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

3

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

15

16 **Abstract**

17 *Endo*-α-1,2-mannosidases and -mannanases, members of glycoside hydrolase family 99
18 (GH99), cleave α-Glc/Man-1,3-α-Man-OR structures within mammalian N-linked glycans
19 and fungal α-mannan, respectively. They are proposed to act through a two-step mechanism
20 involving a 1,2-anhydrosugar 'epoxide' intermediate, involving two conserved catalytic
21 residues. In the first step Glu333 acts as general base to deprotonate the 2-hydroxyl group
22 adjacent to the fissile glycosidic bond, while Glu336 provides general acid assistance to
23 departure of the aglycon. We report the synthesis of two inhibitors designed to interact with
24 either the general base (α-mannosyl-1,3-(2-aminodeoxymannojirimycin); Man2NH_2DMJ) or
25 the general acid (α-mannosyl-1,3-mannoimidazole; ManManIm). Modest affinities were
26 observed for an *endo*-α-1,2-mannanase from *Bacteroides thetaiotaomicron*. Structural studies
27 reveal that Man2NH_2DMJ binds like other iminosugar inhibitors, suggesting that the poor
28 inhibition by this compound is not a result of a failure to achieve the expected interaction
29 with the general base, but rather the reduction in basicity of the endocyclic nitrogen caused
30 by introduction of a vicinal, protonated amine at C2. ManManIm binds with the imidazole
31 headgroup distorted downwards, a result of an unfavourable interaction with a conserved
32 active site tyrosine. This study identifies important limitations associated with mechanism-
33 inspired inhibitor design for GH99 enzymes.
34

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

67 ligand with mildly potent affinity (K_D 15 μM for *Bt*GH99).[17] Computational free energy
68 landscape analysis of the preferred conformation of D-glucal suggested that the inhibition of
69 the glucal-based inhibitors arises from mimicry of the proposed ^{4}E conformation of the
70 transition state, but with no contribution from charge mimicry owing to the neutral nature of
71 this compound.[17]

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

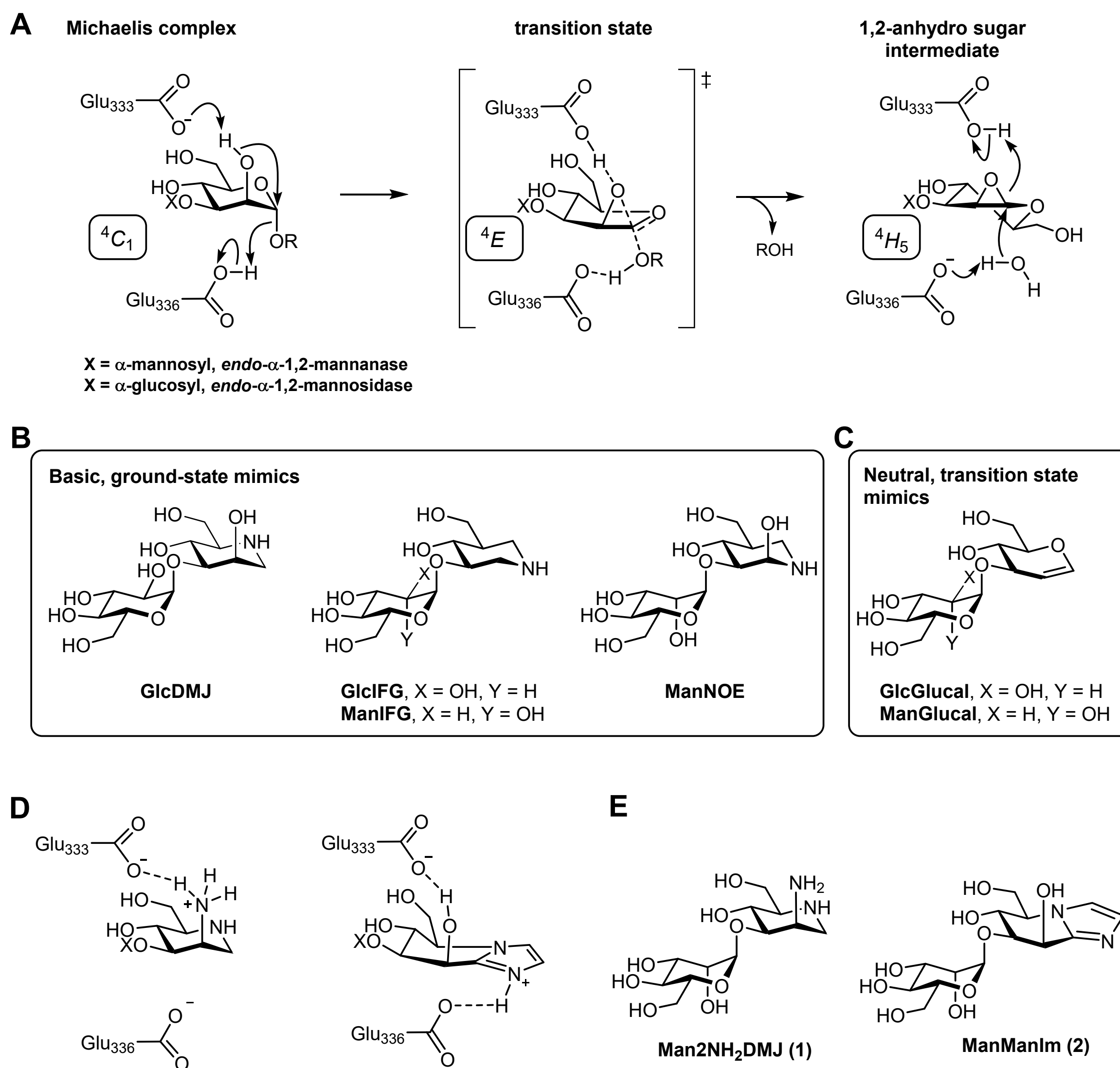
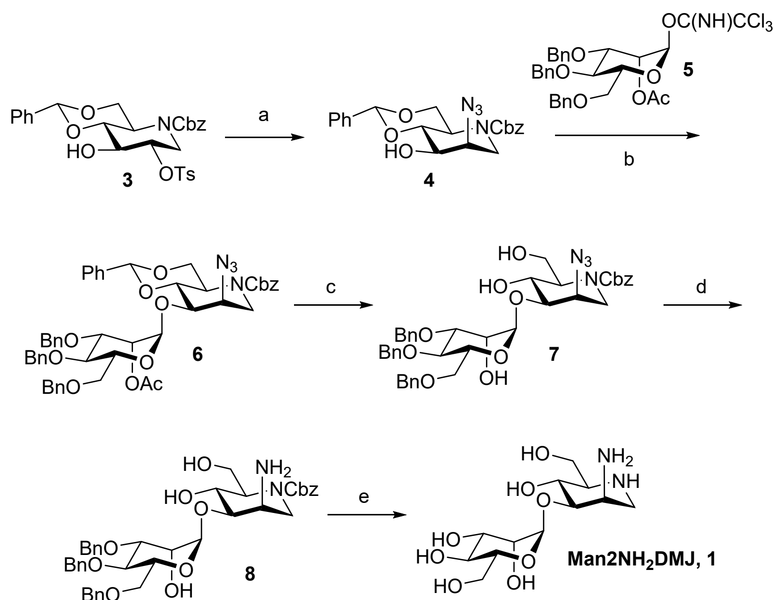


Figure 1. (A) Proposed mechanism for family GH99 retaining endomannosidases/endomannanases. Only the first half of the catalytic cycle is shown. (B) Saturated basic heterocyclic inhibitors for GH99 enzymes mimic ground state conformation. (C) Neutral glycal inhibitors for GH99 enzymes mimic transition state. (D) Two inhibitor design concepts explored herein. (E) Structure of Man2NH2DMJ (**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_2O) 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**.

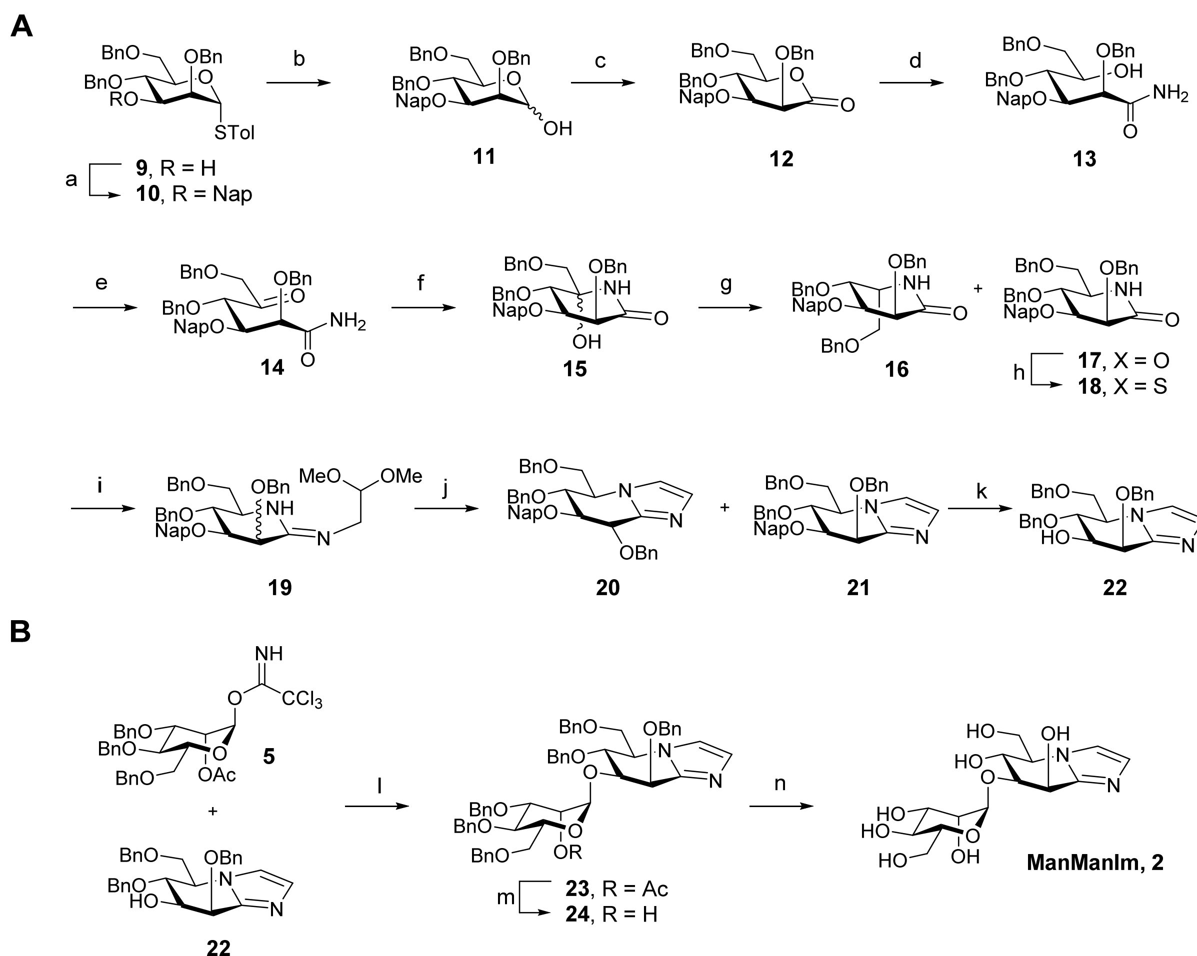


Scheme 1. Reagents and conditions. a) NaN_3, DMF, reflux, 74%; b) TfOH, CH_2Cl_2, -30–0 °C, 87%; c) i) NaOMe, MeOH, ii) 9:1 TFA/H_2O, 83%; d) DTT, pyr, pH 9.2 $NaHCO_3$/Na_2CO_3, 80%; e) H_2, $Pd(OH)_2$, aq HCl, 2:2:1 EtOAc/MeOH/H_2O, 70%.

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_2O/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**.

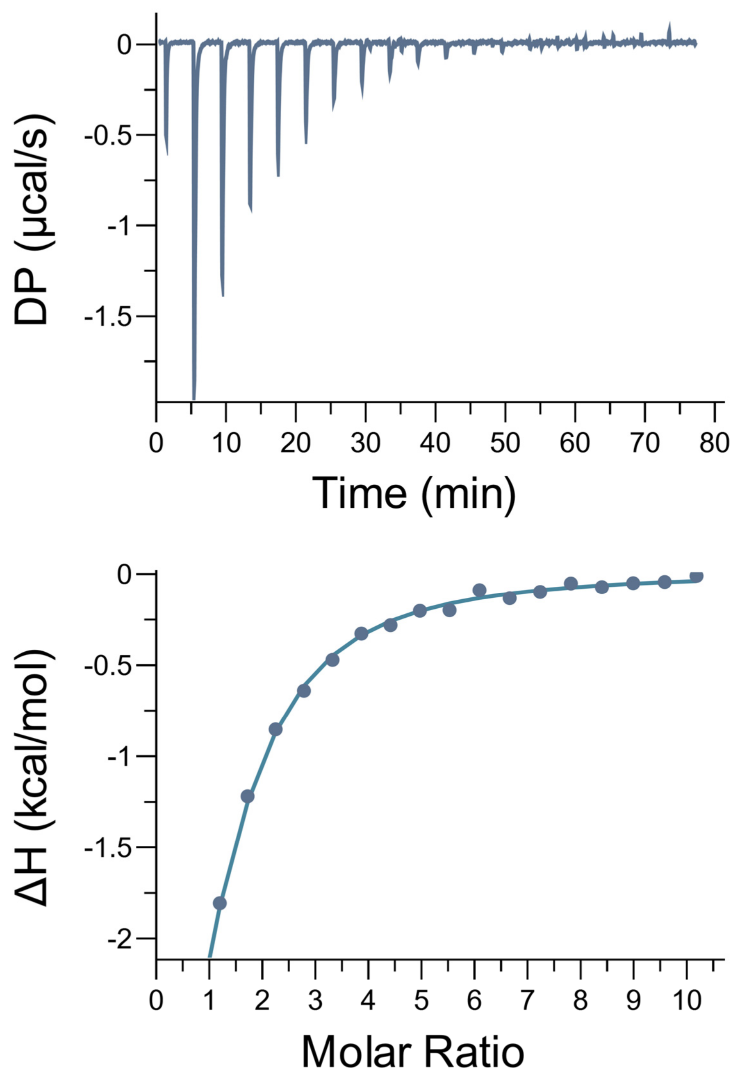


128 **Scheme 2.** A) Preparation of imidazole alcohol **22**. Reagents and conditions. a) NapBr, NaH,
129 DMF, 86%; b) NIS, H_2O, acetone, 0 °C, 99%; c) DMSO, Ac_2O; d) NH_3, THF, reflux; e)
130 DMSO, Ac_2O; f) NH_3, MeOH, 88% over steps c-f; g) HCO_2H, $NaBH_3(CN)$, 38% D-*manno*,
131 33% L-*gulo*; h) Lawesson's reagent, pyridine, 4 Å mol. sieves, toluene, 93%; i)
132 $H_2NCH_2CH(OMe)_2$; j) $TsOH.H_2O$, toluene, 60 °C, yields over steps i and j, 42% D-*gluco*,
133 32% D-*manno*; k) DDQ, CH_2Cl_2/H_2O, 67%. B) Synthesis of ManManI **2**. Reagents and
134 conditions. l) TfOH, 4 Å mol. sieves, toluene, -20 °C, 47%; m) K_2CO_3/MeOH, 46%; n) H_2
135 (34 bar), $Pd(OH)_2$/C, AcOH, EtOAc, MeOH, H_2O, 48%.

136

137 *Binding affinities and 3D structures*

138 Isothermal titration calorimetry (ITC) was used to assess the binding of **1** and **2** to a bacterial
139 endomannosidase. Titration of *Bt*GH99 revealed that $Man2NH_2DMJ$ binds with K_D =
140 97.7±4.9 μM (Figure 2). No binding was evident by ITC for ManManIm. Placed in context, **1**
141 binds worse to *Bt*GH99 than GlcDMJ (K_D = 24 μM);[10] the equivalent data is not available
142 for ManDMJ but as this enzyme prefers to bind Man-configured substrates the difference
143 would be expected to be even greater. 3D structures were obtained for **1** and **2** with *Bx*GH99
144 that diffracted to a resolution of 1.1 and 1.3 Å, respectively (Table 1). Occupancy for the
145 complex with **1** was essentially complete, whereas that with **2**, with prolonged soaking, was
146 estimated at 80%, likely a consequence of the poor affinity of the compound for the enzyme.
147 As predicted, both compounds bound in the −2/−1 subsites of the enzyme (subsite
148 nomenclature from Ref.[27]) and will be discussed in turn.



149

150 **Figure 2.** Isothermal titration calorimetry thermogram showing binding of Man2NH_2DMJ 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

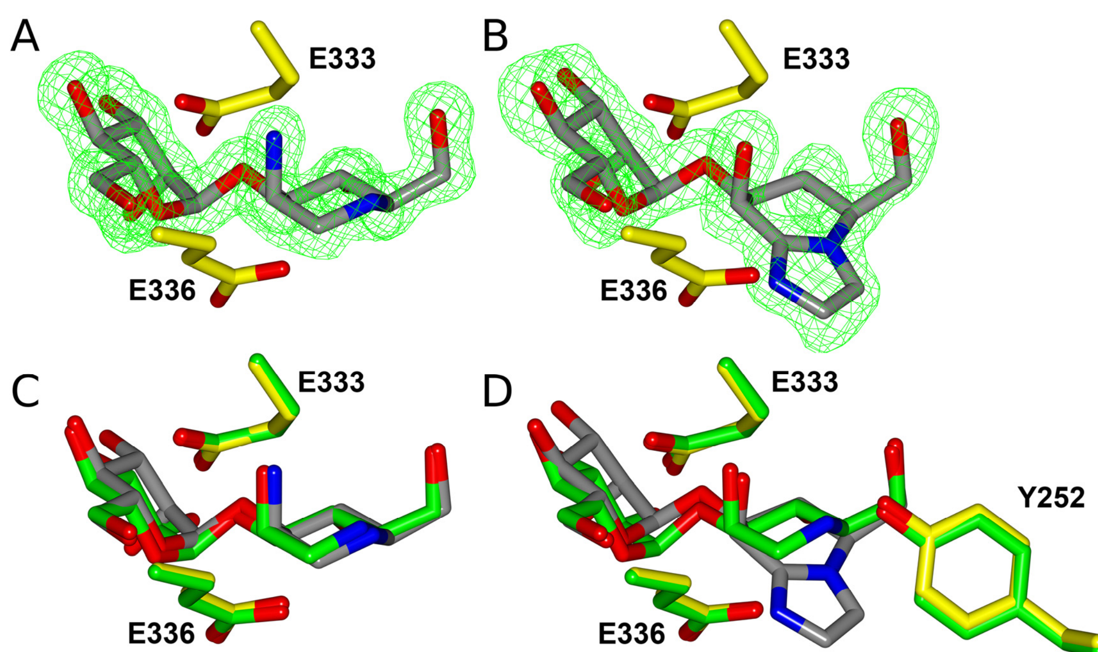
154 **Table 1 Data collection and refinement statistics for complexes of *Bx*GH99 with 1 and 2.**
155

	*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

156 [a] Values in parentheses are for highest-resolution shell.
157
158

159 The *Bx*GH99-**1** complex (Figure 3A) reveals the piperidine ring in a $^4C_1$
160 conformation, matching that seen for complexes of the wildtype enzyme with GlcDMJ and
161 isofagomine-based inhibitors,[8, 10, 17] as well as that of a disabled mutant with substrate.[8] The
162 2-amino group is situated appropriately to interact with the E333 residue that is proposed to
163 act as a general base/acid through deprotonation of the 2-hydroxyl group. Overlay of this
164 complex with that of *Bx*GH99-GlcDMJ reported previously[10] reveals that the positioning and
165 conformation of the rings in the –1 and –2 subsites are essentially identical, and that no
166 amino acid residues undergo significant movements (Figure 3C). In particular, the E333…O2
167 and E333…N2 distances are 2.54 and 2.59 Å, respectively. The poor binding affinity of **1**
168 relative to GlcDMJ therefore does not result from incorrect binding of the inhibitor, and must
169 instead reflect a failure to fully capitalize on the proposed interactions. It is widely

170 acknowledged that iminosugars such as DMJ (and thus GlcDMJ) achieve inhibition through
171 binding to glycosidases in their protonated form;[28] this is supported by first principles
172 consideration of the basicity of these inhibitors and the relevant pK_a values of catalytic
173 residues, and by studies of pH dependence of inhibition. In the case of **1**, this compound has
174 two basic nitrogen residues. However, for vicinal diamines, protonation at one nitrogen has a
175 profound effect on the pK_a value at the second nitrogen; in acyclic systems this effect has
176 been estimated as $\Delta pK_a = 3.6$ units for NH_3^+ or NR_3^+.[29] Moreover, in cyclic systems there
177 are stereoelectronic and conformational contributions, notable examples for various diamines
178 include (pK_{a1}, pK_{a2}): piperazine 9.8, 5.7;[29] *cis*-1,3-diaminocyclohexane 10.3, 8.3;[30] *trans*-
179 1,3-diaminocyclohexane 10.4, 8.5).[30] Finally, vicinal hydroxyl groups can also perturb
180 amine pK_a values; in Man2NH_2DMJ O4 is antiperiplanar to the endocyclic nitrogen and
181 would be expected to reduce its basicity by around 1.3 pK_a units.[30] Collectively, this analysis
182 would suggest that N2 is protonated by the general acid E333, and that it is unlikely that the
183 dication is formed, and therefore Man2NH_2DMJ fails to appropriately mimic an
184 oxocarbenium ion like transition state. A related example of this phenomenon was reported in
185 which introduction of a second amine vicinal to a pre-existing one in apramycin resulted in a
186 dramatic loss of binding to a bacterial ribosome of approximately 100-fold.[31] Additionally,
187 the proposed binding mode of **1** shown in Figure 1D highlights that the 2-amino group has
188 additional hydrogen substituents that may cause an energetic penalty upon binding of the
189 inhibitor.



190

191 **Figure 3.** Three-dimensional structures of *Bt*GH99. (A) Complex with $Man2NH_2DMJ$. (B)
192 Complex with ManManIm. Electron density maps are maximum likelihood/σ_A weight F_o –
193 F_c difference syntheses contoured at 0.5 and 0.3 e$Å^{-3}$ respectively for panels A and B) visible
194 before refining the structure model with the ligand added. (C) Overlay of $Man2NH_2DMJ$ with
195 GlcDMJ (PDB code 4FAM). (D) Overlay of ManManIm with GlcDMJ complex (PDB code
196 4FAR).

197 The *Bx*GH99-**2** complex reveals the piperidine ring of the mannoimidazole moiety to
198 be in an unusual $^2H_3/E_3$ conformation (Figure 3B).[32] Overlay of the complex with that of
199 *Bx*GH99-GlcDMJ[10] reveals that while the –2 sugar residues occupy similar positions, the
200 mannoimidazole headgroup is atypically positioned such that the heterocycle projects
201 downward into the active site, below the plane of the piperidine ring of the GlcDMJ complex
202 (Figure 3D). In this case the E336…N (imidazole ring) distance is 2.65 Å, similar to that seen
203 in related glycoimidazole complexes.[33] In the original formulation by Vasella and co-
204 workers, β-equatorial glycosidases were proposed to perform protonation from the side, in
205 what was termed 'lateral protonation', with the acid either on the same side as the endocyclic
206 oxygen (*syn*) or opposed to it (*anti*).[20] In a subsequent publication Nerinckx formalized this
207 concept by dividing the space around the –1 sugar into *anti* and *syn* hemispheres through a
208 plane defined by the glycosidic oxygen, C1 and H1 of the sugar residue.[34] Analysis of
209 complexes of various *anti*-protonating glycosidases reveals that the acid/base or acid residues
210 responsible for protonating the leaving group are in fact not universally located lateral to the
211 sugar mean plane, but are more commonly positioned above or below it, so as to better
212 protonate the leaving group oxygen. However, this does not prevent glycoimidazoles binding
213 in normal orientations and engaging in hydrogen-bonding interactions with the imidazole
214 nitrogen. For example, in the case of the retaining GH116 β-glucosidase from
215 *Thermoanaerobacterium xylanolyticum*, the acid/base is positioned above the sugar mean
216 plane, but a normal orientation and conformation of glucoimidazole was observed.[35]
217 Mannoimidazole also bound in the normal fashion to an inverting GH47 α-mannosidase from
218 *Caulibacter* sp. in which the acid is below the mean plane of the inhibitor, but instead the
219 inhibitor establishes an interaction with another conserved active site carboxylic acid that lies
220 lateral to the imidazole.[36] *Bx*GH99 is an *anti*-protonating enzyme with its general acid/base
221 Glu336 positioned below the ring plane in order to facilitate classical anti-protonation of the
222 axial glycosidic oxygen (approximate O5–C1–O1 angle is 60 degrees). The distorted mode of
223 binding of the mannoimidazole moiety of **2** seems to be a consequence of the imidazole

224 binding to maximize this interaction with the acid/base. Close examination of the active site
225 of *Bx*GH99 reveals that if the ManIm moiety were to be shifted up to the same position as
226 that of the piperidine of GlcDMJ, a steric interaction would result with Tyr252, a conserved
227 residue. In fact, the distance between the imidazole C=C bond and Tyr252 Cε is only 3.2 Å,
228 causing the wwPDB validation software to report H–H steric clashes in this region. In fact, a
229 ternary complex of GlcDMJ and α-1,2-mannobiose highlighted that the active site of the
230 enzyme involves a sharp bend in the –1 and +1 subsites. The failure of **2** to bind in a typical
231 position in the –1 subsite is thus likely a result of a failure to accommodate the imidazole ring
232 owing to the location of Tyr252.

233 Conclusions

234 In summary, we report the design and synthesis of two 'mechanism-based' inhibitors
235 of family GH99 endomannanases. While Man2NH_2DMJ bound to the bacterial
236 endomannanase *Bx*GH99 in the expected manner, its affinity for *Bt*GH99 did not exceed that
237 seen for GlcDMJ. This appears to be a result of the perturbing effect of the 2-amino
238 substituent, reducing the basicity of the endocyclic nitrogen and its ability to be protonated in
239 the active site and thereby resemble the oxocarbenium-ion-like transition state. On the other
240 hand, binding of ManManIm to *Bt*GH99 could not be detected by ITC, and consistent with
241 this an X-ray structure in complex with *Bx*GH99 displayed incomplete occupancy. The poor
242 binding of this inhibitor appears to be a consequence of an inability of the active site of
243 *Bx*GH99 to accommodate the annulated imidazole ring because of an interaction with a
244 conserved Tyr active site residue. This study provides important insights that will inform
245 future strategies for the developing mechanism-inspired and transition-state mimicking
246 inhibitors of GH99 enzymes.

247

248 **Experimental**

249 General

250 ^{1}H and ^{13}C NMR spectra were recorded using 400, 500 or 600 MHz instruments. All signals
251 were referenced to TMS (δ=0.00 ppm), or solvent peaks ($CDCl_3$: δ=7.26 ppm for ^{1}H or 77.16
252 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
253 ppm for ^{1}H or δ=49.0 ppm for ^{13}C). Melting points were obtained using a Reichert–Jung
254 hotstage apparatus. TLC analysis used aluminium backed Merck Silica Gel 60 F254 sheets,
255 detection was achieved using UV light, 5% H_2SO_4 in MeOH, or ceric ammonium molybdate
256 ("Hanessian's stain") with charring as necessary. Flash chromatography was performed using
257 Geduran silica gel according to the method of Still *et al*.[37] Dry CH_2Cl_2, THF, and Et_2O were
258 obtained from a dry solvent apparatus (Glass Contour of SG Water, Nashua).[38] DMF and
259 DMSO were dried over 4 Å molecular sieves.

260

261 **2-Azido-4,6-*O*-benzylidene-*N*-benzyloxycarbonyl-1,2,5-trideoxy-1,5-imino-D-mannitol**
262 **(4)**

263 Sodium azide (57.8 mg, 0.890 mmol) was added to a solution of 4,6-*O*-(*R*-benzylidene)-*N*-
264 benzyloxycarbonyl-1,5-dideoxy-2-*O*-(*p*-toluenesulfony1)-D-glucitol[21] **3** (120 mg, 0.222
265 mmol) in DMF (1 mL). The suspension was refluxed for 18 h, poured into ice, extracted into
266 EtOAc (3 × 20 mL), washed with brine (2 × 20mL), dried over anhydrous $MgSO_4$ and
267 evaporated to dryness. Column chromatography (AcOEt:pet. spirits 1:5) gave the azide **4** (67.7
268 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
269 (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
270 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
271 (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
272 (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,
273 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
274 (1 C, C=O); HRMS (ESI)$^+$ *m/z* 411.1664 [$C_{21}H_{22}N_4O_5$ $(M+H)^+$ requires 411.1663].

275

276 **2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-azido-4,6-*O*-benzylidene-**
277 ***N*-benzyloxycarbonyl-1,2,5-trideoxy-1,5-imino-D-mannitol (5)**

278

279 TfOH (0.043 µL, 0.0049 mmol) was added to a mixture of acceptor **4** (20 mg, 0.049 mmol)
280 and 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate **5**[22] (37 mg,
281 0.058) in CH_2Cl_2 over 4 Å sieves at -30 °C, The mixture was stirred for 30 min, warmed to 0
282 °C and quenched with Et_3N (7 µL, 0.05 mmol) then concentrated under reduced pressure.
283 Flash chromatography (EtOAc/pet. spirits 25:75) gave the disaccharide **6** (37.4 mg, 87%) as a
284 colourless oil. $[\alpha]_D^{24}$ -4.2 (*c* . 0.89, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.80 (1 H, $J_{1,1}$
285 =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,
286 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* =
287 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*
288 = 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),
289 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
290 (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
291 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,
292 C6), 69.3 (1 C, C6'), 72.2, 73.6, 75.1 (3 C, **C**H$_2$Ph), 78.1 (1 C, C2), 78.2 (C1, H3'), 99.5 (1 C,
293 C1'), 100.90 (1 C, CH), 100.92, 126.0, 127.77, 127.79, 127.83, 127.9, 128.0, 128.2, 128.28,
294 128.29, 128.41, 128.44, 128.5, 128.7, 128.9 (C30, Ph); HRMS (ESI)$^+$ *m/z* 907.3544
295 [$C_{50}H_{52}N_4O_{11}$ (M+Na)$^+$ requires 907.3525].

296

297 **3,4,6-Tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-azido-*N*-benzyloxycarbonyl-1,2,5-**
298 **trideoxy-1,5-imino-D-mannitol (7)**

299 A solution of sodium methoxide in methanol (0.1 M, 10 µL, 1 µmol) was added to **6** (60 mg,
300 0.068 mmol) in methanol (0.5 mL) and stirred for 1 h. The mixture was concentrated under
301 reduced pressure to give an alcohol, which was used without purification. TFA/H_2O 9:1 (100
302 µL) was added to the crude alcohol, the mixture was stirred for 30 min, concentrated and
303 azeotroped with toluene (3 × 10 mL). Flash chromatography (EtOAc/pet. spirits 9:1) gave the
304 triol **7** (42.5 mg, 83%,). $[\alpha]_D^{25}$ 44.6 (*c*. 1.03, MeOH); ^{1}H NMR (500 MHz, CD_3OD), 3.67-
305 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),
306 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),
307 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,
308 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,
309 127.9, 128.0, 128.1, 128.16, 128.19, 128.4, 128.5, 128.6, 128.7, 137.9, 138.0, 138.3 (24 C

310 Ph), 156.5 (1 C, C=O); HRMS (ESI)$^+$ *m/z* 755.3300 [$C_{41}H_{46}N_4O_{10}$ (M+H)$^+$ requires
311 755.3287].

312 **3,4,6-Tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-amino-*N*-benzyloxycarbonyl-1,2,5-**
313 **trideoxy-1,5-imino-D-mannitol (8)**

314 DTT (51 mg, 0.331 mmol) was added to a solution of azide **7** (25 mg, 0.0331 mmol) in
315 pyridine (1 mL) and $NaHCO_3/H_2CO_3$ buffer (0.625 mL, pH 9.16). The mixture was stirred at
316 room temperature for 4 h, concentrated and azeotroped toluene (5 × 10 mL). Flash
317 chromatography (EtOAc/MeOH/H_2O 94:4:2) to give the amine **8** (80%, 19.2 mg). ^{1}H NMR
318 (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$, 1'-
319 6$_b$'), 4.36 (1 H, t, *J* = 7.8 Hz, H4) ,4.46-4.54 (2 H, m, C**H**$_2$Ph), 4.58 (1 H, d, *J* = 12.0 Hz,
320 C**H**$_2$Ph), 4.66 (d, *J* =11.8 Hz, C**H**$_2$Ph), 4.77-4.81 (2 H, m, C**H**$_2$Ph), 4.98 (1 H, d, *J* = 2.5 Hz,
321 H1'), 5.15 (2 H, s, CH_2), 7.16-7.47 (20 H, m, Ph), ^{13}C NMR ($CDCl_3$, 125 MHz) 46.8, 59.9,
322 65.6, 68.5, 69.4, 70.4, 72.6, 73.7, 74.4, 75.4, 75.7, 78.1, 80.1, 100.8 (16 C C1-6, C1'-6', 4 ×
323 CH_2), 128.81, 128.84, 129.2, 129.28, 128.30, 129.3, 129.4, 129.5, 138.0, 139.3, 139.5, 139.6
324 (24 C Ph); HRMS (ESI)$^+$ *m/z* 729.3398 [$C_{41}H_{48}N_2O_{10}$ (M+H)$^+$ requires 729.3385].

325

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

328 The triol **8** (19.2 mg, 0.0264 mmol) in MeOH/H_2O (2:1, 3 mL) and 10% HCl in methanol
329 (0.3 mL) was treated with PdOH/C (50 mg) and H_2 (20 atm, 18h). The suspension was
330 filtered, concentrated and purified with cation and anion resin (eluted with aqueous NH_3) to
331 give ManNH$_2$DMJ **1** (70%, 6.02mg) as a colourless oil. $[\alpha]_D^{25}$ 17.2 (*c*. 0.08, H_2O); ^{1}H NMR
332 (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
333 (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
334 (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
335 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,
336 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
337 325.1605].

338

339 **4-Methylphenyl 2,4,6-tri-*O*-benzyl-3-*O*-(2-naphthylmethyl)-1-thio-α-D-**
340 **mannopyranoside (10)**

341 A dry solution of the alcohol **9**[23] (167 mg, 0.30 mmol) in DMF (5 mL) was cooled to 0 °C.
342 The solution was charged with NaH (60% dispersion in mineral oil, 36 mg, 0.9 mmol) and
343 stirred for 30 min. 2-bromomethylnaphthalene (79.6 mg, 0.36 mmol) was added to the mixture
344 and the reaction was stirred overnight. The mixture was diluted with Et_2O (20 mL), poured into
345 ice water and washed with water (3 × 20 mL) and brine (1× 20 mL). The organic extracts were
346 dried ($MgSO_4$), the solvent was removed under reduced pressure and the resulting residue was
347 subjected to flash chromatography (EtOAc/pet. spirits 15:85) to give the protected
348 thioglycoside **10** (179.3 mg, 86%) as a colourless oil; $[\alpha]_D^{24}$ +65 (*c* 0.69, $CHCl_3$); ^{1}H NMR
349 (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),
350 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),
351 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, $J_{5,6a}$ =
352 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
353 (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,
354 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,
355 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,
356 C6), 71.9 (1 C, **C**H$_2$Ph), 72.2 (1 C, **C**H$_2$Nap), 72.8 (1 C, C5), 73.3 (1 C, **C**H$_2$Ph), 75.1 (1 C,
357 C4), 75.2 (1 C, **C**H$_2$Ph), 76.3 (1 C, C2), 80.3 (1 C, C3), 86.1 (1 C, C1), 125.9-126.5 (4 C, Nap),
358 127.5-128.4 (18 C, 3 × Ph, Nap), 129.8 (2 C, Tol), 132.3 (2 C, Tol), 133.4, 135.8, 137.6, 138.0,
359 138.5, 138.6 (6 C, Cq); HRMS (ESI)$^+$ *m/z* 719.2809 [$C_{45}H_{44}O_5S$ (M+Na)$^+$ requires 719.2802].

360 **2,4,6-Tri-*O*-benzyl-3-*O*-(2-naphthylmethyl)-α-D-mannopyranose (11)**

361 *N*-Iodosuccinimide (216 mg, 0.961 mmol) was added to a solution of the thioglycoside **10** (447
362 mg, 0.641 mmol) in acetone (1% aq., 10 mL) at 0 °C and left to stir for 2.5 h. The solution was
363 quenched with aq. $Na_2S_2O_3$ (0.5 M, 10 mL), diluted with EtOAc (20 mL) and washed with aq.
364 $Na_2S_2O_3$ (0.5 M, 3 × 20 mL), $NaHCO_3$ (2 × 20 mL) and brine (1 × 20 mL). The organic extracts
365 were dried ($MgSO_4$), the solvent was removed under reduced pressure and the resulting residue
366 was subjected to flash chromatography (EtOAc/pet. spirits/Et_3N 30:69.5:0.5) to afford the
367 hemiacetals **11** (344 mg, 91%; *α/β* 3.3:1) as a white powder, α anomer; ^{1}H NMR (500 MHz,
368 $CDCl_3$): 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
369 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
370 (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),
371 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* =
372 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,
373 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,

374 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
375 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
376 (18 C, 3 × Ph, Nap), 133.0, 133.4, 136.1, 138.0, 138.5 (6 C, Cq); HRMS $(ESI)^+$ *m/z* 608.3007
377 [$C_{38}H_{38}O_6$ $(M+NH_4)^+$ requires 608.3007].

378 **2,4,6-Tri-*O*-benzyl-3-*O*-(2-naphthylmethyl)-D-mannonolactone (12)**

379 A solution of the hemiacetal **11** (742 mg, 1.26 mmol) in acetic anhydride (6.1 mL) and dry
380 DMSO (6.6 mL) was stirred under N_2 for 22 h. The reaction was diluted with EtOAc (20 mL),
381 quenched with ice and washed with water (3 × 20 mL) and brine (1× 20 mL). The organic
382 extracts were dried ($MgSO_4$) and the solvent was evaporated. Azeotropic toluene was used to
383 remove any residual AcOH, affording the crude lactone **12** (823 mg), which was used directly
384 in the next step. A portion of **12** obtained from a separate experiment was purified by flash
385 chromatography (EtOAc/pet. spirits 1:9) to yield analytically pure **12** as a colourless oil, $[\alpha]_D^{25}$
386 +4.05 (*c* 0.44, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 3.61 (2 H, m, H6a, H6b), 3.80 (1 H, dd,
387 $J_{2,3}$ = 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),
388 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,
389 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,
390 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,
391 $CDCl_3$) 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,
392 **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,
393 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,
394 137.6 (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
395 606.2850].

396 **2,4,6-Tri-*O*-benzyl-3-*O*-(2-naphthylmethyl)-D-mannonamide (13)**

397 A dry-ice/acetone cold finger cooling trap was used to condense ammonia (50 mL) into a
398 solution of the crude lactone **12** (823 mg) in dry THF (30 mL) at –78 °C. The solution was
399 allowed to reflux at 0 °C for 4 h. The mixture was evaporated to dryness to afford the crude
400 amide **13** (771 mg), which was used directly in the next step. A portion obtained from an
401 independent experiment was purified by flash chromatography (EtOAc/pet. spirits 3:2) to yield
402 analytically pure **13** as a yellow solid, m.p. 120 °C; $[\alpha]_D^{25}$ +7.21 (*c* 0.41, $CHCl_3$); ^1H NMR
403 (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,
404 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),
405 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),
406 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,

407 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,
408 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
409 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
410 (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);
411 HRMS (ESI)$^+$ *m/z* 606.2850 [$C_{38}H_{39}NO_6$ $(M+H)^+$ requires 606.2844].

412 **(3*S*,4*S*,5*S*,6*R*/*S*)-3,5-Bis(benzyloxy)-6-[(benzyloxy)methyl]-6-hydroxy-4-(2-**
413 **naphthylmethyloxy)piperidin-2-one (15)**

414 A solution of the crude amide **13** (771 mg) in acetic anhydride (6.1 mL) and dry DMSO (6.6
415 mL) was stirred under N_2 for 21 h. The reaction mixture was diluted with EtOAc (20 mL),
416 quenched with ice and washed with water (3 × 20 mL) and brine (1 × 20 mL). The organic
417 extracts were dried ($MgSO_4$) and the solvent was evaporated to afford the keto-amide **14** as a
418 white solid. A dry-ice/acetone cold finger was used to condense ammonia (20 mL) into a
419 solution of the crude keto-amide in dry methanol (30 mL) at 0 °C. The solution was allowed
420 to attain rt and was stirred under N_2 for 16 h. The solvent was removed under reduced
421 pressure and the resulting residue was subjected to flash chromatography (EtOAc/pet. spirits
422 1:1) to give a separable mixture of the hydroxyl-lactams **15** (669 mg, 88% over four steps; D-
423 *manno*/L-*gulo* 2.2:1); ^{1}H NMR (500 MHz, $CDCl_3$), partial spectrum of the mixture of
424 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,
425 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,
426 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
427 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* =
428 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*);
429 ^{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*),
430 169.6 (1 C, C=O D-*manno*), 170.2 (1 C, C=O L-*gulo*); HRMS (ESI)$^+$ *m/z* 606.2698
431 [$C_{38}H_{37}NO_6$ $(M+H)^+$ requires 604.2694].

432 **(3*S*,4*S*,5*S*,6*R*)-3,5-Bis(benzyloxy)-6-[(benzyloxy)methyl]-4-(2-**
433 **naphthylmethoxy)piperidin-2-one (16) and (3*S*,4*S*,5*S*,6*S*)-3,5-bis(benzyloxy)-6-**
434 **[(benzyloxy)methyl]-4-(2-naphthylmethoxy)piperidin-2-one (17)**
435 Sodium cyanoborohydride (90.4 mg, 1.44 mmol) was added to a solution of the hydroxy-
436 lactams **15** (86.9 mg, 0.144 mmol) and formic acid (0.52 mL) in dry acetonitrile (3 mL) and
437 left to stir under N_2 for 20 h. Sodium cyanoborohydride (90.4 mg, 1.44 mmol) was added and
438 the reaction mixture was stirred for a further 24 h when TLC analysis (EtOAc/pet. spirits 1:3)

439 indicated complete consumption of the starting material. The mixture was diluted with EtOAc
440 (20 mL) and washed with aq. sat. $NaHCO_3$ (3 × 20 mL) and brine (1 × 20 mL). The aqueous
441 extracts were treated with sodium hypochlorite prior to disposal. The organic extracts were
442 dried ($MgSO_4$), the solvent was removed under reduced pressure and the resulting residue was
443 subjected to flash chromatography (EtOAc/pet. spirits 1:1) to afford the L-*gulo* lactam **16** (28.2
444 mg, 33%) and the D-*manno* lactam **17** (32.5 mg, 38%), both as colourless oils.

445 Characterization for **16:**

446 $[\alpha]_D^{23}$ –57 (*c* 0.535, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.36 (1 H, dd, $J_{6,6a}$ = 4.27, $J_{6a,6b}$ =
447 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,
448 $J_{4,5}$ = 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 ×
449 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,
450 *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,
451 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,
452 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),
453 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
454 (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,
455 133.3, 135.6, 137.0, 137.6, 138.4 (6 C, Cq), 171.3 (1 C, C=O); HRMS (ESI)$^+$ *m/z* 588.2747
456 [$C_{38}H_{37}NO_5$ (M+H)$^+$ requires 588.2749].

457 Characterization for **17:**

458 $[\alpha]_D^{25}$ –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
459 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
460 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,
461 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,
462 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),
463 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
464 (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,
465 **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),
466 126.1-126.3 (3 C, Nap), 127.0 (1 C, Nap), 127.8-128.6 (18 C, 3 × Ph, Nap), 133.2, 133.3, 135.5,
467 137.5, 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)$^+$
468 requires 588.2744].

469 **(3*S*,4*S*,5*S*,6*S*)-3,5-Bis(benzyloxy)-6-[(benzyloxy)methyl]-4-(2-**
470 **naphthylmethoxy)piperidin-2-thione (18)**

471 Lawesson's reagent (202 mg, 0.50 mmol) was added to a mixture containing the
472 mannonolactam **17** (98 mg, 0.167 mmol), pyridine (6.7 uL, 0.083 mmol), freshly activated 4
473 Å molecular sieves and distilled toluene (6 mL) and the reaction was left to stir for 20 h. The
474 mixture was filtered, stirred with MeOH (1.68 mL) for 2 h and the solvent was removed under
475 reduced pressure. The residue obtained was subjected to flash chromatography (EtOAc/pet.
476 spirits 20:80) to afford the thionolactam **18** (94 mg, 93%) as a white solid; m.p. 147 °C; $[\alpha]_D^{23}$
477 –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,
478 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,
479 $J_{3,4}$ = 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
480 (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,
481 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);
482 ^{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),
483 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
484 (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,
485 133.3, 135.4, 137.3, 137.6, 138.0 (6 C, Cq), 200.0 (1 C, C=O); HRMS (ESI)$^+$ *m/z* 604.2524
486 [$C_{38}H_{37}NO_4S$ $(M+H)^+$ requires 604.2516].

487 **(5*R*,6*R*,7*S*,8*R*)-7-(2-Naphthylmethoxy)-6,8-bis(benzyloxy)-5-(benzyloxy)methyl-5,6,7,8-**
488 **tetrahydroimidazo[1,2-*a*]pyridine (21) and (5*R*,6*R*,7*S*,8*S*)-7-(2-naphthylmethoxy)-6,8-**
489 **bis(benzyloxy)-5-(benzyloxy)methyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine (20)**
490 Thionolactam **18** (256 mg, 0.424 mmol) was dissolved in aminoacetaldehyde dimethyl acetal
491 (0.69 mL, 6.33 mmol) and stirred under N_2 for 18 h. The mixture was diluted with Et_2O (20
492 mL) and washed with H_2O (2 × 20 mL) and brine (1 × 20 mL). The organic extracts were dried
493 ($MgSO_4$) and the solvent removed under reduced pressure to afford the amidines **19** as a
494 colourless residue. *p*-Toluenesulfonic acid monohydrate (0.14 g, 0.74 mmol) was added to a
495 solution of the crude amidines in toluene (9.5 mL) and the reaction was stirred at 60 °C
496 overnight. The mixture was diluted with DCM (20 mL) and washed with $NaHCO_3$ (2 × 20 mL)
497 and brine (1 × 20 mL). The organic extracts were dried ($MgSO_4$), the solvent was removed
498 under reduced pressure and the residue was subjected to flash chromatography (EtOAc/pet.
499 spirits 1:1) to afford the glucoimidazole **20** (110 mg, 42% over two steps) as a colourless oil,
500 and the mannoimidazole **21** (83.3 mg, 32% over two steps) as a yellow oil.

501 Characterization for **20:**

502 [α]$_D^{25}$ +52 (c 0.315, $CHCl_3$; lit.[39] +52, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.75 (1 H, dd,
503 $J_{5,5a}$ = 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,
504 $J_{7,8}$ = 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,
505 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 =
506 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
507 (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 ×
508 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,
509 **C**H$_2$(C5)), 72.9 (1 C, **C**H$_2$Nap), 73.4 (1 C, **C**H$_2$Ph), 74.3 (1 C, **C**H$_2$Ph), 74.4 (1 C, **C**H$_2$Ph),
510 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
511 (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,
512 138.4 (6 C, Cq), 144.2 (Cq, imidazole).

513 Characterization for **21:**

514 [α]$_D^{25}$ –24 (c 0.24, $CHCl_3$) (lit.[39] –20, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.57 (1 H, dd,
515 $J_{5,5a}$ = 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
516 (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,
517 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
518 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,
519 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),
520 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
521 (1 C, **C**H$_2$Nap), 71.2 (1 C, **C**H$_2$(C5)), 71.8 (1 C, **C**H$_2$Ph), 73.3 (1 C, **C**H$_2$Ph), 74.3 (1 C, C6),
522 75.0 (1 C, **C**H$_2$Ph), 80.2 (1 C, C3), 119.5 (1 C, C2), 125.2-126.9 (3 C, Nap), 126.7 (1 C, Nap),
523 128.6-127.7 (18 C, 3 × Ph, Nap), 129.4 (1 C, C3), 133.2, 133.3, 135.4, 137.6, 138.2, 138.3 (6
524 C, Cq), 143.0 (Cq, imidazole).

525 **(5*R*,6*R*,7*S*,8*R*)-6,8-Bis(benzyloxy)-5-[(benzyloxy)methyl]-5,6,7,8-tetrahydroimidazo[1,2-**
526 ***a*]pyridin-7-ol (22)**

527 DDQ (25.2 mg, 0.111 mmol) was added to a solution of the mannoimidazole **21** (22.6 mg,
528 0.037 mmol) in DCM/H_2O (9:1, 1 mL) and the reaction mixture was stirred at rt overnight.
529 DDQ (25 mg, 0.11 mmol) was again added and the reaction mixture was stirred for 3 days
530 when TLC analysis (EtOAc/pet. spirits 8:2) indicated complete consumption of the starting
531 material. The reaction was diluted with DCM (20 mL), washed with water (3 × 20 mL) and aq.
532 sat. $NaHCO_3$ (3 × 20 mL), dried ($MgSO_4$), filtered, and concentrated. The crude product was
533 purified by flash chromatography (EtOAc/pet. spirits 80:20 to 100:0) to afford the alcohol **22**

534 (11.7 mg, 67%) as a yellow oil; $[\alpha]_D^{24}$ –35 (*c* 0.585, $CHCl_3$) (lit.[39] -6, $CHCl_3$); ^{1}H NMR (500
535 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,
536 $J_{5a,5b}$ = 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
537 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),
538 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
539 (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
540 (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),
541 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,
542 137.8 (3 C, Cq), 142.3 (Cq, imidazole).

543 **(5*R*,6*R*,7*S*,8*R*)-7-(2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyloxy)-6,8-**
544 **bis(benzyloxy)-5-[(benzyloxy)methyl]-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine (23)**

545 A mixture of the alcohol **22** (13.8 mg, 0.029 mmol), 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-
546 mannopyranosyl trichloroacetimidate **5**[22] (32.5 mg, 0.051 mmol) and freshly activated 4 Å
547 molecular sieves in toluene (1.5 mL) was stirred at rt for 30 min. Triflic acid (1 μL, 0.011
548 mmol) was added to the mixture at –20 °C and the reaction was left to stir for 1 h. The reaction
549 mixture was stirred at 0 °C for 20 min, then at r.t for another 20 min, quenched with pyridine
550 (1 drop) and filtered through a Celite pad. The solvent was removed under reduced pressure
551 and the resulting residue was subjected to flash chromatography (EtOAc/pet. spirits/ Et_3N
552 80:19:1) to recover alcohol **26** (6.4 mg) and afford the disaccharide **23** (12.9 mg, 47%) as a
553 colourless oil; $[\alpha]_D^{23}$ +7.2 (*c* 0.175, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 2.11 (3 H, s, Ac),
554 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,
555 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, $J_{5a,5b}$
556 = 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,
557 $J_{2',3'}$ = 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),
558 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
559 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,
560 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
561 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,
562 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,
563 $CDCl_3$) 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),
564 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),
565 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
566 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,

567 137.7, 137.9, 138.1, 138.2, 138.8 (6 C, Cq), 142.6 (Cq, imidazole), 170.4 (1 C, C=O); HRMS
568 (ESI)$^+$ *m/z* 945.4322 [$C_{58}H_{60}N_2O_{10}$ (M+H)$^+$ requires 945.4321].

569 **(5*R*,6*R*,7*S*,8*R*)-7-(3,4,6-Tri-*O*-benzyl-α-D-mannopyranosyloxy)-6,8-bis(benzyloxy)-5-**
570 **[(benzyloxy)methyl]-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine (24)**

571 K_2CO_3 (1 mg, 0.007 mmol) was added to a solution of the acetate **23** (13.1 mg, 0.014 mmol)
572 in dry methanol (0.3 mL) and the resulting suspension was stirred at rt for 6.5 h. The reaction
573 mixture was quenched with acetic acid (5 µL, 0.087 mmol), the solvent was removed under
574 reduced pressure, and the resulting residue was subjected to flash chromatography (EtOAc/pet.
575 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
576 (*c* 0.305, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 2.40 (1 H, d, $J_{2',OH}$ = 2.5 Hz, OH), 3.49 (1 H,
577 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,
578 $J_{5a,5b}$ = 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
579 (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,
580 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),
581 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,
582 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,
583 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'),
584 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),
585 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),
586 129.6 (1 C, C3), 137.6, 137.8, 138.1, 138.3, 138.7 (6 C, Cq), 142.7 (Cq, imidazole); HRMS
587 (ESI)$^+$ *m/z* 903.4214 [$C_{56}H_{58}N_2O_9$ (M+H)$^+$ requires 903.4215].

588 **(5*R*,6*R*,7*S*,8*R*)-6,8-Dihydroxy-5-[(hydroxy)methyl]-7-(α-D-mannopyranosyloxy)-5,6,7,8-**
589 **tetrahydroimidazo[1,2-*a*]pyridine (2)**

590 $Pd(OH)_2$/C (20%, 24 mg) was added to a solution of the deacetylated disaccharide **24** (12.6 mg,
591 0.014 mol) in EtOAc/MeOH/H_2O (5:17:3, 1.50 ml) and AcOH (0.34 ml). The reaction vessel
592 was filled with H_2 (34 bar) and agitated for 4 d. At this point TLC analysis (EtOAc/MeOH/H_2O
593 7:3:2) indicated complete conversion to a single species along with baseline byproducts. The
594 suspension was filtered through a Celite pad, the solvent was evaporated and the resulting
595 residue was subjected to flash chromatography (EtOAc/MeOH/H_2O 5:2:1) to afford the
596 ManManIm **2** (2.4 mg, 48%) as a colourless residue; $[\alpha]_D^{27}$ +13 (*c* 0.12, H_2O); ^1H NMR (500
597 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
598 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, $J_{5,5a}$
599 = 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$_2$DMJ 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

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725 **Entry for the Table of Contents**

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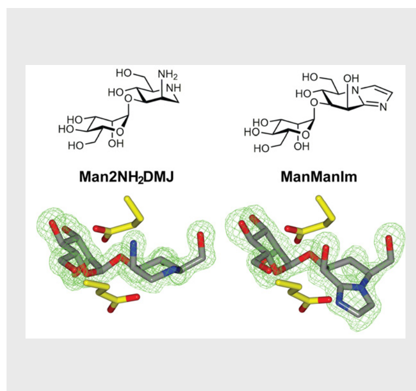
FULL PAPER

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**

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

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