SYNTHESIS AND ACTIVITY AS A GLYCOSIDASE INHIBITOR OF 2,8-DIHYDROXYINDOLIZIDINES

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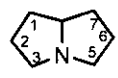
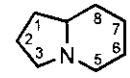
Abstract - The synthesis and enzyme assay of 2,8-dihydroxyindolizidines (**5**) were described. Four diastereomers of these were prepared from *trans*-4-hydroxy-*L*-proline (**6**) which is commercially available amino acid. Among synthetic compounds $\mathbf{5a}_1$ and $\mathbf{5b}_2$ showed medium potent inhibition to α-amyloglycosidase, while $\mathbf{5a}_2$ displayed medium activity to α-glucosidase.

INTRODUCTION

The pyrrolizidine alkaloids possess the 1-azabicyclo[3.3.0]octane skeleton, while the indolizidine alkaloids have the 1-azabicyclo[3.4.0]nonane skeleton. These two classes of 1-azabicyclic alkaloids constitute a very large family of natural products which are widely isolated from plants, insects, animals, oceanic lives and secondary metabolites of microbes.[1] They are often functionalized at a variety of structural sites and demonstrate a wide range of potent biological and pharmacological effects.[2,3] Due to the various pharmacological and biological activities and the intriguing chemical structures, these two classes of alkaloids have attracted considerable synthetic interest. Among their various activities, the indolizidine alkaloids are well known to be an effective and selective inhibitor of glycosidase.[4] Glycosidase is a key enzyme in processing of glycoprotein and in metabolizing glycogen for energy source in living organisms.[5-7]



Pyrrolizidine

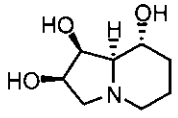


Indolizidine

Swainsonine (**1**), a trihydroxyindolizidine alkaloid comes from the realization that the clinical signs and pathologic effects of *Swainsona* intoxication in cattle resembled those of a hereditary condition in man and other animals known as α-mannosidosis, is a very potent and specific inhibitor of α-mannosidase.[8-11] Castanospermin (**2**), a tetrahydroxyindolizidine alkaloid isolated from *Castanosperum australe*, is a good inhibitor of α- and β-glucosidases.[12,13] This compound has been of great interest because of its inhibitory activity against replication of the human immunodeficiency virus (HIV).[14]

As a part of our research program, we were particularly interested in the synthesis and their activities as a glycosidase inhibitor of dihydroxypyrrolizidines and dihydroxyindolizidines. We have already published the synthesis and activity of 1,6-dihydroxypyrrolizidines (**3**).[15] Few studies have been done for dihydroxyindolizidine alkaloids. However, lentiginosine (**4**), 1,2-dihydroxyindolizidine isolated from *Astragalus lentiginosine var. dipbysus*, showed strong inhibition to amyloglucosidase.[16]

Now we wish to describe the synthesis of 2,8-dihydroxyindolizidines (**5**) using *trans*-4-hydroxy-*L*-proline (**6**) as a starting material which is commercially available, and their activities as a glycosidase inhibitor.



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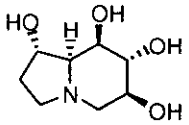

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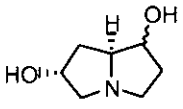

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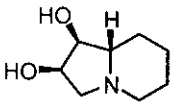

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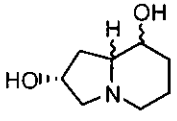

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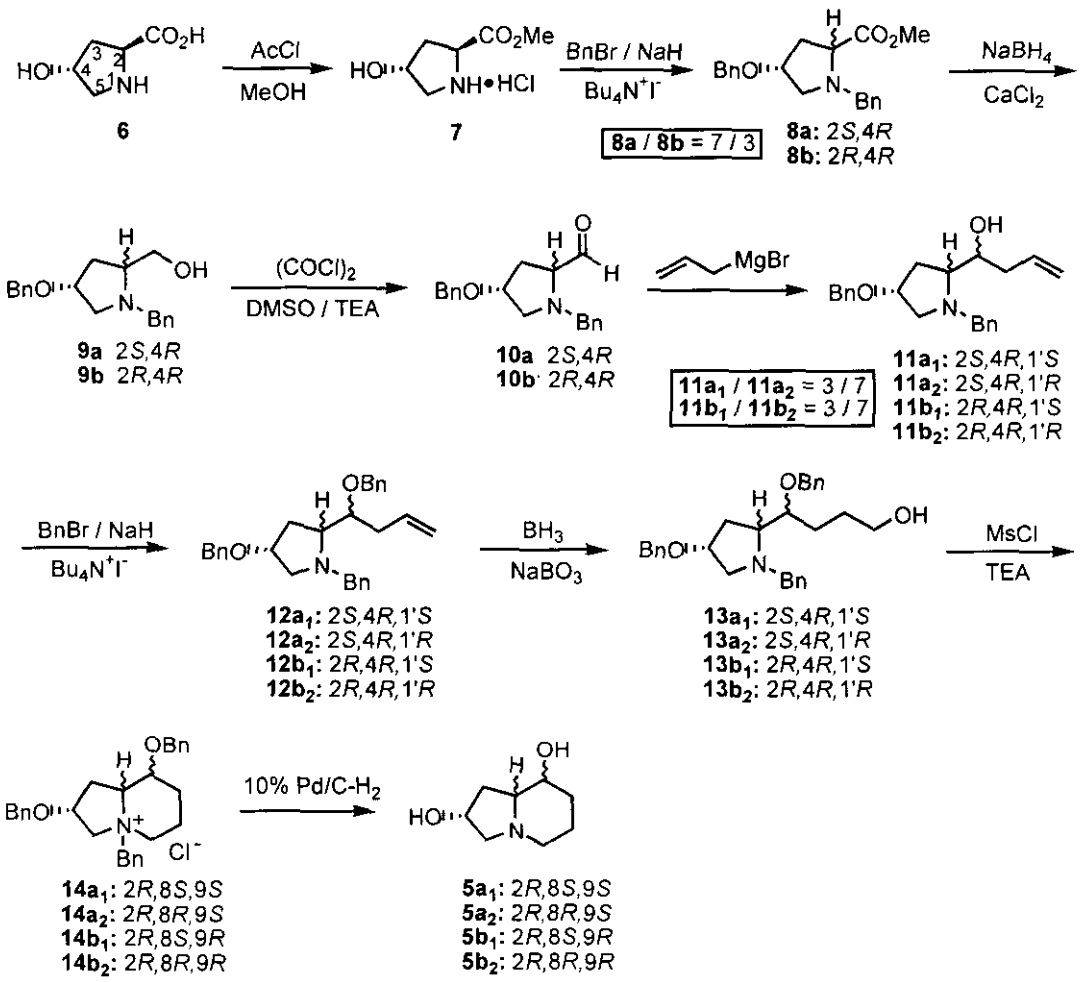
CHEMISTRY

The synthetic route employed for the title compounds, 2,8-dihydroxyindolizidines (**5a$_1$,a$_2$,b$_1$,b$_2$**), was depicted in Scheme 1.

The starting material for the synthetic effort was the optically pure *trans*-4-hydroxy-*L*-proline (**6**), which, *via* esterification with MeOH in the presence of acid generated *in situ* from AcCl and MeOH, was converted to proline methyl ester hydrochloride (**7**) in 96% yield. Subsequent treatment with benzyl bromide and NaH in the presence of tetrabutylammonium iodide at 0 °C afforded the separable mixture of two epimers (**8a**) and (**8b**) in 70% yield in the ratio of about 7:3. Separation of the two isomers was achieved by silica gel chromatography. The absolute stereochemistry of **8a** could be established by the

successful transformation of **7** into **8a** with retention of configuration without epimerization using benzyl bromide and freeze-dried KOH in DMF (Scheme 2). On the ground that our efforts were directed toward the synthesis and activity of varying diastereomers of 2,8-dihydroxyindolizidines, we used this stereo-selective process only for confirming structure configuration not for synthesis.



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7

8a: 2*S*,4*R*
8b: 2*R*,4*R*

9a 2*S*,4*R*
9b 2*R*,4*R*

10a 2*S*,4*R*
10b 2*R*,4*R*

11a$_1$: 2*S*,4*R*,1'*S*
11a$_2$: 2*S*,4*R*,1'*R*
11b$_1$: 2*R*,4*R*,1'*S*
11b$_2$: 2*R*,4*R*,1'*R*

11a$_1$ / **11a**$_2$ = 3 / 7
11b$_1$ / **11b**$_2$ = 3 / 7

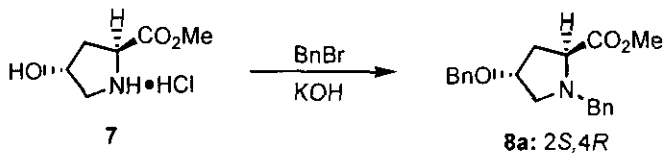
12a$_1$: 2*S*,4*R*,1'*S*
12a$_2$: 2*S*,4*R*,1'*R*
12b$_1$: 2*R*,4*R*,1'*S*
12b$_2$: 2*R*,4*R*,1'*R*

13a$_1$: 2*S*,4*R*,1'*S*
13a$_2$: 2*S*,4*R*,1'*R*
13b$_1$: 2*R*,4*R*,1'*S*
13b$_2$: 2*R*,4*R*,1'*R*

14a$_1$: 2*R*,8*S*,9*S*
14a$_2$: 2*R*,8*R*,9*S*
14b$_1$: 2*R*,8*S*,9*R*
14b$_2$: 2*R*,8*R*,9*R*

5a$_1$: 2*R*,8*S*,9*S*
5a$_2$: 2*R*,8*R*,9*S*
5b$_1$: 2*R*,8*S*,9*R*
5b$_2$: 2*R*,8*R*,9*R*

Scheme 1



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8a: 2*S*,4*R*

Scheme 2

^{1}H NMR and optical rotation of **8a** [C_2-H δ 3.59; $[\alpha]_D^{25}$ = -59.0° (c 0.30, $CHCl_3$)] as a major product in an epimeric mixture are consistent with those of **8a** [C_2-H δ 3.58; $[\alpha]_D^{25}$ = -59.0° (c 0.30, $CHCl_3$)] in Scheme 2. The C_2-H proton of **8b** (δ 3.34) appeared at higher field than that of **8a** and its optical rotation [$[\alpha]_D^{25}$ = +58.0° (c 0.30, $CHCl_3$)] showed the positive value. Thus, the absolute configuration at C_2 of **8a** was confirmed to be *S*. Reduction of the ester functions of **8a,b** by $NaBH_4$ and $CaCl_2$ gave the corresponding alcohols (**9a,b**) in 92 and 82% yields, respectively. The aldehyde (**10a**) was prepared from **9a** by the method of Swern[17] in excellent yield and used to the next reaction without purification. Compound (**10b**) was obtained in similar fashion from the corresponding alcohol (**9b**) in 80% yield. The desired allyl side chain was introduced into **10a** *via* Grignard reaction by allylmagnesium bromide. The reaction product was a separable mixture of two epimeric alcohols (**11a$_1$,a$_2$**), bearing the three asymmetric centers, in 70% yield in the ratio of 3:7. The preferred stereochemical course of the alkylation would be consistent with Grignard reagent attack from the less hindered direction. The stereochemistry of **11a$_1$,a$_2$** was determined by comparison with the title compounds (**5a$_1$,a$_2$**). The assignments of configuration at C_1' in the diastereomers (**5a$_1$,a$_2$**) were based on the proton coupling between the C_2-H and C_1'-H protons in the 2D-COSY spectra. The COSY spectrum of **5a$_1$** showed the proton coupling between the C_2-H and C_1'-H protons at the same direction, while that of **5a$_2$** didn't. Thus, the configuration at C_1' of **5a$_1$** was established as *S*. As a result, the stereochemistry at C_1' of **11a$_1$** also was assigned as *S* by relation with **5a$_1$**. By the same procedure **11b$_1$,b$_2$** were obtained in the similar ratio and yield. Benzyl protection of alcohols (**11a$_1$,a$_2$,b$_1$,b$_2$**) was accomplished by the same manner as described for the preparation of **8a,b** to provide **12a$_1$,a$_2$,b$_1$,b$_2$**, respectively. Hydroboration-oxidation of **12a$_1$** using BH_3-THF complex and $NaBO_3$ in THF provided the alcohol (**13a$_1$**) in 71% yield. Then, activation of alcohol (**13a$_1$**) with methanesulfonyl chloride, followed by displacement of the resulting mesylate by *N*-alkylation led to a bicyclic compound (**14a$_1$**).[18] Similarly **12a$_2$,b$_1$,b$_2$** were converted to the corresponding bicyclic salts (**14a$_2$,b$_1$,b$_2$**) in excellent overall yields, respectively. Finally, these bicyclic salts were debenzylated by catalytic hydrogenation at 50 *psi* in ethanolic HCl and subsequent desalting with Dowex 50W-X8 (H^- form) resin using 0.7*N* NH_4OH to afford the title compounds (**5a$_1$,a$_2$,b$_1$,b$_2$**) in 59-75% yields, respectively.[18] The structures of these title compounds were confirmed by HRMS, IR, ^{1}H- and ^{13}C-NMR spectra.

INHIBITION STUDY

Inhibition study for 2,8-dihydroxyindolizidines (**5**) was performed on various enzymes, α-glucosidase, α-galactosidase, β-glucosidase, β-galactosidase, α-amyloglucosidase, β-cellulase, α-mannosidase, and β-

mannosidase. The enzyme activity was measured by the formation of *p*-nitrophenol at 405 nm. The inhibition constant, *Ki* value was calculated using Dixon plots.[19] Each diastereomer of 2,8-dihydroxy-indolizidines (**5**) showed medium potent inhibition on specific enzyme. Diastereomer (**5b$_2$**) inhibited α-amyloglucosidase, and its *Ki* value was 1970 μM. And also, each *Ki* value of diastereomers (**5a$_1$**) and (**5a$_2$**) was 370 μM on α-amyloglucosidase and 1670 μM on α-glucosidase, respectively. Other enzymes were inhibited very little or not inhibited.

EXPERIMENTAL

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 16F PC FT-IR spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were obtained on a 300 MHz Gemini Varian NMR spectrometer (300 MHz for ^{1}H and 75.5 MHz for ^{13}C) using tetramethylsilane as an internal standard. Optical rotations were determined with an *AUTOPOL III (Rudolph Research) automatic polarimeter.* FAB-MS were obtained with a JMS AX505WA mass spectrometer. HRMS were recorded on a Finnigan 95S spectrometer operating in the electron impact mode. Microanalytical data were obtained by using a EA 1108 Fisons Instruments. Column chromatography was carried out using silica-gel (230-400 mesh).

trans-4-Hydroxy-*L*-proline methyl ester hydrochloride (7)

Acetyl chloride (22.3 mL, 0.32 mol) was added to a solution of **6** (30.0 g, 0.23 mol) in MeOH (200 mL) and the mixture was stirred under reflux for 7-8 h. After cooling to rt, the mixture was solidified with Et_2O (1 L). The solid was filtered and dried to give **7** as a colorless solid, yield 39.4 g (96%); mp 170 °C (ether); IR ($CDCl_3$) ν 3376 (OH), 1742 (C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.03-2.38 (m, 2H, C_3-H), 3.06-3.38 (m, 2H, C_5-H), 3.74 (s, 3H, OCH_3), 4.38-4.47 (m, 2H, C_2-H and C_4-H).

(2*S/R*,4*R*)-*N*-Benzyl-4-benzyloxyproline methyl esters (8a,b)

A suspension of NaH (21.8 g, 0.90 mol, 60% oil suspension) in THF (200 mL) was added at 0 °C to a mixture of **7** (36.0 g, 0.19 mol) and tetrabutylammonium iodide (1.6 g, 0.006 mol) in DMF (500 mL). Stirring was continued at rt for 30 min, then benzyl bromide (66 mL, 0.60 mol) was added. After being stirred at rt for 12 h, the mixture was diluted with EtOAc, washed with saturated aqueous NaCl, and dried over $MgSO_4$. Removal of the solvent gave an oily residue, which was chromatographed on silica gel using EtOAc/*n*-hexane (1:9) to give **8a** and **8b** as the ratio of 7:3.

8a: yield 31.8 g (48%); colorless oil; $[\alpha]_D^{25}$ = -59.0° (c 0.30, $CHCl_3$); IR ($CDCl_3$) ν 3060, 2950, 1748 (C=O), 1604 (C=C) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.23-2.28 (m, 2H, C_3-H), 2.56-2.60 (m, 1H, C_5-H), 3.35-

3.40 (m, 1H, C_5-H), 3.59 (t, 1H, *J*=7.5 Hz, C_2-H), 3.63, 3.97 (d, each 1H, *J*=12.7 Hz, -NC*H*$_2$-aromatic), 3.67 (s, 3H, -OCH_3), 4.22 (m, 1H, C_4-H), 4.44, 4.49 (d, each 1H, *J*=11.5 Hz, -OC*H*$_2$-aromatic), 7.26-7.39 (m, 10H, aromatic-H); ^{13}C-NMR ($CDCl_3$) δ 36.57, 51.72, 58.76, 64.22, 71.15, 71.29, 127.28, 127.42, 127.70, 128.12, 128.36, 129.25, 138.25, 173.96. *Anal.* Calcd for $C_{20}H_{23}NO_3$: C, 73.82; H, 7.12; N, 4.30. Found: C, 73.87; H, 7.08; N, 4.26.

8b: yield 14.2 g (22%); colorless oil; $[\alpha]_D^{25}$ = +58.0° (c 0.30, $CHCl_3$); IR ($CDCl_3$) ν 3062, 2948, 1746 (C=O), 1604 (C=C) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.18-2.24 (m, 1H, C_3-H), 2.14-2.51 (m, 1H, C_3-H), 2.62 (m, 1H, C_5-H), 3.18 (m, 1H, C_5-H), 3.34 (m, 1H, C_2-H), 3.61, 4.05 (d, each 1H, *J*=13.5 Hz, -NC*H*$_2$-aromatic), 3.72 (s, 3H, -OCH_3), 4.11 (m, 1H, C_4-H), 4.42 (s, 2H, -OC*H*$_2$-aromatic), 7.26-7.41 (m, 10H, aromatic-H); ^{13}C-NMR ($CDCl_3$) δ 36.63, 51.78, 58.08, 58.22, 64.14, 70.72, 70.87, 127.23, 127.34, 127.64, 128.06, 128.37, 129.21, 138.11, 138.32, 173.71. *Anal.* Calcd for $C_{20}H_{23}NO_3$: C, 73.82; H, 7.12; N, 4.30. Found: C, 73.92; H, 7.09; N, 4.28.

Preparation of optically pure (2*S*,4*R*)-*N*-benzyl-4-benzyloxyproline methyl ester (8a) from 7

A finely powdered KOH (54.0 mg, 0.96 mmol) prepared by freeze-drying from aqueous KOH was added to a solution of **7** (77.8 mg, 0.43 mmol) in DMF (10 mL) and the mixture was stirred at rt for 30 min. Then, benzyl bromide (0.13 mL, 1.07 mmol) was added and stirred at 60 °C for overnight. After cooling to rt, the mixture was diluted with EtOAc, washed with saturated aqueous NaCl, and dried over $MgSO_4$. Removal of the solvent gave an oily residue, which was chromatographed on silica gel using EtOAc/*n*-hexane (1:3) to give optically pure **8a** as a colorless oil, yield 0.16 g (90%); $[\alpha]_D^{25}$ = -59.0° (c 0.30, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 2.22-2.27 (m, 2H, C_3-H), 2.55-2.61 (m, 1H, C_5-H), 3.35-3.41 (m, 1H, C_5-H), 3.59 (t, 1H, C_2-H), 3.64, 3.96 (d, each 1H, *J*=12.8 Hz, -NC*H*$_2$-aromatic), 3.70 (s, 3H, -OCH_3), 4.23 (m, 1H, C_4-H), 4.45, 4.50 (d, each 1H, *J*=11.6 Hz, -OC*H*$_2$-aromatic), 7.25-7.41 (m, 10H, aromatic-H).

(2*S*,4*R*)-*N*-Benzyl-2-hydroxymethyl-4-benzyloxypyrrolidine (9a)

A mixture of anhydrous $CaCl_2$ (1.0 g, 10.0 mmol) and $NaBH_4$ (0.75 g, 20.0 mmol) in THF (100 mL) was stirred at rt for 24 h. After addition of **8a** (2.4 g, 7.3 mmol) in EtOH (20 mL), the mixture was stirred at rt for 3 h. Then, the reaction was quenched with 10% aqueous NH_4Cl (30 mL) and the mixture was extracted with EtOAc. The organic layer was washed with saturated aqueous NaCl and dried over Na_2SO_4. Removal of the solvent gave an oily residue, which was chromatographed on silica gel using EtOAc/*n*-hexane (2:1) to give **9a** as a colorless oil, yield 2.0 g (92%); $[\alpha]_D^{25}$ = -57.3° (c 0.37, $CHCl_3$); IR ($CDCl_3$) ν 3398 (OH), 3028, 2866 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.00-2.17 (m, 2H, C_3-H), 2.28-2.51 (m, 2H, C_5-H), 3.03 (m, 1H, C_2-H), 3.45 (d, 1H, -C*H*$_2$OH), 3.46, 4.02 (d, each 1H, *J*=11.6 Hz, -NC*H*$_2$-aromatic), 3.71 (dd, 1H, *J*=3.2, 11.0 Hz, -C*H*$_2$OH), 4.07 (m, 1H, C_4-H), 4.45, 4.50 (d, each 1H, *J*=11.0 Hz, -OC*H*$_2$-aromatic), 7.27-7.36 (m, 10H, aromatic-H); ^{13}C-NMR ($CDCl_3$) δ 34.57, 58.57, 59.70, 61.04, 63.38, 71.27,

76.97, 127.59, 127.68, 128.49, 128.77, 138.31, 139.12; MS (FAB) *m/z* 298 [(M+1)$^+$].

(2*R*,4*R*)-*N*-Benzyl-2-hydroxymethyl-4-benzyloxypyrrolidine (9b)

This compound was prepared from **8b** in the same manner as described above for the preparation of **9a**. Yield 82%; colorless oil; $[\alpha]_D^{25}$ = +25.9° (c 0.90, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 2.05-2.38 (m, 2H, C_3-H), 2.42-3.14 (m, 2H, C_5-H), 2.84 (m, 1H, C_2-H), 3.37, 4.02 (d, each 1H, *J*=13.0 Hz, -NC*H*$_2$-aromatic), 3.49 (dd, 1H, *J*=1.8, 11.1 Hz, -C*H*$_2$OH), 3.74 (dd, 1H, *J*=3.0, 11.1 Hz, -C*H*$_2$OH), 4.01 (m, 1H, C_4-H), 4.41, 4.49 (d, each 1H, *J*=11.4 Hz, -OC*H*$_2$-aromatic), 7.26-7.33 (m, 10H, aromatic-H); ^{13}C-NMR ($CDCl_3$) δ 34.92, 58.62, 59.89, 61.80, 64.14, 71.17, 77.21, 127.74, 128.15, 129.01, 129.32, 138.94, 139.42; MS (FAB) *m/z* 298 [(M+1)$^+$].

(2*S*,4*R*)-*N*-Benzyl-4-benzyloxy-2-formylpyrrolidine (10a)

Me_2SO (1.3 mL, 30.0 mmol) was added to a solution of oxalyl chloride (1.13 mL, 13.4 mmol) in CH_2Cl_2 (100 mL) cooled below -65 °C with a dry ice-acetone bath and the mixture was stirred for 15 min. After addition of **9a** (2.0 g, 6.7 mmol) in CH_2Cl_2 (30 mL), the mixture was stirred below -65 °C for 30 min. Triethylamine (6.37 mL, 45.0 mmol) was added and the mixture was stirred for 15 min and then allowed to warm to rt. Water was then added and the aqueous layer was reextracted with additional CH_2Cl_2. The organic layers were combined, washed with saturated aqueous NaCl, and dried over $MgSO_4$. Removal of the solvent gave **10a**, which was used to the next step without purification. Yield 1.8 g (92%); colorless oil; IR ($CDCl_3$) ν 2867, 2807, 1742 (C=O) cm^{-1}.

(2*R*,4*R*)-*N*-Benzyl-4-benzyloxy-2-formylpyrrolidine (10b)

This compound was prepared from **9b** in the same manner as described above for the preparation of **10a**. Yield 80%; colorless oil; IR ($CDCl_3$) ν 2865, 2805, 1740 (C=O) cm^{-1}.

(2*S*,4*R*,1'*S*/*R*)-*N*-Benzyl-4-benzyloxy-2-(1'-hydroxy-3'-butenyl)pyrrolidines (11a$_1$,a$_2$)

A solution of **10a** (1.8 g, 6.1 mmol) in THF (100 mL) was treated with allylmagnesium chloride (12.2 mL, 1M solution in THF) at -78 °C for 4 h. Then, the reaction was quenched with 10% aqueous NH_4Cl (8.1 mL) and the mixture was extracted with EtOAc. The organic layer was washed with saturated aqueous NaCl and dried over Na_2SO_4. Removal of the solvent gave an oily residue, which was chromatographed on silica gel using EtOAc/*n*-hexane (1:4) to give **11a$_1$** and **11a$_2$** as the ratio of 3:7.

11a$_1$: yield 0.48 g (21%); colorless oil; $[\alpha]_D^{25}$ = -70.3° (c 0.20, $CHCl_3$); IR ($CDCl_3$) ν 3447 (OH), 3029, 2863 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.87 (m, 1H, -C*H*$_2$CH=CH_2), 2.07-2.19 (m, 2H, C_3-H and -C*H*$_2$CH=CH_2), 2.36 (m, 1H, C_3-H), 2.49 (m, 1H, C_5-H), 2.97 (m, 1H, C_2-H), 3.27 (m, 1H, C_5-H), 3.39, 4.04 (d, each 1H, *J*=12.7 Hz, -NC*H*$_2$-aromatic), 3.89 (m, 1H, -C*H*OH), 4.03 (m, 1H, C_4-H), 4.47 (d, 2H, *J*=12.4 Hz, C_4-OC*H*$_2$-aromatic), 5.16 (m, 2H, -CH=C*H*$_2$), 5.90 (m, 1H, -C*H*=CH_2), 7.10-7.39 (m, 10H, aromatic-H); ^{13}C-NMR ($CDCl_3$) δ 30.35, 37.71, 58.09, 59.60, 66.22, 67.57, 71.04, 76.74, 116.93, 126.50, 126.88,

127.09, 127.52, 128.33, 128.60, 134.78, 138.21, 138.71. *Anal.* Calcd for $C_{22}H_{27}NO_2$: C, 78.30; H, 8.06; N, 4.15. Found: C, 77.70; H, 8.07; N, 3.87.

11a$_2$: yield 1.08 g (49%); colorless oil; $[\alpha]_D^{25}$ = -30.4° (c 1.00, $CHCl_3$); IR ($CDCl_3$) ν 3432 (OH), 3064, 2922, 1640 (C=C) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.88 (m, 1H, C_3-H), 2.12-2.32 (m, 3H, C_3-H and -C*H*$_2$CH=CH_2), 2.72-3.02 (m, 2H, C_5-H), 3.12 (m, 1H, C_2-H), 3.39 (m, 1H, -C*H*OH), 4.06, 3.80 (d, each 1H, *J*=11.6 Hz, -NC*H*$_2$-aromatic), 4.12 (m, 1H, C_4-H), 4.48 (s, 2H, -OC*H*$_2$-aromatic), 5.13 (m, 2H, -CH=C*H*$_2$), 5.89 (m, 1H, -C*H*=CH_2), 7.25-7.36 (m, 10H, aromatic-H); ^{13}C-NMR ($CDCl_3$) δ 35.42, 39.27, 57.96, 62.30, 67.31, 71.08, 72.78, 78.99, 117.05, 127.14, 127.43, 127.56, 128.37, 128.64, 135.14, 138.22, 139.23. *Anal.* Calcd for $C_{22}H_{27}NO_2$: C, 78.30; H, 8.06; N, 4.15. Found: C, 78.15; H, 8.07; N, 4.05.

(2*R*,4*R*,1'*S*/*R*)-*N*-Benzyl-4-benzyloxy-2-(1'-hydroxy-3'-butenyl)pyrrolidines (11b$_1$,b$_2$)

These compounds were prepared from **10b** in the same manner as described above for the preparation of **11a$_1$,a$_2$**.

11b$_1$: yield 21%; colorless oil; $[\alpha]_D^{25}$ = +34.3° (c 0.17, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 2.13 (m, 2H, C_5-H), 2.28-2.40 (m, 3H, C_5-H and -C*H*$_2$CH=CH_2), 2.73 (m, 1H, C_2-H), 3.11 (m, 1H, C_5-H), 3.30, 4.10 (d, each 1H, *J*=13.2 Hz, -NC*H*$_2$-aromatic), 3.90 (m, 1H, C_2-H), 4.06 (m, 1H, C_4-H), 4.44, 4.49 (d, each 1H, *J*=11.7 Hz, C_4-OC*H*$_2$-aromatic), 5.11 (m, 2H, -CH=C*H*$_2$), 5.91 (m, 1H, -C*H*=CH_2), 7.26-7.35 (m, 10H, aromatic-H); ^{13}C-nmr ($CDCl_3$) δ 30.39, 38.41, 57.73, 59.02, 66.09, 67.97, 70.44, 76.09, 116.85, 127.09, 127.58, 127.64, 128.34, 128.64, 128.75, 134.96. *Anal.* Calcd for $C_{22}H_{27}NO_2$: C, 78.30; H, 8.06; N, 4.15. Found: C, 78.33; H, 8.02; N, 4.14.

11b$_2$: yield 49%; colorless oil; $[\alpha]_D^{25}$ = -3.4° (c 0.15, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.84-2.40 (m, 4H, C_3-H and -C*H*$_2$CH=CH_2), 2.94 (m, 1H, C_2-H), 2.58-3.09 (m, 2H, C_5-H), 3.53, 4.10 (d, each 1H, *J*=13.4 Hz, -NC*H*$_2$-aromatic), 3.66 (m, 1H, -C*H*OH), 4.08 (m, 1H, C_4-H), 4.43, 4.50 (d, each 1H, *J*=12.0 Hz, -OC*H*$_2$-aromatic), 5.14 (m, 2H, -CH=C*H*$_2$), 5.94 (m, 1H, -C*H*=CH_2), 7.22-7.38 (m, 10H, aromatic-H). *Anal.* Calcd for $C_{22}H_{27}NO_2$: C, 78.30; H, 8.06; N, 4.15. Found: C, 78.26; H, 8.04; N, 4.08.

(2*S*,4*R*,1'*S*)-*N*-Benzyl-2-(1'-benzyloxy-3'-butenyl)pyrrolidine (12a$_1$)

A suspension of NaH (0.11 g, 4.5 mmol, 60% oil suspension) in THF (10 mL) was added at 0 °C to a mixture of **11a$_1$** (1.08 g, 3.2 mmol) and tetrabutylammonium iodide (12 mg, 0.036 mmol) in DMF (5 mL) and the mixture was stirred at rt for 30 min. Then, benzyl bromide (0.36 mL, 3.6 mmol) was added. After being stirred at rt for 24 h, the mixture was diluted with EtOAc, washed with 10% aqueous NH_4Cl, and dried over Na_2SO_4. Removal of the solvent gave an oily residue, which was chromatographed on silica gel using EtOAc/*n*-hexane (1:6) to give **12a$_1$** as a colorless oil, yield 0.97 g (70%); $[\alpha]_D^{25}$ = -38.3° (c 0.22, $CHCl_3$); IR ($CDCl_3$) ν 3029, 2861 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.96 (m, 1H, -C*H*$_2$-CH=CH_2), 2.24, 2.27 (m, 4H, -C*H*$_2$CH=CH_2, C_3-H and C_5-H), 3.07 (m, 1H, C_2-H), 3.32 (m, 1H, C_5-H), 3.45, 4.25 (d, each

1H, *J*=13.3 Hz, -NC*H*$_2$-aromatic), 3.70 (m, 1H, C$_1$'-H), 4.18 (m, 1H, C$_4$-H), 4.47, 4.55 (d, each 1H, *J*=12.3 Hz, C$_4$-OC*H*$_2$-aromatic), 4.71, 4.94 (d, each 1H, *J*=11.8 Hz, C$_1$'-OC*H*$_2$-aromatic), 5.14 (m, 2H, -CH= C*H*$_2$), 5.93 (m, 1H, -C*H*=CH$_2$), 7.26-7.45 (m, 15H, aromatic-H); ^{13}C-nmr ($CDCl_3$) δ 32.12, 37.24, 59.49, 66.19, 71.05, 72.85, 77.14, 78.45, 116.52, 127.70, 127.21, 127.39, 127.49, 127.63, 127.79, 128.15, 128.23, 128.47, 135.60, 138.43, 139.19, 139.63. *Anal.* Calcd for $C_{29}H_{33}NO_2$: C, 81.46; H, 7.78; N, 3.28. Found: C, 81.56; H, 7.84; N, 3.30.

The following compounds (**12a$_2$**, **12b$_{1-2}$**) were prepared from **11a$_2$**, **11b$_{1-2}$** in the same manner as described for the preparation of **12a$_1$**, respectively.

(2*S*,4*R*,1'*R*)-*N*-Benzyl-2-(1'-benzyloxy-3'-butenyl)pyrrolidine (12a$_2$)

Yield 74%; colorless oil; $[\alpha]_D^{25}$ = -52.0° (c 0.20, $CHCl_3$); ir ($CDCl_3$) ν 3027, 2863 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.99 (m, 2H, C$_3$-H), 2.25-2.42 (m, 2H, -C*H*$_2$-CH=CH$_2$), 2.53-3.21 (m, 2H, C$_3$-H), 3.21 (m, 1H, C$_2$-H), 3.48, 4.12 (d, each 1H, *J*=11.6 Hz, -NC*H*$_2$-aromatic), 3.53 (m, 1H, C$_1$'-H), 4.05 (m, 1H, C$_4$-H), 4.47 (d, 2H, *J*=11.9 Hz, C$_4$-OC*H*$_2$-aromatic), 4.60 (d, 2H, *J*=11.9 Hz, C$_1$'-OC*H*$_2$-aromatic), 5.08 (m, 2H, -CH= C*H*$_2$), 5.91 (m, 1H, -C*H*=CH$_2$), 7.23-7.35 (m, 15H, aromatic-H); ^{13}C-NMR ($CDCl_3$) δ 33.33, 34.66, 59.74, 60.34, 64.16, 71.11, 72.15, 76.99, 81.22, 116.33, 126.78, 127.54, 127.77, 128.19, 128.26, 128.33, 128.61, 136.08, 138.25, 138.68, 139.77. *Anal.* Calcd for $C_{29}H_{33}NO_2$: C, 81.46; H, 7.78; N, 3.28. Found: C, 81.49; H, 7.85; N, 3.34.

(2*R*,4*R*,1'*S*)-*N*-Benzyl-2-(1'-benzyloxy-3'-butenyl)pyrrolidine (12b$_1$)

Yield 70%; colorless oil; $[\alpha]_D^{25}$ = +18.6° (c 0.13, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 2.12-2.48 (m, 5H, C$_3$-H, C$_5$-H and -C*H*$_2$-CH=CH$_2$), 2.71 (m, 1H, C$_2$-H), 3.14, 4.28 (d, each 1H, *J*=15.0 Hz, -NC*H*$_2$-aromatic), 3.18 (m, 1H, C$_5$-H), 3.73 (m, 1H, C$_1$'-H), 4.04 (m, 1H, C$_4$-H), 4.46 (d, 2H, *J*=12.0 Hz, C$_4$-OC*H*$_2$-aromatic), 4.64, 5.02 (d, each 1H, *J*=21.0 Hz, C$_1$'-OC*H*$_2$-aromatic), 5.12 (m, 2H, -CH=C*H*$_2$), 5.91 (m, 1H, -C*H*=CH$_2$), 7.01-7.48 (m, 15H, aromatic-H); ^{13}C-NMR ($CDCl_3$) δ 32.48, 37.12, 59.14, 59.86, 66.91, 70.47, 72.75, 76.67, 78.40, 116.45, 127.22, 127.32, 127.44, 127.55, 127.80, 127.92, 128.12, 128.55, 128.63, 136.04; MS (FAB) *m/z* 428 [(M+1)$^+$].

(2*R*,4*R*,1'*R*)-*N*-Benzyl-2-(1'-benzyloxy-3'-butenyl)pyrrolidine (12b$_2$)

Yield 75%; colorless oil; $[\alpha]_D^{25}$ = +11.7° (c 0.16, $CHCl_3$); IR ($CDCl_3$) ν 3062, 2862, 1640 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.84-2.37 (m, 4H, C$_3$-H and -C*H*$_2$-CH=CH$_2$), 2.88 (m, 1H, C$_2$-H), 3.34, 4.18 (d, each 1H, *J*=13.7 Hz, -NC*H*$_2$-aromatic), 3.63 (m, 1H, C$_1$'-H), 3.99 (m, 1H, C$_4$-H), 4.45 (d, 2H, *J*=12.2 Hz, C$_4$-OC*H*$_2$-aromatic), 4.63 (d, 2H, *J*=12.3 Hz, C$_1$'-OC*H*$_2$-aromatic), 5.08 (m, 2H, -CH=C*H*$_2$), 5.94 (m, 1H, -C*H*=CH$_2$), 7.13-7.32 (m, 15H, aromatic-H); MS (FAB) *m/z* 428 [(M+1)$^+$].

(2*S*,4*R*,1'*S*)-*N*-Benzyl-2-(1'-benzyloxy-4'-hydroxybutyl)pyrrolidine (13a$_1$)

BH_3-THF complex (4.74 mL, 1.0M solution in THF) was added to a solution of **12a$_1$** (1.03 g, 2.37 mmol)

in THF (50 mL) and the mixture was stirred at 40 °C for 2 h. After addition of $NaBO_3$ (0.47 g, 4.74 mmol) in H_2O (50 mL), the mixture was stirred at rt for 2 h. Then, the reaction was quenched with 10% aqueous NH_4Cl and the mixture was extracted with EtOAc. The organic layer was washed with saturated aqueous NaCl and dried over Na_2SO_4. Removal of the solvent gave an oily residue, which was chromatographed on silica gel using EtOAc/*n*-hexane (1:1) to give **13a$_1$** as a colorless oil, yield 0.76 g (71%); $[\alpha]_D^{25}$ = -44.6° (c 0.50, $CHCl_3$); IR ($CDCl_3$) ν 3412 (OH), 3028, 2868 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.54-1.77 (m, 4H, -C*H*$_2$C*H*$_2$CH$_2$OH), 1.91-2.23 (m, 2H, C$_3$-H), 2.32-3.25 (m, 2H, C$_5$-H), 3.02 (m, 1H, C$_2$-H), 3.38, 4.23 (d, each 1H, *J*=13.2 Hz, -NC*H*$_2$-aromatic), 3.61 (m, 3H, -C*H*$_2$OH and C$_1$'-H), 4.12 (m, 1H, C$_4$-H), 4.45 (d, 2H, *J*=14.4 Hz, C$_4$-OC*H*$_2$-aromatic), 4.59, 5.00 (d, each 1H, *J*=11.7 Hz, C$_1$'-OC*H*$_2$-aromatic), 7.23-7.33 (m, 15H, aromatic-H); ^{13}C-NMR ($CDCl_3$) δ 29.17, 29.69, 32.37, 59.74, 62.62, 66.93, 71.14, 73.21, 77.07, 78.61, 126.82, 127.50, 127.57, 127.81, 128.27, 128.31, 128.54, 138.63, 138.99, 139.60. *Anal.* Calcd for $C_{29}H_{35}NO_3$: C, 78.17; H, 7.92; N, 3.14. Found: C, 77.91; H, 7.92; N, 3.12.

The following compounds (**13a$_2$**, **13b$_{1-2}$**) were prepared from **12a$_2$**, **12b$_{1-2}$** in the same manner as described for the preparation of **13a$_1$**, respectively.

(2*S*,4*R*,1'*R*)-*N*-Benzyl-2-(1'-benzyloxy-4'-hydroxybutyl)pyrrolidine (13a$_2$)

Yield 70%; colorless oil; $[\alpha]_D^{25}$ = -90.0° (c 0.70, $CHCl_3$); IR ($CDCl_3$) ν 3424 (OH), 3020, 2940 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.49-2.05 (m, 6H, C$_3$-H and -C*H*$_2$C*H*$_2$CH$_2$OH), 2.45-3.23 (m, 2H, C$_5$-H), 3.23 (m, 1H, C$_2$-H), 3.41 (m, 1H, C$_1$'-H), 3.47, 4.03 (d, each 1H, *J*=12.0 Hz, -NC*H*$_2$-aromatic), 3.59 (t, 2H, *J*=6.0 Hz, -C*H*$_2$OH), 4.04 (m, 1H, C$_4$-H), 4.47, 4.65 (d, each 1H, *J*=11.3 Hz, C$_4$-OC*H*$_2$-aromatic), 4.46 (d, 2H, *J*=11.7 Hz, C$_1$'-OC*H*$_2$-aromatic), 7.25-7.34 (m, 15H, aromatic-H); ^{13}C-NMR ($CDCl_3$) δ 25.94, 29.43, 32.99, 59.95, 60.44, 62.91, 63.70, 71.18, 72.03, 77.13, 80.80, 126.89, 127.57, 127.67, 127.95, 128.24, 128.37, 128.66, 138.43, 139.69. *Anal.* Calcd for $C_{29}H_{35}NO_3$: C, 78.17; H, 7.92; N, 3.14. Found: C, 78.06; H, 7.86; N, 3.12.

(2*R*,4*R*,1'*S*)-*N*-Benzyl-2-(1'-benzyloxy-4'-hydroxybutyl)pyrrolidine (13b$_1$)

Yield 72%; colorless oil; $[\alpha]_D^{25}$ = +18.3° (c 0.23, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.62-1.82 (m, 4H, -C*H*$_2$C*H*$_2$CH$_2$OH), 2.21-2.38 (m, 3H, C$_5$-H, C$_3$-H), 2.76 (m, 1H, C$_2$-H), 3.21 (m, 1H, C$_5$-H), 3.23, 4.31 (d, each 1H, *J*=13.6 Hz, -NC*H*$_2$-aromatic), 4.11 (m, 1H, C$_4$-H), 4.45 (d, 2H, *J*=12.0 Hz, C$_4$-OC*H*$_2$-aromatic), 4.62 (m, 2H, -C*H*$_2$OH), 4.78 (m, 1H, C$_1$'-H), 4.57, 5.13 (d, each 1H, *J*=11.2 Hz, C$_1$'-OC*H*$_2$-aromatic), 7.21-7.60 (m, 15H, aromatic-H); ^{13}C-NMR ($CDCl_3$) δ 29.14, 29.62, 32.45, 59.30, 59.84, 62.59, 67.63, 70.38, 72.89, 76.47, 78.15, 126.69, 127.23, 127.29, 127.50, 128.01, 128.17, 128.32, 138.55, 139.00, 139.32. *Anal.* Calcd for $C_{29}H_{35}NO_3$: C, 78.17; H, 7.92; N, 3.14. Found: C, 77.75; H, 7.90; N, 2.83.

(2*R*,4*R*,1'*R*)-*N*-Benzyl-2-(1'-benzyloxy-4'-hydroxybutyl)pyrrolidine (13b$_2$)

Yield 75%; colorless oil; $[\alpha]_D^{25}$ = +40.6° (c 0.16, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.58-2.34 (m, 6H, C$_3$-H and

-C*H*$_2$C*H*$_2$CH$_2$OH), 2.41 (m, 1H, C$_5$-H), 3.02 (m, 1H, C$_2$-H), 3.21 (m, 1H, C$_5$-H), 3.43, 4.19 (d, each 1H, *J*=13.5 Hz, -NC*H*$_2$-aromatic), 3.62 (m, 3H, -C*H*$_2$OH and C$_1$'-H), 4.07 (m, 1H, C$_4$-H), 4.45 (d, 2H, *J*=11.8 Hz, C$_4$-OC*H*$_2$-aromatic), 4.59, 4.78 (d, each 1H, *J*=11.1 Hz, C$_1$'-OC*H*$_2$-aromatic), 7.24-7.58 (m, 15H, aromatic-H); ^{13}C-NMR (CDCl$_3$) δ 26.37, 29.20, 33.28, 59.81, 62.67, 64.43, 70.41, 72.06, 76.28, 81.06, 126.67, 127.26, 127.39, 127.45, 127.74, 128.06, 128.14, 128.19, 128.49, 138.19, 138.39. *Anal.* Calcd for C$_{29}$H$_{35}$NO$_3$: C, 78.17; H, 7.92; N, 3.14. Found: C, 78.02; H, 8.00; N, 3.15.

(2*R*,8*S*,9*S*)-*N*-Benzyl-2,8-dibenzyloxyindolizidinium chloride (14a$_1$)

Triethylamine (0.34 mL, 2.47 mmol) and methanesulfonyl chloride (0.15 mL, 1.98 mmol) were added to a solution of **13a$_1$** (0.75 g, 1.60 mmol) in CH_2Cl_2 (30 mL) and the mixture was stirred at rt for 4 h. Then, the mixture was treated with 10% aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with saturated aqueous NaCl, dried over Na_2SO_4 and evaporated off *in vacuo* to give **14a$_1$** as an amorphous solid, yield 0.70 g (90%); IR (CDCl$_3$) ν 3379, 2957 cm^{-1}; ^{1}H-nmr (CDCl$_3$) δ 1.86-2.81 (m, 6H, C$_1$-H, C$_6$-H and C$_7$-H), 3.63 (m, 1H, C$_5$-H), 3.86 (m, 3H, C$_5$-H, C$_3$-H and C$_9$-H), 4.19 (m, 2H, C$_8$-H and C$_3$-H), 4.36 (d, 2H, *J*=20.2 Hz, C$_2$-OC*H*$_2$-aromatic), 4.63 (m, 1H, C$_2$-H), 4.70, 4.53 (d, each 1H, *J*=11.5Hz, -NC*H*$_2$-aromatic), 5.12, 4.82 (d, each 1H, *J*=12.7 Hz, C$_8$-OC*H*$_2$-aromatic), 7.12-7.55 (m, 15H, aromatic-H); ^{13}C-NMR (CDCl$_3$) δ 14.44, 22.24, 35.03, 54.69, 59.98, 66.49, 71.61, 72.12, 72.47, 73.54, 75.88, 127.43, 127.89, 127.95, 128.32, 128.37, 128.71, 128.90, 130.37, 133.85, 136.57, 136.75.

The following compounds (**14a$_2$**, **14b$_{1-2}$**) were prepared from **13a$_2$**, **13b$_{1-2}$** in the same manner as described for the preparation of **14a$_1$**, respectively.

(2*R*,8*R*,9*S*)-*N*-Benzyl-2,8-dibenzyloxyindolizidinium chloride (14a$_2$)

Yield 92%; amorphous solid; IR (CDCl$_3$) ν 3375, 2951 cm^{-1}; ^{1}H-NMR (CDCl$_3$) δ 1.76-2.46 (m, 6H, C$_1$-H, C$_6$-H and C$_7$-H), 2.97 (m, 1H, C$_9$-H), 3.61-3.43 (m, 2H, C$_5$-H), 3.78 (m, 1H, C$_8$-H), 4.25 (d, 1H, *J*=11.1 Hz, -NC*H*$_2$-aromatic), 4.39 (m, 1H, C$_2$-H), 4.41-4.58 (m, 4H, C$_2$-OC*H*$_2$-aromatic, -NC*H*$_2$-aromatic and C$_3$-H), 5.10 (m, 1H, C$_3$-H), 5.61, 4.69 (d, each 1H, *J*=12.1 Hz, C$_8$-OC*H*$_2$-aromatic), 7.17-7.63 (m, 15H, aromatic-H); ^{13}C-NMR (CDCl$_3$) δ 15.99, 20.65, 33.39, 56.59, 64.45, 67.43, 69.54, 71.53, 71.81, 72.37 74.12, 127.75, 127.86, 127.91, 128.24, 128.42, 128.64, 129.09, 130.50 133.25, 136.73, 136.94.

(2*R*,8*S*,9*R*)-*N*-Benzyl-2,8-dibenzyloxyindolizidinium chloride (14b$_1$)

Yield 93%; amorphous solid; ^{1}H-NMR (CDCl$_3$) δ 1.72-2 10 (m, 6H, C$_1$-H, C$_6$-H and C$_7$-H), 2.41-3.06 (m, 2H, C$_5$-H), 3.07 (m, 1H, C$_9$-H), 3.25-3.54 (m, 2H, C$_3$-H), 3.88 (s, 1H, C$_8$-H), 4.41 (d, 2H, *J*=15.3 Hz, C$_2$-OC*H*$_2$-aromatic), 4.68, 4.88 (d, each 1H, *J*=11.2 Hz, -NC*H*$_2$-aromatic), 4.74 (m, 1H, C$_2$-H), 5.22, 5.33 (d, each 1H, *J*=11.2 Hz, C$_8$-OC*H*$_2$-aromatic), 7.19-7.59 (m, 15H, aromatic-H).

(2*R*,8*R*,9*R*)-*N*-Benzyl-2,8-dibenzyloxyindolizidinium chloride (14b$_2$)

Yield 91%; amorphous solid; ^{1}H-NMR (CDCl$_3$) δ 1.52-2.18 (m, 6H, C$_1$-H, C$_6$-H and C$_7$-H), 2.34 (m, 1H,

C_5-H), 3.17 (m, 2H, C_9-H and C_5-H), 3.32-3.61 (m, 2H, C_3-H), 4.12 (m, 1H, C_8-H), 4.45 (d, 2H, *J*=11.2 Hz, C_2-OC*H*$_2$-aromatic), 4.82 (m, 1H, C_2-H), 4.61, 4.80 (d, 2H, *J*=10.5 Hz, -NC*H*$_2$-aromatic), 5.42, 5.73 (d, each 1H, C_8-OC*H*$_2$-aromatic), 7.08-7.62 (m, 15H, aromatic-H).

(2*R*,8*S*,9*S*)-2,8-Dihydroxyindolizidine (5a$_1$)

10% Pd/C (151 mg, 1.26 mmol) was added to a solution of **14a$_1$** (200 mg, 0.42 mmol) in saturated ethanolic HCl (10 mL) and the mixture was stirred at rt for 2 h under hydrogen at 50 *psi*. The mixture was filtered through cellite and concentrated *in vacuo*. The residue was placed on a Dowax 50W-X8 (H^+ form) column and eluted with 0.7*N* NH_4OH. Freezing drying of the appropriate fractions gave **5a$_1$** as a white solid, yield 80 mg (75%); mp 162.0-165.0 °C; $[\alpha]_D^{25}$ = -44.5° (c 0.20, $CHCl_3$); ^{1}H-NMR (D_2O) δ 4.42 (m, 1H, C_2-H), 3.48 (m, 1H, C_8-H), 3.41 (m, 1H, C_3-H), 2.98 (m, 1H, C_5-H), 2.25 (m, 1H, C_9-H), 2.21 (m, 1H, C_5-H), 2.12 (m, 1H, C_3-H), 2.08 (m, 1H, C_1-H), 1.82 (m, 1H, C_1-H), 1.56 (m, 2H, C_6-H), 1.32 (m, 2H, C_7-H); ^{13}C-nmr (D_2O) δ 23.33, 32.29, 38.56, 50.78, 61.82, 67.46, 67.97, 71.93; HRMS *m/z* 157.1102 (M^+, $C_8H_{15}NO_2$ requires M, 157.1102).

The following compounds (**5a$_2$**, **5b$_{1-2}$**) were prepared from **14a$_2$**, **14b$_{1-2}$** in the same manner as described for the preparation of **5a$_1$**, respectively.

(2*R*,8*R*,9*S*)-2,8-Dihydroxyindolizidine (5a$_2$)

Yield 70%; white solid; mp 54.0-58.0 °C; $[\alpha]_D^{25}$ = -4.5° (c 0.20, CH_3OH); ^{1}H-NMR (D_2O) δ 4.50 (m, 1H, C_2-H) 4.13 (m, 1H, C_8-H), 3.59 (m, 1H, C_3-H), 3.20 (m, 1H, C_5-H), 2.81 (m, 1H, C_9-H), 2.47 (m, 1H, C_5-H), 2.38 (m, 1H, C_3-H), 2.13 (m, 1H, C_1-H), 1.96 (m, 1H, C_1-H), 1.66-1.85 (m, 4H, C_6-H and C_7-H); ^{13}C-NMR (D_2O) δ 18.95, 29.68, 35.09, 51.98, 61.73, 64.60, 65.39, 67.58; HRMS *m/z* 157.1103 (M^+, $C_8H_{15}NO_2$ requires M, 157.1102).

(2*R*,8*S*,9*R*)-2,8-Dihydroxyindolizidine (5b$_1$)

Yield 75%; colorless oil; $[\alpha]_D^{25}$ = +50.5° (c 0.20, CH_3OH); ^{1}H-NMR (D_2O) δ 4.43 (m, 1H, C_4-H), 3.52 (m, 1H, C_8-H), 3.01 (m, 2H, C_3-H and C_5-H), 2.58 (m, 2H, C_9-H and C_5-H), 2.06 (m, 3H, C_1-H and C_3-H), 1.24-1.82 (m, 4H, C_6-H and C_7-H); ^{13}C-NMR (D_2O) δ 22.97, 32.15, 38.27, 50.91, 62.24, 68.25, 69.05, 71.75; HRMS *m/z* 157.1107 (M^+, $C_8H_{15}NO_2$ requires M, 157.1102).

(2*R*,8*R*,9*R*)-2,8-Dihydroxyindolizidine (5b$_2$)

Yield 59%; white solid; mp 97.0-99.0 °C; $[\alpha]_D^{25}$ = +1.4° (c 0.42, CH_3OH); ^{1}H-NMR (D_2O) δ 4.42 (m, 1H, C_4-H), 3.92 (s, 1H, C_8-H), 3.01 (m, 1H, C_3-H), 2.91 (m, 1H, C_5-H), 2.45 (m, 1H, C_9-H), 2.25 (m, 2H, C_5-H and C_3-H), 2.11 (m, 1H, C_1-H), 1.56-1.93 (m, 5H, C_6-H, C_7-H and C_1-H); ^{13}C-NMR (D_2O) δ 19.30, 30.11, 35.51, 52.00, 62.46, 65.22, 66.49, 68.57; HRMS *m/z* 157.1103 (M^+, $C_8H_{15}NO_2$ requires M, 157.1102).

ENZYME ASSAY

Enzyme assay was performed with commercially available as follows: α-glucosidase (G-6136)[†], α-galactosidase (G-4408), α-glucosidase (G-0395), β-galactosidase (G-5635), α-amyloglucosidase (A-7420), β-cellulase (C-0901), α-mannosidase (M-7257), β-mannosidase (M-9400) were purchased from Sigma Chemical Company. Corresponding substrates were *p*-nitrophenylated glycopyranosides from Sigma Chemical Company and used without further purification. The enzyme assay was performed on 10 mM MES[‡], 100 mM NaCl and adjusted to the optimum pH designated on each glycosidase (α-glucosidase at 6.5, α-galactosidase at 6.5, β-glucosidase at 5.0, β-galactosidase at 7.0, α-glucoamylase at 5.0, β-cellulase at 5.0, α-mannosidase at 4.5, β-mannosidase at 3.8). To 0.90 mL of glycosidase solution, 0.1 mL of 10X substrate solution was added and incubated at 37 °C for 2 h. In every 30 min, 75 μL of aliquot was taken and quenched with 75 μL of 0.1M of Na_2CO_3 solution. The concentration of the product, *p*-nitrophenol was measured by absorbance at 405 nm The initial velocity was determined at not less than 10% of the substrate was converted to the product. The *Ki* value was calculated using Dixon plots. Two concentrations of the substrate solutions were chosen as *Km* (Michaelis-Menten constant) and a half of the *Km* values of the enzyme, respectively. Five different concentrations of inhibitor were used for the Dixon plots. The inhibition values more than 5 mM were not measured.

[†]Sigma Cat. No.

[‡]MES=2-*N*-morpholinoethanesulfonic acid

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