Synthesis and glycosidase-inhibitory activity of novel polyhydroxylated quinolizidines derived from D-glycals†

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Received 5th January 2009, Accepted 9th February 2009
First published as an Advance Article on the web 26th March 2009
DOI: 10.1039/b900011a

A number of structurally novel polyhydroxylated quinolizidines have been prepared starting from 2-deoxyglycosylamines which in turn were derived from D-glycals by following a methodology developed in our laboratory. In our strategy, Grignard reaction and ring-closing metathesis (RCM) reactions are the key steps to construct the desired skeletons. All synthesized final molecules were checked for glycosidase inhibition activity, and some were found to be selective for certain glycosidases.

Introduction

The synthesis of polyhydroxylated indolizidine,[1b] pyrrolizidine and quinolizidine[1c] aza-bicylic alkaloids is a subject of current research.[1,2] Among others, swainsonine **1**,[3] castanospermine **2**[4] and alexine **3**[5] (Fig. 1) have attracted significant interest due to their biological activities as glycosidase inhibitors. Since glycosidases are involved in various metabolic pathways, these compounds exhibit a wide range of pharmacological activities including anti-viral, anti-HIV, anti-feedant and immunoregulator.[3] To study the structure–activity relationships within this series of glycomimetics, it is important to develop efficient and diverse synthetic routes towards them as well as their stereoisomers and analogues. However, only few reports of polyhydroxylated quinolizidines (which can be regarded as higher analogues of indolizidines) are known.[6] Ganem and coworkers[6a] reported the first synthesis of derivatives **4** and **5** (Fig. 2), which was followed by several syntheses of these molecules by other laboratories.[6b,6c] Several stereoisomers and analogues have been also synthesized[6d–i] and tested for their glycosidase inhibition activities.

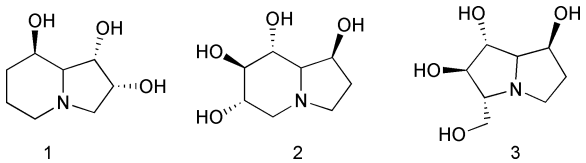

Fig. 1 Some naturally occurring polyhydroxylated azabicyclic alkaloids.

Interestingly, the analogue of homonojirimycin **6** reported by Liu *et al.*[6j] was found to be a potent inhibitor of α-glucosidase I from pig kidney (IC_{50} = 0.15 μM). Vogel *et al.*[6k] reported the synthesis of quinolizidines **7** and **8** bearing a polyhydroxy side chain, which, however, were found to be weak inhibitors of glycosidases.

As part of our on going research towards the synthesis of natural azasugars, their analogues and hybrid sugars,[7] we have recently reported the synthesis of D- and L-fagomine,[9] and some analogues starting from D-glycals, using a methodology[8] developed in our laboratory for the formation of 2-deoxyglycosylamine derivatives. Herein, we report the synthesis of pentahydroxylated 6-hydroxymethyl-quinolizidines **9** and **10**, and trihydroxylated 6-hydroxymethyl-quinolizidines **11** and **12**, using the same 2-deoxyglycosylamine intermediate. These molecules can be regarded as analogues of L-1,2-dideoxyhomonojirimycin.

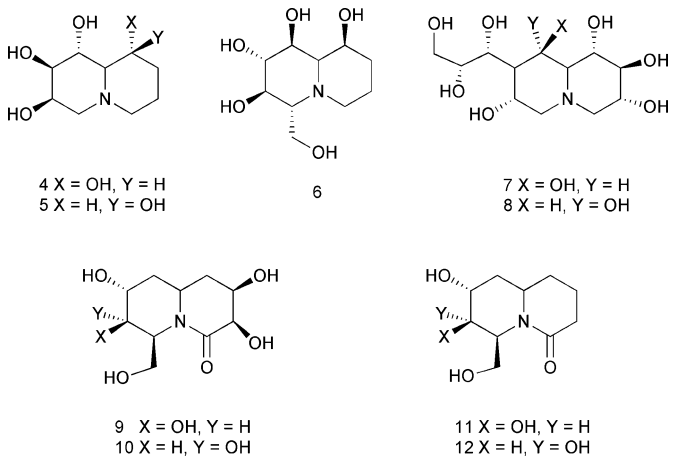

Fig. 2 Polyhydroxylated quinolizidines.

Results and discussion

The synthesis began with the *tert*-butyloxycarbonyl-protected glycosylamine derivatives **14a** and **14b**, obtained from the D-glucal and D-galactal derivatives **13a** and **13b** respectively, which were treated with seven equivalents of allyl magnesium bromide (following a literature report on the opening of *N*-glycosides by Grignard reagents[10]) to give ring-opened amino alcohols **15a** (70% yield) and **15b** (75% yield), each as an inseparable 1:1 mixture of diastereoisomers. The free hydroxyl groups of amino alcohols **15a** and **15b** were mesylated using $MsCl/Et_3N$ to afford **16a** and **16b** respectively in 98 and 95% yield. These mesylates underwent intramolecular S_N2 cyclization (after removal of the Boc group using trifluoroacetic acid in dichloromethane and

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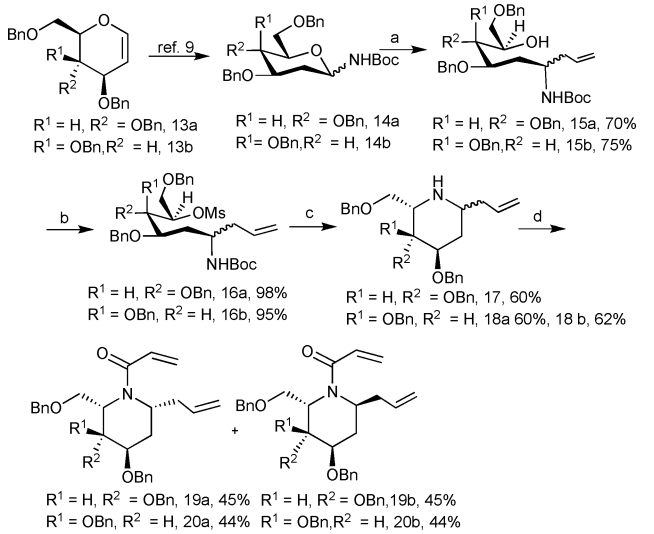
† Electronic supplementary information (ESI) available: Spectroscopic data of new compounds and copies of spectra for compounds **21–30**. See DOI: 10.1039/b900011a

further treatment with potassium carbonate), leading to the pieperidine derivatives **17** and **18**[11] respectively (Scheme 1).



Scheme 1 *Reagents and conditions*: (a) allylMgBr, THF, 0 °C, 1 h; (b) MsCl, Et_3N, CH_2Cl_2, 0 °C, 2 h; (c) (i) TFA, CH_2Cl_2, 0 °C → rt, 2 h; (ii) K_2CO_3, CH_3CN, 8–10 h; (d) Et_3N, acryloyl chloride, CH_2Cl_2, 1 h.

In the case of **16b**, the diastereomers were separated chromatographically by using a chromatotron to give **18a** and **18b**, while in the case of **16a** the diastereomers could not be separated at this stage. With the key precursors **17** and **18** in hand, we pursued the synthetic sequence to the quinolizidines. Acryloylation of the free NH group in **17** and **18** with acryloyl chloride and triethylamine afforded **19a,b** and **20a,b** respectively in good yields. The diasteromers **19a** and **19b** were separated at this stage chromatographically by using a chromatotron.

We next examined the RCM reaction in order to prepare bicyclic compounds. Unlike the first-generation Grubbs' ruthenium catalyst, the second generation catalyst proved to be suitable for the RCM reaction of the α,β-unsaturated amides **19** and **20**, which proceeded smoothly in refluxing dichloromethane using 5 mol% of the catalyst. This led to the formation of bicyclic quinolizidines **21** and **22** respectively, which were characterized by ^1H, ^{13}C NMR and COSY experiments. The stereochemistry at the newly formed stereocenters, generated from the Grignard reaction, was further confirmed by NOE experiments. In the case of **21a** and **22a** irradiation of signals for H-6 led to the enhancement of the signals for H-1, suggesting a *cis* relationship between them (Fig. 3). For **21b** and **22b**, the irradiation of the signals for H-1 led to the enhancement of signals for H-8, thus confirming the assigned stereochemistry. Further, in neither **21b** nor **22b** did irradiation of H-6 lead to enhancement of the signals for H-1, indicating a *trans* relationship between H-1 and H-6 (Fig. 3).

Dihydroxylation of the double bond in **21a**, **21b**, **22a** and **22b** was carried out using a catalytic amount of OsO_4 in the presence of NMO, which gave a single diastereomer (viz. **23a**, **23b**, **24a** and **24b**) in all cases, in almost quantitative yield. Subsequent deprotection of benzyl ethers using $Pd(OH)_2$ in MeOH under H_2 afforded the fully deprotected compounds **9a**, **9b**, **10a** and **10b** respectively. These products were characterized by ^1H, ^{13}C NMR and mass spectrometry, and also by spectroscopic analysis of the

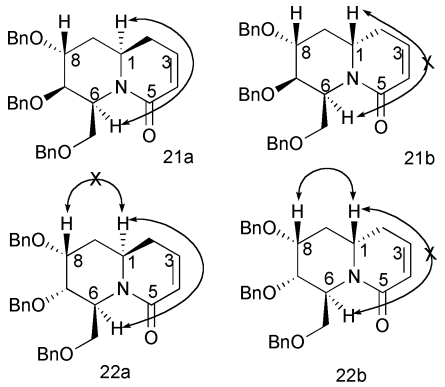
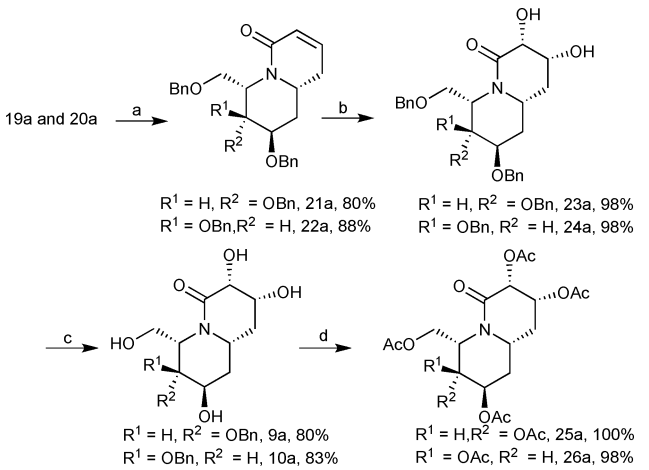
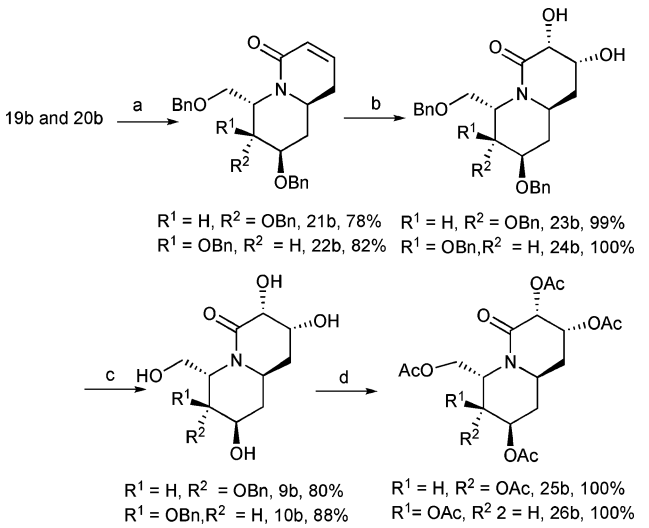


Fig. 3 NOE correlations.

corresponding acetates (**25a**, **25b**, **26a** and **26b**) (Schemes 2 and 3). The stereochemistry of the *cis*-diol formed was confirmed through



Scheme 2 *Reagents and conditions*: (a) 5 mol% Grubbs' catalyst, CH_2Cl_2, reflux, 5–7 h; (b) OsO_4 cat., NMO, *t*-BuOH–acetone–water (1 : 2 : 2), rt, 12 h; (c) $Pd(OH)_2$, MeOH, 50 psi H_2, rt, 24 h. (d) Ac_2O, pyridine, DMAP, 24 h.



Scheme 3 *Reagents and conditions*: (a) Grubbs' catalyst, CH_2Cl_2, reflux, 5–7 h; (b) OsO_4 cat., NMO, *t*-BuOH–acetone–water (1 : 2 : 2), rt, 12 h; (c) $Pd(OH)_2$, MeOH, 50 psi H_2, rt, 24 h. (d) Ac_2O, pyridine, DMAP, 24 h.

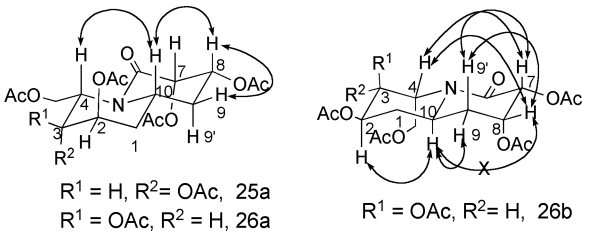


Fig. 4 NOE correlations.

NOE and NOESY experiments (Fig. 4). Thus, irradiation of the signal for H-10 led to the enhancement of the signals for protons H-7, H-8 and H-4 for **25a** and **26a**. In **26b**, on the other hand, irradiation of signals for H-7 and H-8 led to the enhancement of signal for H-4, and irradiation of the signal for H-10 led to the enhancement of H-2 signals. The stereochemistry shown in Fig. 4 for **26b** was also confirmed by a NOESY spectrum, which showed a *cis* relation for H-7 and H-8 with H-9′, while H-10 showed a *cis* relation with H-9, thus confirming the stereochemistry assigned.

To confirm the stereochemistry of diol in the case of **23b**, the diol was protected as the corresponding acetate derivative **29**. The NOESY spectrum of **29** showed a *cis* relation for H-8 and H-7 with H-9′, while H-10 showed a *cis* relation with H-9. This stereochemistry was further supported by the NOE, in which irradiation of signals for H-7 and H-8 did not lead to the enhancement of H-10 signal, indicating that H-10 is *trans* to H-7 and H-8, and consequently that the -OH groups are *cis*-oriented with respect to H-10.

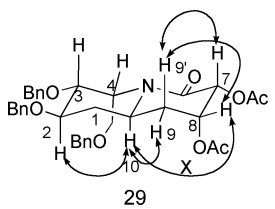
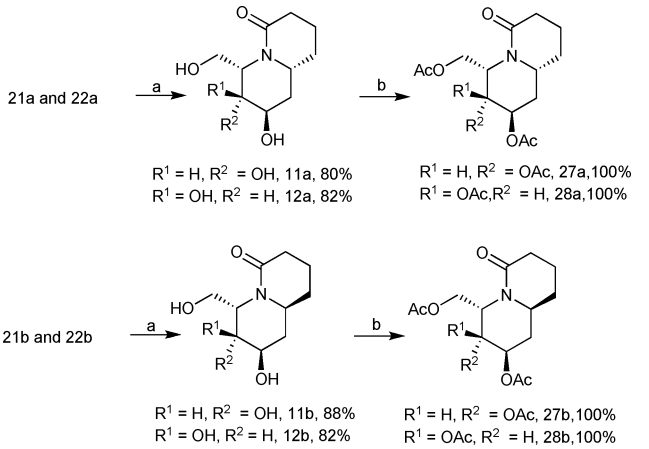


Fig. 5 NOE correlations.

The intermediates **21** and **22** were also used as starting materials for the synthesis of the quinolizidine derivatives **11** and **12**, as shown in Scheme 4. Thus, compounds **21a**, **21b**, **22a** and **22b** were subjected to hydrogenation conditions ($Pd(OH)_2$, H_2 atm), whereby saturation of the double bond proceeded along with deprotection of benzyl ethers. Compounds **11a**, **11b**, **12a** and **12b** thus formed were characterized by ^1H, ^{13}C NMR and MS, and also by spectroscopic analysis of the corresponding acetate derivatives (**27a**, **27b**, **28a** and **28b**).



Scheme 4 *Reagents and conditions*: (a) $Pd(OH)_2$, MeOH, 50 psi H_2, rt, 24 h. (b) Ac_2O, pyridine, DMAP, 24 h.

The inhibitory activity of all new bicyclic quinolizidines **9–12** was tested against several glycosidases,[12] and the IC_{50} values are collected in Table 1. Pentahydroxylated quinolizidines **9a**, **9b** and **10a** showed only very weak activity while **10b** showed moderate inhibition of β-glucosidase and β-galactosidase. On the other hand, trihydroxyquinolizidines **11a**, **11b**, **12a** and **12b** were found to be selective inhibitors of glycosidases.

Conclusion

In conclusion, we have demonstrated the utility of 2-deoxyglycosylamine derivatives, obtained from glycals, in the synthesis of new quinolizidine molecules **9–12**, some of which were found be moderate but selective glycosidase inhibitors.

Experimental

General

Infrared spectra were recorded on Bruker FT/IR Vector 22 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on JEOL LA-400 and JEOL ECX-500 spectrometer in solution of $CDCl_3$ using tetramethylsilane as the internal standard. The mass spectra were recorded on a Waters HAB213 Q Tof Premier Micromass spectrometer and Microscopic II triple Quadrupole mass spectrometer. Rotation values were recorded on Autopol II automatic polarimeter at the wavelength of sodium D-line (589 nm) at 25 °C. Column chromatography was performed on silica gel (100–200 mesh) and thin layer chromatography (TLC) was performed on Silica gel plates made by using grade G silica gel obtained

Table 1 IC_{50} values for compounds **9–12** in mM, carried out at optimal pH for the enzymes, at 37 °C

Enzymes	9a	9b	10a	10b	11a	11b	12a	12b
α-Glucosidase (Bakers' yeast)	1.58	—[a]	6.40	—[a]	—[a]	—[a]	—[a]	—[a]
β-Glucosidase (Almond)	1.98	—[a]	—[a]	0.75	—[a]	—[a]	3.80	—[a]
α-Galactosidase (Coffee bean)	—[a]	0.98	2.10	—[a]	—[a]	—[a]	—[a]	—[a]
β-Galactosidase (Bovine liver)	—[a]	—[a]	1.35	0.29	1.40	—[a]	—[a]	1.58
α-Mannosidase (Jack bean)	2.50	2.80	—[a]	—[a]	—[a]	0.37	—[a]	—[a]
α-Glucosidase (Rice)	—[a]	2.80	—[a]	—[a]	—[a]	—[a]	—[a]	—[a]

[a] No inhibition at <1.0 mM concentration.

from s.d.fine-chem ltd., Mumbai. Melting points were determined using a Fischer-John melting point apparatus. All solvents and common reagents were purified by established procedures.

Starting material. Starting materials **14a** and **14b** were prepared according to a reported procedure.[8,9]

General procedure for Grignard reagent opening of 2-deoxyglycosylamine derivatives. In a two-necked round-bottomed flask were placed magnesium turnings (1.1 g, 45.8 mmol), THF (20 mL) and iodine (63 mg, 0.5 mmol). To this suspension, allyl chloride (1.8 g, 23.5 mmol) was added very slowly and the temperature of the flask was kept at 0 °C. Within 5 min vigorous reaction started, and the external temperature was maintained at 0 °C. The mixture was stirred for 1 h, after which the reagent was transferred slowly to another round-bottomed flask cooled to 0–10 °C containing a solution of 2-deoxyglycosylamine derivative (2.5 g, 4.67 mmol in 10 mL THF). The reaction was stirred at the same temperature for 1–1.5 h and then quenched by adding saturated aq. NH_4Cl solution (25 mL) and extracted with ethyl acetate (3 × 30 mL). The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated on a rotary evaporator to obtain a crude product which was purified by column chromatography.

***tert*-Butyl (6*R*,7*R*,8*R*)-6,7,9-tris(benzyloxy)-8-hydroxynon-1-en-4-ylcarbamate (15a).** Yield: 70% (1.76 g, viscous liquid). R_f: 0.50 (hexane–ethyl acetate, 9:1) IR (CH_2Cl_2) ν_{max}: 1605, 1695, 3300 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) (1:1 mixture of diastereomers): δ 7.23–7.37 (30H, m, aromatic), 5.64–5.75 (2H, m, -C*H*=CH_2), 5.01–5.05 (4H, m, -CH=CH_2), 4.42–4.75 (12H, m, -OCH_2Ph), 3.55–3.92 (8H, m), 3.43–3.55 (4H, m), 3.00 (1H, br.s, OH), 2.95 (1H, br.s, OH), 2.20–2.25 (4H, m), 1.69–1.85 (4H, m), 1.44 (18H, s) ppm. ^{13}C NMR (125.7 MHz): δ 155.6, 138.1, 137.9, 137.6 134.8, 134.5, 127.7–128.4 (m, aromatic), 118.1, 118.0, 80.1, 79.8, 78.9, 77.9, 73.8, 73.6, 73.4, 72.8, 70.9, 70.1, 69.9, 48.8, 48.4, 41.4, 39.6, 36.3, 36.1, 28.5, 28.1 ppm. MS/ESI : $[M + H]^+$ Calcd; 536.3021; found: 536.3020.

General procedure for mesylation

Alcohol (2.7 g, 4.11 mmol) **15a** or **15b** was dissolved in dry CH_2Cl_2 (30 mL) and cooled to 0 °C using an ice bath. Triethylamine (1.04 g, 10.3 mmol) and DMAP (25 mg, 0.2 mmol) were added to the reaction flask followed by slow addition of methanesulfonyl chloride (517 mg, 4.52 mmol). The reaction was stirred for 1 h at 0 °C to complete the reaction (TLC monitoring), quenched by adding saturated aq. $NaHCO_3$ solution (10 mL) and the mixture was extracted with CH_2Cl_2 (3 × 25 mL). The organic layer was washed with brine and dried over anhydrous sodium sulfate. Concentration of the organic layer on a rotary evaporator gave a crude product which was purified by column chromatography.

(2*R*,3*S*,4*R*)-1,3,4-Tris(benzyloxy)-6-(*tert*-butoxycarbonyl amino)non-8-en-2-yl methanesulfonate (16a). Yield: 98% (3.23 g, viscous liquid). R_f: 0.50 (hexane–ethyl acetate, 9:1) IR (CH_2Cl_2) ν_{max}: 1600, 1700 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) (1:1 mixture of diastereomers): δ 7.25–7.39 (30H, m, aromatic), 5.73–5.78 (2H, m, -C*H*=CH_2), 4.84–5.06 (4H, m, -CH=CH_2), 4.43–4.76 (12H, m, -OCH_2Ph), 4.01 (1H, d, J = 4.5 Hz), 3.91–3.93 (2H, dd, J = 1.5, 5.5 Hz), 3.32–3.78 (9H, m), 2.90 (3H, s, -OMs), 2.88 (3H, s, -OMs), 2.16–2.24 (4H, m), 1.88–1.94 (2H, m), 1.79–1.82 (1H, m), 1.56–1.61 (1H, m), 1.43 (18H, s) ppm. ^{13}C NMR (125.7 MHz): δ 153.9, 153.7 136.3, 136.1, 136.0, 135.5, 135.4, 132.9, 132.6, 125.2–126.8 (m, aromatic), 116.2, 115.8, 80.6, 80.1, 77.4, 77.1, 77.0, 76.8, 72.5, 71.9, 71.8, 71.2, 70.8, 70.2, 68.1, 67.7, 66.8, 47.0, 45.5, 38.7, 37.8, 36.8, 36.6, 33.5, 33.2, 26.7, 26.6 ppm. MS/ESI : $[M + H]^+$ Calcd; 654.3101; found: 654.3100.

General procedure for intramolecular cyclization

To a stirred solution of mesylate **16a** or **16b** (268 mg, 0.41 mmol) in dry CH_2Cl_2 (4 ml) at 0 °C was added trifluoroacetic acid (0.08 ml, 1.05 mmol) dropwise over 5 min. Immediately the reaction mixture was warmed to room temperature and stirred for a further 45 min. After cooling the reaction mixture to 0 °C and diluting it with CH_2Cl_2 (10 mL), 2 M K_2CO_3 solution (5 mL) was added carefully. This mixture was partitioned and the aqueous phase was extracted with CH_2Cl_2 (5 mL × 4). The combined organic phase was dried over anhydrous K_2CO_3 and filtered through Celite. The solvent was removed on a rotary evaporator. The residue was then dissolved in CH_3CN (15 mL), and K_2CO_3 (283 mg, 2.05 mmol) was added in two portions over 2 h. After stirring the mixture for 8 h, it was gradually heated up to 70 °C over 1 h. The consumption of primary amine was confirmed by TLC analysis, and the mixture was filtered through Celite and concentrated *in vacuo* to give the crude cyclized product, which was purified by column chromatography. The diastereomers **18a** and **18b** were separated using a chromatotron, collecting small fractions using hexane–ethyl acetate (1:1).

(2*S*,3*R*,4*R*)-6-Allyl-3,4-bis(benzyloxy)-2-(benzyloxymethyl) piperidine(17). Yield: 60% (112 mg, viscous liquid). R_f: 0.50 (hexane–ethyl acetate, 3:7). IR (CH_2Cl_2) ν_{max}: 1605, 3450 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) (1:1 mixture of diastereomers): δ 7.23–7.37 (30H, m, aromatic), 5.66–5.80 (2H, m, -C*H*=CH_2), 5.05–5.13 (4H, m, -CH=CH_2), 4.38–4.69 (12H, m, -OCH_2Ph), 3.51–3.73 (8H, m, H-7, H-7′, H-4, H-3 for both isomers), 3.30–3.42 (m, 2H, H-2 for both isomers), 2.96–2.97 (1H, m, H-6 for one isomer), 2.84–2.87 (1H, m, for one isomer), 1.99–2.13 (5H, m, -CH_2-CH=CH_2, H-5 for one isomer), 1.75–1.80 (1H, m, H-5 for one isomer), 1.65–1.70 (1H, m, H-5′ for one isomer), 1.25–1.28 (1H, m, H-5′ for one isomer) ppm. ^{13}C NMR (125.7 MHz): δ 142.3, 142.0, 139.1, 138.8, 131.1–132.1 (m, aromatic), 121.4, 121.1, 84.5, 81.0, 80.7, 80.6, 80.5, 77.1, 76.9, 76.5, 76.3, 76.0, 75.8, 75.4, 74.3, 69.4, 58.4, 58.1, 53.4, 50.6, 44.7, 44.6, 40.0, 36.1 ppm. MS/ESI : $[M + H]^+$ Calcd; 458.2695; found: 458.2690.

General procedure for acryloylation

To a stirred solution of amine **17** or **18** (96 mg, 0.211 mmol) in dry CH_2Cl_2 at 0 °C was added dropwise Et_3N (26 mg, 0.323 mmol) followed by acryloyl chloride (20 μL, 0.248 mmol). The reaction mixture was stirred for 1 h and after completion of reaction (TLC monitoring), it was extracted with CH_2Cl_2 (2 × 10 mL). Usual work-up gave a crude product which was purified by column chromatography to give a diene. The diastereomers **19a** and **19b** were separated using a chromatotron, collecting small fractions using hexane–ethyl acetate (7:3).

***N*-Acryloyl (2*S*,3*R*,4*R*,6*R*)-6-allyl-3,4-bis(benzyloxy)-2-(benzyloxymethyl) piperidine (19a).** Yield: 45% (48 mg, viscous liquid). R_f: 0.45 (hexane–ethyl acetate, 7:3, after 4 times elution on a 7 cm long TLC plate). $[\alpha]_D^{25} = -36$ (*c* 1.71, CH_2Cl_2). IR (CH_2Cl_2)

ν_{max}: 1625, 1655 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.22–7.32 (m, 15H), 6.67 (1H, br s, -CO-CH=CH_2), 6.34 (1H, br d, J = 8.60 Hz, -CO-CH=CH_2), 5.77–5.79 (1H, m, -CH=CH_2), 5.65 (1H, br d, J = 9.80 Hz, -CO-CH=CH_2), 5.05–5.09 (2H, m, -CH=CH_2), 4.62–4.73 (3H, m, -OCH_2Ph, H-2), 4.42–4.54 (4H, m, -OCH_2Ph), 4.01 (2H, br t, J = 6.09, H-7, H-7′), 3.86 (2H, br s, H-4, H-3), 3.61 (1H, br s, H-6), 2.48 (2H, br s, -CH_2-CH=CH_2), 2.02 (2H, br d, J = 12.4 Hz, H-5,H-5′) ppm.^{13}C NMR (125.7 MHz): δ 166.7, 151.8, 138.5, 138.2, 135.1, 127.5–128.9 (m, aromatic), 117.9, 79.3, 73.2, 72.4, 71.2, 69.1, 53.1, 51.3, 40.1, 28.2 ppm. MS/ESI : $[M + H]^+$ Calcd; 512.2801; found: 512.2800.

General procedure for RCM

To a stirred solution of compound **19** or **20** (384 mg, 0.751 mmol) in dry CH_2Cl_2 (15 mL) at room temperature was added the second-generation Grubbs catalyst (13 mg, 0.015 mmol). The mixture was refluxed for 5–7 h and after completion of reaction, the solvent was evaporated and residue purified by column chromatography.

(6*S*,7*R*,8*R*,9a*R*)-7,8-Bis(benzyloxy)-6-(benzyloxymethyl)-7,8,9, 9a-tetrahydro-1*H*-quinolizin-4(6*H*)-one (21a). Yield: 80% (290 mg, viscous liquid). R_f: 0.30 (hexane–ethyl acetate, 5:5). $[\alpha]_D^{25} = -55$ (c 2.2, CH_2Cl_2). IR (CH_2Cl_2) ν_{max}: 1605 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 7.25–7.35 (15H, m, aromatic), 6.45–6.49 (m, 1H, H-3), 5.93 (d, J = 6.5 Hz, 1H, H-4), 5.11–5.13 (m, 1H, H-6), 4.77 (d, J = 11.5 Hz, 1H, -OCH_2Ph), 4.75 (d, J = 3.0 Hz, 2H, -OCH_2Ph), 4.67 (d, J = 12.0 Hz, 1H, -OCH_2Ph), 4.52 (d, J = 12.0 Hz, 1H, -OCH_2Ph), 4.45 (d, J = 12.0 Hz, 1H, -OCH_2Ph), 4.06–4.13 (m, 1H, H-10′), 3.96–4.00 (m, 1H, H-1), 3.92–3.95 (m, 1H, H-8), 3.84–3.92 (dd, J = 10.0, 3.0 Hz, 1H, H-7), 3.64–3.67 (dd, J = 9.50, 7.0 Hz, 1H, H-10), 2.43–2.48 (m, 1H, H-2′), 2.05–2.18 (m, 2H, H-9′, H-2),1.49–1.56 (q, J = 12.0 Hz, 1H, H-9) ppm. ^{13}C NMR (125.7 MHz): δ 164.9, 138.9, 138.4, 137.7, 127.4–128.3 (m, aromatic), 124.6, 80.6, 75.9, 73.1, 73.0, 72.9, 72.6, 67.1, 51.3, 50.1, 38.5, 30.8, 29.6 ppm. MS/ESI : $[M + H]^+$ Calcd; 484.2488; found: 484.2485.

General procedure for dihydroxylation

To a stirred solution of cyclized olefin **21** or **22** (200 mg, 0.42 mmol) in acetone–water–*t*-BuOH (4 mL, 1:1:0.4) at 35 °C, were added $NMO{\cdot}H_2O$ (60 mg, 0.49 mmol) and OsO_4 (1mg, 0.004 mmol). The reaction mixture was stirred for 24 h and then it was treated with $Na_2S_2O_5$ (123 mg, 0.65 mmol). It was stirred for further 1 h and extracted with ethyl acetate (3 × 15 mL). The organic layer was washed with 1 N HCl, water and finally with brine. Evaporation of the organic layer followed by purification through column chromatography gave pure product.

(6*S*,7*S*,8*R*,9a*S*)-7,8-Bis(benzyloxy)-6-(benzyloxymethyl)-7,8,9, 9a-tetrahydro-1*H*-quinolizin-4(6*H*)-one (23a). Yield: 98% (210 mg, viscous liquid). R_f: 0.30 (hexane–ethyl acetate, 1:9). $[\alpha]_D^{25} = -56$ (c 2.9, CH_2Cl_2). IR (CH_2Cl_2) ν_{max}: 1660, 3400 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.25–7.35 (15H, m, aromatic), 4.98–5.01 (1H, m, H-4), 4.71–4.75 (3H, m, -OCH_2Ph), 4.63 (1H, d, J = 11.5 Hz, -OCH_2Ph), 4.51 (1H, d, J = 12.5 Hz, -OCH_2Ph), 4.43 (1H, d, J = 12.2 Hz, -OCH_2Ph), 4.21 (1H, br s), 3.99–4.15 (3H, m), 3.96 (1H, d, J = 2.7 Hz), 3.85–3.89 (1H, m), 3.74–3.78 (1H, dd, J = 10.2, 3.2 Hz), 3.54–3.58 (1H, dd, J = 9.5, 6.6 Hz), 2.97 (1H, br s, -OH), 2.15–2.21 (1H, m), 2.01–2.07 (1H m), 1.49–1.56 (1H, m), 1.22–1.33 (1H, m) ppm. ^{13}C NMR (100 MHz): δ 171.2, 138.7, 138.2, 127.2–128.3 (m, aromatic), 80.5, 75.9, 73.2, 72.9, 72.5, 70.2, 67.7, 65.3, 51.9, 48.6, 38.5, 33.3, 29.6 ppm. MS/ESI : $[M + H]^+$ Calcd; 518.2543; found: 518.2540.

General procedure for debenzylation

A solution of compound **23** or **24** (440 mg, 0.85 mmol) in 6 mL of MeOH was placed in a Parr hydrogenation apparatus vessel. $Pd(OH)_2/C$ (40 mg) was added and the mixture was hydrogenated (H_2, 50 psi) for 12 h. The reaction mixture was then filtered through a pad of Celite and the slurry was washed repeatedly with more MeOH.

(2*R*,3*R*,4*S*,7*R*,8*R*,9a*S*)-4-(Hydroxymethyl)-6-oxooctahydro-1*H*-quinolizine-2,3,7,8-tetraol (9a). Yield: 80% (168 mg, viscous liquid). R_f: 0.50 (methanol00ethyl acetate, 4:6). $[\alpha]_D^{25} = +12$ (c 1.0, CH_2Cl_2). IR (CH_2Cl_2) ν_{max}: 1660, 3400 cm^{-1}. ^1H NMR (400 MHz, D_2O): δ 4.05–4.09 (2H, m), 3.69–3.72 (5H, m), 3.43 (1H, br s), 2.07–2.10 (1H, m), 1.91–1.93 (1H, m), 1.51–1.66 (1H, m), 1.16–1.25 (1H, m) ppm. ^{13}C NMR (100 MHz): δ 173.4, 73.9, 71.4, 69.1, 67.4, 58.8, 56.9, 48.4, 41.8, 34.1 ppm. MS/ESI : $[M + H]^+$ Calcd; 248.1134; found: 248.1130.

General procedure for acetylation

The diol **9** or **10** (50 mg, 0.19 mmol) was placed in a round-bottomed flask, and Ac_2O (58 mg, 0.56 mmol), pyridine (505 mg, 5 mmol) and DMAP (3 mg, 0.025 mmol) were added successively. The reaction mixture was stirred for 24 h to complete the reaction (TLC monitoring). The solvent was then removed on rotary evaporator, the mixture diluted with water and CH_2Cl_2, and extracted with CH_2Cl_2 (3 × 10 mL). The organic layer was washed with 1% HCl (5 ml), water and brine. After drying the organic layer with anhydrous sodium sulfate, the solvent was evaporated on rotary evaporator to obtain a crude product, which was further purified by column chromatography to obtain the product as a pale yellow oil.

(2*R*,3*R*,4*S*,7*R*,8*R*,9a*S*)-4-(Acetoxymethyl)-6-oxooctahydro-1*H*quinolizine-2,3,7,8-tetrayl tetraacetate (25a). Yield: 100% (93 mg, viscous liquid). R_f: 0.50 (ethyl acetate). $[\alpha]_D^{25} = -26$ (c 1.50, CH_2Cl_2). IR (CH_2Cl_2) ν_{max}: 1670, 1735 cm^{-1}.^1H NMR (500 MHz, $CDCl_3$): δ 5.45–5.47 (1H, m, H-8), 5.41–5.43 (1H, dd, J = 8.0, 6.0 Hz, H-3), 5.38 (1H, d, J = 8.0 Hz, H-7), 5.18 (1H, t, J = 8.0 Hz, H-2), 4.63–4.66 (1H, m, H-4), 4.34–4.38 (1H, dd, J = 12.0, 3.0 Hz, H-11), 4.21–4.25 (1H, dd, J = 12.0, 4.0 Hz, H-11′), 3.93–3.98 (1H, dt, J = 10.0, 2.5 Hz, H-10), 2.26–2.36 (1H, m, H-1), 2.17–2.22 (1H, m, H-9), 2.16 (3H, s, OAc), 2.12 (3H, s, OAc), 2.09 (3H, s, OAc), 2.08 (3H, s, OAc), 2.05 (3H, s, OAc), 1.76–1.81 (2H, m, H-9′, H-1′) ppm. ^{13}C NMR (100 MHz): δ 170.4, 170.3, 170.1, 169.8, 165.7, 69.2, 69.1, 68.2, 66.8, 60.9, 50.9, 46.2, 36.6, 32.7, 21.2, 20.9, 20.7 ppm. MS/ESI : $[M + H]^+$ Calcd; 458.1662; found: 458.1661.

(6*S*,7*R*,8*R*,9a*R*)-7,8-Dihydroxy-6-(hydroxymethyl)hexahydro-1*H*-quinolizin-4(6*H*)-one (11a). The same experimental procedure for the synthesis of **9** from **23** was followed. Yield: 80% (170 mg, viscous liquid). R_f: 0.60 (ethyl acetate). $[\alpha]_D^{25} = -6$ (c 2.8, CH_2Cl_2). IR ν_{max}: 1670, 3400 cm^{-1}.^1H NMR (500 MHz, D_2O): δ 3.63–3.75 (4H, m), 3.52–3.56 (1H, m), 3.36–3.39 (1H, dd, J = 10.0,

6.0 Hz), 2.16–2.28 (2H, m), 1.88–1.93 (2H, m), 1.62–1.66 (1H, m), 1.48–1.55 (1H, m), 1.37–1.44 (1H, m), 1.21–1.29 (1H, dd, J = 24.0, 12.0 Hz) ppm. ^{13}C NMR (100 MHz): δ 174.3, 72.4, 67.9, 56.2, 54.9, 49.7, 39.5, 32.1, 28.5, 17.6 ppm. MS/ESI : $[M + H]^+$ Calcd;216.1236; found: 216.1235.

(2*R*,3*R*,4*S*,9a*S*)-4-(Acetoxymethyl)-6-oxooctahydro-1*H*-quinolizine-2,3-diyl diacetate (27a). The same experimental procedure for the synthesis of **25a** from **9** was followed. Yield: 98% (92 mg, viscous liquid). R_f: 0.50 (ethyl acetate). $[\alpha]_D^{25}$ = −13 (*c* 1.1, CH_2Cl_2). IR ν_{max}: 1670, 3400 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.45–5.47 (1H, m), 5.40–5.43 (1H, m), 4.23 (1H, d, J = 8.0 Hz), 4.29–4.32 (1H, dd, J = 6.3, 4.45 Hz), 3.79–3.85 (1H, m), 2.42–2.48 (1H, m), 2.31–2.38 (1H, m), 2.07 (3H, s, OAc), 2.06 (3H, s, OAc), 2.03 (3H, s, OAc), 1.74–1.96 (2H, m), 1.36–1.43 (1H, m) ppm. ^{13}C NMR (125.7 MHz): δ 171.0, 170.4, 169.7, 169.5, 67.7, 64.6, 62.0, 53.7, 51.7, 35.9, 32.6, 30.0, 20.8, 20.7, 19.7 ppm. MS/ESI : $[M + H]^+$ Calcd; 342.1553; found: 342.1551.

(2*R*,3*R*,6*S*,7*R*,8*R*,9a*R*)-7,8-Bis(benzyloxy)-6-(benzyloxymethyl)-4-oxooctahydro-1*H*-quinolizine-2,3-diyl diacetate (29). The same experimental procedure for the synthesis of **25a** from **9** was followed. Yield: 99% (99 mg, viscous liquid). R_f: 0.50 (1:1 hexane–ethyl acetate). $[\alpha]_D^{25}$ = −40 (*c* 1.0, CH_2Cl_2). IR ν_{max}: 1670, 3400 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.40 (1H, br.s, H-7), 5.36 (1H, br.s, H-8), 5.03 (1H, br.d J = 3.0 Hz, H-4), 4.7–4.76 (3H, m, -OCH_2Ph), 4.66 (1H, d, J = 11.5 Hz, -OCH_2Ph), 4.50 (1H, d, J = 12.0 Hz, -OCH_2Ph) 4.43 (1H, d, J = 11.5 Hz, -OCH_2Ph), 3.97–4.12 (3H, m, H-10, H-10, H-2), 3.79–3.82 (1H, br.t, J = 4.5 Hz, H-11′), 3.57–3.60 (1H, t, J = 8.5 Hz, H-3), 2.25–2.28 (1H, m, H-9) 2.17 (3H, s, OAc), 2.04–2.08 (1H, m, H-1), 1.86 (3H, s, OAc), 1.69–1.74 (1H, m, H-9′), 1.24–1.41 (1H, m, H-1′) ppm. ^{13}C NMR (125.7 MHz): δ 170.1, 165.4, 138.7, 138.3, 128.4, 128.3, 127.6, 127.5, 127.4, 127.3, 80.4, 75.7, 73.4, 72.6, 68.8, 68.4, 67.6, 51.5, 48.7, 38.5, 32.4, 20.7 ppm. MS/ESI : $[M + H]^+$ Calcd; 602.2754; found: 602.2752.

Acknowledgements

We thank the Department of Science and Technology (DST), New Delhi, for financial support to Y.D.V. in the form of a Ramanna Fellowship (Grant No. SR/S1/RFOC-04/2006). N.K. thanks the University Grant Commission, New Delhi, for a Senior Research Fellowship.

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