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Polyhydroxylated pyrrolizidines. Part 8: Enantiospecific synthesis of looking-glass analogues of hyacinthacine A_5 from DADP☆

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Abstract—(1*R*,2*S*,3*S*,5*R*,7a*R*)-1,2-Dihydroxy-3-hydroxymethyl-5-methylpyrrolizidine[(−)-3-*epi*hyacinthacine A_5, **1a**] and (1*S*,2*R*,3*R*,5*S* 7a*S*)-1,2-dihydroxy-3-hydroxymethylpyrrolizidine[(+)-3-*epi*hyacinthacine A_5, **1b**] have been synthesized either by Wittig's or Horner–Wadsworth–Emmond's (HWE's) methodology using aldehydes **4** and **9**, both prepared from (2*S*,3*S*,4*R*,5*R*)-3,4-dibenzyloxy-2′-*O*-*tert*-butyldiphenylsilyl-2,5-bis(hydroxymethyl)pyrrolidine (**2**, partially protected DADP), and the appropriate ylides, followed by cyclization through an internal reductive amination process of the resulting α,β-unsaturated ketones **5** and **10**, respectively, and total deprotection.

1. Introduction

We have recently reported on the stereocontrolled transformation of D-fructose into a suitably protected derivative of 2,5-dideoxy-2,5-imino-D-allitol (**2**, DADP),[2] which could be considered as an excellent and versatile key intermediate for the enantiosynthesis of polyhydroxylated pyrrolizidines. The necessity for new preparations of enantiomerically pure looking-glass hyacinthacines such as **1a** and **1b** arose from the discovery[3] that synthetic L-DMDP (2,5-dideoxy-2,5-imino-L-mannitol), is a more powerful and more specific α-glucosidase inhibitor than the enantiomeric natural product DMDP (see Fig. 1) one of the most widespread of secondary metabolite sugar mimics.[4] This behaviour occurs in other polyhydroxylated pyrrolidinic and piperidinic alkaloids.[5]

According to Figure 1 below, the pivotal character of compound **2**, would allow the syntheses of (−)-3-*epi* (**1a**) and (+)-3-*epi* isomers of hyacinthacine A_5, a natural polyhydroxylated pyrrolizidinic alkaloid and moderate inhibitor (IC_{50}=110 μM) of amyloglucosidase isolated from an extract of the bulbs of *Scilla sibirica* (Liliaceae),[6] by building-up the bicyclic skeleton from either C(5′) or C(2′).

The former synthetic strategy was successfully achieved and (−)-3-*epi*hyacinthacine A_5 (**1a**) was obtained from **2** in five steps (28% yield), whereas the application of the second strategy, consisting in a C(5′) *O*-protection and C(2′) *O*-deprotection, adequately functionalized chain-lengthening in this position and finally cyclization to the pyrrolizidine skeleton, afforded the mirror image (+)-3-*epi*-hyacinthacine A_5 (**1b**).

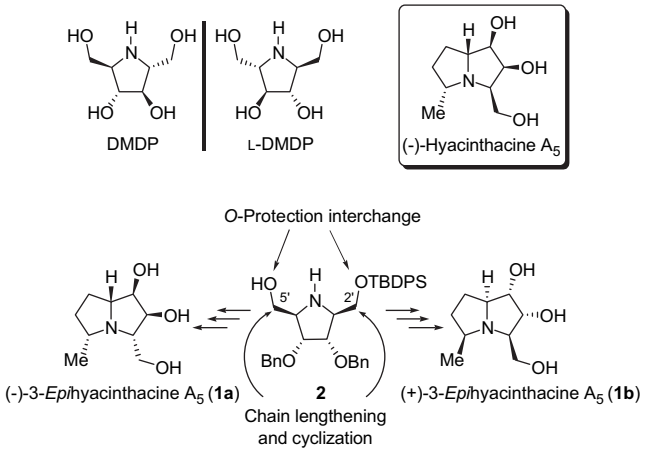

Figure 1. Synthetic strategy for the preparation of (−)-3-*epi* (**1a**) and (+)-3-*epi*hyacinthacine A_5 (**1b**) from orthogonally protected DADP (**2**).

2. Results and discussion

In the synthesis described herein, the starting pyrrolidine **2** was previously *N*-protected as its Cbz derivative **3** that was then oxidized (TPAP/NMO) to the pyrrolidinic aldehyde **4**

☆ For Part 7, see Ref. 1.

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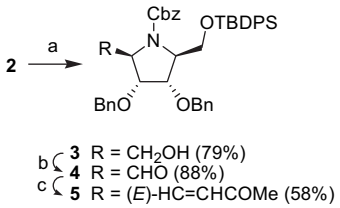
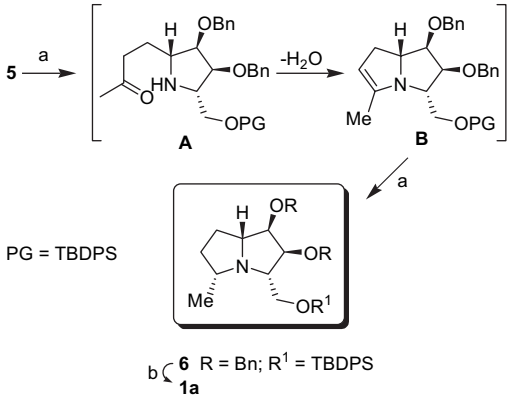
and finally allowed to react with 1-triphenylphosphoranylidene-2-propanone to afford, in a highly stereoselective manner, 4-[(3*E*,2′*R*,3′*R*,4′*S*,5′*S*)-3′,4′-dibenzyloxy-*N*-benzyloxycarbonyl-5′-*tert*-butyldiphenylsilyloxymethylpyrrolidin-2′-yl]but-3-en-2-one (**5**), in accordance with the $J_{3,4}$ values of 16 and 15.6 Hz, showed by H-3 in the mixture of rotamers (Scheme 1).



Scheme 1. Synthesis of pyrrolidinic α,β-unsaturated ketone **5**. Reagents and conditions: (a) $CbzCl/Me_2CO/K_2CO_3$; (b) TPAP/NMO/CH_2Cl_2/4 Å MS and (c) $Ph_3P{=}CHCOCH_3$/MePh, 80 °C.

Catalytic hydrogenation (10% Pd–C)–cyclization of **5** afforded, in only one step, the fully protected (1*R*,2*S*,3*S*,5*R*,7a*R*)-1,2-dibenzyloxy-3-*tert*-butyldiphenylsilyloxymethyl-5-methylpyrrolizidine (**6**).

According to Scheme 2, formation of **6** must take place as follows: concomitant hydrogenation and *N*-deprotection of **5** gave the saturated ketone **A**, not isolated, which on subsequent intramolecular condensation gave the intermediate Δ^5-pyrrolizine **B** that was finally hydrogenated to **6**.



Scheme 2. Synthesis of (−)-3-*epi*hyacinthacine A_5 (**1a**). Reagents and conditions: (a) 10% Pd–C/H_2/MeOH and (b) (i) 10% Pd–C/H_2/HCl, then Amberlite IRA-400 (OH^- form), (ii) TBAF·$3H_2O$/THF.

The stereochemistry of the new C(5) stereogenic centre was established on the basis of extensive NOE experiments. The NOE interactions are shown in Figure 2. The definite NOE effects between C(3)H and C(5)H, and Me(5)H and C(8)H were crucial in order to establish the *R*-configuration at C-5. In addition, the rest of the NOE interactions also confirmed the total stereochemistry of **6** and made possible to assign the resonance signals for H-6α,6β,7α,7β.

Removal of the protecting groups in **6** gave the target molecule (−)-3-*epi*hyacinthacine A_5 (**1a**), in accordance with its analytical and spectroscopic data.

The high stereoselective formation of **6** can be attributed, according to our previous results[7] and to Figure 2, to the

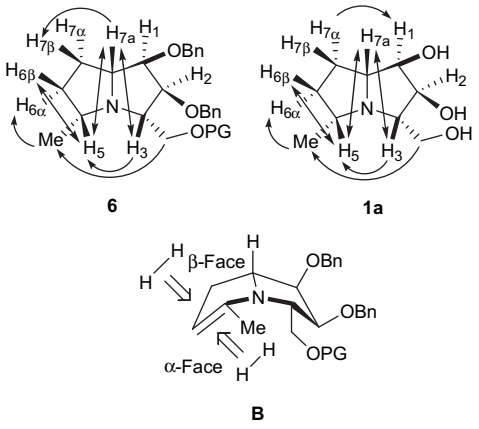
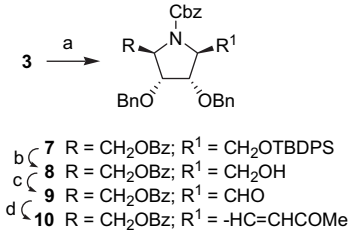


Figure 2. NOE interactions in **6** and **1a** and hydrogenation pathway of intermediate Δ^5-pyrrolizine (**B**).

peculiar shape of Δ^5-pyrrolizine **B**,[6,8] where it is appreciated that the β-face is less hindered for hydrogen attack that the α-face is, affording only compound **6**.

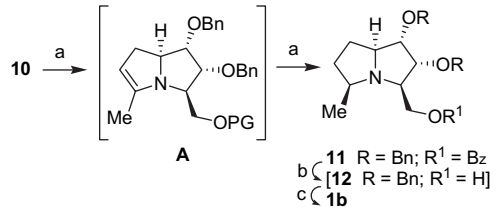
The above mentioned pivotal chiral character of **3**, allows the synthesis of looking-glass molecules, was probed in the synthesis of the mirror image of **1a**, (+)-3-*epi*hyacinthacine A_5 (**1b**). Thus, conventional benzoylation of **3** gave the fully protected derivative **7** (see Scheme 3). O-desilylation of **7** to the corresponding partially protected pyrrolidine **8** and subsequent oxidation (TPAP/NMO) afforded the pyrrolidinic aldehyde **9** that was not investigated, but used in the next step. In order to explore new synthetic possibilities for chain-lengthening and functionalization at C(5′) in **9**, the former C(2′) in **3**, the HWE's methodology was applied. Thus, aldehyde **9** readily reacted with diethyl (2-oxopropyl)phosphonate giving 4-[(2′*S*,3′*S*,4′*R*,5′*R*)-5′-benzoyloxymethyl-3′,4′-dibenzyloxy-*N*-benzyloxycarbonylpyrrolidin-2′-yl]but-3-en-2-one (**10**). On the contrary of compound **5**, the stereochemistry at the carbon–carbon double bond in **10** could not be determined in this case, due to an extensive broadening of the resonance signals.



Scheme 3. Synthesis of pyrrolidinic α,β-unsaturated ketone **10**. Reagents and conditions: (a) BzCl/CH_2Cl_2/TEA/DMAP (cat.); (b) *n*-$Bu_4N^+F^-\cdot 3H_2O$/THF and (c) TPAP/NMO/CH_2Cl_2/4 Å MS; (d) $(EtO)_2P(O)CH_2COCH_3$/NaH/THF, rt.

In Scheme 4 below and as for **5**, catalytic hydrogenation of **10** afforded a single isomeric pyrrolizidine identified as (1*S*,2*R*,3*R*,5*S*,7a*S*)-3-benzoyloxymethyl-1,2-dibenzyloxy-5-methylpyrrolizidine (**11**).

The absolute configuration of the new stereogenic centre C(5) was established on the basis of the NOE effects found (see Fig. 3). Thus, the definite NOE effects between C(3)H–C(5)H, C(3)H–C(7a)H, C(5)H–C(7a)H and Me(5)H–C(8)H



Scheme 4. Synthesis of (+)-3-*epi*hyacinthacine A_5 (**1b**). Reagents and conditions: (a) 10% Pd–C/H_2/MeOH; (b) MeONa (cat.)/MeOH; (c) 10% Pd–C/H_2/HCl, then Amberlite IRA-400 (OH^- form).

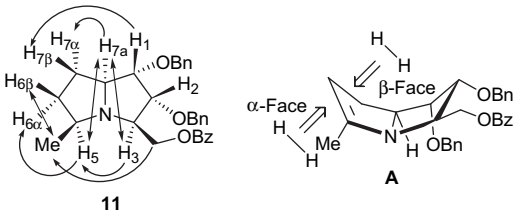


Figure 3. NOE interactions in **11** and hydrogenation pathway of intermediate Δ^5-pyrrolizine (**A**) in Scheme 4.

were essential in order to establish the *S*-configuration at C-5. In addition, the rest of the NOE interactions also confirmed the total stereochemistry of **11** and made possible to assign the resonance signals for H-6α,6β,7α,7β (Fig. 3).

As above, the configuration at the new stereogenic centre C(5), was again controlled by that existing at C(7a) in such a way that Me(5) and C(7a)H had a trans-disposition.[9]

Finally, compounds **1a** and **1b** were tested on a range of glycosidases and their IC_{50} values are included in the Table 1. Thus, **1a** was inhibitor to β-galactosidase (from bovine liver) at 62.4 μM, whereas both (**1a** and **1b**) were shown to be inhibitors to α-mannosidase (from jack beans) at 62.4 μM (**1a**) and 89.6 μM (**1b**). Compound **1a** was a more potent inhibitor (K_i=250 μM) of jack beans α-mannosidase than **1b** (K_i=420 μM). However, only **1a** inhibited of bovine liver β-galactosidase activity (K_i=300 μM). The alkaloids were not inhibitors of α-glucosidase (from baker's yeast), β-glucosidase (from almonds), α-galactosidase (green coffee), β-galactosidase (from *Aspergillus oryzae*) at 62.4 μM (**1a**) and 89.6 μM (**1b**).

Table 1. IC_{50} values for compounds **1a** and **1b** versus different glycosidases[a]

Enzyme	IC_{50} (μM)	
	1a	**1b**
α-Glucosidase (baker's yeast)	NI	NI
β-Glucosidase (almond)	NI	NI
α-Galactosidase (green coffee)	NI	NI
β-Galactosidase (bovine liver)	329	NI
β-Galactosidase (*A. oryzae*)	NI	NI
α-Mannosidase (Jack bean)	253	417

[a] NI=inhibition not observed under assay conditions.

3. Conclusions

Three conclusions can be stated from the above results: (i) that partially protected polyhydroxylated pyrrolidines, derived from common hexuloses, together with classical Wittig's or HWE's methodologies are both suitable for the enantiosynthesis of complex polyhydroxylated pyrrolizidines alkaloids; (ii) the configuration at C(5) in 5-methylpyrrolizidines is controlled by that existing at C(7a), in such a way that C(5)Me group and C(7a)H are in a trans-disposition and (iii) finally, that (−)-3-*epi*hyacinthacine A_5 (**1a**) and its (+)-enantiomer (**1b**) were shown as moderate inhibitors towards Jack bean α-mannosidase (IC_{50} 253 and 417 μM, respectively), whereas the former was specific of bovine liver β-galactosidase.

4. Experimental

4.1. General

Solutions were dried over $MgSO_4$ before concentration under reduced pressure. The ^1H and ^{13}C NMR spectra were recorded with Bruker AMX-300, AM-300 and ARX-400 spectrometers for solutions in $CDCl_3$ (internal Me_4Si). IR spectra were recorded with a Perkin–Elmer FTIR Spectrum One instrument, UV–vis measurements in a Spectronic® Genesys 5 spectrophotometer and mass spectra were recorded with a Hewlett–Packard HP-5988-A and Fisons mod. Platform II and VG Autospec-Q mass spectrometers. Optical rotations were measured for solutions in $CHCl_3$ (1-dm tube) with a Jasco DIP-370 polarimeter. TLC was performed on precoated silica gel 60 F_{254} aluminium sheets and detection by employing a mixture of 10% ammonium molybdate (w/v) in 10% aqueous sulfuric acid containing 0.8% cerium sulfate (w/v) and heating. Column chromatography was performed on silica gel (Merck, 7734). The noncrystalline compounds were shown to be homogeneous by chromatographic methods and characterized by NMR, MS and HRMS.

4.1.1. (2*S*,3*S*,4*R*,5*R*)-3,4-Dibenzyloxy-*N*-benzyloxycarbonyl-2′-*O*-*tert*-butyldiphenylsilyl-2,5-bis(hydroxymethyl)pyrrolidine (3). To a well stirred solution of **2**[2] (1.8 g, 3 mmol) in dry acetone (20 mL), anhydrous potassium carbonate (3 g) and a solution of benzyl chloroformate (600 μL, 4.2 mmol) in the same solvent (10 mL) were added and the mixture kept at rt for 30 min. TLC (Et_2O) then revealed the presence of a faster-running compound. The mixture was filtered and the solid thoroughly washed with acetone and the filtrate and washings concentrated to a residue that was submitted to chromatography (Et_2O–hexane, 1:2) to give **3** as colourless syrup. Yield: 1.7 g (79%); $[\alpha]_D^{25}$ +10 (*c*, 1.8). IR (neat): 3448 (OH), 3067 and 3031 (aromatic), 1704 (C=O, Cbz), 740 and 700 cm^{-1} (aromatic). NMR data (300 MHz, inter alia): ^1H, δ 7.68–7.12 (m, 25H, 5Ph), 5.09 and 5.01 (2br d, 2H, *J*=12 Hz, CH_2Ph), 4.61–4.51 (br m, 4H, $2CH_2Ph$), 4.28–3.60 (3br m, 8H, H-2,2′a,2′b,3,4,5,5′a,5′b), 1.05 (s, 9H, CMe_3); ^{13}C (inter alia), δ 155.73 (C=O, Cbz), 77.66 and 76.74 (C-3,4), 71.96 and 71.79 ($2CH_2Ph$), 67.57 (CH_2Ph, Cbz), 64.63 and 62.82 (C-2′,5′), 64.56 and 63.59 (C-2,5), 26.98 (CMe_3) and 19.23 (CMe_3). HRMS (LSIMS): *m*/*z* 738.3221 [M^++Na]. For $C_{44}H_{49}NO_6NaSi$ 738.3227 (deviation +0.9 ppm).

4.1.2. 4-[(3*E*,2′*R*,3′*R*,4′*S*,5′*S*)-3′,4′-Dibenzyloxy-*N*-benzyloxycarbonyl-5′-*tert*-butyldiphenylsilyloxymethylpyrrolidin-2′-yl]but-3-en-2-one (5). To a stirred solution of **3**

(835 mg, 1.17 mmol) in dry CH_2Cl_2 (10 mL) were added activated 4 Å molecular sieves (0.6 g), *N*-oxide-*N*-methylmorpholine (NMO, 213 mg, 1.82 mmol) and tetra-*n*-propylammonium perruthenate (TPAP, 50 mg) and the reaction mixture was kept at rt for 15 min. TLC (Et_2O/hexane, 1:1) then indicated the absence of the starting material and the presence of a faster-running compound. The reaction was diluted with ether (30 mL), filtered through a bed of Silica gel 60 (Scharlau, 230–400 mesh) and thoroughly washed with ether. The combined filtrate and washings were concentrated to aldehyde **4** (730 mg, 88%); $[\alpha]_D^{25}$ −9 (*c*, 0.85). IR (neat): 3068 and 3032 (aromatic), 1735 (CHO), 1710 (C=O, Cbz), 738 and 700 cm^{-1} (aromatic). This material was used in the next step.

To a solution of **4** (730 mg, 1 mmol) in dry toluene (20 mL) was added 1-triphenylphosphoranylidene-2-propanone (1.07 g, 3.36 mmol) and the mixture was heated at 80 °C for 3 h. TLC (ether/hexane, 2:1) then revealed the presence of a slightly slower-running compound. The reaction mixture was filtered and supported on silica gel, then chromatographed (ether/hexane, 1:2) to afford **5** (730 mg, 83%) as a thick syrup; $[\alpha]_D^{26}$ +27 (*c* 1). IR (neat): 3068 and 3032 (aromatic), 1705, 1679 and 1633 (C=O, conjugated ketone, Cbz and C=C conjugated), and 700 cm^{-1} (aromatic). NMR data (400 MHz): ^1H, δ 7.57–7.14 (m, 25H, 5Ph), 6.64 and 6.56 (2br dd, $J_{2',4}$=6.5 and 7.1 Hz, H-4, two rotamers), 6.26 and 6.12 (2br d, $J_{3,4}$=16 and 15.6 Hz, H-3, two rotamers), 5.20–4.97 and 4.64–3.74 (4br m, 12H, 3PhCH_2 and H-2′,3′,4′,5′,5″a,5″b), 2.05 and 1.90 (2br s, 3H, H-1,1,1, two rotamers) and 1.01 (s, 9H, CMe_3). ^{13}C (inter alia), δ 198.16 (C-2), 155.62 (Cbz), 81.52 and 80.47 (C-3′,4′), 72.27, 71.66 and 67.30 (2Ph*C*H_2 and Cbz), 64.21, 63.56, 62.49 and 62.26 (C-2′,5′, two rotamers), 62.72 (C-5″), 27.01 (C-1 and C*Me*$_3$) and 19.33 (*C*Me_3). HRMS (LSIMS): *m*/*z* 776.3381 [M^++Na]. For $C_{47}H_{51}NO_6NaSi$ 776.3383 (deviation +0.3 ppm).

4.1.3. (1*R*,2*S*,3*S*,5*R*,7a*R*)-1,2-Dibenzyloxy-3-*tert*-butyldiphenylsilyloxymethyl-5-methylpyrrolizidine (6). Compound **5** (690 mg, 0.92 mmol) in methanol (30 mL) was hydrogenated at 60 psi over 10% Pd–C (200 mg) for 18 h. TLC (ether/hexane 1:2) then showed the presence of a new compound of higher mobility. The catalyst was filtered off, washed with methanol and the filtrate and washings concentrated to a residue that was submitted to column chromatography (ether/hexane 1:2) to afford pure syrupy **6** (290 mg, 52%), which had $[\alpha]_D^{25}$ +15 (*c* 1.3). IR (neat): 3068, 3030, 738 and 700 cm^{-1} (aromatic). NMR data (400 MHz): ^1H, δ 7.70–7.25 (2m, 20H, 4Ph), 4.70 and 4.63 (2d, 2H, J=11.8 Hz, CH_2Ph), 4.60 and 4.57 (2d, 2H, J=12.8 Hz, CH_2Ph), 4.06 (dd, 1H, $J_{1,2}$=6.2, $J_{2,3}$=3.4 Hz, H-2), 3.75 (dd, 1H, $J_{3,8}$=5.0, $J_{8,8'}$=10.5 Hz, H-8), 3.60 (dd, 1H, H-1), 4.56 (dd, 1H, $J_{3,8'}$=6.6 Hz, H-8′), 3.11 (dt, 1H, $J_{7\beta,7a}$=5.4, $J_{1,7a}$=$J_{7\alpha,7a}$=10.0 Hz, H-7a), 2.83 (m, 1H, H-3), 2.52 (sex, 1H, $J_{5,6\beta}$=$J_{5,6\alpha}$=$J_{5,Me}$=6.2 Hz, H-5), 2.18 (dq, 1H, $J_{6\beta,7\beta}$=$J_{6\beta,7\alpha}$=8.3, $J_{6\alpha,6\beta}$=13 Hz, H-6β), 1.77 (m, 1H, H-7β), 1.58 (m, 1H, H-6α), 1.39 (dq, 1H, $J_{6\alpha,7\alpha}$=7.6, $J_{7\alpha,7\beta}$=10.7 Hz, H-7α), 1.08 (s, 9H, CMe_3) and 0.98 (d, 3H, Me); ^{13}C (inter alia), δ 84.91 (C-2), 79.36 (C-1), 72.20 and 71.73 (2*C*H_2Ph), 72.09 (C-7a), 68.27 (C-3), 65.86 (C-8), 55.03 (C-5), 37.10 (C-6), 26.99 (C*Me*$_3$), 25.21 (C-7), 21.33 (Me) and 19.34 (*C*Me_3). Mass spectrum (LSIMS): *m*/*z* 628.3227 [M^++Na]. For $C_{39}H_{47}NO_3NaSi$ 628.3223 (deviation −0.6 ppm).

4.1.4. (1*R*,2*S*,3*S*,5*R*,7a*R*)-1,2-Dihydroxy-3-hydroxymethyl-5-methylpyrrolizidine [(−)-3-*epi*hyacinthacine A_5, 1a]. A solution of **6** (260 mg, 0.43 mmol) in methanol (30 mL) was acidified (concd HCl) and hydrogenated (10% Pd–C, 110 mg) at 60 psi for 15 h. The catalyst was filtered off, washed with methanol and the filtrate and washings neutralized with Amberlite IRA-400 (OH^- form) and concentrated. ^1H NMR of the residue showed the absence of benzyl group and that the TBDPS group still remains. The residue was dissolved in THF (5 mL) and treated with a solution of TBAF·$3H_2O$ (350 mg) in the same solvent (5 mL) at rt overnight. TLC (ether–methanol–aq 30% NH_4OH, 5:1:0.1) then revealed a new compound with R_f 0.46. The solvent was eliminated and the residue chromatographed (ether → ether–methanol–aq 30% NH_4OH, 5:1:0.1) to afford pure **1a** (75 mg, 93%), which had $[\alpha]_D^{29}$ −15 and $[\alpha]_{405}^{29}$ −20 (*c* 0.44, methanol). NMR data (400 MHz, methanol-d_4): ^1H, δ 4.10 (dd, 1H, $J_{1,2}$=7.0, $J_{2,3}$=4.8 Hz, H-2), 3.73 (dd, 1H, $J_{3,8}$=4.4, $J_{8,8'}$=11.9 Hz, H-8), 3.70 (dd, 1H, $J_{3,8'}$=4.6 Hz, H-8′), 3.62 (dd, 1H, $J_{1,7a}$=8.5 Hz, H-1), 2.77 (ddd, 1H, $J_{7a,7\beta}$=5.8, $J_{7a,7\alpha}$=10.4 Hz, H-7a), 2.53 (br sex, 1H, $J_{5,6\alpha}$=$J_{5,6\beta}$=$J_{5,Me}$=6.3 Hz, H-5), 2.43 (q, 1H, H-3), 2.23 (ddt, 1H, $J_{6\beta,7\beta}$=9.0, $J_{6\beta,7\alpha}$=7.9, $J_{6\alpha,6\beta}$=12.8 Hz, H-6β), 1.79 (dddd, 1H, $J_{6\alpha,7\beta}$=2.6, $J_{7\alpha,7\beta}$=11.7 Hz, H-7β), 1.65 (dddd, 1H, $J_{6\alpha,7\alpha}$=10.7 Hz, H-6α), 1.45 (br dq, 1H, H-7α) and 1.19 (d, 3H, Me); ^{13}C, δ 78.26 (C-2), 76.14 (C-7a), 72.45 (C-1), 71.70 (C-3), 63.22 (C-8), 56.95 (C-5), 37.69 (C-6), 24.98 (C-7) and 21.19 (Me). Mass spectrum (LSIMS): *m*/*z* 156.1025 [M^+-CH_2OH]. For $C_8H_{14}NO_2$ 156.1025 (deviation −0.1 ppm).

4.1.5. (2*R*,3*R*,4*S*,5*S*)-2′-*O*-Benzoyl-3,4-dibenzyloxy-*N*-benzyloxycarbonyl-5′-*O*-*tert*-butyldiphenylsilyl-2,5-bis(hydroxylmethyl)pyrrolidine (7). To a stirred solution of **3** (835 mg, 1.17 mmol) in dry dichloromethane (10 mL) were added triethylamine (TEA, 150 μL, 1.8 mmol), DMAP (50 mg) and benzoyl chloride (150 μL, 1.4 mmol) and the mixture left at rt for 20 h. TLC (ether/hexane 2:1) then revealed a faster-running compound. Conventional work-up of the reaction mixture and column chromatography (ether/hexane 1:3) afforded pure **7** (910 mg, 95%) as a colourless syrup, which had $[\alpha]_D^{25}$ +10 and $[\alpha]_{405}^{26}$ +27 (*c* 1.8). IR (neat): 3088 and 3067 (aromatic), 1722 (COPh and >NCO_2Bn), 740 and 700 cm^{-1} (aromatic). NMR data (400 MHz): ^1H, δ 8.14–7.18 (m, 30H, 6Ph), 5.21–5.01 and 4.69–3.68 (2m, 14H, 3CH_2Ph, H-2,2′a,2′b,3,4,5,5′a,5′b), and 1.03 (s, 9H, CMe_3); ^{13}C (inter alia), δ 166.03 (*C*OPh), 155.74 (>N*C*O_2Bn), 76.97, 76.07, 75.78 and 75.07 (C-3,4, two rotamers), 71.85, 71.71 and 71.43 (2O*C*H_2Ph, two rotamers), 67.35 and 67.13 (two rotamers), 63.58, 62.99, 60.41 and 59.88 (C-2,5, two rotamers), 62.57, 62.23, 62.02 and 61.69 (C-2′,5′, two rotamers), 26.98 (C*Me*$_3$) and 19.26 (*C*Me_3). Mass spectrum (LSIMS): *m*/*z* 842.3485 [M^++Na]. For $C_{51}H_{53}NO_7NaSi$ 842.3489 (deviation +0.4 ppm).

4.1.6. (2*R*,3*R*,4*S*,5*S*)-2′-*O*-Benzoyl-3,4-dibenzyloxy-*N*-benzyloxycarbonyl-2,5-bis(hydroxymethyl)pyrrolidine (8). To a stirred solution of **7** (840 mg, 1.03 mmol) in THF (15 mL) was added TBAF·$3H_2O$ (490 mg, 1.55 mmol) and the mixture was kept at rt. TLC (ether/hexane 3:1)

then showed a new compound of lower mobility. The mixture was neutralized with acetic acid, concentrated to a residue that was dissolved in ether, washed with brine, concentrated, and then submitted to column chromatography (ether/hexane 2:1) to yield pure **8** (500 mg, 84%) as a colourless syrup, which had $[\alpha]_D^{26}$ −12 (*c* 0.8). IR (neat): 3470 (OH), 3064 and 3032 (aromatic), 1719 (COPh and >NCO_2Bn), 712 and 698 cm^{-1} (aromatic). NMR data (300 MHz): ^{1}H, δ 7.93–7.27 (m, 20H, 4Ph), 5.26 and 5.12 (2d, 2H, *J*=12.3 Hz, OCH_2Ph), 4.62–3.85 (m, 11H, 2CH_2Ph, H-2,2′a,2′b,3,4,5,5′a) and 3.63 (dd, 1H, $J_{4,5'b}$=4.9, $J_{5'a,5'b}$=11.6 Hz, H-5′b); ^{13}C (inter alia), δ 77.10 and 76.68 (C-3,4), 72.15 and 71.90 (2O*C*H_2Ph), 67.80 (>$NCO_2C H_2$Ph), 64.32 and 61.30 (C-2,5), 63.82 and 63.17 (C-2′,5′). Mass spectrum (LSIMS): *m*/*z* 604.2312 [M$^+$+Na]. For $C_{35}H_{35}NO_7Na$ 604.2311 (deviation −0.1 ppm).

4.1.7. 4-[(2′*S*,3′*S*,4′*R*,5′*R*)-5′-Benzoyloxymethyl-3′,4′-dibenzyloxy-*N*-benzyloxycarbonylpyrrolidin-2′-yl]but-3-en-2-one (10). To a solution of **8** (1.08 g, 1.9 mmol) in dry dichloromethane (10 mL) were added activated powdered 4 Å molecular sieve (700 mg), *N*-methylmorpholine *N*-oxide (325 mg, 2.8 mmol) and TPAP (40 mg) and the reaction mixture kept at rt for 1 h. TLC (ether/hexane 4:1) then showed a faster-running compound. The reaction was diluted with ether (30 mL), filtered through a bed of Silica gel 60 (Scharlau, 230–400 mesh) and thoroughly washed with ether. The combined filtrate and washings were concentrated to aldehyde **9**, that was used in the next step.

To a well stirred suspension of sodium hydride (60% 150 mg, 3.7 mmol) in anhydrous THF (15 mL), diethyl(2-oxopropyl)phosphonate (660 μL, 3.7 mmol) was added and the mixture was left at rt for 1 h, when a solution of aldehyde **9** in THF (10 mL) was added. After 5 min TLC (ether/hexane 4:1) revealed the presence of a new compound of slightly lower mobility. The solvent was eliminated and the residue was partitioned into ether/water. The organic phase was separated and concentrated to a residue that was submitted to column chromatography with ether/hexane (3:1) as eluent to give pure **10** (520 mg, 45% from **8**) as a colourless syrup, which had $[\alpha]_D^{23}$ −15 and $[\alpha]_{405}^{24}$ −46 (*c* 2.1). IR (neat): 3088 and 3063 (aromatic), 1718 and 1678 ($PhCO_2$, >NCO_2Bn and α,β-unsaturated ketone), 713 and 699 cm^{-1} (aromatic). NMR data (300 MHz): ^{1}H, δ 7.85–7.25 (m, 20H, 4Ph), 6.52 (br m, 1H, H-4), 6.20–6.07 (br m, 1H, H-3), 5.22–4.25 (2m, 10H, 3CH_2Ph, H-3′,4′,5″a,5″b), 4.01 (br t, 1H, *J*=4 Hz) and 3.91 (br t, 1H, *J*=5.2 Hz) for H-2′,5′ and 1.90 (br s, 3H, H-1,1,1); ^{13}C (inter alia), δ 197.58 (C-2), 166.09 (*C*OPh), 155.72 (>N*C*O_2Bn), 72.11 (2O*C*H_2Ph), 67.58 (>$NCO_2C H_2$Ph), 62.56 (C-5″) and 27.41 (C-1). Mass spectrum (LSIMS): *m*/*z* 642.2470 [M$^+$+Na]. For $C_{38}H_{37}NO_7Na$ 642.2468 (deviation −0.3 ppm).

4.1.8. (1*S*,2*R*,3*R*,5*S*,7a*S*)-3-Benzoyloxymethyl-1,2-dibenzyloxy-5-methylpyrrolizidine (11). Compound **10** (500 mg, 0.8 mmol) in dry methanol (15 mL) was hydrogenated at 60 psi over 10% Pd–C (100 mg) for 24 h. TLC (ether/hexane 4:1) then showed the presence of a new compound of lower mobility. The catalyst was filtered off, washed with methanol and the filtrate and washings concentrated to a residue that was submitted to column chromatography (ether/hexane 2:1) to afford pure syrupy **11** (280 mg, 74%), which had $[\alpha]_D^{25}$ +8.5 and $[\alpha]_{405}^{25}$ +19 (*c* 1). IR (neat): 3063 and 3031 (aromatic), 1720 (CO benzoate), 712 and 697 cm^{-1} (aromatic). NMR data (400 MHz): ^{1}H, δ 8.03 (d, 2H, $J_{o,m}$=7.5 Hz, H-*ortho* Bz), 7.58 (t, 1H, $J_{m,p}$=7.5 Hz, H-*para* Bz), 7.44 (t, 2H, H-*meta* Bz), 7.39–7.27 (m, 10H, 2CH_2Ph), 4.77 and 4.58 (2d, 2H, *J*=11.7 Hz, CH_2Ph), 4.65 and 4.59 (2d, 2H, *J*=12.0 Hz, CH_2Ph), 4.43 (dd, 1H, $J_{3,8}$=4.8, $J_{8,8'}$=11.4 Hz, H-8), 4.30 (dd, 1H, $J_{3,8'}$=6.3 Hz, H-8′), 4.14 (dd, 1H, $J_{1,2}$=6.4, $J_{2,3}$=4.2 Hz, H-2), 3.68 (dd, 1H, $J_{1,7a}$=8.7 Hz, H-1), 3.10 (dt, 1H, $J_{7a,7\alpha}$=5.5, $J_{7a,7\beta}$=9.9 Hz, H-7a), 3.00 (br q, 1H, H-3), 2.62 (br sex, 1H, $J_{5,6\alpha}$=$J_{5,6\beta}$=7.0 Hz, H-5), 2.22 (dq, 1H, $J_{6\alpha,7\alpha}$=$J_{6\alpha,7\beta}$=8.5, $J_{6\alpha,6\beta}$=13.0 Hz, H-6α), 1.81 (dddd, 1H, $J_{7\alpha,6\beta}$=2.2 Hz, H-7α), 1.65 (dddd, 1H, $J_{6\beta,7\beta}$=10.0 Hz, H-6β), 1.47 (dq, 1H, $J_{7\alpha,7\beta}$=10.9 Hz, H-7β) and 1.16 (d, 3H, $J_{Me,5}$=6.0 Hz, Me); ^{13}C (inter alia), δ 166.46 (*C*OPh), 84.82 (C-2), 79.07 (C-1), 72.73 (C-7a), 72.73 and 71.83 (2*C*H_2Ph), 65.93 (C-8), 65.07 (C-3), 54.81 (C-5), 37.13 (C-6), 25.36 (C-7) and 21.47 (Me). Mass spectrum (LSIMS): *m*/*z* 494.2309 [M$^+$+Na]. For $C_{30}H_{33}NO_4Na$ 494.2307 (deviation −0.4 ppm).

4.1.9. (1*S*,2*R*,3*R*,5*S*,7a*S*)-1,2-Dihydroxy-3-hydroxymethylpyrrolizidine [(+)-3-*epi*hyacinthacine A_5, 1b]. Conventional debenzoylation of **11** (270 mg, 1 mmol) in 0.2 N sodium methoxide in dry methanol (7 mL) gave after work-up compound **12** (280 mg, 0.8 mmol) that was dissolved in dry methanol (30 mL) and hydrogenated (10% Pd–C, 170 mg) in acid medium (concd HCl, four drops) at 50 psi for 48 h. TLC (ether/methanol 3:1) then showed a not mobile compound. The catalyst was filtered off, washed with methanol and the filtrate and washings neutralized with Amberlite IRA-400 (OH^- form), then concentrated. Column chromatography (ether/methanol/TEA 5:1:0.1) of the residue gave pure **1b** (110 mg, 80%), which had $[\alpha]_D^{26}$ +14.5 (*c* 0.8, methanol) and NMR data identical to **1a**.

4.2. Glycosidase inhibitory activities

All pNP-pyranoside substrates, α-glucosidase (from baker's yeast), β-glucosidase (from almonds), α-galactosidase (from green coffee), β-galactosidase (from bovine liver), β-galactosidase (from *A. oryzae*) and α-mannosidase (from Jack beans) were purchased from Sigma Chemical Company. Kinetic studies were performed at 37 °C in 50 mM sodium citrate/phosphate buffer. Enzyme concentrations ranging from 0.5 μg mL^{-1} to 0.1 μg mL^{-1} were used, depending on the substrate studied. The activities of enzymes were determined using *p*-nitrophenyl glycosides as substrates at the optimum pH of each enzyme. Substrates, suitably diluted enzyme solutions and inhibitors were incubated together for 30 min at 37 °C. Reactions were followed in an UV–vis spectrophotometer by measuring the change in the absorbance of light at 400 nm. Data were analyzed using the programme GraFit.[10]

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Supplementary data

^{1}H and ^{13}C NMR (six pages) for compounds **1a**, **6** and **11**. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.04.003.

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