Article:
Fernandes, Pearl Z., Petricevic, Marija, Sobala, Lukasz orcid.org/0000-0002-3807-6452 et al. (2 more authors) (2018) Exploration of Strategies for Mechanism-Based Inhibitor Design for Family GH99 endo-α-1,2-Mannanases. Chemistry : A European Journal. pp. 7464-7473. ISSN: 0947-6539

https://doi.org/10.1002/chem.201800435

Exploration of strategies for mechanism-based inhibitor design for family GH99 *endo*-α-1,2-mannanases

Pearl Z. Fernandes,[a] Marija Petricevic,[a] Lukasz Sobala,[a] Gideon J. Davies,*[b] Spencer J. Williams*[a]

[a] School of Chemistry and Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, Vic 3010 (Australia)

[b] York Structural Biology Laboratory, Department of Chemistry, University of York, Heslington, YO10 5DD (UK)

sjwill@unimelb.edu.au, gideon.davies@york.ac.uk

Keywords: glycosidase, X-ray crystallography, enzymes, inhibitors

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, involving two conserved catalytic residues. In the first step Glu333 acts as general base to deprotonate the 2-hydroxyl group adjacent to the fissile glycosidic bond, while Glu336 provides general acid assistance to departure of the aglycon. We report the synthesis of two inhibitors designed to interact with either the general base (α-mannosyl-1,3-(2-aminodeoxymannojirimycin); Man2NH$_2$DMJ) 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 reveal that Man2NH$_2$DMJ binds like other iminosugar inhibitors, suggesting that the poor inhibition 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 identifies important limitations associated with mechanism-inspired inhibitor design for GH99 enzymes.

Introduction

Glycoside hydrolases of 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).[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 utilize an unusual mechanism involving neighboring group participation by the substrate 2-hydroxyl to form a 1,2-anhydro sugar intermediate.[10] In this proposed mechanism, a conserved active site residue acts as a general base to deprotonate the 2-OH group, facilitating its nucleophilic attack on C1. 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 colleagues revealed α-mannosyl-1,3-deoxymannojirimycin ManDMJ to be a slightly weaker inhibitor for this enzyme.[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, leading to the development of α-mannosyl-1,3-isofagomine (ManIFG; K_D 0.14 μM for *Bacteroides thetaiotaomicron* GH99).[8] Furthermore, reintroduction of the 'missing' 2-OH of IFG into ManIFG gave α-mannosyl-1,3-noeuromycin (ManNOE), which was shown to be 5-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 conformation of substrate within the Michaelis complex, suggesting that potent inhibition of GH99 enzymes can be achieved simply by mimicry of charge in the transition state.[17]

Separately, Spiro and coworkers showed that the neutral compound GlcGlucal was a modest inhibitor of mammalian GH99 (rat Golgi preparation, IC_{50} 2.3 μM; for GlcDMJ IC_{50} 1.7 μM);[14, 18] the equivalent molecule targeting bacterial GH99, ManGlucal was also a

ligand with mildly potent affinity (K_D 15 μM 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, but with no contribution from charge mimicry owing to the neutral nature of this compound.[17]

In this study we report our efforts to explore two new inhibitor design strategies for inhibition of GH99 enzymes. Considering the role of the basic residue implicated in the 1,2-anhydro sugar mechanism of GH99 enzymes, we speculated that introduction of an amino group into the structure of ManDMJ to give Man-2NH_2DMJ (**1**) could promote the formation of a favourable ionic interaction upon inhibitor binding. Separately, the glycoimidazole class of inhibitors were developed following the discovery of the natural product nagstatin,[19] and are believed to derive their potency through the ability to mimic the shape of the oxocarbenium-ion-like transition state as well as through the ability of the imidazole glycosidic nitrogen to engage in a hydrogen bond with an appropriately situated carboxylate residue in the active site.[20] For the present work this would require the synthesis of ManManIm (**2**). We report on the synthesis of these two target inhibitors, structural characterization of their binding modes and measurement of their binding constants.

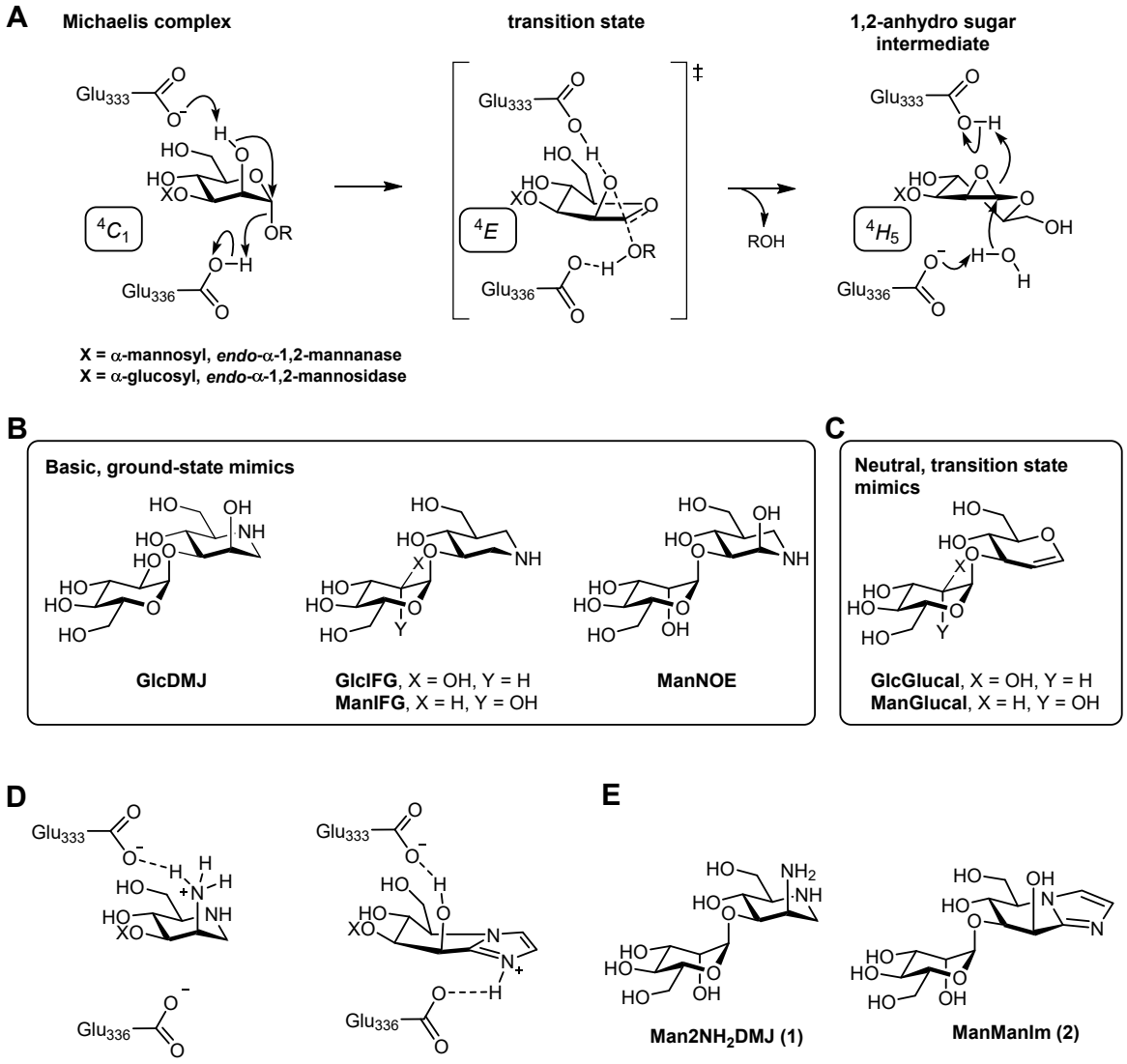
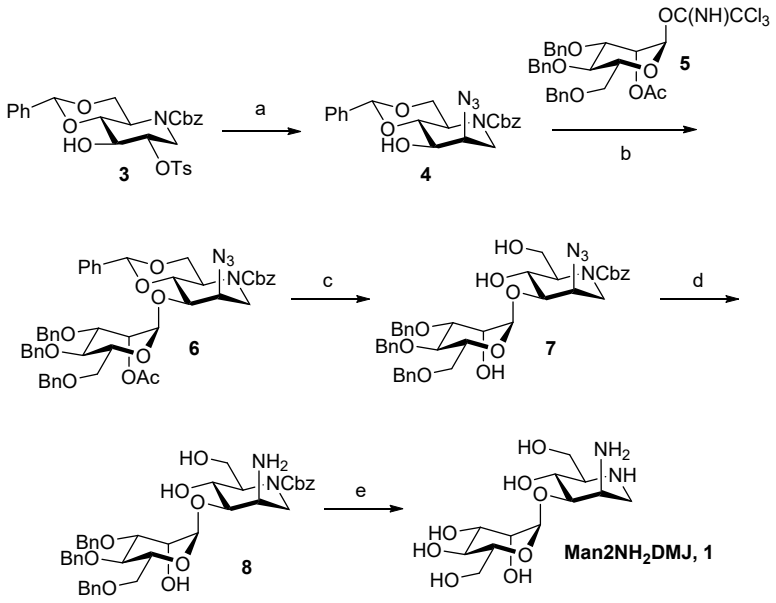


Figure 1. (A) Proposed mechanism for family GH99 retaining endomannosidases/endomannanases. Only the first half of the catalytic cycle is shown. (B) Saturated basic heterocyclic inhibitors for GH99 enzymes mimic ground state conformation. (C) Neutral glycal inhibitors for GH99 enzymes mimic transition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

Results and Discussion

Synthesis of Man2NH$_2$DMJ and ManManIm

Preparation of Man2NH$_2$DMJ (**1**) was achieved 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 83% yield. Deprotection of **6** was achieved in a stepwise manner, as attempts to perform a global deprotection that involved simultaneously removing Cbz, benzylidene, benzyl ethers and reducing the azide were unsuccessful. Deacetylation of **6** (NaOMe/MeOH) and then hydrolysis of the benzaldehyde acetal (TFA/H$_2$O) afforded triol **7**. Reduction of the azide group was achieved with DTT/pyr buffer to afford amine **8**. Removal of the Cbz and benzyl groups then proceeded smoothly using H$_2$ and Pearlman's catalyst, affording **1**.

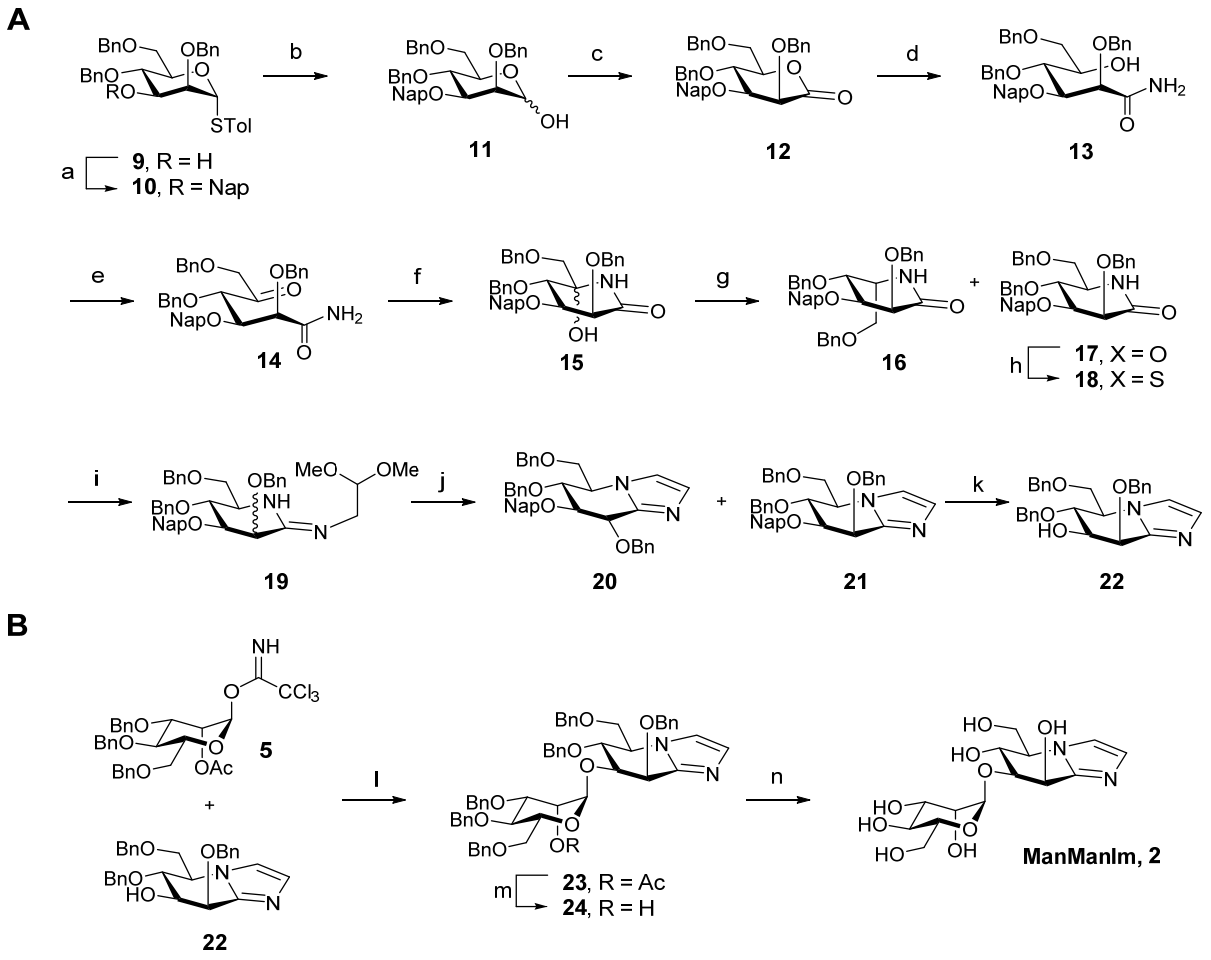


Scheme 1. Reagents and conditions. a) NaN$_3$, DMF, reflux, 74%; b) TfOH, CH$_2$Cl$_2$, -30–0 °C, 87%; c) i) NaOMe, MeOH, ii) 9:1 TFA/H$_2$O, 83%; d) DTT, pyr, pH 9.2 NaHCO$_3$/Na$_2$CO$_3$, 80%; e) H$_2$, Pd(OH)$_2$, aq HCl, 2:2:1 EtOAc/MeOH/H$_2$O, 70%.

The preparation of ManManIm was achieved through a sequence involving preparation of the protected mannoimidazole alcohol **22**, followed by elaboration to the disaccharide (Scheme 2). The known alcohol **9**[23] was treated with NapBr/NaH in DMF, affording **10**. Hydrolysis of the thioglycoside using NIS in H$_2$O/acetone gave the hemiacetal **11**, which was oxidized to the lactone **12** under Albright-Goldman conditions.[24] For conversion of the lactone **12** to the lactam **17** we followed the protocol developed by Overkleeft and co-workers,[25] involving 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 2:1 mixture of the D-*manno* and L-*gulo* lactams, from which the D-*manno* lactam **17** was isolated in 38% yield. Conversion of the lactam to the thionolactam **18** was achieved using Lawesson's reagent in toluene. Annulation of the imidazole ring followed 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 under catalysis of TsOH, providing a mixture of D-*gluco* and D-*manno* imidazoles in a 2:1 ratio, from which the D-*manno* imidazole **21** was isolated in 32% yield over two steps. Removal of the Nap group was achieved under the agency of DDQ, CH_2Cl_2/H_2O, affording the alcohol **22**.

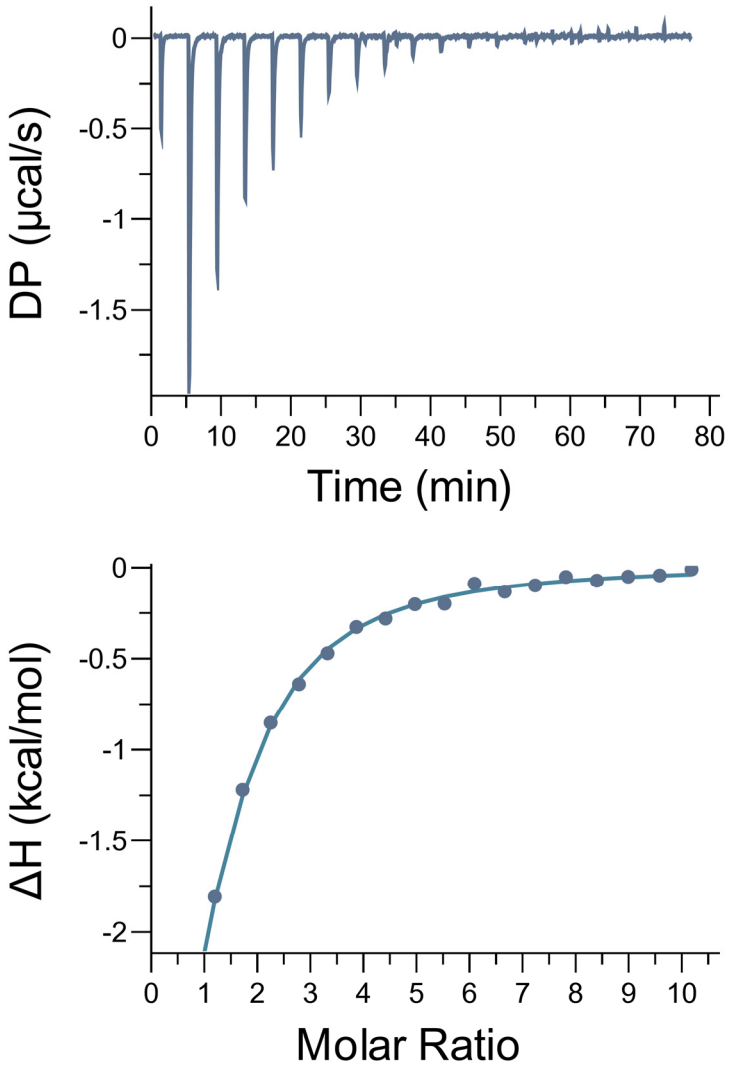
Coupling of **22** with trichloroacetimidate **5**[22] catalyzed by TfOH afforded the disaccharide **23** in 47% yield. Deprotection was achieved in two steps, under conditions chosen to avoid epimerization at C2. Treatment of **23** with K_2CO_3/MeOH afforded the alcohol **24**, and hydrogenation with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



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 mol. 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 ManManI **2**. Reagents and conditions. l) TfOH, 4 Å mol. 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%.

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 revealed that Man2NH$_2$DMJ binds with K_D = 97.7±4.9 μM (Figure 2). No binding was evident by ITC for ManManIm. Placed in context, **1** binds worse to *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. 3D structures were obtained for **1** and **2** with *Bx*GH99 that diffracted to a resolution of 1.1 and 1.3 Å, respectively (Table 1). Occupancy for the complex with **1** was essentially complete, whereas that with **2**, with prolonged soaking, was estimated at 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 (subsite nomenclature from Ref.[27]) and will be discussed in turn.



149

150 **Figure 2.** Isothermal titration calorimetry thermogram showing binding of Man2NH$_2$DMJ to

151 *Bacteroides thetaiotaomicron endo*-α-1,2-mannanase (*Bt*GH99). DP = differential power.

152 Binding parameters K_D = 97.7±4.9 µM, N = 1 (fixed) and ΔH= –5.9±0.1 kcal mol^{-1}.

153

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

	*Bx*GH99 in complex with aminoDMJ	*Bx*GH99 in complex with ManManIm
Data collection		
Space group	I 4	I 4
Cell dimensions		
a, *b*, *c* (Å)	108.1, 108.1, 67.5	108.6, 108.6, 67.8
α, β, γ (°)	90, 90, 90	90, 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
$I / \sigma I$	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 highest-resolution shell.

The *Bx*GH99-**1** complex (Figure 3A) reveals the piperidine ring in a $^4C_1$ conformation, matching that seen for complexes of the wildtype 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 is proposed to act as a general base/acid through deprotonation of the 2-hydroxyl group. Overlay of this complex with that of *Bx*GH99-GlcDMJ reported previously[10] reveals that the positioning and conformation of the rings in the –1 and –2 subsites are essentially identical, and that no amino acid residues undergo significant movements (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** relative to GlcDMJ therefore does not result from incorrect binding of the inhibitor, and must instead reflect a failure to fully capitalize 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 form;[28] this is supported by first principles consideration of the basicity of these inhibitors and the relevant pK_a values of catalytic residues, and by studies of pH dependence of inhibition. In the case of **1**, this compound has 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 as $\Delta pK_a = 3.6$ units for NH_3^+ or NR_3^+.[29] Moreover, in cyclic systems there are stereoelectronic and conformational contributions, notable examples for various diamines include (pK_{a1}, pK_{a2}): piperazine 9.8, 5.7;[29] *cis*-1,3-diaminocyclohexane 10.3, 8.3;[30] *trans*-1,3-diaminocyclohexane 10.4, 8.5).[30] Finally, vicinal hydroxyl groups can also perturb amine pK_a values; in Man2NH$_2$DMJ O4 is antiperiplanar 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$_2$DMJ fails to appropriately mimic an oxocarbenium ion 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 1D highlights that the 2-amino group has additional hydrogen substituents that may cause an energetic penalty upon binding of the inhibitor.

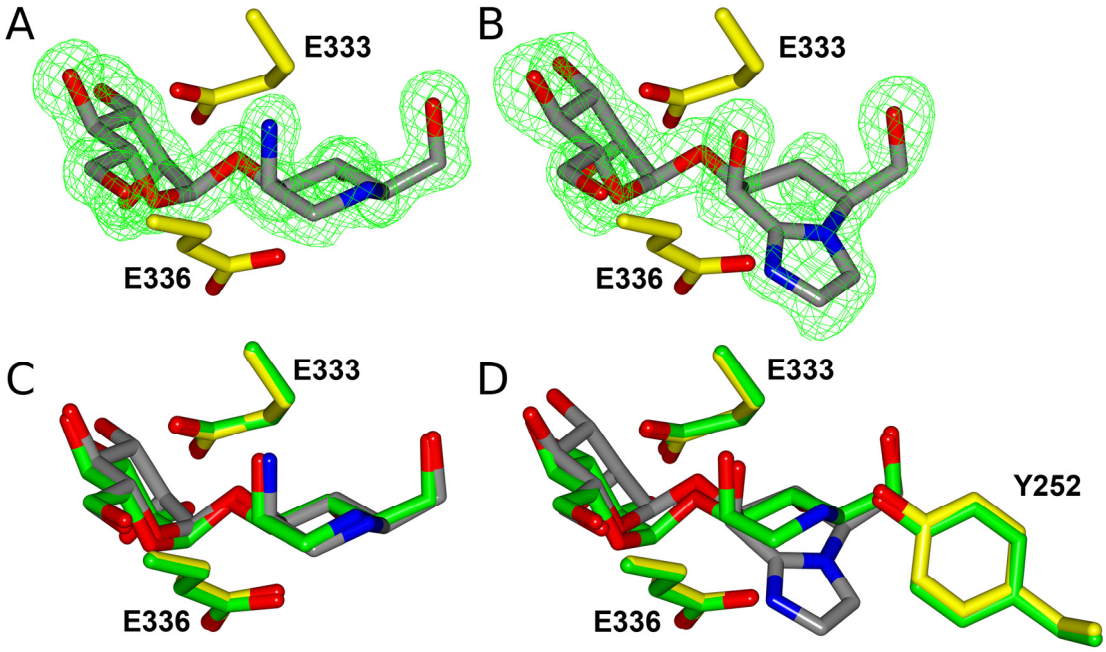


Figure 3. Three-dimensional structures of *Bt*GH99. (A) Complex with Man2NH$_2$DMJ. (B) Complex with ManManIm. Electron density maps are maximum likelihood/σ_A weight F_o – F_c difference syntheses contoured at 0.5 and 0.3 eÅ^{-3} respectively for panels A and B) visible before refining the structure model with the ligand added. (C) Overlay of Man2NH$_2$DMJ with GlcDMJ (PDB code 4FAM). (D) Overlay of ManManIm with GlcDMJ complex (PDB code 4FAR).

The *Bx*GH99-**2** complex reveals the piperidine ring of the mannoimidazole moiety to be in an unusual $^2H_3/E_3$ conformation (Figure 3B).[32] Overlay of the complex with that of *Bx*GH99-GlcDMJ[10] reveals that while 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 3D). 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 Vasella and co-workers, 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 formalized 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 reveals that the acid/base or acid residues responsible for protonating the leaving group are in fact not universally located lateral to the sugar mean plane, 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 sugar mean plane, but a normal orientation and conformation of glucoimidazole was observed.[35] Mannoimidazole also bound 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 ring plane in order to facilitate classical anti-protonation of the axial glycosidic oxygen (approximate O5–C1–O1 angle is 60 degrees). The distorted mode of binding of the mannoimidazole moiety of **2** seems to be a consequence of the imidazole

binding to maximize this interaction with the acid/base. Close examination of the active site of *Bx*GH99 reveals 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, causing the wwPDB validation software to report H–H steric clashes in this region. In fact, a ternary complex of GlcDMJ and α-1,2-mannobiose highlighted that the active site of the enzyme involves a sharp bend in the –1 and +1 subsites. The failure of **2** to bind in a typical position in the –1 subsite is thus likely a result of a failure to accommodate the imidazole ring owing to the location of Tyr252.

Conclusions

In summary, we report the design and synthesis of two 'mechanism-based' inhibitors of family GH99 endomannanases. While Man2NH$_2$DMJ 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, reducing the basicity of the endocyclic nitrogen and its ability to be protonated in the active site and thereby resemble the oxocarbenium-ion-like transition state. On the other hand, binding of ManManIm to *Bt*GH99 could not be detected by ITC, and consistent with this an X-ray structure in complex 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 developing mechanism-inspired and transition-state mimicking inhibitors of GH99 enzymes.

Experimental

General

^{1}H and ^{13}C NMR spectra were recorded using 400, 500 or 600 MHz instruments. All signals were referenced to TMS (δ=0.00 ppm), or solvent peaks ($CDCl_3$: δ=7.26 ppm for ^{1}H or 77.16 ppm for ^{13}C; D_2O: δ =4.80 ppm for ^{1}H or TMS: δ = 0.00 ppm for ^{13}C; [D_4]MeOH: δ =3.49 ppm for ^{1}H or δ=49.0 ppm for ^{13}C). Melting points were obtained using a Reichert–Jung hotstage apparatus. TLC analysis used aluminium backed Merck Silica Gel 60 F254 sheets, detection was achieved using UV light, 5% H_2SO_4 in MeOH, or ceric ammonium molybdate ("Hanessian's stain") with charring as necessary. Flash chromatography was performed using Geduran silica gel according to the method of Still *et al*.[37] Dry CH_2Cl_2, THF, and Et_2O were obtained from a dry solvent apparatus (Glass Contour of SG Water, Nashua).[38] 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 refluxed for 18 h, poured into ice, extracted into EtOAc (3 × 20 mL), washed with brine (2 × 20mL), dried over anhydrous $MgSO_4$ and evaporated to dryness. Column chromatography (AcOEt:pet. spirits 1:5) gave the azide **4** (67.7 mg, 74%) as a white solid; $[\alpha]_D^{24}$ –21.9 (*c* 1.12, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.74 (s, 1 H, NH), 2.82 (1 H, d, *J* = 1.6, 14.5 Hz, H1a), 3.06 (1 H, td, *J* = 4.6, 10.2 Hz, H5), 3.74 (1 H, dd, *J* = 3.8, 9.2 Hz, H3), 3.79-3.93 (2 H, m, H2,4), 4.31 (1 H, dd, *J* = 3.0, 14.5 Hz, $H1_b$) 4.46 (t, *J* = 1, 11 Hz, $H6_a$), 4.66 (1 H, dd, *J* = 4.6, 11.6 Hz, $H6_b$), 5.01 (2 H, d, *J* = 3.1 Hz, CH_2), 5.48 (1 H, s, CH). ^{13}C NMR ($CDCl_3$, 125 MHz) 48.1, 55.8, 60.1, 67.8, 69.2, 73.6, 78.2 (7 C, C1-6, CH_2, 101.8 (1 C, CH), 126.3, 128.3, 128.4, 128.5, 128.7, 129.4, 136.0, 137.3 (12 C Ph), 155.0 (1 C, C=O); HRMS $(ESI)^+$ *m/z* 411.1664 [$C_{21}H_{22}N_4O_5$ $(M+H)^+$ requires 411.1663].

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 (5)

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) then concentrated under reduced pressure. Flash chromatography (EtOAc/pet. spirits 25:75) gave the disaccharide **6** (37.4 mg, 87%) as a colourless oil. $[\alpha]_D^{24}$ -4.2 (*c* . 0.89, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.80 (1 H, $J_{1,1}$ =14.4, $J_{1,2}$ = 0.9, H1a), 3.15 (1 H, dt, *J* = 10.1, 4.6, 1 Hz, H5), 3.70-4.00 (6 H, m, H3,4,4',5',6a',6b'), 4.03 (1 H, dd, *J* = 9.3, 3.4, H3'), 4.17-4.20 (1 H, m, H2), 4.28 (1 H, dd, *J* = 14.5, 2.2, H1b), 4.47-4.52 (3 H, m, 3 × C**H**$_2$Ph), 4.60-4.64 (2 H, m, H6a,CH_2), 4.69 (1 H, d, *J* = 11 Hz, C**H**$_2$Ph), 4.76 (1 H, dd, *J* = 11.6, 4.5 Hz, H6b), 4.86 (1 H, d, *J* = 11 Hz, C**H**$_2$Ph), 5.12 (2 H, *J* = 3.6, CH_2), 5.28 (1 H, d, *J* = 1.6 Hz, H1'), 5.59 (1 H, *J* = 3.3, 1.8 Hz, H2'), 5.64 (1 H, s, CH), 7.17-7.46 (25 H, m, Ph); ^{13}C NMR ($CDCl_3$, 125 MHz) 48.3 (1 C, C1), 56.3 (1 C, C5), 60.0, 72.7, 74.4, 77.8 (4 C, C3,4,4',5), 67.7 (1 C, CH_2), 68.5 (1 C, C2'), 69.1 (1 C, C6), 69.3 (1 C, C6'), 72.2, 73.6, 75.1 (3 C, **C**H_2Ph), 78.1 (1 C, C2), 78.2 (C1, H3'), 99.5 (1 C, C1'), 100.90 (1 C, 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 (C30, Ph); HRMS $(ESI)^+$ *m/z* 907.3544 [$C_{50}H_{52}N_4O_{11}$ $(M+Na)^+$ requires 907.3525].

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 stirred for 1 h. The mixture was 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, the mixture was stirred for 30 min, concentrated and azeotroped with toluene (3 × 10 mL). Flash chromatography (EtOAc/pet. spirits 9:1) gave the triol **7** (42.5 mg, 83%,). $[\alpha]_D^{25}$ 44.6 (*c*. 1.03, MeOH); ^{1}H NMR (500 MHz, CD_3OD), 3.67-4.20 (13 H, $H1_a$-6_b, H2'-H6'$_b$), 4.43-4.46 (2 H, m, CH_2), 4.58 (1 H, d, *J* = 12.0 Hz, C**H**$_2$Ph), 4.67 (2 H, s, *J* = 12.4 Hz, C**H**$_2$Ph), 4.78 (1 H, d, *J* = 11.0 Hz, C**H**$_2$Ph), 5.12 (2 H, s, CH_2), 5.15 (1 H, apt. s, H1'), 7.03-7.42 (20 H, m, 4 × Ph), ^{13}C NMR ($CDCl_3$, 125 MHz) 59.5, 68.0, 68.9, 69.0, 71.9, 72.5, 73.5, 74.2, 74.9, 79.5 (13 C C1,2,3,4,5,6,1',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 (24 C

Ph), 156.5 (1 C, C=O); HRMS (ESI)$^+$ *m/z* 755.3300 [$C_{41}H_{46}N_4O_{10}$ (M+H)$^+$ requires 755.3287].

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 toluene (5 × 10 mL). Flash chromatography (EtOAc/MeOH/H_2O 94:4:2) to give the amine **8** (80%, 19.2 mg). ^{1}H NMR (500 MHz, CD_3OD), 2.89 (1 H, t, *J* = 12.4 Hz, H2), 3.21-4.13 (13 C m, H1$_a$,1$_b$,3,5,6$_a$6$_b$, 1'-6$_b$'), 4.36 (1 H, t, *J* = 7.8 Hz, H4) ,4.46-4.54 (2 H, m, C**H**$_2$Ph), 4.58 (1 H, d, *J* = 12.0 Hz, C**H**$_2$Ph), 4.66 (d, *J* =11.8 Hz, C**H**$_2$Ph), 4.77-4.81 (2 H, m, C**H**$_2$Ph), 4.98 (1 H, d, *J* = 2.5 Hz, H1'), 5.15 (2 H, s, CH_2), 7.16-7.47 (20 H, m, Ph), ^{13}C NMR ($CDCl_3$, 125 MHz) 46.8, 59.9, 65.6, 68.5, 69.4, 70.4, 72.6, 73.7, 74.4, 75.4, 75.7, 78.1, 80.1, 100.8 (16 C C1-6, C1'-6', 4 × CH_2), 128.81, 128.84, 129.2, 129.28, 128.30, 129.3, 129.4, 129.5, 138.0, 139.3, 139.5, 139.6 (24 C Ph); HRMS (ESI)$^+$ *m/z* 729.3398 [$C_{41}H_{48}N_2O_{10}$ (M+H)$^+$ requires 729.3385].

α-D-Mannopyranosyl-(1→3)-2-amino-*N*-benzyloxycarbonyl-1,2,5-trideoxy-1,5-imino-D-mannitol (1)

The triol **8** (19.2 mg, 0.0264 mmol) in MeOH/H_2O (2:1, 3 mL) and 10% HCl in methanol (0.3 mL) was treated with PdOH/C (50 mg) and H_2 (20 atm, 18h). The suspension was filtered, concentrated and purified with cation and anion resin (eluted with aqueous NH_3) to give ManNH_2DMJ **1** (70%, 6.02mg) as a colourless oil. $[\alpha]_D^{25}$ 17.2 (*c*. 0.08, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 2.78-2.84 (1 H, m, H5), 3.09 (1 H, dd, $J_{1a,1b}$ = 14.0, $J_{1a,2}$ = 2.1, H1a), 3.25 (1 H, dd, $J_{1a,1b}$ = 14.0, $J_{1a,2}$ = 3.2 Hz, H1b), 3.62-3.95 (9 H, m, H2,3,4,4',5',6a,6a',6b,6b'), 3.98 (1 H, dd, $J_{3',4'}$ = 9.2, $J_{2',3'}$ = 4.3 Hz, H3'), 4.09 (1 H, dd, $J_{2',3'}$ = 3.3, $J_{1',2'}$ = 1.8 Hz, H2'), 5.24 (1 H, d, $J_{1',2'}$ = 1.6 Hz, H1'); ^{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; HRMS (ESI)$^+$ *m/z* 325.1606 [$C_{12}H_{24}N_2O_8$ (M+H)$^+$ requires 325.1605].

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 stirred for 30 min. 2-bromomethylnaphthalene (79.6 mg, 0.36 mmol) was added to the mixture and the reaction was 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. spirits 15:85) to give the protected thioglycoside **10** (179.3 mg, 86%) as a colourless oil; $[\alpha]_D^{24}$ +65 (*c* 0.69, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.28 (3 H, s, TolMe), 3.78 (1 H, dd, $J_{5,6a}$ = 1.8, $J_{6a,6b}$ = 10.9 Hz, H6a), 3.87 (1 H, dd, $J_{5,6b}$ = 5.2, $J_{6a,6b}$ = 10.9 Hz, H6b), 3.97 (1 H, dd, $J_{2,3}$ = 3.0, $J_{3,4}$ = 9.3 Hz, H3), 4.04 (1 H, t, $J_{1,2}$ = 3.0, $J_{2,3}$ = 1.8 Hz, H2), 4.11 (1 H, m, H4), 4.33 (1 H, ddd, $J_{4,5}$ = 9.8, $J_{5,6a}$ = 5.1, $J_{5,6b}$ = 1.6 Hz, H5), 4.49 (1 H, d, *J* = 11.9 Hz, C**H**$_2$Ph), 4.57-4.67 (3 H, m, 3 × C**H**$_2$Ph), 4.74 (3 H, m, C**H**$_2$Ph, 2 × C**H**$_2$Nap), 4.96 (1 H, d, *J* = 10.9 Hz, C**H**$_2$Ph), 5.58 (1 H, d, $J_{1,2}$ = 1.5 Hz, H1), 7.02 (2 H, apt. d, *J* = 7.9 Hz, Tol), 7.21-7.37 (17 H, m, 3 × Ph, Tol), 7.44-7.47 (3 H, m, Nap), 7.74-7.83 (4 H, m, Nap); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.2 (1 C, TolMe), 69.3 (1 C, C6), 71.9 (1 C, **C**H_2Ph), 72.2 (1 C, **C**H_2Nap), 72.8 (1 C, C5), 73.3 (1 C, **C**H_2Ph), 75.1 (1 C, C4), 75.2 (1 C, **C**H_2Ph), 76.3 (1 C, C2), 80.3 (1 C, C3), 86.1 (1 C, C1), 125.9-126.5 (4 C, Nap), 127.5-128.4 (18 C, 3 × Ph, Nap), 129.8 (2 C, Tol), 132.3 (2 C, Tol), 133.4, 135.8, 137.6, 138.0, 138.5, 138.6 (6 C, Cq); HRMS (ESI)$^+$ *m/z* 719.2809 [$C_{45}H_{44}O_5S$ (M+Na)$^+$ requires 719.2802].

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. spirits/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 (1 H, dd, $J_{5,6a}$ = 6.6, $J_{6a,6b}$ = 10.5 Hz, H6a), 3.74 (1 H, dd, $J_{5,6b}$ = 2.0, $J_{6a,6b}$ = 10.4 Hz, H6b), 3.83 (1 H, dd, $J_{1,2}$ = 2.0, $J_{2,3}$ = 2.8 Hz, H2), 3.91 (1 H, t, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H4), 4.05 (1 H, dd, $J_{2,3}$ = 3.0, $J_{3,4}$ = 9.4 Hz, H3), 4.10 (1 H, ddd, $J_{4,5}$ = 8.7, $J_{5,6a}$ = 5.8, $J_{5,6b}$ = 1.9 Hz, H5), 4.51-4.59 (3 H, m, 3 × C**H**$_2$Ph), 4.74-4.76 (4 H, m, 2 × C**H**$_2$Ph, 2 × C**H**$_2$Nap), 4.94 (1 H, d, *J* = 11.0 Hz, C**H**$_2$Ph), 5.27 (1 H, d, $J_{1,2}$ = 1.8 Hz, H1), 7.18-7.41 (17 H, m, 3 × Ph), 7.45-7.47 (3 H, m, Nap), 7.72-7.83 (4 H, m, Nap); ^{13}C NMR (125 MHz, $CDCl_3$) δ 69.7 (1 C, C6), 71.4 (1 C,

C5), 72.2 (1 C, **C**H_2Nap), 72.7 (1 C, **C**H_2Ph), 73.3 (1 C, **C**H_2Ph), 75.1 (1 C, **C**H_2Ph), 75.1 (1 C, C2), 75.3 (1 C, C4), 79.8 (1 C, C3), 92.6 (1 C, C1), 125.8-126.3 (4 C, Nap), 127.6-128.5 (18 C, 3 × Ph, Nap), 133.0, 133.4, 136.1, 138.0, 138.5 (6 C, Cq); HRMS $(ESI)^+$ *m/z* 608.3007 [$C_{38}H_{38}O_6$ $(M+NH_4)^+$ requires 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 reaction 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, affording 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. spirits 1:9) to yield analytically pure **12** as a colourless oil, $[\alpha]_D^{25}$ +4.05 (*c* 0.44, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.61 (2 H, m, H6a, H6b), 3.80 (1 H, dd, $J_{2,3}$ = 1.5, $J_{3,4}$ = 7.2 Hz, H3), 4.09 (1 H, dd, $J_{1,2}$ = 2.6, $J_{2,3}$ = 1.6 Hz, H2), 4.23 (2 H, m, H5, H4), 4.38 (1 H, d, *J* = 2.6 Hz, C**H**$_2$Ph), 4.48 (2 H, apt. d, 2 × C**H**$_2$Ph), 4.56 (1 H, d, *J* = 11.8 Hz, C**H**$_2$Ph), 4.77 (1 H, d, *J* = 12.5 Hz, C**H**$_2$Ph), 4.94 (1 H, d, *J* = 12.5 Hz, C**H**$_2$Ph), 5.06 (2 H, m, 2 × C**H**$_2$Nap), 6.96-7.45 (18 H, m, 3 × Ph, Nap), 7.69-7.78 (4 H, m, Nap); ^{13}C NMR (125 MHz, $CDCl_3$) δ 69.0 (1 C, C6), 71.6 (1 C, C4), 72.8 (1 C, **C**H_2Ph), 72.9 (1 C, **C**H_2Nap), 73.3 (1 C, **C**H_2Ph), 75.5 (1 C, **C**H_2Ph), 75.8 (1 C, C3), 76.5 (1 C, C2), 78.4 (1 C, C5), 125.9-126.1 (3 C, Nap), 126.9 (1 C, Nap), 127.6-128.9 (18 C, 3 × Ph, Nap), 132.9, 133.0, 135.0, 136.7, 137.3, 137.6 (6 C, Cq), 169.3 (1 C, C=O); HRMS $(ESI)^+$ *m/z* 606.2853 [$C_{38}H_{36}O_6$ $(M+NH_4)^+$ requires 606.2850].

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 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. spirits 3:2) to yield analytically pure **13** as a yellow solid, m.p. 120 °C; $[\alpha]_D^{25}$ +7.21 (*c* 0.41, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.20 (1 H, d, $J_{5,OH}$ = 6.2 Hz, OH), 3.61 (2 H, m, H6a, H6b), 3.87 (1 H, dd, $J_{3,4}$ = 5.9, $J_{4,5}$ = 7.3 Hz, H4), 3.98 (1 H, m, H5), 4.13 (1 H, dd, $J_{2,3}$ = 3.5, $J_{3,4}$ = 5.8 Hz, H3), 4.33 (1 H, d, $J_{2,3}$ = 3.5 Hz, H2), 4.43-4.60 (6 H, m, 6 × C**H**$_2$Ph), 4.82 (2 H, s, 2 × C**H**$_2$Nap), 5.50 (1 H, broad s, NH), 6.54 (1 H, broad s, NH), 7.11-7.27 (15 H, m, 3 × Ph), 7.38-7.43 (3 H,

m, Nap), 7.68-7.76 (4 H, m, Nap); ^{13}C NMR (125 MHz, $CDCl_3$) δ 71.1 (1 C, C5), 71.4 (1 C, C6), 72.9 (1 C, **C**H_2Ph), 73.6 (1 C, **C**H_2Ph), 74.6 (1 C, **C**H_2Ph), 75.0 (1 C, **C**H_2Nap), 79.1 (1 C, C4), 80.2 (1 C, C2), 81.6 (1 C, C3), 126.0-126.3 (3 C, Nap), 126.9 (1 C, Nap), 127.8-128.7 (18 C, 3 × Ph, Nap), 133.1, 133.4, 135.7, 137.2, 138.2, 138.4 (6 C, Cq), 173.4 (1 C, C=O); HRMS (ESI)$^+$ *m/z* 606.2850 [$C_{38}H_{39}NO_6$ $(M+H)^+$ requires 606.2844].

(3*S*,4*S*,5*S*,6*R*/*S*)-3,5-Bis(benzyloxy)-6-[(benzyloxy)methyl]-6-hydroxy-4-(2-naphthylmethyloxy)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 attain rt and was 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. spirits 1:1) to give a separable mixture of the hydroxyl-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 (1 H, d, *J* = 9.8 Hz, C**H**$_2$(C6) D-*manno*), 3.43 (1 H, d, *J* = 9.6 Hz, C**H**$_2$(C6) L-*gulo*), 3.47 (1 H, d, *J* = 9.8 Hz, C**H**$_2$(C6) D-*manno*), 3.57 (1 H, d, *J* = 9.6 Hz, C**H**$_2$(C6) L-*gulo*), 3.72 (1 H, broad s, OH), 4.22 (1 H, d, $J_{3,4}$ = 3.0 Hz, H3 D-*manno*), 4.26 (1 H, d, $J_{3,4}$ = 3.1 Hz, H3 L-*gulo*), 4.98 (1 H, d, *J* = 12.5 Hz, C**H**$_2$Ph D-*manno*), 5.10 (1 H, d, *J* = 12.3 Hz, C**H**$_2$Ph L-*gulo*), 6.33 (1 H, broad s, NH L-*gulo*), 6.22 (1 H, broad s, NH D-*manno*); ^{13}C NMR (125 MHz, $CDCl_3$) δ 74.0 (1 C, **C**H_2(C6) D-*manno*), 74.5 (1 C, C3 D-*manno*), 169.6 (1 C, C=O D-*manno*), 170.2 (1 C, C=O L-*gulo*); HRMS (ESI)$^+$ *m/z* 606.2698 [$C_{38}H_{37}NO_6$ $(M+H)^+$ requires 604.2694].

(3*S*,4*S*,5*S*,6*R*)-3,5-Bis(benzyloxy)-6-[(benzyloxy)methyl]-4-(2-naphthylmethoxy)piperidin-2-one (16) and (3*S*,4*S*,5*S*,6*S*)-3,5-bis(benzyloxy)-6-[(benzyloxy)methyl]-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 left to stir 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. spirits 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. spirits 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.

Characterization for **16:**

$[\alpha]_D^{23}$ –57 (*c* 0.535, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.36 (1 H, dd, $J_{6,6a}$ = 4.27, $J_{6a,6b}$ = 9.11 Hz, C**H**$_2$(C6)), 3.46 (2 H, m, H6, C**H**$_2$(C6)), 3.57 (1 H, m, H3), 3.91 (1 H, dd, $J_{3,4}$ = 3.1, $J_{4,5}$ = 4.4 Hz, H4), 3.95 (1 H, m, H6), 4.08-4.19 (3 H, m, 2 × C**H**$_2$Ph, H5), 4.40 (2 H, m, 2 × C**H**$_2$Ph), 4.66 (1 H, d, *J* = 12.4 Hz, C**H**$_2$Ph), 4.71 (1 H, d, *J* = 12.3 Hz, C**H**$_2$Nap), 4.93 (1 H, d, *J* = 12.3 Hz, C**H**$_2$Nap), 5.10 (1 H, d, *J* = 12.4 Hz, C**H**$_2$Ph), 5.83 (1 H, broad s, NH), 6.84 (2 H, apt. d, *J* = 7.05 Hz, Ph), 7.07-7.45 (16 H, m, Ph, Nap), 7.62 (1 H, s, Nap), 7.72-7.79 (3 H, m, Nap); ^{13}C NMR (100 MHz, $CDCl_3$) δ 52.8 (1 C, C6), 70.3 (1 C, **C**H$_2$(C6)), 72.5 (1 C, **C**H$_2$Nap), 73.6 (1 C, **C**H$_2$Ph), 73.6 (1 C, **C**H$_2$Ph), 73.7 (1 C, **C**H$_2$Ph), 74.2 (1 C, C5), 74.3 (1 C, C3), 74.8 (1 C, C4), 126.0-126.3 (3 C, Nap), 126.8 (1 C, Nap), 127.8-128.6 (18 C, 3 × Ph, Nap), 133.2, 133.3, 135.6, 137.0, 137.6, 138.4 (6 C, Cq), 171.3 (1 C, C=O); HRMS (ESI)$^+$ *m/z* 588.2747 [$C_{38}H_{37}NO_5$ (M+H)$^+$ requires 588.2749].

Characterization for **17:**

$[\alpha]_D^{25}$ –9.49 (*c* 0.715, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.41 (1 H, m, C**H**$_2$(C6)), 3.54 (2 H, m, H6, C**H**$_2$(C6)), 3.66 (1 H, t, $J_{4,5}$ = $J_{5,6}$ = 5.2 Hz, H5), 3.98 (1 H, dd, $J_{3,4}$ = 2.9, $J_{4,5}$ = 5.0 Hz, H4), 4.18 (1 H, d, $J_{3,4}$ = 2.9 Hz, H3), 4.38 (1 H, d, *J* = 11.6 Hz, C**H**$_2$Ph), 4.42-4.49 (2 H, m, 2 × C**H**$_2$Ph), 4.55 (1 H, d, *J* = 11.6 Hz, C**H**$_2$Ph), 4.69 (1 H, d, *J* = 12.1 Hz, C**H**$_2$Ph), 4.74 (1 H, d, *J* = 12.2 Hz, C**H**$_2$Nap), 4.88 (1 H, d, *J* = 12.2 Hz, C**H**$_2$Nap), 5.06 (1 H, d, *J* = 12.2 Hz, C**H**$_2$Ph), 5.91 (1 H, broad s, NH), 7.08-7.49 (18 H, m, 3 × Ph, Nap), 7.72-7.84 (4 H, m, Nap); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.5 (1 C, C6), 71.5 (1 C, **C**H$_2$(C6)), 72.9 (1 C, **C**H$_2$Nap), 72.9 (1 C, **C**H$_2$Ph), 73.4 (1 C, **C**H$_2$Ph), 73.5 (1 C, **C**H$_2$Ph), 75.0 (1 C, C5), 75.2 (1 C, C3), 77.8 (1 C, C4), 126.1-126.3 (3 C, Nap), 127.0 (1 C, Nap), 127.8-128.6 (18 C, 3 × Ph, Nap), 133.2, 133.3, 135.5, 137.5, 138.1 (6 C, Cq), 169.6 (1 C, C=O); HRMS (ESI)$^+$ *m/z* 588.2747 [$C_{38}H_{37}NO_5$ (M+H)$^+$ requires 588.2744].

(3*S*,4*S*,5*S*,6*S*)-3,5-Bis(benzyloxy)-6-[(benzyloxy)methyl]-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 uL, 0.083 mmol), freshly activated 4 Å molecular sieves and distilled toluene (6 mL) and the reaction was left to stir for 20 h. The mixture was filtered, stirred with MeOH (1.68 mL) for 2 h and the solvent was removed under reduced pressure. The residue obtained was subjected to flash chromatography (EtOAc/pet. spirits 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, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.43 (1 H, m, C**H**$_2$(C6)), 3.56 (2 H, m, H6, C**H**$_2$(C6)), 3.83 (1 H, apt. t, H5), 3.91 (1 H, dd, $J_{3,4}$ = 2.6, $J_{4,5}$ = 7.2 Hz, H4), 4.42 (1 H, d, $J_{3,4}$ = 2.5 Hz, H3), 4.44-4.52 (3 H, m, 3 × C**H**$_2$Ph), 4.68-4.73 (2 H, m, C**H**$_2$Nap, C**H**$_2$Ph), 4.79 (1 H, d, *J* = 12.1 Hz, C**H**$_2$Nap), 4.83 (1 H, d, *J* = 12.0 Hz, C**H**$_2$Ph), 5.08 (1 H, d, *J* = 12.1 Hz, C**H**$_2$Ph), 7.14-7.52 (18 H, m, 3 × Ph, Nap), 7.73-7.85 (4 H, m, Nap), 8.13 (1 H, broad s, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 59.8 (1 C, C6), 70.6 (1 C, C **C**H$_2$(C6)), 72.5 (1 C, **C**H$_2$Nap), 73.2 (1 C, **C**H$_2$Ph), 73.5 (1 C, **C**H$_2$Ph), 73.7 (1 C, **C**H$_2$Ph), 74.2 (1 C, C5), 78.3 (1 C, C4), 79.8 (1 C, C3), 125.9-126.3 (3 C, Nap), 126.8 (1 C, Nap), 127.8-128.7 (18 C, 3 × Ph, Nap), 133.1, 133.3, 135.4, 137.3, 137.6, 138.0 (6 C, Cq), 200.0 (1 C, C=O); HRMS $(ESI)^+$ *m/z* 604.2524 [$C_{38}H_{37}NO_4S$ $(M+H)^+$ requires 604.2516].

(5*R*,6*R*,7*S*,8*R*)-7-(2-Naphthylmethoxy)-6,8-bis(benzyloxy)-5-(benzyloxy)methyl-5,6,7,8-tetrahydroimidazo[1,2-*α*]pyridine (21) and (5*R*,6*R*,7*S*,8*S*)-7-(2-naphthylmethoxy)-6,8-bis(benzyloxy)-5-(benzyloxy)methyl-5,6,7,8-tetrahydroimidazo[1,2-*α*]pyridine (20)

Thionolactam **18** (256 mg, 0.424 mmol) was dissolved in aminoacetaldehyde dimethyl acetal (0.69 mL, 6.33 mmol) and 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 reaction was stirred at 60 °C overnight. The mixture was 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. spirits 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.

Characterization for **20:**

$[\alpha]_D^{25}$ +52 (*c* 0.315, $CHCl_3$; lit.[39] +52, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ 3.75 (1 H, dd, $J_{5,5a}$ = 5.0, $J_{5a,5b}$ = 10.3 Hz, C**H**$_2$(C5)), 3.87 (2 H, m, H6, C**H**$_2$(C5)), 4.13 (1 H, dd, $J_{6,7}$ = 7.5, $J_{7,8}$ = 5.8 Hz, H7), 4.18 (1 H, m, H5), 4.45 (2 H, apt. d, 2 × C**H**$_2$Ph), 4.51 (1 H, d, *J* = 11.2 Hz, C**H**$_2$Ph), 4.78 (1 H, d, $J_{7,8}$ = 5.8 Hz, H8), 4.84 (1 H, d, *J* = 11.6 Hz, C**H**$_2$Ph), 4.86 (1 H, d, *J* = 11.2 Hz, C**H**$_2$Ph), 4.89 (1 H, d, *J* = 11.5 Hz, C**H**$_2$Nap), 4.97 (1 H, d, *J* = 11.5 Hz, C**H**$_2$Ph), 5.19 (1 H, d, *J* = 11.5 Hz, C**H**$_2$Nap), 7.04 (1 H, s, H2), 7.12 (1 H, s, H3), 7.14-7.48 (18 H, m, 3 × Ph, Nap), 7.68-7.83 (4 H, m, Nap); ^{13}C NMR (125 MHz, $CDCl_3$) δ 58.3 (1 C, C5), 68.5 (1 C, **C**H_2(C5)), 72.9 (1 C, **C**H_2Nap), 73.4 (1 C, **C**H_2Ph), 74.3 (1 C, **C**H_2Ph), 74.4 (1 C, **C**H_2Ph), 74.5 (1 C, C8), 76.2 (1 C, C6), 82.2 (1 C, C7), 117.4 (1 C, C2), 126.1-126.9 (3 C, Nap), 127.7 (1 C, Nap), 127.8-128.6 (18 C, 3 × Ph, Nap), 129.5 (1 C, C3), 133.2, 133.4, 135.5, 137.4, 137.7, 138.4 (6 C, Cq), 144.2 (Cq, imidazole).

Characterization for **21:**

$[\alpha]_D^{25}$ –24 (*c* 0.24, $CHCl_3$) (lit.[39] –20, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ 3.57 (1 H, dd, $J_{5,5a}$ = 7.1, $J_{5a,5b}$ = 10.1 Hz, C**H**$_2$(C5)), 3.71 (1 H, dd, $J_{5,5a}$ = 3.4, $J_{5a,5b}$ = 10.1 Hz, C**H**$_2$(C5)), 3.84 (1 H, dd, $J_{6,7}$ = 9.3, $J_{7,8}$ = 3.1 Hz, H7), 4.06 (1 H, m, H5), 4.25 (1 H, dd, $J_{5,6}$ = 9.3, $J_{6,7}$ = 7.2 Hz, H6), 4.39 (2 H, m, 2 × C**H**$_2$Ph), 4.56-4.66 (3 H, m, 2 × C**H**$_2$Ph, C**H**$_2$Nap), 4.69 (1 H, d, *J* = 12.2 Hz, C**H**$_2$Nap), 4.74 (1 H, d, *J* = 12.0 Hz, C**H**$_2$Ph), 4.78 (1 H, d, $J_{7,8}$ = 3.0 Hz, H8), 4.96 (1 H, d, *J* = 11.2 Hz, C**H**$_2$Ph), 6.98 (1 H, s, H3), 7.09 (1 H, s, H2), 7.17-7.39 (18 H, m, 3 × Ph, Nap), 7.62-7.74 (4 H, m, Nap); ^{13}C NMR (125 MHz, $CDCl_3$) δ 60.0 (1 C, C5), 68.3 (1 C, C8), 70.6 (1 C, **C**H_2Nap), 71.2 (1 C, **C**H_2(C5)), 71.8 (1 C, **C**H_2Ph), 73.3 (1 C, **C**H_2Ph), 74.3 (1 C, C6), 75.0 (1 C, **C**H_2Ph), 80.2 (1 C, C3), 119.5 (1 C, C2), 125.2-126.9 (3 C, Nap), 126.7 (1 C, Nap), 128.6-127.7 (18 C, 3 × Ph, Nap), 129.4 (1 C, C3), 133.2, 133.3, 135.4, 137.6, 138.2, 138.3 (6 C, Cq), 143.0 (Cq, imidazole).

(5*R*,6*R*,7*S*,8*R*)-6,8-Bis(benzyloxy)-5-[(benzyloxy)methyl]-5,6,7,8-tetrahydroimidazo[1,2-*α*]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 rt overnight. DDQ (25 mg, 0.11 mmol) was again added and the reaction mixture was stirred for 3 days when TLC analysis (EtOAc/pet. spirits 8:2) indicated complete consumption of the starting material. The reaction was 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. spirits 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, $CHCl_3$) (lit.[39] -6, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 3.64 (1 H, dd, $J_{5,5a}$ = 5.9, $J_{5a,5b}$ = 10.2 Hz, C**H**$_2$(C5)), 3.78 (1 H, dd, $J_{5,5a}$ = 2.5, $J_{5a,5b}$ = 10.2 Hz, C**H**$_2$(C5)), 4.03 (3 H, m, H7, H6, H5), 4.42 (2 H, apt. s, 2 × C**H**$_2$Ph), 4.54 (1 H, d, *J* = 11.2 Hz, C**H**$_2$Ph), 4.65 (1 H, d, *J* = 11.6 Hz, C**H**$_2$Ph), 4.70 (1 H, d, $J_{7,8}$ = 3.3 Hz, H8), 4.85 (1 H, d, *J* = 11.6 Hz, C**H**$_2$Ph), 4.90 (1 H, d, *J* = 11.2 Hz, C**H**$_2$Ph), 7.05 (1 H, s, H3), 7.13 (1 H, s, H2), 7.19-7.28 (15 H, m, 3 × Ph); ^{13}C NMR (125 MHz, $CDCl_3$) δ 59.1 (1 C, C5), 70.2 (1 C, **C**H$_2$(C5)), 71.2 (2 C, C8, **C**H$_2$Ph), 72.4 (1 C, C6), 73.2 (1 C, **C**H$_2$Ph), 74.6 (1 C, **C**H$_2$Ph), 75.3 (1 C, C7), 118.9 (1 C, C2), 127.7-128.5 (15 C, 3 × Ph), 129.6 (1 C, C3), 137.5, 137.7, 137.8 (3 C, Cq), 142.3 (Cq, 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-[(benzyloxy)methyl]-5,6,7,8-tetrahydroimidazo[1,2-*α*]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 rt for 30 min. Triflic acid (1 µL, 0.011 mmol) was added to the mixture at –20 °C and the reaction was left to stir for 1 h. The reaction mixture was stirred at 0 °C for 20 min, then at r.t for another 20 min, quenched with pyridine (1 drop) and filtered through a Celite pad. The solvent was removed under reduced pressure and the resulting residue was subjected to flash chromatography (EtOAc/pet. spirits/ 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, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ 2.11 (3 H, s, Ac), 3.49 (1 H, dd, $J_{5',5a'}$ = 1.7, $J_{5a',5b'}$ = 10.9 Hz, C**H**$_2$(C5')), 3.55 (1 H, dd, $J_{5,5a}$ = 6.7, $J_{5a,5b}$ = 10.2 Hz, C**H**$_2$(C5)), 3.63 (1 H, dd, $J_{5',5b'}$ = 3.5, $J_{5a',5b'}$ = 10.8 Hz, C**H**$_2$(C5')), 3.67 (1 H, dd, $J_{5,5b}$ = 3.2, $J_{5a,5b}$ = 10.2 Hz, C**H**$_2$(C5)), 3.87 (1 H, m, H5'), 3.93 (1 H, t, $J_{3',4'} = J_{4',5'}$ = 9.5 Hz, H4'), 4.01 (1 H, dd, $J_{2',3'}$ = 3.3, $J_{3',4'}$ = 9.5 Hz, H3'), 4.07 (1 H, dd, $J_{6,7}$ = 9.5, $J_{7,8}$ = 3.1 Hz, H7), 4.13 (1 H, m, H5), 4.29 (1 H, dd, $J_{5,6}$ = 7.1, $J_{6,7}$ = 9.5 Hz, H6), 4.41 (2 H, m, 2 × C**H**$_2$Ph), 4.46 (1 H, d, *J* = 10.9 Hz, C**H**$_2$Ph), 4.51 (1 H, d, *J* = 11.3 Hz, C**H**$_2$Ph), 4.54 (1 H, d, *J* = 12.0 Hz, C**H**$_2$Ph), 4.57 (1 H, d, *J* = 11.3 Hz, C**H**$_2$Ph), 4.64 (3 H, apt. d, 3 × C**H**$_2$Ph), 4.81 (1 H, d, $J_{2,3}$ = 3.1 Hz, H2), 4.84 (2 H, m, 2 × C**H**$_2$Ph), 5.19 (1 H, d, $J_{1',2'}$ = 1.6 Hz, H1'), 5.48 (1 H, dd, $J_{1',2'}$ = 1.6, $J_{2',3'}$ = 3.3 Hz, H2'), 7.07 (1 H, s, H3), 7.14 (1 H, s, H2), 7.08-7.34 (30 H, m, 6 × Ph); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.2 (1 C, Me), 60.0 (1 C, C5), 68.5 (1 C, C6'), 69.1 (1 C, C2'), 70.3 (1 C, **C**H$_2$Ph), 70.8 (1 C, **C**H$_2$(C5)), 70.9 (1 C, C8), 72.1 (1 C, **C**H$_2$Ph), 72.4 (1 C, C5'), 73.4 (1 C, **C**H$_2$Ph), 73.7 (1 C, **C**H$_2$Ph), 74.2 (1 C, C4'), 74.4 (1 C, C6), 75.1 (2 C, **C**H$_2$Ph), 78.2 (1 C, C3'), 80.3 (1 C, C7), 100.1 (1 C, C1'), 119.4 (1 C, C2), 127.6-128.7 (30 C, 6 × Ph), 129.5 (1 C, C3), 137.6,

137.7, 137.9, 138.1, 138.2, 138.8 (6 C, Cq), 142.6 (Cq, imidazole), 170.4 (1 C, C=O); HRMS (ESI)$^+$ *m/z* 945.4322 [$C_{58}H_{60}N_2O_{10}$ $(M+H)^+$ requires 945.4321].

(5*R*,6*R*,7*S*,8*R*)-7-(3,4,6-Tri-*O*-benzyl-α-D-mannopyranosyloxy)-6,8-bis(benzyloxy)-5-[(benzyloxy)methyl]-5,6,7,8-tetrahydroimidazo[1,2-*α*]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 rt 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. spirits/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, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.40 (1 H, d, $J_{2',OH}$ = 2.5 Hz, OH), 3.49 (1 H, dd, $J_{5',6a'}$ = 1.8, $J_{6a',6b'}$ = 10.8 Hz, H6a'), 3.58 (2 H, m, C**H**$_2$(C5), H6b'), 3.70 (1 H, dd, $J_{5,5a}$ = 3.2, $J_{5a,5b}$ = 10.1 Hz, C**H**$_2$(C5)), 3.87 (1 H, m, H5'), 3.91 (2 H, m, H4', H3'), 4.03 (1 H, m, H2'), 4.08 (1 H, dd, $J_{6,7}$ = 9.6, $J_{7,8}$ = 3.1 Hz, H7), 4.13 (1 H, m, H5), 4.28 (1 H, dd, $J_{5,6}$ = 7.3, $J_{6,7}$ = 9.6 Hz, H6), 4.40-4.53 (5 H, m, 5 × C**H**$_2$Ph), 4.57-4.68 (5 H, m, 5 × C**H**$_2$Ph), 4.79 (2 H, m, 2 × C**H**$_2$Ph), 4.85 (1 H, d, $J_{7,8}$ = 3.1 Hz, H8), 5.23 (1 H, d, $J_{1',2'}$ = 1.5 Hz, H1'), 7.08 (1 H, s, H3), 7.14 (1 H, s, H2), 7.11-7.35 (30 H, m, 6 × Ph); ^{13}C NMR (125 MHz, $CDCl_3$) δ 60.0 (1 C, C5), 68.6 (1 C, C6'), 69.0 (1 C, C2'), 70.3 (1 C, **C**H$_2$Ph), 70.7 (1 C, C8), 71.1 (1 C, **C**H$_2$(C5)), 72.0 (1 C, C5'), 72.4 (1 C, **C**H$_2$Ph), 73.4 (1 C, **C**H$_2$Ph), 73.7 (1 C, **C**H$_2$Ph), 74.3 (2 C, C6,3'), 75.1 (2 C, **C**H$_2$Ph), 80.1 (1 C, C4'), 80.4 (1 C, C7), 101.8 (1 C, C1'), 119.3 (1 C, C2), 127.6-128.7 (30 C, 6 × Ph), 129.6 (1 C, C3), 137.6, 137.8, 138.1, 138.3, 138.7 (6 C, Cq), 142.7 (Cq, imidazole); HRMS (ESI)$^+$ *m/z* 903.4214 [$C_{56}H_{58}N_2O_9$ $(M+H)^+$ requires 903.4215].

(5*R*,6*R*,7*S*,8*R*)-6,8-Dihydroxy-5-[(hydroxy)methyl]-7-(α-D-mannopyranosyloxy)-5,6,7,8-tetrahydroimidazo[1,2-*α*]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 d. At this point TLC analysis (EtOAc/MeOH/H_2O 7:3:2) indicated complete conversion to a single species along with baseline byproducts. The suspension was filtered through a Celite pad, the solvent was evaporated and the resulting residue was subjected to flash chromatography (EtOAc/MeOH/H_2O 5:2:1) to afford the ManManIm **2** (2.4 mg, 48%) as a colourless residue; $[\alpha]_D^{27}$ +13 (*c* 0.12, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 3.57 (1 H, t, $J_{3',4'} = J_{4',5'}$ = 9.8 Hz, H4'), 3.66 (1 H, dd, $J_{5',6a'}$ = 6.3, $J_{6a',6b'}$ = 12.1 Hz, H6a'), 3.77 (1 H, m, H5'), 3.83 (2 H, m, H3', H6b'), 3.91 (1 H, m, H5), 3.95 (1 H, dd, $J_{5,5a}$ = 3.3, $J_{5a,5b}$ = 12.7 Hz, C**H**$_2$(C5)), 3.99 (1 H, dd, $J_{6,7}$ = 10.2, $J_{7,8}$ = 3.7 Hz, H7), 4.02 (1 H, dd,

$J_{1',2'}$ = 3.4, $J_{2',3'}$ = 1.7 Hz, H2'), 4.13 (1 H, dd, $J_{5,5b}$ = 2.6, $J_{5a,5b}$ = 12.7 Hz, C**H**$_2$(C5)), 4.27 (1 H, dd, $J_{5,6}$ = 8.6, $J_{6,7}$ = 10.2 Hz, H6), 4.97 (1 H, d, $J_{7,8}$ = 3.7 Hz, H8), 5.23 (1 H, d, $J_{1',2'}$ = 1.6 Hz, H1'), 7.01 (1 H, s, H3), 7.20 (1 H, s, H2); ^{13}C NMR (125 MHz, D_2O) δ 59.3 (1 C, **C**H_2(C5)), 60.9 (1 C, C5,6'), 63.5 (1 C, C8), 63.9 (1 C, C6), 66.7 (1 C, C4'), 69.9 (1 C, C2'), 70.3 (2 C, C4,3'), 73.5 (1 C, C5'), 78.1 (1 C, C7), 102.1 (1 C, C1'), 118.3 (1 C, C2), 128.7 (1 C, C3), 144.7 (Cq, imidazole); HRMS (ESI)$^+$ *m/z* 363.1398 [$C_{14}H_{22}N_2O_9$ $(M+H)^+$ requires 363.1398].

Isothermal titration calorimetry (ITC)

The binding affinity of Man2NH_2DMJ to *Bt*GH99 was determined 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. Due to the low affinity of the ligand, which prevented the observation of a sigmoidal binding isotherm, N was fixed at 1.[40] An initial ITC experiment was conducted 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 was calculated using the MicroCal PEAQ-ITC Analysis Software (Malvern Instruments).

Crystallization and Data Collection

*Bx*GH99 protein[10] was crystallized using a vapour diffusion–hanging drop method in 3 M sodium acetate, 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 pH 7.0, 100 mM NaCl) with 1 µl of the crystallant solution. Crystals were fished from the droplet using a nylon cryoloop, without cryoprotection. Data were collected at Diamond Light Source beamline i04 using X-rays at a wavelength of 0.979 Å.

Structure solution and Refinement

Images containing diffraction patterns were indexed and integrated by using DIALS[41] through xia2.[42] The HKL index of each data set was then matched to a previous solution in Aimless.[43] Refinement was performed in Refmac5[44] and real-space model building in

630 Coot.[45] Model geometry and agreement with electron density was validated in Coot and
631 Edstats.[46] Quality of the carbohydrates and the nitrogen heterocycles was checked using
632 Privateer.[47] The modelling and refinement process was aided by using ccp4i2 interface.[48]

633

Acknowledgments

The Australian Research Council is thanked for financial support (DP120101396, FT130100103). We thank Diamond Light Source for access to beamline i04 (proposal mx13587) that contributed to the results presented here. GJD and LFS were supported by the European Research Council (ERC-2012-AdG-32294 'Glycopoise'). GJD thanks the Royal Society for the Ken Murray Research Professorship.

References

[1] V. Lombard, H. Golaconda Ramulu, E. Drula, P. M. Coutinho, B. Henrissat, *Nucleic Acids Res.* **2014**, *42*, D490-495.

[2] *Glycobiology* **2018**, *28*, 3-8.

[3] C. Rabouille, R. G. Spiro, *J. Biol. Chem.* **1992**, *267*, 11573-11578.

[4] S. E. Moore, R. G. Spiro, *J. Biol. Chem.* **1992**, *267*, 8443-8451.

[5] S. E. Moore, R. G. Spiro, *J. Biol. Chem.* **1990**, *265*, 13104-13112.

[6] W. A. Lubas, R. G. Spiro, *J. Biol. Chem.* **1988**, *263*, 3990-3998.

[7] W. A. Lubas, R. G. Spiro, *J. Biol. Chem.* **1987**, *262*, 3775-3781.

[8] Z. Hakki, A. J. Thompson, S. Bellmaine, G. Speciale, G. J. Davies, S. J. Williams, *Chem. Eur. J.* **2015**, *21*, 1966-1977.

[9] F. Cuskin, E. C. Lowe, M. J. Temple, Y. Zhu, E. A. Cameron, N. A. Pudlo, N. T. Porter, K. Urs, A. J. Thompson, A. Cartmell, A. Rogowski, B. S. Hamilton, R. Chen, T. J. Tolbert, K. Piens, D. Bracke, W. Vervecken, Z. Hakki, G. Speciale, J. L. Munoz-Munoz, A. Day, M. J. Pena, R. McLean, M. D. Suits, A. B. Boraston, T. Atherly, C. J. Ziemer, S. J. Williams, G. J. Davies, D. W. Abbott, E. C. Martens, H. J. Gilbert, *Nature* **2015**, *517*, 165-169.

[10] A. J. Thompson, R. J. Williams, Z. Hakki, D. S. Alonzi, T. Wennekes, T. M. Gloster, K. Songsrirote, J. E. Thomas-Oates, T. M. Wrodnigg, J. Spreitz, A. E. Stutz, T. D. Butters, S. J. Williams, G. J. Davies, *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 781-786.

[11] J. Munoz-Munoz, A. Cartmell, N. Terrapon, B. Henrissat, H. J. Gilbert, *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 4936-4941.

[12] G. Speciale, M. Farren-Dai, F. S. Shidmoossavee, S. J. Williams, A. J. Bennet, *J. Am. Chem. Soc.* **2016**, *138*, 14012-14019.

[13] U. Spohr, M. Bach, R. G. Spiro, *Can. J. Chem.* **1993**, *71*, 1928-1942.

[14] S. Hiraizumi, U. Spohr, R. G. Spiro, *J. Biol. Chem.* **1993**, *268*, 9927-9935.

[15] H. Ardron, T. D. Butters, F. M. Platt, M. R. Wormald, R. A. Dwek, G. W. J. Fleet, G. S. Jacob, *Tetrahedron Asymmetry* **1993**, *4*, 2011-2024.

[16] D. S. Alonzi, N. V. Kukushkin, S. A. Allman, Z. Hakki, S. J. Williams, L. Pierce, R. A. Dwek, T. D. Butters, *Cell. Mol. Life Sci.* **2013**, *70*, 2799-2814.

[17] M. Petricevic, L. F. Sobala, P. Fernandes, L. Raich, A. J. Thompson, G. Bernardo-Seisdedos, O. Millet, S. Zhu, M. Sollogoub, J. Jimenez-Barbero, C. Rovira, G. J. Davies, S. J. Williams, *J. Am. Chem. Soc.* **2017**, *139*, 1089–1097.

[18] U. Spohr, M. Bach, R. G. Spiro, *Can. J. Chem.* **1993**, *71*, 1919-1927.

[19] T. Aoyagi, H. Suda, K. Uotani, F. Kojima, T. Aoyama, K. Horiguchi, M. Hamada, T. Takeuchi, *J. Antibiot.* **1992**, *45*, 1404-1408.

[20] T. D. Heightman, A. T. Vasella, *Angew. Chem. Int. Ed.* **1999**, *38*, 750-770.

[21] I. K. Khanna, F. J. Koszyk, M. A. Stealey, R. M. Weier, J. Julien, R. A. Mueller, S. N. Rao, L. Swenton, D. P. Getman, G. A. DeCrescenzo, R. M. Heintz, *J. Carbohydr. Chem.* **1995**, *14*, 843-878.

[22] M. Hoch, E. Heinz, R. R. Schmidt, *Carbohydr. Res.* **1989**, *191*, 21-28.

[23] T. Oshitari, M. Shibasaki, T. Yoshizawa, M. Tomita, K.-i. Takao, S. Kobayashi, *Tetrahedron* **1997**, *53*, 10993-11006.

[24] J. D. Albright, L. Goldman, *J. Am. Chem. Soc.* **1967**, *89*, 2416-2423.

[25] H. S. Overkleeft, J. van Wiltenburg, U. K. Pandit, *Tetrahedron* **1994**, *50*, 4215-4224.

[26] T. Granier, N. Panday, A. Vasella, *Helv. Chim. Acta* **1997**, *80*, 979-987.

[27] G. J. Davies, K. S. Wilson, B. Henrissat, *Biochem. J.* **1997**, *321*, 557-559.

[28] D. L. Zechel, A. B. Boraston, T. Gloster, C. M. Boraston, J. M. Macdonald, D. M. G. Tilbrook, R. V. Stick, G. J. Davies, *J. Am. Chem. Soc.* **2003**, *47*, 14313-14323.

[29] J. Clark, D. D. Perrin, *Q. Rev.* **1964**, *18*, 295-320.

[30] S. Inouye, *Chem. Pharm. Bull.* **1968**, *16*, 1134-1137.

[31] A. R. Mandhapati, D. Shcherbakov, S. Duscha, A. Vasella, E. C. Böttger, D. Crich, *ChemMedChem* **2014**, *9*, 2074-2083.
[32] J. Agirre, J. Iglesias-Fernandez, C. Rovira, G. J. Davies, K. S. Wilson, K. D. Cowtan, *Nat. Struct. Mol. Biol.* **2015**, *22*, 833-834.
[33] A. Varrot, M. Schülein, M. Pipelier, A. Vasella, G. J. Davies, *J. Am. Chem. Soc.* **1999**, *121*, 2621-2622.
[34] W. Nerinckx, T. Desmet, K. Piens, M. Claeyssens, *FEBS Lett.* **2005**, *579*, 302-312.
[35] R. Charoenwattanasatien, S. Pengthaisong, I. Breen, R. Mutoh, S. Sansenya, Y. Hua, A. Tankrathok, L. Wu, C. Songsiriritthigul, H. Tanaka, S. J. Williams, G. J. Davies, G. Kurisu, J. R. Cairns, *ACS Chem. Biol.* **2016**, *11*, 1891-1900.
[36] A. J. Thompson, J. Dabin, J. Iglesias-Fernandez, A. Ardevol, Z. Dinev, S. J. Williams, O. Bande, A. Siriwardena, C. Moreland, T. C. Hu, D. K. Smith, H. J. Gilbert, C. Rovira, G. J. Davies, *Angew. Chem. Int. Ed.* **2012**, *51*, 10997-11001.
[37] W. C. Still, M. Kahn, A. M. Mitra, *J. Org. Chem.* **1978**, *43*, 2923-2925.
[38] A. B. Pangborn, M. A. Giardello, R. H. Grubbs, R. K. Rosen, F. J. Timmers, *Organometallics* **1996**, *15*, 1518-1520.
[39] C. Ouairy, T. Cresteil, B. Delpech, D. Crich, *Carbohydrate Research* **2013**, *377*, 35-43.
[40] W. B. Turnbull, A. H. Daranas, *J. Am. Chem. Soc.* **2003**, *125*, 14859-14866.
[41] D. G. Waterman, G. Winter, R. J. Gildea, J. M. Parkhurst, A. S. Brewster, N. K. Sauter, G. Evans, *Acta Crystallogr. Sect. D* **2016**, *72*, 558-575.
[42] G. Winter, *J. Appl. Crystallogr.* **2010**, *43*, 186-190.
[43] P. R. Evans, G. N. Murshudov, *Acta Crystallogr. Sect. D* **2013**, *69*, 1204-1214.
[44] G. N. Murshudov, P. Skubak, A. A. Lebedev, N. S. Pannu, R. A. Steiner, R. A. Nicholls, M. D. Winn, F. Long, A. A. Vagin, *Acta Crystallogr. D* **2011**, *67*, 355-367.
[45] P. Emsley, B. Lohkamp, W. G. Scott, K. Cowtan, *Acta Crystallogr. Sect. D* **2010**, *66*, 486-501.
[46] I. Tickle, *Acta Crystallogr. Sect. D* **2012**, *68*, 454-467.
[47] J. Agirre, J. Iglesias-Fernández, C. Rovira, G. J. Davies, K. S. Wilson, K. D. Cowtan, *Nat. Struct. Mol. Biol.* **2015**, *22*, 833.
[48] L. Potterton, J. Agirre, C. Ballard, K. Cowtan, E. Dodson, P. R. Evans, H. T. Jenkins, R. Keegan, E. Krissinel, K. Stevenson, A. Lebedev, S. J. McNicholas, R. A. Nicholls, M. Noble, N. S. Pannu, C. Roth, G. Sheldrick, P. Skubak, V. Uski, F. von Delft, D. Waterman, K. Wilson, M. Winn, M. Wojdyr, *Acta Crystallographica Section D: Structural Biology* **2018**, *in press*.

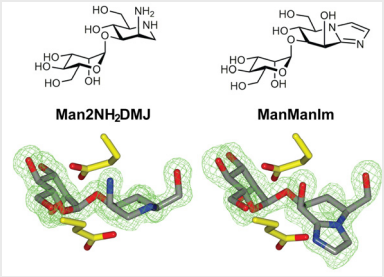
725 **Entry for the Table of Contents**

726

727

FULL PAPER

Mechanism-inspired inhibitor design: Compounds targeting bacterial endomannanase were synthesized to interact with conserved, mechanistically-important residues. X-ray crystallography revealed that binding achieved the anticipated polar interactions, yet sub-optimal affinities were observed. This study identifies challenges associated with mechanism-inspired inhibitor design for GH99 enzymes.



Pearl Z. Fernandes, Marija Petricevic, Lukasz Sobala, Gideon J. Davies, Spencer J. Williams**

Page No. – Page No.

Exploration of strategies for charge and shape mimicry in inhibitor design for family GH99 *endo*-α-1,2-mannanases

728