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β-Mannosidase and β-hexosaminidase inhibitors: synthesis of 1,2-bis-*epi*-valienamine and 1-*epi*-2-acetamido-2-deoxy-valienamine from D-mannose

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ABSTRACT

A partially protected C-5=C-5a unsaturated carbasugar with α-*lyxo* configuration is synthesised in five steps and 26% overall yield from a known mannose-derived hemiacetal, using ring-closing metathesis as a key step. This carbasugar is converted into valienamine derivatives with β-*lyxo* (i.e., corresponding to β-*manno* at C-1–C-4), α-*lyxo* (i.e., corresponding to α-*manno* at C-1–C-4) and β-2-acetamido-2-deoxy-*xylo* (i.e., corresponding to β-GlcNAc at C-1–C-4) configurations. This is the first report of the synthesis of the β-*lyxo* compound, 1,2-bis-*epi*-valienamine, which was found to inhibit *Cellulomonas fimi* β-mannosidase (CfMan2A) with K_i 140 μM. We report the crystal structures of three protected C-5=C-5a unsaturated carbasugars with *lyxo* configuration.

1. Introduction

Valienamine **1** (Fig. 1) is an unsaturated carbahexopyranose that has the same configuration at C-1–C-4 as α-glucose (i.e., α-*xylo* configuration),[†] occurring in nature as a component of larger structures, for example, acarbose[1a] or the validomycins.[1b] It has moderate activity as an inhibitor of α-glucosidases (IC_{50} 18 μM–1 mM),[2] while larger oligosaccharide analogues containing an N-substituted valienamine moiety may have higher inhibitory activity (e.g., acarbose, K_i 260 nM against sucrase[1]). Some epimers of valienamine as well as N-substituted variants have been synthesised in attempts to inhibit other glycosidases, with varying degrees of success.[3] For example, *N*-octyl 1-*epi*-valienamine has been shown to inhibit β-glucocerebrosidase (IC_{50} 30 nM),[4] *N*-octyl 1,4-bis-*epi*-valienamine has been shown to inhibit bovine liver β-galactosidase (IC_{50} 870 nM)[5] and recently, 2-acetamido-2-deoxy-1-*epi*-valienamine (i.e., corresponding to β-GlcNAc configuration at C-1–C-4) has been shown to inhibit various β-hexosaminidases.[6]

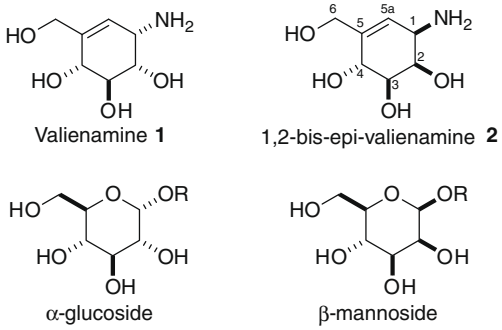

Figure 1.

Glycosidases have been classified into families based on primary sequence and structural similarities.[‡] β-Mannosidases are retaining *exo*-glycosidases that hydrolyse β-mannosidic linkages, and are classified as belonging to glycosidase families 1, 2 or 5. Some microbial β-mannosidases[7,8] are part of the hydrolytic enzyme systems that hydrolyse mannans and heteromannans, major components of hemicellulose.[9] The active site architecture may vary among β-mannosidases, as shown by the two high-resolution crystal structures that

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† Valienamine and other carbocyclic derivatives are treated as carbasugars and numbered as such (see IUPAC recommendations, *Carbohydr. Res.* **1997**, *297*, 1–90; Ref. 3; Yamagishi, K.; Ogawa, S. *Liebigs Ann.* **1995**, 279–284); numbering is shown in Figure 1. Hence, the carbocyclic derivatives with C-1–C-4 configurational match with α-mannose have the α-*lyxo* configuration; C-1–C-4 configurational match with β-mannose have the β-*lyxo* configuration (e.g., 1,2-bis-*epi*-valienamine **2**); C-1–C-4 configurational match with β-glucose have the β-*xylo* configuration (e.g., 2-acetamido-2-deoxy-1-*epi*-valienamine **27**); Open-chain derivatives are numbered as shown in Scheme 1.

‡ Carbohydrate Active Enzymes (CAZy) database (http://www.cazy.org/). See: Coutinho, P. M.; Henrissat, B. In *Recent Advances in Carbohydrate Bioengineering*, Gilbert, H. J., Davies, G., Henrissat, B., Svensson, B., Eds.; The Royal Society of Chemistry: Cambridge, 1999, pp 3–12.

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have been published.[10] The family 2 β-mannosidase of the human gut bacterium *Bacteroides thetaiotaomicron* has an active site formed by several protein domains,[10a] whereas the family 5 β-mannosidase of the soil bacterium *Cellvibrio mixtus* has an active site within a single domain,[10b] as do *endo*-β-1,4-mannanases of the same family.[11] To further investigate catalytic mechanisms and enzyme–carbohydrate interactions of β-mannosidases,[7,8] we became interested in potential inhibitors of these enzymes.[12] It has been proposed that several β-mannoside hydrolases of families 2, 5 and 26 operate with a boat-like ($B_{2,5}$) substrate conformation at the transition state,[13] a suggestion that has been further strengthened by very recent work with a number of monosaccharide-mimicking inhibitors.[14] Of the various β-mannosidase inhibitors that have been reported in the literature, some have been described as transition-state analogues where a six-membered ring has a fixed or low-energy conformation resembling a postulated transition state in the cleavage of a β-mannoside linkage,[15] while for others (e.g., polyhydroxylated pyrrolidines), the resemblance to β-mannose is less obvious.[16] We undertook to synthesise the as yet unknown 1,2-bis-*epi*-valienamine **2** (Fig. 1; i.e., corresponding to a β-*manno* configuration at C-1–C-4, whose potential biological relevance has been noted[3]) and test for β-mannosidase inhibition.

Conversion of carbohydrates to unsaturated carbocycles has been described before using, for example, an intramolecular Wadsworth–Emmons reaction[17] or ring-closing metathesis.[18,19] We planned the synthesis around a ring-closing metathesis reaction that would form the cyclohexene with the trisubstituted C-5=C-5a double bond. The required polyhydroxylated diene should be accessible from mannose, which necessarily has the required configuration at C-2–C-4. Introduction of the two alkenes could then be achieved by vinyl Grignard addition and Wittig methylenation; related approaches to carbocyclic carbohydrate derivatives have been reported before.

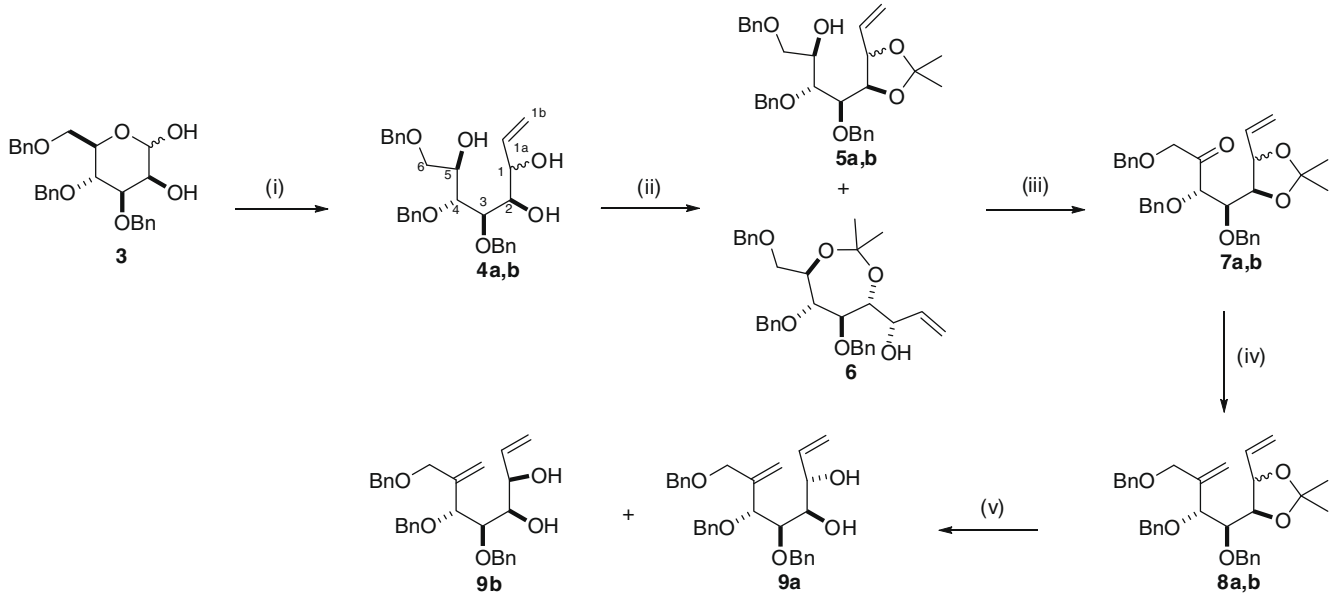
Starting from a partially protected mannose hemiacetal **3** with OH-2 free has several potential benefits:[19j] firstly, we may expect a diastereoselective Grignard addition arising from a chelate effect;[20] secondly, the product of Grignard addition would contain a 1,2-diol that it should be possible to selectively protect leaving OH-5 free[21] and finally, it should be possible to access GlcNAc valienamine analogues[6,22] by S_N2 substitution of OH-2 of the carbocyclic product with a nitrogen nucleophile.

2. Results and discussion

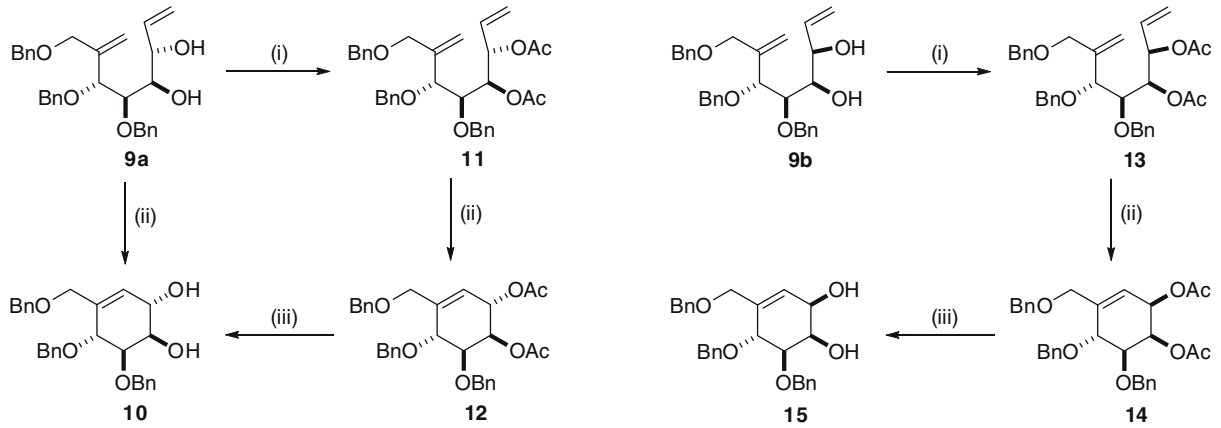
Hemiacetal **3** is synthesised from mannose in six steps on a multigram scale without the need for chromatography, following literature procedures.[23] Vinyl Grignard addition gave the triols **4a**,**b** as an inseparable diastereomeric mixture (**4a**:**4b**, 3:1, Scheme 1). Protection with an isopropylidene acetal (2,2-dimethoxypropane, DMF, CSA, rt; heating to 70 °C did not change the product distribution) gave the required five-ring compound **5a** as the major component of an inseparable mixture (**5a**:**5b**:**6**, 3.8:1.2:1).[21] Oxidation to the ketones **7a**,**b** was followed by Wittig methylenation to give the inseparable dienes **8a**,**b**. Before attempting metathesis, the isopropylidene protection was removed to give diastereomers **9a** and **9b**, which could now easily be separated by flash chromatography.

Treatment of diene **9a** with the Grubbs' second generation ruthenium complex gave variable yields (22–76%) of carbocycle **10** along with unidentified by-products. The diol **9a** was protected as a diacetate **11**, which was ring-closed by Grubbs' second generation complex in toluene at 60 °C to give carbocycle **12** in consistently good yield (82%, Scheme 2). The Hoveyda–Grubbs second generation ruthenium complex gave good cyclisation results with both the diacetate (**11**→**12**; 87–96%) and the diol (**9a**→**10**; 81%) under the same conditions. The minor diastereomer **9b** was protected as its diacetate **13**, and treatment with Hoveyda–Grubbs second generation complex in toluene at 60 °C gave the carbocycle **14** (Scheme 2). The acetates were removed from **12** and **14** by methanolysis to give the respective carbocyclic diols **10** and **15**.

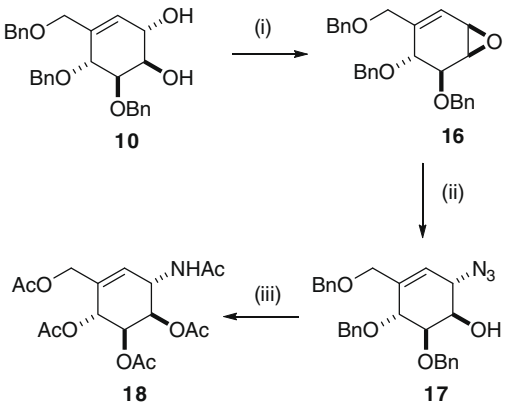
To reach 1,2-bis-*epi*-valienamine **2** from the carbocyclic diol **10**, it was necessary to introduce nitrogen at C-1 with inversion of configuration. We first attempted this under Mitsunobu conditions,[24] but treatment with triphenylphosphine, DIAD and phthalimide in THF gave no nitrogen substitution, and the epoxide **16** was identified as the major product (42%). Neither did a different nitrogen source, diphenylphosphoryl azide (DPPA) give any substitution, but a better yield of the epoxide **16** (71%, Scheme 3) was obtained. Epoxide opening with azide gave the known α-*lyxo* configured derivative **17**, which has been converted into 2-*epi*-valienamine and its peracetate **18** by Shing.[25]



Scheme 1. Reagents and conditions: (i) vinylmagnesium bromide, THF, 70 °C, 82% (**4a**:**4b**, 3:1); (ii) 2,2-dimethoxypropane, DMF, CSA, 88% (**5a**:**5b**:**6**, 3.8:1.2:1); (iii) oxalyl chloride, DMSO, CH_2Cl_2, −78 °C, then Et_3N, −78 °C→rt, 81% (**7a**:**7b**, 3.3:1); (iv) ^tBuOK, Ph_3PCH_3Br, toluene, 80%; (v) AcOH, H_2O, HCl (1 M), 80 °C; **9a**, 69%; **9b**, 19%.



Scheme 2. Reagents and conditions: (i) Ac_2O, py, DMAP; **11**, 92%; **13**, 93%; (ii) Hoveyda–Grubbs second generation complex, toluene, 60 °C; **12**, 87%; **10**, 81%; **14** 88%; (iii) NaOMe, MeOH; **10**, 88%; **15**, 95%.

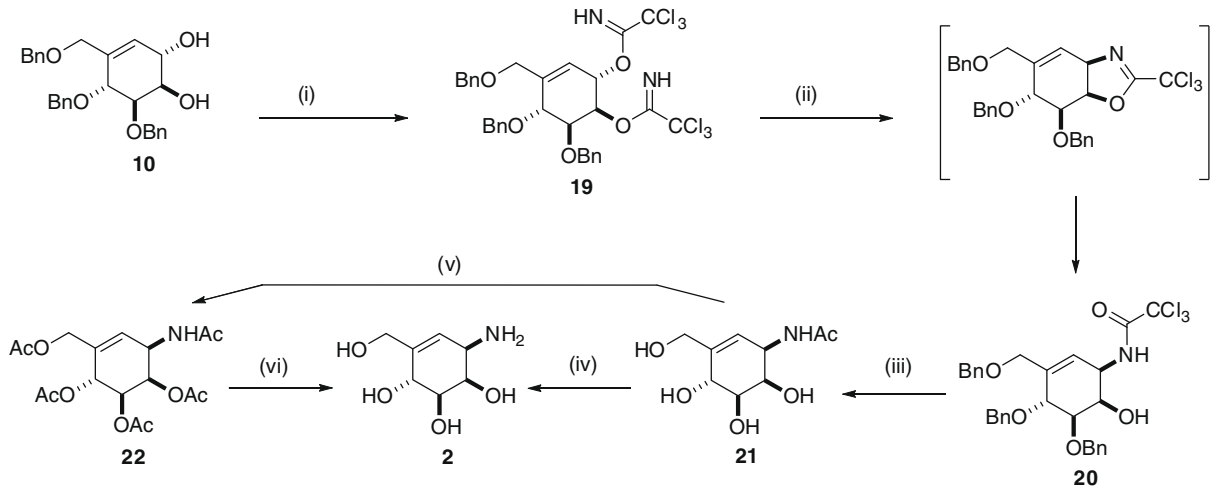


Scheme 3. Reagents and conditions: (i) DPPA, PPh_3, THF, 0 °C→rt, 71%; (ii) NaN_3, NH_4Cl, H_2O, MeOH, THF, 0 °C, 77%; (iii) Ref. 25.

We therefore tried a different approach: Danishefsky has converted the allylic alcohol of a *trans* 1,2-diol into a *cis* amino alcohol, with nitrogen introduced at the allylic position, by elimination of a trichloroacetamide from a diimidate, a five-membered intermediate oxazoline ring ensuring *cis* stereochemistry in the product.[26] We attempted the analogous reaction on our system: diol **10** was converted into its diimidate **19** (Scheme 4). Treatment of the diimidate with a Lewis acid at low temperature resulted in the formation of a major product. This material could be isolated and gave NMR data consistent with the oxazoline, but it was somewhat unstable during the work-up, and the hydrolysis product, the amide **20**, was also isolated. Adding water to the reaction mixture after the complete consumption of diimidate **19** resulted in hydrolysis of the first-formed oxazoline to give the amide **20**.

Subjection of the trichloroacetamide **20** to Birch conditions cleaved the benzyl ethers and reduced the trichloroacetamide group to an acetamide (→**21**). Peracetylation of **21** gave the pentaacetate **22**. A minor by-product from the Birch reduction was identified as the amine **21a**, presumably formed by incomplete reduction of the CCl_3 group and nucleophilic substitution of one chloride by ammonia. Peracetylation of this compound gave **22a** (9% from **19**, Fig. 2). The acetamide in **21** was cleaved with LiOH to give the 1,2-bis-*epi*-valienamine **2**; the peracetate **22** could also be completely deprotected to give **2** under the same conditions.

The C-1 stereochemistry of the carbocycles could be assigned using $^3J_{1,2}$ ^{1}H–^{1}H coupling constants according to Shing:[27] the carbocycles derived from the major diastereomer from the Grignard addition (i.e., **12**, **10**) were assigned with α-*lyxo* configuration ($J_{1,2}$ 7–8 Hz); those derived from the minor diastereomer from Grignard addition (i.e., **14**, **15**) were assigned β-*lyxo* stereochemistry ($J_{1,2}$ ca. 2 Hz). This treatment implies that α-*lyxo* compounds



Scheme 4. Reagents and conditions: (i) Cl_3CCN, DBU, CH_2Cl_2, 99%; (ii) $BF_3{\cdot}OEt_2$, CH_2Cl_2, −78 °C, then H_2O, rt; (iii) Na, $NH_{3(l)}$, THF, −78 °C, 83% from **19**; (iv) LiOH, H_2O, 33%; (v) Ac_2O, pyridine, 61% from **19**; (vi) LiOH, THF, H_2O, 77%.

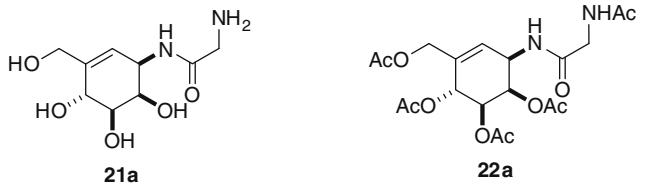


Figure 2.

have a $^3H_2$ solution conformation in which H-1 and H-2 have a pseudoaxial–axial relationship. We solved crystal structures of three compounds: **15**, **18** and **22** (Fig. 3), confirming this stereochemical assignment and confirming that the major product of the Grignard addition was that predicted from a chelation-controlled model; that epoxide-opening occurred with inversion of configuration at C-1 to give the 1,2-*trans* product; and that the oxazoline route indeed gave a 1,2-*cis* arrangement of the substituents on the carbocyclic ring. In both of the crystal structures of the protected β-*lyxo* configured unsaturated carbasugars **15** and **22**, the carbocyclic rings have $^2H_3$ conformations. The α-*lyxo* compound **18** in contrast, crystallises with the carbocycle in a $^3H_2$ conformation. The conformation of the carbasugars can have important consequences for the biological activity, although the bound conformation is not always identical to unbound solution or solid-phase conformations.[6,28]

We also demonstrate that nitrogen can be introduced at C-2 of the *lyxo* configured cyclohexenes with inversion of configuration to give GlcNAc analogues. Trichloroacetamide **20** was triflated and azide introduced at C-2 to give the β-*xylo* configured azide **23** (Scheme 5). This was converted into the β-GlcNAc valienamine **27** recently synthesised by Stubbs et al.[6] as follows: A protecting group swap at N-1 gave the Boc derivative **24**. Subjection of this compound to Birch conditions cleaved the benzyl ethers and reduced the azide, and acetylation of the crude reaction mixture gave the tetraacetate **25**. The ester protection was removed with NaOMe in MeOH to give **26**, and then the Boc protection was cleaved with HCl to give the GlcNAc valienamine **27** as its HCl salt.

The glycosidase inhibitory effects of GlcNAc analogue **27** against various β-hexosaminidases have already been investigated.[6] We evaluated the inhibitory effect of 1,2-bis-*epi*-valienamine **2** towards β-mannosidases by incubating it separately with a glycosidase family 2 β-mannosidase (recombinant *Cellulomonas fimi* β-mannosidase; CfMan2A)[8a] and also a β-glucosidase (*Aspergillus niger* β-glucosidase; AnGlu) under steady-state conditions. The CfMan2A-catalysed initial rates of hydrolysis of *p*NPMan were decreased in the presence of 1,2-bis-*epi*-valienamine **2** at lower than 1.0 mM concentrations, hence we carried out full steady-state inhibition analysis to evaluate the inhibition pattern and the inhibition constant K_i. The Lineweaver-Burk double-reciprocal plot (Fig. 4A) shows a pattern consistent with a reversible competitive inhibition scheme[29] suggesting that β-*manno*-valienamine **2** serves as a competitive inhibitor of CfMan2A with respect to *p*NPMan substrate. The dissociation constant K_i was determined to be 140 ± 30 μM from a replot of apparent K_m (K_m^{app}) values versus 1,2-bis-*epi*-valienamine **2** concentrations (Fig. 4B). In contrast, the AnGlu-catalysed initial rates of hydrolysis of *p*NPGlc were unchanged in the presence of up to 2.0 mM 1,2-bis-*epi*-valienamine **2** (data not shown) suggesting that **2** may be a β-mannosidase-specific inhibitor.

We also compared the effect of 1,2-bis-*epi*-valienamine **2** on the hydrolytic activity of CfMan2A to that of the substrate analogue D-mannose. The CfMan2A-catalysed hydrolysis of *p*NPMan was not inhibited by D-mannose at concentrations up to 1.0 mM and indeed the overall initial rates slightly increased in the presence of D-mannose (data not shown), possibly due to transglycosylation in addition to hydrolysis.[8c] The inhibition of CfMan2A by **2** and not by the monosaccharide D-mannose (substrate analogue) suggests that **2** binds to the active site of CfMan2A more strongly than to just a monosaccharide. To put the inhibitory potency of the monosaccharide-mimicking inhibitor **2** into context, we compare its K_i against CfMan2A with the K_i values of other reported monosaccharide-mimicking inhibitors against the related GH family 2 β-mannosidase BtMan2A: the K_i value of **2** is two orders of magnitude lower than that of deoxymannojirimycin (K_i = 33 mM), but on the other hand, two orders of magnitude higher than that of noeuromycin (K_i = 1 μM).[14]

In conclusion, a metathesis approach may be used to transform D-mannose into a versatile carbocyclic diol **10**, which may then be converted into both α- and β-mannose- and β-GlcNAc-mimicking C-5=C-5a unsaturated carbasugars. Work to further demonstrate the utility of this approach by the synthesis of carbasugar-containing pseudodisaccharides is in progress, and the results will be reported in due course. 1,2-bis-*epi*-Valienamine **2**, synthesised here for the first time, does indeed act as a β-mannosidase inhibitor, and fails to inhibit a β-glucosidase. Further work is necessary to understand the structural basis for inhibition.

3. Experimental

3.1. General methods

Melting points were measured using a Gallenkamp melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^1H) spectra were recorded on Bruker Avance II 500 (500 MHz), Bruker Avance II 400 (400 MHz) or Varian Mercury 400 (400 MHz) spectrometers; multiplicities are quoted as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), apparent triplet (at), doublet of apparent triplets (dat) or multiplet (m). Carbon nuclear magnetic resonance (^{13}C) spectra were recorded on Bruker Avance II 500 (125 MHz), Bruker Avance II 400 (100 MHz) or Varian Mercury 400 (100 MHz) spectrometers. ^1H and ^{13}C spectra and ^{13}C multiplicities were assigned using COSY, HSQC and DEPT experiments. All chemical shifts are quoted on the δ-scale in parts per million (ppm). Residual solvent signals or TMS were used as an internal reference. Low- and high-resolution (HRMS) electrospray (ESI) mass spectra were recorded using a Bruker Microtof instrument. Infra-red spectra were recorded on a Perkin–Elmer Spectrum One FT-IR spectrometer using the thin film method on NaCl plates. Optical rotations were measured on a Perkin–Elmer 241 polarimeter with a path length of 1 dm; concentrations are given in g/100 mL. Thin layer chromatography (TLC) was carried out on Merck Kieselgel sheets, pre-coated with $60F_{254}$ silica. Plates were visualised with UV light and developed using 10% sulfuric acid, or an ammonium molybdate (10% w/v) and cerium(IV) sulfate (2% w/v) solution in 10% sulfuric acid. Flash column chromatography was carried out on silica gel (35–70 μm, Grace). Grubbs' second generation complex (CAS No.: 246047-72-3) was bought from Sigma Aldrich and coated in wax (complex 12% by weight) before use.[30] Hoveyda–Grubbs second generation complex (CAS No.: 301224-40-8) was bought from Sigma Aldrich and used as supplied. Dichloromethane was distilled from calcium hydride. Diethyl ether and THF were distilled from sodium benzophenone ketyl radical. Toluene was distilled from sodium. Reactions performed under an atmosphere of nitrogen or argon were maintained by an inflated balloon.

3.1.1. (2*R*,3*R*,4*R*,5*R*,6*RS*)-1,3,4-Tri-*O*-benzyl-1,2,3,4,5, 6-hexahydroxy-oct-7-ene 4a,b

Hemiacetal **3** (6.95 g, 15.4 mmol) was dissolved in THF (30 mL, freshly distilled) under Ar. Vinylmagnesium bromide (0.7 M in THF, 92 mL, 64.4 mmol) was added and the yellow reaction mix-

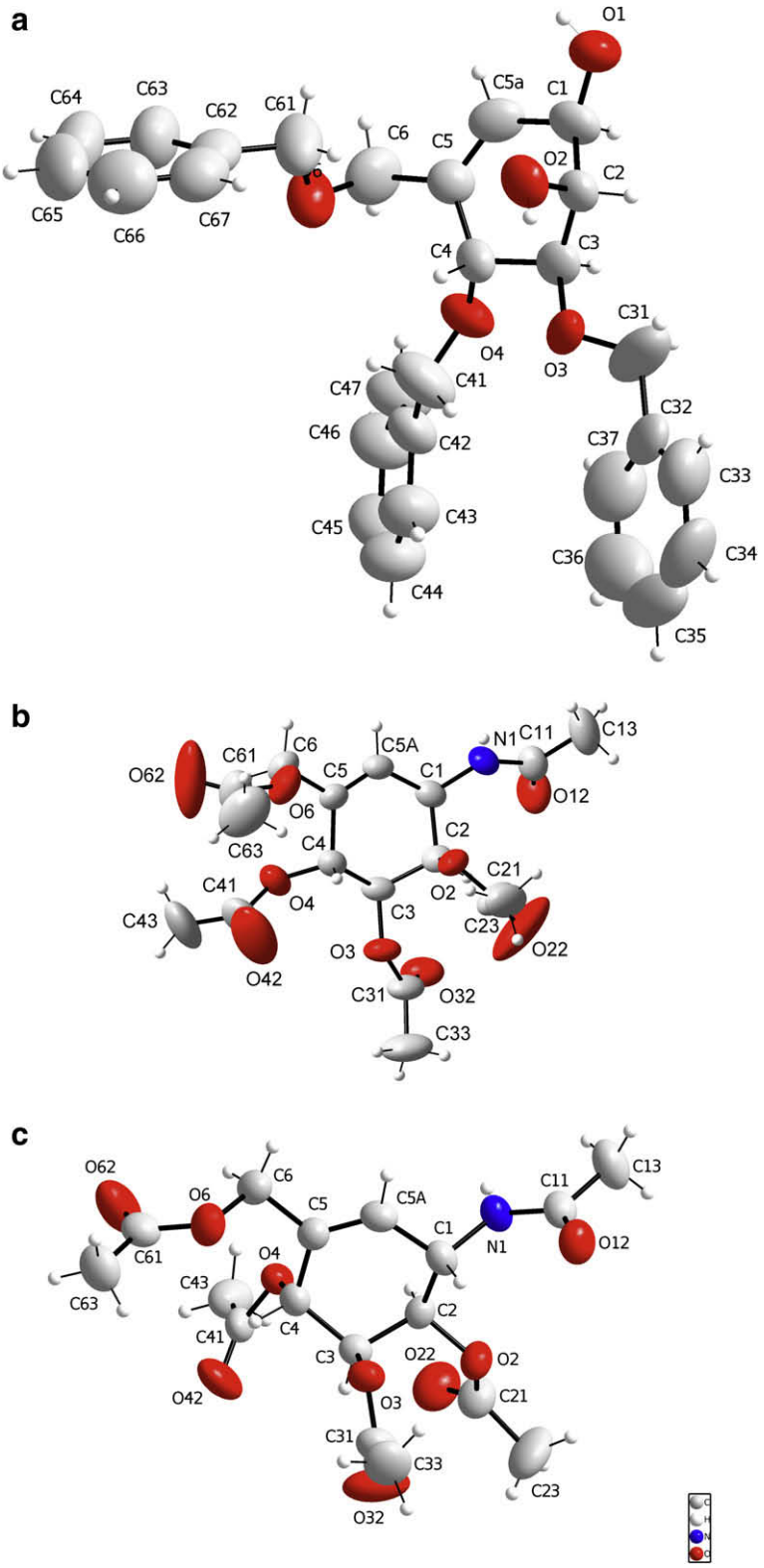
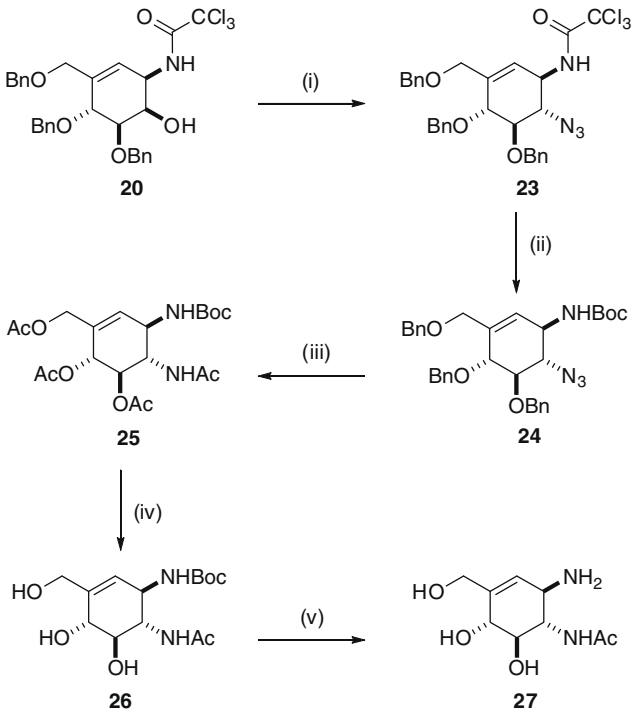


Figure 3. X-ray structures drawn as thermal ellipsoids at 50% probability and showing crystallographic atom numbering scheme. (a) Compound **15**; (b) compound **22** and (c) compound **18**.

ture was warmed to 65 °C and left at reflux for 20 h. TLC (toluene/EtOAc, 1:2) indicated the formation of a major product (R_f 0.7) and the presence of some starting material (R_f 0.5). The reaction was quenched by addition of ammonium chloride (25 mL, satd aq) at 0 °C and diluted with EtOAc (100 mL). The solution was washed with water (3 × 100 mL) and brine (2 × 100 mL). The aqueous phases were combined and extracted with EtOAc (3 × 50 mL). The organic phases were combined, dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (pentane/EtOAc, 2:1→1:1) to yield recovered



Scheme 5. Reagents and conditions: (i) Tf_2O, py, CH_2Cl_2, 0 °C; then NaN_3, DMF, 70 °C, 63% from **19**; (ii) KOH, ^iPrOH, 40 °C; then Boc_2O, CH_2Cl_2, 68%; (iii) Na, $NH_{3(l)}$, THF, −78 °C; then Ac_2O, py, 87%; (iv) NaOMe, MeOH, 79%; (v) HCl, H_2O, 93%.

starting material **3** (381 mg, 5%) and triols **4a**,**b** (6.07 g, 82%, inseparable diastereomeric mixture, **4a**:**4b**, 2.9:1) as a colourless oil; HRMS (ESI$^+$) calcd for $C_{29}H_{34}O_6Na$ (M+Na$^+$) 501.2248; found 501.2230.

Selected data for major diastereomer **4a**: δ_H (500 MHz, $CDCl_3$) 2.21 (1H, d, $J_{OH,1}$ 6.9 Hz, OH-1), 2.91 (1H, d, $J_{OH,2}$ 5.5 Hz, OH-2), 2.94 (1H, d, $J_{OH,5}$ 5.0 Hz, OH-5), 3.81 (1H, m, H-2), 4.30 (1H, ddd, $J_{1,2}$ 1.5 Hz, $J_{1,1a}$ 5.1 Hz, H-1), 5.22 (1H, obs dat, $J_{1a,1b}$ 10.5 Hz, $J_{1b,1b'}$ 1.5 Hz, H-1b), 5.33 (1H, obs dat, $J_{1a,1b'}$ 17.5 Hz, H-1b′), 5.90 (1H, obs ddd, H-1a), 7.21–7.31 (15H, m, Ar-H); δ_C (125 MHz, $CDCl_3$) 70.4 (d, C-5), 71.2 (t, C-6), 71.5 (d, C-1), 72.9 (d, C-2), 73.7, 73.9, 74.0 (3 × t, 3 × PhCH_2), 77.5, 78.0 (2 × d, C-3, C-4), 116.2 (t, C-1b), 128.0, 128.1, 128.2, 128.5, 128.6, 128.6, 128.6, 128.7 (8 × d, Ar-CH), 136.8, 137.6, 137.7, 138.0 (4 × s, Ar-C), 138.3 (d, C-1a); *m/z* (ESI$^+$) 501 (M+Na$^+$, 100%).

Selected data for minor diastereomer **4b**: δ_H (500 MHz, $CDCl_3$) 2.51 (1H, d, $J_{OH,1}$ 5.0 Hz, OH-1), 3.01 (1H, d, $J_{OH,5}$ 5.5 Hz, OH-5), 3.04 (1H, d, $J_{OH,2}$ 4.5 Hz, OH-2); δ_C (125 MHz, $CDCl_3$) 70.6 (d, C-5), 71.3 (t, C-6), 73.3, 73.6 (2 × t, 2 × PhCH_2), 74.1 (d, C-1), 117.7 (t, C-1b).

3.1.2. (2*R*,3*R*,4*S*,5*R*,6*RS*)-1,3,4-Tri-*O*-benzyl-5,6-*O*-isopropylidene-1,2,3,4,5,6-hexahydroxy-oct-7-ene 5a,b and (2*R*,3*S*,4*S*,5*R*,6*S*)-1,3,4-tri-*O*-benzyl-2,5-*O*-isopropylidene-1,2,3,4,5,6-hexahydroxy-oct-7-ene 6

Triols **4a**,**b** (6.05 g, 12.6 mmol) were dissolved in DMF (60 mL) under Ar, and 2,2-dimethoxypropane (12.5 mL, 101 mmol) was added. Camphorsulfonic acid (2.8 g, 12.1 mmol) was added and the reaction mixture was stirred at rt for 2 h. TLC (toluene/EtOAc, 3:1) indicated the formation of a major product (R_f 0.6) and a minor product (R_f 0.5), and the complete consumption of starting material (R_f 0.1). The reaction was quenched by addition of NEt_3 until the pH was raised from 2 to 8. The reaction mixture was diluted with EtOAc (100 mL) and washed with brine (3 × 80 mL). The brine fractions were combined and extracted with EtOAc (2 × 50 mL). The combined organic phases were dried (Na_2SO_4), filtered and concentrated in vacuo. The crude product was purified by flash column chromatography (pentane/EtOAc, 5:1→3:1) to yield an inseparable mixture of acetals **5a**,**b** and **6** (5.78 g, 88%) as a colourless oil; ν_{max}/cm^{-1} 3480 (br, OH); *m/z* (ESI$^+$) 541 (M+Na$^+$, 100%); HRMS (ESI$^+$) calcd for $C_{32}H_{38}O_6Na$ (M+Na$^+$) 541.2561; found 541.2572.

Selected data for major diastereomer **5a**: δ_H (500 MHz, $CDCl_3$) 1.40, 1.44 (6H, 2 × s, C(CH_3)$_2$), 2.60 (1H, d, $J_{OH,5}$ 5.5 Hz, OH-5), 3.56 (1H, dd, $J_{6,6'}$ 9.5 Hz, $J_{5,6}$ 5.5 Hz, H-6), 3.66 (1H, dd, $J_{5,6'}$ 3.0 Hz, H-6′), 3.75 (1H, dd, $J_{4,5}$ 8.0 Hz, $J_{3,4}$ 3.0 Hz, H-4), 3.94 (1H, dd, $J_{2,3}$ 7.5 Hz, H-3), 3.99 (1H, m, H-5), 4.08 (1H, at, *J* 7.8 Hz, H-2), 4.42 (1H, at, *J* 7.0 Hz, H-1), 5.16 (1H, d, $J_{1a,1b}$ 10.5 Hz, H-1b), 5.36 (1H, d, $J_{1a,1b'}$ 17.1 Hz, H-1b′), 5.91 (1H, ddd, $J_{1,1a}$ 6.4 Hz, H-1a), 7.24–7.36 (15H, m, Ar-H); δ_C (125 MHz, $CDCl_3$) 27.1, 27.3 (2 × q, C(CH_3)$_2$), 109.0 (s, *C*(CH_3)$_2$ five-ring), 117.6 (t, C-1b), 136.3 (d, C-1a), 138.0, 138.2, 138.5 (3 × s, 3 × Ar-C).

Selected data for minor diastereomer **5b**: δ_H (500 MHz, $CDCl_3$) 1.36, 1.51 (6H, 2 × s, C(CH_3)$_2$), 2.58 (1H, OH-5), 6.00 (1H, ddd, $J_{1,1a}$ 7.7 Hz, $J_{1a,1b}$ 10.4 Hz, $J_{1a,1b'}$ 17.1 Hz, H-1a); δ_C (125 MHz, $CDCl_3$) 25.8, 28.3 (2 × q, C(CH_3)$_2$), 108.6 (s, *C*(CH_3)$_2$ five-ring), 118.4 (t, C-1b), 135.1 (d, C-1a).

Selected data for regioisomer **6**: δ_H (500 MHz, $CDCl_3$) 1.28, 1.34 (6H, 2 × s, C(CH_3)$_2$), 2.14 (1H, d, $J_{OH,1}$ 10.5 Hz, OH-1); δ_C (125 MHz, $CDCl_3$) 24.6, 25.2 (2 × q, C(CH_3)$_2$), 100.8 (s, *C*(CH_3)$_2$ seven-ring), 115.4 (t, C-1b).

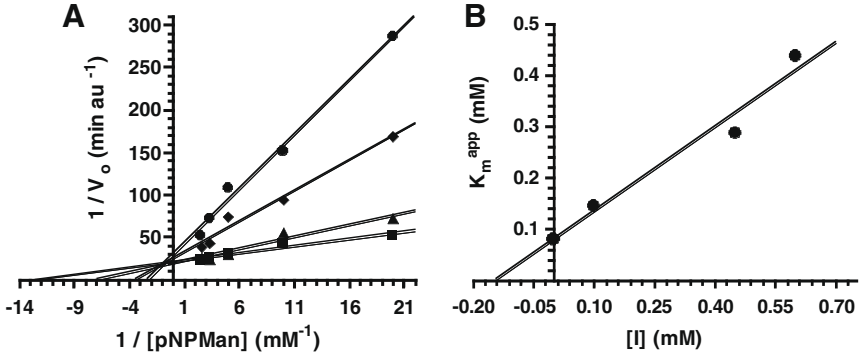


Figure 4. Inhibition of the CfMan2A-catalysed hydrolysis of *p*NPMan by β-*manno*-valienamine **2**: (A) Lineweaver-Burk double-reciprocal plot showing a competitive inhibition pattern. β-*manno*-valienamine concentrations were 0 (■), 100 (▲), 450 (♦) and 600 (●) μM. The lines are the linear regression fits of data. (B) Replot of K_m^{app} as a function of β-*manno*-valienamine concentration [I]. The line is the linear regression fit of data. K_i value was determined according to the relationship: $K_m^{app} = K_m \cdot (1 + [I]/K_i)$.

3.1.3. (3*S*,4*R*,5*R*,6*RS*)-1,3,4-Tri-*O*-benzyl-5,6-*O*-isopropylidene-1,3,4,5,6-pentahydroxy-oct-7-ene-2-one 7a,b

Oxalyl chloride (3.6 mL, 42 mmol) was dissolved in CH_2Cl_2 (40 mL, freshly distilled) under Ar at −78 °C. DMSO (6.4 mL, 90 mmol) was dissolved in CH_2Cl_2 (20 mL, freshly distilled) under Ar at −78 °C and then transferred to the oxalyl chloride solution by cannula. After 45 min, a solution of alcohols **5a**,**b** and **6** (5.67 g, 10.9 mmol) in CH_2Cl_2 (30 + 10 + 10 mL) was transferred to the reaction vessel at −78 °C. After 1.5 h, NEt_3 (16 mL, 114.8 mmol) was added to the reaction mixture. After an additional 1 h 15 min, TLC (toluene/EtOAc, 3:1) indicated the complete consumption of starting material (R_f 0.6) and the formation of a major product (R_f 0.7). The reaction mixture was diluted with CH_2Cl_2 (150 mL), transferred to a separatory funnel and washed with brine (3 × 100 mL). The aqueous phases were combined and extracted with CH_2Cl_2 (2 × 50 mL). The organic phases were combined, dried (Na_2SO_4), filtered and concentrated in vacuo, to yield the crude product (6.8 g) as a slightly yellow oil. The crude product was purified by flash column chromatography (toluene→toluene/EtOAc, 10:1→8:1) to yield the ketones **7a**,**b** (4.57 g, 81%, inseparable diastereomers, **7a**:**7b**, 3.3:1) as a yellow oil; ν_{max}/cm^{-1} 1730 (s, C=O); *m/z* (ESI$^+$) 539 (M+Na$^+$,100%); HRMS (ESI$^+$) calcd for $C_{32}H_{36}O_6Na$ (M+Na$^+$) 539.2404; found 539.2420.

Selected data for major diastereomer **7a**: δ_H (500 MHz, $CDCl_3$) 1.38, 1.40 (6H, 2 × s, 2 × CH_3), 4.00–4.04 (2H, m, H-2, H-4), 4.24, 4.36 (2H, ABq, $J_{6,6'}$ 18.5 Hz, H-6, H-6′), 4.27 (1H, d, $J_{3,4}$ 3.5 Hz, H-3), 4.36 (1H, m, H-1), 5.19 (1H, d, $J_{1a,1b}$ 10.5 Hz, H-1b), 5.36 (1H, obs d, $J_{1a,1b'}$ 17.3 Hz, H-1b′), 5.88 (1H, ddd, $J_{1,1a}$ 6.5 Hz, H-1a), 7.15–7.34 (15H, m, Ar-H); δ_C (125 MHz, $CDCl_3$) 27.1, 27.1 (2 × q, 2 × CH_3), 73.3, 74.7, 74.7 (3 × t, 3 × PhCH_2), 74.8 (t, C-6), 79.0 (d, C-2), 80.1 (d, C-1), 81.9 (d, C-4), 84.2 (d, C-3), 109.3 (s, $C(CH_3)_2$ five-ring), 118.0 (t, C-1b), 135.9 (d, C-1a), 137.0, 137.3, 137.3 (3 × s, Ar-C), 208.8 (s, C=O).

Selected data for minor diastereomer **7b**: δ_H (500 MHz, $CDCl_3$) 1.35, 1.47 (6H, 2 × s, 2 × CH_3), 5.23 (1H, br d, $J_{1a,1b}$ 10.5 Hz, H-1b), 5.94 (1H, ddd, $J_{1,1a}$ 7.0 Hz, $J_{1a,1b'}$ 17.5 Hz, H-1a); δ_C (125 MHz, $CDCl_3$) 25.7, 28.1 (2 × q, 2 × CH_3), 108.7 (s, $C(CH_3)_2$) five-ring), 118.1 (t, C-1b), 134.1 (d, C-1a), 209.3 (s, C=O).

3.1.4. (3*R*,4*S*,5*R*,6*RS*)-1,3,4-Tri-*O*-benzyl-5,6-*O*-isopropylidene-1,3,4,5,6-pentahydroxy-2-methylene-oct-7-ene 8a,b

Triphenylmethylphosphonium bromide (18.7 g, 52 mmol) was suspended in freshly distilled toluene (80 mL) under Ar. After 20 min, potassium *tert*-butoxide (5.6 g, 50 mmol) was added, and the resulting suspension was heated to 80 °C. After a few minutes, the suspension turned intensely yellow. After a further 2 h 40 min, the suspension containing the ylid was allowed to cool to rt, and ketones **7a**,**b** (4.51 g, 8.73 mmol) were dissolved in toluene (freshly distilled, 40 + 10 + 10 mL) and added to the ylid by cannula. After 45 min, TLC (toluene/EtOAc, 6:1) indicated the complete consumption of starting material (R_f 0.5) and the formation of a major product (R_f 0.6). The reaction mixture was diluted with toluene (80 mL), filtered through Celite and concentrated in vacuo. The crude product (15 g) was purified by flash column chromatography (pentane/EtOAc, 20:1→10:1) to yield dienes **8a**,**b** (3.61 g, 80%, inseparable diastereomeric mixture, **8a**:**8b**, 3.3:1) as a yellow oil *m/z* (ESI$^+$) 537 (M+Na$^+$, 100%); HRMS (ESI$^+$) calcd for $C_{33}H_{38}O_5Na$ (M+Na$^+$) 537.2611; found 537.2612.

Selected data for major diastereomer **8a**: δ_H (500 MHz, $CDCl_3$) 1.35, 1.38 (6H, 2 × s, 2 × CH_3), 3.89 (1H, dd, $J_{3,4}$ 6.5 Hz, $J_{2,3}$ 4.9 Hz, H-3), 3.99 (1H, dd, $J_{1,2}$ 7.8 Hz, H-2), 4.03–4.08 (2H, m, H-4, H-6), 4.13 (1H, d, $J_{6,6'}$ 13.0 Hz, H-6′), 4.35 (1H, d, *J* 11.5 Hz, PhC*H*H′), 4.48–4.57 (4H, m, PhCH_2, PhCHH′, H-1), 4.67, 4.76 (2H, ABq, J_{AB} 11.0 Hz, PhCH_2), 5.14 (1H, d, $J_{1a,1b}$ 10.5 Hz, H-1b), 5.29–5.32 (2H, m, H-5a, H-1b′), 5.46 (1H, d, $J_{5a,5a'}$ 1.0 Hz, H-5a′), 5.80 (1H, ddd, $J_{1,1a}$ 7.1 Hz, $J_{1a,1b'}$ 17.1 Hz, H-1a), 7.22–7.36 (15H, m, Ar-H); δ_C (125 MHz, $CDCl_3$) 27.0, 27.1 (2 × q, 2 × CH_3), 70.2 (t, C-6), 71.0, 72.7, 75.4 (3 × t, 3 × PhCH_2), 79.0 (d, C-1), 80.0 (d, C-2), 80.8 (d, C-3), 81.6 (d, C-4), 108.6 (s, $C(CH_3)_2$ five-ring), 117.4 (t, C-5a), 118.2 (t, C-1b), 127.6, 127.6, 127.7, 127.7, 128.2, 128.3, 128.3, 128.3, 128.5 (9 × d, Ar-CH), 136.7 (d, C-1a), 138.3, 138.3, 138.6 (3 × s, 3 × Ar-C), 142.3 (s, C-5).

Selected data for minor diastereomer **8b**: δ_H (500 MHz, $CDCl_3$) 1.32, 1.45 (6H, 2 × s, 2 × CH_3), 3.79 (1H, dd, $J_{3,4}$ 3.6 Hz, $J_{2,3}$ 7.8 Hz, H-3), 4.27 (1H, d, H-4), 6.00 (1H, ddd, $J_{1,1a}$ 7.5 Hz, $J_{1a,1b}$ 10.0 Hz, $J_{1a,1b'}$ 17.0 Hz, H-1a); δ_C (125 MHz, $CDCl_3$) 25.5, 28.1 (2 × q, 2 × CH_3), 108.3 (s, $C(CH_3)_2$), 135.3 (d, C-1a), 138.3, 138.5, 138.7 (3 × s, 3 × Ar-C), 143.1 (s, C-5).

3.1.5. (3*R*,4*R*,5*R*,6*S*)-1,3,4-Tri-*O*-benzyl-1,3,4,5,6-pentahydroxy-2-methylene-oct-7-ene 9a and (3*R*,4*R*,5*R*,6*R*)-1,3,4-tri-*O*-benzyl-1,3,4,5,6-pentahydroxy-2-methylene-oct-7-ene 9b

Acetals **8a**,**b** (3.59 g, 6.98 mmol) were suspended in AcOH (45 mL) and water (15 mL), and HCl (1 M aq, 1 mL) was added, and the resulting suspension was heated to 80 °C. After 1 h, TLC (pentane/EtOAc, 4:1) indicated the complete consumption of starting material (R_f 0.7) and the formation of a major product (R_f 0.1). The heating was turned off and the reaction mixture concentrated in vacuo by co-evaporation of solvent with toluene. The crude product (3.66 g) was purified by flash column chromatography (pentane/EtOAc, 4:1→1:1) to yield **9b** (635 mg, 19%) as a colourless oil; $[\alpha]_D^{23} = -0.3$ (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 3436 (br, OH); δ_H (400 MHz, $CDCl_3$) 2.75 (1H, d, $J_{OH,1}$ 4.4 Hz, OH-1), 3.03 (1H, d, $J_{OH,2}$ 4.8 Hz, OH-2), 3.67 (1H, dd, $J_{3,4}$ 4.3 Hz, $J_{2,3}$ 6.6 Hz, H-3), 3.80 (1H, m, H-2), 4.00, 4.12 (2H, ABq, $J_{6,6'}$ 12.6 Hz, H-6, H-6′), 4.18 (1H, m, H-1), 4.34 (1H, d, *J* 11.6 Hz, PhC*H*H′), 4.38 (1H, d, H-4), 4.45–4.54 (3H, m, J_{AB} 11.8 Hz, PhC*H*H′, PhCH_2), 4.61–4.65 (2H, m, 2 × PhCH*H*′), 5.24 (1H, dat, *J* 1.5 Hz, $J_{1a,1b}$ 10.6 Hz, H-1b), 5.31 (1H, dat, *J* 1.5 Hz, $J_{1a,1b'}$ 17.2 Hz, H-1b′), 5.52 (1H, s, H-5a), 5.56 (1H, d, *J* 1.6 Hz, H-5a′), 5.90 (1H, ddd, $J_{1,1a}$ 6.4 Hz, H-1a), 7.22–7.35 (15H, m, Ar-H); δ_C (100 MHz, $CDCl_3$) 71.2, 72.7, 73.7 (3 × t, 3 × PhCH_2), 71.2 (t, C-6), 73.5 (d, C-2), 73.8 (d, C-1), 80.0 (d, C-4), 80.6 (d, C-3), 116.8 (t, C-5a), 116.9 (t, C-1b), 127.8, 127.8, 128.0, 128.1, 128.3, 128.4, 128.5, 128.6 (8 × d, Ar-CH), 137.0 (d, C-1a), 137.6, 137.8, 138.0 (3 × s, 3 × Ar-C), 142.2 (s, C-5). *m/z* (ESI$^+$) 497 (M+Na$^+$, 100%); HRMS (ESI$^+$) calcd for $C_{30}H_{34}O_5Na$ (MNa$^+$) 497.2298; found 497.2310.

And **9a** (2.32 g, 69%) as a colourless oil; $[\alpha]_D^{23} = -22.9$ (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 3431 (br, OH); δ_H (400 MHz, $CDCl_3$) 2.62 (1H, d, $J_{OH,1}$ 6.4 Hz, OH-1), 2.99 (1H, d, $J_{OH,2}$ 5.2 Hz, OH-2), 3.68 (1H, ddd, $J_{2,3}$ 6.7 Hz, $J_{1,2}$ 2.0 Hz, H-2), 3.79 (1H, dd, $J_{3,4}$ 5.0 Hz, H-3), 4.02, 4.11 (2H, ABq, $J_{6,6'}$ 12.4 Hz, H-6, H-6′), 4.26–4.30 (2H, m, H-1, H-4), 4.34, 4.63 (2H, ABq, J_{AB} 12.2 Hz, PhCH_2), 4.49, 4.55 (2H, ABq, J_{AB} 11.6 Hz, PhCH_2), 4.60, 4.70 (2H, ABq, J_{AB} 11.0 Hz, PhCH_2), 5.17 (1H, dat, *J* 1.6 Hz, $J_{1a,1b}$ 10.5 Hz, H-1b), 5.28 (1H, dat, *J* 1.6 Hz, $J_{1a,1b'}$ 17.2 Hz, H-1b′), 5.41 (1H, s, H-5a), 5.49 (1H, d, *J* 1.2 Hz, H-5a′), 5.86 (1H, ddd, $J_{1,1a}$ 5.0 Hz, H-1a), 7.25–7.37 (15H, m, Ar-H). δ_C (100 MHz, $CDCl_3$) 71.1 (t, C-6), 71.2, 72.8, 74.8 (3 × t, 3 × PhCH_2), 71.7 (d, C-1), 72.6 (d, C-2), 80.3 (d, C-4), 80.7 (d, C-3), 115.8 (t, C-1b), 116.7 (t, C-5a), 127.8, 127.8, 128.1, 128.1, 128.3, 128.4, 128.6, 128.6 (8 × d, Ar-CH), 137.7, 137.9, 138.2 (3 × s, 3 × Ar-C), 138.3 (d, C-1a), 142.3 (s, C-5). *m/z* (ESI$^+$) 497 (M+Na$^+$, 100%); HRMS (ESI$^+$) calcd for $C_{30}H_{34}O_5Na$ (MNa$^+$) 497.2298; found 497.2307.

3.1.6. 3,4,6-Tri-*O*-benzyl-5a-carba-α-D-*lyxo*-hex-5(5a)-enopyranose 10

Method 1: Cyclisation of unprotected diol. Diene **9a** (24 mg, 0.05 mmol) was dissolved in toluene (2 mL, freshly distilled) under Ar and heated to 60 °C. Hoveyda–Grubbs' second generation complex (3 mg, 0.005 mmol) was added. After 50 min, TLC (toluene/EtOAc, 1:1) indicated the complete consumption of starting

material (R_f 0.7) and the formation of a major product (R_f 0.2). The reaction mixture was concentrated in vacuo and the residue purified by flash column chromatography (toluene→toluene/EtOAc, 6:1→1:1) to yield carbocycle **10** (18 mg, 81%) as a colourless oil; $[\alpha]_D^{23} = -15.3$ (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 3394 (br, OH); δ_H (500 MHz, $CDCl_3$) 2.57 (1H, br d, $J_{OH,1}$ 5.5 Hz, OH-1), 2.62 (1H, br d, $J_{OH,2}$ 7.6 Hz, OH-2), 3.81 (1H, dat, *J* 7.8 Hz, $J_{2,3}$ 2.8 Hz, H-2), 3.85 (1H, d, $J_{6,6'}$ 12.0 Hz, H-6), 3.88 (1H, at, *J* 3.0 Hz, H-3), 4.04 (1H, d, $J_{3,4}$ 2.5 Hz, H-4), 4.11 (1H, dat, *J* 1.4 Hz, H-6′), 4.32–4.34 (2H, m, H-1, PhC*H*H′), 4.44, 4.55 (2H, ABq, J_{AB} 11.5 Hz, PhCH_2), 4.45 (1H, d, *J* 11.5 Hz, PhCH*H*′), 4.55, 4.58 (2H, ABq, J_{AB} 12.0 Hz, PhCH_2), 5.79 (1H, m, H-5a), 7.22–7.33 (15H, m, Ar-H); δ_C (125 MHz, $CDCl_3$) 69.7 (d, C-1), 70.4 (t, C-6), 71.8, 72.7, 73.5 (3 × t, 3 × PhCH_2), 72.7 (d, C-2), 73.6 (d, C-4), 78.0 (d, C-3), 127.7, 127.9, 127.9, 128.0, 128.1, 128.4, 128.5, 128.6, 128.6 (9 × d, Ar-CH), 129.6 (d, C-5a), 134.2 (s, C-5), 137.8, 138.1, 138.2 (3 × s, 3 × Ar-C); *m/z* (ESI^+) 469 ($M+Na^+$, 100%), HRMS (ESI^+) calcd for $C_{28}H_{30}O_5Na$ ($M+Na^+$) 469.1985; found 469.1991.

Method 2: Deacetylation of carbocycle **12**. Diacetate **12** (2.01 g, 3.80 mmol) was dissolved in MeOH (25 mL) under Ar. Sodium (40 mg, 1.7 mmol) was dissolved in MeOH (5 mL) and added to the reaction mixture. After 1 h, TLC (toluene/EtOAc, 1:1) indicated the complete consumption of starting material (R_f 0.8) and the formation of a major product (R_f 0.4). The reaction mixture was concentrated in vacuo by co-evaporation with toluene and the residue purified by flash column chromatography (pentane/EtOAc, 6:1→EtOAc) to yield diol **10** (1.50 g, 88%) identical to that described above.

3.1.7. (3*R*,4*S*,5*R*,6*S*)-5,6-Di-*O*-acetyl-1,3,4-tri-*O*-benzyl-pentahydroxy-2-methylene-oct-7-ene 11

Diol **9a** (2.32 g, 4.89 mmol) was dissolved in acetic anhydride (20 mL, 212 mmol), and pyridine (20 mL, 248 mmol) and dimethylaminopyridine (50 mg, 0.4 mmol) were added. After 2.5 h, TLC (pentane/EtOAc, 4:1) indicated the complete consumption of starting material (R_f 0.1) and the formation of a major product (R_f 0.6). The reaction mixture was concentrated in vacuo by co-evaporation with toluene. The residue was purified by flash column chromatography (pentane/EtOAc, 7:1→4:1) to yield diacetate **11** (2.52 g, 92%) as a yellow oil; $[\alpha]_D^{23} = -28.3$ (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 1745 (s, C=O); δ_H (500 MHz, $CDCl_3$) 1.93, 2.03 (6H, 2 × s, 2 × CH_3), 3.84 (1H, dd, $J_{3,4}$ 5.4 Hz, $J_{2,3}$ 6.9 Hz, H-3), 4.08, 4.18 (2H, ABq, $J_{6,6'}$ 13.0 Hz, H-6, H-6′), 4.10 (1H, d, H-4), 4.28, 4.51 (2H, ABq, J_{AB} 11.0 Hz, PhCH_2), 4.42, 4.69 (2H, ABq, J_{AB} 10.5 Hz, PhCH_2), 4.54, 4.57 (2H, ABq, J_{AB} 12.3 Hz, PhCH_2), 5.14–5.20 (2H, m, H-1b, H-1b′), 5.29 (1H, dd, $J_{1,2}$ 2.5 Hz, H-2), 5.38 (1H, s, H-5a), 5.49 (1H, s, H-5a′), 5.69–5.76 (2H, m, H-1, H-1a), 7.23–7.35 (15H, m, Ar-H); δ_C (125 MHz, $CDCl_3$) 21.0, 21.1 (2 × q, 2 × CH_3), 70.4 (t, C-6), 71.1, 72.8, 75.1 (3 × t, 3 × PhCH_2), 72.1 (d, C-2), 72.7 (d, C-1), 78.6 (d, C-3), 81.2 (d, C-4), 116.4 (t, C-5a), 117.1 (t, C-1b), 127.6, 127.7, 127.8, 128.4, 128.5 (5 × d, Ar-CH), 133.4 (d, C-1a), 137.9, 138.4 (2 × s, Ar-C), 142.2 (s, C-5), 169.7, 169.8 (2 × s, 2 × C=O); *m/z* (ESI^+) 597 ($M+K^+$, 12), 581 ($M+Na^+$, 100%); HRMS (ESI^+) calcd for $C_{34}H_{38}O_7Na$ ($M+Na^+$) 581.2510; found 581.2524.

3.1.8. 1,2-Di-*O*-acetyl-3,4,6-tri-*O*-benzyl-5a-carba-α-D-*lyxo*-hex-5(5a)-enopyranose 12

Protected diene **11** (2.48 g, 4.44 mmol) was dissolved in freshly distilled toluene (40 mL) under Ar. Hoveyda–Grubbs' second generation complex (30 mg, 0.05 mmol, 1 mol %) was added and the yellow-green reaction mixture was heated to 60 °C. After 15 h, TLC (pentane/EtOAc, 4:1) indicated the formation of a major product (R_f 0.5) and the presence of starting material (R_f 0.6). Three additional portions of Hoveyda–Grubbs' second generation complex (10 + 10 + 15 mg, 0.06 mmol, 1.3 mol %) were added to the reaction mixture over the course of 5 h, and after a further 4 h, TLC indicated the consumption of starting material and the formation of product. The reaction mixture was concentrated in vacuo and the residue purified by flash column chromatography (pentane/EtOAc, 6:1→4:1) to yield starting material **11** (256 mg, 10%), and carbocycle **12** (2.05 g, 87%) as a colourless oil; $[\alpha]_D^{23} = +63.9$ (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 1741 (s, C=O); δ_H (500 MHz, $CDCl_3$) 2.04, 2.05 (6H, 2 × s, 2 × CH_3), 3.89, 4.15 (2H, ABq, $J_{6,6'}$ 13.0 Hz, H-6, H-6′), 4.02 (1H, dd, $J_{2,3}$ 2.3 Hz, $J_{3,4}$ 4.2 Hz, H-3), 4.07 (1H, d, H-4), 4.37, 4.48 (2H, ABq, J_{AB} 11.5 Hz, PhCH_2), 4.48, 4.65 (2H, ABq, J_{AB} 11.3 Hz, PhCH_2), 4.54, 4.60 (2H, ABq, J_{AB} 12.0 Hz, PhCH_2), 5.36 (1H, dd, $J_{1,2}$ 7.4 Hz, H-2), 5.64 (1H, d, H-1), 5.78 (1H, m, H-5a), 7.27–7.35 (15H, m, Ar-H); δ_C (125 MHz, $CDCl_3$) 21.2, 21.2 (2 × q, 2 × CH_3), 69.2 (d, C-1), 70.3 (t, C-6), 71.3 (d, C-2), 72.0, 72.8, 73.9 (3 × t, PhCH_2), 74.0 (d, C-4), 75.6 (d, C-3), 124.4 (d, C-5a), 127.8, 127.9, 128.0, 128.1, 128.1, 128.2, 128.4, 128.5, 128.6 (9 × d, Ar-CH), 137.5 (s, C-5), 138.0, 138.2 (2 × s, Ar-C), 170.5, 170.6 (2 × s, 2 × C=O); *m/z* (ESI^+) 569 ($M+K^+$, 14), 553 ($M+Na^+$, 100%); HRMS (ESI^+) calcd for $C_{32}H_{34}O_7Na$ ($M+Na^+$) 553.2197; found 553.2210.

3.1.9. (3*R*,4*S*,5*R*,6*R*)-5,6-Di-*O*-acetyl-1,3,4-tri-*O*-benzyl-pentahydroxy-2-methylene-oct-7-ene 13

Diol **9b** (620 mg, 1.31 mmol) was dissolved in acetic anhydride (3 mL, 32 mmol), and pyridine (3 mL, 37 mmol) and dimethylaminopyridine (25 mg, 0.2 mmol) were added. After 6 h, TLC (pentane/EtOAc, 4:1) indicated the complete consumption of starting material (R_f 0.1) and the formation of a major product (R_f 0.5). The reaction mixture was concentrated in vacuo by co-evaporation with toluene. The residue was purified using flash column chromatography (pentane/EtOAc, 6:1→EtOAc) to yield diacetate **13** (677 mg, 93%) as a yellow oil; $[\alpha]_D^{22} = -12.9$ (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 1744 (s, C=O); δ_H (500 MHz, $CDCl_3$) 1.88, 1.95 (6H, 2 × s, 2 × CH_3), 3.79 (1H, at, *J* 6.3 Hz, H-3), 4.07, 4.16 (2H, ABq, $J_{6,6'}$ 13.3 Hz, H-6, H-6′), 4.11 (1H, d, $J_{3,4}$ 5.5 Hz, H-4), 4.29 (1H, d, *J* 11.5 Hz, PhC*H*H′), 4.51–4.57 (3H, m, PhCH*H*′, PhCH_2), 4.62, 4.77 (2H, ABq, J_{AB} 11.3 Hz, PhCH_2), 5.18–5.25 (2H, m, H-1b, H-1b′), 5.31 (1H, dd, $J_{2,3}$ 6.0 Hz, $J_{1,2}$ 4.3 Hz, H-2), 5.35, 5.48 (2H, 2 × s, H-5a, H-5a′), 5.65 (1H, dd, $J_{1,1a}$ 6.7 Hz, H-1), 5.85 (1H, ddd, $J_{1a,1b}$ 10.6 Hz, $J_{1a,1b'}$ 17.4 Hz, H-1a), 7.24–7.35 (15H, m, Ar-H); δ_C (125 MHz, $CDCl_3$) 21.0, 21.1 (2 × q, 2 × CH_3), 70.4 (t, C-6), 70.9, 74.2 (2 × t, 2 × PhCH_2), 72.8 (d, t, C-2, PhCH_2), 73.7 (d, C-1), 79.2 (d, C-3), 81.3 (d, C-4), 116.6 (t, C-5a), 119.2 (t, C-1b), 127.6, 127.7, 127.7, 127.7, 127.9, 128.3, 128.4, 128.4, 128.5 (9 × d, Ar-CH), 132.6 (d, C-1a), 138.0, 138.4, 138.4 (3 × s, 3 × Ar-C), 142.1 (s, C-5), 169.7, 169.7 (2 × s, 2 × C=O); *m/z* (ESI^+) 581 ($M+Na^+$, 100%); HRMS (ESI^+) calcd for $C_{34}H_{38}O_7Na$ ($M+Na^+$) 581.2510; found 581.2520.

3.1.10. 1,2-Di-*O*-acetyl-3,4,6-tri-*O*-benzyl-5a-carba-β-D-*lyxo*-hex-5(5a)-enopyranose 14

Protected diene **13** (650 mg, 1.16 mmol) was dissolved in freshly distilled toluene (5 mL) under Ar. Hoveyda–Grubbs' second generation complex (12 mg, 0.02 mmol, 2 mol %) was added and the yellow-green reaction mixture was heated to 60 °C. After 1 h, TLC (pentane/EtOAc, 3:1) indicated the formation of a major product (R_f 0.5) and the presence of starting material (R_f 0.7). Two additional portions of Hoveyda–Grubbs' second generation complex (8 + 3 mg, 0.02 mmol, 2 mol %) were added to the reaction mixture over the course of 4 h, after which time TLC analysis indicated the consumption of starting material and the formation of product. The reaction mixture was then concentrated in vacuo and the residue purified by flash column chromatography (pentane/EtOAc, 5:1→3:1) to yield carbocycle **14** (545 mg, 88%) as a yellow oil; $[\alpha]_D^{23} = -75.1$ (*c* 1.0 $CHCl_3$); ν_{max}/cm^{-1} 1745 (s, C=O); δ_H (500 MHz, $CDCl_3$) 2.05, 2.11 (6H, 2 × s, 2 × CH_3), 3.88 (1H, dd, $J_{2,3}$ 2.0 Hz, $J_{3,4}$ 7.5 Hz, H-3), 3.97, 4.27 (2H, ABq, $J_{6,6'}$ 12.5 Hz, H-6, H-6′), 4.38 (1H, br d, H-4), 4.46, 4.55 (2H, ABq, J_{AB} 12.0 Hz, PhCH_2),

4.53, 4.75 (2H, ABq, J_{AB} 11.5 Hz, PhCH_2), 4.66, 4.84 (2H, ABq, J_{AB} 11.0 Hz, PhCH_2), 5.54 (1H, m, H-1), 5.62 (1H, m, H-5a), 5.82 (1H, m, H-2), 7.25–7.34 (15H, m, Ar-H); δ_C (125 MHz, $CDCl_3$) 21.0, 21.1 (2 × q, 2 × CH_3), 68.3 (d, C-1), 68.7 (d, C-2), 69.9 (t, C-6), 71.7, 72.3, 75.3 (3 × t, 3 × PhCH_2), 76.6 (d, C-4), 79.7 (d, C-3), 122.5 (d, C-5a), 127.8, 127.8, 127.8, 128.0, 128.3, 128.5, 128.5, 128.6 (8 × d, Ar-CH), 137.6, 138.2, 138.5, 138.6 (4 × s, 3 × Ar-C, C-5), 170.3, 170.7 (2 × s, 2 × C=O); *m/z* (ESI^+) 569 ($M+K^+$, 26), 553 ($M+Na^+$, 100%); HRMS (ESI^+) calcd for $C_{32}H_{34}O_7Na$ ($M+Na^+$) 553.2197; found 553.2206.

3.1.11. 3,4,6-Tri-*O*-benzyl-5a-carba-β-D-*lyxo*-hex-5(5a)-enopyra-nose 15

Diacetate **14** (520 mg, 0.98 mmol) was dissolved in MeOH (7 mL) under Ar. Sodium (16 mg, 0.7 mmol) was dissolved in MeOH (3 mL) and added to the reaction mixture. After 1 h, TLC (pentane/EtOAc, 3:1) indicated the complete consumption of starting material (R_f 0.5) and the formation of a major product (R_f 0.1). The reaction mixture was concentrated in vacuo by co-evaporation with toluene and the residue purified by flash column chromatography (pentane/EtOAc, 1:1→1:2) to yield diol **15** (415 mg, 95%) as white crystals, mp 85–86 °C (EtOAc/pentane), (lit.[25] 79–85 °C). $[\alpha]_D^{23} = -69.1$ (*c* 1.0, $CHCl_3$) (lit.[25] −75.7); ν_{max}/cm^{-1} 3417 (br s, OH); δ_H (500 MHz, $CDCl_3$) 2.64 (1H, d, $J_{OH,1}$ 10.9 Hz, OH-1), 3.12 (1H, d, $J_{OH,2}$ 8.4 Hz, OH-2), 3.89, 4.13 (2H, ABq, $J_{6,6'}$ 12.5 Hz, H-6, H-6′), 3.92 (1H, ddd, *J* 1.0 Hz, $J_{2,3}$ 2.0 Hz, $J_{3,4}$ 3.6 Hz, H-3), 4.03–4.06 (2H, m, H-1, H-2), 4.16 (1H, d, H-4), 4.36, 4.48 (2H, ABq, J_{AB} 11.9 Hz, PhCH_2), 4.45, 4.59 (2H, ABq, J_{AB} 11.4 Hz, PhCH_2), 4.54, 4.57 (2H, ABq, J_{AB} 11.7 Hz, PhCH_2), 5.99 (1H, d, $J_{1,5a}$ 4.2 Hz, H-5a), 7.22–7.34 (15H, m, Ar-H); δ_C (125 MHz, $CDCl_3$) 66.7, 67.3 (2 × d, C-1, C-2), 70.5 (t, C-6), 72.0, 73.3, 73.5 (3 × t, 3 × PhCH_2), 73.8 (d, C-4), 79.8 (d, C-3), 127.7, 127.7, 128.0, 128.2, 128.3, 128.3, 128.3, 128.4, 128.5, 128.7 (10 × d, Ar-CH, C-5a), 135.6 (s, C-5), 137.6, 137.9, 138.1 (3 × s, 3 × Ar-C); *m/z* (ESI^+) 485 ($M+K^+$, 54), 469 ($M+Na^+$, 100%); HRMS (ESI^+) calcd for $C_{28}H_{30}O_5Na$ ($M+Na^+$) 469.1985; found 469.1987.

3.1.12. 1,2-Anhydro-3,4,6-tri-*O*-benzyl-5a-carba-β-D-*lyxo*-hex-5(5a)-enopyranose 16

Triphenylphosphine (85 mg, 0.32 mmol) was dissolved in THF (2 mL) and cooled to 0 °C. DIAD (70 μL, 0.36 mmol) was added and the reaction mixture was left for 25 min before DPPA (325 μL, 1.5 mmol) and diol **10** (41 mg, 0.09 mmol, dissolved in 1 + 1 mL THF) were added and the cooling was removed. After 17 h, TLC (pentane/EtOAc, 1:1) indicated the formation of a major product (R_f 0.8) and the presence of some starting material (R_f 0.1). The reaction mixture was concentrated in vacuo and the residue purified by flash column chromatography (pentane/EtOAc, 4:1→2:1) to yield epoxide **16** (28 mg, 71%) as a colourless oil; $[\alpha]_D^{22} = +21.5$ (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.41 (1H, at, *J* 4.2 Hz, H-1), 3.57 (1H, dd, $J_{1,2}$ 4.4 Hz, $J_{2,3}$ 1.2 Hz, H-2), 3.96 (1H, dd, $J_{3,4}$ 7.6 Hz, H-3), 4.05 (1H, d, $J_{6,6'}$ 13.6 Hz, H-6), 4.18 (1H, dat, *J* 1.5 Hz, H-6′), 4.26 (1H, dat, *J* 1.2 Hz, H-4), 4.47, 4.52 (2H, ABq, J_{AB} 12.0 Hz, PhCH_2), 4.69, 4.87 (2H, ABq, J_{AB} 10.8 Hz, PhCH_2), 4.82, 4.86 (2H, ABq, J_{AB} 11.8 Hz, PhCH_2), 6.02 (1H, m, H-5a), 7.25–7.43 (15H, m, Ar-H); δ_C (100 MHz, $CDCl_3$) 49.1 (d, C-1), 52.6 (d, C-2), 69.3 (t, C-6), 72.2, 72.4, 75.5 (3 × t, 3 × PhCH_2), 77.6 (d, C-4), 81.5 (d, C-3), 119.4 (d, C-5a), 127.7, 127.7, 127.7, 127.8, 127.9, 128.1, 128.4, 128.5 (8 × d, Ar-CH), 138.2, 138.3, 138.5 (3 × s, 3 × Ar-C), 144.0 (s, C-5); *m/z* (ESI^+) 451 ($M+Na^+$, 12), 469 ($M+H_2O+Na^+$, 100%); HRMS (ESI^+) calcd For $C_{28}H_{28}O_4Na$ ($M+Na^+$) 451.1880; found 451.1902.

3.1.13. 1-Azido-3,4,6-tri-*O*-benzyl-1-deoxy-5a-carba-α-D-*lyxo*-hex-5(5a)-enopyranose 17

NaN_3 (26 mg, 0.40 mmol) and NH_4Cl (9 mg, 0.17 mmol) were dissolved in MeOH (3 mL) and H_2O (0.4 mL) and cooled to 0 °C. A solution of epoxide **16** (36 mg, 0.08 mmol) in THF (2 × 0.75 mL) was added by cannula. After 14 h, TLC (toluene/EtOAc, 3:1) indicated the formation of a major product (R_f 0.6) and the complete consumption of starting material (R_f 0.7). The reaction was quenched by addition of $NaHCO_3$ (satd aq, 20 mL) and extracted with EtOAc (20 mL). The organic phase was washed with $NaHCO_3$ (satd aq, 2 × 20 mL) and the combined aqueous fractions were extracted with EtOAc (2 × 20 mL). The combined organic phases were dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (pentane/EtOAc, 10:1→6:1→4:1) to give the azide **17** (32 mg, 77%) as a colourless oil; $[\alpha]_D^{23} = +50.5$ (*c* 1.0, $CHCl_3$) (lit.[25] +36 (*c*, 1.3 in $CHCl_3$)); ν_{max}/cm^{-1} 2100 (s, N_3); δ_H (400 MHz, $CDCl_3$) 2.22 (1H, d, $J_{OH,2}$ 7.8 Hz, OH-2), 3.87–3.95 (3H, m, H-2, H-3, H-6), 4.05 (1H, d, $J_{3,4}$ 2.9 Hz, H-4), 4.10 (1H, dat, *J* 1.5 Hz, $J_{6,6'}$ 12.4 Hz, H-6′), 4.15 (1H, br d, $J_{1,2}$ 8.0 Hz, H-1), 4.36, 4.48 (2H, ABq, J_{AB} 11.8 Hz, PhCH_2), 4.47 (1H, d, *J* 11.8 Hz, PhC*H*H′), 4.53–4.62 (3H, m, 2 × PhCH_2, PhCH*H*′), 5.74 (1H, m, H-5a), 7.23–7.36 (15H, m, Ar-H); δ_C (100 MHz, $CDCl_3$) 61.3 (d, C-1), 70.3 (t, C-6), 71.0 (d, C-2), 72.1, 72.8, 73.7 (3 × t, 3 × PhCH_2), 73.3 (d, C-4), 78.0 (d, C-3), 124.5 (d, C-5a), 127.8, 127.9, 128.0, 128.2, 128.2, 128.3, 128.5, 128.6, 128.7 (9 × d, Ar-CH), 136.6 (s, C-5), 137.8, 137.9, 138.2 (3 × s, Ar-C); *m/z* (ESI^+) 510 ($M+K^+$, 20), 494 ($M+Na^+$, 100%); HRMS (ESI^+) calcd for $C_{28}H_{29}N_3O_4Na$ 494.2050 ($M+Na^+$); found 494.2027.

3.1.14. 1,2-Di-*O*-trichloroacetimidoyl-3,4,6-tri-*O*-benzyl-5a-carba-α-D-*lyxo*-hex-5(5a)-enopyranose 19

Diol **10** (375 mg, 0.84 mmol) was dissolved in freshly distilled CH_2Cl_2 (15 mL) under Ar. Trichloroacetonitrile (420 μL, 4.2 mmol) and DBU (150 μL, 1.0 mmol) were added, and the reaction mixture turned from colourless to orange. After 1 h 20 min, the reaction mixture turned dark brown and TLC (toluene/EtOAc, 5:1) indicated the complete consumption of starting material (R_f 0.1) and the formation of a major product (R_f 0.8). The reaction mixture was concentrated in vacuo and the residue purified by flash column chromatography (toluene with 1% Et_3N) to yield diimidate **19** (610 mg, 0.83 mmol, 99%) as a colourless oil; $[\alpha]_D^{23} = +38.1$ (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 3341 (s, N–H), 1662 (s, C=N); δ_H (500 MHz, $CDCl_3$) 3.92, 4.22 (2H, ABq, $J_{6,6'}$ 12.7 Hz, H-6, H-6′), 4.15 (1H, d, $J_{3,4}$ 3.6 Hz, H-4), 4.35–4.37 (2H, m, H-3, PhC*H*H′), 4.45, 4.64 (2H, ABq, J_{AB} 11.1 Hz, PhCH_2), 4.51 (1H, d, *J* 11.8 Hz, PhCH*H*′), 4.57, 4.79 (2H, ABq, J_{AB} 11.9 Hz, PhCH_2), 5.75 (1H, d, $J_{1,2}$ 7.6 Hz, H-2), 5.96 (1H, m, H-5a), 6.04 (1H, d, H-1), 7.28–7.35 (15H, m, Ar-H), 8.44, 8.54 (2H, 2 × s, 2 × NH); δ_C (125 MHz, $CDCl_3$) 70.3 (t, C-6), 71.8, 73.3, 73.7 (3 × t, 3 × PhCH_2), 73.9 (d, C-1), 74.4 (d, C-3, C-4), 76.5 (d, C-2), 91.4, 91.4 (2 × s, 2 × CCl_3), 123.6 (d, C-5a), 127.7, 127.9, 128.0, 128.1, 128.1, 128.5, 128.5, 128.6, 128.6 (9 × d, Ar-CH), 137.6 (s, C-5), 137.9, 138.1, 138.3 (3 × s, 3 × Ar-C), 162.0, 162.2 (2 × s, 2 × C=N); *m/z* (ESI^+) Isotope distribution $M+Na^+$: 762.0 (11), 761.0 (34), 760.0 (28), 759.0 (84), 758.0 (39), 757.0 (100), 756.0 (19), 755.0 (47). $C_{32}H_{30}O_7N_2Cl_6Na$ requires 762.0 (12), 761.0 (34), 760.0 (28), 759.0 (79), 758.0 (35), 757.0 (100), 756.0 (19), 755.0 (52%); HRMS (ESI^+) calcd for $C_{32}H_{30}O_5N_2Cl_6Na$ ($M+Na^+$) 755.0178; found 755.0199.

3.1.15. 3,4,6-Tri-*O*-benzyl-1-deoxy-1-trichloroacetamido-5a-carba-β-D-*lyxo*-hex-5(5a)-enopyranose 20

Diimidate **19** (582 mg, 0.79 mmol) was dissolved in freshly distilled CH_2Cl_2 (15 mL) under Ar and cooled to −78 °C. Boron trifluoride diethyletherate (200 μL, 1.6 mmol) was added, and after 2 h 30 min, TLC (toluene/EtOAc, 4:1) indicated the presence of starting material (R_f 0.8) and the formation of a major product (R_f 0.6) and a minor product (R_f 0.3). Further boron trifluoride diethyletherate (50 μL, 0.4 mmol) was added, and after a further 1 h 45 min, TLC indicated the complete consumption of starting material (R_f 0.8) and the formation of major (R_f 0.6) and minor (R_f 0.3) products.

The reaction mixture was allowed to warm to rt and in this process it changed from colourless to yellow. Water (150 μL, 8.2 mmol) was added, and after 20 min, TLC indicated the complete conversion of the product with R_f 0.6 to that with R_f 0.3. The reaction was quenched by addition of $NaHCO_3$ (10 mL, satd aq). The reaction mixture was diluted with EtOAc (30 mL), transferred to a separatory funnel and washed with $NaHCO_3$ (3 × 30 mL, satd aq). The aqueous fractions were combined and extracted with EtOAc (2 × 30 mL). The combined organic phases were dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified twice by flash column chromatography; first (pentane/EtOAc, 5:1→1:1) and then (CH_2Cl_2/ether, 15:1) to yield amide **20** contaminated with trichloroacetamide (473 mg), as a yellow oil; ν_{max}/cm^{-1} 3385 (br, N–H), 1710 (s, C=O); δ_H (500 MHz, $CDCl_3$) 2.52 (1H, d, $J_{OH,2}$ 6.9 Hz, OH-2), 3.91–3.94 (2H, m, H-3 or H-4 and H-6), 4.15 (1H, dat, *J* 1.3 Hz, $J_{6,6'}$ 12.5 Hz, H-6′), 4.24 (1H, d, $J_{3,4}$ 3.6 Hz, H-3 or H-4), 4.27 (1H, ddd, *J* 5.0 Hz, *J* 2.1 Hz, H-2), 4.40 (1H, d, *J* 11.8 Hz, PhC*H*H′), 4.50–4.54 (3H, PhCH*H*′, 2 × PhC*H*H′), 4.59 (1H, d, *J* 11.0 Hz, PhCH*H*′), 4.64–4.68 (2H, m, H-1, PhCH*H*′), 5.80 (1H, d, $J_{1,5a}$ 4.6 Hz, H-5a), 7.24–7.35 (15H, m, Ar-H), 7.56 (1H, d, $J_{1,NH}$ 9.3 Hz, NH); δ_C (125 MHz, $CDCl_3$) 49.1 (d, C-1), 66.4 (d, C-2), 70.3 (t, C-6), 72.3, 73.3, 73.7 (3 × t, 3 × PhCH_2), 73.5 (d, C-4), 79.6 (d, C-3), 92.6 (s, CCl_3), 124.4 (d, C-5a), 127.9, 128.2, 128.3, 128.5, 128.5, 128.5, 128.6, 128.7 (8 × d, Ar-CH), 136.9, 137.2, 137.7, 137.9 (4 × s, C-5, 3 × Ar-C), 162.4 (s, C=O); *m/z* (ESI^+) Isotope distribution M+Na^+: 616.1 (31), 615.1 (31), 614.1 (100), 613.1 (33), 612.1 (97). 759.0 (84), 758.0 (39), 757.0 (100), 756.0 (19), 755.0 (47). $C_{30}H_{30}O_5NCl_3Na$ requires 616.1 (31), 615.1 (32), 614.1 (97), 613.1 (33), 612.1 (100%). HRMS (ESI^+) calcd for $C_{30}H_{30}O_5NCl_3Na$ (M+Na^+) 612.1082; found 612.1062.

NMR data for the oxazoline intermediate: δ_H (400 MHz, $CDCl_3$) 3.94 (1H, d, $J_{6,6'}$ 12.4 Hz, H-6), 3.98 (1H, dd, $J_{2,3}$ 3.9 Hz, $J_{3,4}$ 5.2 Hz, H-3), 4.04 (1H, d, H-4), 4.12 (1H, dat, *J* 1.7 Hz, H-6′), 4.38 (1H, d, *J* 12.4 Hz, PhC*H*H′), 4.43–4.45 (3H, m, PhCH_2, PhCH*H*′), 4.54, 4.75 (2H, ABq, J_{AB} 12.4 Hz, PhCH_2), 4.82 (1H, dat, $J_{1,2}$ 9.3 Hz, *J* 1.6 Hz, H-1), 5.28 (1H, dd, H-2), 6.15 (1H, m, H-5a), 7.15–7.34 (15H, m, Ar-H); δ_C (100 MHz, $CDCl_3$) 64.5 (d, C-1), 71.3 (t, C-6), 71.9, 73.7, 73.9 (3 × t, 3 × PhCH_2), 72.8 (d, C-4), 73.8 (d, C-3), 81.1 (d, C-2), 94.4 (s, CCl_3), 124.3 (d, C-5a), 127.7, 127.8, 128.0, 128.1, 128.1, 128.2, 128.5, 128.5, 128.6 (9 × d, Ar-CH), 136.6 (s, C-5), 137.8, 137.9, 138.2 (3 × s, 3 × Ar-C), 162.9 (s, C=N).

3.1.16. 1-Acetamido-1-deoxy-5a-carba-β-D-*lyxo*-hex-5(5a)-enopyranose 21

Amide **20** (131 mg, 0.22 mmol) was dissolved in THF (freshly distilled, 5 mL) under Ar and cooled to −78 °C. Ammonia (ca. 15 mL) was condensed into the reaction flask and sodium was added (115 mg, 5 mmol). After a few minutes, the reaction mixture turned dark blue. After 50 min, the reaction mixture turned yellow, and more sodium (18 mg, 0.8 mmol) was added, after which the blue colour reappeared. After another 30 min, the reaction mixture was quenched by addition of solid NH_4Cl (312 mg, 5.8 mmol) and left to warm to rt in order for the ammonia to evaporate. The crude residue was purified by flash column chromatography (MeOH/$CHCl_3$/NH_4OH (25%, aq), 5:5:1), to give major **21** (67 mg, R_f 0.6) and minor **21a** (8 mg, R_f 0.2) products. The major product was purified from salts by repeating the flash column chromatography (MeOH/$CHCl_3$/NH_4OH (25%, aq), 5:5:1) yielding **21** (40 mg, 83% from **19**) as a pale yellow solid. δ_H (500 MHz, D_2O) 2.05 (3H, s, CH_3), 3.82 (1H, dd, $J_{2,3}$ 2.2 Hz, $J_{3,4}$ 7.9 Hz, H-3), 4.12 (1H, ddd, $J_{2,5a}$ 1.5 Hz, $J_{1,2}$ 3.7 Hz, H-2), 4.16 (1H, m, H-6), 4.23 (1H, m, H-6′), 4.31 (1H, d, H-4), 4.65 (1H, m, H-1), 5.50 (1H, m, H-5a); δ_C (125 MHz, D_2O) 22.0 (q, CH_3), 49.2 (d, C-1), 61.4 (t, C-6), 69.1 (d, C-4), 70.6 (d, C-2), 74.0 (d, C-3), 122.1 (d, C-5a), 139.3 (s, C-5), 173.8 (s, C=O); *m/z* (ESI^+) 240 (M+Na^+, 100%); HRMS (ESI^+) calcd for $C_9H_{15}NO_5Na$ (M+Na^+) 240.0842; found 240.0839.

Data for minor compound **21a**: δ_H (500 MHz, D_2O) 3.79–3.81 (3H, m, H-3, CH_2), 4.10–4.15 (2H, m, H-2, H-6), 4.19 (1H, d, $J_{6,6'}$ 14.0 Hz, H-6′), 4.27 (1H, d, $J_{3,4}$ 7.5 Hz, H-4), 4.70 (1H, m, H-1), 5.48 (1H, m, H-5a); *m/z* (ESI^+) 255 (M+Na^+, 100), 233 (M+H^+, 96%); HRMS (ESI^+) calcd for $C_9H_{17}N_2O_5$ 233.1132 (M+H^+); found 233.1120. Calcd for $C_9H_{16}N_2O_5Na$ 255.0951 (M+Na^+); found 255.0940.

The minor product was acetylated as follows: **21a** (8 mg, containing salts) was suspended in Ac_2O and pyridine at rt and left for 5 days. The reaction mixture was diluted with EtOAc (20 mL) and washed with 1 M HCl (3 × 10 mL). The combined aqueous phases were extracted with EtOAc (2 × 10 mL) and the combined organic phases washed with $NaHCO_3$ (satd aq, 2 × 10 mL), dried (Na_2SO_4), filtered and concentrated in vacuo. The crude product was purified by flash column chromatography (EtOAc) to yield **22a** (7 mg, 0.02 mmol) as a thin yellow film. δ_H (500 MHz, $CDCl_3$) 2.02, 2.03, 2.07, 2.07, 2.16 (15H, 5 × s, 5 × CH_3), 3.76 (1H, dd, $J_{CHH',NH}$ 5.5 Hz, J_{gem} 15.5 Hz, C*H*H′), 3.96 (1H, dd, $J_{CHH',NH}$ 5.5 Hz, CH*H*′), 4.40, 4.68 (2H, ABq, $J_{6,6'}$ 13.3 Hz, H-6, H-6′), 5.03 (1H, m, H-1), 5.18 (1H, dd, $J_{2,3}$ 2.5 Hz, $J_{3,4}$ 7.5 Hz, H-3), 5.49 (1H, m, H-2), 5.69 (1H, m, H-5a), 5.76 (1H, d, H-4), 6.39 (1H, at, *J* 5.3 Hz, NH), 6.73 (1H, d, $J_{NH,1}$ 9.5 Hz, NH-1); δ_H (125 MHz, $CDCl_3$) 20.9, 20.9, 21.0, 23.0 (4 × q, CH_3), 44.2 (t, CH_2), 46.3 (d, C-1), 63.2 (t, C-6), 68.0 (d, C-4), 69.5 (d, C-2), 71.9 (d, C-3), 127.5 (d, C-5a), 133.0 (s, C-5), 169.0, 170.0, 170.3, 170.5, 170.5, 171.4 (6 × s, 6 × C=O); *m/z* (ESI^+) 465.2 (M+Na^+, 100%); HRMS (ESI^+) calcd for $C_{19}H_{26}N_2O_{10}Na$ (M+Na^+) 465.1485; found 465.1533.

3.1.17. 1,2-Bis-*epi*-valienamine 2

Method 1: Deprotection of acetamide **21**. Acetamide **21** (40 mg, 0.18 mmol) was dissolved in water (4 mL, Milli-Q), LiOH (22 mg, 0.9 mmol) was added, and the reaction mixture was heated to 70 °C. After 1.5 h, TLC (MeOH/$CHCl_3$/NH_4OH (25%, aq), 5:5:1) indicated the presence of mostly starting material (R_f 0.6) and the formation of small amounts of a product (R_f 0.1). Further LiOH (15 mg, 0.6 mmol) was added, and after a further 18 h, TLC indicated the presence of small amounts of starting material and the formation of a major product (R_f 0.1). Further LiOH (7 mg, 0.3 mmol) was added and the reaction mixture stirred at 70 °C for another 7 h until TLC indicated almost complete consumption of starting material (R_f 0.6) and the formation of a major product, which stained with ninhydrin (R_f 0.1). The reaction mixture was concentrated in vacuo by co-evaporation with toluene. The crude yellow residue (67 mg) was suspended in MeOH (2.5 mL) and filtered through a plug of cotton wool to remove salts. The procedure was repeated twice (2 × 1 mL, MeOH). The yellow residue (51 mg) was purified by flash column chromatography (MeOH/$CHCl_3$/NH_4OH (25%, aq), 5:5:1) to yield impure material (13 mg) as a pale yellow film. This material was purified by reverse phase flash column chromatography (Waters Sep-Pak C18) eluting with water, to yield **2** (12 mg, 27% over 3 steps from **19**) as a pale yellow solid. To ensure reproducible NMR data, AcOH was added to form the acetate salt before analysis: δ_H (500 MHz, D_2O) 2.05 (s, CH_3), 3.87 (1H, dd, $J_{2,3}$ 2.1 Hz, $J_{3,4}$ 6.4 Hz, H-3), 4.10 (1H, m, H-1), 4.20–4.22 (3H, m, H-2, H-6, H-6′), 4.28 (1H, br d, H-4), 5.70 (1H, m, H-5a); δ_C (125 MHz, D_2O) 21.0 (q, CH_3), 49.7 (d, C-1), 61.7 (t, C-6), 67.4 (d, C-2), 68.9 (d, C-4), 73.7 (d, C-3), 117.4 (d, C-5a), 143.4 (s, C-5), 177.5 (s, C=O); *m/z* (ESI^+) 198 (M+Na^+, 73), 176 (M+H^+, 100%); HRMS (ESI^+) calcd for $C_7H_{14}NO_4$ (M+H^+) 176.0917; found 176.0923. Calcd for $C_7H_{13}NO_4$-Na (M+Na^+) 198.0737; found 198.0741.

Method 2: Deprotection of pentaacetate **22**. Pentaacetate **22** (17 mg, 0.04 mmol) was dissolved in THF (1 mL), and water (1 mL) was added. LiOH (19 mg, 0.8 mmol) was added and the reaction mixture was heated to 70 °C. After 15 h, TLC (MeOH/$CHCl_3$/NH_4OH (25%, aq), 5:5:1) indicated the complete consumption of starting material (R_f 0.9) and the formation of a major prod-

uct (R_f 0.1), which stained with ninhydrin. The reaction mixture was concentrated in vacuo and the crude product purified by flash column chromatography (MeOH/$CHCl_3$/NH_4OH (25%, aq), 5:5:1) to yield the free base **2** (6 mg, 77%) as a pale yellow film. Addition of AcOH to form the acetate salt gave NMR spectral data identical to that described above.

3.1.18. 1-Acetamido-2,3,4,6-tetra-*O*-acetyl-1-deoxy-5a-carba-β-D-*lyxo*-hex-5(5a)-enopyranose 22

Trichloroacetamide **20** (50 mg, 0.08 mmol) was dissolved in THF (freshly distilled, 3 mL) under Ar and cooled to −78 °C. Ammonia (ca. 10 mL) was condensed into the reaction flask and sodium was added (50 mg, 2.2 mmol). After a few minutes, the reaction mixture turned dark blue. After a further 10 min, the reaction mixture turned yellow and further sodium (30 mg, 1.3 mmol) was added, after which the blue colour reformed. After a further 20 min, the reaction was quenched by addition of NH_4Cl (390 mg, 7.3 mmol) and left to warm to rt for the ammonia to evaporate.

The solid residue, crude **21**, was suspended in pyridine (1 mL, 12 mmol) and acetic anhydride (1 mL, 11 mmol) and left for 5 h. The reaction mixture was diluted with EtOAc and washed with 1 M HCl (3 × 10 mL). The aqueous phases were extracted with EtOAc (2 × 10 mL) and the combined organic phases were washed with satd aq $NaHCO_3$ (2 × 10 mL), dried ($MgSO_4$), filtered and concentrated in vacuo. The crude product was purified by flash column chromatography (toluene/EtOAc, 1:2) to yield **22** (20 mg, 61%) as a yellow oil, which was recrystallised to give white crystals, mp 177–178 °C (EtOAc/pentane); δ_H (400 MHz, $CDCl_3$) 1.99, 2.04, 2.08, 2.08, 2.14 (15H, 5 × s, 5 × CH_3), 4.43, 4.66 (2H, ABq, $J_{6,6'}$ 13.4 Hz, H-6, H-6′), 5.09 (1H, m, H-1), 5.20 (1H, dd, $J_{2,3}$ 2.1 Hz, $J_{3,4}$ 6.8 Hz, H-3), 5.51 (1H, dd, $J_{1,2}$ 4.4 Hz, H-2), 5.67–5.74 (3H, m, H-4, H-5a, NH); δ_C (100 MHz, $CDCl_3$) 20.9, 20.9, 20.9, 21.0, 23.3 (5 × q, 5 × *C*H_3), 45.9 (d, C-1), 63.3 (t, C-6), 67.8 (d, C-4), 69.6 (d, C-2), 71.9 (d, C-3), 128.0 (d, C-5a), 132.7 (s, C-5), 169.5, 169.7, 170.2, 170.2, 170.5 (5 × s, 5 × C═O); *m/z* (ESI^+) 408 ($M+Na^+$, 100%); HRMS (ESI^+) calcd for $C_{17}H_{23}NO_9Na$ 408.1265; found 408.1265.

3.1.19. 2-Azido-3,4,6-tri-*O*-benzyl-1,2-dideoxy-1-trichloroacetylamido-5a-carba-β-D-*xylo*-hex-5(5a)-enopyranose 23

Amide **20** (153 mg, 0.26 mmol, containing traces of trichloroacetamide) was dissolved in CH_2Cl_2 (5 mL, freshly distilled) under Ar and cooled to 0 °C. Pyridine (65 μL, 0.8 mmol) and trifluoromethanesulfonic anhydride (130 μL, 0.8 mmol) were added, and the colourless solution instantly turned yellow. After 1 h 15 min, TLC (toluene/EtOAc, 5:1) indicated the formation of a major product (R_f 0.8) and the presence of starting material (R_f 0.3). Further pyridine (50 μL, 0.6 mmol) and trifluoromethanesulfonic anhydride (100 μL, 0.6 mmol) were added, and the reaction mixture changed from yellow to orange-brown. After 20 min, TLC (toluene/EtOAc, 5:1) indicated the formation of a major product (R_f 0.8) and the complete consumption of starting material (R_f 0.3). The reaction mixture was diluted with CH_2Cl_2 (30 mL) and washed with ice-water (3 × 30 mL). The aqueous phases were combined and extracted with CH_2Cl_2 (2 × 30 mL). The combined organic phases were dried (Na_2SO_4), filtered and concentrated in vacuo to yield crude triflate (227 mg) as a yellow oil, which was used without further purification.

Crude triflate (227 mg, 0.26 mmol) was dissolved in DMF (5 mL) under Ar. Sodium azide (170 mg, 2.6 mmol) was added, and the reaction mixture was heated to 70 °C. After 9.5 h, TLC (toluene/EtOAc, 5:1) indicated the formation of a major product (R_f 0.6) and the complete consumption of starting material (R_f 0.7). The reaction mixture was diluted with CH_2Cl_2 (40 mL) and washed with brine (3 × 30 mL). The combined aqueous phases were extracted with CH_2Cl_2 (2 × 30 mL) and the combined organic phases were dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (pentane/EtOAc, 5:1→4:1) to yield azide **23** (98 mg, 0.16 mmol, 63% from **19**) as a yellow oil; $[\alpha]_D^{23} = -97.8$ (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 3328 (br, N–H), 2110 (s, N_3), 1696 (s, C═O); δ_H (500 MHz, $CDCl_3$) 3.75 (1H, dd, $J_{1,2}$ 6.3 Hz, $J_{2,3}$ 7.7 Hz, H-2), 3.85 (1H, dd, $J_{3,4}$ 5.3 Hz, H-3), 3.94 (1H, d, $J_{6,6'}$ 12.1 Hz, H-6), 4.19–4.24 (2H, m, H-4, H-6′), 4.45–4.52 (3H, m, H-1, PhCH_2), 4.65–4.70 (3H, m, PhC*H*H′, PhCH_2), 4.74 (1H, d, *J* 11.3 Hz, PhCH*H*′), 5.70 (1H, m, H-5a), 6.98 (1H, d, $J_{NH,1}$ 9.0 Hz, NH), 7.26–7.36 (15H, m, Ar-H); δ_C (100 MHz, $CDCl_3$) 50.6 (d, C-1), 62.7 (d, C-2), 70.1 (t, C-6), 72.8, 74.5, 74.7 (3 × t, 3 × PhCH_2), 75.7 (d, C-4), 79.9 (d, C-3), 92.4 (s, CCl_3), 122.7 (d, C-5a), 128.0, 128.0, 128.2, 128.3, 128.4, 128.5, 128.6, 128.7, 128.7 (9 × d, Ar-CH), 137.1, 137.8, 137.9, 138.4 (4 × s, 3 × Ar-C, C-5), 161.6 (s, C═O); *m/z* (ESI^+) Isotope distribution M+Na^+: 641.1 (28), 640.1 (28), 639.1 (100), 638.1 (30), 637.1 (100). $C_{30}H_{29}O_4N_4Cl_3Na$ requires 641.1 (31), 640.1 (32), 639.1 (96), 638.1 (33), 637.1 (100%); HRMS (ESI^+) calcd for $C_{30}H_{29}Cl_3N_4O_4Na$ ($M+Na^+$) 637.1147; found 637.1123.

3.1.20. 2-Azido-3,4,6-tri-*O*-benzyl-1-(*tert*-butyloxycarbonylamino)-1,2-dideoxy-5a-carba-β-D-*xylo*-hex-5(5a)-enopyranose 24

Trichloroacetamide **23** (70 mg, 0.11 mmol) was dissolved in *i*-PrOH (3 mL) under Ar. KOH (38 mg, 0.7 mmol) was added, and the reaction mixture was heated to 40 °C. After 17.5 h, TLC (toluene/EtOAc, 1:1) indicated the formation of a major product (R_f 0.1) and the presence of starting material (R_f 0.95). Further KOH (22 mg, 0.4 mmol) was added, and the reaction mixture was stirred at 40 °C for a further 1 h 50 min. The solvent was then removed by co-evaporation with toluene, and the residue was suspended in CH_2Cl_2 (5 mL) under Ar. Di-*tert*-butyl dicarbonate (240 μL, 1.0 mmol) was added, and after 2.5 h, TLC (toluene/EtOAc, 1:1) indicated the formation of a major product (R_f 0.9). The reaction mixture was concentrated in vacuo and the residue purified by flash column chromatography (toluene→toluene/EtOAc, 10:1) yielding **24** (44 mg, 68%) as a white solid; $[\alpha]_D^{23} = -98.1$ (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 3342 (br, N–H), 2107 (s, N_3), 1691 (br, C═O); δ_H (500 MHz, $CDCl_3$) 1.47 (9H, s, $C(CH_3)_3$), 3.53 (1H, at, *J* 9.3 Hz, H-2), 3.73 (1H, dd, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 7.7 Hz, H-3), 3.88 (1H, d, $J_{6,6'}$ 11.5 Hz, H-6), 4.17–4.27 (3H, m, H-1, H-4, H-6′), 4.46, 4.49 (2H, ABq, J_{AB} 12.0 Hz, PhCH_2), 4.66–4.68 (2H, m, NH, PhC*H*H′), 4.77, 4.88 (2H, ABq, J_{AB} 10.8 Hz, PhCH_2), 4.78 (1H, d, *J* 10.5 Hz, PhCH*H*′), 5.60 (1H, s, H-5a), 7.25–7.38 (15H, m, Ar-H); δ_C (125 MHz, $CDCl_3$) 28.5 (q, $C(CH_3)_3$), 51.8 (d, C-1), 66.2 (d, C-2), 70.1 (t, C-6), 72.7, 74.8, 75.4 (3 × t, 3 × PhCH_2), 78.8 (d, C-4), 80.3 (s, *C*$(CH_3)_3$), 82.6 (d, C-3), 126.2 (d, C-5a), 128.0, 128.0, 128.2, 128.3, 128.4, 128.5, 128.6, 128.7, 128.8 (9 × d, Ar-CH), 137.0, 137.8, 138.1, 138.2 (4 × s, 3 × Ar-C, C-5), 155.3 (s, C═O); *m/z* (ESI^+) 609 ($M+K^+$, 20), 593 ($M+Na^+$, 100%); HRMS (ESI^+) calcd for $C_{33}H_{38}N_4O_5Na$ ($M+Na^+$) 593.2734; found 593.2735.

3.1.21. 2-Acetamido-3,4,6-tri-*O*-acetyl-1-(*tert*-butyloxycarbonylamino)-1,2-dideoxy-5a-carba-β-D-*xylo*-hex-5(5a)-enopyranose 25

Azide **24** (15 mg, 0.026 mmol) was dissolved in THF (1 mL) under Ar and cooled to −78 °C. Ammonia (5 mL) was condensed into the flask and sodium (13 mg) was added, after which the reaction mixture turned dark blue within a couple of minutes. Over the next 45 min, sodium (10 mg + 6 mg, in total 1.3 mmol) was added twice in order to maintain the blue colour of the reaction mixture, after which the reaction was quenched by addition of solid NH_4Cl (67 mg, 1.3 mmol). The ammonia was allowed to evaporate, and the reaction mixture was concentrated in vacuo. The residue was suspended in acetic anhydride (1 mL) and pyridine (1 mL). After 3 h 15 min, TLC (toluene/EtOAc, 1:2) indicated formation of a major product (R_f 0.1), a by-product (R_f 0) and the complete consump-

tion of starting azide **24** (R_f 0.9). The reaction was quenched by the addition of HCl (1 M aq, 20 mL) and extracted with EtOAc (20 mL). The organic phase was washed with HCl (1 M aq, 2 × 20 mL) and the aqueous phases were extracted with EtOAc (2 × 20 mL). The combined organic phases were then washed with $NaHCO_3$ (satd aq, 3 × 20 mL), dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (toluene/EtOAc, 1:2→1:3, with 1% NEt_3) to yield **25** (10 mg, 87%) as a white solid; $[\alpha]_D^{23} = -65.6$ (*c* 0.7, $CHCl_3$); ν_{max}/cm^{-1} 3363 (s, N–H), 3263 (s, N–H), 1748 (s, C=O), 1688 (s, C=O), 1653 (s, C=O); δ_H (500 MHz, $CDCl_3$) 1.42 (9H, s, $C(CH_3)_3$), 1.94, 2.04, 2.05, 2.06 (12H, 4 × s, 4 × $C(O)CH_3$), 4.25 (1H, aq, *J* 10.3 Hz, H-2), 4.36, 4.66 (2H, ABq, $J_{6,6'}$ 12.8 Hz, H-6, H-6′), 4.42 (1H, at, *J* 9.5 Hz, H-1), 4.91 (1H, d, $J_{NH,1}$ 9.5 Hz, NH), 5.11 (1H, dd, $J_{2,3}$ 11.5 Hz, $J_{3,4}$ 7.8 Hz, H-3), 5.80 (1H, s, H-5a), 5.84 (1H, d, H-4), 5.89 (1H, d, $J_{NH,2}$ 9.5 Hz, NH); δ_C (125 MHz, $CDCl_3$) 20.8, 20.8, 20.9, 23.3 (4 × q, 4 × $C(O)CH_3$), 28.4 (q, $C(CH_3)_3$), 52.0 (d, C-1), 53.9 (d, C-2), 62.9 (t, C-6), 70.6 (d, C-4), 73.4 (d, C-3), 80.4 (s, $C(CH_3)_3$), 130.3 (d, C-5a), 132.8 (s, C-5), 156.2 (s, C=O), 170.0, 170.6, 171.0, 171.5 (4 × s, 4 × C=O); *m/z* 465 ($M+Na^+$, 100%); HRMS (ESI^+) calcd for $C_{20}H_{30}N_2O_9Na$ ($M+Na^+$) 465.1844; found 465.1862.

3.1.22. 2-Acetamido-1-(*tert*-butyloxycarbonylamino)-1, 2-dideoxy-5a-carba-β-D-*xylo*-hex-5(5a)-enopyranose 26

Tetraacetate **25** (14 mg, 0.03 mmol) was dissolved in MeOH (1.5 mL). Sodium (8 mg, 0.17 mmol) was dissolved in MeOH (0.5 mL) and added to the reaction mixture. After 1 h, TLC (toluene/EtOAc, 1:1) indicated the complete consumption of starting material (R_f 0.2) and the formation of a major product (R_f 0). The reaction mixture was neutralised by the addition of Dowex 50WX8 (H^+) resin. After filtration, the reaction mixture was concentrated in vacuo and the residue was purified by flash column chromatography (MeOH/EtOAc, 1:4) to yield triol **26** (8 mg, 79%) as a colourless oil; δ_H (500 MHz, D_2O) 1.41 (9H, s, $C(CH_3)_3$), 2.01 (3H, s, $C(O)CH_3$), 3.66 (1H, dd, $J_{2,3}$ 11.3 Hz, $J_{3,4}$ 8.2 Hz, H-3), 3.89 (1H, at, *J* 10.5 Hz, H-2), 4.10 (1H, d, $J_{6,6'}$ 13.5 Hz, H-6), 4.18–4.23 (2H, m, H-1, H-6′), 4.27 (1H, d, H-4), 5.55 (1H, s, H-5a); δ_C (125 MHz, D_2O) 22.2 (q, $C(O)CH_3$), 27.6 (q, $C(CH_3)_3$), 51.8 (d, C-1), 55.2 (d, C-2), 61.1 (t, C-6), 72.0 (d, C-4), 73.9 (d, C-3), 81.2 (s, $C(CH_3)_3$), 124.3 (d, C-5a), 139.0 (s, C-5), 157.6 (s, C=O), 174.6 (s, C=O).

3.1.23. 1-*epi*-2-Acetamido-2-deoxy-valienamine hydrochloride 27

Boc-protected compound **26** (8 mg, 0.025 mmol) was dissolved in water (1.5 mL), and HCl (12 M, 1.0 mL) was added dropwise. After 15 min, TLC (EtOAc/MeOH, 1:2) indicated the complete consumption of starting material (R_f 0.9) and the formation of a major product (R_f 0.3). The reaction mixture was concentrated in vacuo and the residue purified twice by reverse phase flash column chromatography (Waters Sep-Pak aminopropyl and C18 cartridges) to yield the GlcNAc analogue **27** (5.9 mg, 93%) as a pale yellow powder; $[\alpha]_D^{23} = -65.6$ (*c* 0.7, H_2O); (lit.[6] −59.6); δ_H (500 MHz, D_2O) 2.09 (3H, s, $C(O)CH_3$), 3.72 (1H, dd, $J_{2,3}$ 10.9 Hz, $J_{3,4}$ 8.0 Hz, H-3), 4.04 (1H, m, H-1), 4.12 (1H, dd, $J_{1,2}$ 9.8 Hz, H-2), 4.20, 4.27 (2H, ABq, $J_{6,6'}$ 14.8 Hz, H-6, H-6′), 4.32 (1H, d, H-4), 5.69 (1H, m, H-5a); δ_C (125 MHz, D_2O) 22.2 (q, $C(O)CH_3$), 52.5 (d, C-1), 52.6 (d, C-2), 60.7 (t, C-6), 71.6 (d, C-4), 72.8 (d, C-3), 117.0 (d, C-5a), 143.6 (s, C-5), 175.5 (s, C=O); *m/z* (ESI^+) 239 ($M+Na^+$, 100), 217 ($M+H^+$, 54%); HRMS (ESI^+) calcd for $C_9H_{17}N_2O_4$ 217.1183 ($M+H^+$); found 217.1180. Calcd for $C_9H_{16}N_2O_4Na$ 239.1002 ($M+Na^+$); found 239.0999.

3.2. Enzyme assay: General materials and methods

Recombinant *C. fimi* β-mannosidase (CfMan2A),[8a] (Lot. 30901) and *A. niger* β-glucosidase (AnGlu), (Lot. 90301) were purchased from Megazyme (Bray, Ireland). Each enzyme was dialysed into the respective assay buffer. Both enzyme preparations appeared as single bands when analysed by SDS–PAGE (Coomassie staining) using a 6 cm 4–12% gradient gel (Invitrogen Life Sciences, Carlsbad, CA, USA). Protein concentrations were determined using the Micro BCA™ Protein Assay Reagent Kit with BSA standards (Pierce, Rockford, IL, USA). All other chemicals were obtained from Sigma Aldrich Chemical Co. (Schnelldorf, Germany) and were of reagent grade.

3.3. Steady-state initial velocity kinetics

All kinetic experiments were performed using 1 cm path length cuvettes in a Varian CARY 4000 spectrophotometer attached to a temperature control unit. CfMan2A initial velocity assays were carried out in 50 mM sodium phosphate buffer, pH 7.0 at 25 °C. The enzyme and substrate concentrations in the assays were 1.53 μg mL^{-1} CfMan2A and 0.05–0.40 mM *para*-nitrophenyl β-mannoside (*p*NPMan) at each of a series of the 1,2-bis-*epi*-valienamine inhibitor concentrations of 0.00, 0.10, 0.45 and 0.60 mM. *p*NPMan concentrations lower than 0.5 mM were used in this study to avoid complications arising from substrate inhibition at higher concentrations as reported previously.[8c] The enzyme and the inhibitor in the assay buffer were pre-incubated at 25 °C for 10 min before addition of the substrate. CfMan2A-catalysed initial rates of hydrolysis of *p*NPMan were determined by following the increase in the absorbance at 400 nm due to the release of *para*-nitrophenolate into solution. The initial velocity curves (V_o vs [S]) were hyperbolic with respect to substrate concentration and fitted to the standard Michaelis–Menten equation using nonlinear regression with the program GraphPad Prism 4 (GraphPad Software, San Diego, CA, USA). The initial velocity data were also plotted in the form of a Lineweaver-Burk double-reciprocal plot ($1/V_o$ vs 1/[S]) using linear regression with the same software. The values of apparent K_m (K_m^{app}) were then determined from the *x*-intercepts of the double-reciprocal plot, replotted versus inhibitor concentrations and fitted using linear regression with the same software.

Possible inhibition of CfMan2A activity by D-mannose was also tested in the assay buffer at 25 °C. The enzyme and substrate concentrations in the assays were 1.53 μg mL^{-1} CfMan2A and 0.40 mM pNPMan at each of a series of the inhibitor (D-mannose) concentrations of 0.00, 0.50 and 1.00 mM. CfMan2A and D-mannose in the assay buffer were pre-incubated at 25 °C for 10 min before addition of the substrate.

AnGlu initial velocity assays were carried out in 50 mM sodium acetate buffer, pH 4.0 at 40 °C. The enzyme and substrate concentrations in the assays were 6.33 μg mL^{-1} AnGlu and 1.00 mM *para*-nitrophenyl β-glucoside (*p*NPGlc) at each of a series of the 1,2-bis-*epi*-valienamine inhibitor concentrations of 0.00, 0.60 and 2.00 mM. The enzyme and the inhibitor in the assay buffer were pre-incubated at 40 °C for 15 min before addition of the substrate. AnGlu-catalysed initial rates of hydrolysis of *p*NPGlc were determined by following the increase in the absorbance at 400 nm due to the release of *para*-nitrophenolate into solution.

3.4. X-ray data collection, structure solution and refinement

Crystals for diffraction were grown by slow diffusion of pentane into a EtOAc solution of the compound for each of **15**, **22** and **18**. The resulting crystals were measured with an Oxford Diffraction Excalibur-II κ diffractometer equipped with a sapphire-3 CCD using Mo Kα (λ = 0.71073 Å) radiation (DIAMOND (Version 2.1e, Crystal Impact, Germany), CRYSALIS CCD and CRYSALIS RED (Version 1.170, Oxford Diffraction Ltd, UK)). All measurements were made at ambient temperature. The structures were solved using standard direct methods using SHELXS-97 and refined with full-matrix least-square

Table 1
Crystal data for compounds **15**, **18** and **22**

Compound	**15**	**18**	**22**
Formula	$C_{28}H_{30}O_5$	$C_{17}H_{23}NO_9$	$C_{17}H_{23}NO_9$
Formula weight	446.52	385.36	385.36
Temperature (K)	293(2)	293(2)	293(2)
Space group	$P22_12_1$ (18)	$P2_1$ (4)	$P2_12_12_1$ (19)
a (Å)	5.8194(3)	9.1983(2)	7.93420(10)
b (Å)	18.9944(10)	8.5360(2)	11.4114(2)
c (Å)	22.2329(11)	12.8687(3)	22.9755(4)
α (°)	90	90	90
β (°)	90	98.731(2)	90
γ (°)	90	90	90
V (Å^3)	2457.5(2)	998.70(4)	2080.21(6)
Z	4	2	4
Density	1.207	1.281	1.230
μ (mm^{-1})	0.082	0.105	0.100
N_{meas}, N_{unique}, N_{obs}	15,542, 4337, 1237	12,985, 3251, 2381	39,175, 7001, 2680
R_{int}	0.1091	0.0375	0.0503
N_{par}	265	250	249
wR_2(all), R_1(all)	0.1293, 0.2014	0.0856, 0.0590	0.1097, 0.1230
wR_2(obs), R_1(obs)	0.1043, 0.0514	0.0809, 0.0384	0.1025, 0.0436
Completeness, θ	0.996, 25.03	0.995, 25.03	0.965, 32.25
Residual density (e/Å^3)	−0.199 to 0.288	−0.112 to 0.142	−0.182 to 0.247

methods using SHELXL-97.[31] Compound **15** was the weakest scatterer of the three compounds, a fact that can describe the rather high internal *R*-value for this compound. However, this is mainly a result of the large amount of weak reflections. No extra absorption correction except the scaling applied by the diffractometer software was applied due to the low value of the linear absorption coefficient. The absolute configuration was set from the known chirality of the starting material, D-mannose.

The rings defined by C-1–C-2–C-3–C-4–C-5a can be described by the Cremer–Pople parameters[32] for each compound, calculated using the PLATON program.[33] For **15**: $Q(2) = 0.408(5)$ Å, $Q(3) = -0.328(5)$ Å, $\varphi(2) = 252.5(7)°$, Puckering Amplitude (Q) = 0.524(5) Å, $\theta = 128.8(5)°$, $\varphi = 252.5(7)°$. For **18**: $Q(2) = 0.384(2)$ Å, $Q(3) = 0.325(2)$ Å, $\varphi(2) = 92.3(4)°$, Puckering Amplitude (Q) = 0.504(2) Å, $\theta = 49.8(2)°$, $\varphi = 92.3(4)°$. For **22**: $Q(2) = 0.3768(17)$ Å, $Q(3) = -0.3146(17)$ Å, $\varphi(2) = 263.8(3)°$, Puckering Amplitude (Q) = 0.4909(17) Å, $\theta = 129.9(2)°$, $\varphi = 263.8(3)°$. The ring in **18** is described with the half-chair pucker descriptor (Table 1).

Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 718023 (**15**), CCDC 718024 (**22**) and CCDC 718025 (**18**). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

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