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Biosynthetic studies on the α-glucosidase inhibitor acarbose: the chemical synthesis of isotopically labeled 2-*epi*-5-*epi*-valiolone analogs

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Abstract

2-*epi*-5-*epi*-Valiolone is a cyclization product of the C_7 sugar phosphate, sedoheptulose 7-phosphate, involved in the biosynthesis of the aminocyclitol moieties of acarbose, validamycin, and pyralomicin. As part of our investigation into the pathway from 2-*epi*-5-*epi*-valiolone to the valienamine moiety of acarbose, we prepared 1-*epi*-5-*epi*-(6-$^2H_2$)valiolol [(6-$^2H_2$)-**6**], 5-*epi*-(6-$^2H_2$)valiolol [(6-$^2H_2$)-**17**], 1-*epi*-2-*epi*-5-*epi*-(6-$^2H_2$)valiolol [(6-$^2H_2$)-**12**] and 2-*epi*-5-*epi*-(6-$^2H_2$)valiolamine [(6-$^2H_2$)-**11**]. Compounds (6-$^2H_2$)-**6** and (6-$^2H_2$)-**17** were synthesized from 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose in 10 and seven steps, respectively, whereas (6-$^2H_2$)-**12** and (6-$^2H_2$)-**11** were synthesized from 2,3,4,6-tetra-*O*-benzyl-D-mannopyranose in eight and 10 steps, respectively.

Keywords: 2-*epi*-5-*epi*-Valiolone analogs; Acarbose biosynthesis; Valiolol; Valiolamine

1. Introduction

The α-glucosidase inhibitor acarbose and related compounds are members of the C_7N aminocyclitol family of natural products that are increasingly gaining recognition due to their significant biomedical and agricultural uses.[1–4] Their chemical structures most commonly contain an unsaturated aminocyclitol moiety, the so called valienamine moiety (Fig. 1). In our earlier work on the biosynthesis of acarbose (**1**),[5,6] validamycin A (**2**),[5,7] and pyralomicin (**3**)[8] we demonstrated that the valienamine moiety of these compounds is derived from 2-*epi*-5-*epi*-valiolone (**4**), an intramolecular aldol cyclization product of sedoheptulose 7-phosphate. The cyclization reaction has been proposed to occur via a dehydroquinate (DHQ) synthase-like mechanism.[9] In the biosynthesis of validamycin A (**2**) by *Streptomyces hygroscopicus* var *limoneus*, the 2-*epi*-5-*epi*-valiolone is epimerized at the C-2 stereocenter to give 5-*epi*-valiolone followed by dehydration between C-5 and C-6 to yield valienone.[7,10] However, feeding experiments with (6-$^2H_2$)-5-*epi*-valiolone and (1-^{13}C)valienone to the acarbose (**1**) producer revealed that neither of these ketocyclitols was incorporated into the valienamine moiety of **1**.[6] Further studies using isotopically labeled 2-*epi*-valiolone, 2-*epi*-valienone, as well as valiolamine and valienamine yielded similar results, leaving the pathway from 2-*epi*-5-*epi*-valiolone to the valienamine moiety of **1** purely conjectural.

One explanation that we previously proposed was that the transformation of 2-*epi*-5-*epi*-valiolone (**4**) to the valienamine moiety of **1** involves a substrate-channeling mechanism in which enzyme-bound intermediates are directly transferred from one enzyme active site to the next in a multi-enzyme complex. However, while this proposal is mechanistically attractive, there is no hard evidence to support it. On the other hand, several alternatives are certainly also plausible, for example, one cannot rule out the possibility that the nitrogen is introduced into the cyclitol moiety in an

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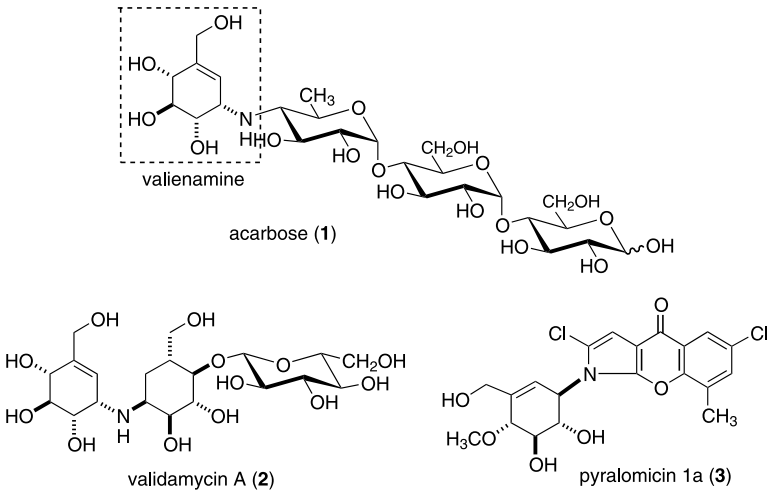


Fig. 1. Chemical structures of acarbose (**1**), validamycin A (**2**), and pyralomicin 1a (**3**).

earlier step (Scheme 1). Alternatively, the coupling may occur by an S_N2 displacement of an activated hydroxyl group on the cyclitol by the nitrogen of the amino sugar moiety.[11] The most plausible intermediate for this mechanism was 1-*epi*-valienol, which is found in the acarbose production culture at the early stage of fermentation, qualifying it as a biosynthetic intermediate. However, when 1-*epi*-(1-^{13}C)valienol was synthesized and fed, no incorporation into acarbose was observed.[6] Thus, if this mode of coupling does operate in **1** biosynthesis, the coupling must occur at an earlier stage, that of 1-*epi*-5-*epi*-valiolol or 1-*epi*-2-*epi*-5-*epi*-valiolol (Scheme 1). On such a hypothetical pathway, the coupling product might undergo epimerization and dehydration catalyzed by flavin-containing redox enzyme which transiently generates an imine in its active site. In the present paper, we report the synthesis of isotopically labeled 1-*epi*-5-*epi*-valiolol (**6**), 5-*epi*-valiolol (**17**), 1-*epi*-2-*epi*-5-*epi*-valiolol (**12**), and 2-*epi*-5-*epi*-valiolamine (**11**). The examination of their possible involvement in the biosynthesis of acarbose is also discussed.

2. Results and discussion

2.1. Synthesis of 1-*epi*-5-*epi*-(6-$^2H_2$)valiolol [(6-$^2H_2$)-6] and 5-*epi*-(6-$^2H_2$)valiolol [(6-$^2H_2$)-17]

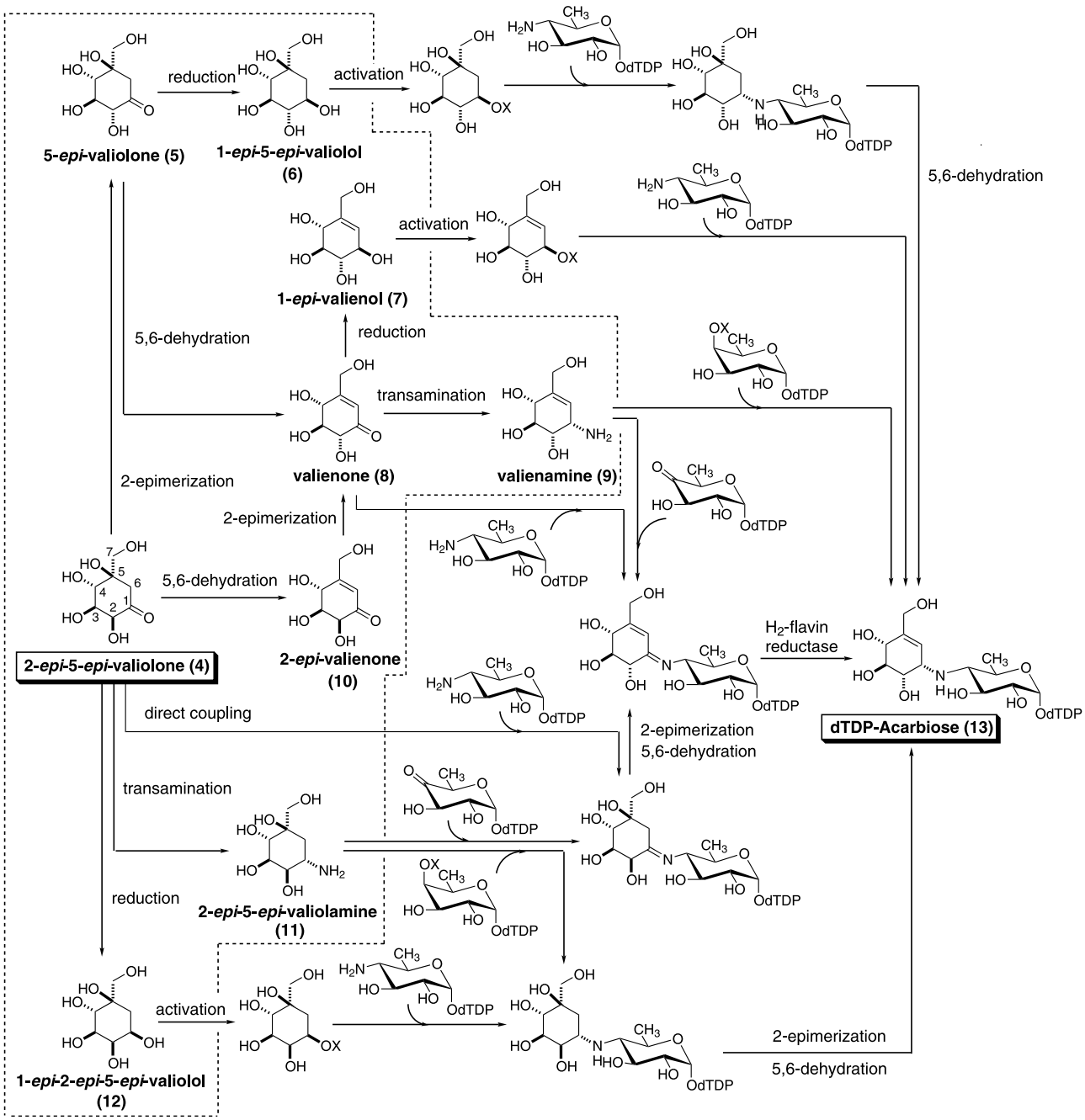
1-*epi*-5-*epi*-(6-$^2H_2$)Valiolol [(6-$^2H_2$)-**6**] and 5-*epi*-(6-$^2H_2$)valiolol [(6-$^2H_2$)-**17**] were prepared from 2,3,4,7-tetra-*O*-benzyl-5-*epi*-(6-$^2H_2$)valiolone [(6-$^2H_2$)-**15**] (Scheme 2). The latter compound was generated from 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (**14**) in five steps.[10] Reduction of the ketone (6-$^2H_2$)-**15** with sodium borohydride gave predominantly the undesired cyclitol (6-$^2H_2$)-**16** in 86% yield. The stereochemistry at C-1 was determined based on the coupling constant between H-1 (4.01 ppm, d, *J* 2.6 Hz) and H-2 (3.48 ppm, dd, *J* 2.6 and 7.8 Hz), as well as the strong nOe correlation between H-1 and H-2 in (6-$^2H_2$)-**16**. Large coupling constants between H-2 and H-3 (3.69 ppm, dd, *J* 7.8, 8.3 Hz) and between H-3 and H-4 (3.50 ppm, d, *J* 8.3 Hz) suggest that (6-$^2H_2$)-**16** adopts a chair conformation.

The stereoselectivity of the borohydride reduction of ketone (6-$^2H_2$)-**15** is most likely due to steric hindrance, especially by the C-2 benzyloxyl group, that allows the nucleophilic attack to proceed only from the β face. A small quantity of β-alcohol was also isolated, but all attempts to increase the proportion of the β-alcohol using different reducing agents and reaction conditions were unsuccessful. It was therefore necessary to invert the stereochemistry at the 1-position by acetate displacement of a C-1 tosylate. Thus, α-alcohol (6-$^2H_2$)-**16** was reacted with *p*-toluenesulfonyl chloride in pyridine to give tosylate (6-$^2H_2$)-**18** in 82% yield. Subsequent reaction with KOAc in DMF afforded acetate (6-$^2H_2$)-**19** along with elimination product (6-$^2H_2$)-**20**. The mixture of (6-$^2H_2$)-**19** and (6-$^2H_2$)-**20** was reacted with NaOMe–MeOH to give alcohol (6-$^2H_2$)-**21**. A large coupling constant (*J* 8.8 Hz) between H-1 and H-2 of alcohol (6-$^2H_2$)-**21** confirms the β-OH configuration.

Finally, debenzylation of (6-$^2H_2$)-**21** with Pd–C under an atmosphere of hydrogen gave 1-*epi*-5-*epi*-(6-$^2H_2$)valiolol [(6-$^2H_2$)-**6**] in 96% yield. Likewise, α-alcohol (6-$^2H_2$)-**16** was debenzylated under identical conditions to give 5-*epi*-(6-$^2H_2$)valiolol [(6-$^2H_2$)-**17**].

2.2. Synthesis of 1-*epi*-2-*epi*-5-*epi*-(6-$^2H_2$)valiolol [(6-$^2H_2$)-12] and 2-*epi*-5-*epi*-(6-$^2H_2$)valiolamine [(6-$^2H_2$)-11]

2,3,4,7-Tetra-*O*-benzyl-2-*epi*-5-*epi*-(6-$^2H_2$)valiolone [(6-$^2H_2$)-**23**], prepared from commercially available 2,3,4,6-tetra-*O*-benzyl-D-mannopyranose,[6] was used for the synthesis of 1-*epi*-2-*epi*-5-*epi*-(6-$^2H_2$)valiolol [(6-$^2H_2$)-**12**] and 2-*epi*-5-*epi*-(6-$^2H_2$)valiolamine [(6-$^2H_2$)-**11**]

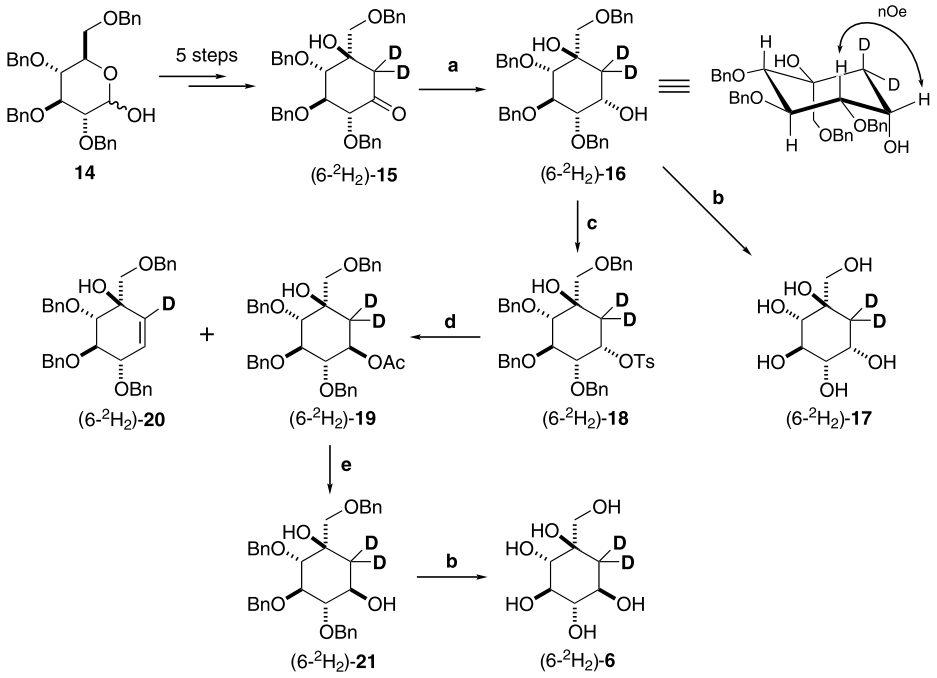


Scheme 1. Originally considered possible pathways from 2-*epi*-5-*epi*-valiolone (**4**) to dTDP-acarbiose (**13**), the precursor of acarbose (**1**).

(Scheme 3). Compound (6-$^2H_2$)-**23** was treated with sodium borohydride in THF–methanol to give predominantly the desired alcohol (6-$^2H_2$)-**24** in 86% yield. This stereoselectivity may be due to the strong steric effect of the benzyloxyl group at C-2 that shields the β face and directs the hydride attack at C-1 to proceed exclusively from the α face.

Alcohol (6-$^2H_2$)-**24** was subsequently subjected to catalytic hydrogenation to afford (6-$^2H_2$)-**12** in quantitative yield. The stereochemistry at C-1 in (6-$^2H_2$)-**12** was concluded to be as shown in Scheme 3 based on a comparison of its NMR data with those of racemic compound **12** and its 1-epimer, synthesized from *p*-benzoquinone[12] and provided by Dr Oliver Block.

The synthesis of 2-*epi*-5-*epi*-(6-$^2H_2$)valiolamine [(6-$^2H_2$)-**11**] also proceeded from alcohol (6-$^2H_2$)-**24**. The latter compound was tosylated to afford (6-$^2H_2$)-**25** in 90% yield. Displacement of the C-1 tosylate with azide was surprisingly difficult, given that the stereoconfigurations of the C-1 tosyloxyl and C-2 benzyloxyl moieties would usually allow an S_N2 substitution to proceed from the α face. After many attempts, (6-$^2H_2$)-

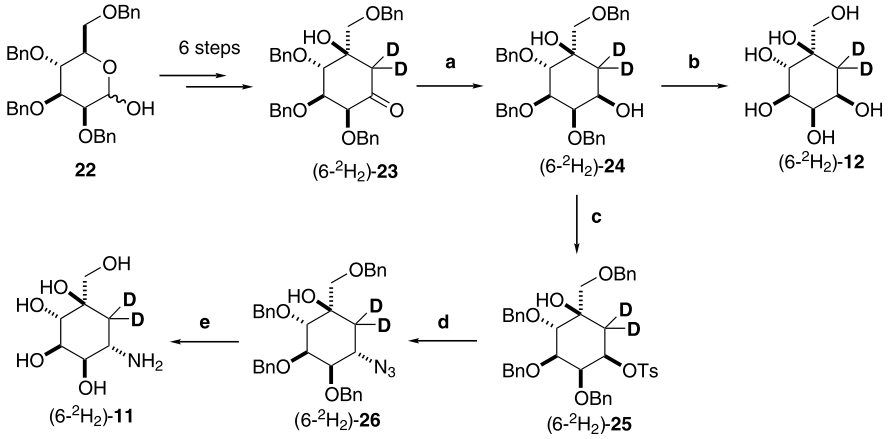


Scheme 2. The synthesis of 1-*epi*-5-*epi*-(6-$^2H_2$)valiolol [(6-$^2H_2$)-**6**] and 5-*epi*-(6-$^2H_2$)valiolol [(6-$^2H_2$)-**17**]. Reagents: (a) $NaBH_4$/THF–EtOH, 86%; (b) H_2, 10% Pd–C/95% EtOH, 96%; (c) *p*TsCl, DMAP/pyridine, 82%; (d) KOAc/DMF; (e) NaOMe/methanol, 34% in 2 steps.

25 was eventually treated with a large excess of sodium azide in the presence of crown ether[13] and the reaction was carried out under vigorous reaction conditions (90 °C for 52 h) to furnish azidocyclitol (6-$^2H_2$)-**26** in 32% yield. Further extension of the reaction time or increasing the temperature leads to extensive decomposition. Similar conditions applied to the triflate derivative of alcohol (6-$^2H_2$)-**24** did not give the desired product. Finally, azide (6-$^2H_2$)-**26** was subjected to catalytic hydrogenation with 20% $Pd(OH)_2$ on charcoal to afford (6-$^2H_2$)-**11** as an amorphous solid in quantitative yield.

2.3. Feeding experiments in resting cells with labeled compounds

The cyclitols (6-$^2H_2$)-**6**, (6-$^2H_2$)-**12**, and (6-$^2H_2$)-**17**, as well as the aminocyclitol (6-$^2H_2$)-**11** (15 mg each) were fed to resting cell cultures of *Actinoplanes* sp. SN223/29 (60 mL each) along with (U-$^{13}C_3$)glycerol and 2-*epi*-5-



Scheme 3. The synthesis of 1-*epi*-2-*epi*-5-*epi*-(6-$^2H_2$)valiolol [(6-$^2H_2$)-**12**] and 2-*epi*-5-*epi*-(6-$^2H_2$)valiolamine [(6-$^2H_2$)-**11**]. Reagents: (a) $NaBH_4$/THF–EtOH, 86%; (b) H_2, 10% Pd–C/95% EtOH, quantitative; (c) *p*TsCl, DMAP/pyridine, 90%; (d) NaN_3, 18-crown-6/DMF, 32% (38% recovered); (e) H_2, 20% $Pd(OH)_2$–C/95% EtOH, quantitative.

epi-(6-$^2H_2$)valiolone as positive controls. After 24 h incubation, the resulting acarbose was isolated and purified as described in the previous paper.[6] Analysis of the acarbose by ESI-MS indicated that except the positive controls, none of the cyclitols was significantly incorporated into acarbose. The non-incorporation of 1-*epi*-2-*epi*-5-*epi*-(6-$^2H_2$)valiolol [(6-$^2H_2$)-**12**] rules out the possibility of an immediate reduction of 2-*epi*-5-*epi*-valiolone (**4**) prior to a S_N2 coupling between the alcohol (or its activated form) with dTDP-4-amino-4,6-dideoxy-D-glucose[11] (Scheme 1). Likewise, the non-incorporation of 1-*epi*-5-*epi*-(6-$^2H_2$)valiolol [(6-$^2H_2$)-**6**] and its C-1 isomer, 5-*epi*-(6-$^2H_2$)valiolol [(6-$^2H_2$)-**17**], excludes the possibility of **4** being epimerized at C-2 to give 5-*epi*-valiolone (**5**) and subsequently reduced to **6** prior to the coupling reaction. Evidence suggesting that **5** is not directly involved in the biosynthesis of acarbose has also been obtained in previous feeding experiments with 5-*epi*-(6-$^2H_2$)valiolone to the acarbose producer, which yielded a negative incorporation result (T. Mahmud, J. Xu, Y. U. Choi, unpublished data).

After none of the possible cyclitols used in this study nor the ketocyclitols described in our previous report[6] (except 2-*epi*-5-*epi*-valiolone) was incorporated into acarbose, only a very short list of compounds could still be considered as plausible intermediates. Although a reduction of 2-*epi*-5-*epi*-valiolone (**4**) in theory will give either 1-*epi*-2-*epi*-5-*epi*-valiolol (**12**) or its 1-epimer, 2-*epi*-5-*epi*-valiolol, the involvement of the latter compound in acarbose biosynthesis is less likely since a coupling between 2-*epi*-5-*epi*-valiolol or its activated form with dTDP-4-amino-4,6-dideoxy-D-glucose will lead to the opposite C-1 stereochemistry in the product as in acarbose. Although this inverted stereochemistry could be corrected by a further imine formation, which will also facilitate the 2-epimerization and 5,6-dehydration to give dTDP-acarbiose, such inefficiency is unusual in a biological system. In addition, it is evident that hydride reduction of the protected 2-*epi*-5-*epi*-valiolone from the *pro-S* face to give protected 2-*epi*-5-*epi*-valiolol is rather difficult, presumably due to steric hindrance, which most likely will also be the case if the reduction of 2-*epi*-5-*epi*-valiolone has to occur in the biological system.

The non-incorporation of all plausible cyclitols that have been tested suggests the involvement of a rather unusual mechanism in the biosynthesis of acarbose. Recently, the group of Piepersberg reported the identification of a kinase activity in the acarbose producer, which modifies 2-*epi*-5-*epi*-valiolone to its 7-phosphate derivative.[14] The product is then epimerized at the C-2 position to give 5-*epi*-valiolone 7-phosphate.[15] These results suggest that, the intermediates involved in the biosynthesis of the valienamine moiety of acarbose are phosphorylated cyclitols which, except for 2-*epi*-5-*epi*-valiolone 7-phosphate, cannot be generated directly from their unphosphorylated counterparts. A similar instance, where none of the plausible intermediates, except 2-*epi*-5-*epi*-valiolone and to some extent 5-*epi*-valiolone, was incorporated has also been observed in the biosynthesis of pyralomicin 1a.[8]

3. Experimental

3.1. General

The ^1H, ^2H, and ^{13}C NMR spectra were recorded on Bruker AF-300 or AM500 NMR spectrometers with *Mac* NMR 5.5 PCI as the instrument controller and data processor. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. Low-resolution electrospray mass spectra were recorded on a Bruker–Esquire Liquid Chromatograph–Ion Trap Mass Spectrometer. A PerSeptive Biosystems Mariner Electrospray Time of Flight Mass Spectrometer (ESI-TOF) was used for the high resolution mass spectrometry. A ISF-4-V shaker, Adolf Kuhner AG was used for the fermentations. All synthetic reactions were carried out under an atmosphere of dry Ar at room temperature (rt) in oven-dried glassware unless otherwise noted. Reactions were monitored by TLC (silica gel 60 F_{254}, Merck) with detection by UV light or by alkaline permanganate spray or $Ce(SO_4)_2/H_2SO_4$ solution. Column chromatography was performed on 230–400 mesh silica gel (Aldrich), Sephadex LH-20 (Sigma) or Sephadex CM-25 (Pharmacia).

3.2. Materials

Actinoplanes sp. SN223/29 was obtained from Bayer AG, Wuppertal, Germany. All chemicals were purchased from Aldrich or Sigma and used without further purification unless otherwise noted. Ingredients for fermentations, soybean meal (fat free), maltzin powder, and yeast extract, were obtained from Bayer AG, Germany and NZ-Amine A from ICN Biochemicals.

3.3. Culture conditions for feeding experiments in resting cells

For the resting cell experiments, production cultures in complex medium[6] were incubated for 28–30 h and centrifuged (4000*g* for 5 min) to remove the supernatant. The remaining cells were washed twice with cold 100 mM potassium phosphate buffer (pH 6.9) and then suspended in 50 mM potassium phosphate (pH 6.9) containing maltose (1.5%) and bactopeptone (0.15%) to make 75 mL of cell suspension from 50 mL of production culture. Each 60–75 mL of resting cells suspension in a 500-mL flask was incubated for 22–24 h on a rotary shaker at 220 rpm and 28 °C with labeled substrates added at 0 h (1/3) and the other 2/3 at 8 h.

Isolation and purification procedures of acarbose were same as described in our previous paper.[6]

3.4. Synthesis of labeled substrates

3.4.1. 2,3,4,7-Tetra-*O*-benzyl-5-*epi*-(6-$^2H_2$)valiolol [(6-$^2H_2$)-16]. 2,3,4,7-Tetra-*O*-benzyl-5-*epi*-(6-$^2H_2$)valiolone [(6-$^2H_2$)-**15**] (926 mg, 1.67 mmol) was dissolved in THF–EtOH (50 mL) and $NaBH_4$ (250 mg, 6.64 mmol) was added. The mixture was stirred for 30 min after which TLC [eluent 2:1 (v/v) hexane–EtOAc] indicated complete conversion to a lower running product. The solution was diluted with EtOAc (100 mL) and washed with 1 M HCl (2 × 50 mL), satd $NaHCO_3$ (3 × 50 mL), and brine (50 mL), dried ($MgSO_4$), filtered and the filtrate concentrated under reduced pressure. The resulting syrup was purified by column chromatography [eluent 5:1–4:1 (v/v) hexane–EtOAc] and concentration of the appropriate fractions afforded (6-$^2H_2$)-**16** (797 mg, 1.43 mmol, 86%) as a clear syrup; $[\alpha]_D^{23}$ −1.8 (*c* 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 3.48 (dd, 1 H, $J_{1,2}$ 2.6, $J_{2,3}$ 7.8 Hz, H-2), 3.50 (d, 1 H, $J_{3,4}$ 8.3 Hz, H-4), 3.69 (dd, 1 H, $J_{2,3}$ 7.8, $J_{3,4}$ 8.3 Hz, H-3), 3.71 (s, 2 H, H-7a, H-7b), 4.01 (d, 1 H, $J_{1,2}$ 2.6 Hz, H-1), 4.57–4.81 (m, 8 H, C*H*$_2$Ph × 4), 7.19–7.48 (m, 20 H, C_6H_5 × 4); ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 36.2, 65.7, 72.9, 73.4, 73.7, 75.4, 75.5, 75.6, 79.2, 81.8, 84.3, 127.7–128.7, 138.7, 138.4, 138.5, 138.9. Electrospray-MS: *m*/*z* 557.3 $[M+H]^+$. Electrospray-HRMS: *m*/*z* 579.2686 $[M+Na]^+$ Calcd for $C_{35}H_{36}D_2NaO_6$: 579.2689.

3.4.2. 2,3,4,7-Tetra-*O*-benzyl-1-*O*-(*p*-tolylsulfonyl)-5-*epi*-(6-$^2H_2$)valiolol [(6-$^2H_2$)-18]. 2,3,4,7-Tetra-*O*-benzyl-5-*epi*-(6-$^2H_2$)valiolol [(6-$^2H_2$)-**16**] (497 mg, 893 μmol) was dissolved in Py (80 mL) and *p*-toluenesulfonyl chloride (0.85 g, 4.47 mmol) and DMAP (54 mg, 447 μmol) were added. The reaction mixture was stirred for 15 h at rt followed by the addition of more *p*-toluenesulfonyl chloride (0.85 g, 4.47 mmol) and DMAP (54 mg, 447 μmol). The mixture was stirred at 70 °C for 5 h after which TLC [eluent 2:1 (v/v) hexane–EtOAc] indicated that the reaction was complete. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and water (20 mL) was added. The organic layer was washed with 1 M HCl (2 × 50 mL), $NaHCO_3$ (2 × 50 mL), and brine (50 mL), dried ($MgSO_4$), filtered and the filtrate concentrated under reduced pressure. The resulting residue was purified by column chromatography [eluent 5:1 (v/v) hexane–EtOAc] and concentration of the appropriate fractions afforded (6-$^2H_2$)-**18** (523 mg, 736 μmol, 82%) as a clear syrup; $[\alpha]_D^{23}$ +8.5° (*c* 0.6, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 2.35 (s, 3 H, C*H*$_3$), 3.41–3.48 (m, 2 H), 3.52 (dd, 1 H, $J_{1,2}$ 3.1, $J_{2,3}$ 7.3 Hz, H-2), 3.59–3.72 (m, 2 H), 4.36–4.59 (m, 8 H, C*H*$_2$Ph × 4), 4.84 (d, 1 H, $J_{1,2}$ 3.1 Hz, H-1), 7.12–7.36 (m, 22 H, Ar*H*), 7.76 (d, 2 H, *J* 8.1 Hz, Ar*H*); ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 21.8, 73.0, 73.3, 73.7, 74.7, 75.7, 76.4, 77.9, 78.0, 78.1, 78.3, 127.7–129.9, 134.1, 137.8, 138.1, 138.3, 138.6, 144.7. Electrospray-MS: *m*/*z* 733.4 $[M+Na]^+$. Electrospray-HRMS: *m*/*z* 733.2787 $[M+Na]^+$ Calcd for $C_{42}H_{42}D_2NaO_8S$: 733.2778.

3.4.3. 2,3,4,7-Tetra-*O*-benzyl-1-*epi*-5-*epi*-(6-$^2H_2$)valiolol [(6-$^2H_2$)-21]. 2,3,4,7-Tetra-*O*-benzyl-1-*O*-(*p*-tolylsulfonyl)-5-*epi*-(6-$^2H_2$)valiolol [(6-$^2H_2$)-**18**] (497 mg, 699 μmol) was dissolved in DMF (50 mL) and KOAc (207 mg, 2.11 mmol), was added. The reaction mixture was stirred at 60 °C for 48 h after which TLC [eluent 2:1 (v/v) hexane–EtOAc] indicated complete consumption of the starting material. The solution was diluted with water (20 mL) and extracted with EtOAc (3 × 50 mL), the organic layer was washed with water (2 × 25 mL), and brine (25 mL), dried ($MgSO_4$), filtered and the filtrate concentrated under reduced pressure. The resulting residue was purified by column chromatography [eluent 4:1 (v/v) hexane–EtOAc] and concentration of the appropriate fractions afforded (6-$^2H_2$)-**19**; TLC R_f 0.61 [eluent 2:1 (v/v) hexane–EtOAc], contaminated with elimination product (6-^2H)-**20**; R_f 0.55. This mixture was dissolved in MeOH (20 mL) and NaOMe (5 mg, 92.5 μmol) was added. The reaction mixture was stirred at rt for 18 h after which CH_2Cl_2 (100 mL) was added. The resulting solution was washed with 1 M HCl (50 mL), satd $NaHCO_3$ (2 × 50 mL), brine (50 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated under reduced pressure. The resulting residue was purified by column chromatography [2:1 (v/v) hexane–EtOAc] and concentration of the appropriate fractions afforded (6-$^2H_2$)-**21** (132 mg, 237 μmol, 34% over two steps) as a white solid; TLC R_f 0.21 [2:1 (v/v) hexane–EtOAc); $[\alpha]_D^{23}$ −39.8° (*c* 0.6, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 2.16 (d, 1 H, $J_{1,OH}$ 2.0 Hz, O*H*), 2.56 (s, 1 H, O*H*), 3.32 (dd, 1 H, $J_{1,2}$ 8.8, $J_{2,3}$ 9.3 Hz, H-2), 3.38 (d, 1 H, $J_{7a,7b}$ 9.3 Hz, H-7b), 3.42 (dd, 1 H, $J_{3,4}$ 9.8, $J_{2,3}$ 9.3 Hz, H-3), 3.47 (dd, 1 H, $J_{1,2}$ 8.8, $J_{1,OH}$ 2.0 Hz, H-1), 3.58 (d, 1 H, $J_{7a,7b}$ 9.3 Hz, H-7a), 3.65 (d, 1 H, $J_{3,4}$ 9.8 Hz, H-4), 4.44, 4.55, 4.60, 4.65, 4.70, 4.78, 4.81, 4.88 (8 H, 8 × 1/2 AB, C*H*$_2$Ph × 4), 7.53–7.10 (m, 20 H, Ar*H*); ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 68.3, 71.5, 73.8, 74.5, 75.5, 75.6, 75.7, 75.9, 82.4, 86.4, 127.7–128.9, 138.5, 137.9, 138.6, 138.9. Electrospray-MS: *m*/*z* 579.3 $[M+Na]^+$. Electrospray-HRMS: *m*/*z* 579.2690 $[M+Na]^+$ Calcd for $C_{35}H_{36}D_2NaO_6$: 579.2689.

3.4.4. 1-*epi*-5-*epi*-(6-$^2H_2$)Valiolol [(6-$^2H_2$)-6]. 2,3,4,7-Tetra-*O*-benzyl-1-*epi*-5-*epi*-(6-$^2H_2$)-valiolol [(6-$^2H_2$)-**21**] (107 mg, 192 μmol) was dissolved in 95% EtOH–H_2O (10 mL) and Pd–C (107 mg) was added. The suspension was stirred under an atmosphere of H_2 at rt for 28 h after which TLC [eluent 4:1:1 (v/v/v) 2-propanol–water–AcOH] indicated complete conversion to a slower running product. The product was filtered

through Celite and concentrated under reduced pressure to give [(6-$^2H_2$)-**6**] (36 mg, 184 μmol, 96%) as a white solid; TLC R_f 0.63 [eluent 4:1:1 (v/v/v) 2-propanol–water–AcOH]; $[\alpha]_D^{23}$ −7.3° (*c* 0.3, H_2O); ^{1}H NMR (300 MHz, CD_3OD): δ_H 3.28 (dd, 1 H, $J_{2,3}$ 9.3, $J_{1,2}$ 8.6 Hz, H-2), 3.40–3.45 (m, 1 H, H-3), 3.52 (d, 1 H, $J_{3,4}$ 9.9 Hz, H-4), 3.57 (d, 1 H, $J_{7a,7b}$ 11.7 Hz, H-7b), 3.59 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 3.85 (d, 1 H, $J_{7a,7b}$ 11.7 Hz, H-7a); ^{13}C NMR (75 MHz, CD_3OD): δ_C 39.2 (br, m), 64.6, 69.8, 74.5, 75.3, 79.8, 80.5. Electrospray-MS: *m*/*z* 218.9 $[M+Na]^+$. Electrospray-HRMS: *m*/*z* 219.0817 $[M+Na]^+$ Calcd for $C_7H_{12}D_2NaO_6$: 219.0812.

3.4.5. 5-*epi*-(6-$^2H_2$)Valiolol [(6-$^2H_2$)-17]. 2,3,4,7-Tetra-*O*-benzyl-5-*epi*-(6-$^2H_2$)-valiolol [(6-$^2H_2$)-**16**] (101 mg, 181 μmol) was dissolved in 95% EtOH–H_2O (10 mL) and Pd–C (101 mg) was added. The suspension was stirred under an atmosphere of H_2 at rt for 24 h after which TLC [4:1:1 (v/v/v) 2-propanol–water–AcOH] indicated complete conversion to a lower running product. The product was filtered through Celite and concentrated under reduced pressure to give (6-$^2H_2$)-**17** (34 mg, 173 μmol, 96%) as a white solid; TLC R_f 0.60 [eluent 4:1:1 (v/v/v) 2-propanol–water–AcOH]; $[\alpha]_D^{23}$ +18.0° (*c* 0.6, H_2O); ^{1}H NMR (300 MHz, CD_3OD): δ_H 3.52 (d, 1 H, $J_{3,4}$ 7.3 Hz, H-4), 3.60 (dd, 1 H, $J_{1,2}$ 3.6, $J_{2,3}$ 6.7 Hz, H-2), 3.63 (d, 1 H, $J_{7a,7b}$ 10.9 Hz, H-7b), 3.74 (d, 1 H, $J_{7a,7b}$ 10.9 Hz, H-7a), 3.83 (dd, 1 H, $J_{2,3}$ 6.7, $J_{3,4}$ 7.3 Hz, H-3), 4.02 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1); ^{13}C NMR (75 MHz, CD_3OD): δ_C 36.5 (br, m), 66.8, 68.3, 73.2, 75.4, 76.4, 78.5. Electrospray-MS: *m*/*z* 219.0 $[M+Na]^+$. Electrospray-HRMS: *m*/*z* 219.0816 $[M+Na]^+$ Calcd for $C_7H_{12}D_2NaO_6$: 219.0812.

3.4.6. 2,3,4,7-Tetra-*O*-benzyl-1-*epi*-2-*epi*-5-*epi*-(6-$^2H_2$)valiolol [(6-$^2H_2$)-24]. To a solution of 2,3,4,7-tetra-*O*-benzyl-2-*epi*-5-*epi*-(6-$^2H_2$)valiolone [(6-$^2H_2$)-**23**] (696 mg, 1.25 mmol) in THF–MeOH (7 mL, 1:1 v/v) was added $NaBH_4$ (200 mg, 5.28 mmol) in THF–MeOH (3 mL, 1:1 v/v) at 0 °C, and the mixture was stirred at rt for 2.5 h. The mixture was concentrated in vacuo, and the resulting residue was diluted with ether and water. The organic phase was washed with 2 M HCl, satd $NaHCO_3$, and brine, dried (Na_2SO_4), filtered and concentrated to dryness. The residue was chromatographed over silica gel with hexanes–EtOAc (4:1–2:1) to give alcohol (6-$^2H_2$)-**24** (600 mg, 86%) as a colorless syrup; $[\alpha]_D^{24}$ −11.3° (*c* 0.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.80 (1 H, br, 1-OH), 3.39 (d, 1 H, $J_{7a,7b}$ 9.3 Hz, H-7a), 3.56 (d, 1 H, $J_{7a,7b}$ 9.3 Hz, H-7b), 3.75 (t, 1 H, $J_{1,2/2,3}$ 3.1 Hz, H-2), 3.80 (dd, 1 H, $J_{3,4}$ 5.7, $J_{2,3}$ 3.1 Hz, H-3), 4.01 (d, 1 H, $J_{3,4}$ 5.7 Hz, H-4), 4.05 (br, 1 H, H-1), 4.55–4.73 ($CH_2Ph \times 4$), 7.10–7.45 (Ar*H*); ^{13}C NMR (75 MHz, $CDCl_3$): δ 34.9, 67.2, 71.7, 72.9, 73.4, 73.5, 73.5, 74.0, 76.3, 77.2, 78.5, 127.6–128.2, 137.9–138.2. Electrospray-MS: *m*/*z* 557 $[M+H]^+$. Electrospray-HRMS: *m*/*z* 557.2874 $[M+H]^+$ Calcd for $C_{35}H_{37}D_2O_6$: 557.2870.

3.5. 1-*epi*-2-*epi*-5-*epi*-(6-$^2H_2$)Valiolol [(6-$^2H_2$)-12]

A mixture of benzyl ether (6-$^2H_2$)-**24** (200 mg, 0.359 mmol) and wet 10% Pd–C (180 mg) in 95% aq EtOH (20 mL) was stirred under a hydrogen atmosphere at rt for 14 h. The mixture was passed through a pad of Celite with 1:1 MeOH–water, and the filtrate and washings were concentrated to dryness. The residue was dissolved in water and was passed through a membrane filter. The filtrate was concentrated to dryness to afford (6-$^2H_2$)-**12** (69.0 mg, quant.) as a white solid; $[\alpha]_D^{25}$ −6.03° (*c* 0.318, H_2O); ^{1}H NMR (300 MHz, D_2O): δ 3.56 (d, 1 H, $J_{7a,7b}$ 12 Hz, H-7a), 3.57 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 10.4 Hz, H-3), 3.75 (d, 1 H, $J_{7a,7b}$ 12 Hz, H-7b), 3.78 (d, 1 H, $J_{3,4}$ 10.4 Hz, H-4), 3.79 (d, 1 H, $J_{1,2}$ 3.0 Hz, H-1), 4.03 (t, 1 H, $J_{1,2/2,3}$ 3.0 Hz, H-2); ^{13}C NMR (75 MHz, D_2O–CD_3OD = 2:1): δ_C 34.3 (br, m), 63.3, 67.0, 72.0, 73.6, 74.6, 76.7. Electrospray-MS: 219 $[M+Na]^+$. Electrospray-HRMS: *m*/*z* 219.0814 $[M+Na]^+$ Calcd for $C_7H_{12}D_2NaO_6$: 219.0812.

3.5.1. 2,3,4,7-Tetra-*O*-benzyl-1-*O*-(*p*-tolylsulfonyl)-1-*epi*-2-*epi*-5-*epi*-(6-$^2H_2$)valiolol [(6-$^2H_2$)-25]. To a solution of alcohol (6-$^2H_2$)-**24** (373 mg, 0.671 mmol) and DMAP (165 mg, 1.35 mmol) in Py (13 mL) was added *p*-toluenesulfonyl chloride (1.41 g, 7.40 mmol) at rt, and the mixture was stirred at rt for 10 h. Water (10 mL) was added to the mixture, and the product was extracted with EtOAc. The combined organic phase was washed with 2 M HCl, satd $NaHCO_3$, and brine, dried (Na_2SO_4), filtered and concentrated to dryness. The residue was chromatographed over silica gel with 4:1 hexanes–EtOAc to give tosylate (6-$^2H_2$)-**25** (429 mg, 90%) as a colorless syrup; $[\alpha]_D^{26}$ −12.83° (*c* 0.6, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.37 (s, 3 H, CH_3–C_6H_4), 3.42 (d, 1 H, $J_{7a,7b}$ 9.3 Hz, H-7a), 3.49 (d, 1 H, $J_{7a,7b}$ 9.3 Hz, H-7b), 3.67 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 8.8 Hz, H-3), 3.96 (d, 1 H, $J_{3,4}$ 8.8 Hz, H-4), 3.98 (br, 1 H), 4.48–4.81 (m, 10 H), 7.10–7.70 (Ar*H*); ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 21.4, 34.3 (br, m), 71.6, 72.8, 72.9, 73.0, 75.1, 76.1, 76.5, 78.6, 127.3, 127.4, 127.4, 127.5, 127.5, 127.6, 128.0, 128.1, 128.1, 128.3, 129.5, 133.8, 137.6, 138.0, 138.3, 138.5, 144.4. Electrospray-MS: *m*/*z* 711 $[M+H]^+$. Electrospray-HRMS: *m*/*z* 733.2780 $[M+Na]^+$ Calcd for $C_{42}H_{42}D_2NaO_8S$: 733.2778.

3.5.2. 2,3,4,7-Tetra-*O*-benzyl-1-azido-1-deoxy-2-*epi*-5-*epi*-(6-$^2H_2$)valiolol [(6-$^2H_2$)-26]. A mixture of tosylate (6-$^2H_2$)-**25** (43.5 mg, 0.0612 mmol), NaN_3 (250 mg, 3.85 mmol), and 18-crown-6 (15 mg, 0.0567 mmol) in DMF (1 mL) was stirred at 90 °C. Additional NaN_3 (250 mg, 3.85 mmol) and 18-crown-6 (15 mg, 0.0757 mmol) were added to the reaction mixture every 16 h period. After

52 h stirring, water (3 mL) was added to the mixture, and the product was extracted with EtOAc. The combined organic phase was washed with brine, dried (Na_2SO_4), filtered and concentrated to dryness. The residue was chromatographed over silica gel with 4:1 hexanes–EtOAc to give azide (6-$^2H_2$)-**26** (11.3 mg, 32%) as a colorless syrup and unreacted tosylate (6-$^2H_2$)-**25** (16.7 mg, 38%); $[\alpha]_D^{23}$ −15.6° (*c* 0.7, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.35 (d, 1 H, $J_{7a,7b}$ 9.9 Hz, H-7a), 3.56 (dd, 1 H, $J_{7b,5\text{-OH}}$ 2.1, $J_{7a,7b}$ 9.9 Hz, H-7b), 3.67 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 9.9 Hz, H-3), 3.78 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 3.84 (dd, 1 H, $J_{2,3}$ 3.1, $J_{1,2}$ 3.6 Hz, H-2), 4.08 (d, 1 H, $J_{3,4}$ 9.9 Hz, H-4), 4.29–4.74 (CH_2Ph × 4), 4.39 (d, 1 H, $J_{5\text{-OH},7b}$ 2.1 Hz, 5-OH), 7.10–7.45 (Ar*H*); ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 57.0, 72.5, 73.2, 73.7, 74.0, 74.1, 74.9, 75.3, 75.8, 79.7, 127.6–128.5, 136.9, 137.8, 137.8, 138.1. Electrospray-MS: *m*/*z* 582 $[M+H]^+$ and 554 $[M+H-N_2]^+$. Electrospray-HRMS: *m*/*z* 582.2932 $[M+H]^+$ Calcd for $C_{35}H_{36}D_2N_3O_5$: 582.2935.

3.5.3. 2-*epi*-5-*epi*-(6-$^2H_2$)Valiolamine [(6-$^2H_2$)-11]. A mixture of azide (6-$^2H_2$)-**26** (110 mg, 0.189 mmol) and wet 20% $Pd(OH)_2$ on charcoal (315 mg) in 95% aq EtOH (14 mL) was stirred under hydrogen atmosphere at rt for 36 h. The mixture was passed through a pad of Celite with 1:1 MeOH–water , and the filtrate and washings were concentrated to dryness. The residue was dissolved in water and passed through a membrane filter. The filtrate was concentrated to dryness to afford (6-$^2H_2$)-**11** (37.0 mg, quant.) as an amorphous solid; $[\alpha]_D^{27}$ +45.9° (*c* 0.7, H_2O); ^{1}H NMR (300 MHz, D_2O): δ 3.30 (d, 1 H, $J_{7a,7b}$ 12 Hz, H-7a), 3.46 (d, 1 H, $J_{3,4}$ 9.4 Hz, H-4), 3.49 (d, 1 H, $J_{7a,7b}$ 12 Hz, H-7b), 3.75–3.79 (m, 2 H, H-2 and H-3), 3.91 (brs, 1 H, H-1); ^{13}C NMR (75 MHz, D_2O–CD_3OD = 8:1): δ_C 32.0 (br, m), 48.4, 66.8, 69.6, 70.3, 74.1, 75.6. Electrospray-MS: *m*/*z* 196 $[M+H]^+$. Electrospray-HRMS: *m*/*z* 196.1152 $[M+H]^+$ Calcd for $C_7H_{14}D_2NO_5$: 196.1152.

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