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Synthesis and evaluation of 1-deoxy-8-*epi*-castanospermine, 1-deoxy-8-hydroxymethyl castanospermine, and (6*S*,7*S*,8*R*,8a*R*)-8-amino-octahydroindolizine-6,7-diol

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Abstract—A short, versatile, and enantioselective synthesis of 1-deoxy-8-*epi*-castanospermine (**5**), 1-deoxy-8-hydroxymethyl castanospermine (**6**), and (6*S*,7*S*,8*R*,8a*R*)-8-amino-octahydroindolizine-6,7-diol (**7**) is achieved from a common template **12**. The key step utilized is PET provoked amine radical cyclization of **11** to **12** in excellent diastereoselectivity. The exocyclic double bond at C-8 of the template is functionalized to obtain **5**–**7** as exclusive diastereomers. 1-Deoxy-8-*epi*-castanospermine exhibited inhibition of α- and β-galactosidase and β-glucosidase. Compounds **6** and **7** were found to be weak inhibitors of β-glucosidase.

1. Introduction

The polyhydroxylated 1-azabicyclo[4.3.0]nonane skeleton comprising indolizidine alkaloids such as (+)-castanospermine[1] (**1**), (+)-6-*epi*-castanospermine[2] (**2**), and swainsonine[3] (**8**) (Fig. 1), which are regarded as a bicyclic derivatives of 1-deoxynojirimycin (1-DNJ) with an ethylene bridge between the hydroxymethyl group and the nitrogen at the ring junction, are known to exhibit potent activity against glucosidases and antiviral properties against a number of viruses.[4–7] However, unfortunately, castanospermine (**1**) is also found to inhibit intestinal sucrases causing osmotic diarrhea.[8] In an attempt to minimize the side effects of castanospermine and to establish possible structure–activity relationship, various stereoisomers of **1**[9–11] and its derivatives such as *O*-acyl-[9–10] and several analogs of deoxy-castanospermines (**4**)[12–22] have been synthesized and evaluated. From the success of these studies, Celgosivir (6-*O*-butanoyl derivative of castanospermine, **3**) is currently under clinical phase II trials[23] (Fig. 1). Though 1-deoxy-8-*epi*-castanospermine (**5**) has been synthesized by Martin et al.[24] and Majewski et al.,[25] no effort was made to study its enzyme inhibition properties. Moreover, the synthetic steps used by these workers are also lengthy and low yielding. Considering the unexplored biological significance of this molecule and as a part of our ongoing program on developing novel glycomimetics,[26] we have developed a short and versatile synthesis of **5** and two other new analogs such as 1-deoxy-8-hydroxymethyl castanospermine (**6**) and its *C*-8-amino derivative **7**, and describe herein the synthetic details along with the enzyme inhibition study.

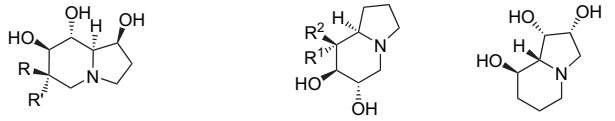

Figure 1. Polyhydroxylated indolizidine alkaloids.

2. Results and discussion

The synthesis of **5** was carried out through the steps as outlined in Scheme 1. The designed template **12** was obtained by the photoinduced electron transfer (PET) cyclization of the acetylene tethered amine **11**, a protocol well established by our group.[27] The amine **11** was prepared via reductive amination of aldehyde **9** with amine **10** using sodium triacetoxyborohydride as a reducing agent[28] (Scheme 1). The preparation of aldehyde **9**[29] and amine **10**[30] is already described earlier by our group. The PET cyclization of **11**

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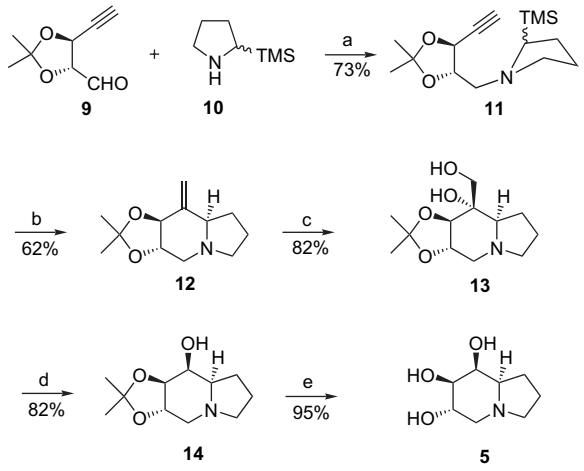
essentially involved the irradiation of a dilute solution of **11** (3 mmol) and 1,4-dicyanonaphthalene (0.4 mmol) in *iso*-propanol (250 mL) through a Pyrex filtered light emanating from a 450 W Hanovia medium pressure lamp. Usual work-up and purification of the photolyzate by column chromatography produced **12** as a single diastereomer in 62% yield (Scheme 1), which was fully characterized by extensive ^{1}H NMR, ^{13}C NMR, and ^{1}H–^{1}H NOESY and HETCOR ^{1}H–^{13}C spectral analyses.



Scheme 1. Synthesis of 1-deoxy-8-*epi*-castanospermine. Reagents and conditions: (a) $Na(OAc)_3BH$, 1,2-dichloroethane, rt, 8 h, rt; (b) hν, DCN, *iso*-propanol, 30 min; (c) OsO_4, $K_3Fe(CN)_6$, K_2CO_3, *t*-BuOH–H_2O (1:1), 12 h, rt; (d) (i) $NaIO_4$, silica gel, CH_2Cl_2, 10 min; (ii) $NaBH_4$, MeOH, rt, 6 h; (e) 1 M HCl, MeOH, 3 h.

Osmium tetroxide dihydroxylation of **12** afforded **13** in 82% yield. The stereochemical outcome of **13** was adjudged by ^{1}H–^{1}H COSY, HETCOR ^{1}H–^{13}C, and ^{1}H–^{1}H NOESY as shown in Figure 2 and Table 1. The diol **13** upon sodium periodate oxidation afforded the corresponding ketone, which on sodium borohydride reduction gave **14** in 82% yield as the only diastereomer. The ^{1}H NMR data for **14** are in full

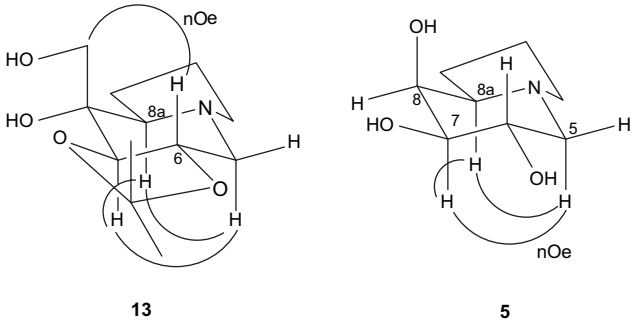
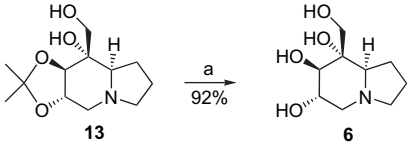


Figure 2. Graphical summary of NOESY spectrum observed for **13** and **5**.

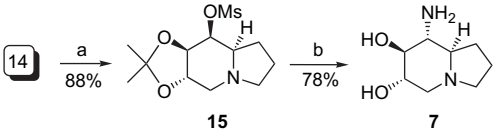
harmony with the literature data.[25] Removal of the acetonide protecting group from **14** afforded 1-deoxy-8-*epi*-castanospermine (**5**) in 95% yield. Although, the ^{1}H NMR data of **5** matched well with the values reported by Martin et al.,[24] to complete the stereochemical assignments we carried out extensive ^{1}H–^{1}H COSY, HETCOR ^{1}H–^{13}C, and ^{1}H–^{1}H NOESY spectral studies and details are summarized in Figure 2 and Table 1.

Having developed a simple strategy for synthesizing **13** as an intermediate for the synthesis of **5**, we realized that it would be interesting to obtain a new compound **6**, hitherto unknown, for evaluation as a glycosidase inhibitor. In this context, acetonide deprotection of **13**, brought about by stirring with 1 M HCl in methanol for 3 h, gave **6** in 92% yield (Scheme 2).



Scheme 2. Reagents and conditions: (a) 1 M HCl, MeOH, 3 h.

Furthermore, another new analog **7** having a basic amine moiety at C-8 was also visualized to be easily affordable from **14** for evaluation as a new glycosidase inhibitor. Toward this end, C-8 hydroxy moiety of **14** was first converted to the corresponding mesylate derivative **15**, which upon nucleophilic displacement with azide followed by catalytic hydrogenation (Pd–C, 10%) and acetonide deprotection afforded (6*S*,7*S*,8*R*,8a*R*)-8-amino-octahydroindolizine-6,7-diol (**7**) in 78% yield (Scheme 3).



Scheme 3. Reagents and conditions: (a) MsCl, Py, rt, 4 h; (b) (i) LiN_3, DMF, 110 °C, 16 h; (ii) H_2, Pd–C, atm, MeOH, 7 h; (iii) 1 M HCl, MeOH, 4 h.

3. Glycosidase inhibitory study

The inhibitory activities of **5–7** were screened against β-galactosidase (*Aspergillus oryzae*), α-galactosidase (coffee beans), β-glucosidase/β-mannosidase (almonds), α-glucosidase (yeast), and α-mannosidase (jack beans), and the results are summarized in Table 2.

None of the prepared compounds inhibited α-glucosidase and α-/β-mannosidases. The 1-deoxy-8-*epi*-castanospermine

Table 1. Selective coupling constants *J* (Hz) for **13** and **5**

	H_5-Axial	H_5-Equatorial	H_6-Axial	H_7-Axial	H_8-Equatorial	H_{8a}-Axial
13	2.16 (app t), $J_{5\alpha,5\beta}=J_{5\alpha,6\beta}=9.8$	3.26 (dd), $J_{5\beta,5\alpha}=9.6$, $J_{5\beta,6\beta}=4.1$	3.80 (dt), $J_{6\beta,7\alpha}=9.8$	3.49 (d), $J_{7\alpha,6\beta}=9.5$	No hydrogen	2.19–2.26, merged with H_3
5	2.94 (app. t), $J_{5\alpha,5e}=J_{5\alpha,6\beta}=11.5$	3.74 (dd), $J_{5\beta,5\alpha}=9.8$, $J_{5\beta,6\beta}=5.3$	4.10 (ddd), $J_{6\beta,7\alpha}=7.7$, $J_{6\beta,5\alpha}=11.4$, $J_{6\beta,7\beta}=5.4$	3.76 (dd), $J_{7\alpha,6\beta}=9.5$, $J_{7\alpha,8\alpha}=3.0$	4.30 (dd), $J_{8\alpha,7\alpha}=3.0$, $J_{8\alpha,8a\alpha}=1.0$	3.53 (ddd), $J_{8a\alpha,8\alpha}=1.0$

Table 2. Inhibition of various glycosidases by **5–7** (K_i in μM)

Inhibitor	Glycosidase					
	β-Gal	α-Gal	β-Glc	α-Glc	β-Man	α-Man
1[a]	—	—	1.5	0.015	—	—
4[b]	—	—	31	7	—	21
5	73	71	33	n.i.	n.i.	n.i.
6	4000	n.i.	942	n.i.	n.i.	n.i.
7	n.i.	n.i.	1400	n.i.	n.i.	n.i.

n.i., No Inhibition at 1 mM; —, means not measured.

[a] Inhibition value K_i, α-glucosidase (rice) and β-glucosidase (almonds) for castanospermine, is referred from Ref. 31.

[b] Percentage inhibition at 1 mM for α-/β-glucosidase (lysosomal) and α-mannosidase (lysosomal), the data for 1-deoxycastanospermine are from Ref. 32.

(**5**) exhibited non-specific competitive inhibition against α-galactosidase (K_i=71 μM), β-galactosidase (K_i=73 μM), and β-glucosidase (K_i=33 μM). To our dismay, 1-deoxy-8-hydroxymethyl castanospermine (**6**) in which C-8 has an additional hydroxymethyl moiety showed weak non-competitive inhibition against β-glucosidase. Similarly, compound **7** in which hydroxy group at C-8 of castanospermine is replaced by an amino group showed weak inhibition against β-glucosidase (Table 2). In the case of **7**, the weak inhibitory activity could be attributed to the more basic amino group at C-8 position than the ring nitrogen thereby forbidding it from binding to active site of α-/β-glucosidase in correct orientation.

4. Conclusions

In conclusion, we have demonstrated a general synthetic strategy to access polyhydroxylated 1-azabicyclo[4.3.0]-nonane skeletons by synthesizing **5–7**. Further inhibitory study will help in understanding the structure–activity relationship of polyhydroxylated indolizidine alkaloids.

5. Experimental

5.1. General

All commercially available reagents were used without further purification. Enzymes were purchased from commercial sources. Reactions requiring dry conditions were performed under an argon atmosphere. 1,2-Dichloroethane and CH_2Cl_2 were distilled from CaH_2 under argon. Column chromatography was performed with silica gel (100–200 mesh). The combined organic layers were dried over Na_2SO_4. Solvents were evaporated under reduced pressure. All yields that are given refer to isolated yields. Optical rotations were measured on a precision automated polarimeter. NMR spectra were recorded on a 200, 400, and 500 MHz spectrometer. Chemical shifts are reported in parts per million. Internal reference for ^{1}H NMR: $SiMe_4$ (δ=0.00) or $CDCl_3$ (δ=7.27), D_2O (δ=4.80); ^{13}C NMR: $CDCl_3$ (δ=77.0). Coupling constants (J values) are reported in hertz. ^{13}C peak multiplicity assignments were made based on DEPT data. IR spectra were recorded on an FTIR spectrometer. MS experiments were performed on a low/high-resolution magnetic sector mass spectrometer.

5.2. 1-{[(4*S*,5*S*)-5-Ethynyl-2,2-dimethyl-1,3-dioxolan-4-yl]methyl}-2-(trimethylsilyl)pyrrolidine (11)

To a solution of **9** (4.5 g, 29.09 mmol) in dry 1,2-dichloroethane (180 mL), amine **10** (4.57 g, 32.00 mmol) followed by sodium triacetoxyborohydride (8.15 g, 38.46 mmol) was added. The mixture was stirred at rt under an argon atmosphere for 8 h. The reaction mixture was cooled with ice and quenched by adding 1 M NaOH till the aqueous layer was basic. The reaction mixture was extracted with ethyl acetate (2×100 mL) and the combined organic extracts were dried over anhydrous Na_2SO_4. The solvent was removed by rotary-evaporation and the residue was purified by column chromatography (silica, pet. ether–ethyl acetate, 9:1) to afford a 1:1 diastereomeric mixture of *title compound* **11** (6.6 g, 73%) as a colorless oil. [Found: C, 64.12; H, 9.71; N, 5.01. $C_{15}H_{27}NO_2Si$ requires: C, 64.01; H, 9.67; N, 4.98%.] R_f (10% EtOAc–hexane) 0.52; ν_{max} (neat) 3311, 2985, 2956, 2119, 1730, 1691, 1456 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 0.00+0.01 (9H, two s, *Me*$_3$Si–), 1.37+1.39+1.43+1.44 (6H, four s for acetonide methyl), 1.50–1.85 (5H, m), 1.90–2.12 (1H, m), 2.28–2.40 (1H, m), 2.42–2.51 (1H, m), 2.80–3.02 (1H, m), 3.12–3.34 (1H, m), 4.09–4.27 (1H, m), 4.32–4.47 (1H, two sets of dd, J 2.2, 7.8 Hz); δ_C (50 MHz, $CDCl_3$) −3.0 (CH_3), 24.2+24.5 (CH_2), 25.5+25.9 (CH_3), 26.6+26.9 (CH_3), 26.7 (CH_2), 55.6+56.1 (CH), 56.2+56.7 (CH_2), 57.0+58.6 (CH_2), 67.5 (CH), 68.9 (CH), 73.6+74.2 (C), 80.7+81.0 (CH), 109.9+110.1 (C). MS (*m*/*z*, %) 304 (M+Na$^+$, 15%), 282 (MH$^+$, 100%).

5.3. (3a*S*,8a*R*,9a*S*)-2,2-Dimethyl-9-methylene-octahydro[1,3]dioxolo[4,5-*f*]indolizine (12)

A solution containing **11** (1.0 g, 3.55 mmol) and 1,4-dicyanonaphthalene (0.12 g, 0.67 mmol) in *iso*-propanol (250 mL) was irradiated in an open vessel using a 450 W Hanovia medium pressure mercury vapor lamp. The lamp was immersed in a Pyrex water-jacketed immersion well to allow only wavelengths greater than 280 nm to pass through. After about 20 min of irradiation, the consumption of the starting material was found to be almost complete (monitored by GC) and the irradiation was discontinued. The solvent was removed under reduced pressure and the residue was column chromatographed (silica, pet. ether–acetone, 9:1) to afford *title compound* **12** (0.46 g, 62%) as a yellow liquid. [Found: C, 68.97; H, 9.20; N, 6.54. $C_{12}H_{19}NO_2$ requires: C, 68.87; H, 9.15; N, 6.69%.] R_f (10% EtOAc–hexane) 0.25; $[\alpha]_D^{27}$ +56.1 (*c* 2.3, CH_2Cl_2); ν_{max} ($CHCl_3$) 3093, 2985, 2935, 1674, 1461, 1380, 1371, 1228 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.44 (3H, s, C*Me*), 1.46 (3H, s, C*Me*), 1.76–1.84 (3H, m, N–CH_2–C*H*$_2$–C*H*H–CH), 1.85–1.99 (1H, m, N–CH_2–CH_2–CH*H*–CH), 2.33–2.41 (2H, m, 4-*H* and 6-*H*), 2.59 (1H, app t, J 6.4, 8.9 Hz, 8a-*H*), 3.04 (1H, dt, J 1.9, 9.2 Hz, 6-*H*), 3.40 (1H, dd, J 4.1, 9.6 Hz, 4-*H*), 3.60 (1H, dt, J 4.1, 9.6 Hz, 3a-*H*), 3.85 (1H, td, J 1.8, 9.2 Hz, 9a-*H*) 4.83 (1H, s, =C*H*H), 5.02 (1H, s, =CH*H*); δ_C (125 MHz, $CDCl_3$) 22.0 (CH_2), 24.6 (CH_2), 26.7 (CH_3), 26.9 (CH_3), 52.6 (CH_2), 52.7 (CH_2), 64.5 (CH), 78.2 (CH), 82.6 (CH), 102.7 (CH_2), 110.6 (C), 142.7 (C); MS (*m*/*z*, %) 209 (M$^+$, 100%).

5.4. (3a*S*,8a*R*,9*S*,9a*R*)-9-(Hydroxymethyl)-2,2-dimethyl-octahydro[1,3]dioxolo[4,5-*f*]indolizin-9-ol (13)

To a mixture of potassium ferricyanide (2.83 g, 8.61 mmol) and potassium carbonate (1.19 g, 8.61 mmol) in water (36 mL) at 5 °C, **12** (0.45 g, 2.15 mmol) dissolved in *t*-BuOH (28 mL) followed by osmium tetroxide (2 mL of 1% solution of OsO_4 in *t*-BuOH) was added. The reaction mixture was allowed to warm to rt and stirred for 12 h. Solid Na_2SO_3 of 0.4 g was added to the stirring solution and clear separation of two layers was noticed. The aqueous layer was extracted with ethyl acetate (5×20 mL) and the combined organic extracts were dried over anhydrous Na_2SO_4. The solvent was removed by rotary-evaporation and the residue was purified by column chromatography (silica, pet. ether–ethyl acetate, 3:7) to afford *title compound* **13** (0.43 g, 82%) as a white gummy liquid. R_f (55% EtOAc–hexane) 0.20; $[\alpha]_D^{27}$ +30.7 (*c* 1.35, CH_2Cl_2); ν_{max} ($CHCl_3$) 3448, 2985, 2937, 1460, 1373, 1234, 1130, 1114 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.39 (6H, s, two *Me*), 1.61–1.86 [4H, m, C(7)H_2–C(8)H_2], 2.16 (1H, app t, *J* 9.6, 11.2 Hz, 4α-*H*), 2.19–2.26 (2H, m, 8a-*H* and 6α-*H*), 2.96 (1H, dt, *J* 2.3, 8.2 Hz, 6β-*H*), 3.26 (1H, dd, *J* 4.3, 9.3 Hz, 4β-*H*), 3.49 (1H, d, *J* 9.5 Hz, 9a-*H*), 3.75 (1H, d, *J* 11.0 Hz, C*H*H–OH), 3.80 (1H, dt, *J* 4.3, 9.8 Hz, 3a-*H*), 3.92 (1H, d, *J* 11.4 Hz, CH*H*–OH); δ_C (125 MHz, $CDCl_3$) 22.1 (CH_2), 22.9 (CH_2), 26.5 (CH_3), 26.6 (CH_3), 52.8 (for two CH_2), 61.8 (CH_2), 70.0 (CH), 71.2 (C), 73.7 (CH), 87.1 (CH), 111.4 (C). HRMS found: 243.1475. $C_{12}H_{21}NO_4$ requires: 243.1470.

5.5. 1-Deoxy-8-hydroxymethylcastanospermine or (6*S*,7*R*,8*S*,8a*R*)-8-(hydroxymethyl)-octahydroindolizine-6,7,8-triol (6)

To a solution of **13** (25 mg, 0.102 mmol) in distilled methanol (3 mL) was added 1 mL of 1 M HCl and the reaction mixture was stirred at rt for 3 h. The solvent was evaporated to dryness to afford **6** as the HCl salt, which was further purified by column chromatography as the free base [silica, chloroform–methanol–aq NH_3, (8.0:2:0.5) and finally eluted with MeOH–chloroform (4:6)] to afford *title compound* **6** (19 mg, 92%) as a colorless gummy liquid. [Found: C, 53.32; H, 8.48; N, 6.74. $C_9H_{17}NO_4$ requires: C, 53.19; H, 8.43; N, 6.89%.] R_f (15% MeOH–$CHCl_3$) 0.2; $[\alpha]_D^{27}$ +31.7 (*c* 1.35, MeOH); δ_H (400 MHz, D_2O) 1.82–2.03 [4H, m, C(1)H_2–C(2)H_2], 2.20 (1H, app t, *J* 10.3, 11.9 Hz, 5-*H*), 2.30–2.41 (1H, m, 8a-*H*/3-*H*), 2.42–2.52 (1H, m, 8a-*H*/3-*H*), 3.12 (1H, app t, *J* 8.4, 10.1 Hz, 3-*H*), 3.31 (1H, dd, *J* 5.3, 11.0 Hz, 5-*H*), 3.49 (1H, d, *J* 9.3 Hz, 7-*H*) 3.87 (2H, s, CH_2–OH), 3.88 (1H, dt, *J* 5.3, 9.8 Hz, 6-*H*); δ_C (100 MHz, D_2O) 20.9 (CH_2), 22.9 (CH_2), 53.6 (CH_2), 55.2 (CH_2), 60.4 (CH_2), 68.5 (CH), 70.1 (CH), 72.3 (C), 80.7 (CH); MS (*m*/*z*, %) 226 ($M+Na^+$, 30%), 203 (M^+, 100%), 121 (37%).

5.6. (3a*S*,8a*R*,9*S*,9a*S*)-2,2-Dimethyl-octahydro[1,3]dioxolo[4,5-*f*]indolizin-9-ol (14)

A solution of **13** (0.162 g, 0.666 mmol) in CH_2Cl_2 (10 mL) was added to a suspension of silica gel supported sodium periodate [prepared by dissolving $NaIO_4$ (0.285 g, 1.33 mmol) in 0.65 mL water and 1.31 g of silica gel] in CH_2Cl_2 (5 mL). The suspension was stirred for 10 min and filtered. The solvent was evaporated off and the brownish pasty mass was extracted with ethyl acetate (3×3 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The crude mixture, keto **13(i)** (crude weight 0.130 g, 95%) was pure enough and was used as such for the next step. R_f (55% EtOAc–hexane) 0.30; $[\alpha]_D^{27}$ +70.6 (*c* 0.80, CH_2Cl_2); δ_H (200 MHz, $CDCl_3$) 1.43 (3H, s), 1.44 (3H, s), 1.65–1.90 (3H, m), 2.00–2.25 (1H, m), 2.58–2.75 (1H, m), 2.78–2.96 (2H, m), 3.13 (1H, app t, *J* 9.2, 9.8 Hz), 3.54 (1H, dd, 4.2, 9.6 Hz), 3.87 (1H, dt, *J* 4.0, 9.6 Hz), 4.29 (1H, d, *J* 9.6 Hz); δ_C (50 MHz, $CDCl_3$) 21.9 (CH_2), 22.2 (CH_2), 26.0 (CH_3), 26.4 (CH_3), 50.7 (CH_2), 52.1 (CH_2), 67.1 (CH), 762 (CH), 83.7 (CH), 112.1 (C), 199.3 (C). This ketone was found to be unstable and used immediately for the next step.

Sodium borohydride (47 mg, 1.24 mmol) was added to a solution of ketone **13(i)** (0.13 g, 0.622 mmol) in methanol (4 mL). The resulting mixture was stirred for 6 h at rt and then quenched by adding an excess of saturated NaCl. This brownish suspension was stirred overnight and then extracted with ethyl acetate (4×3 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica, pet. ether–ethyl acetate, 1:4) to afford *title compound* **14** (0.119 g, 85%) as a colorless oil. [Found: C, 62.02; H, 8.91; N, 6.50. $C_{11}H_{19}NO_3$ requires: C, 61.95; H, 8.98; N, 6.57%.] R_f (65% EtOAc–hexane) 0.30; $[\alpha]_D^{27}$ +17.2 (*c* 0.45, $CHCl_3$), lit.[25] $[\alpha]_D^{27}$ +25 (*c* 1.2, $CHCl_3$); δ_H (200 MHz, $CDCl_3$) 1.42 (3H, s, *Me*), 1.44 (3H, s, *Me*), 1.62–1.97 [4H, m, C(7)H_2–C(8)H_2], 2.14–2.36 (3H, m, 8a-*H*, 6-*H*, and 4-*H*), 3.01 (1H, dt, *J* 1.9, 8.1 Hz, 6-*H*), 3.31–3.43 (2H, m, 9a-*H* and 4-*H*), 4.01 (1H, dt, *J* 4.2, 9.8 Hz, 3a-*H*), 4.11 (1H, app t, *J* 1.9, 2.7 Hz, 9-*H*); δ_C (50 MHz, $CDCl_3$) 22.4 (CH_2), 23.7 (CH_2), 26.5 (CH_3), 26.8 (CH_3), 52.6 (CH_2), 52.8 (CH_2), 65.6 (CH), 65.5 (CH), 71.2 (CH), 83.1 (CH), 110.9 (C).

5.7. 1-Deoxy-8-*epi*-castanospermine or (6*S*,7*R*,8*S*,8a*R*)-octahydroindolizine-6,7,8-triol (5)

To a solution of **14** (20 mg, 0.093 mmol) in distilled methanol (3 mL) was added 1 mL of 1 M HCl and the reaction mixture was stirred at rt for 3 h. The solvent was evaporated to dryness to afford **5**·HCl as a white foam, which was further purified by column chromatography as a free base [silica, chloroform–methanol–aq NH_3, (8.0:2:0.5) and finally eluted with MeOH–chloroform (4:6)] to afford *title compound* **5** (15 mg, 95%) as a white solid. Mp 149–151 °C, lit.[24] mp 149–150 °C. [Found: C, 55.52; H, 8.77; N, 8.15. $C_8H_{15}NO_3$ requires: C, 55.47; H, 8.73; N, 8.09%.] R_f (15% MeOH–$CHCl_3$) 0.25; $[\alpha]_D^{27}$ +26.1 (*c* 0.45, MeOH), lit.[24] $[\alpha]_D^{27}$ +41.8 (*c* 0.0057, MeOH); δ_H (500 MHz, D_2O) 1.99–2.30 [4H, m, C(1)H_2–C(2)H_2], 2.94 (1H, t, *J* 11.5, 11.5 Hz, 5α-*H*), 3.14–3.23 (1H, m, 3α-*H*), 3.53 (1H, ddd, *J* 1.0, 6.6, 11.6 Hz, 8a-*H*), 3.67 (1H, ddd, *J* 3.0, 8.3, 11.4 Hz, 3β-*H*), 3.74 (1H, dd, *J* 5.3, 9.8 Hz, 5β-*H*), 3.76 (1H, dd, *J* 3.0, 9.5 Hz, 7α-*H*), 4.10 (1H, ddd, *J* 5.4, 7.7, 11.4 Hz, 6β-*H*), 4.27 (1H, dd, *J* 1.0, 3.0 Hz, 8α-*H*); δ_C (125 MHz, D_2O) 22.2 (CH_2), 24.3 (CH_2), 53.9 (CH_2), 54.3 (CH_2), 66.8 (CH), 67.8 (CH), 69.6 (CH), 75.0 (CH); MS (*m*/*z*, %) 174 (MH^+, 100%), 118 (5%), 99 (55%).

5.8. (3a*S*,8a*R*,9*S*,9a*R*)-2,2-Dimethyl-octahydro[1,3]-dioxolo[4,5-*f*]indolizin-9-yl methanesulfonate (15)

To a solution of **14** (70 mg, 0.33 mmol) in pyridine (2 mL) at 0 °C was added mesyl chloride (40 mg, 0.35 mmol, in 1 mL CH_2Cl_2). The reaction mixture was stirred at rt for 4 h. When TLC revealed no starting material, the solution was diluted with dichloromethane (10 mL) and washed with water (3×5 mL) and brine (5 mL), and then dried over Na_2SO_4. Removal of the solvent followed by column chromatographic purification (ethyl acetate–pet. ether, 3:7) gave *title compound* **15** (83 mg, 88%) as a semi-solid. [Found: C, 49.52; H, 7.30; N, 4.74. $C_{12}H_{21}NO_5S$ requires: C, 49.47; H, 7.26; N, 4.815%.] R_f (35% EtOAc–hexane) 0.30; ν_{max} ($CHCl_3$) 1670, 1382, 1371, 1363, 1352, 1215 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.42 (3H, s, *Me*), 1.43 (3H, s, *Me*), 1.72–1.99 (4H, m, C(7)H_2–C(8)H_2), 2.26–2.39 (2H, m, 4-*H* and 3-*H*), 2.48 (1H, app t, *J* 6.4, 9.1 Hz, 8a-*H*), 2.98–3.08 (1H, m, 6-*H*), 3.10 (3H, s, *Me*SO_3), 3.42 (1H, dd, *J* 4.0, 9.6 Hz, 4-*H*), 3.49 (1H, dd, *J* 2.2, 9.6 Hz, 9a-*H*), 4.02 (1H, dt, *J* 4.2, 9.8 Hz, 3a-*H*), 5.09 (1H, app t, *J* 1.6, 2.4 Hz, 9-*H*); δ_C (50 MHz, $CDCl_3$) 22.2 (CH_2), 24.3 (CH_2), 26.4 (CH_3), 26.8 (CH_3), 39.0 (CH_3), 52.1 (CH_2), 52.3 (CH_2), 63.6 (CH), 71.5 (CH), 75.4 (CH), 80.5 (CH), 111.5 (C); MS (*m*/*z*, %) 314 (M+Na^+, 18%), 291 (M^+, 100%), 252 (50%).

5.9. (6*S*,7*S*,8*R*,8a*R*)-8-Amino-octahydroindolizine-6,7-diol (7)

To a solution of **15** (70 mg, 0.24 mmol) in DMF (3 mL) was added LiN_3 (117 mg, 2.44 mmol) and heated to 110 °C for 16 h. When TLC revealed the absence of starting material, the reaction mixture was diluted with water (15 mL) and extracted with ethyl acetate (3×15 mL). The ethyl acetate layer was washed with water, dried over Na_2SO_4, and concentrated to give the corresponding azide derivative of **15**. The synthesized azide in methanol (3 mL) was hydrogenated for 7 h at atmospheric pressure in the presence of Pd on charcoal (10%) (3 mg). The reaction mixture was passed through a short pad of Celite and the solvent was removed under reduced pressure to afford the corresponding amine derivative (43 mg, 85%) as a gummy liquid. R_f (80% EtOAc–hexane) 0.20; $[\alpha]_D^{27}$ +14 (*c* 0.3, MeOH); ν_{max} ($CHCl_3$) 3410, 2985, 2935, 2800, 1642, 1419, 1363, 1249 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.43 (6H, s), 1.53–1.66 (1H, m), 1.74–1.86 (1H, m), 1.87–1.98 (1H, m), 1.99–2.26 (2H, m), 2.31 (1H, app t, *J* 9.9, 10.7 Hz), 2.35–2.42 (1H, m), 2.87 (1H, app t, *J* 9.0, 10.1 Hz), 3.05 (1H, dt, *J* 2.5, 9.6 Hz) 3.29 (1H, app t, *J* 9.5, 10.1 Hz), 3.34 (1H, dd, *J* 4.0, 9.8 Hz), 3.71 (1H, ddd, *J* 4.0, 8.8, 10.0 Hz); δ_C (100 MHz, $CDCl_3$) 22.3 (CH_2), 26.7 (CH_3), 26.8 (CH_3), 27.2 (CH_2), 52.1 (CH_2), 52.5 (CH_2), 55.1 (CH), 68.5 (CH), 75.6 (CH), 84.8 (CH), 111.5 (C); MS (*m*/*z*, %) 212 (M^+, 100%).

To a solution of the amine (43 mg, 0.20 mmol) in methanol (3 mL) was added 1 mL of 1 M HCl and the reaction mixture was stirred at rt for 4 h. The solvent was evaporated to dryness to afford **7**·HCl as the white foam, which was further purified by column chromatography as the free base [silica, chloroform–methanol–aq NH_3, (8.0:2:0.5) and finally eluted with MeOH–chloroform (4:6)] to afford *title compound* **7** (32 mg, 92%) as a light yellow gummy liquid. [Found: C, 55.81; H, 9.31; N, 16.38. $C_8H_{16}N_2O_2$ requires: C, 55.79; H, 9.36; N, 16.27%.] R_f (20% MeOH–$CHCl_3$) 0.20; $[\alpha]_D^{27}$ +4.5 (*c* 0.3, CH_2Cl_2); δ_H (400 MHz, D_2O) 1.62–1.74 (1H, m, 1-*H*), 1.96–2.05 [2H, m, C(2)H_2], 2.16–2.26 (1H, m, 1-*H*), 2.43 (1H, app t, *J* 11.0, 12.3 Hz, 5-*H*), 2.54–2.73 (m, 2H, 8-*H* and 8a-*H*), 3.05 (1H, app t, *J* 10.2, 11.2 Hz, 7-*H*), 3.19–3.27 (1H, m, 3-*H*), 3.41 (1H, dd, *J* 5.0, 11.4 Hz, 5-*H*), 3.53 (1H, app t, *J* 9.2, 10.1 Hz, 3-*H*), 3.72–3.79 (1H, m, 6-*H*); δ_C (100 MHz, D_2O) 20.6 (CH_2), 26.5 (CH_2), 52.3 (CH_2), 53.4 (CH_2), 54.9 (CH), 63.7 (CH), 69.3 (CH), 74.0 (CH); MS (*m*/*z*, %) 173 (MH^+, 100%).

5.10. Inhibition assay procedure

The inhibition assay for the inhibitory potencies of polyhydroxylated indolizidine alkaloids **5–7** was determined by measuring the residual hydrolytic activities of the glycosidases of the corresponding *p*-nitrophenyl glycosides in the presence of azasugars spectrophotometrically.

In the case of β-galactosidase, each assay was performed in citrate buffer (50 mM, pH 4.5) with *p*-nitrophenyl β-D-galactosidase as the substrate. Varying concentrations of the substrate (50–150 μL, 10 mM) and inhibitors were employed. The reaction was initiated by the addition of 100 mL of appropriately diluted enzyme and the reaction mixture, which had a final volume of 1 mL, was incubated for 20 min at 30 °C, and then quenched by the addition of 2 mL of 1 M Na_2CO_3 solution. The absorbance of the resulting solution was read at 405 nm.

In the case of α-galactosidase (green coffee beans), the assay was performed in a citrate phosphate buffer (50 mM, pH 6.5) with *p*-nitrophenyl α-D-galactopyranoside as the substrate, and the reaction was carried out at 25 °C for 20 min and then quenched by Na_2CO_3 solution.

In the case of β-glucosidase (almond), the assay was performed in a citrate phosphate buffer (50 mM, pH 5.5) with *p*-nitrophenyl β-D-glucopyranoside as the substrate, and the reaction was carried out at 37 °C for 30 min and then quenched by Na_2CO_3 solution.

In the case of α-glucosidase (yeast), the assay was performed in a citrate phosphate buffer (50 mM, pH 6.8) with *p*-nitrophenyl α-D-glucopyranoside as the substrate, and the reaction was carried out at 37 °C for 20 min and then quenched by Na_2CO_3 solution.

In the case of β-mannosidase (snail acetone), the assay was performed in an acetate buffer (50 mM, pH 4.0) with *p*-nitrophenyl β-D-mannopyranoside as the substrate, and the reaction was carried out at 25 °C for 20 min and then quenched by Na_2CO_3 solution.

In the case of α-mannosidase (jack bean), the assay was performed in an acetate buffer (50 mM, pH 4.5) with *p*-nitrophenyl α-D-mannopyranoside as the substrate, and the reaction was carried out at 25 °C for 20 min and then quenched by Na_2CO_3 solution.

The K_i values were determined from the Lineweaver–Burke double reciprocal plots of 1/*v* versus 1/[S]. K_i

for competitive inhibition was determined using the formula:

$$K_i = \frac{[I]}{\left(\frac{K_{MI}}{K_M}\right) - 1}.$$

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2007.03.085.

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