Enzymes

Exploration of Strategies for Mechanism-Based Inhibitor Design for Family GH99 *endo*-α-1,2-Mannanases

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Abstract: *endo*-α-1,2-Mannosidases and -mannanases, members of glycoside hydrolase family 99 (GH99), cleave α-Glc/Man-1,3-α-Man-OR structures within mammalian *N*-linked glycans and fungal α-mannan, respectively. They are proposed to act through a two-step mechanism involving a 1,2-anhydrosugar "epoxide" intermediate incorporating two conserved catalytic carboxylates. In the first step, one carboxylate acts as a general base to deprotonate the 2-hydroxy group adjacent to the fissile glycosidic bond, and the other provides general acid assistance to the departure of the aglycon. We report herein the synthesis of two inhibitors designed to interact with either the general base (α-mannosyl-1,3-(2-aminodeoxymannojirimycin), Man2NH_2DMJ) or the general acid (α-mannosyl-1,3-mannoimidazole, ManManIm). Modest affinities were observed for an *endo*-α-1,2-mannanase from *Bacteroides thetaiotaomicron*. Structural studies revealed that Man2NH_2DMJ binds like other iminosugar inhibitors, which suggests that the poor inhibition shown by this compound is not a result of a failure to achieve the expected interaction with the general base, but rather the reduction in basicity of the endocyclic nitrogen caused by introduction of a vicinal, protonated amine at C2. ManManIm binds with the imidazole headgroup distorted downwards, a result of an unfavourable interaction with a conserved active site tyrosine. This study has identified important limitations associated with mechanism-inspired inhibitor design for GH99 enzymes.

Introduction

Glycoside hydrolases of the carbohydrate-active enzyme (see www.cazy.org; www.cazypedia.org)[1,2] family GH99 are *endo*-acting mannosidases that cleave α-mannoside linkages within mammalian high mannose *N*-glycans (*endo*-α-1,2-mannosidases)[3–7] and fungal α-mannans (*endo*-α-1,2-mannanases, Figure 1 A).[8,9] Inhibitor design for these enzymes is driven by their potential use to understand glycoprotein biosynthesis and maturation in the secretory pathway, and to manipulate fungal mannan degradation processes in the human gut microbiota. Structural and mechanistic studies of family GH99 enzymes suggest that they utilise an unusual mechanism involving neighbouring group participation by the substrate 2-hydroxy to form a 1,2-anhydrosugar intermediate.[10] In this proposed mechanism, a conserved active site residue acts as a general base to deprotonate the 2-OH group, thereby facilitating its nucleophilic attack on C1 (Figure 1 A). This process has little biological precedent (for a related proposal see Ref. [11]), but occurs in the base-promoted solvolysis of α-mannosides.[12]

Efforts to develop inhibitors of GH99 enzymes have relied upon appending 1,3-linked α-glucosyl (to target mammalian *endo*-α-1,2-mannosidases) or 1,3-linked α-mannosyl (to target bacterial *endo*-α-1,2-mannanases) groups to various sugar-shaped heterocycles. Spiro and co-workers reported the discovery of α-glucosyl-1,3-deoxymannojirimycin (GlcDMJ) as an effective inhibitor of the mammalian enzyme,[13,14] and follow-on studies by Fleet and co-workers revealed α-mannosyl-1,3-deoxymannojirimycin (ManDMJ) to be a slightly weaker inhibitor for this enzyme (Figure 1 B).[15] The potency of GlcDMJ was subsequently exceeded by α-glucosyl-1,3-isofagomine (GlcIFG).[10,16] Equivalent results have been noted for bacterial GH99 enzymes, which led to the development of α-mannosyl-1,3-isofagomine (ManIFG; dissociation constant, $K_D = 0.14$ μM for *Bacteroides thetaiotaomicron* GH99).[8] Furthermore, reintroduction of the "missing" 2-OH of 1,3-isofagomine (IFG) into ManIFG gave α-mannosyl-1,3-noeuromycin (ManNOE), which was shown to be five-fold more potent towards the *B. thetaiotaomicron* GH99 enzyme ($K_D = 0.03$ μM).[17] These compounds bind in a ground-state $^4C_1$ conformation, as seen in complexes of inactive enzyme with substrate and thus proposed for the

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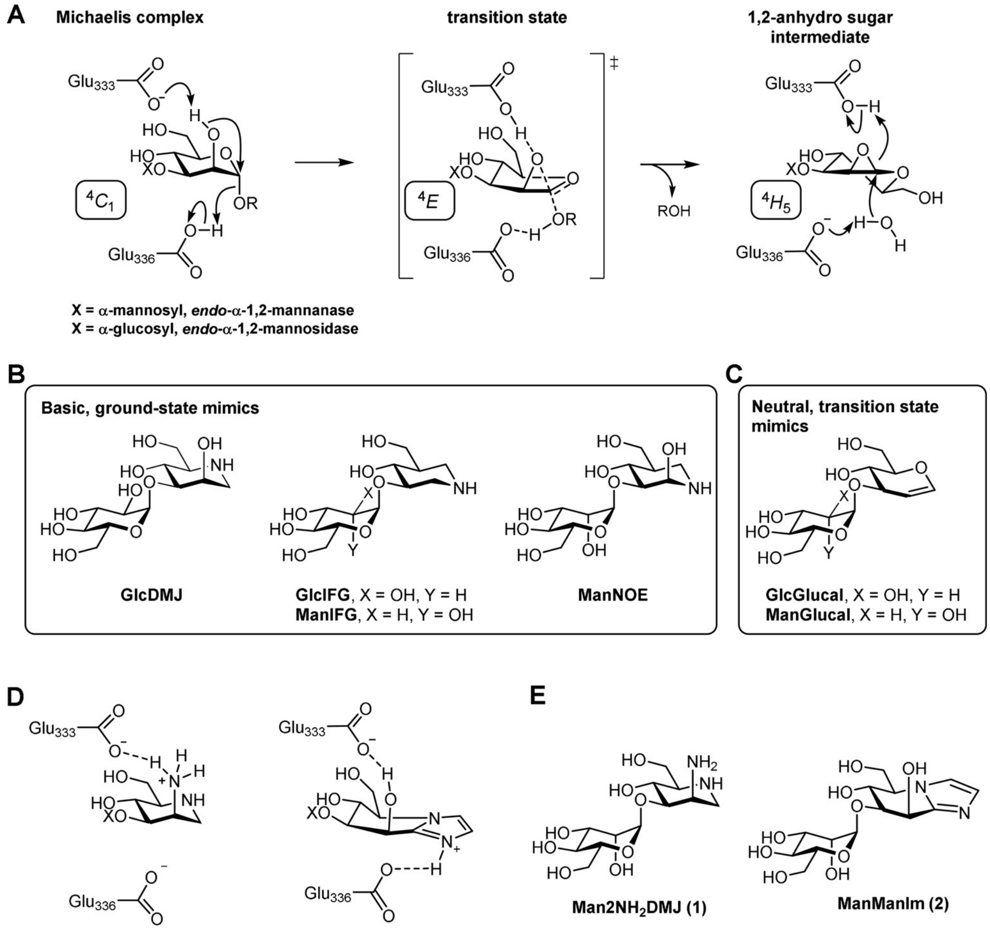

Figure 1. (A) Proposed mechanism for family GH99 enzymes retaining endomannosidases/endomannanases. Only the first half of the catalytic cycle is shown. (B) Saturated basic heterocyclic inhibitors for GH99 enzymes mimicking the ground state conformation. (C) Neutral glycal inhibitors for GH99 enzymes mimicking the transition state. (D) Two inhibitor design concepts explored herein. (E) Structures of Man2NH$_2$DMJ (**1**) and ManManIm (**2**).

conformation of substrate within the Michaelis complex (Figure 1 A), which suggests that potent inhibition of GH99 enzymes can be achieved simply by mimicry of the charge in the transition state.[17]

Separately, Spiro and co-workers showed that the neutral compound GlcGlucal (Figure 1 C) was a modest inhibitor of mammalian GH99 (rat Golgi preparation, $IC_{50}=2.3$ μм; for GlcDMJ $IC_{50}=1.7$ μм);[14,18] the equivalent molecule targeting bacterial GH99, ManGlucal, was also a ligand with mildly potent affinity ($K_D=15$ μм for *Bt*GH99).[17] Computational free-energy landscape analysis of the preferred conformation of D-glucal suggested that the inhibition of the glucal-based inhibitors arises from mimicry of the proposed ^4E conformation of the transition state or the proposed $^4H_5$ conformation of the 1,2-anhydro sugar intermediate, but with no contribution from charge mimicry owing to the neutral nature of this compound.[17]

We report here our efforts to explore two new inhibitor design strategies for the inhibition of GH99 enzymes. Considering the role of the basic residue implicated in the 1,2-anhydrosugar mechanism of GH99 enzymes, we speculated that introduction of an amino group into the structure of ManDMJ to give Man-2NH$_2$DMJ (**1**; Figure 1 E) could promote the formation of a favourable ionic interaction upon inhibitor binding (Figure 1 D). Separately, the glycoimidazole class of inhibitors were developed following the discovery of the natural product nagstatin,[19] and are believed to derive their potency from their ability to mimic the shape of the oxocarbenium-like transition state as well as from the ability of the imidazole glycosidic nitrogen to engage in a hydrogen bond with an appropriately situated carboxylate residue in the active site (Figure 1 D).[20] For the present work, this would require the synthesis of ManManIm (**2**; Figure 1 E). Thus, we report herein on the synthesis of these two target inhibitors, the structural characterisation of their binding modes and measurement of their binding constants.

Results and Discussion

Synthesis of Man2NH$_2$DMJ and ManManIm

Man2NH$_2$DMJ (**1**) was prepared by substitution of known tosylate **3**[21] with sodium azide in DMF to afford azide **4** (Scheme 1). Coupling of azide **4** with trichloroacetimidate **5**[22] under the agency of TfOH afforded the disaccharide **6** in a yield of 83 %. The deprotection of **6** was achieved in a stepwise

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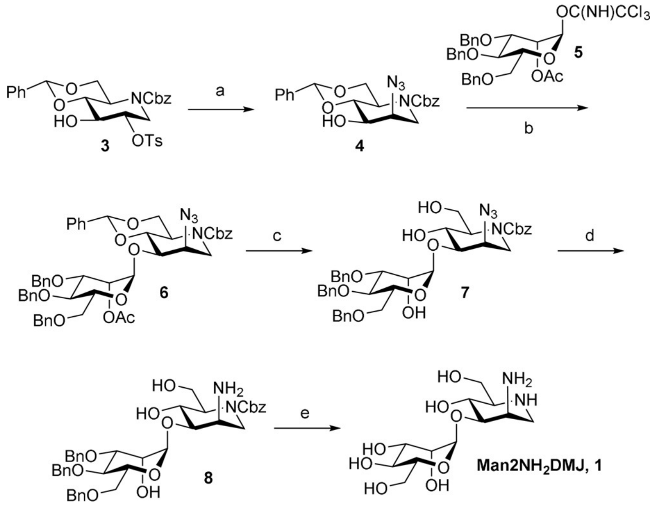
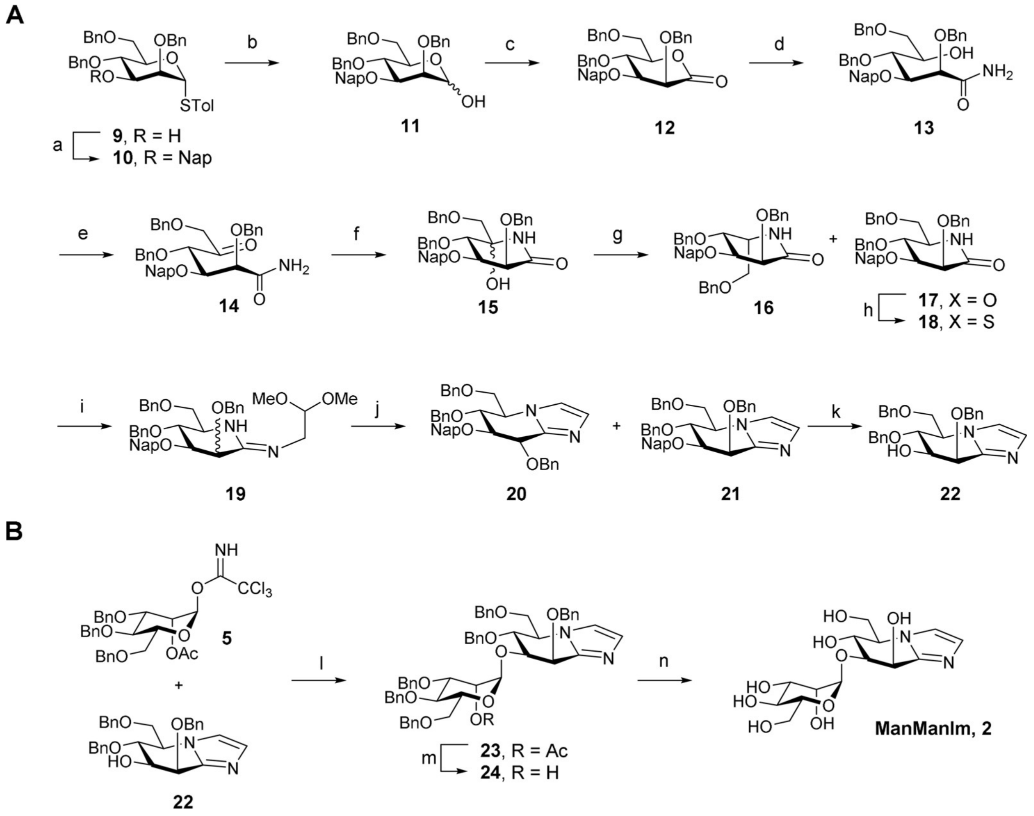


Scheme 1. Reagents and conditions: a) NaN_3, DMF, reflux, 74 %; b) TfOH, CH_2Cl_2, −30 to 0 °C, 87 %; c) i. NaOMe, MeOH, ii. 9:1 TFA/H_2O, 83 %; d) DTT, pyr, pH 9.2 $NaHCO_3/Na_2CO_3$, 80 %; e) H_2, $Pd(OH)_2$/C, aq. HCl, 2:2:1 EtOAc/MeOH/H_2O, 70 %.

manner, as attempts to perform a global deprotection that involved simultaneous removal of benzyloxycarbonyl (Cbz), benzylidene and benzyl ethers as well as the reduction of the azide was unsuccessful. Deacetylation of **6** (NaOMe/MeOH) and then hydrolysis of the benzaldehyde acetal (TFA/H_2O) afforded triol **7**. The azide group was reduced with dithiothreitol (DTT)/pyridine buffer to afford amine **8**. Removal of the Cbz and benzyl groups then proceeded smoothly by using H_2 and Pearlman's catalyst to afford **1**.

ManManIm (**2**) was synthesised through a sequence involving the preparation of the protected mannoimidazole alcohol **22**, followed by elaboration to the disaccharide (Scheme 2). The known alcohol **9**[23] was treated with 2-naphthylmethyl bromide (NapBr)/NaH in DMF to afford **10**. Hydrolysis of the thioglycoside with *N*-iodosuccinimide (NIS) in H_2O/acetone gave the hemiacetal **11**, which was oxidised to the lactone **12** under Albright–Goldman conditions.[24] For the conversion of the lactone **12** to the lactam **17** we followed the protocol developed by Overkleeft et al.,[25] which involved aminolysis to the acyclic amide **13**, Albright–Goldman oxidation (→**14**) and ring closure promoted by ammonia/MeOH (→**15**). Reduction of the hemiaminals **15** with $NaCNBH_3$ afforded a 2:1 mixture of the D-*manno* and L-*gulo* lactams, from which the D-*manno* lactam **17** was isolated in a yield of 38 %. Conversion of the lactam to the thionolactam **18** was achieved by using Lawesson's reagent and pyridine in toluene. Annulation of the imidazole ring was achieved by following the general approach of Vasella and co-workers.[26] Reaction of the thionolactam **18** with aminoacetaldehyde dimethyl acetal afforded the amidine **19**, and imidazole ring formation was achieved by catalysis with TsOH to provide a mixture of D-*gluco* and D-*manno* imida-



Scheme 2. A) Preparation of imidazole alcohol **22**. Reagents and conditions: a) NapBr, NaH, DMF, 86 %; b) NIS, H_2O, acetone, 0 °C, 99 %; c) DMSO, Ac_2O; d) NH_3, THF, reflux; e) DMSO, Ac_2O; f) NH_3, MeOH, 88 % over steps c–f; g) HCO_2H, $NaBH_3(CN)$, 38 % D-*manno*, 33 % L-*gulo*; h) Lawesson's reagent, pyridine, 4 Å molecular sieves, toluene, 93 %; i) $H_2NCH_2CH(OMe)_2$; j) TsOH·H_2O, toluene, 60 °C, yields over steps i and j: 42 % D-*gluco*, 32 % D-*manno*; k) DDQ, CH_2Cl_2/H_2O, 67 %. B) Synthesis of ManManIm (**2**). Reagents and conditions: l) TfOH, 4 Å molecular sieves, toluene, −20 °C, 47 %; m) K_2CO_3/MeOH, 46 %; n) H_2 (34 bar), $Pd(OH)_2$/C, AcOH, EtOAc, MeOH, H_2O, 48 %.

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zoles in a 2:1 ratio, from which the D-*manno* imidazole **21** was isolated in a yield of 32% over two steps. The naphthylmethyl group was removed under the agency of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and CH_2Cl_2/H_2O to afford the alcohol **22**.

Coupling of **22** with trichloroacetimidate **5**[22] catalysed by TfOH afforded the disaccharide **23** in a yield of 47%. Deprotection was achieved in two steps under conditions chosen to avoid epimerisation at C2. Treatment of **23** with K_2CO_3/MeOH afforded the alcohol **24**, and hydrogenation with Pearlman's catalyst afforded **2**.

Binding affinities and 3D structures

Isothermal titration calorimetry (ITC) was used to assess the binding of **1** and **2** to a bacterial endomannosidase. Titration of *Bt*GH99 with $Man2NH_2DMJ$ (**1**) revealed binding with $K_D = 97.7 \pm 4.9$ μM (Figure 2), whereas no binding with ManManIm (**2**) was evident by ITC. Placed in context, **1** has a poorer binding affinity towards *Bt*GH99 than GlcDMJ ($K_D = 24$ μM);[10] the equivalent data is not available for ManDMJ, but as this enzyme prefers to bind Man-configured substrates, the difference would be expected to be even greater.

Three-dimensional structures were obtained for **1** and **2** bound to the *endo*-α-1,2-mannanase *Bx*GH99 from *Bacteroides xylanisolvens*, which is closely related to *Bt*GH99 but more amenable to complex formation. These complexes diffracted to a resolution of 1.1 and 1.3 Å, respectively (Table 1). Occupancy of the active site for the complex with **1** was essentially complete, whereas that with **2**, with prolonged soaking, was estimated to be 80%, likely a consequence of the poor affinity of the compound for the enzyme. As predicted, both compounds bound in the −2/−1 subsites of the enzyme (sub-site nomenclature from Ref. [27]) and will be discussed in turn.

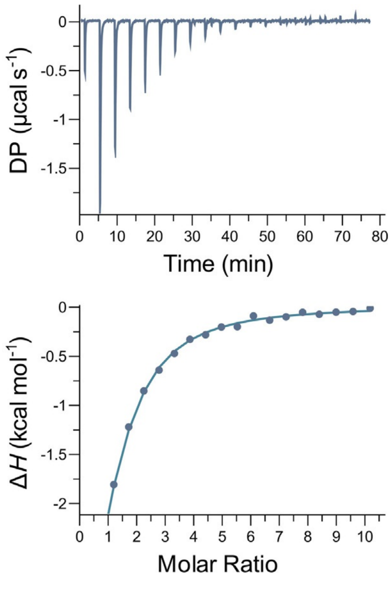

Figure 2. Isothermal titration calorimetry thermogram showing the binding of $Man2NH_2DMJ$ (**1**) to *Bacteroides thetaiotaomicron endo*-α-1,2-mannanase (*Bt*GH99). DP = differential power. Binding parameters $K_D = 97.7 \pm 4.9$ μM, $N = 1$ (fixed) and $\Delta H = -5.9 \pm 0.1$ kcal mol^{-1}.

Table 1. Data collection and refinement statistics for the complexes of *Bx*GH99 with **1** and **2**.

	*Bx*GH99 complexed with aminoDMJ (**1**)	*Bx*GH99 complexed with ManManIm (**2**)
	Data collection	
Space group	*I*4	*I*4
Cell dimensions		
a [Å]	108.1	108.6
b [Å]	108.1	108.6
c [Å]	67.5	67.8
α [°]	90	90
β [°]	90	90
γ [°]	90	90
resolution [Å]	76.44–1.13 (1.15–1.13)[a]	76.81–1.30 (1.32–1.30)[a]
R_{merge}	0.069 (1.501)	0.054 (1.224)
R_{pim}	0.026 (0.735)	0.020 (0.610)
CC(1/2)	0.999 (0.400)	(0.999) 0.486
I/σ*I*	10.2 (1.0)	14.0 (0.9)
completeness [%]	99.1 (86.0)	99.5 (92.7)
redundancy	7.5 (4.8)	7.5 (4.6)
	Refinement	
resolution [Å]	76.44–1.13	76.81–1.30
no. reflections all/free	143544/7133	96144/4810
R_{work}/R_{free}	0.122/0.144	0.134/0.162
no. atoms		
protein	3188	3146
ligand/ion	22	25
water	467	427
B factors [Å^2]		
protein	17.2	20.5
ligand/ion	20.3	22.4
water	35.1	36.7
r.m.s. deviations		
bond lengths [Å]	0.0101	0.011
bond angles [°]	1.495	1.497
PDB ID	6FAM	6FAR

[a] Values in parentheses are for the highest-resolution shell.

Structural analysis of the *Bx*GH99–**1** complex (Figure 3A) revealed the piperidine ring in a $^4C_1$ conformation, which matches that seen for complexes of the wild-type enzyme with GlcDMJ and isofagomine-based inhibitors[8,10,17] as well as that of a disabled mutant with substrate.[8] The 2-amino group is situated appropriately to interact with the E333 residue, that which is proposed to act as a general base/acid through deprotonation of the 2-hydroxy group. Overlay of this complex with that of *Bx*GH99–GlcDMJ reported previously[10] revealed that the positioning and conformations of the rings in the −1 and −2 sub-sites are essentially identical, and that no amino acid residues undergo significant movement (Figure 3C). In particular, the E333···O2 and E333···N2 distances are 2.54 and 2.59 Å, respectively. The poor binding affinity of **1** compared with GlcDMJ therefore does not result from incorrect binding of the inhibitor, and must instead reflect a failure to fully capitalise on the proposed interactions. It is widely acknowledged that iminosugars such as DMJ (and thus GlcDMJ) achieve inhibition through binding to glycosidases in their protonated

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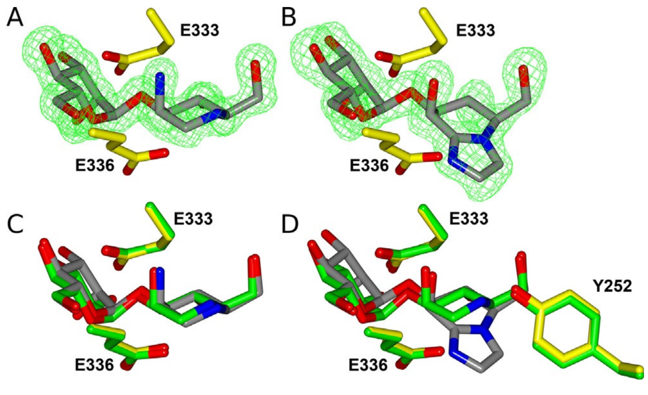

Figure 3. Three-dimensional structures of *Bt*GH99 complexed with A) Man2NH_2DMJ (**1**) and B) ManManIm (**2**). Electron density maps are maximum likelihood/σ_A weight F_o-F_c difference syntheses contoured at 0.5 and 0.3 e$Å^{-3}$ for panels A and B, respectively, visible before refining the structure model with the ligand added. (C) Overlay of Man2NH_2DMJ (**1**) with GlcDMJ (PDB code 4FAM). (D) Overlay of ManManIm (**2**) with GlcDMJ (PDB code 4FAR).

form;[28] this is supported by first-principles consideration of the basicity of these inhibitors and the relevant pK_a values of the catalytic residues, as well as by studies of the pH dependence of inhibition. In the case of **1**, there are two basic nitrogen residues. However, for vicinal diamines, protonation at one nitrogen has a profound effect on the pK_a value at the second nitrogen; in acyclic systems this effect has been estimated to be $\Delta pK_a = 3.6$ units for NH_3^+ and NR_3^+.[29] Moreover, in cyclic systems there are stereoelectronic and conformational contributions, notable examples for various diamines (pK_{a1}, pK_{a2}) include piperazine (9.8, 5.7),[29] *cis*-1,3-diaminocyclohexane (10.3, 8.3)[30] and *trans*-1,3-diaminocyclohexane (10.4, 8.5).[30] Finally, vicinal hydroxy groups can also perturb amine pK_a values; in Man2NH_2DMJ, O4 is antiperiplanar with respect to the endocyclic nitrogen and would be expected to reduce its basicity by around 1.3 pK_a units.[30] Collectively, this analysis would suggest that N2 is protonated by the general acid E333, and that it is unlikely that the dication is formed, and therefore Man2NH_2DMJ fails to appropriately mimic an oxocarbenium-like transition state. A related example of this phenomenon was reported in which introduction of a second amine vicinal to a pre-existing one in apramycin resulted in a dramatic loss of binding to a bacterial ribosome of approximately 100-fold.[31] Additionally, the proposed binding mode of **1** shown in Figure 1 D highlights the fact that the 2-amino group has additional hydrogen substituents that may cause an energy penalty upon binding of the inhibitor.

Structural analysis of the *Bx*GH99–**2** complex revealed the piperidine ring of the mannoimidazole moiety to be in an unusual $^2H_3/E_3$ conformation (Figure 3 B).[32] Overlay of the complex with that of *Bx*GH99–GlcDMJ[10] revealed that although the −2 sugar residues occupy similar positions, the mannoimidazole headgroup is atypically positioned such that the heterocycle projects downward into the active site, below the plane of the piperidine ring of the GlcDMJ complex (Figure 3 D). In this case the E336···N (imidazole ring) distance is 2.65 Å, similar to that seen in related glycoimidazole complexes.[33] In the original formulation by Heightman and Vasella, β-equatorial glycosidases were proposed to perform protonation from the side, in what was termed “lateral protonation”, with the acid either on the same side as the endocyclic oxygen (*syn*) or opposed to it (*anti*).[20] In a subsequent publication Nerinckx et al. formalised this concept by dividing the space around the −1 sugar into *anti* and *syn* hemispheres through a plane defined by the glycosidic oxygen, C1 and H1 of the sugar residue.[34] Analysis of complexes of various *anti*-protonating glycosidases revealed that the acid/base or acid residues responsible for protonating the leaving group are in fact not universally located lateral to the mean plane of the sugar, but are more commonly positioned above or below it, so as to better protonate the leaving group oxygen. However, this does not prevent glycoimidazoles binding in normal orientations and engaging in hydrogen-bonding interactions with the imidazole nitrogen. For example, in the case of the retaining GH116 β-glucosidase from *Thermoanaerobacterium xylanolyticum*, the acid/base is positioned above the mean plane of the sugar, but a normal orientation and conformation of glucoimidazole was observed.[35] Mannoimidazole also binds in the normal fashion to an inverting GH47 α-mannosidase from *Caulibacter* sp. in which the acid is below the mean plane of the inhibitor, but instead the inhibitor establishes an interaction with another conserved active site carboxylic acid that lies lateral to the imidazole.[36] *Bx*GH99 is an *anti*-protonating enzyme with its general acid/base Glu336 positioned below the plane of the ring to facilitate classical *anti* protonation of the axial glycosidic oxygen (O5-C1-O1 angle is approximately 60°). The distorted mode of binding of the mannoimidazole moiety of **2** seems to be a consequence of the imidazole binding to maximise this interaction with the acid/base. Close examination of the active site of *Bx*GH99 revealed that if the ManIm moiety were to be shifted up to the same position as that of the piperidine of GlcDMJ, a steric interaction would result with Tyr252, a conserved residue. In fact, the distance between the imidazole C= C bond and Tyr252 Cε is only 3.2 Å, which causes the wwPDB validation software[37] to report H/H steric clashes in this region. In fact, a ternary complex of GlcDMJ and α-1,2-mannobiose highlighted the fact that the active site of the enzyme involves a sharp bend in the −1 and +1 sub-sites. The failure of **2** to bind in a typical position in the −1 sub-site is thus likely a result of a failure to accommodate the imidazole ring owing to the location of Tyr252.

Conclusions

We have reported here the design and synthesis of two “mechanism-based” inhibitors of family GH99 endomannanases. Although Man2NH_2DMJ (**1**) bound to the bacterial endomannanase *Bx*GH99 in the expected manner, its affinity for *Bt*GH99 did not exceed that seen for GlcDMJ. This appears to be a result of the perturbing effect of the 2-amino substituent, which reduces the basicity of the endocyclic nitrogen and its ability to be protonated in the active site and thereby resemble the oxocarbenium-like transition state. On the other hand, the binding of ManManIm (**2**) to *Bt*GH99 could not be detect-

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ed by ITC and, consistent with this, the X-ray structure of **2** complexed with *Bx*GH99 displayed incomplete occupancy. The poor binding of this inhibitor appears to be a consequence of an inability of the active site of *Bx*GH99 to accommodate the annulated imidazole ring because of an interaction with a conserved Tyr active-site residue. This study provides important insights that will inform future strategies for the development of mechanism-inspired and transition-state mimicking inhibitors of GH99 enzymes.

Experimental Section

General: ^{1}H and ^{13}C NMR spectra were recorded by using 400, 500 or 600 MHz Varian INOVA spectrometers. All signals were referenced to TMS (δ = 0.00 ppm) or solvent peaks ($CDCl_3$: δ = 7.26 ppm for ^{1}H and 77.16 ppm for ^{13}C; D_2O: δ = 4.80 ppm for ^{1}H and TMS: δ = 0.00 ppm for ^{13}C; [D_4]MeOH: δ = 3.49 ppm for ^{1}H and δ = 49.0 ppm for ^{13}C). Melting points were obtained by using a Reichert-Jung hot-stage apparatus. TLC analysis was performed with aluminium-backed Merck Silica Gel 60 F254 sheets, detection was achieved by using UV light, 5% H_2SO_4 in MeOH or ceric ammonium molybdate ("Hanessian's stain") with charring as necessary. Flash chromatography was performed by using Geduran silica gel according to the method of Still et al.[38] Dry CH_2Cl_2, THF and Et_2O were obtained from a dry solvent apparatus (Glass Contour of SG Water, Nashua).[39] DMF and DMSO were dried over 4 Å molecular sieves.

2-Azido-4,6-*O*-benzylidene-*N*-benzyloxycarbonyl-1,2,5-trideoxy-1,5-imino-D-mannitol (4): Sodium azide (57.8 mg, 0.890 mmol) was added to a solution of 4,6-*O*-[(*R*)-benzylidene]-*N*-benzyloxycarbonyl-1,5-dideoxy-2-*O*-(*p*-toluenesulfony1)-D-glucitol[21] (**3**; 120 mg, 0.222 mmol) in DMF (1 mL). The suspension was heated at reflux for 18 h, poured into ice, extracted into EtOAc (3×20 mL), washed with brine (2×20 mL), dried over anhydrous $MgSO_4$ and evaporated to dryness. Column chromatography (AcOEt/pet. ether 40-60, 1:5) gave the azide **4** (67.7 mg, 74%) as a white solid. $[\alpha]_D^{24}$ = −21.9 (*c* = 1.12 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 2.74 (s, 1H; NH), 2.82 (dd, *J* = 1.6, 14.5 Hz, 1H; 1-H$_a$), 3.06 (td, *J* = 4.6, 10.2 Hz, 1H; 5-H), 3.74 (dd, *J* = 3.8, 9.2 Hz, 1H; 3-H), 3.79–3.93 (m, 2H; 2,4-H), 4.31 (dd, *J* = 3.0, 14.5 Hz, 1H; 1-H$_b$) 4.46 (t, *J* = 11 Hz, 1H; 6-H$_a$), 4.66 (dd, *J* = 4.6, 11.6 Hz, 1H; 6-H$_b$), 5.01 (d, *J* = 3.1 Hz, 2H; CH_2), 5.48 ppm (s, 1H; CH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 48.1, 55.8, 60.1, 67.8, 69.2, 73.6, 78.2 (7C; C1–C6, CH_2), 101.8 (1C; CH), 126.3, 128.3, 128.4, 128.5, 128.7, 129.4, 136.0, 137.3 (12C; Ph), 155.0 ppm (1C; C=O); HRMS (ESI, +ve): *m/z* calcd for $C_{21}H_{22}N_4O_5$: 411.1663 $[M+H]^+$; found: 411.1664.

2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-azido-4,6-*O*-benzylidene-*N*-benzyloxycarbonyl-1,2,5-trideoxy-1,5-imino-D-mannitol (6): TfOH (0.043 μL, 0.0049 mmol) was added to a mixture of acceptor **4** (20 mg, 0.049 mmol) and 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate (**5**;[22] 37 mg, 0.058) in CH_2Cl_2 over 4 Å sieves at −30 °C, The mixture was stirred for 30 min, warmed to 0 °C and quenched with Et_3N (7 μL, 0.05 mmol) and then concentrated under reduced pressure. Flash chromatography (EtOAc/pet. ether, 25:75) gave the disaccharide **6** (37.4 mg, 87%) as a colourless oil. $[\alpha]_D^{24}$ = −4.2 (*c* = 0.89 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 2.80 (dd, $J_{1,1}$ = 14.4, $J_{1,2}$ = 0.9 Hz, 1H; 1-H$_a$), 3.15 (dt, *J* = 10.1, 4.6 Hz, 1H; 5-H), 3.70–4.00 (m, 6H; 3,4,4′,5′-H, 6″-H$_a$, 6′-H$_b$), 4.03 (dd, *J* = 9.3, 3.4 Hz, 1H; 3′-H), 4.17–4.20 (m, 1H; 2-H), 4.28 (dd, *J* = 14.5, 2.2 Hz, 1H; 1-H$_b$), 4.47–4.52 (m, 3H; 3×C*H*$_2$Ph), 4.60–4.64 (m, 2H; 6-H$_a$, C*H*$_2$Ph), 4.69 (d, *J* = 11 Hz, 1H; C*H*$_2$Ph), 4.76 (dd, *J* = 11.6, 4.5 Hz, 1H; 6-H$_b$), 4.86 (d, *J* = 11 Hz, 1H; C*H*$_2$Ph), 5.12 (d, *J* = 3.6 Hz, 2H; CH_2), 5.28 (d, *J* = 1.6 Hz, 1H; 1′-H), 5.59 (dd, *J* = 3.3, 1.8 Hz, 1H; 2′-H), 5.64 (s, 1H; CH), 7.17–7.46 ppm (m, 25H; Ph); ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 48.3 (1C; C-1), 56.3 (1C; C-5), 60.0, 72.7, 74.4, 77.8 (4C; C-3,4,4′,5), 67.7 (1C; CH_2), 68.5 (1C; C-2′), 69.1 (1C; C-6), 69.3 (1C; C-6′), 72.2, 73.6, 75.1 (3C; C*H*$_2$Ph), 78.1 (1C; C-2), 78.2 (1C; C-3′), 99.5 (1C; C-1′), 100.90 (1C; CH), 100.92, 126.0, 127.77, 127.79, 127.83, 127.9, 128.0, 128.2, 128.28, 128.29, 128.41, 128.44, 128.5, 128.7, 128.9 ppm (30C; Ph); HRMS (ESI, +ve): *m/z* calcd for $C_{50}H_{52}N_4O_{11}$: 907.3525 $[M+Na]^+$; found: 907.3544.

3,4,6-Tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-azido-*N*-benzyloxycarbonyl-1,2,5-trideoxy-1,5-imino-D-mannitol (7): A solution of sodium methoxide in methanol (0.1 M, 10 μL, 1 μmol) was added to **6** (60 mg, 0.068 mmol) in methanol (0.5 mL) and the mixture was stirred for 1 h and then concentrated under reduced pressure to give an alcohol, which was used without purification. TFA/H_2O (9:1, 100 μL) was added to the crude alcohol and the mixture was stirred for 30 min, concentrated and azeotroped with toluene (3×10 mL). Flash chromatography (EtOAc/pet. ether, 9:1) gave the triol **7** (42.5 mg, 83%,). $[\alpha]_D^{25}$ = 44.6 (*c* = 1.03 in MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ = 2.67–4.20 (13H; 1-H$_a$–6-H$_b$, 2′-H–6′-H$_b$), 4.43–4.46 (m, 2H; 2×C*H*$_2$Ph), 4.52 (d, *J* = 12.0 Hz, 1H; C*H*$_2$Ph), 4.70 (d, *J* = 12.7 Hz, 1H; C*H*$_2$Ph), 4.72 (d, *J* = 11.2 Hz, 1H; C*H*$_2$Ph), 4.89 (d, *J* = 2.1 Hz, 1H; 1′-H), 5.12 (s, 2H; CH_2), 5.15 (app. s, 1H; 1′-H), 7.03–7.42 ppm (m, 20H; 4×Ph); ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 59.5, 68.0, 68.9, 69.0, 71.9, 72.5, 73.5, 74.2, 74.9, 79.5 (13C; C-1,2,3,4,5,6,1′,2′,3′,4′,5′,6′, CH_2) 127.8, 127.9, 128.0, 128.1, 128.16, 128.19, 128.4, 128.5, 128.6, 128.7, 137.9, 138.0, 138.3 (24C; Ph), 156.5 ppm (1C; C=O); HRMS (ESI, +ve): *m/z* calcd for $C_{41}H_{46}N_4O_{10}$: 755.3287 $[M+H]^+$; found: 755.3300.

3,4,6-Tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-amino-*N*-benzyloxycarbonyl-1,2,5-trideoxy-1,5-imino-D-mannitol (8): DTT (51 mg, 0.331 mmol) was added to a solution of azide **7** (25 mg, 0.0331 mmol) in pyridine (1 mL) and $NaHCO_3/H_2CO_3$ buffer (0.625 mL, pH 9.16). The mixture was stirred at room temperature for 4 h, concentrated and azeotroped with toluene (5×10 mL). Flash chromatography (EtOAc/MeOH/H_2O, 94:4:2) gave the amine **8** (80%, 19.2 mg). ^{1}H NMR (500 MHz, CD_3OD): δ = 2.89 (t, *J* = 12.4 Hz, 1H; 2-H), 3.21–4.13 (13C; m, 1-H$_a$, 1-H$_b$, 3,5-H, 6-H$_a$, 6-H$_b$, 1′–6$_b$′-H), 4.36 (t, *J* = 7.8 Hz, 1H; 4-H),4.46–4.54 (m, 2H; 2×C*H*$_2$Ph), 4.58 (d, *J* = 12.0 Hz, 1H; C*H*$_2$Ph), 4.66 (d, *J* = 11.8 Hz, 1H; C*H*$_2$Ph), 4.77–4.81 (m, 2H; 2×C*H*$_2$Ph), 4.98 (d, *J* = 2.5 Hz, 1H; 1′-H), 5.15 (s, 2H; CH_2), 7.16–7.47 ppm (m, 20H; Ph); ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 46.8, 59.9, 65.6, 68.5, 69.4, 70.4, 72.6, 73.7, 74.4, 75.4, 75.7, 78.1, 80.1, 100.8 (16C; C-1–6, C1′–6′, 4×CH_2), 128.81, 128.84, 129.2, 129.28, 128.30, 129.3, 129.4, 129.5, 138.0, 139.3, 139.5, 139.6 ppm (24C; Ph); HRMS (ESI, +ve): *m/z* calcd for $C_{41}H_{48}N_2O_{10}$: 729.3385 $[M+H]^+$; found: 729.3398.

α-D-Mannopyranosyl-(1→3)-2-amino-1,2,5-trideoxy-1,5-imino-D-mannitol (1): The triol **8** (19.2 mg, 0.0264 mmol) in EtOAc/MeOH/H_2O (2:2:1, 3 mL) and 10% HCl in methanol (0.3 mL) was treated with $Pd(OH)_2$/C (50 mg) and H_2 (20 atm, 18 h). The suspension was filtered, concentrated and purified with cation and anion resin (eluted with aqueous NH_3) to give ManNH$_2$DMJ (**1**; 70%, 6.02 mg) as a colourless oil. $[\alpha]_D^{25}$ = 17.2 (*c* = 0.08 in H_2O); ^{1}H NMR (500 MHz, D_2O): δ = 2.78–2.84 (m, 1H; 5-H), 3.09 (dd, $J_{1a,1b}$ = 14.0, $J_{1a,2}$ = 2.1 Hz, 1H; 1-H$_a$), 3.25 (dd, $J_{1a,1b}$ = 14.0, $J_{1a,2}$ = 3.2 Hz, 1H; 1-H$_b$), 3.62–3.95 (m, 9H; 2,3,4,4′,5′-H, 6-H$_a$, 6′-H$_a$, 6-H$_b$, 6′-H$_b$), 3.98 (dd, $J_{3',4'}$ = 9.2, $J_{2',3'}$ = 4.3 Hz, 1H; 3′-H), 4.09 (dd, $J_{2',3'}$ = 3.3, $J_{1',2'}$ = 1.8 Hz, 1H; 2′-H), 5.24 ppm (d, $J_{1',2'}$ = 1.6 Hz, 1H; 1′-H); ^{13}C NMR (125 MHz, D_2O): δ = 44.5, 50.4, 60.0, 60.8, 61.0, 66.6, 67.3, 69.7, 70.1, 73.7, 77.3, 101.6 ppm; HRMS (ESI, +ve): *m/z* calcd for $C_{12}H_{24}N_2O_8$: 325.1605 $[M+H]^+$; found: 325.1606.

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4-Methylphenyl 2,4,6-tri-*O*-benzyl-3-*O*-(2-naphthylmethyl)-1-thio-α-D-mannopyranoside (10): A dry solution of the alcohol **9**[23] (167 mg, 0.30 mmol) in DMF (5 mL) was cooled to 0 °C. The solution was charged with NaH (60% dispersion in mineral oil, 36 mg, 0.9 mmol) and the mixture stirred for 30 min. 2-Bromomethylnaphthalene (79.6 mg, 0.36 mmol) was added and the mixture stirred overnight. The mixture was diluted with Et_2O (20 mL), poured into ice/water and washed with water (3×20 mL) and brine (1×20 mL). The organic extracts were dried ($MgSO_4$), the solvent was removed under reduced pressure and the resulting residue was subjected to flash chromatography (EtOAc/pet. ether, 15:85) to give the protected thioglycoside **10** (179.3 mg, 86%) as a colourless oil. $[\alpha]_D^{24}=+65$ (c=0.69 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ=2.28 (s, 3H; TolMe), 3.78 (dd, $J_{5,6a}$=1.8, $J_{6a,6b}$=10.9 Hz, 1H; 6-H_a), 3.87 (dd, $J_{5,6b}$=5.2, $J_{6a,6b}$=10.9 Hz, 1H; 6-H_b), 3.97 (dd, $J_{2,3}$=3.0, $J_{3,4}$=9.3 Hz, 1H; 3-H), 4.04 (dd, $J_{1,2}$=3.0, $J_{2,3}$=1.8 Hz, 1H; 2-H), 4.11 (m, 1H; 4-H), 4.33 (ddd, $J_{4,5}$=9.8, $J_{5,6a}$=5.1, $J_{5,6b}$=1.6 Hz, 1H; 5-H), 4.49 (d, J=11.9 Hz, 1H; CH_2Ph), 4.57–4.67 (m, 3H; 3×CH_2Ph), 4.74 (m, 3H; CH_2Ph, 2×CH_2Nap), 4.96 (d, J=10.9 Hz, 1H; CH_2Ph), 5.58 (d, $J_{1,2}$=1.5 Hz, 1H; 1-H), 7.02 (app. d, J=7.9 Hz, 2H; Tol), 7.21–7.37 (m, 17H; 3×Ph, Tol), 7.44–7.47 (m, 3H; Nap), 7.74–7.83 ppm (m, 4H; Nap); ^{13}C NMR (125 MHz, $CDCl_3$): δ=21.2 (1C; TolMe), 69.3 (1C; C-6), 71.9 (1C; CH_2Ph), 72.2 (1C; CH_2Nap), 72.8 (1C; C-5), 73.3 (1C; CH_2Ph), 75.1 (1C; C-4), 75.2 (1C; CH_2Ph), 76.3 (1C; C-2), 80.3 (1C; C-3), 86.1 (1C; C-1), 125.9–126.5 (4C; Nap), 127.5–128.4 (18C; 3×Ph, Nap), 129.8 (2C; Tol), 132.3 (2C; Tol), 133.4, 135.8, 137.6, 138.0, 138.5, 138.6 ppm (6C; C_q); HRMS (ESI, +ve): m/z calcd for $C_{45}H_{44}O_5S$: 719.2802 $[M+Na]^+$; found: 719.2809.

2,4,6-Tri-*O*-benzyl-3-*O*-(2-naphthylmethyl)-α-D-mannopyranose (11): *N*-Iodosuccinimide (216 mg, 0.961 mmol) was added to a solution of the thioglycoside **10** (447 mg, 0.641 mmol) in acetone (1% aq., 10 mL) at 0 °C and left to stir for 2.5 h. The solution was quenched with aq. $Na_2S_2O_3$ (0.5 M, 10 mL), diluted with EtOAc (20 mL) and washed with aq. $Na_2S_2O_3$ (0.5 M, 3×20 mL), $NaHCO_3$ (2×20 mL) and brine (1×20 mL). The organic extracts were dried ($MgSO_4$), the solvent was removed under reduced pressure and the resulting residue was subjected to flash chromatography (EtOAc/pet. ether/Et_3N, 30:69.5:0.5) to afford the hemiacetals **11** (344 mg, 91%; α/β 3.3:1) as a white powder. α anomer: ^{1}H NMR (500 MHz, $CDCl_3$): δ=3.69 (dd, $J_{5,6a}$=6.6, $J_{6a,6b}$=10.5 Hz, 1H; 6-H_a), 3.74 (dd, $J_{5,6b}$=2.0, $J_{6a,6b}$=10.4 Hz, 1H; 6-H_b), 3.83 (dd, $J_{1,2}$=2.0, $J_{2,3}$=2.8 Hz, 1H; 2-H), 3.91 (t, $J_{3,4}=J_{4,5}$=9.6 Hz, 1H; 4-H), 4.05 (dd, $J_{2,3}$=3.0, $J_{3,4}$=9.4 Hz, 1H; 3-H), 4.10 (ddd, $J_{4,5}$=8.7, $J_{5,6a}$=5.8, $J_{5,6b}$=1.9 Hz, 1H; 5-H), 4.51–4.59 (m, 3H; 3×CH_2Ph), 4.74–4.76 (m, 4H; 2×CH_2Ph, 2×CH_2Nap), 4.94 (d, J=11.0 Hz, 1H; CH_2Ph), 5.27 (d, $J_{1,2}$=1.8 Hz, 1H; 1-H), 7.18–7.41 (m, 17H; 3×Ph), 7.45–7.47 (m, 3H; Nap), 7.72–7.83 ppm (m, 4H; Nap); ^{13}C NMR (125 MHz, $CDCl_3$): δ=69.7 (1C; C-6), 71.4 (1C; C-5), 72.2 (1C; CH_2Nap), 72.7 (1C; CH_2Ph), 73.3 (1C; CH_2Ph), 75.1 (1C; CH_2Ph), 75.1 (1C; C-2), 75.3 (1C; C-4), 79.8 (1C; C-3), 92.6 (1C; C-1), 125.8–126.3 (4C; Nap), 127.6–128.5 (18C; 3×Ph, Nap), 133.0, 133.4, 136.1, 138.0, 138.5 ppm (6C; C_q); HRMS (ESI, +ve): m/z calcd for $C_{38}H_{38}O_6$: 608.3007 $[M+NH_4]^+$; found: 608.3007.

2,4,6-Tri-*O*-benzyl-3-*O*-(2-naphthylmethyl)-D-mannonolactone (12): A solution of the hemiacetal **11** (742 mg, 1.26 mmol) in acetic anhydride (6.1 mL) and dry DMSO (6.6 mL) was stirred under N_2 for 22 h. The mixture was diluted with EtOAc (20 mL), quenched with ice and washed with water (3×20 mL) and brine (1×20 mL). The organic extracts were dried ($MgSO_4$) and the solvent was evaporated. Azeotropic toluene was used to remove any residual AcOH to afford the crude lactone **12** (823 mg), which was used directly in the next step. A portion of **12** obtained from a separate experiment was purified by flash chromatography (EtOAc/pet. ether, 1:9) to yield analytically pure **12** as a colourless oil. $[\alpha]_D^{25}=+4.05$ (c=0.44 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ=3.61 (m, 2H; 6-H_a, 6-H_b), 3.80 (dd, $J_{2,3}$=1.5, $J_{3,4}$=7.2 Hz, 1H; 3-H), 4.09 (dd, $J_{1,2}$=2.6, $J_{2,3}$=1.6 Hz, 1H; 2-H), 4.23 (m, 2H; 5-H, 4-H), 4.38 (d, J=2.6 Hz, 1H; CH_2Ph), 4.48 (app. d, 2H; 2×CH_2Ph), 4.56 (d, J=11.8 Hz, 1H; CH_2Ph), 4.77 (d, J=12.5 Hz, 1H; CH_2Ph), 4.94 (d, J=12.5 Hz, 1H; CH_2Ph), 5.06 (m, 2H; 2×CH_2Nap), 6.96–7.45 (m, 18H; 3×Ph, Nap), 7.69–7.78 ppm (m, 4H; Nap); ^{13}C NMR (125 MHz, $CDCl_3$): δ=69.0 (1C; C-6), 71.6 (1C; C-4), 72.8 (1C; CH_2Ph), 72.9 (1C; CH_2Nap), 73.3 (1C; CH_2Ph), 75.5 (1C; CH_2Ph), 75.8 (1C; C-3), 76.5 (1C; C-2), 78.4 (1C; C-5), 125.9–126.1 (3C; Nap), 126.9 (1C; Nap), 127.6–128.9 (18C; 3×Ph, Nap), 132.9, 133.0, 135.0, 136.7, 137.3, 137.6 (6C; C_q), 169.3 ppm (1C; C=O); HRMS (ESI, +ve): m/z calcd for $C_{38}H_{36}O_6$: 606.2850 $[M+NH_4]^+$; found: 606.2853.

2,4,6-Tri-*O*-benzyl-3-*O*-(2-naphthylmethyl)-D-mannonamide (13): A dry-ice/acetone cold finger cooling trap was used to condense ammonia (50 mL) into a solution of the crude lactone **12** (823 mg) in dry THF (30 mL) at −78 °C. The solution was allowed to reflux at 0 °C for 4 h. The mixture was then evaporated to dryness to afford the crude amide **13** (771 mg), which was used directly in the next step. A portion obtained from an independent experiment was purified by flash chromatography (EtOAc/pet. ether, 3:2) to yield analytically pure **13** as a yellow solid. M.p. 120 °C; $[\alpha]_D^{25}=+7.21$ (c=0.41 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ=3.20 (d, $J_{5,OH}$=6.2 Hz, 1H; OH), 3.61 (m, 2H; 6-H_a, 6-H_b), 3.87 (dd, $J_{3,4}$=5.9, $J_{4,5}$=7.3 Hz, 1H; 4-H), 3.98 (m, 1H; 5-H), 4.13 (dd, $J_{2,3}$=3.5, $J_{3,4}$=5.8 Hz, 1H; 3-H), 4.33 (d, $J_{2,3}$=3.5 Hz, 1H; 2-H), 4.43–4.60 (m, 6H; 6×CH_2Ph), 4.82 (s, 2H; 2×CH_2Nap), 5.50 (brs, 1H; NH), 6.54 (brs, 1H; NH), 7.11–7.27 (m, 15H; 3×Ph), 7.38–7.43 (m, 3H; Nap), 7.68–7.76 ppm (m, 4H; Nap); ^{13}C NMR (125 MHz, $CDCl_3$): δ=71.1 (1C; C-5), 71.4 (1C; C-6), 72.9 (1C; CH_2Ph), 73.6 (1C; CH_2Ph), 74.6 (1C; CH_2Ph), 75.0 (1C; CH_2Nap), 79.1 (1C; C-4), 80.2 (1C; C-2), 81.6 (1C; C-3), 126.0–126.3 (3C; Nap), 126.9 (1C; Nap), 127.8–128.7 (18C; 3×Ph, Nap), 133.1, 133.4, 135.7, 137.2, 138.2, 138.4 (6C; C_q), 173.4 ppm (1C; C=O); HRMS (ESI, +ve): m/z calcd for $C_{38}H_{39}NO_6$: 606.2844 $[M+H]^+$; found: 606.2850 ppm.

(3*S*,4*S*,5*S*,6*R*/*S*)-3,5-Bis(benzyloxy)-6-(benzyloxymethyl)-6-hydroxy-4-(2-naphthylmethoxy)piperidin-2-one (15): A solution of the crude amide **13** (771 mg) in acetic anhydride (6.1 mL) and dry DMSO (6.6 mL) was stirred under N_2 for 21 h. The reaction mixture was diluted with EtOAc (20 mL), quenched with ice and washed with water (3×20 mL) and brine (1×20 mL). The organic extracts were dried ($MgSO_4$) and the solvent was evaporated to afford the keto-amide **14** as a white solid. A dry-ice/acetone cold finger was used to condense ammonia (20 mL) into a solution of the crude keto-amide in dry methanol (30 mL) at 0 °C. The solution was allowed to warm to room temperature and stirred under N_2 for 16 h. The solvent was removed under reduced pressure and the resulting residue was subjected to flash chromatography (EtOAc/pet. ether, 1:1) to give a separable mixture of the hydroxy-lactams **15** (669 mg, 88% over four steps; D-*manno*/L-*gulo* 2.2:1). ^{1}H NMR (500 MHz, $CDCl_3$), partial spectrum of the mixture of diastereomers: δ=3.38 (d, J=9.8 Hz, 1H; CH_2(C6) D-*manno*), 3.43 (d, J=9.6 Hz, 1H; CH_2(C6) L-*gulo*), 3.47 (d, J=9.8 Hz, 1H; CH_2(C6) D-*manno*), 3.57 (d, J=9.6 Hz, 1H; CH_2(C6) L-*gulo*), 3.72 (brs, 1H; OH), 4.22 (d, $J_{3,4}$=3.0 Hz, 1H; 3-H D-*manno*), 4.26 (d, $J_{3,4}$=3.1 Hz, 1H; 3-H L-*gulo*), 4.98 (d, J=12.5 Hz, 1H; CH_2Ph D-*manno*), 5.10 (d, J=12.3 Hz, 1H; CH_2Ph L-*gulo*), 6.33 (brs, 1H; NH L-*gulo*), 6.22 ppm (brs, 1H; NH D-*manno*); ^{13}C NMR (125 MHz, $CDCl_3$): δ=74.0 (1C; CH_2(C6) D-*manno*), 74.5 (1C; C-3 D-*manno*), 169.6 (1C; C=O D-*manno*), 170.2 ppm (1C; C=O L-*gulo*); HRMS (ESI, +ve): m/z calcd for $C_{38}H_{37}NO_6$: 604.2694 $[M+H]^+$; found: 606.2698 ppm.

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(3*S*,4*S*,5*S*,6*R*)-3,5-Bis(benzyloxy)-6-(benzyloxymethyl)-4-(2-naphthylmethoxy)piperidin-2-one (16) and (3*S*,4*S*,5*S*,6*S*)-3,5-bis(benzyloxy)-6-(benzyloxymethyl)-4-(2-naphthylmethoxy)piperidin-2-one (17): Sodium cyanoborohydride (90.4 mg, 1.44 mmol) was added to a solution of the hydroxy-lactams **15** (86.9 mg, 0.144 mmol) and formic acid (0.52 mL) in dry acetonitrile (3 mL) and the mixture stirred under N_2 for 20 h. Sodium cyanoborohydride (90.4 mg, 1.44 mmol) was added and the reaction mixture was stirred for a further 24 h when TLC analysis (EtOAc/pet. ether, 1:3) indicated complete consumption of the starting material. The mixture was diluted with EtOAc (20 mL) and washed with aq. sat. $NaHCO_3$ (3×20 mL) and brine (1×20 mL). The aqueous extracts were treated with sodium hypochlorite prior to disposal. The organic extracts were dried ($MgSO_4$), the solvent was removed under reduced pressure and the resulting residue was subjected to flash chromatography (EtOAc/pet. ether, 1:1) to afford the L-*gulo* lactam **16** (28.2 mg, 33%) and the D-*manno* lactam **17** (32.5 mg, 38%), both as colourless oils.

Characterisation for **16**: $[\alpha]_D^{23}=-57$ (c=0.535 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ=3.36 (dd, $J_{6,6a}$=4.27, $J_{6a,6b}$=9.11 Hz, 1H; CH_2(C6)), 3.46 (m, 2H; 6-H, CH_2(C6)), 3.57 (m, 1H; 3-H), 3.91 (dd, $J_{3,4}$=3.1, $J_{4,5}$=4.4 Hz, 1H; 4-H), 3.95 (m, 1H; 6-H), 4.08–4.19 (m, 3H; 2×CH_2Ph, 5-H), 4.40 (m, 2H; 2×CH_2Ph), 4.66 (d, J=12.4 Hz, 1H; CH_2Ph), 4.71 (d, J=12.3 Hz, 1H; CH_2Nap), 4.93 (d, J=12.3 Hz, 1H; CH_2Nap), 5.10 (d, J=12.4 Hz, 1H; CH_2Ph), 5.83 (brs, 1H; NH), 6.84 (app. d, J=7.05 Hz, 2H; Ph), 7.07–7.45 (m, 16H; Ph, Nap), 7.62 (s, 1H; Nap), 7.72–7.79 ppm (m, 3H; Nap); ^{13}C NMR (100 MHz, $CDCl_3$): δ=52.8 (1C; C-6), 70.3 (1C; CH_2(C6)), 72.5 (1C; CH_2Nap), 73.6 (1C; CH_2Ph), 73.6 (1C; CH_2Ph), 73.7 (1C; CH_2Ph), 74.2 (1C; C-5), 74.3 (1C; C-3), 74.8 (1C; C-4), 126.0–126.3 (3C; Nap), 126.8 (1C; Nap), 127.8–128.6 (18C; 3×Ph, Nap), 133.2, 133.3, 135.6, 137.0, 137.6, 138.4 (6C; C_q), 171.3 ppm (1C; C=O); HRMS (ESI, +ve): m/z calcd for $C_{38}H_{37}NO_5$: 588.2749 $[M+H]^+$; found: 588.2747.

Characterisation for **17**: $[\alpha]_D^{25}=-9.49$ (c=0.715 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ=3.41 (m, 1H; CH_2(C6)), 3.54 (m, 2H; 6-H, CH_2(C6)), 3.66 (t, $J_{4,5}$=$J_{5,6}$=5.2 Hz, 1H; 5-H), 3.98 (dd, $J_{3,4}$=2.9, $J_{4,5}$=5.0 Hz, 1H; 4-H), 4.18 (d, $J_{3,4}$=2.9 Hz, 1H; 3-H), 4.38 (d, J=11.6 Hz, 1H; CH_2Ph), 4.42–4.49 (m, 2H; 2×CH_2Ph), 4.55 (d, J=11.6 Hz, 1H; CH_2Ph), 4.69 (d, J=12.1 Hz, 1H; CH_2Ph), 4.74 (d, J=12.2 Hz, 1H; CH_2Nap), 4.88 (d, J=12.2 Hz, 1H; CH_2Nap), 5.06 (d, J=12.2 Hz, 1H; CH_2Ph), 5.91 (brs, 1H; NH), 7.08–7.49 (m, 18H; 3×Ph, Nap), 7.72–7.84 ppm (m, 4H; Nap); ^{13}C NMR (100 MHz, $CDCl_3$): δ=55.5 (1C; C-6), 71.5 (1C; CH_2(C6)), 72.9 (1C; CH_2Nap), 72.9 (1C; CH_2Ph), 73.4 (1C; CH_2Ph), 73.5 (1C; CH_2Ph), 75.0 (1C; C-5), 75.2 (1C; C-3), 77.8 (1C; C-4), 126.1–126.3 (3C; Nap), 127.0 (1C; Nap), 127.8–128.6 (18C; 3×Ph, Nap), 133.2, 133.3, 135.5, 137.5, 138.1 (6C; C_q), 169.6 ppm (1C; C=O); HRMS (ESI, +ve): m/z calcd for $C_{38}H_{37}NO_5$: 588.2744 $[M+H]^+$; found: 588.2747.

(3*S*,4*S*,5*S*,6*S*)-3,5-Bis(benzyloxy)-6-(benzyloxymethyl)-4-(2-naphthylmethoxy)piperidin-2-thione (18): Lawesson's reagent (202 mg, 0.50 mmol) was added to a mixture containing the mannonolactam **17** (98 mg, 0.167 mmol), pyridine (6.7 µL, 0.083 mmol), freshly activated 4 Å molecular sieves and distilled toluene (6 mL) and the mixture was stirred for 20 h. The mixture was then filtered, stirred with MeOH (1.68 mL) for 2 h and the solvent removed under reduced pressure. The residue obtained was subjected to flash chromatography (EtOAc/pet. ether, 20:80) to afford the thionolactam **18** (94 mg, 93%) as a white solid. M.p. 147°C; $[\alpha]_D^{23}=-52$ (c=0.215 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ=3.43 (m, 1H; CH_2(C6)), 3.56 (m, 2H; 6-H, CH_2(C6)), 3.83 (apt. t, 1H; 5-H), 3.91 (dd, $J_{3,4}$=2.6, $J_{4,5}$=7.2 Hz, 1H; 4-H), 4.42 (d, $J_{3,4}$=2.5 Hz, 1H; 3-H), 4.44–4.52 (m, 3H; 3×CH_2Ph), 4.68–4.73 (m, 2H; CH_2Nap, CH_2Ph), 4.79 (d, J=12.1 Hz, 1H; CH_2Nap), 4.83 (d, J=12.0 Hz, 1H; CH_2Ph), 5.08 (d, J=12.1 Hz, 1H; CH_2Ph), 7.14–7.52 (m, 18H; 3×Ph, Nap), 7.73–7.85 (m, 4H; Nap), 8.13 ppm (brs, 1H; NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ=59.8 (1C; C-6), 70.6 (1C; CH_2(C6)), 72.5 (1C; CH_2Nap), 73.2 (1C; CH_2Ph), 73.5 (1C; CH_2Ph), 73.7 (1C; CH_2Ph), 74.2 (1C; C-5), 78.3 (1C; C-4), 79.8 (1C; C-3), 125.9–126.3 (3C; Nap), 126.8 (1C; Nap), 127.8–128.7 (18C; 3×Ph, Nap), 133.1, 133.3, 135.4, 137.3, 137.6, 138.0 (6C; C_q), 200.0 ppm (1C; C=O); HRMS (ESI, +ve): m/z calcd for $C_{38}H_{37}NO_4S$: 604.2516 $[M+H]^+$; found: 604.2524 [].

(5*R*,6*R*,7*S*,8*S*)-7-(2-Naphthylmethoxy)-6,8-bis(benzyloxy)-5-(benzyloxymethyl)-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine (20) and (5*R*,6*R*,7*S*,8*R*)-7-(2-naphthylmethoxy)-6,8-bis(benzyloxy)-5-(benzyloxymethyl)-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine (21): Thionolactam **18** (256 mg, 0.424 mmol) was dissolved in aminoacetaldehyde dimethyl acetal (0.69 mL, 6.33 mmol) and the mixture stirred under N_2 for 18 h. The mixture was diluted with Et_2O (20 mL) and washed with H_2O (2×20 mL) and brine (1×20 mL). The organic extracts were dried ($MgSO_4$) and the solvent removed under reduced pressure to afford the amidines **19** as a colourless residue. *p*-Toluenesulfonic acid monohydrate (0.14 g, 0.74 mmol) was added to a solution of the crude amidines in toluene (9.5 mL) and the mixture was stirred at 60°C overnight. The mixture was then diluted with DCM (20 mL) and washed with $NaHCO_3$ (2×20 mL) and brine (1×20 mL). The organic extracts were dried ($MgSO_4$), the solvent was removed under reduced pressure and the residue was subjected to flash chromatography (EtOAc/pet. ether, 1:1) to afford the glucoimidazole **20** (110 mg, 42% over two steps) as a colourless oil and the mannoimidazole **21** (83.3 mg, 32% over two steps) as a yellow oil.

Characterisation for **20**: $[\alpha]_D^{25}=+52$ (c=0.315 in $CHCl_3$; lit.:[39] +52 (in $CHCl_3$)); ^{1}H NMR (600 MHz, $CDCl_3$): δ=3.75 (dd, $J_{5,5a}$=5.0, $J_{5a,5b}$=10.3 Hz, 1H; CH_2(C5)), 3.87 (m, 2H; 6-H, CH_2(C5)), 4.13 (dd, $J_{6,7}$=7.5, $J_{7,8}$=5.8 Hz, 1H; 7-H), 4.18 (m, 1H; 5-H), 4.45 (app. d, 2H; 2×CH_2Ph), 4.51 (d, J=11.2 Hz, 1H; CH_2Ph), 4.78 (d, $J_{7,8}$=5.8 Hz, 1H; 8-H), 4.84 (d, J=11.6 Hz, 1H; CH_2Ph), 4.86 (d, J=11.2 Hz, 1H; CH_2Ph), 4.89 (d, J=11.5 Hz, 1H; CH_2Nap), 4.97 (d, J=11.5 Hz, 1H; CH_2Ph), 5.19 (d, J=11.5 Hz, 1H; CH_2Nap), 7.04 (s, 1H; 2-H), 7.12 (s, 1H; 3-H), 7.14–7.48 (m, 18H; 3×Ph, Nap), 7.68–7.83 ppm (m, 4H; Nap); ^{13}C NMR (125 MHz, $CDCl_3$): δ=58.3 (1C; C-5), 68.5 (1C; CH_2(C5)), 72.9 (1C; CH_2Nap), 73.4 (1C; CH_2Ph), 74.3 (1C; CH_2Ph), 74.4 (1C; CH_2Ph), 74.5 (1C; C-8), 76.2 (1C; C-6), 82.2 (1C; C-7), 117.4 (1C; C-2), 126.1–126.9 (3C; Nap), 127.7 (1C; Nap), 127.8–128.6 (18C; 3×Ph, Nap), 129.5 (1C; C-3), 133.2, 133.4, 135.5, 137.4, 137.7, 138.4 (6C; C_q), 144.2 ppm (C_q, imidazole).

Characterisation for **21**: $[\alpha]_D^{25}=-24$ (c=0.24 in $CHCl_3$: lit.:[39] −20 (in $CHCl_3$)); ^{1}H NMR (600 MHz, $CDCl_3$): δ=3.57 (dd, $J_{5,5a}$=7.1, $J_{5a,5b}$=10.1 Hz, 1H; CH_2(C5)), 3.71 (dd, $J_{5,5a}$=3.4, $J_{5a,5b}$=10.1 Hz, 1H; CH_2(C5)), 3.84 (dd, $J_{6,7}$=9.3, $J_{7,8}$=3.1 Hz, 1H; 7-H), 4.06 (m, 1H; 5-H), 4.25 (dd, $J_{5,6}$=9.3, $J_{6,7}$=7.2 Hz, 1H; 6-H), 4.39 (m, 2H; 2×CH_2Ph), 4.56–4.66 (m, 3H; 2×CH_2Ph, CH_2Nap), 4.69 (d, J=12.2 Hz, 1H; CH_2Nap), 4.74 (d, J=12.0 Hz, 1H; CH_2Ph), 4.78 (d, $J_{7,8}$=3.0 Hz, 1H; 8-H), 4.96 (d, J=11.2 Hz, 1H; CH_2Ph), 6.98 (s, 1H; 3-H), 7.09 (s, 1H; 2-H), 7.17–7.39 (m, 18H; 3×Ph, Nap), 7.62–7.74 ppm (m, 4H; Nap); ^{13}C NMR (125 MHz, $CDCl_3$): δ=60.0 (1C; C-5), 68.3 (1C; C8), 70.6 (1C; CH_2Nap), 71.2 (1C; CH_2(C5)), 71.8 (1C; CH_2Ph), 73.3 (1C; CH_2Ph), 74.3 (1C; C-6), 75.0 (1C; CH_2Ph), 80.2 (1C; C-3), 119.5 (1C; C-2), 125.2–126.9 (3C; Nap), 126.7 (1C; Nap), 128.6–127.7 (18C; 3×Ph, Nap), 129.4 (1C; C-3), 133.2, 133.3, 135.4, 137.6, 138.2, 138.3 (6C; C_q), 143.0 ppm (C_q, imidazole).

(5*R*,6*R*,7*S*,8*R*)-6,8-Bis(benzyloxy)-5-(benzyloxymethyl)-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridin-7-ol (22): DDQ (25.2 mg, 0.111 mmol) was added to a solution of the mannoimidazole **21** (22.6 mg, 0.037 mmol) in DCM/H_2O (9:1, 1 mL) and the reaction mixture was stirred at room temperature overnight. DDQ (25 mg,

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0.11 mmol) was again added and the mixture stirred for 3 days when TLC analysis (EtOAc/pet. ether, 8:2) indicated complete consumption of the starting material. The mixture was then diluted with DCM (20 mL), washed with water (3×20 mL) and aq. sat. $NaHCO_3$ (3×20 mL), dried ($MgSO_4$), filtered and concentrated. The crude product was purified by flash chromatography (EtOAc/pet. ether, 80:20 to 100:0) to afford the alcohol **22** (11.7 mg, 67%) as a yellow oil. $[\alpha]_D^{24} = -35$ ($c = 0.585$ in $CHCl_3$; lit.:[40] −6 (in $CHCl_3$)); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 3.64$ (dd, $J_{5,5a} = 5.9$, $J_{5a,5b} = 10.2$ Hz, 1H; CH_2(C5)), 3.78 (dd, $J_{5,5a} = 2.5$, $J_{5a,5b} = 10.2$ Hz, 1H; CH_2(C5)), 4.03 (m, 3H; 7-H, 6-H, 5-H), 4.42 (app. s, 2H; 2×CH_2Ph), 4.54 (d, $J = 11.2$ Hz, 1H; CH_2Ph), 4.65 (d, $J = 11.6$ Hz, 1H; CH_2Ph), 4.70 (d, $J_{7,8} = 3.3$ Hz, 1H; 8-H), 4.85 (d, $J = 11.6$ Hz, 1H; CH_2Ph), 4.90 (d, $J = 11.2$ Hz, 1H; CH_2Ph), 7.05 (s, 1H; 3-H), 7.13 (s, 1H; 2-H), 7.19–7.28 ppm (m, 15H; 3×Ph); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 59.1$ (1C; C-5), 70.2 (1C; CH_2(C5)), 71.2 (2C; C-8, CH_2Ph), 72.4 (1C; C-6), 73.2 (1C; CH_2Ph), 74.6 (1C; CH_2Ph), 75.3 (1C; C-7), 118.9 (1C; C-2), 127.7–128.5 (15C; 3×Ph), 129.6 (1C; C-3), 137.5, 137.7, 137.8 (3C; C_q), 142.3 ppm (C_q, imidazole).

(5*R*,6*R*,7*S*,8*R*)-7-(2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyloxy)-6,8-bis(benzyloxy)-5-(benzyloxymethyl)-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine (23): A mixture of the alcohol **22** (13.8 mg, 0.029 mmol), 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate (**5**;[22] 32.5 mg, 0.051 mmol) and freshly activated 4 Å molecular sieves in toluene (1.5 mL) was stirred at room temperature for 30 min. Triflic acid (1 μL, 0.011 mmol) was added to the mixture at −20 °C and the mixture was stirred for 1 h, then at 0 °C for 20 min, and at room temperature for another 20 min, quenched with pyridine (1 drop) and filtered through a pad of Celite. The solvent was removed under reduced pressure and the resulting residue was subjected to flash chromatography (EtOAc/pet. ether/ Et_3N 80:19:1) to recover alcohol **26** (6.4 mg) and afford the disaccharide **23** (12.9 mg, 47%) as a colourless oil. $[\alpha]_D^{23} = +7.2$ ($c = 0.175$ in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 2.11$ (s, 3H; Ac), 3.49 (dd, $J_{5',5a'} = 1.7$, $J_{5a'',5b'} = 10.9$ Hz, 1H; CH_2(C5′)), 3.55 (dd, $J_{5,5a} = 6.7$, $J_{5a,5b} = 10.2$ Hz, 1H; CH_2(C5)), 3.63 (dd, $J_{5',5b'} = 3.5$, $J_{5a'',5b'} = 10.8$ Hz, 1H; CH_2(C5′)), 3.67 (dd, $J_{5,5b} = 3.2$, $J_{5a,5b} = 10.2$ Hz, 1H; CH_2(C5)), 3.87 (m, 1H; 5′-H), 3.93 (t, $J_{3',4'} = J_{4',5'} = 9.5$ Hz, 1H; 4′-H), 4.01 (dd, $J_{2',3'} = 3.3$, $J_{3',4'} = 9.5$ Hz, 1H; 3′-H), 4.07 (dd, $J_{6,7} = 9.5$, $J_{7,8} = 3.1$ Hz, 1H; 7-H), 4.13 (1H, m, 5-H), 4.29 (dd, $J_{5,6} = 7.1$, $J_{6,7} = 9.5$ Hz, 1H; 6-H), 4.41 (m, 2H; 2×CH_2Ph), 4.46 (d, $J = 10.9$ Hz, 1H; CH_2Ph), 4.51 (d, $J = 11.3$ Hz, 1H; CH_2Ph), 4.54 (d, $J = 12.0$ Hz, 1H; CH_2Ph), 4.57 (d, $J = 11.3$ Hz, 1H; CH_2Ph), 4.64 (app. d, 3H, 3×CH_2Ph), 4.81 (d, $J_{2,3} = 3.1$ Hz, 1H; 2-H), 4.84 (m, 2H; 2×CH_2Ph), 5.19 (d, $J_{1',2'} = 1.6$ Hz, 1H; 1′-H), 5.48 (dd, $J_{1',2'} = 1.6$, $J_{2',3'} = 3.3$ Hz, 1H; 2′-H), 7.07 (s, 1H; 3-H), 7.14 (s, 1H; 2-H), 7.08–7.34 ppm (m, 30H; 6×Ph); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 21.2$ (1C; Me), 60.0 (1C; C-5), 68.5 (1C; C-6′), 69.1 (1C; C-2′), 70.3 (1C; CH_2Ph), 70.8 (1C; CH_2(C5)), 70.9 (1C; C-8), 72.1 (1C; CH_2Ph), 72.4 (1C; C-5′), 73.4 (1C; CH_2Ph), 73.7 (1C; CH_2Ph), 74.2 (1C; C-4′), 74.4 (1C; C-6), 75.1 (2C; CH_2Ph), 78.2 (1C; C-3′), 80.3 (1C; C-7), 100.1 (1C; C-1′), 119.4 (1C; C-2), 127.6–128.7 (30C; 6×Ph), 129.5 (1C; C-3), 137.6, 137.7, 137.9, 138.1, 138.2, 138.8 (6C; C_q), 142.6 (C_q, imidazole), 170.4 ppm (1C; C=O); HRMS (ESI, +ve): *m*/*z* calcd for $C_{58}H_{60}N_2O_{10}$: 945.4321 $[M+H]^+$; found: 945.4322.

(5*R*,6*R*,7*S*,8*R*)-7-(3,4,6-Tri-*O*-benzyl-α-D-mannopyranosyloxy)-6,8-bis(benzyloxy)-5-(benzyloxymethyl)-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine (24): K_2CO_3 (1 mg, 0.007 mmol) was added to a solution of the acetate **23** (13.1 mg, 0.014 mmol) in dry methanol (0.3 mL) and the resulting suspension was stirred at room temperature for 6.5 h. The reaction mixture was quenched with acetic acid (5 μL, 0.087 mmol), the solvent was removed under reduced pressure and the resulting residue was subjected to flash chromatography (EtOAc/pet. ether/Et_3N 50:49.5:0.5) to afford the alcohol **24** (5.8 mg, 46%) as a colourless oil. $[\alpha]_D^{24} = +13$ ($c = 0.305$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 2.40$ (d, $J_{2',OH} = 2.5$ Hz, 1H; OH), 3.49 (dd, $J_{5',6a'} = 1.8$, $J_{6a'',6b'} = 10.8$ Hz, 1H; 6′-H_a), 3.58 (m, 2H; CH_2(C5), 6′-H_b), 3.70 (dd, $J_{5,5a} = 3.2$, $J_{5a,5b} = 10.1$ Hz, 1H; CH_2(C5)), 3.87 (m, 1H; 5′-H), 3.91 (m, 2H; 4′,3′-H), 4.03 (m, 1H; 2′-H), 4.08 (dd, $J_{6,7} = 9.6$, $J_{7,8} = 3.1$ Hz, 1H; 7-H), 4.13 (1H, m, 5-H), 4.28 (dd, $J_{5,6} = 7.3$, $J_{6,7} = 9.6$ Hz, 1H; 6-H), 4.40–4.53 (m, 5H; 5×CH_2Ph), 4.57–4.68 (m, 5H; 5×CH_2Ph), 4.79 (m, 2H; 2×CH_2Ph), 4.85 (d, $J_{7,8} = 3.1$ Hz, 1H; 8-H), 5.23 (d, $J_{1',2'} = 1.5$ Hz, 1H; 1′-H), 7.08 (s, 1H; 3-H), 7.14 (s, 1H; 2-H), 7.11–7.35 ppm (m, 30H; 6×Ph); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 60.0$ (1C; C-5), 68.6 (1C; C-6′), 69.0 (1C; C-2′), 70.3 (1C; CH_2Ph), 70.7 (1C; C-8), 71.1 (1C; CH_2(C5)), 72.0 (1C; C-5′), 72.4 (1C; CH_2Ph), 73.4 (1C; CH_2Ph), 73.7 (1C; CH_2Ph), 74.3 (2C; C-6,3′), 75.1 (2C; CH_2Ph), 80.1 (1C; C-4′), 80.4 (1C; C-7), 101.8 (1C; C-1′), 119.3 (1C; C-2), 127.6–128.7 (30C; 6×Ph), 129.6 (1C; C-3), 137.6, 137.8, 138.1, 138.3, 138.7 (6C; C_q), 142.7 ppm (C_q, imidazole); HRMS (ESI, +ve): *m*/*z* calcd for $C_{56}H_{58}N_2O_9$: 903.4215 $[M+H]^+$; found: 903.4214.

(5*R*,6*R*,7*S*,8*R*)-6,8-Dihydroxy-5-(hydroxymethyl)-7-(α-D-mannopyranosyloxy)-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine (2): Pd(OH)2/C (20%, 24 mg) was added to a solution of the deacetylated disaccharide **24** (12.6 mg, 0.014 mol) in EtOAc/MeOH/H_2O (5:17:3, 1.50 mL) and AcOH (0.34 mL). The reaction vessel was filled with H_2 (34 bar) and agitated for 4 days. At this point TLC analysis (EtOAc/MeOH/H_2O, 7:3:2) indicated complete conversion to a single species along with baseline by-products. The suspension was filtered through a pad of Celite, the solvent was evaporated and the resulting residue was subjected to flash chromatography (EtOAc/MeOH/H_2O, 5:2:1) to afford ManManIm (**2**; 2.4 mg, 48%) as a colourless residue. $[\alpha]_D^{27} = +13$ ($c = 0.12$ in H_2O); ^{1}H NMR (500 MHz, D_2O): $\delta = 3.57$ (t, $J_{3',4'} = J_{4',5'} = 9.8$ Hz, 1H; 4′-H), 3.66 (dd, $J_{5',6a'} = 6.3$, $J_{6a'',6b'} = 12.1$ Hz, 1H; 6′-H_a), 3.77 (m, 1H; 5′-H), 3.83 (m, 2H; 3′-H, 6′-H_b), 3.91 (m, 1H; 5-H), 3.95 (dd, $J_{5,5a} = 3.3$, $J_{5a,5b} = 12.7$ Hz, 1H; CH_2(C5)), 3.99 (dd, $J_{6,7} = 10.2$, $J_{7,8} = 3.7$ Hz, 1H; 7-H), 4.02 (dd, $J_{1',2'} = 3.4$, $J_{2',3'} = 1.7$ Hz, 1H; 2′-H), 4.13 (dd, $J_{5,5b} = 2.6$, $J_{5a,5b} = 12.7$ Hz, 1H; CH_2(C5)), 4.27 (dd, $J_{5,6} = 8.6$, $J_{6,7} = 10.2$ Hz, 1H; 6-H), 4.97 (d, $J_{7,8} = 3.7$ Hz, 1H; 8-H), 5.23 (d, $J_{1',2'} = 1.6$ Hz, 1H; 1′-H), 7.01 (s, 1H; 3-H), 7.20 ppm (s, 1H; 2-H); ^{13}C NMR (125 MHz, D_2O): $\delta = 59.3$ (1C; CH_2(C5)), 60.9 (1C; C-5,6′), 63.5 (1C; C-8), 63.9 (1C; C-6), 66.7 (1C; C-4′), 69.9 (1C; C-2′), 70.3 (2C; C-4,3′), 73.5 (1C; C-5′), 78.1 (1C; C-7), 102.1 (1C; C-1′), 118.3 (1C; C-2), 128.7 (1C; C-3), 144.7 ppm (C_q, imidazole); HRMS (ESI, +ve): *m*/*z* calcd for $C_{14}H_{22}N_2O_9$: 363.1398 $[M+H]^+$; found: 363.1398.

Isothermal titration calorimetry (ITC): The binding affinity of Man2NH2DMJ (**1**) to *Bt*GH99 was determined by using a Microcal iTC200 calorimeter (GE Healthcare/Malvern Instruments). The assay was carried out at 25 °C with 18×2 μL injections of the inhibitor (6 mM) titrated into the ITC cell containing 117 μM *Bt*GH99. Owing to the low affinity of the ligand, which prevented the observation of a sigmoidal binding isotherm, *N* was fixed at 1.[41] An initial ITC experiment was conducted by using 1 M inhibitor in the syringe and 52 μM protein with 24×1.5 μL injections. The dissociation constant (K_D), change in enthalpy (ΔH) and measurement uncertainty were calculated by using the MicroCal PEAQ-ITC Analysis Software (Malvern Instruments).

Crystallisation and data collection: *Bx*GH99 protein[10] was crystallised by using the vapour diffusion hanging drop method in 3 M sodium acetate at pH 7.4. Crystals were grown at 19 °C in a 24-well plate with 500 μL of reservoir solution in each well and sealed with vacuum grease. The droplet was created by mixing 1 μL of *Bx*GH99 solution (34 mg mL^{-1} in 25 mM HEPES buffer, pH 7.0, 100 mM NaCl) with 1 μL of the crystallant solution. Crystals were fished from the droplet by using a nylon cryoloop, without cryoprotection. Data

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were collected at Diamond Light Source beamline i04 using X-rays with a wavelength of 0.979 Å.

Structure solution and refinement: Images containing diffraction patterns were indexed and integrated by using DIALS[42] through xia2.[43] The *hkl* index of each data set was then matched to a previous solution in Aimless.[44] Refinement was performed by using Refmac5[45] and real-space model building in Coot.[46] Model geometry and agreement with electron density were validated in Coot and Edstats.[47] The quality of the carbohydrates and nitrogen heterocycles were verified by using Privateer.[32] The modelling and refinement processes were aided by using ccp4i2 interface.[48]

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Conflict of interest

The authors declare no conflict of interest.

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