Concise synthesis of (-)-steviamine and analogues and their glycosidase inhibitory activities

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A concise synthesis of steviamine is reported along with the synthesis of its analogues 10-*nor*-steviamine, 10-*nor*-*ent*-steviamine and 5-*epi*-*ent*-steviamine. These compounds were tested against twelve glycosidases (at 143 μg/mL concentrations) and were found to have in general poor inhibitory activity against most enzymes. The 10-*nor* analogues however, showed 50-54% inhibition of α-L-rhamnosidase from *Penicillium decumbens* while one of these, 10-*nor*-steviamine, showed 51% inhibition of *N*-acetyl-β-D-glucosaminidase (from Jack bean) at the same concentration (760 μM).

Introduction

(-)-Steviamine **1** is the most recent member of the polyhydroxylated indolizidine natural products (Fig. 1). Steviamine was isolated from the leaves of *Stevia rebaudiana* (Asteraceae) and its absolute configuration was established by X-ray crystallographic analysis of its hydrobromide salt.[1,2] (-)-Steviamine **1** is the first polyhydroxylated indolizidine to have a methyl group at C-5 and a hydroxymethyl group at C-3. This group of alkaloids which includes, swainsonine **2**, castanosperimine **3** and lentiginosine **4** (Fig. 1) have potential utility as antidiabetic, antiviral, anticancer and immunoregulatory agents.[3] Unlike swainsonine **2**, steviamine **1** and its synthesised enantiomer ((+)-steviamine)), have shown relatively weak to modest glycosidase inhibitory activity against a number of different glycosidases.[4] The most potent activity found in this study was against β-galactosidase (from rat intestinal lactase), where *ent*-steviamine had an IC_{50} value of 35 μM.[4] While, *ent*-steviamine[4] and some of its analogues, including 10-nor-steviamine **5** (and some of its 1,2,3,8a-epimers,),[5] 5-*epi*-*ent*-steviamine **7**[4] and 1,3-di*epi*-10-(4- methoxyphenyl)steviamine[6]

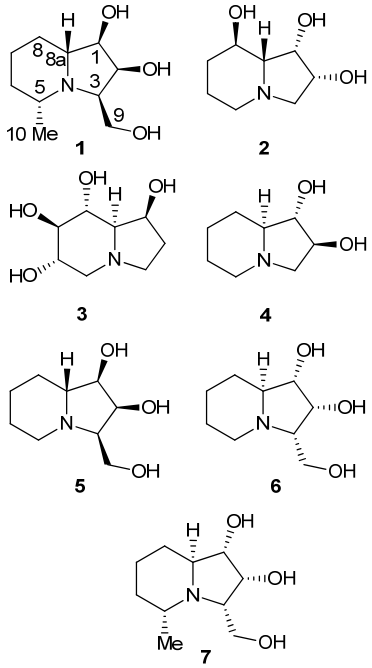

Fig. 1 Representative polyhydroxylated indolizidine natural products (**1**-**4**) and synthetic analogues (**5**-**7**).

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†Electronic Supplementary Information (ESI) available: [copies of the ^{1}H and ^{13}C NMR spectra of all new compounds, NOESY/ROSY spectra of compound x, y and z]. See DOI: 10.1039/b000000x/

have been synthesized recently, (-)-steviamine **1** itself has not been previously prepared. We report here a concise synthesis of steviamine **1** and the synthesis of three analogues, 10-*nor*-steviamine **5**, 10-*nor*-*ent*-steviamine **6** and 5-*epi*-*ent*-steviamine **7** (Fig. 1) and their activities against a panel of twelve glycosidases.

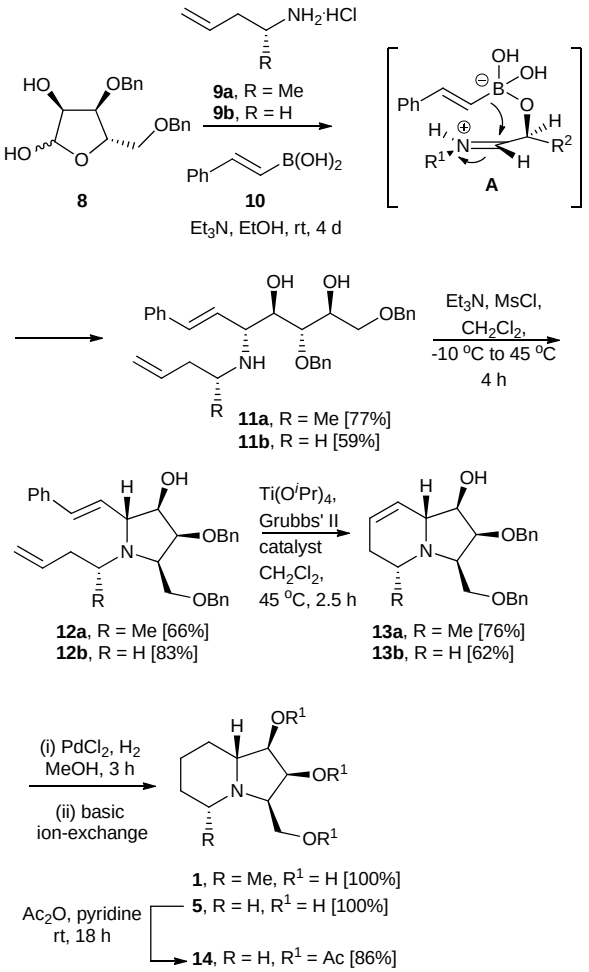
Results and discussion

The synthesis of (-)-steviamine **1** started with a Petasis boronic acid Mannich reaction (PBAMR)[7,8] between the known L-β-ribofuranose derivative **8**[9] ((3*S*,4*R*,5*S*)-4-(benzyloxy)-5-(benzyloxymethyl)tetrahydrofuran-2,3-diol) and commercially available (*R*)-4-penten-2-amine.HCl **9a** and *E*-styrylboronic acid **10** (Scheme 1). Stirring these three components in the presence of triethylamine (to generate the free amine of **9a**) in ethanol at rt for 4 d, gave, after purification of the crude reaction mixture by column chromatography, the amino alcohol **11** in 77% yield, as a single diastereomer. Shorter reaction times (1-2 d) and other solvents (e.g. MeOH, CH_2Cl_2 and MeCN) gave lower yields. The configuration at the newly created, amino group bearing, stereogenic centre in **11a**, that would become C-8a in the final target **1**, was assumed to the desired one based on reports that the

PBAMR normally provides 1,2-*anti*-amino alcohol products via a boronate intermediate, similar to **A**, as shown in Scheme 1.[7,8] This assumption was later confirmed to be correct in the eventual execution of the synthesis of **1**. Treatment of **11a** with 1.07 equivalents of methanesulfonyl chloride and 3.5 equivalents of triethylamine,[8(g)] followed by warming of the *O*-mesylate intermediate to 40−45 °C for 4 h provided the fully substituted pyrrolidine **12a** in 66% yield after separation of small amounts of *O*,*N*-dimesylated **11a** and unreacted **11a** by column chromatography. A ring-closing metathesis reaction of diene **11a** using 18 mol% Grubbs' second generation catalyst, in the presence of $Ti(O^iPr)_4$ (0.2 equivalents)[8b,10] to deactivate the amino group in **11a**, gave the indolizidine **12a** in 76% yield. Hydrogenation/hydrogenolysis of **12a**, over $PdCl_2/H_2$[8c] gave (-)-steviamine **1** in quantitative yield after neutralization/purification by basic ion-exchange chromatography.

The NMR spectroscopic data of synthetic **1**, matched very closely (^{1}H NMR 0.1 ppm consistent differences, ^{13}C NMR .04 ppm consistent differences) to those of the natural product[2] (see the Electronic Supplementary Information (ESI)). Further the specific rotation of the synthetic material, $[\alpha]_D^{25}$ -23.8 (*c* 1.0, MeOH), was of the same sign and close in magnitude to that of the natural product (lit.[2] $[\alpha]_D^{20}$ -22 (*c* 1.0, MeOH). Thus the first synthesis of (-)-steviamine **1** has been achieved in four synthetic steps from compounds **8**, **9a** and **10**. Since compound **8** was prepared in four steps (45% overall yield (see ESI)) from commercially available β-L-ribofuranose-1,2,3,5-tetra-*O*-acetate, this synthesis represents an eight step total synthesis of steviamine **1** from commercially available starting materials with an over yield of 17%. This concise strategy was further employed to prepare the analogues **5**, **6** and **7**.

Treatment of a mixture of **8**, 4-buten-1-amine.HCl **9b** and **10** under the aforementioned PBAMR conditions gave the amino diol **11b** as a single diastereomer in 59% yield (Scheme 1). This compound was converted to 10-nor-steviaimine **5**, in an analogues fashion, in 30% overall yield (Scheme 1). While this compound had a specific rotation of $[\alpha]_D^{25}$ -7.7 (*c* 1.0, H_2O), similar to that reported in the literature ($[\alpha]_D^{25}$ -8.7 (*c* 1.2, H_2O),[5] there were significant differences in the ^{1}H NMR spectral data recorded in D_2O (see ESI). The most significant difference was the relative chemical shifts for the protons H-1 and H-9a and H-9b in the range of δ ~ 3.8-3.9. In our sample the H9 protons were observed as dd resonances (*J* = 12.0, 5.0-5.5 Hz) at δ 3.87 and δ 3.81 while the H-1 resonance at δ 3.82 (apparent t, *J* = 6.0 Hz) was observed at a chemical shift in between those of the two H-9 resonances. The literature, however, reported the H9 protons as dd resonances (*J* = 12.2, 5.0-5.5 Hz) at δ 3.83 and δ 3.77 with the



Scheme 1 Synthesis of steviamine **1** and 10-*nor*-steviamine **5** and its triacetate derivative **14**.

H-1 signal being the most downfield of this group at δ 3.87 (apparent t, *J* = 5.5 Hz). Further, H-8a resonated at δ 2.67-2.72 (m) in our sample while the literature value for the chemical shift of this proton was δ 2.97 (s). The ^{13}C NMR chemical shifts were also significantly different with chemical shift differences varying from -1.9 to 0.2 ppm (see ESI). Since NOESY and ROESY NMR experiments on our sample of **5** were not unequivocal in defining the stereochemistry of our compound, because of the closeness of the individual resonances, and because the NMR chemical shifts of these types of polyhydroxylated compounds in D_2O can vary with pH and concentration,[8d,f] we prepared **14**, the triacetate derivative of **5** (Scheme 1). ROESY NMR experiments in $CDCl_3$ clearly indicated the assigned stereochemistry of **14**. Significant cross peaks were observed between H-8a and both H-5β and H-9, which clearly supported the relative *syn*-stereochemical relationship between these three protons (Fig. 2).

For the synthesis of 5-*epi*-*ent*-steviamine **7** the known D-β-ribofuranose derivative **15**[9] ((3*R*,4*S*,5*R*)-4-(benzyloxy)-5-(benzyloxymethyl)tetrahydrofuran-2,3-diol) was treated with (*R*)-4-penten-2-amine.HCl **9a**, triethylamine and *E*-styrylboronic acid **10** in ethanol solution at rt for 4 d to give the amino diol **16a** in

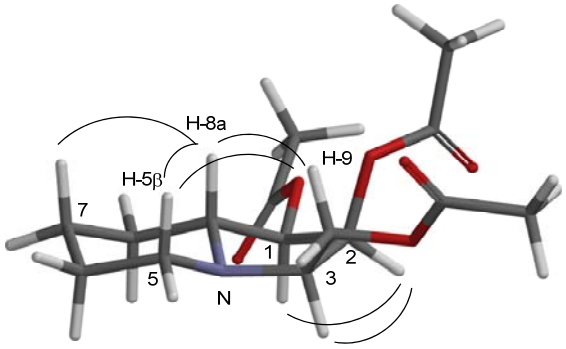
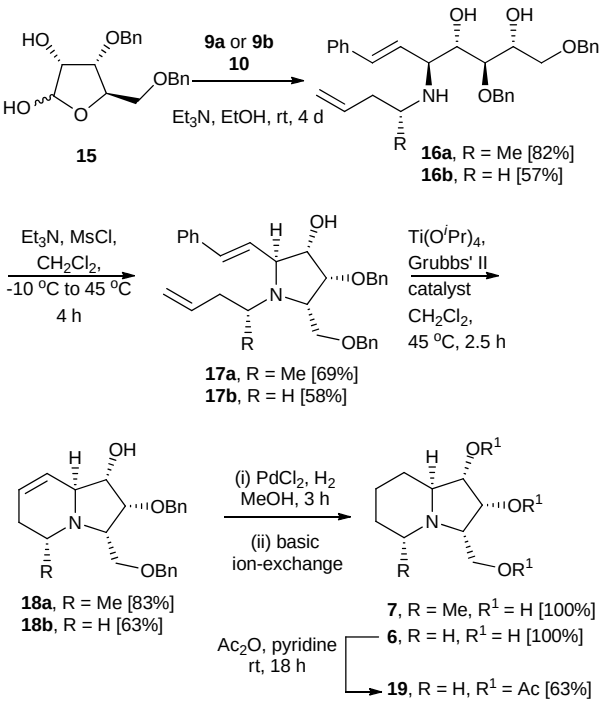


Fig. 2 Significant ROESY cross-peaks of compound **14** (SPARTAN generated structure using a DFT calculation (B3LYP/6-31G** level)).

82% yield (Scheme 2). This compound was readily converted to 5-*epi*-*ent*-steviamine **7** in three efficient steps according to the protocols developed in Scheme 1. The overall yield of **7** was 38% from **15** or 24% from D-β-ribofuranose. The NMR spectroscopic data of **7** agreed well with those reported[2] (see ESI), the specific rotation of **7** ($[\alpha]_D^{25}$ -4.6 (*c* 1.0, MeOH)) was of the same sign and of similar low magnitude to that reported ($[\alpha]_D^{20}$ -1.2 (*c* 1.0, MeOH)).[2]



Scheme 2 Synthesis of 5-*epi*-*ent*-steviamine **7**, 10-*nor*-*ent*-steviamine **6** and its triacetate derivative **19**.

10-Nor-*ent*-steviamine **6** and its triacetate derivative **19** were prepared in an analogous fashion from **15** (Scheme 2). The NMR spectroscopic data of **5** and **6** and those of **14** and **19** were identical, allowing for slight spectrometer variations. While the optical rotations of **5** and **6** were opposite in sign they varied significantly in magnitude (see Experimental section), however those of compounds **14** and **19**, which could be purified on silica gel using organic solvents, were essentially equal and opposite in sign (**14**: $[\alpha]_D^{25}$ +9.4 (*c* 0.2, $CHCl_3$); **19**: $[\alpha]_D^{25}$ -9.2 (*c* 0.2, $CHCl_3$)). These results suggested that the samples of **5** or **6** may be different hydrates resulting in incorrect mass measurements. Repeated purifications of these samples did not provide more closely matching specific rotations.

Glycosidase inhibition studies

The results of our glycosidase inhibitor testing for (-)-steviamine **1**, its analogues **5**-**7** and swainsonine **2**, *ent*-**2** and castanosperimine **3** against twelve glycosidases are shown in

Table 1 The glycosidase inhibition of compounds **1**, and **5**-**7** (Mean % Inhibition at 143 μg/mL).

Enzyme	**Cpd**						
(Source, pH)	**1**	**7**	**5**	**6**	**2**	***ent*-2**	**3**
α-D-glucosidase (*Saccharomyces cerevisiae*, 6.8)	0	0	0	5	0	3	0
α-D-glucosidase (*Bacillus sterothermophilus*, 6.8)	**-27**	18	22	**-38**	0	15	**80**
α–D-glucosidase (rice, 4.0)	-6	-9	-8	-6	12	2	6
β- D-glucosidase (Almond (*Prunus* sp.), 5.0)	0	0	11	0	0	2	**90**
α- D-galactosidase (Green coffee bean (*Coffea* sp.), 6.5)	0	-15	12	0	-4	1	-3
β- D-galactosidase (Bovine liver, 7.3)	0	6	19	20	-3	7	16
α- D-mannosidase (Jack bean (*Canavalia ensiformis*), 4.5)	11	0	31	9	11	**100**	9
β- D-mannosidase (*Cellullomonas fimi*, 6.5)	-13	0	0	0	2	-5	-
α–L-rhamnosidase (*Penicillium decumbens*,	6	20	**53**	**50**	**100**	4	39

4.0)							
N-acetyl-β- D-glucosaminidase (Bovine kidney, 4.25)	0	0	-9	-19	13	5	-
N-acetyl-β- D-glucosaminidase (Jack bean, 5.0)	11	30	**51**	-17	3	24	-
β-glucuronidase (Bovine liver, 5.0)	-5	0	0	0	-	-	-

Table 1. These mean % inhibition tests were determined for each compound at 143 μg/mL according to previously published protocols.[11] In general compounds **1** and **5**–**7** were found to have poor inhibitory activity against most enzymes. None were as active as (-)-swainsonine **2** against α-L-rhamnosidase (from *Penicillium decumbens*) or (+)-swainsonine (*ent*-**2**) against α-D-mannosidase (from Jack bean) or castanospermine **3** against α–D-glycosidase (from *Bacillus sterothermophilus*) and almond β-D-glycosidase. The 10-*nor* analogues, **5** and **6**, however, showed 50-54% inhibition of α-L-rhamnosidase from *Penicillium decumbens* while 10-*nor*-steviamine **5**, showed 51% inhibition of *N*-acetyl-β-D-glucosaminidase (from bovine kidney) at the same concentration (760 μM). It is interesting that the enantiomeric compounds **5** and **6** give almost equal inhibition of α-L-rhamnosidase whereas **7**, with the extra methyl group, is a much weaker inhibitor. Both **5** and **6** have two equivalent hydroxyls to (+)-swainsonine (*ent*-**2**) and (-)-swainsonine **2**, respectively and yet they both follow (-)-swainsonine **2** in inhibition of α-L-rhamnosidase and not α-mannosidase. (-)-Steviamine **1** does not show significant inhibition of any glycosidase tested; it could be that it has a biological function in the source plant inhibiting a glycosidase we have not tested against or it is clear that iminosugars can be functional without glycosidase inhibition and in fact this lack of glycosidase inhibition (or high selectivity) may make them more suitable as pharmaceutical products.[12]
Interestingly, all compounds appeared to promote the activities of certain enzymes. In particular, compounds **1** and **6** seemed to significantly promote the activity of α-D-glucosidase (from *Bacillus sterothermophilus*). This promotion of activity could be due to enzyme stabilisation or improved folding of the enzyme via non-catalytic site binding.

The inhibitory activity of compound **5**, which was prepared previously[5,] and had different NMR properties to ours, was reported to have no inhibitory activity against two α-D-glucosidases (from Baker's yeast and rice), one β-D-glucosidase (from sweet almonds) and a β-D-galactosidase (from bovine liver). These results are consistent with our results shown in Table 1. The earlier report showed that compound **5** was a more significant inhibitor of α-L-rhamnosidase (from *Penicillium decumbens*, IC_{50} 35 μM)) and α-D-mannosidase (from Jack bean, IC_{50} 82 μM)) and a significantly weaker inhibitor of α-L-fucosidase (from bovine kidney, IC_{50} 593 μM)). Our compound **5** also showed some, although very weak, activity against α-D-mannosidase (from Jack bean, only 31% inhibition at 708, μM Table 1) and an IC_{50} of approximately 708 μM (53% inhibition) against the α-L-rhamnosidase from *Penicillium decumbens*.

Conclusions

In conclusion, a concise and efficient four step synthesis of natural (-)-steviamine **1** has been developed from the readily accessible L-β-ribofuranose derivative **8**. This synthetic protocol involves a highly *anti*-selective Petasis reaction, and efficient ring-closing metathesis and *O*-mesylate cyclization reactions to prepare the piperidine and pyrrolidine rings, respectively. This synthetic protocol allowed for the synthesis of the (-)-steviamine analogues 10-*nor*-steviamine, 10-*nor*-*ent*-steviamine and 5-*epi*-*ent*-steviamine. These compounds were tested against twelve glycosidases (at 143 μg/mL concentrations) and were found in general to have poor inhibitory activity against most enzymes. The 10-nor analogues however, showed 50-54% inhibition of α-L-rhamnosidase from *Penicillium decumbens* while one of these, 10-nor-steviamine, showed 51% inhibition of *N*-acetyl-β-D-glucosaminidase (from bovine kidney) at the same concentration (457 μM).

Experimental Section

General Information

All reagents were used as received from commercial sources without further purification. Solvents were purchased as Analytical Reagents (AR) grade. Petrol refers to the hydrocarbon fraction of bp 40-60 °C. Tetrahydrofuran was stored over KOH pellets until needed, then distilled over sodium wire under nitrogen, using benzophenone as indicator. Anhydrous CH_2Cl_2 and MeOH were purchased from Aldrich. Reactions were stirred using Teflon-coated magnetic stirring bars. Analytical TLC was performed with aluminium backed Merck F_{254} sorbent silica gel. TLC plates were visualized by ultraviolet light or by treatment with acidified, aqueous solution of ammonium molybdate and cerium(IV) sulfate, followed by development with a 1400 Watt heat gun. Chromatographic purification of products was carried out by flash column chromatography on silica gel (70-230 mesh). Basic ion-exchange chromatography was performed using Amberlyst A-26(OH) resin. Infrared spectra were recorded as neat samples on a MIRacle 10 Shimadzu Spectrophotometer. NMR spectra were measured in $CDCl_3$ (with TMS as internal standard) or D_2O (with MeOH as internal standard) on a Varian VNMRS PS54-500 or a Varian INOVA-500 (^{1}H at 500 MHz, ^{13}C at 125 MHz) magnetic resonance spectrometer. Chemical shifts (δ) are reported in ppm, and coupling constants (J) are in Hz. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Low-resolution mass spectra were obtained on a Waters LCZ single quadropole (ESI). High-resolution mass spectra (HRMS) were recorded on a Waters QTOF (ESI), a Waters Xevo (ESI) or a Waters Xevo (ASAP). Polarimetry was carried out using a JASCO P-2000 Digital Polarimeter and the measurements were made at the sodium D-line with a 1 dm path length cell. Concentrations (c) are given in grams per 100 mL.

(2*S*,3*R*,4*R*,*E*)-1,3-Bis(benzyloxy)-5-((*R*)-pent-4-en-2-ylamino)-7-phenylhept-6-ene-2,4-diol (11a). To a solution of **8** (1.00 g, 3.03 mmol, see ESI for synthesis details) in absolute ethanol (25 mL) was added (*R*)-pent-4-en-2-amine.hydrochloride **9a** (368 mg,

3.03 mmol, a commercial sample from NetChem, Inc. USA, >95% ee, $[\alpha]_D^{25}$ +4.0 (*c* 1.0, EtOH)) followed by Et_3N (0.42 mL, 3.03 mmol) and *trans*-2-phenylvinyl boronic acid (**10**) (448 mg, 3.03 mmol, commercial sample from Aldrich). The mixture was stirred at rt for 4 d, followed by the evaporation of all volatiles *in vacuo*. The residue was dissolved in CH_2Cl_2 (10 mL) and washed with sat. aq. $NaHCO_3$ (2×10 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford a brown foam. Purification by flash column chromatography (increasing polarity from 0:100 to 20:80 MeOH/CH_2Cl_2 as eluent) afforded the title compound (1.16 g, 77%) as a yellow foam. R_f 0.48 (10:90 MeOH/CH_2Cl_2). $[\alpha]_D^{25}$ +47.9 (*c* 2.0, $CHCl_3$). IR (cm^{-1}): 3289, 3072, 1452, 1072, 1028. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41−7.20 (m, 15H), 6.47 (d, *J* = 16.0 Hz, 1H), 6.16 (dd, *J* = 16.0, 9.0 Hz, 1H), 5.75−5.67 (m, 1H), 5.05 (d, *J* = 16.0 Hz, 1H), 5.04 (d, *J* = 12.0 Hz, 1H), 4.63, 4.54 (AB_q, J_{AB} = 12.0 Hz, 2H), 4.56, 4.48 (AB_q, J_{AB} = 11.5 Hz, 2H), 4.06−4.03 (m, 1H), 3.95 (t, *J* = 4.5 Hz, 1H), 3.78 (dd, *J* = 9.0, 5.0 Hz, 1H), 3.75-3.66 (m[a], 3H), 2.88−2.76 (m, 1H), 2.22−2.10 (m[a], 2H), 1.04 (d, *J* = 6.5 Hz, 3H) [[a] indicates the overlapping of signals]. ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.4, 138.2, 136.5, 135.1, 133.9, 128.7, 128.5, 128.0, 127.9, 127.7, 126.5, 127.5, 117.9, 80.0, 74.0, 73.7, 73.0, 71.7, 69.1, 59.6, 49.2, 42.1, 19.2. MS (ESI +ve) *m/z* 502.4 ($M+H^+$, 100%). HRMS (ESI +ve) calculated for $C_{32}H_{40}NO_4$ ($M+H^+$) 502.2957, found 502.2938.

(2*R*,3*R*,4*S*,5*R*)-4-(Benzyloxy)-5-(benzyloxymethyl)-1-((*R*)-pent-4-en-2-yl)-2-styrylpyrrolidin-3-ol (12a). To a solution of **11a** (1.02 g, 2.02 mmol) in anhydrous CH_2Cl_2 (6 mL) at 0 °C was added Et_3N (0.99 mL, 7.08 mmol) under an atmosphere of N_2. The mixture was then cooled to -10 °C followed by the addition of a 0.13 M solution of methanesulfonyl chloride in anhydrous CH_2Cl_2 (17.0 mL, 2.18 mmol $MeSO_2Cl$) . After complete addition the reaction mixture was gradually warmed to 40 °C over 3 h and stirred for further 30 min with heating under a gentle reflux. The solution was subsequently concentrated *in vacuo* to afford a brown oil. Purification by flash column chromatography (increasing polarity from 10:90 to 20:80 EtOAc/petrol and 20:80 MeOH/CH_2Cl_2 as eluent) afforded the title compound (642.2 mg, 66%) as a yellow oil. R_f 0.62 (3:7 EtOAc/petrol). $[\alpha]_D^{25}$ -29.4 (*c* 1.0, $CHCl_3$). IR (cm^{-1}): 3393, 3062, 3027, 2906, 2869, 1496, 1452, 1365, 1178, 1138, 1116, 1098, 1055, 1026. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35−7.19 (m, 15H), 6.52 (d, *J* = 16.0 Hz, 1H), 5.97 (dd, *J* = 16.0, 9.0 Hz, 1H), 5.84−5.76 (m, 1H), 5.03 (d, *J* = 17.0 Hz, 1H), 4.97 (d, *J* = 10.0 Hz, 1H), 4.76, 4.48 (AB_q, J_{AB} = 12.0 Hz, 2H), 4.62 (d, *J* = 10.5 Hz, 1H), 4.59, 4.55 (AB_q, J_{AB} = 12.0 Hz, 2H), 4.15 (t, *J* = 5.5 Hz, 1H), 3.97 (dd, *J* = 10.5, 5.5 Hz, 1H), 3.88 (d, *J* = 9.0 Hz, 1H), 3.62−3.56 (m[a], 3H), 3.08-3.02 (m, 1H), 2.40−2.34 (m, 1H), 2.17−2.11 (m, 1H), 1.03 (d, *J* = 6.5 Hz, 1H) [[a] indicates the overlapping of signals]. ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.5, 137.6, 137.3, 137.2, 132.5, 130.5, 128.7, 128.5, 128.4, 127.9, 127.7, 127.4, 126.4, 115.7, 77.9, 74.9, 73.8, 71.4, 70.0, 68.0, 58.9, 51.4, 39.6, 17.9. MS (ESI +ve) *m/z* 484.3 ($M+H^+$, 100%). HRMS (ESI +ve) calculated for $C_{32}H_{38}NO_3$ ($M+H^+$) 484.2852, found 484.2832.

((1*R*,2*S*,3*R*,5*R*,8a*R*)-2-(Benzyloxy)-3-(benzyloxymethyl)-5-methyl-1,2,3,5,6,8a-hexahydroindolizin-1-ol (13a). To a solution of **12a** (490.7 mg, 1.02 mmol) in anhydrous CH_2Cl_2 (36 mL) was added via syringe a solution of $Ti(O^i\text{-}Pr)_4$ (0.06 mL, 0.203 mmol) in anhydrous CH_2Cl_2 (11 mL). The above solution was stirred at rt for 0.5 h, then added Grubbs II catalyst (155.3 mg, 0.183 mmol). The reaction mixture was heated at reflux at 45 °C for 2.5 h, when TLC analysis showed complete consumption of **12a**. The reaction mixture was then diluted with CH_2Cl_2 (125 mL) and washed with sat. aq. $NaHCO_3$ (87 mL). The aqueous layer was further extracted with CH_2Cl_2 (125 mL). The organic layers were dried ($MgSO_4$) and concentrated *in vacuo* to afford a dark brown oil as a crude product. Purification by flash column chromatography (increasing polarity from 50:50 to 0:100 petrol/EtOAc as eluent) afforded the title compound (294 mg, 76%) as a brown oil. R_f 0.28 (1:4 petrol/EtOAc). $[\alpha]_D^{25}$ +38.6 (*c* 1.4, $CHCl_3$). IR (cm^{-1}): 3382, 3015, 2928, 2874, 2316, 1496, 1451, 1152, 1055. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34−7.21 (m, 10H), 5.79−5.76 (m, 1H), 5.53 (d, *J* = 10.5 Hz, 1H), 4.93 (d, *J* = 9.5 Hz, 1H), 4.71, 4.45 (AB_q, J_{AB} = 11.5 Hz, 2H), 4.69, 4.54 (AB_q, J_{AB} = 12.0 Hz, 2H), 4.12 (dd, *J* = 9.5, 4.5 Hz, 1H), 3.97 (dd, *J* = 9.5, 4.5 Hz, 1H), 3.91 (brs, 1H), 3.58 (d, *J* = 9.0 Hz, 1H), 3.41 (dd, *J* = 9.5, 3.0 Hz, 1H), 3.34 (brd, *J* = 9.5 Hz, 1H), 3.27−3.20 (m, 1H), 2.04−1.98 (m, 1H), 1.77−1.72 (m, 1H), 1.17 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.4, 137.5, 129.1, 128.4, 128.3, 128.2, 127.8, 127.6, 127.7, 78.2, 74.0, 71.5, 71.5, 70.6, 66.7, 55.7, 50.6, 26.1, 21.3. MS (ESI +ve) *m/z* 380.2 ($M+H^+$, 100%). HRMS (ESI +ve) calculated for $C_{24}H_{30}NO_3$ ($M+H^+$) 380.2226, found 380.2214.

(1*R*,2*S*,3*R*,5*R*,8a*R*)-3-(Hydroxymethyl)-5-methyloctahydroindolizine-1,2-diol ((-)-Steviamine) (1). To a solution of **13a** (183.5 mg, 0.484 mmol) in MeOH (10 mL) was added $PdCl_2$ (171.5 mg, 0.967 mmol). The mixture was stirred at rt under an atmosphere of H_2 (balloon) for 3 h. The mixture was filtered through a pad of Celite and the solids were washed with MeOH. The combined filtrates were evaporated *in vacuo* and the residue was dissolved in water (10 mL) and applied to a column of Amberlyst A-26 (OH^-) resin (3 cm). Elution with water followed by evaporation *in vacuo* afforded the title compound (98.0 mg, 100%) as a brown oil. $[\alpha]_D^{25}$ - 23.8 (*c* 1.0, MeOH) (lit.[2] $[\alpha]_D^{25}$ -22.0 (*c* 1.0, MeOH)). IR (cm^{-1}): 3329, 2929, 2855, 1631, 1441, 1379, 1315, 1214, 1137, 1097, 1079, 1036, 1006.
^{1}H NMR (500 MHz, D_2O) δ 4.33 (t, *J* = 7.5 Hz, 1H, H-2), 3.96 (dd, *J* = 12.5, 5.5 Hz, 1H, H-9), 3.91 (dd, *J* = 12.0, 3.5 Hz, 1H, H-9'), 3.80 (t, *J* = 7.0 Hz, 1H, H-1), 3.52 (dd, *J* = 9.5, 6.5 Hz, 1H, H-3), 2.85−2.82 (m, 1H, H-5), 2.67−2.64 (m, 1H, H-8a), 2.00 (brd, *J* = 12.5 Hz, 1H, H-8), 1.81 (brd, *J* = 13.0 Hz, 1H, H-7), 1.74 (brd, *J* = 13.0 Hz, 1H, H-6), 1.42−1.34 (m, 1H, H-7'), 1.21−1.12 (m[a], 5H, H-6', H-8' and CH_3) [[a] = overlapping of signals].^{13}C NMR (125 MHz, D_2O) δ 74.1 (C-1), 69.3 (C-2), 66.9 (C-8a), 61.5 (C-3), 56.7 (C-9), 52.8 (C-5), 33.6 (C-6), 29.5 (C-8), 23.9 (C-7), 19.4 (CH_3). MS (ESI +ve) *m/z* 202.0 ($M+H^+$, 100%). HRMS (ESI +ve) calculated for $C_{10}H_{20}NO_3$ ($M+H^+$) 202.1443, found 202.1465.

((2*S*,3*R*,4*R*,*E*)-1,3-Bis(benzyloxy)-5-(but-3-enylamino)-7-phenylhept-6-ene-2,4-diol (11b) and its enantiomer 16b. To a solution of **8** (30.4 mg, 0.09 mmol) in absolute EtOH (0.75 mL) was added 3-butenylamine.hydrochloride (**9b**) (9.7 mg, 0.09 mmol) followed by Et_3N (0.013 mL, 0.09 mmol) and *trans*-2-phenylvinyl boronic acid (**10**) (13.3 mg, 0.09 mmol). The mixture was stirred at rt for 4 d, followed by the evaporation of all volatiles *in vacuo*. The residue was dissolved in CH_2Cl_2 (4 mL) and washed with sat. aq. $NaHCO_3$ (2×4 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford a brown foam. Purification by flash column chromatography (increasing polarity from 0:100 to 20:80 MeOH/CH_2Cl_2 as eluent) afforded the title compound (26.3 mg, 59%) as a brown foam. R_f 0.45 (10:90 MeOH/CH_2Cl_2). $[\alpha]_D^{25}$ +51.5 (*c* 1.3, $CHCl_3$). IR (cm^{-1}): 3376, 3029, 1452, 1088, 1072, 1028. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39−7.19 (m, 15H), 6.51 (d, *J* = 16.0 Hz, 1H), 6.23 (dd, *J* = 16.0, 9.0, 1H), 5.77−5.67 (m, 1H), 5.07 (d, *J* = 18.0

Hz, 1H), 5.03 (d, J = 10.5 Hz, 1H), 4.64, 4.56 (ABq, J_{AB} = 12.5 Hz, 2H), 4.55, 4.47 (ABq, J_{AB} = 11.5 Hz, 2H), 4.04−4.00 (m[a], 2H), 3.75−3.68 (m[a], 3H), 3.65 (dd, J = 7.0, 4.5 Hz, 1H), 2.76−2.71 (m, 1H), 2.60−2.55 (m, 1H), 2.26 (q, J = 7.0 Hz, 2H) [a = overlapping of signals]. ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.5, 138.0, 136.4, 135.7, 134.6, 128.8, 128.5, 128.1, 128.0, 127.9, 127.7, 126.8, 126.8, 117.1, 79.7, 73.8, 73.5, 72.9, 71.7, 68.8, 62.8, 46.0, 33.8. MS (ESI +ve) *m/z* 488.5 ($M+H^+$, 100%). HRMS (ESI +ve) calculated for $C_{31}H_{38}NO_4$ ($M+H^+$) 488.2801, found 488.2784.

Its enantiomer (**16b**) was prepared as described above using **15** (see ESI for syntheses details) as a starting material (0.605 mmol scale). Compound **16b** was obtained as a brown foam (167.9 mg, 57%). $[\alpha]_D^{25}$ -58.7 (*c* 1.0, $CHCl_3$).

(2*R*,3*R*,4*S*,5*R*)-4-(Benzyloxy)-5-(benzyloxymethyl)-1-(but-3-enyl)-2-styrylpyrrolidin-3-ol (12b) and its enantiomer 17b. To a solution of **11b** (80.0 mg, 0.1641 mmol) in anhydrous CH_2Cl_2 (9 mL) at 0 °C was added Et_3N (0.023 mL, 0.164 mmol) under an atmosphere of N_2. The mixture was then cooled to -10 °C followed by the addition of a 0.13 M solution of methanesulfonyl chloride in anhydrous CH_2Cl_2 (1.51 mL, 0.197 mmol $MeSO_2Cl$). After complete addition the reaction mixture was gradually warmed to 40 °C over 3 h and stirred for further 30 min under gentle reflux. The solution was subsequently concentrated *in vacuo* to afford a brown oil. Purification by flash column chromatography (increasing polarity from 10:90 to 20:80 EtOAc/petrol and 20:80 MeOH/CH_2Cl_2 as eluent) afforded the title compound (63.9 mg, 83%) as a yellow oil. R_f 0.52 (3:7 EtOAc/petrol). $[\alpha]_D^{25}$ -18.2 (*c* 0.7, $CHCl_3$). MS (ESI +ve) *m/z* 470.4 ($M+H^+$, 100%). HRMS (ESI +ve) calculated for $C_{31}H_{36}NO_3$ ($M+H^+$) 470.2695, found 470.2674. IR (cm^{-1}): 3405, 3061, 3028, 2863, 1640, 1599, 1495, 1452, 1363, 1099, 1055. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35−7.20 (m, 15H), 6.56 (d, J = 16.0 Hz, 1H), 6.03 (dd, J = 16.0, 9.0 Hz, 1H), 5.79−5.72 (m, 1H), 4.99 (d, J = 17.0 Hz, 1H), 4.95 (d, J = 10.0 Hz, 1H), 4.76, 4.51 (AB_q, J_{AB} = 12.0 Hz, 2H), 4.60, 4.57 (AB_q, J_{AB} = 12.0 Hz, 2H), 4.18 (t, J = 7.0 Hz, 1H), 4.01 (brs, 1H), 3.69−3.61 (m[a], 3H), 3.43 (d, J = 8.0 Hz, 1H), 2.78−2.66 (m, 2H), 2.25−2.14 (m, 2H) [[a] indicates the overlapping of signals]. ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.4, 137.6, 137.1, 136.9, 131.8, 130.3, 128.7, 128.6, 128.5 , 128.0, 127.9, 127.8, 127.6, 126.5, 115.6, 77.6, 74.7, 73.8, 73.4, 71.8, 66.4, 61.4, 47.9, 33.1.

Its enantiomer (**17b**) was prepared from **16b** (0.3341 mmol scale) as described above. Compound **17b** was obtained as a yellow oil (90.5 mg, 58%). $[\alpha]_D^{25}$ +35.4 (*c* 0.8, $CHCl_3$).

(1*R*,2*S*,3*R*,8a*R*)-2-(Benzyloxy)-3-(benzyloxymethyl)-1,2,3,5,6,8a-hexahydroindolizin-1-ol (13b) and its enantiomer 18b. To a solution of **12b** (63.9 mg, 0.136 mmol) in anhydrous CH_2Cl_2 (4.8 mL) was added via syringe a solution of $Ti(O^i\text{-}Pr)_4$ (0.008 mL, 0.027 mmol) in anhydrous CH_2Cl_2 (1.6 mL). The above solution was stirred at rt for 0.5 h, then Grubbs II catalyst was added (13.84 mg, 0.016 mmol). The reaction mixture was heated at reflux at 45 °C for 2.5 h, when TLC analysis showed complete consumption of **12b**. The reaction mixture was then diluted with CH_2Cl_2 (17 mL) and washed with sat. aq. $NaHCO_3$ (11 mL). The aqueous layer was further extracted with CH_2Cl_2 (17 mL). The organic layers were dried ($MgSO_4$) and concentrated *in vacuo* to afford a dark brown oil. Purification by flash column chromatography (increasing polarity from 20:80 to 10:90 petrol/EtOAc and 20:80 MeOH/CH_2Cl_2 as eluent) afforded the title compound (30.9 mg, 62%) as a brown oil. R_f 0.28 (1:4 petrol: EtOAc). $[\alpha]_D^{25}$ +108.9 (*c* 0.2, $CHCl_3$). MS (ESI +ve) *m/z* 366.3 ($M+H^+$, 100%). HRMS (ESI +ve) calculated for $C_{23}H_{28}NO_3$ ($M+H^+$) 366.2069, found 366.2053. IR (cm^{-1}): 3259, 2922, 2854, 2364, 1731, 1631, 1452, 1362, 1143, 1084, 1025. 1H NMR (500 MHz, $CDCl_3$) δ 7.36−7.25 (m, 10H), 5.84−5.82 (m, 1H), 5.62 (d, J = 9.0 Hz, 1H), 4.68, 4.54 (ABq, J_{AB} = 11.5 Hz, 2H),4.62, 4.53 (ABq, J_{AB} = 11.5 Hz, 2H), 4.15 (dd, J = 7.5, 4.0 Hz, 1H), 3.96 (brs, 1H), 3.77 (brs, 1H), 3.63 (dd, J = 9.5, 4.5 Hz, 1H), 3.55 (dd, J = 9.5, 3.0 Hz, 1H), 3.29−3.27 (m, 1H), 3.04−3.00 (ma, 2H), 2.24−2.22 (m, 1H), 1.77 (d, J = 17.5 Hz, 1H). [[a] = overlapping of signals] ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.2, 137.9, 128.5, 128.4, 128.4, 128.1, 127.9, 127.8, 127.7, 127.2, 78.6, 73.9, 73.4, 72.6, 68.8, 64.2, 60.9, 44.6, 19.3.

Its enantiomer (**18b**) was prepared from **17b** (0.182 mmol scale) as described as above. Compound **18b** was obtained as a brown oil (41.8 mg, 63%). $[\alpha]_D^{25}$ -114.5 (*c* 0.2, $CHCl_3$).

(1*R*,2*S*,3*R*,8a*R*)-3-(Hydroxymethyl)octahydroindolizine-1,2-diol (10-nor-steviamine) (5) and its enantiomer 6. To a solution of **13b** (28.6 mg, 0.078 mmol) in MeOH (1.7 mL) was added $PdCl_2$ (20.8 mg, 0.117 mmol). The mixture was stirred at rt under an atmosphere of H_2 (balloon) for 3 h. The mixture was filtered through a pad of Celite and the solids were washed with MeOH. The combined filtrates were evaporated *in vacuo* and the residue was dissolved in water (1.5 mL) and applied to a column of Amberlyst A-26 (OH^-) resin (3 cm). Elution with water followed by evaporation *in vacuo* afforded the title compound (14.6 mg, 100%) as a brown oil. $[\alpha]_D^{25}$ -7.7 (*c* 0.6, H_2O), $[\alpha]_D^{25}$ -11.4 (*c* 0.6, MeOH) (lit.[5] $[\alpha]_D^{22}$ -8.7 (*c* 1.2, H_2O)). IR (cm^{-1}): 3324, 2929, 1636, 1596, 1445, 1141, 1105, 1083, 1049, 1007. ^{1}H NMR (500 MHz, D_2O) δ 4.40 (t, J = 6.5 Hz, 1H, H-2), 3.87 (dd, J = 12.0, 5.5 Hz, 1H, H-9), 3.82 (t, J = 6.0 Hz, 1H, H-1), 3.81 (dd, J = 12.0, 5.0 Hz, 1H, H-9'), 3.30 (dd, J = 12.5, 5.5 Hz, 1H, H-3), 2.96−2.93 (m, 1H, H-5α), 2.76-2.74 (m, 1H, H-8a), 2.72-2.67 (m, 1H, H-5β), 1.87−1.84 (m, 1H, H-8), 1.80−1.76 (m, 1H, H-7), 1.62−1.58 (m, 1H, H-6), 1.54−1.46 (m, 1H, H-6'), 1.41−1.32 (m, 1H, H-7'), 1.29−1.21 (m, 1H, H-8'). ^{13}C NMR (125 MHz, D_2O) δ 74.5 (C-1), 70.1 (C-2), 64.0 (C-3), 63.8 (C-8a), 58.5 (C-9), 47.3 (C-5), 27.2 (C-8), 23.1 (C-6), 22.7 (C-7). MS (ESI +ve) *m/z* 188.2 ($M+H^+$, 100%). HRMS (ESI +ve) calculated for $C_9H_{18}NO_3$ ($M+H^+$) 188.1287, found 188.1288.

Its enantiomer **6** (10-nor-*ent*-steviamine) was prepared from **18b** (0.102 mmol scale) as described above. Compound **6** was obtained as a brown oil (19.2 mg, 100%). $[\alpha]_D^{25}$ +23.8 (*c* 0.6, MeOH).

(1*R*,2*S*,3*R*,8a*R*)-3-(Acetoxymethyl)octahydroindolizine-1,2-diyldiacetate (14) and its enantiomer 19. To a solution of **5** (3.0 mg, 0.0160 mmol) in dry pyridine (0.06 mL, 0.68 mmol) was added Ac_2O (0.06 mL, 0.64 mmol). The mixture was stirred at rt for 18 h followed by the evaporation of all volatiles. The oily residue was purified by flash column chromatography (increasing polarity from 100:0 to 0:100 petrol/EtOAc and 20:80 MeOH/CH_2Cl_2 as eluent) to afford the title compound (4.3 mg, 86%) as a yellow oil. R_f 0.38 (1:1 petrol: EtOAc). $[\alpha]_D^{25}$ +9.4 (*c* 0.2, $CHCl_3$). MS (ESI +ve) *m/z* 314.3 ($M+H^+$, 100%). HRMS (ESI +ve) calculated for $C_{15}H_{23}NO_6$ ($M+H^+$) 314.1604, found 314.1595. IR (cm^{-1}): 2935, 2855, 1738, 1440, 1369, 1220, 1148, 1131, 1091, 1038. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.44 (t, J = 6.5 Hz, 1H, H-2), 4.98 (dd, J = 6.5, 4.0 Hz, 1H, H-1), 4.26 (dd, J = 11.5, 6.5 Hz, 1H, H-9), 4.09 (dd, J = 11.5, 5.5 Hz, 1H, H-9'), 3.59 (dd, J = 13.0, 6.0 Hz, 1H, H-3), 3.04 (d, J = 12.5 Hz, 1H, H-5α), 2.93 (dt, J = 11.5, 4.0 Hz, 1H, H-8a), 2.75−2.69 (m, 1H, H-5β), 2.07 (s, 3H, Ac), 2.05 (s, 6H, 2Ac), 1.82−1.75 (m[a], 2H, H-6, H8), 1.49-1.42 (m[a], 2H, H-7), 1.36−1.26 (m, 1H, H-6'), 1.23−1.15 (m,

1H, H-8') [[a] indicates the overlapping of signals]. ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.8 (C=O), 170.4 (C=O), 169.9 (C=O), 75.3 (C-1), 70.2 (C-2), 62.8 (C-8a), 61.7 (C-9), 59.6 (C-3), 47.7 (C-5), 28.3 (C-8), 23.9 (C-6), 23.1 (C-7), 21.1 (CH_3), 20.9 (CH_3), 20.7 (CH_3).

Its enantiomer **19** was prepared from **6** (0.027 mmol scale) as described above. Compound **19** was obtained as a yellow oil (5.3 mg, 63%)). $[\alpha]_D^{25}$ -9.2 (*c* 0.2, $CHCl_3$).

(2*R*,3*S*,4*S*,*E*)-1,3-Bis(benzyloxy)-5-((*R*)-pent-4-en-2-ylamino)-7-phenylhept-6-ene-2,4-diol (16a). To a solution of **15** (200 mg, 0.605 mmol) in absolute ethanol (5 mL) was added (*R*)-pent-4-en-2-amine.hydrochloride (**9a**) (73.6 mg, 0.605 mmol) followed by Et_3N (0.084 mL, 0.605 mmol) and *trans*-2-phenylvinyl boronic acid (**10**) (89.6 mg, 0.605 mmol). The mixture was stirred at rt for 4 d, followed by the evaporation of all volatiles *in vacuo*. The residue was dissolved in CH_2Cl_2 (5 mL) and washed with sat. aq. $NaHCO_3$ (2×5 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford a brown foam. Purification by flash column chromatography (increasing polarity from 0:100 to 20:80 MeOH/CH_2Cl_2 as eluent) afforded the title compound (248 mg, 82%) as a brown oil. R_f 0.45 (10:90 MeOH/CH_2Cl_2). $[\alpha]_D^{25}$ -35.7 (*c* 0.8, $CHCl_3$). IR (cm^{-1}): 3366, 3062, 3029, 2925, 2863, 1641, 1599, 1495, 1452, 1373, 1092, 1072. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39−7.16 (m, 15H), 6.48 (d, *J* = 16.0 Hz, 1H), 6.24 (dd, *J* = 16.0, 9.0 Hz, 1H), 5.78−5.69 (m, 1H), 5.08 (d, *J* = 11.5 Hz, 1H), 5.07 (d, *J* = 14.5 Hz, 1H), 4.63, 4.55 (AB_q, J_{AB} = 12.5 Hz, 2H), 4.52, 4.45 (AB_q, J_{AB} = 11.0 Hz, 2H), 4.05−4.02 (m, 1H), 3.95 (t, *J* = 4.0 Hz, 1H), 3.82 (dd, *J* = 8.5, 4.0 Hz, 1H), 3.76−3.69 (m[a], 2H), 3.64 (dd, *J* = 7.5, 4.5 Hz, 1H), 2.82−2.78 (m, 1H), 2.25−2.20 (m, 1H), 2.12−2.07 (m, 1H), 1.06 (d, *J* = 6.5 Hz, 3H) [[a] indicates the overlapping of signals]. ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.4, 138.0, 136.5, 134.6, 133.8, 128.7, 128.5, 128.4, 128.0, 127.9, 127.5, 126.7, 126.5, 127.6, 118.0, 79.5, 73.8, 73.7, 72.8, 71.6, 68.6, 60.2, 49.4, 39.5, 21.2. MS (ESI +ve) *m/z* 502.3 (M+H$^+$, 100%). HRMS (ESI +ve) calculated for $C_{32}H_{40}NO_4$ (M+H$^+$) 502.2957, found 502.2941.

(2*S*,3*S*,4*R*,5*S*)-4-(Benzyloxy)-5-(benzyloxymethyl)-1-((*R*)-pent-4-en-2-yl)-2-styrylpyrrolidin-3-ol (17a). To a solution of **16a** (241 mg, 0.481 mmol) in anhydrous CH_2Cl_2 (1.6 mL) at 0 °C was added Et_3N (0.23 mL, 1.68 mmol) under an atmosphere of N_2. The mixture was then cooled to -10 °C followed by the addition of a 0.13 M solution of methanesulfonyl chloride in anhydrous CH_2Cl_2 (4.10 mL, 0.529 mmol $MeSO_2Cl$). After complete addition the reaction mixture was gradually warmed to 40 °C over 3 h and stirred for further 30 min under gentle reflux. The solution was subsequently concentrated *in vacuo* to afford a brown oil. Purification by flash column chromatography (increasing polarity from 10:90 to 20:80 EtOAc/petrol and 20:80 MeOH/CH_2Cl_2 as eluent) afforded the title compound (161 mg, 69%) as a brown oil. R_f 0.62 (3:7 EtOAc/petrol). $[\alpha]_D^{25}$ +20.0 (*c* 0.5 $CHCl_3$). IR (cm^{-1}): 3384, 3062, 3028, 2931, 2869, 1725, 1640, 1495, 1452, 1174, 1140, 1087, 1054, 1026. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37−7.20 (m, 15H), 6.51 (d, *J* = 16.5 Hz, 1H), 5.98 (dd, *J* = 16.0, 9.0 Hz, 1H), 5.81−5.72 (m, 1H), 4.97 (d, *J* = 15.5 Hz, 1H), 4.95 (d, *J* = 11.5 Hz, 1H), 4.77, 4.49 (AB_q, J_{AB} = 12.0 Hz, 2H), 4.71 (d, *J* = 10.0 Hz, 1H), 4.61, 4.57 (AB_q, J_{AB} = 11.5 Hz, 2H), 4.19 (dd, *J* = 8.0, 5.5 Hz, 1H), 3.99 (dd, *J* = 8.0, 6.5 Hz, 1H), 3.91 (d, *J* = 9.0 Hz, 1H), 3.62−3.54 (m[a], 3H), 3.06−2.99 (m, 1H), 2.43−2.38 (m, 1H), 1.98−1.92 (m, 1H), 1.12 (d, *J* = 7.0 Hz, 1H) [[a] indicates the overlapping of signals]. ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.5, 137.5, 137.3, 137.3, 132.5, 130.6, 128.7, 128.6, 128.5, 128.0, 127.9, 127.7, 127.5, 126.4, 116.0, 77.8, 74.9, 73.8, 71.5, 70.5, 68.1, 59.8, 52.1, 40.4, 18.5. MS (ESI +ve) *m/z* 484.3 (M+H$^+$, 100%). HRMS (ESI +ve) calculated for $C_{32}H_{38}NO_3$ (M+H$^+$) 484.2852, found 484.2837.

(1*S*,2*R*,3*S*,5*R*,8a*S*)-2-(Benzyloxy)-3-(benzyloxymethyl)-5-methyl-1,2,3,5,6,8a-hexahydroindolizin-1-ol (18a). To a solution of **17a** (62.5 mg, 0.129 mmol) in anhydrous CH_2Cl_2 (4.4 mL) was added via syringe a solution of Ti(O^i-Pr)$_4$ (0.008 mL, 0.026 mmol) in anhydrous CH_2Cl_2 (1.5 mL). The above solution was stirred at rt for 0.5 h, then Grubbs II catalyst was added (19.8 mg, 0.023 mmol). The reaction mixture was heated at reflux at 45 °C for 2.5 h, when TLC analysis showed complete consumption of **17a**. The reaction mixture was then diluted with CH_2Cl_2 (16 mL) and washed with sat. aq. $NaHCO_3$ (11 mL). The aqueous layer was further extracted with CH_2Cl_2 (16 mL). The organic layers were dried ($MgSO_4$) and concentrated *in vacuo* to afford a dark brown oil. Purification by flash column chromatography (increasing polarity from 20:80 to 10:90 petrol/EtOAc as eluent) afforded the title compound (23.8 mg, 83%) as a brown oil. R_f 0.28 (1:4 EtOAc/petrol). $[\alpha]_D^{25}$ -7.7 (*c* 0.9, $CHCl_3$). IR (cm^{-1}): 3376, 3030, 2925, 2869, 2358, 1636, 1453, 1375, 1265, 1207, 1087, 1060, 1027. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36-7.25 (m, 10H), 5.73-5.70 (m, 1H), 5.60 (dd, *J* = 10.0, 1.0 Hz, 1H), 4.68, 4.57 (AB_q, J_{AB} = 12.0 Hz, 2H), 4.68, 4.60 (AB_q, J_{AB} = 12.0 Hz, 2H), 4.13 (dd, *J* = 8.0, 4.0 Hz, 1H), 3.96 (brs, 1H), 3.71 (brs, 1H), 3.63 (dd, *J* = 9.5, 5.0 Hz, 1H), 3.55 (brd, *J* = 8.5 Hz, 1H), 3.25−3.21 (m ,1H), 3.04 (brs, 1H), 2.37−2.32 (m, 1H), 1.63 (m, 1H), 1.18 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.4, 138.2, 128.5, 128.4, 127.9, 127.8, 127.7, 125.3, 78.4, 73.8, 73.2, 72.6, 69.3, 62.3, 60.6, 48.8, 25.6, 20.0. MS (ESI +ve) *m/z* 380.2 (M+H$^+$, 100%). HRMS (ESI +ve) calculated for $C_{24}H_{30}NO_3$ (M+H$^+$) 380.2226, found 380.2227.

(1*S*,2*R*,3*S*,5*R*,8a*S*)-3-(Hydroxymethyl)-5-methyloctahydroindolizine-1,2-diol (7). To a solution of **18a** (37.0 mg, 0.098 mmol) in MeOH (2 mL) was added $PdCl_2$ (34.6 mg, 0.195 mmol). The mixture was stirred at rt under an atmosphere of H_2 (balloon) for 3 h. The mixture was filtered through a pad of Celite and the solids were washed with MeOH. The combined filtrates were evaporated *in vacuo* and the residue was dissolved in water (1.5 mL) and applied to a column of Amberlyst A-26 (OH^-) resin (3 cm). Elution with water followed by evaporation *in vacuo* afforded the title compound (19.6 mg, 100.0%) as a brown oil. $[\alpha]_D^{25}$ -4.6 (*c* 1.0, MeOH), lit.[4] $[\alpha]_D^{20}$ -1.2 (*c* 1.0, MeOH). IR (cm^{-1}): 3352, 2931, 2863, 1629, 1596, 1455, 1381, 1339, 1121, 1096, 1059, 1028. ^{1}H NMR (500 MHz, D_2O) δ 4.37 (t, *J* = 6.0 Hz, 1H, H-2), 4.09 (dd, *J* = 10.5, 5.5 Hz, 1H, H-1), 3.77 (dd, *J* = 11.0, 9.5 Hz, 1H, H-9), 3.60 (dd, *J* = 11.0, 5.5 Hz, 1H, H-9'), 3.29−3.25 (m, 1H, H-3), 3.00−2.97 (m,1H, H-8a), 2.55−2.51 (m, 1H, H-5), 1.77−1.75 (m, 1H, H-8), 1.71−1.63 (m[a], 2H, H-6, H-8'), 1.56−1.52 (m, 1H, H-7), 1.45−1.37 (m, 1H, H-7'), 1.16−1.08 (m, 1H, H-6'), 1.02 (d, *J* = 6.0 Hz, 3H, CH_3) [[a] indicates the overlapping of signals]. ^{13}C NMR (125 MHz, D_2O) δ 70.6 (C-1), 69.8 (C-2), 66.2 (C-3), 60.4 (C-9), 58.5 (C-8a), 55.1 (C-5), 32.0 (C-6), 22.7 (C-8), 20.4 (CH_3), 18.1 (C-7). MS (ESI +ve) *m/z* 202.1 (M+H$^+$, 100%). HRMS (ESI +ve) calculated for $C_{10}H_{20}NO_3$ (M+H$^+$) 202.1443, found 202.1450.

Glycosidase inhibition assay[11]

All enzymes and *para*-nitrophenyl substrates were purchased from Sigma, with the exception of β-mannosidase which came from Megazyme. Enzymes were assayed at 27 ^{o}C in 0.1M citric acid / 0.2M disodium hydrogen phosphate buffers at the optimum pH for the enzyme. The incubation mixture consisted of 10μL

enzyme solution, 10μL of 1 mg/mL aqueous solution of test compound and 50μL of the appropriate 5 mM *para*-nitrophenyl substrate made up in buffer at the optimum pH for the enzyme. The reactions were stopped by addition of 70μL 0.4M glycine (pH 10.4) during the exponential phase of the reaction, which had been determined at the beginning using uninhibited assays in which water replaced inhibitor. Final absorbances were read at 405 nm using a Versamax microplate reader (Molecular Devices). Assays were carried out in triplicate, and the values given are means of the three replicates per assay.

Notes and references

1 A. L. Thompson, A. Michalik, R. J. Nash, F. X. Wilson, R. van Well, P. Johnson, G. W. J. Fleet, C.-Y. Yu, X.-G. Hu, R. I. Cooper and D. J. Watkin, *Acta Cryst.,* 2009, E**65**, o2904−o2905.

2 A. Michalik, J. Hollinshead, L. Jones, G. W. J. Fleet, C.-Y. Yu, X.-G. Hu, R. van Well, G. Horne, F. X. Wilson, A. Kato, S. F. Jenkinson and R. J. Nash, *Phytochem. Lett.,* 2010, **3**, 136−138.

3 N. Asano, in *Modern Alkaloids: Structure, Isolation, Synthesis and Biology*, E. Fattorusso and O. Taglialatela-Scafati, O., Eds. Wiley-VCH Verlag, Weinheim, 2008; pp 111-138.

4 X.-G. Hu, B. Bartholomew, R. J. Nash, F. X. Wilson, G. W. J. Fleet, S. Nakagawa, A. Kato, Y.-M. Jia, R. van Well and C.-Y. Yu, *Org. Lett.* 2010, **12**, 2562−2565.

5 L. Gómez, X. Garrabou, J. Joglar, J. Bujons, T. Paralla, C. Vilaplana, P. J. Cardona and P. Clapés, *Org. Biomol. Chem.,* 2012, **10**, 6309−6321.

6 A. Chronowska, E. Gallienne, C. Nicolas, A. Kato, I. Adachi and O. R. Martin, *Tetrahedron Lett.*, 2011, **52**, 6399−6402.

7 N. A. Petasis and I. A. Zavialov, *J. Am. Chem. Soc*., 1998, **120**, 11798−11799.

8 For our previous use of the Petasis boronic acid Mannich reaction in alkaloid synthesis see: (a) A. S. Davis, S. G. Pyne, B. W. Skelton and A. H. White, *J. Org. Chem.*, 2004, **69**, 3139−3143. (b) C. W. G. Au and S. G. Pyne, *J. Org. Chem.* 2006, **71**, 7097−7099. (c) A. D. Davis, T. Ritthiwigrom and S. G. Pyne, *Tetrahedron*, 2008, **64**, 4868−4879. (d) T. Ritthiwigrom and S. G. Pyne, *Org. Lett.*, 2008, **10**, 2769−2771. (e) T. Machan, A. S. Davis, B. Liawruangrath and S. G. Pyne, *Tetrahedron*, 2008, **64**, 2725−2732. (f) T. Ritthiwigrom, A. C. Willis and S. G. Pyne, *J. Org. Chem.* 2010, **75**, 815−824. (g) C. W. G. Au, R. J. Nash and S. G. Pyne. *Chem. Comm*. 2010, **46**, 713−715.

9 S. Bachir-Lesage, P. Gode´, G. Goethals, P. Villa and P. Martin, *J. Carbohydr. Chem.*, 2003, **22**, 35−46.

10 Q. Yang, W.-J. Xiao, Z. Yu, *Org. Lett.*, 2005, **7**, 871−874.

11 A. A. Watson, R. J. Nash, M. R. Wormald, D. J. Harvey, S. Dealler, E. Lees, N. Asano, H. Kizu, A. Kato, R. C. Griffiths, A. J. Cairns, A. J. and G. W. J. Fleet, *Phytochemistry* 1997, **46**, 255−259.

12 R. J. Nash, A. Kato, C.-Y. Yu, G. W. J. Fleet, *Future Med. Chem*. 2011, **3**, 1513−1521.