solution of the product in methanol (5 mL) was stirred under hydrogen with a catalytic amount of palladium black. After 15 h, t.l.c. (methanol–dichloromethane, 1:9) revealed only one product, R_F 0.6. The mixture was filtered through Celite which was washed with methanol. The solvents from the combined filtrate and washings were removed under reduced pressure and a solution of the residue in trifluoroacetic acid–water (1:1, 5 mL) was stored for 2 h when t.l.c. (ethanol–chloroform–ammonia, 45:45:10) revealed one product, R_F 0.2. The solvent was removed under reduced pressure and toluene (2 × 5 mL) was evaporated from the residue. Flash-column chromatography (ethanol–chloroform–ammonia, 45:45:10) followed by ion-exchange chromatography yielded **3** (41 mg, 82%), $[\alpha]^{24}$ (*c* 0.7, methanol): +2° (589), +2° (578), +3° (546), +4° (436), +11° (365). D.c.i.-mass spectrum: *m/z* 190 ($M^+ + H$). N.m.r.

data (D_2O): ^1H, δ 4.28 (ddd, 1 H, H-1), 3.88 (dd, 1 H, H-6), 3.76 (dd, 1 H, H-8), 3.42 (dd, 1 H, H-7), 3.00 (m, 2 H, H-3,5), 2.21 (m, 2 H, H-2′, 5′), 2.07 (dd, 1 H, H-3), 1.84 (dd, 1 H, H-4), 1.61 (m, 1 H, H-2′); ^{13}C, δ 78.0 (d), 74.5 (d), 72.7 (d), 71.5 (d), 69.8 (d), 58.0 (t), 54.6 (t), 35.4 (t).

The hydrochloride of **3** had m.p. 160° (dec.) (from ethanol–water), $[\alpha]_D^{20} \sim 0°$ (*c* 1.5, water). N.m.r. data (D_2O): ^1H, δ 1.90 (m, 1 H), 2.41 (m, 1 H), 3.00 (br.m., 3 H), 3.47 (dd, 1 H), 3.60 (dd and m, 2 H), 3.97 (dd, 1 H), 4.09 (d, 1 H), 4.51 (m, 1 H); ^{13}C, δ 74.3 (d), 72.7 (d), 68.4 (d), 67.9 (d), 65.8 (d), 55.7 (t), 52.9 (t), 32.5 (t).

(*1S,6R,7R,8R,8aR*)-*1,6,7,8-Tetrahydroxyoctahydroindolizine (1,6-diepicastanospermine*, **4**). — To a solution of **15** (207 mg, 0.56 mmol) in dry dichloromethane (5 mL) were added triethylamine (78.5 μL, 0.69 mmol) and methanesulphonyl chloride (43 μL, 0.69 mmol), and the mixture was stirred under dry nitrogen for 15 h when t.l.c. (ethyl acetate–hexane, 1:3) revealed baseline material only. The solvents were removed under reduced pressure and the residue was purified by flash-column chromatography (methanol–dichloromethane, 1:9).

A solution of the residue in methanol (5 mL) was stirred under hydrogen with a catalytic amount of palladium black. After 15 h, t.l.c. (methanol–dichloromethane, 1:9) revealed no **15** (R_F 0.2) and one product, R_F 0.6. The mixture was worked-up as for **3**, to give **4** (43 mg, 70%), $[\alpha]^{24}$ (*c* 0.7, methanol) −72° (589), −75° (578), −85° (546), −140° (436), −212° (365). D.c.i.-mass spectrum: *m/z* 190 ($M^+ + H$). N.m.r. data (D_2O): ^1H, δ 4.13 (ddd, 1 H, H-1), 3.88 (dd, 1 H, H-6), 3.54 (dd, 1 H, H-8), 3.38 (dd, 1 H, H-7), 2.92 (dd, 1 H, H-5), 2.80 (dd, 1 H, H-3), 2.36 (dd, 1 H, H-3′), 2.15 (d, 1 H, H-2′), 1.88 (dd, 1 H, H-4), 1.51 (dddd, 1 H, H-2); ^{13}C, δ 75.7 (d), 74.5 (d), 74.0 (d), 72.0 (d), 69.3 (d), 55.67 (t), 51.8 (t), 32.8 (t).

N-*Benzyl-4*-O-*benzyl-6*-O-tert-*butyldimethylsilyl-2,3*-O-*isopropylidene*-L-*manno-no-δ-lactam* (**18**). — Sodium hydride (1.09 g) was washed with hexane (2 × 10 mL), and to a suspension in dry oxolane (100 mL) was added a solution of **17** (3.62 g, 10.9 mmol) in oxolane (50 mL) with stirring under dry nitrogen for 15 min. Benzyl bromide (2.73 mL, 22.89 mmol) and tetrabutylammonium iodide (500 mg) were added, and the mixture was stirred under dry nitrogen for 15 h when t.l.c. (ethyl acetate) revealed no **17** (R_F 0.4) and one major product, R_F 0.8. Methanol (5 mL) was added, the mixture was diluted with ether (200 mL) and filtered through Celite which was then washed with ether (2 × 100 mL), and the solvent was removed under reduced pressure from the combined filtrate and washings. Flash-column chromatography (ethyl acetate–hexane, 1:5) of the residue gave **18** (5.22 g, 94%), m.p. 72–73°, $[\alpha]_D^{20}$ −53° (*c* 0.45, chloroform); ν_{max} ($CHCl_3$) 1670 cm^{-1} (C=O). D.c.i.-mass spectrum: *m/z* 512 ($M^+ + H$), 454 ($M^+ + H - Me_2CO$), 81 (100%, $C_7H_7^+$). N.m.r. data ($CDCl_3$): ^1H, δ 7.29 and 7.07 (2 m, 10 H), 5.53 (d, 1 H), 4.69 (d, 1 H), 4.49 (m, 1 H), 4.15 (m, 3 H), 3.88 (m, 3 H), 3.71 (m, 1 H), 1.52 and 1.37 (2 s, each 3 H), 0.90 (s, 9 H), 0.06 (s, 6 H); ^{13}C, δ 166.61 (s, C-1), 137.29 and 136.90 (2 s, Ar), 128.63, 128.49, 128.32, 127.94, 127.81, and 127.56 (6 d, Ar), 109.89 (s, CMe_2), 75.53, 73.45, and 71.86 (3 d, C-2,3,4), 71.01 (t, C-6), 63.42 (t, $ArCH_2O$), 59.52 (d, C-5), 48.95 (t, $ArCH_2N$), 25.74 (q, $SiCMe_3$), 25.55 and 23.34 [2 q, $C(CH_3)_2$], 17.98 [S, $SiC(CH_3)_3$], −5.57 (q, CH_3Si).

Anal. Calc. for $C_{29}H_{41}NO_5Si$: C, 68.10; H, 8.02; N, 2.74. Found: C, 68.25; H, 8.32; N, 2.68.

N-*Benzyl-4*-O-*benzyl-1,5-dideoxy-1,5-imino-2,3*-O-*isopropylidene*-L-*mannitol* (**19**). — To a solution of **18** (3.00 g, 5.4 mmol) in dry oxolane (50 mL) was added lithium aluminium hydride (413 mg, 10.8 mmol). The mixture was stirred under dry nitrogen for 15 min when t.l.c. (ethyl acetate–hexane, 1:5) showed no **18** (R_F 0.2) and one major product, R_F 0.3. Aluminium trichloride (720 mg, 5.4 mmol) was added, and the mixture was stirred for a further 15 min when t.l.c. (ethyl acetate–hexane, 1:5) showed one major product, R_F 0.4. The reaction was quenched with saturated aqueous sodium sulphate (25 mL), the aqueous phase was extracted with dichloromethane (3 × 25 mL), the combined organic phases were dried ($MgSO_4$), and the solvents were removed under reduced pressure. Flash-column chromatography (ethyl acetate–hexane, 1:3) of the residue yielded **19** as a colourless oil (1.73 g, 82%), $[\alpha]_D^{20}$ −6.9° (*c* 1.6, chloroform); ν_{max} (film) 3300 cm^{-1} (br, OH). D.c.i.-mass spectrum: *m/z* 384 ($M^+ + H$), 352 ($M^+ + H - MeOH$). N.m.r. data ($CDCl_3$): ^1H, δ 7.32 (m, 10 H, Ar), 4.85 and 4.66 (2 d, 2 H, $ArCH_2O$), 4.32 (m, 2 H), 3.98 and 3.60 (2 d, 2 H, $ArCH_2N$), 3.80 (m, 3 H), 2.93 (dd, 1 H), 2.71 (m, 3 H), 1.55 and 1.37 (2 s, each 3 H, CMe_2); ^{13}C, δ 138.64 and 138.52 (2 s), 129.11, 128.64, 128.13, 127.98, and 127.49 (5 d), 109.27 (d), 77.99 (d), 76.73 (d), 72.76 (t), 72.28 (d), 62.83 (d), 60.46 (t), 57.16 (t), 49.22 (t), 27.46 and 25.39 (2 q).

Anal. Calc. for $C_{23}H_{29}NO_4$: C, 72.06; H, 7.57; N, 3.65. Found: C, 71.95; H, 7.47; N, 3.45.

N-*Benzyl-4*-O-*benzyl-1,5,7,8-tetradeoxy-1,5-imino-2,3*-O-*isopropylidene*-DL-glycero-L-manno-*oct-7-enitol* (**20**). — To methyl sulphoxide (1.64 mL, 23.1 mmol) in dry dichloromethane (25 mL) under dry nitrogen at −40° was added oxalyl chloride (1.03 mL, 11.8 mmol) dropwise with stirring. After 15 min, a solution of **19** (1.50 g, 3.9 mmol) in dry dichloromethane (25 mL) was added dropwise and the mixture was stirred for a further 15 min. Triethylamine (4.7 mL, 33.7 mmol) was added, the mixture was stirred for 30 min, then washed with saturated aqueous copper sulphate (100 mL) and saturated aqueous sodium hydrogencarbonate (100 mL), and dried ($MgSO_4$), and the solvent was removed under reduced pressure.

To a solution of the residue in dry oxolane (50 mL) was added M vinylmagnesium bromide in oxolane (11.7 mL, 11.7 mmol). The mixture was stirred under dry nitrogen for 2 h when t.l.c. (ethyl acetate–hexane, 1:3) showed one product, R_F 0.7. Saturated aqueous ammonium chloride (25 mL) was added, the aqueous phase was extracted with dichloromethane (3 × 50 mL), the combined organic solutions were dried ($MgSO_4$), and the solvent was removed under reduced pressure. Flash-column chromatography of the residue yielded **20** as a mixture of diastereomers (1.17 g, 73%). D.c.i.-mass spectrum: *m/z* 410 (100%, $M^+ + H$), 352 ($M^+ + H - Me_2CO$).

N-*Benzyl-4*-O-*benzyl-6*-O-tert-*butyldimethylsilyl-1,5,7,8-tetradeoxy-1,5-imino-2,3*-O-*isopropylidene*-DL-glycero-L-manno-*oct-7-enitol* (**21**). — A solution of **20** (1.17 g, 2.9 mmol) in dry *N,N*-dimethylformamide (50 mL) under dry nitrogen was stirred with imidazole (390 mg, 5.7 mmol) and *tert*-butyldimethylsilyl chloride (650 mg, 4.3 mmol) for 15 h when t.l.c. (ethyl acetate–hexane, 1:5) revealed no **20** (R_F 0.3) and one major

product, R_F 0.8. Methanol (5 mL) was added, the solvents were removed under reduced pressure, a solution of the residue in brine (100 mL) was extracted with dichloromethane (3 × 100 mL), the combined extracts were dried ($MgSO_4$), and the solvent was removed under reduced pressure. Flash-column chromatography (ether–hexane, 1:10) of the residue yielded **21** as a mixture of diastereomers (1.27 g, 85%). D.c.i.-mass spectrum: *m/z* 524 ($M^+ + H$).

Anal. Calc for $C_{31}H_{45}NO_4Si$: C, 71.51; H, 8.75; N, 2.61. Found: C, 71.40; H, 8.75; N, 2.40.

N-*Benzyl-4*-O-*benzyl-6*-O-tert-*butyldimethylsilyl-1,5,7-trideoxy-1,5-imino-2,3*-O-*isopropylidene*-L-(**22**) *and*-D-glycero-L-manno-*octitol* (**23**). — To a stirred solution of **21** (390 mg, 0.75 mmol) in dry oxolane (15 mL) was added M diborane in oxolane (2.5 mL). The mixture was stirred for 30 min when t.l.c. (ethyl acetate–hexane, 1:7) revealed no **21** (R_F 0.8) and two close-running products, R_F 0.6. Water was added dropwise to the mixture until all effervescence had ceased, followed by 3M hydroxide (750 μL) and aqueous 30% hydrogen peroxide (250 μL), and the mixture was stirred for 15 h when t.l.c. (ethyl acetate–hexane, 1:7) revealed only two close-running products, R_F 0.2. The mixture was treated with brine (20 mL), and extracted with dichloromethane (3 × 25 mL), the combined extracts were dried ($MgSO_4$), and the solvent was removed under reduced pressure. Flash-column chromatography (ethyl acetate–hexane, 1:10) of the residue yielded **22** (97 mg, 24%) and **23** (93 mg, 23%).

Compound **23** had $[\alpha]_D^{20}$ −5° (*c* 1, chloroform); ν_{max} 3300 cm^{-1} (br, OH). D.c.i.-mass spectrum: *m/z* 542 ($M^+ + H$). N.m.r. data ($CDCl_3$): ^{13}C, δ 138.86 and 138.53 (2 s), 129.21, 128.56, 128.23, 127.91, and 127.41 (5 d), 109.06 (s), 78.97 (d), 76.84 (d), 72.54 (t), 71.81 (d), 69.93 (d), 65.85 (d), 60.17 (t), 58.68 (t), 47.69 (t), 34.99 (t), 27.62 and 25.43 (2 q), 25.81 (q), 17.84 (s), −4.51 (q).

Anal. Calc. for $C_{31}H_{47}NO_5Si$: C, 68.76; H, 8.69; N, 2.59. Found: C, 68.89; H, 8.79; N, 2.34.

Compound **22** had $[\alpha]_D^{20}$ −20° (*c* 0.8, chloroform). D.c.i.-mass spectrum: *m/z* 542 ($M^+ + H$). N.m.r. data ($CDCl_3$): ^{13}C, δ 138.87 and 138.46 (2 s), 129.73, 128.62, 128.42, 127.51, and 127.38 (5 d), 109.02 (s), 79.69 (d), 74.49 (d), 73.50 (t), 71.67 (d), 70.52 (d), 64.55 (d), 58.46 (t), 55.68 (t), 47.81 (t), 37.94 (t), 27.76 and 25.34 (2 q), 25.89 (q), 17.99 (s), −4.06 (q).

Anal. Found: C, 68.54; H, 8.79; N, 2.34.

N-*Benzyl-4*-O-*benzyl-6*-O-tert-*butyldimethylsilyl-2,3*-O-*isopropylidene-(1S,6S,7S,8S,8aS)-1,6,7,8-tetrahydroxyoctahydroindolizinium methanesulphonate* (**24**). — To a solution of **22** (58 mg, 0.11 mmol) in dry dichloromethane (5 mL) were added triethylamine (22 μL, 0.16 mmol) and methanesulphonyl chloride (12 μL, 0.16 mmol). The mixture was stirred under dry nitrogen for 15 h when t.l.c. (ethyl acetate–hexane, 1:3) revealed baseline material only. The solvents were removed under reduced pressure and flash-column chromatography (methanol–dichloromethane, 1:9) of the residue yielded **24**. F.a.b.-mass spectrum: *m/z* 524 (M^+), 91 (100%, $C_7H_7^+$).

*(1S,6S,7S,8S,8aS)-1,6,7,8-tetrahydroxyoctahydroindolizine (*L-*6-epicastanospermine,* **5**). — A solution of **24** (56 mg) in methanol (5 mL) was stirred under hydrogen

with a catalytic amount of palladium black. After 15 h, t.l.c. (methanol–dichloromethane, 1:9) revealed no **24** (R_F 0.2) and one product, R_F 0.6. The mixture was filtered through Celite which was then washed with methanol. The solvents were removed under reduced pressure from the combined filtrate and washings, and a solution of the residue in trifluoroacetic acid–water (1:1, 5 mL) was left to stand for 2 h when t.l.c. (ethanol–chloroform–ammonia, 45:45:10) revealed one product, R_F 0.2. The solvent was removed under reduced pressure and toluene (2 × 5 mL) was evaporated from the residue. Flash-column chromatography (ethanol–chloroform–ammonia, 45:45:10) followed by ion-exchange chromatography yielded **5** (12 mg, 79%), $[\alpha]^{24}$ (*c* 0.25, methanol) −2° (589), −2° (578), −2° (546), −5° (436), −12° (365). D.c.i.-mass spectrum: *m/z* 190 ($M^+ + H$). N.m.r. data (D_2O): ^1H, δ 4.28 (ddd, 1 H, H-1), 3.88 (dd, 1 H, H-6), 3.76 (dd, 1 H, H-8), 3.42 (dd, 1 H, H-7), 3.00 (m, 2 H, H-3,5), 2.21 (m, 2 H, H-2′, 5′), 2.07 (dd, 1 H, H-3), 1.84 (dd, 1 H, H-4), 1.61 (m, 1 H, H-2′); ^{13}C, δ 78.0 (d), 74.5 (d), 72.7 (d), 71.5 (d), 69.8 (d), 58.0 (t), 54.6 (t), 35.4 (t).

N-*Benzyl-4*-O-*benzyl-6*-O-tert-*butyldimethylsilyl-2,3*-O-*isopropylidene-(1*R,*6*S,*7*S,*8*S,*8a*S)-*1,6,7,8-tetrahydroxyoctahydroindolizinium methanesulphonate* (**25**). — To a solution of **23** (48 mg, 0.13 mmol) in dry dichloromethane (5 mL) were added triethylamine (22 μL, 0.16 mmol) and methanesulphonyl chloride (12 μL, 0.16 mmol). The mixture was stirred under dry nitrogen for 15 h when tl.c. (ethyl acetate–hexane, 1:3) revealed baseline material only. The solvents were removed under reduced pressure and flash-column chromatography (methanol–dichloromethane, 1:9) of the residue gave **25**. F.a.b.-mass spectrum: *m/z* 524 (M^+), 91 (100%, $C_7H_7^+$).

(*1*R,*6*S,*7*S,*8*S,*8a*S)-*1,6,7,8-Tetrahydroxyoctahydroindolizine* (L-*1,6-diepicastanospermine*, **6**). — A solution of **25** (34 mg) in methanol (5 mL) was stirred under hydrogen with a catalytic amount of palladium black. After 15 h, t.l.c. (methanol–dichloromethane, 1:9) revealed no **25** (R_F 0.2) and one product, R_F 0.6. The mixture was filtered through Celite which was then washed with methanol. The solvents were removed under reduced pressure from the combined filtrate and washings, and a solution of the residue in trifluoroacetic acid–water (1:1, 5 mL) was stored for 2 h when t.l.c. (ethanol–chloroform–ammonia, 45:45:10) revealed one product, R_F 0.2. The solvent was removed under reduced pressure and toluene (2 × 5 mL) was evaporated from the residue. Flash-column chromatography (ethanol–chloroform–ammonia, 45:45:10) followed by ion-exchange chromatography yielded **6** (7.8 mg, 85%), $[\alpha]^{24}$ (*c* 0.5, methanol) +70° (589), +73° (578), +83° (546), +137° (436), +209° (365). D.c.i.-mass spectrum: *m/z* 190 ($M^+ + H$). N.m.r. data (D_2O): ^1H, δ 4.13 (ddd, 1 H, H-1), 3.88 (dd, 1 H, H-6), 3.54 (dd, 1 H, H-8), 3.38 (dd, 1 H, H-7), 2.92 (dd, 1 H, H-5), 2.80 (dd, 1 H, H-3), 2.36 (dd, 1 H, H-3′), 2.30 (d, 1 H, H-5′), 2.15 (ddd, 1 H, H-2′), 1.88 (dd, 1 H, H-4), 1.51 (dddd, 1 H, H-2); ^{13}C, δ 75.7 (d), 74.5 (d), 74.0 (d), 72.0 (d), 69.3 (d), 55.7 (t), 51.8 (t), 32.7 (t).

Enzyme inhibition studies. — Each assay mixture consisted of mM *p*-nitrophenyl α-D-glucopyranoside, 25mM sodium citrate buffer (pH 5.0), and an appropriate amount of amyloglucosidase (EC 3.2.1.3; Sigma) in a total assay volume of 1 mL. The mixture was incubated at 37° for 15 min, then quenched with 0.1M sodium carbonate (2 mL), and

the *p*-nitrophenol released was determined spectrophotometrically at 400 nm. From plots of percentage inhibition against inhibitor concentration, the values for 50% inhibition were obtained.

The isolation of the human liver glycosidases and the methods of assay of their inhibition have been described[35].

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