A potent bicyclic inhibitor of a family 27 α-galactosidase†

Yi Wang and Andrew J. Bennet*

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Two isomeric bicyclo[4.1.0]heptane analogues of the glycosidase inhibitor *galacto*-validamine, (1*R**,2*S*,3*S*,4*S*,5*S*,6*S**)-5-amino-1-(hydroxymethyl)bicyclo[4.1.0]heptane-2,3,4-triol, have been synthesized in 13 steps from 2,3,4,6-tetra-*O*-benzyl-D-galactose. The inhibitory activities of the two conformationally restricted amines, and their corresponding acetamides, were measured against commercial α-galactosidase enzymes from coffee bean and *E. coli*. The activity of the glycosyl hydrolase family GH27 enzyme (coffee bean) was competitively inhibited by the 1*R*,6*S*-amine (**7**), a binding interaction that was characterized by a K_i value of 0.541 μM. The GH36 *E. coli* α-galactosidase exhibited a much weaker binding interaction with the 1*R*,6*S*-amine ($IC_{50} = 80$ μM). The diastereomeric 1*S*,6*R*-amine (**9**) bound weakly to both galactosidases, (coffee bean, $IC_{50} = 286$ μM) and (*E. coli*, $IC_{50} = 2.46$ mM).

Introduction

An important field in glycobiology involves design, synthesis, and biological evaluation of inhibitors for various glycosidase enzymes.[1] Tight-binding reversible inhibitors of glycosidases have the potential to be therapeutic agents for the treatment of various medical conditions, including diabetes,[2] cancer,[3] and influenza.[4] Of particular importance with respect to this report, it is known that Fabry disease is a lysosomal storage disorder caused by a deficiency of α-galactosidase A (α-Gal A).[5] This enzyme activity deficiency results from impaired trafficking of misfolded α-Gal A variants to the lysosome.[5] Of note, the use of low concentrations of galactosidase inhibitors, as chemical chaperones, ameliorates the trafficking of mutant enzymes presumably by stabilizing the correctly folded enzymatic structure.[6,7]

At the present time, α-galactosidase enzymes are classified into four glycosidase families based on protein sequence alignment methods.[8] These families have been assigned glycosyl hydrolase (GH) numbers 4, 27, 36, and 57. All of these categorized α-galactosidases (EC 3.2.1.22) are retaining hydrolases, that is, the first product formed during the catalysed hydrolysis of an α-galactopyranoside is α-galactopyranose. Moreover, as family GH 4 members operate *via* an atypical mechanism involving oxidation of the 3-OH group followed by an $E1_{CB}$ elimination reaction,[9] compounds that are designed to inhibit glycopyranosylium ion-like transitions states are not expected to inhibit enzymes from glycosidase family GH 4. Consequently, discussion will be limited to retaining glycosidases that hydrolyse sugar acetal linkages *via* a glycosylated enzyme intermediate.[10,11] Both enzyme-catalysed glycosylation and deglycosylation steps occur *via* transition states (TSs) that have oxacarbenium ion character and a distorted six-membered ring.

While no single compound effectively inhibits all galactosidases[12–14] most inhibitors possess structural features that mimic certain facets of the galactopyranosylium ion (**1**), a high-energy intermediate formed during the acid-catalysed hydrolysis of galactosides in aqueous solution.[15–17] Regardless of whether the enzyme-catalysed glycosylation and deglycosylation steps are dissociative (D_N * A_N)[18,19] or "exploded" associative ($A_N D_N$)[19,20] reactions, it is clear that there is a sizable degree of cationic character on the sugar moiety at the critical enzymatic TSs,[21,22] which therefore must bear some resemblance to the galactopyranosylium ion. Thus, 1-deoxy-*galacto*-nojirimycin (**2**),[23,24] *galacto*-isofagomine (**3**)[12,25] and *galacto*-validamine (**4**)[26] mimic charge development at the TS by incorporating a basic nitrogen atom in place of *O*-5, *C*-1, and *O*-1, respectively (Fig. 1). Whereas, *galacto*-valienamine (**5**) mimics both the ring distortion and charge development present in galactopyranosylium ions.[27]

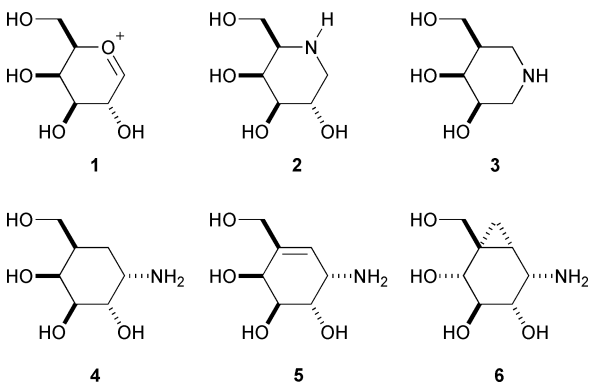

Fig. 1 Structures of the galactopyranosylium ion (**1**) and selected glycosidase inhibitors that contain a basic amino group (**2**–**6**).

In 2001, Tanaka *et al.* reported that sub-micromolar inhibition was obtained for yeast α-glucosidase using the carbocyclic bicyclo[4.1.0]heptylamine α-glucopyranose mimic **6**.[28] The current report details the synthesis and inhibitory activity of the structurally similar four cyclopropane containing bicyclic galactose analogues **7**-**10** (Fig. 2).

Department of Chemistry, Simon Fraser University, 8888 University Drive, Burnaby, V5A 1S6, British Columbia, Canada. E-mail: bennet@sfu.ca
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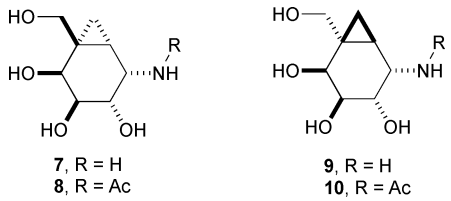


Fig. 2 Structures of the designed bicyclo[4.1.0]heptane-based potential galactosidase inhibitors (**7**–**10**).

Results and discussion

The route chosen for the synthesis of these inhibitors utilized 2,3,4,6-tetra-*O*-benzyl-D-galactose (**11**), which can be made in two steps from methyl α-D-galactopyranoside,[29] as starting material (Scheme 1).

After reduction of the protected hemiacetal, selective protection of the primary alcohol as a trityl ether, followed by a Swern oxidation and a Wittig reaction gave olefin **12** in a yield of 47% over these 4 steps. Removal of the trityl ether using mild acid-catalysis and subsequent Swern oxidation and addition of vinylmagnesium bromide gave a 1 : 2 ratio of the *R* : *S* diastereomers of an acyclic octadienol (**13-*R*,*S***). Of note, the assignment of stereochemistry is based on the ratio of **14-*R*** to **14-*S*** that is formed by a ring closing metathesis reaction catalysed by a second generation Grubbs' catalyst.[30] Specifically, the stereochemistry of **14-*R*** was assigned based on the larger coupling constant observed between H-1 and H-6 in the ^{1}H NMR spectrum (6.4 Hz) relative to that of the **14-*S*** diastereomer (4.3 Hz).[31] In addition, an NOE contact was observed for **14-*R*** between H-1 and H-5. Following separation of the *R*-diastereomer **14-*R*** from the pseudo-axial isomer **14-*S***, a process that was accomplished by the use of radial chromatography,[32] incorporation of an azide functionality was accomplished with inversion of configuration to give the allylic azide **15**, along with a small amount of the S_N2' product (**16**). Reduction of the azide, followed by acetylation gave the acetamide **17**.

Subsequent use of the Furukawa modification of the Simmons–Smith reaction gave the two cyclopropyl isomers in a total yield of 88%.[33] Of note, the ratio of the D-*galacto* (**18**) and L-*altro* (**19**) diastereomers formed in this reaction was unpredictable, that is, it varied between 2.5 : 1.0 and 1.0 : 1.5. The absolute stereochemistry for the two diastereomers was assigned based on observed NOE contacts between one of the H-7 protons and either H-3 or H-4 in the 1D NOE difference spectra (Fig. 3).

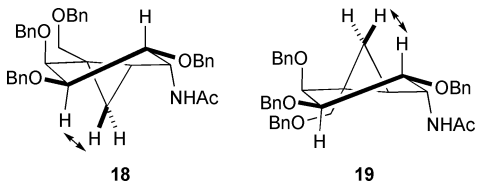
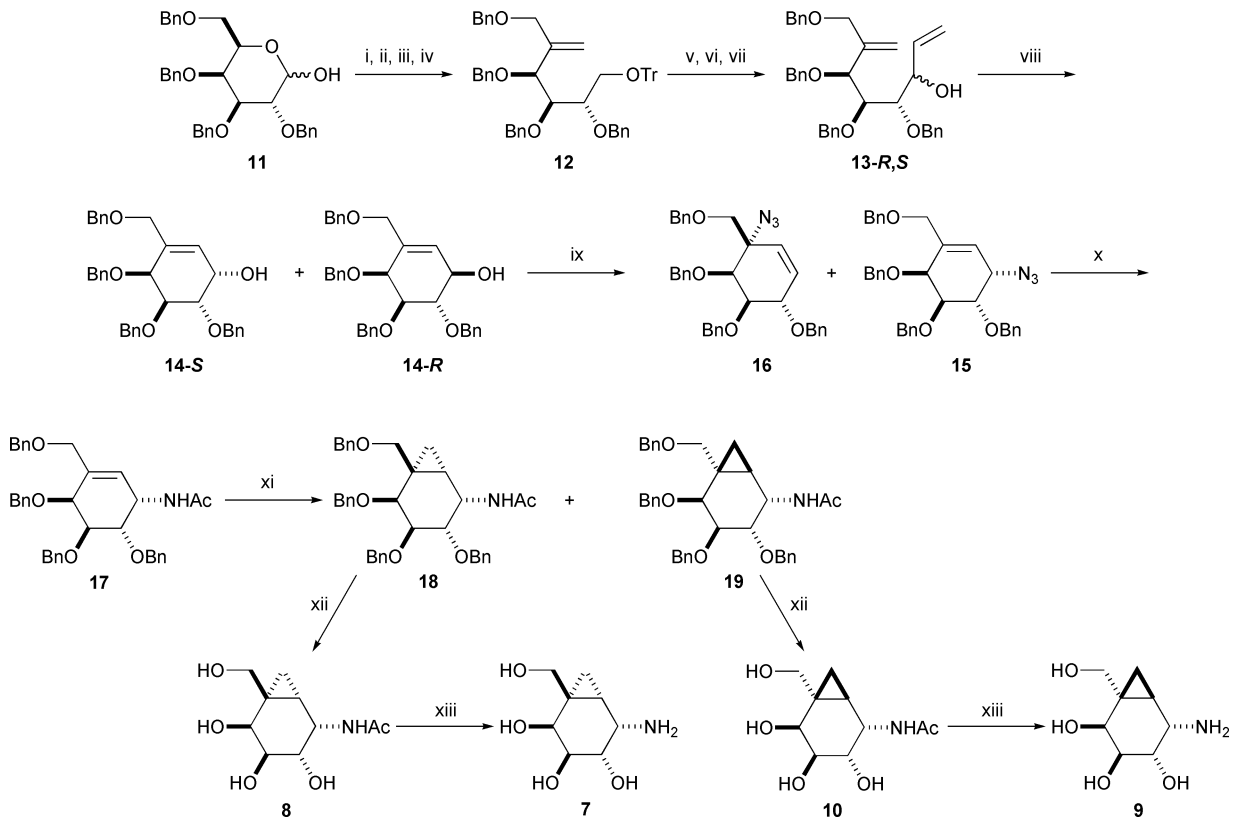


Fig. 3 Observed NOE contacts for the protected bicyclo[4.1.0]heptane-based galactoside analogues (**18** and **19**).



Scheme 1 *Reagents and conditions*: i, $NaBH_4$, EtOH, rt, 93%; ii, TrCl, pyridine, rt, 74%; iii, DMSO, Ac_2O, rt, 86%; iv, Ph_3PCH_3I, BuLi, −78 to 0 °C, 79%; v, HCl·H_2O, AcOH, rt, 61%; vi, $(COCl)_2$, DMSO, −78 °C, then Et_3N, vii, $CH_2CHMgBr$, −78 °C, *R* : *S* 1 : 2, 60%; viii, Grubbs' catalyst, CH_2Cl_2, 40 °C, *R*: 32%, *S*: 54%; ix, $(PhO)_2P(O)N_3$, $PhCH_3$, DBU, 60 °C, **15**: 76%, **16**: 21%; x, H_2S, Et_3N, H_2O, rt, then AcCl, pyridine, 80%; xi, $ZnMe_2$, CH_2I_2, 10 °C, **18** : **19** 1 : 1, 88%; xii, 10% Pd–C, H_2, rt, xiii, LiOH, THF : H_2O 1 : 1, 70 °C.

Table 1 Inhibitory activity (IC_{50}/μM) of **2**, **3**, **4**, **5**, **7**, **8**, **9**, and **10** against two α-galactosidases

Enzyme	**2**	**3**	**4**	**5**	**7**	**8**	**9**	**10**
Coffee bean	0.0016[a]	200[b]	500[c]	56[d]	(0.541 ± 0.018)[e]	343 ± 14	286 ± 15	N.I.[f]
E. coli	0.24[a]	N.R.[g]	890[c]	N.R.[g]	80 ± 6	N.I.[f]	2460 ± 130	N.I.[h]

[a] Reference 24. [b] Reference 25. [c] Reference 26. [d] Reference 27. [e] K_i value. [f] No inhibition at 1.0 mM. [g] Not reported. [h] No inhibition at 5.0 mM.

Removal of the benzyl protecting groups was accomplished using conventional hydrogenation conditions to give the amides **8** and **10** in good yields. Subsequent hydrolysis of the amide groups, using LiOH in THF–H_2O gave the corresponding amines **7** and **9**. It was not possible to obtain crystals of either **7** or **9**, however, their conformations in solution are likely similar to their respective *gluco*-analogues[28] because of the similarity of ^1H–^1H coupling constants for the pseudo-anomeric hydrogen atom.

Amines **7** and **9**, and their respective acetamido compounds **8** and **10** were tested as inhibitors against two commercially available α-galactosidase enzymes. Measured inhibition parameters (K_i or IC_{50} values) for these four bicyclo[4.1.0]heptane derivatives and the reported values for **2**, **3**, **4** and **5** are listed in Table 1.

The plot (Fig. 4) for inhibition of 4-nitrophenyl α-D-galactopyranoside hydrolysis catalysed by coffee bean α-galactosidase on the addition of **7** shows that this compound binds competitively to the active site of the enzyme. Specifically, fitting the kinetic data displayed in Fig. 4 to three standard models of inhibition, namely: competitive, non-competitive, and uncompetitive results in the following "goodness of fit" (R^2 values) of 0.999, 0.983, and 0.956, respectively.

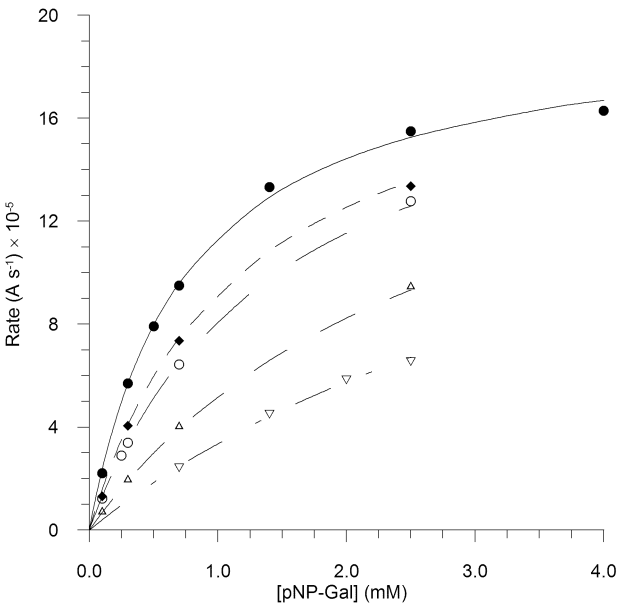


Fig. 4 Plot for inhibition of coffee bean α-galactosidase by **7**. Drawn lines are for the fit of the data to a competitive inhibition model. Concentrations of inhibitor **7** are: ● 0.0 μM, ♦ 0.3 μM, ○ 0.5 μM, △ 1.5 μM, and ▽ 3.0 μM.

Moreover, the measured K_i value (541 ± 18 nM) for inhibition of coffee bean α-galactosidase shows that **7** is the tightest binding inhibitor of those in which the basic nitrogen atom of the inhibitor is located in the position of a substrate's glycosidic oxygen atom (*cf.*, **4**, **5**, and **7** in Table 1), an observation that matches the inhibition of yeast α-glucosidase by compound **6**, valienamine and validamine.[28]

Given that all IC_{50} values were measured at low substrate concentration (between 0.20 and 0.25 times K_m) the reported IC_{50} values are between 1.20 and 1.25 times larger than the true inhibition constant K_i,[34] assuming that all inhibitors bind competitively to the enzyme. With respect to inhibition of the GH27 enzyme (coffee bean), the comparison between the *galacto*-bicyclic inhibitor **7** and both **5** and **9** is particularly interesting since these compounds are structurally very similar, and **7** is ~100 fold (11.9 kJ mol^{-1}) tighter binder than either the alkene (**5**) or the *altro*-isomer (**9**). This observation suggests that in compound **7** the hydroxymethyl group (CH_2OH) has more optimal interactions with the coffee bean enzyme, but that in both **5** and **9** no severe steric interactions are introduced by changing the position of this group relative to the carbasugar portion of the inhibitors.

Also, the contrast between the potency of amine **7** and acetamide **8** supports the idea that a positive charge enhances inhibitor binding to the enzymatic active site, in this case the difference in the free energy of binding is 14.5 kJ mol^{-1}.

Given that both glycosyl hydrolase families GH27 and GH36 share a commonality of mechanism,[35] it is not surprising that inhibitor **7** is also the tightest binder to the GH36 enzyme from *E. coli.* (*cf.*, **4**, **7**, and **9** in Table 1 bottom row). An extended discussion on the differences in binding affinity of **7** between the two GH family members is unwarranted until structural data is available.

Also of note, the weakest binding inhibitor of the three compounds that are present in $^4C_1$ conformations (**2**, **3** and **4**; Table 1) is **4**, the inhibitor where the basic nitrogen atom is incorporated in place *O*-1. It remains to be seen whether analogues of **2** and **3** in which the pyranosyl ring is held in non-chair conformations are tighter binding inhibitors than the parent compounds themselves as is the case for **7**.

Conclusions

A tight binding competitive inhibitor of coffee bean α-galactosidase (**7**) was made in 13 steps from 2,3,4,6-tetra-*O*-benzyl-D-galactose in an overall yield of 0.6%.

Experimental

Thin-layer chromatography (TLC) was performed on aluminium-backed TLC plates pre-coated with Merck silica gel 60 F_{254}. Compounds were visualized with UV light and/or staining with phosphomolybdic acid (5% solution in EtOH). Flash chromatography was performed using Avanco silica gel 60 (230–400 mesh).

Radial chromatography was performed on a Harrison Research Inc. model 8924 chromatotron. Solvents used for anhydrous reactions were dried and distilled immediately prior to use. Methanol was dried and distilled over magnesium methoxide. Dichloromethane was dried and distilled over calcium hydride. Glassware for anhydrous reactions was flame-dried and cooled under a nitrogen atmosphere immediately prior to use. NMR spectra were recorded on a Varian Unity 500 MHz spectrometer. Chemical shifts (δ) are listed in ppm downfield from TMS using the residual solvent peak as an internal reference. ^{1}H and ^{13}C NMR peak assignments are made based on ^{1}H–^{1}H COSY and ^{1}H–^{13}C HMQC experiments. Coupling constants are reported in Hz. IR spectra were recorded on a Bomem IR spectrometer and samples were prepared as cast evaporative films on NaCl plates from CH_2Cl_2. Optical rotations were measured using a Perkin-Elmer 341 polarimeter and are reported in units of deg cm^2 g^{-1} (concentrations reported in units of g per 100 cm^3).

Green coffee bean (GH27) and *E. coli* (GH36) α-galactosidases were purchased from Sigma-Aldrich and Calbiochem, respectively.

2,3,4,6-Tetra-*O*-benzyl-D-galactitol

$NaBH_4$ (2.8 g, 74 mmol) was added to a solution of **11**[29] (17.5 g, 32.4 mmol) in ethanol (180 cm^3). After the reaction mixture had been stirred for 15 h it was diluted with ether (300 cm^3), and it was then washed with water (2 × 100 cm^3) and brine (100 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by flash column chromatography (hexane : EtOAc, 2 : 1 v/v) to give a colourless syrup (16.3 g, 93%): $[\alpha]_D^{20}$ −5.5 (c = 1.85, CH_2Cl_2), IR 3443 cm^{-1} (br, O–H), ^{1}H NMR ($CDCl_3$) δ 3.50 (dd, 1 H, $J_{6,6'}$ = 9.1, $J_{5,6}$ = 6.7, H-6), 3.54 (dd, 1 H, $J_{5,6'}$ = 5.5, H-6′), 3.68–3.73 (m, 2 H, H-3, H-1), 3.79 (m, 1 H, H-1′), 3.86–3.91 (m, 2 H, H-2, H-4), 4.03 (m, 1 H, H-5), 4.42 (d, 1 H, $J_{A,B}$ = 11.9, CH_AH$_B$C$_6$H$_5$), 4.47 (d, 1 H, CH$_A$$H_BC_6H_5$), 4.49 (d, 1 H, $J_{C,D}$ = 11.3, CH_CH$_D$C$_6$H$_5$), 4.60 (d, 1 H, $J_{E,F}$ = 11.6, CH_EH$_F$C$_6$H$_5$), 4.64 (d, 1 H, CH$_C$$H_DC_6H_5$), 4.67 (d, 1 H, CH$_E$$H_FC_6H_5$), 4.68 (d, 1 H, $J_{G,H}$ = 11.3, CH_GH$_H$C$_6$H$_5$), 4.75 (d, 1 H, CH$_G$$H_HC_6H_5$), 7.22–7.36 (m, 20 H, H-Ar), ^{13}C NMR ($CDCl_3$) δ 60.8, 69.7, 70.8, 72.2, 73.1, 73.6, 74.2, 77.3, 79.0, 79.9, 127.6, 127.7, 128.0, 128.2, 128.3, 137.7, 137.8, 137.9.

2,3,4,6-Tetra-*O*-benzyl-1-*O*-trityl-D-galactitol

To a solution of 2,3,4,6-tetra-*O*-benzyl-D-galactitol (5.4 g, 10.0 mmol) in dry pyridine (17 cm^3) chlorotriphenylmethane (3.1 g, 11.0 mmol) was added. The reaction mixture was stirred at rt for 72 h, and it was then poured onto ice (200 cm^3). The resultant slurry was stirred for 2 h at 0 °C, and the aqueous layer was decanted from the syrup, which was then dissolved in chloroform (120 cm^3) and washed with aqueous acetic acid (10%, 30 cm^3), saturated $NaHCO_3$, brine, dried ($MgSO_4$), and concentrated under reduced pressure. The resultant residue was purified by flash column chromatography (hexane : EtOAc, 6 : 1 v/v) to give a light yellow syrup (5.8 g, 74%): $[\alpha]_D^{20}$ +22.5 (c = 1.32, CH_2Cl_2), IR 3529 cm^{-1} (br, O–H), ^{1}H NMR ($CDCl_3$) δ 3.23 (dd, 1 H, $J_{1,1'}$ = 10.3, $J_{1,2}$ = 4.9, H-1), 3.41 (dd, 1 H, $J_{6,6'}$ = 9.4, $J_{6,5}$ = 6.0, H-6), 3.45 (dd, 1 H, $J_{1',2}$ = 4.7, H-1′), 3.53 (dd, 1 H, $J_{6',5}$ = 6.4, H-6′), 3.69 (dd, 1 H, $J_{4,5}$ = 1.7, $J_{4,3}$ = 5.2, H-4), 3.84 (q, 1H, $J_{2,3} + J_{2,1} + J_{2,1'}$ = 14.5, H-2), 4.00 (dt, 1 H, H-5), 4.18 (t, 1 H, H-3), 4.31 (d, 1 H, $J_{A,B}$ = 11.7, CH_AH$_B$C$_6$H$_5$), 4.37 (d, 1 H, $J_{C,D}$ = 12.0, CH_CH$_D$C$_6$H$_5$), 4.42 (d, 1 H, CH$_C$$H_DC_6H_5$), 4.44 (d, 1 H, CH$_A$$H_BC_6H_5$), 4.51 (d, 1 H, $J_{E,F}$ = 11.7, CH_EH$_F$C$_6$H$_5$), 4.62 (d, 1 H, $J_{G,H}$ = 11.0, CH_GH$_H$C$_6$H$_5$), 4.66 (d, 1 H, CH$_E$$H_FC_6H_5$), 4.73 (d, 1 H, CH$_G$$H_HC_6H_5$), 7.09–7.44 (m, 35 H, H-Ar), ^{13}C NMR ($CDCl_3$) δ 63.3 (C-1), 70.2 (C-5), 71.5 (C-6), 72.8, 73.0, 73.4, 75.2 (4 × CH$_2$C$_6$H$_5$), 77.1 (C-4), 78.9 (C-2), 79.8 (C-3), 87.1 (CPh$_3$), 127.2, 127.7, 127.8, 127.9, 128.0, 128.2, 128.3, 128.4, 128.4$_4$, 128.8, 138.2, 138.2, 138.4, 138.5, 144.0. Found, C, 80.5; H, 6.6. $C_{53}H_{52}O_6$ requires C, 81.1; H, 6.7%.

(3*R*,4*R*,5*S*)-1,3,4,5-Tetrabenzyloxy-6-trityloxy-2-hexanone

Acetic anhydride (17.4 cm^3) was added to a solution of 2,3,4,6-tetra-*O*-benzyl-1-*O*-trityl-D-galactitol (6.7 g, 8.5 mmol) in dry DMSO (27 cm^3). The reaction mixture was stirred for 16 h at rt under N_2. Following the addition of water (40 cm^3) to the reaction mixture, aqueous NH_3 was added. The resultant mixture was extracted with Et_2O (3 × 30 cm^3) and the combined organic layers were washed with brine (60 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by flash column chromatograph (hexane : EtOAc, 9 : 1 v/v) to give a yellow syrup (5.72 g, 86%): $[\alpha]_D^{20}$ +0.17 (c = 0.60, CH_2Cl_2), IR 1731 cm^{-1} (C=O), ^{1}H NMR ($CDCl_3$) δ 3.33 (dd, 1 H, $J_{6,6'}$ = 10.4, $J_{5,6}$ = 5.3, H-6), 3.46 (dd, 1 H, $J_{5,6'}$ = 3.7, H-6′), 3.84 (m, 1 H, H-5), 3.88 (d, 1 H, $J_{3,4}$ = 3.9, H-3), 4.22 (dd, 1 H, $J_{4,5}$ = 5.7, H-4), 4.25–4.46 (m, 6 H, CH_2C$_6$H$_5$), 4.51 (d, 1 H, $J_{A,B}$ = 11.6, CH_AH$_B$C$_6$H$_5$), 4.58 (d, 1 H, CH$_A$$H_BC_6H_5$), 4.60 (br s, 2 H, H-1, H-1′), 7.01–7.44 (m, 35 H, H-Ar), ^{13}C NMR ($CDCl_3$) δ 63.3 (C-6), 72.4, 73.0, 73.0$_5$, 74.4 (4 × CH$_2$C$_6$H$_5$), 74.5 (C-1), 79.0 (C-5), 80.4 (C-4), 82.6 (C-3), 86.8 (CPh$_3$), 127.0, 127.4, 127.5, 127.6, 127.7, 127.7$_7$, 127.8, 127.9, 128.1, 128.2, 128.2$_6$, 128.2$_8$, 128.3, 128.4, 128.6, 137.3, 137.6, 137.9, 138.3, 143.9, 207.4 (C-2). Found, C, 80.95; H, 6.7. $C_{53}H_{50}O_6$ requires C, 81.3; H, 6.4%.

(3*S*,4*S*,5*S*)-3,4,5-Tribenzyloxy-2-((benzyloxy)methyl)-6-trityloxy-1-hexene (12)

To a solution of methyltriphenylphosphonium iodide (1.13 g, 2.8 mmol) in THF (10 cm^3), *n*-BuLi (2.5 M, 1.12 cm^3, 2.8 mmol) was added at −78 °C. After stirring the reaction at this temperature for 1 h, the mixture was warmed up to 0 °C, and a solution of the fully protected 2-hexanone (1.00 g, 1.28 mmol) in THF (5 cm^3) was added dropwise. The resulting mixture was allowed to warm to rt over a period of 16 h. The reaction was quenched by the addition of saturated NH_4Cl, and it was then diluted with water (20 cm^3) and extracted with CH_2Cl_2 (3 × 20 cm^3). The combined organic layers were washed with aqueous H_2SO_4 (10%), saturated $NaHCO_3$, brine, dried ($MgSO_4$), and concentrated at reduced pressure. The resultant crude syrup was purified by flash column chromatography (hexane : EtOAc, 9 : 1 v/v) to give the product as a light yellow syrup (0.784 g, 79%): $[\alpha]_D^{20}$ +22.5 (c = 1.32, CH_2Cl_2), IR 1650 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$) δ 3.25 (dd, 1 H, $J_{6,6'}$ = 10.0, $J_{6,5}$ = 5.6, H-6), 3.27 (dd, $J_{6',5}$ = 5.3, H-6′), 3.81 (br q, 1 H, $J_{6,5} + J_{6',5} + J_{5,4}$ = 14.7, H-5), 3.86 (dd, 1 H, $J_{4,5}$ = 3.9, $J_{4,3}$ = 7.1, H-4), 4.04–4.16 (m, 4 H, H-3, H-7, H-7′, CH_AH$_B$C$_6$H$_5$), 4.43 (d, 1 H, $J_{C,D}$ = 11.0, CH_CH$_D$C$_6$H$_5$), 4.47 (d, 1 H, $J_{B,A}$ = 11.6, CH$_A$$H_BC_6H_5$), 4.48 (d, 1 H, $J_{E,F}$ = 12.0, CH_EH$_F$C$_6$H$_5$), 4.49 (d, 1 H, $J_{G,H}$ = 11.6, CH_GH$_H$C$_6$H$_5$), 4.52 (d, 1 H, CH$_E$$H_FC_6H_5$),

4.57 (d, 1 H, $CH_CH_DC_6H_5$), 4.64 (d, 1 H, $CH_GH_HC_6H_5$), 5.13 (br s, 1 H, H-1), 5.44 (m, 1 H, H-1′), 7.07–7.43 (m, 35 H, H-Ar), ^{13}C NMR ($CDCl_3$) δ 63.7 (C-6), 70.4 (C-7), 71.0, 72.8, 73.7, 74.8 (4 × $CH_2C_6H_5$), 78.6 (C-5), 79.8 (C-3), 81.2 (C-4), 87.0 (CPh_3), 115.9 (C-1), 127.0, 127.4, 127.5, 127.6, 127.7, 127.8, 127.9, 128.1, 128.3, 128.3$_8$, 128.4, 128.8, 138.5, 138.5$_3$, 138.6, 139.0, 144.0 (C-2), 144.1. Found, C, 82.8; H, 6.8. $C_{54}H_{52}O_5$ requires C, 83.05; H, 6.7%.

(2*S*,3*S*,4*S*)-2,3,4-Tribenzyloxy-5-((benzyloxy)methyl)-5-hexen-1-ol

Aqueous HCl (1 M, 1 cm^3) was added to a solution of **12** (10.2 g, 13.1 mmol) in acetic acid (90%, 145 cm^3). After stirring this mixture for 40 min, the reaction was quenched by the addition of saturated $NaHCO_3$. The resultant solution was extracted with ether (3 × 50 cm^3) and the combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure. The crude product was purified by flash column chromatography (hexane : EtOAc, 3 : 1 v/v) to give a light yellow syrup (4.2 g, 61%): $[\alpha]_D^{20}$ + 22.8 (c = 0.71, CH_2Cl_2), IR 3470 cm^{-1} (br, O–H), ^{1}H NMR ($CDCl_3$) δ 3.59 (dd, 1 H, $J_{1,2}$ = 4.8, $J_{1,1'}$ = 11.7, H-1), 3.66 (dd, 1 H, $J_{1',2}$ = 4.5, H-1′), 3.70–3.76 (m, 2 H, H-2, H-3), 4.06–4.18 (m, 3 H, H-4, H-7, H-7′), 4.22 (d, 1 H, $J_{A,B}$ = 11.4, $CH_AH_BC_6H_5$), 4.48–4.62 (m, 7 H, $CH_2C_6H_5$), 5.45 (br s, 1 H, H-6), 5.55 (br s, 1 H, H-6′), 7.20–7.34 (m, 20 H, H-Ar), ^{13}C NMR ($CDCl_3$) δ 62.0 (C-1), 70.6 (C-7), 70.5, 72.8, 73.2, 74.4 (4 × $CH_2C_6H_5$), 79.7, 81.0 (C-3, C-2), 80.1 (C-4), 116.2 (C-6), 127.6, 127.7, 127.7, 127.9, 128.0, 128.3, 128.4, 138.0, 138.2, 138.3, 138.6, 143.7 (C-5). Found, C, 77.78; H, 6.87. $C_{35}H_{38}O_5$ requires C, 78.0; H, 7.1%.

(2*S*,3*S*,4*S*)-2,3,4-Tribenzyloxy-5-((benzyloxy)methyl)-5-hexenal

To a solution of oxalyl chloride (0.230 cm^3, 2 M in CH_2Cl_2, 0.46 mmol) in CH_2Cl_2 (2.6 cm^3) at −78 °C, DMSO (0.11 cm^3) was added slowly. This mixture was stirred for 10 min, and then a solution of the hexenol (206 mg, 0.38 mmol) in CH_2Cl_2 (1.5 cm^3) was added dropwise. After 10 min, triethylamine (0.26 cm^3) was added to the reaction mixture, which was kept at −78 °C for 40 min. The solvent was then evaporated under reduced pressure to give a light yellow syrup of aldehyde (195.6 mg), which was used in the next step without being purified further.

(3*R**,4*S*,5*S*,6*S*)-4,5,6-Tribenzyloxy-7-((benzyloxy)methyl)octa-1,7-dien-3-ol (13-*R* and 13-*S*)

To a solution of the freshly prepared aldehyde in dry THF (50 cm^3) at −78 °C under N_2 was slowly added a solution of vinylmagnesium bromide in THF (0.30 cm^3, 1.0 M, 0.30 mmol). The reaction mixture was then stirred for 1 h at rt, after which it was quenched by the addition of a saturated aqueous NH_4Cl. The resultant solution was extracted with EtO_2 (3 × 60 cm^3) and the combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure to give crude product as an orange coloured syrup. This material was purified by flash column chromatography (hexane : EtOAc, 4 : 1 v/v) to give a pale yellow syrup (129 mg, ratio **13-*R*** : **13-*S*** = 1 : 2, 60% over 2 steps): IR 3463 cm^{-1} (br, O–H). Found, C, 78.7; H, 7.05. $C_{37}H_{40}O_5$ requires C, 78.7; H, 7.1%. The NMR spectral assignments given below were made on the mixture of diastereomers.

Compound 13-*R*. ^{1}H NMR ($CDCl_3$): δ 2.66 (d, 1 H, $J_{OH,3}$ = 5.6, OH), 3.66 (t, 1 H, $J_{4,5} + J_{4,3}$ = 9.0, H-4), 3.80 (dd, 1 H, $J_{5,4}$ = 4.8, $J_{5,6}$ = 7.1, H-5), 4.05–4.18 (m, 3 H, H-6, H-9, H-9′), 4.24–4.31 (m, 2 H, H-3, $CH_AH_BC_6H_5$), 4.45–4.70 (m, 7 H, $CH_2C_6H_5$), 5.15 (dt, 1 H, $J_{1E,2}$ = 10.5, $J_{1E,1Z} + J_{1E,3}$ = 2.7, H-1$_E$), 5.26 (dt, 1 H, $J_{1Z,2}$ = 17.2, $J_{1Z,1E} + J_{1Z,3}$ = 2.9, H-1$_Z$), 5.44 (br s, 1 H, H-8), 5.56 (d, 1 H, $J_{8,8'}$ = 1.5, H-8′), 5.86 (m, 1 H, H-2), 7.36–7.21 (m, 20 H, H-Ar), ^{13} C NMR ($CDCl_3$) δ 70.5 (C-3), 81.1 (C-5), 82.1 (C-4), 116.3 (C-1), 116.4 (C-8), 138.2 (C-2), 143.8 (C-7).

Compound 13-*S*. ^{1}H NMR ($CDCl_3$): δ 2.62 (d, 1 H, $J_{OH,3}$ = 5.8, OH), 3.71 (dd, 1 H, $J_{4,5}$ = 3.1, $J_{4,3}$ = 5.5, H-4), 3.86 (dd, 1 H, $J_{5,6}$ = 8.0, H-5), 4.05–4.19 (m, 4H, H-6, H-9, H-9′, $CH_AH_BC_6H_5$), 4.41 (m, 1 H, H-3), 4.45–4.70 (m, 7 H, $CH_2C_6H_5$), 5.21 (dt, 1 H, $J_{1E,2}$ = 10.6, $J_{1E,1Z} + J_{1E,3}$ = 3.2, H-1$_E$), 5.37 (dt, 1 H, $J_{1Z,2}$ = 17.2, $J_{1Z,1E} + J_{1Z,3}$ = 3.3, H-1$_Z$), 5.47 (br s, 1 H, H-8), 5.58 (d, 1 H, $J_{6,8'}$ = 1.5, H-8), 5.94 (m, 1 H, H-2), 7.36–7.21 (m, 20 H, H-Ar), ^{13}C NMR ($CDCl_3$) δ 72.2 (C-3), 80.4 (C-5), 80.6 (C-4), 116.2 (C-1), 116.5 (C-8), 138.1 (C-2), 143.8 (C-7).

(1*R**,4*S*,5*S*,6*S*)-4,5,6-Tribenzyloxy-3-((benzyloxy)methyl)-cyclohex-2-enol (14-*R* and 14-*S*)

To a solution of the diastereomeric mixture of **13-*R*** and **13-*S*** (2.3 g, 4.1 mmol) in dry CH_2Cl_2 (1.1 L) was added second generation Grubbs' catalyst (174.3 mg, 0.21 mmol). This reaction mixture was heated to reflux for 3 h under N_2, and then the volatiles were removed under reduced pressure. The resultant residue was purified by flash column chromatography (hexane : EtOAc, 2 : 1 v/v) to give a colourless syrup. The two diastereomers were separated by radial chromatography (CH_2Cl_2 : EtOAc, 25 : 1 v/v) to give **14-*R*** (0.7 g, 32%) and **14-*S*** (1.2 g 54%) as colourless syrups.

Compound 14-*R*. $[\alpha]_D^{20}$ +15.8 (c = 1.61, CH_2Cl_2), IR 3420 cm^{-1} (br, O–H), ^{1}H NMR ($CDCl_3$): δ 3.64 (dd, 1 H, $J_{5,4}$ = 3.5, $J_{5,6}$ = 9.2, H-5), 3.88 (d, 1 H, $J_{7,7'}$ = 12.1, H-7), 3.96 (dd, 1 H, $J_{6,1}$ = 6.4, H-6), 4.08 (d, 1 H, H-7′), 4.14 (m, 1 H, H-1), 4.27 (d, 1 H, H-4), 4.38 (d, 1 H, $J_{A,B}$ = 11.8, $CH_AH_BC_6H_5$), 4.44 (d, 1 H, $CH_AH_BC_6H_5$), 4.58 (d, 1 H, $J_{C,D}$ = 11.3, $CH_CH_DC_6H_5$), 4.75 (d, 1 H, $J_{E,F}$ = 11.6, $CH_EH_FC_6H_5$), 4.78–4.81 (m, 2 H, $CH_2C_6H_5$), 4.89 (d, 1 H, $CH_CH_DC_6H_5$), 4.91 (d, 1 H, $CH_EH_FC_6H_5$), 5.76 (d, 1 H, $J_{2,1}$ = 2.7, H-2), 7.25–7.40 (m, 20 H, H-Ar), ^{13}C NMR ($CDCl_3$) δ 70.6 (C-7), 71.1 (C-1), 72.4 ($CH_AH_BC_6H_5$), 73.3 ($CH_GH_HC_6H_5$), 73.5 (C-4), 74.3 ($CH_EH_FC_6H_5$), 74.4 ($CH_CH_DC_6H_5$), 79.5 (C-5), 80.6 (C-6), 127.6, 127.6$_4$, 127.6$_6$, 127.7, 127.8, 128.0, 128.0$_1$ (C-2), 128.1, 128.3, 128.4, 128.4$_2$, 128.4$_8$, 135.4, 138.1, 138.4, 138.6, 138.7. Found, C, 78.0; H, 6.85. $C_{35}H_{36}O_5$ requires C, 78.3; H, 6.8%.

(3*S*,4*S*,5*S*,6*S*)-3-Azido-4,5,6-tribenzyloxy-1-((benzyloxy)methyl)-cyclohexene (15) and (3*S*,4*S*,5*R*,6*S*)-3-azido-4,5,6-tribenzyloxy-3-((benzyloxy)methyl)cyclohexene (16)

To a solution of **14-*R*** (754.2 mg, 1.4 mmol) in dry toluene (62 cm^3) was added diphenyl phosphoryl azide (1.8 cm^3, 8.4 mmol) followed by the slow addition of DBU (1.2 cm^3, 8.0 mmol) at 0 °C under a N_2 atmosphere. The reaction mixture was stirred for 1.5 h at 0 °C, after which sodium azide (374 mg, 5.8 mmol) and 15-crown-5 (0.6 cm^3, 3 mmol) were added. The reaction mixture was subsequently heated to 60 °C for 14 h. The resultant mixture was then diluted with EtOAc (120 cm^3), and washed with aqueous H_2SO_4 (10%), saturated aqueous $NaHCO_3$, brine, dried (Na_2SO_4),

and concentrated under reduced pressure. The syrupy residue was purified by flash column chromatography (hexane : EtOAc, 8 : 1 v/v) to give colourless syrups of **15** (600 mg, 76%), and the S_N2' tertiary azide product **16** (160 mg, 21%).

Compound 15. $[\alpha]_D^{20}$ +76.0 (c = 1.23, CH_2Cl_2), IR 2030 cm^{-1} (N_3), ^1H NMR ($CDCl_3$) δ 3.86 (dd, 1 H, $J_{5,6}$ = 3.5, $J_{5,4}$ = 8.1, H-5), 3.89 (d, 1 H, $J_{7,7'}$ = 12.9, H-7), 4.07–4.15 (m, 3 H, H-7′, H-3, H-4), 4.24 (d, 1 H, H-6), 4.39 (d, 1 H, $J_{A,B}$ = 11.9, CH_AH$_B$C$_6$H$_5$), 4.46 (d, 1 H, CH$_A$$H_BC_6H_5$), 4.52 (d, 1 H, $J_{C,D}$ = 11.3, CH_CH$_D$C$_6$H$_5$), 4.68–4.79 (m, 4 H, CH_2C$_6$H$_5$), 4.81 (d, 1 H, CH$_C$$H_DC_6H_5$), 5.72 (d, 1 H, $J_{2,3}$ = 3.7, H-2), 7.19–7.42 (m, 20 H, H-Ar), ^{13}C NMR ($CDCl_3$) δ 58.5 (C-3), 70.1 (C-7), 72.0 (CH$_A$H$_B$C$_6$H$_5$), 73.4 (CH$_2$C$_6$H$_5$), 73.8 (C-6), 73.8$_3$ (CH$_2$C$_6$H$_5$), 74.3 (CH$_C$H$_D$C$_6$H$_5$), 76.5 (C-4), 76.6 (C-5), 120.1, 120.1$_4$, 122.2 (C-2), 125.6, 127.6$_3$, 127.6$_5$, 127.7$_1$, 127.7$_3$, 127.7$_8$, 127.8, 127.9, 128.2, 128.3$_1$, 128.3$_8$, 128.4, 129.8, 138.0, 138.1, 138.4, 138.5, 139.0. Found, C, 74.5; H, 6.2; N, 7.3. $C_{35}H_{35}N_3O_4$ requires C, 74.8; H, 6.3; N, 7.5%.

Compound 16. IR 2099 cm^{-1}, ^1H NMR ($CDCl_3$) δ 3.65 (d, 1 H, $J_{7,7'}$ = 9.7, H-7), 3.69 (d, 1 H, H-7′), 3.91 (m, 1 H, H-4), 3.98 (dd, 1H, $J_{5,6}$ + $J_{5,4}$ = 9.6, H-5), 4.40 (m, 1 H, H-6), 4.52 (d, 1 H, $J_{A,B}$ = 12.0, CH_AH$_B$C$_6$H$_5$), 4.56 (d, 1 H, CH$_A$$H_BC_6H_5$), 4.59 (d, 1 H, $J_{C,D}$ = 11.2, CH_CH$_D$C$_6$H$_5$), 4.64–4.76 (m, 4 H, CH_2C$_6$H$_5$), 4.90 (d, 1 H, CH$_C$$H_DC_6H_5$), 5.61–5.67 (m, 1 H, H-2), 6.01 (dd, 1 H, $J_{1,2}$ = 10.0, $J_{1,6}$ = 2.2, H-1), 7.19–7.40 (m, 20 H, H-Ar).

(3*S*,4*S*,5*S*,6*S*)-3-Acetamido-4,5,6-tribenzyloxy-1-((benzyloxy)methyl)cyclohexene (17)

Through a solution containing **15** (77 mg, 0.14 mmol) in pyridine, Et_3N, and H_2O (4 : 1 : 1 v/v/v, 2 cm^3) H_2S gas was bubbled for 3 h at rt. Then N_2 gas was used to purge the aqueous reaction mixture, and this resulting mixture was concentrated under reduced pressure. The syrupy residue was dissolved in dry toluene (2.6 cm^3) that contained pyridine (0.260 cm^3). Acetyl chloride (0.052 cm^3) was then added slowly. The reaction mixture was stirred for 1 h at rt, after which it was diluted with EtOAc (10 cm^3), and washed with H_2SO_4 (10%), saturated aqueous $NaHCO_3$, brine, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by flash column chromatography (hexane : EtOAc, 2 : 1 v/v) to give **17** as a colourless syrup (63 mg, 80%): $[\alpha]_D^{20}$ +69.7 (c = 3.76, CH_2Cl_2), IR 3291 cm^{-1} (N–H), 1650 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 1.92 (s, 3 H, CH_3), 3.81 (dd, 1 H, $J_{5,4}$ + $J_{5,6}$ = 9.8, H-5), 3.88–3.93 (m, 2 H, H-7, H-4), 4.25 (d, 1 H, $J_{7',7}$ = 12.1, H-7′), 4.30 (br s, 1 H, H-6), 4.42 (d, 1 H, $J_{A,B}$ = 11.8, CH_AH$_B$C$_6$H$_5$), 4.43 (d, 1 H, $J_{C,D}$ = 11.8, CH_CH$_D$C$_6$H$_5$), 4.45 (d, 1 H, CH$_A$$H_BC_6H_5$), 4.48 (d, 1 H, $J_{E,F}$ = 11.5, CH_EH$_F$C$_6$H$_5$), 4.49 (d, 1 H, CH$_C$$H_DC_6H_5$), 4.60 (d, 1 H, CH$_E$$H_FC_6H_5$), 4.67 (d, 1 H, $J_{G,H}$ = 12.3, CH_GH$_H$C$_6$H$_5$), 4.71 (d, 1 H, CH$_G$$H_HC_6H_5$), 4.91–4.97 (m, 1 H, H-3), 5.63 (br s, 1 H, H-2), 6.73 (d, 1 H, $J_{NH,3}$ = 8.9, NH), 7.16–7.37 (m, 20 H, H-Ar), ^{13}C NMR ($CDCl_3$) δ: 23.4 (CH_3), 45.8 (C-3), 70.1 (C-7), 72.4 (CH$_E$H$_F$C$_6$H$_5$), 72.8 (CH$_C$H$_D$C$_6$H$_5$), 72.9 (CH$_G$H$_H$C$_6$H$_5$), 73.0 (CH$_A$H$_B$C$_6$H$_5$), 73.2 (C-6), 73.7 (C-5), 75.8 (C-4), 125.4 (C-2), 127.5, 127.6, 127.7, 127.8, 128.0, 128.0$_2$, 128.0$_5$, 128.0$_8$, 128.3, 128.4, 128.5, 135.9 (C-1), 137.8, 138.3, 138.3$_2$, 138.4, 169.4 (C=O). Found, C, 76.7; H, 7.0; N, 2.5. $C_{35}H_{39}NO_5$ requires C, 76.9; H, 6.8; N, 2.4%.

(1*R**,2*S*,3*S*,4*S*,5*S*,6*S**)-5-Acetamido-2,3,4-tribenzyloxy-1-((benzyloxy)methyl)bicyclo[4.1.0]heptane (18: 1*R*,6*S*) and (19: 1*S*,6*R*)

Under N_2, a solution of compound **17** (523 mg, 0.91 mmol) in dry toluene (125 cm^3) was cooled down to −10 °C. Dimethylzinc (2 M in toluene, 8.2 cm^3, 16.3 mmol) was added dropwise. The reaction was stirred at −10 °C for 15 min, then CH_2I_2 (1.68 cm^3, 2.083 mmol) was added slowly. The reaction mixture was stirred for 16 h while being allowed to warm up to 20 °C. The reaction was quenched by the addition of aqueous H_2SO_4 (10%: 4 cm^3), and subsequently EtOAc (200 cm^3) was added. The resulting organic layer was washed with saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$), and concentrated under reduced pressure. The two cyclopropyl isomers were separated and purified by radial chromatography (CH_2Cl_2 : MeOH 200 : 1 v/v) to give colourless syrups of **18** and **19** (total weight 470 mg, 88%).

Compound 18. $[\alpha]_D^{20}$ +67.2 (c = 0.661, CH_2Cl_2), ^1H NMR ($CDCl_3$) δ 0.63–0.71 (m, 2 H, H-7, H-7′), 1.33 (m, 1 H, H-6), 1.92 (s, 3 H, CH_3), 2.72 (d, 1 H, $J_{8,8'}$ = 9.5, H-8), 3.54 (dd, 1 H, $J_{3,2}$ = 2.6, $J_{3,4}$ = 6.8, H-3), 3.95 (t, 1 H, $J_{4,5}$ + $J_{4,3}$ = 13.7, H-4), 4.09 (d, 1 H, H-8′), 4.29 (d, 1 H, H-2), 4.38–4.42 (d, 2 H, CH_2C$_6$H$_5$), 4.45 (d, 1 H, $J_{A,B}$ = 12.0, CH_AH$_B$C$_6$H$_5$), 4.49 (d, 1 H, $J_{C,D}$ = 11.5, CH_CH$_D$C$_6$H$_5$), 4.60 (d, 1 H, $J_{E,F}$ = 11.3, CH_EH$_F$C$_6$H$_5$), 4.65 (d, 1 H, $J_{G,H}$ = 11.9, CH_GH$_H$C$_6$H$_5$), 4.74 (m, 1 H, H-5), 4.76 (d, 1 H, CH$_G$$H_HC_6H_5$), 4.83 (d, 1 H, CH$_E$$H_FC_6H_5$), 6.00 (d, 1 H, $J_{5,NH}$ = 7.1, NH), 7.18–7.37 (m, 20 H, H-Ar), ^{13}C NMR ($CDCl_3$) δ 13.8 (C-7), 20.2 (C-6), 23.6 (CH_3), 26.1 (C-1), 45.4 (C-5), 72.6, 73.1, 73.3 (3 × CH$_2$C$_6$H$_5$), 74.3 (C-8), 74.7 (CH$_2$C$_6$H$_5$), 75.0 (C-2), 77.1 (C-4), 79.3 (C-3), 127.5, 127.6$_2$, 127.6$_7$, 127.7, 127.9$_5$, 127.9$_7$, 128.0, 128.4, 128.5, 128.6, 138.0, 138.4, 138.6, 139.2, 169.8 (C=O). Found, C, 76.9; H, 7.1; N, 2.65. $C_{38}H_{41}NO_5$ requires C, 77.1; H, 7.0; N, 2.4%.

Compound 19. $[\alpha]_D^{20}$ +18.7 (c = 1.20, CH_2Cl_2), ^1H NMR ($CDCl_3$) δ 0.63 (dd, 1 H, $J_{6,7}$ = 9.4, $J_{7,7'}$ = 5.1 H-7), 0.91–0.96 (m, 1 H, H-6), 1.28 (t, 1 H, $J_{7',6}$ + $J_{7',7}$ = 10.8, H-7′), 1.93 (s, 3 H, CH_3), 2.81 (d, 1 H, $J_{8,8'}$ = 10.2, H-8), 3.66 (m, 1 H, H-4), 3.75–3.80 (m, 2 H, H-3, H-8′), 4.32 (d, 1 H, $J_{2,3}$ = 4.3, H-2), 4.37–4.43 (m, 3 H, H-5, 2 × CH_2C$_6$H$_5$), 4.45–4.60 (m, 6 H, CH_2C$_6$H$_5$), 6.10 (d, 1 H, $J_{NH,5}$ = 8.5, NH), 7.22–7.36 (m, 20 H, H-Ar), ^{13}C NMR ($CDCl_3$) δ 13.1 (C-7), 22.7 (C-6), 23.6 (CH_3), 24.2 (C-1), 45.4 (C-5), 72.6 (CH$_2$C$_6$H$_5$), 71.9 (C-2), 72.5, 72.6, 72.7 (3 × CH$_2$C$_6$H$_5$), 74.8 (C-3), 75.9 (C-8), 76.8 (C-4), 127.6, 127.7, 127.8, 128.0, 128.1, 128.4, 128.5, 128.7, 138.0, 138.3, 138.6, 138.7, 169.6 (C=O). Found, C, 77.2; H, 7.0; N, 2.1. $C_{38}H_{41}NO_5$ requires C, 77.1; H, 7.0; N, 2.4%.

(1*R*,2*S*,3*S*,4*S*,5*S*,6*S*)-5-Acetamido-1-(hydroxymethyl)bicyclo-[4.1.0]heptane-2,3,4-triol (8)

A mixture of **18** (467 mg, 0.79 mmol) and 10% Pd–C (217 mg) in MeOH (90 cm^3) was stirred at room temperature under an atmosphere of H_2 for 14 h. The mixture was filtered through a Celite pad, which was washed thoroughly with MeOH (50 cm^3). The filtrate and washings were combined and concentrated under pressure to give a colourless oil. This material was purified by flash column chromatography (CH_2Cl_2–MeOH, 4 : 1 v/v) to give compound **8** (151 mg, 83%) as a colourless syrup: $[\alpha]_D^{20}$ +92.7 (c = 0.52, MeOH), ^1H NMR (D_2O) δ 0.58–0.70 (m, 2 H, H-7, H-7′),

1.35 (m, 1 H, H-6), 2.01 (s, 3 H, CH_3), 2.89 (d, 1 H, $J_{8,8'} = 11.5$, H-8), 3.48 (dd, 1 H, $J_{3,4} = 10.0$, $J_{3,2} = 3.0$, H-3), 3.91 (dd, 1 H, $J_{4,5} = 7.3$, H-4), 4.0 (d, 1 H, H-8′), 4.34 (d, 1 H, H-2), 4.60 (t, 1 H, H-5), ^{13}C NMR (D_2O) δ 10.9 (C-7), 19.9 (C-6), 22.0 (CH_3), 28.0 (C-1), 46.5 (C-5), 65.9 (C-8), 66.6 (C-4), 68.6 (C-2), 68.8 (C-3), 174.3 (C=O). Found, C, 51.6; H, 7.6; N, 6.1. $C_{10}H_{17}NO_5$ requires C, 51.9; H, 7.4; N, 6.1%.

(1*S*,2*S*,3*S*,4*S*,5*S*,6*R*)-5-Acetamido-1-(hydroxymethyl)bicyclo-[4.1.0]heptane-2,3,4-triol (10)

A mixture of **19** (533 mg, 0.90 mmol) and 10% Pd–C (247 mg) in MeOH (100 cm^3) was stirred at rt under an atmosphere of H_2 for 14 h. The reaction mixture was filtered through a Celite pad, which was then washed thoroughly with MeOH (50 cm^3). The filtrate and washings were combined and concentrated under pressure to give a colourless syrup. The resultant crude product was purified by flash column chromatography (CH_2Cl_2–MeOH, 4 : 1 v/v) to yield **10** (185 mg, 88%) as a colourless syrup: $[\alpha]_D^{20}$ +64.5 ($c = 0.53$, H_2O), ^{1}H NMR (D_2O) δ 0.60 (dd, 1 H, $J_{7,7'} = 5.7$, $J_{7,6} = 9.8$, H-7), 0.83 (t, 1 H, $J_{7',6} + J_{7,7'} = 11.7$, H-7′), 1.10 (ddd, 1 H, $J_{6,5} = 1.4$, H-6), 2.03 (s, 3 H, CH_3), 3.06 (d, 1 H, $J_{8,8'} = 11.7$, H-8), 3.65–3.67 (m, 2 H, H-3, H-4), 3.71 (d, 1H, H-8′), 4.42 (m, 1 H, H-2), 4.48 (br s, 1 H, H-5), ^{13}C NMR (D_2O) δ 9.7 (C-7), 22.2 (CH_3), 22.6 (C-6), 27.6 (C-1), 48.7 (C-5), 66.0 (C-4), 68.1 (C-2), 68.2 (C-8), 69.2 (C-3), 174.4 (C=O). Found, C, 52.2; H, 7.5; N, 5.9. $C_{10}H_{17}NO_5$ requires C, 51.9; H, 7.4; N, 6.1%.

(1*R*,2*S*,3*S*,4*S*,5*S*,6*S*)-5-Amino-1-(hydroxymethyl)bicyclo-[4.1.0]heptane-2,3,4-triol (7)

LiOH (32 mg, 0.76 mmol) was added to a solution of amide **8** (82 mg, 0.35 mmol) in THF–H_2O (1 : 1, 12 cm^3). This mixture was heated at 70 °C for 32 h. The solution was then neutralized by the addition of Amberlite IR resin (H^+ form), and filtered. The resin was washed thoroughly with water and the product was eluted with NH_4OH (5%, 100 cm^3). The eluted solution was concentrated under pressure to give a light yellow oil. This substance was further purified by flash chromatography (MeOH–$CHCl_3$–28% NH_3 in H_2O 5 : 5 : 1 v/v/v) to give **7** as a colourless syrup (35 mg, 52%): $[\alpha]_D^{20}$ +76.5 ($c = 0.51$, MeOH), ^{1}H NMR (CD_3OD) δ 0.67 (dd, 1 H, $J_{7,7'} = 5.5$, $J_{7,6} = 9.3$, H-7), 0.75 (m, 1 H, H-7′), 1.33 (m, 1 H, H-6), 3.41 (d, 1 H, $J_{8,8'} = 11.3$, H-8), 3.54 (dd, 1 H, $J_{3,4} = 8.9$, $J_{3,2} = 3.1$, H-3), 3.74 (d, 1 H, H-8′), 3.82 (t, 1 H, $J_{5,4} + J_{5,6} = 13.9$, H-5), 3.93 (dd, 1 H, $J_{4,5} = 6.6$, H-4), 4.30 (d, 1 H, H-2), ^{13}C NMR (CD_3OD) δ 11.6 (C-7), 20.8 (C-6), 29.1 (C-1), 48.7 (C-5), 67.6 (C-8), 68.5 (C-4), 70.5 (C-3), 70.5$_2$ (C-2). Found 190.1074 (ESI-HRMS) $C_8H_{16}NO_4$ (M + H^+) requires 190.1079.

(1*S*,2*S*,3*S*,4*S*,5*S*,6*R*)-5-Amino-1-(hydroxymethyl)bicyclo-[4.1.0]heptane-2,3,4-triol (9)

LiOH (15 mg, 0.36 mmol) was added to a solution of amide **10** (30 mg, 0.13 mmol) in THF–H_2O (1 : 1, 4 cm^3). This mixture was heated at 70 °C for 32 h, when it was neutralized by adding Amberlite IR resin (H^+ form). After filtration the resin was washed thorough by water. The product was eluted using NH_4OH (5%, 20 cm^3) and the eluent was concentrated under pressure to give a light yellow oil. This material was purified by flash chromatography (MeOH–$CHCl_3$–28% NH_3 in H_2O 5 : 5 : 1 v/v/v) to give **9** as a colourless syrup (20.7 mg, 84%): $[\alpha]_D^{20}$ +72.7 ($c = 0.85$, MeOH : H_2O 1 : 1 v/v), ^{1}H NMR (CD_3OD) δ 0.54 (dd, 1 H, $J_{7,7'} = 5.2$, $J_{7,6} = 9.7$, H-7), 0.92 (t, 1 H, $J_{6,7'} + J_{7,7'} = 11.3$, H-7′), 1.05 (m, 1 H, H-6), 2.99 (d, 1 H, $J_{8,8'} = 11.2$, H-8), 3.49 (m, 1 H, H-5), 3.61 (dd, 1 H, $J_{4,5} = 4.2$, $J_{4,3} = 9.2$, H-4), 3.66 (dd, 1 H, $J_{3,2} = 4.7$, H-3), 3.79 (d, 1 H, H-8′), 4.37 (d, 1 H, H-2), ^{13}C NMR (CD_3OD) δ 9.2 (C-7), 22.3 (C-6), 28.0 (C-1), 49.5 (C-5), 66.1 (C-4), 66.5 (C-2), 68.0 (C-8), 68.9 (C-3). Found 190.1074 (ESI-HRMS) $C_8H_{16}NO_4$ (M + H^+) requires 190.1079.

Enzyme kinetics

The activity of both the coffee bean and *E. coli* enzymes were assayed by monitoring the rate of hydrolysis of *p*-nitrophenyl α-D-galactopyranoside (PNPG). For the measurement of K_i values 4 different concentrations of substrate and 4 of inhibitor were used. Whereas, for IC_{50} values a single concentration of PNPG was utilized and this was 0.14 and 0.01 mM for the coffee bean and *E. coli* enzymes, respectively. The corresponding Michaelis constants (K_m) for the PNPG substrate are 0.7 and 0.04 mM for the coffee bean and *E. coli* enzymes, respectively.

The coffee bean α-galactosidase activity assay solutions contained 0.62 mU cm^{-3} enzyme in 50 mM sodium phosphate buffer (pH 6.52) with 0.1% bovine serum albumin in total volume of 0.400 cm^3. Each experiment was initiated by the addition of enzyme to an equilibrated assay solution held at 37 °C.

The activity of the *E. coli* enzyme was assayed using a solution that comprised of 5 mU cm^{-3} enzyme containing 0.1% bovine serum albumin in 50 mM sodium phosphate buffer (pH 7.26), the total volume of the assay solution was 0.400 cm^3. Each analysis began by the addition of a stock solution of enzyme to an equilibrated assay solution held at 25 °C.

For both enzymes, the absorbance at 400 nM was monitored for 10 min using a Cary 3E spectrophotometer equipped with a Peltier temperature controller. The measured initial rate *versus* inhibitor concentration data were fit to standard enzyme kinetic equations using a nonlinear least squares program (Prism).

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