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Exploring a Multivalent Approach to α-L-Fucosidase Inhibition

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Dedicated to Professor Pierre Vogel on the occasion of his 70th birthday

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To probe the utility of a multivalent approach for fucosidase inhibition, a series of di- and tri-valent imino sugars based on L-*fuco*-configured 1,4-imino- and 1,4-bis(imino)-cyclitol epitopes has been synthesized and analyzed for fucosidase inhibition with the best trivalent species yielding a modest improvement in binding constant. Structural analysis of a representative pair of mono- and tri-valent imino sugars has been performed on a bacterial fucosidase, *Bt*Fuc2970. The 3D structures show binding of the imino-cyclitol in the ^{3}E conformation, consistent with the known pathway for fucosidase action.

Introduction

Fucose is a 6-deoxyhexose commonly incorporated into glycoconjugates, by either the direct attachment to proteins or lipids, or attachment to *N*-linked glycans. Such glycoconjugates have roles in a number of physiological activities, such as oncogenesis,[1] the blood coagulation cascade and clot dissolution,[2] antigenic determination[3] and host-microbial interactions.[4] α-L-Fucosidases (EC 3.2.1.51) catalyze the hydrolytic cleavage of fucose residues located at the non-reducing end of glycoconjugates and, like other glycosidases, also catalyze glycosylation reactions. The accumulation of glycoconjugates containing fucose, due to the absence or deficiency of α-L-fucosidases, induces the recognition of the α-L-fucose moieties by specific lectins that leads to the neurovisceral disorder known as fucosidosis.[5] In mammals, these enzymes, which may be lysosomally compartmentalised (FUCA1), or secreted (FUCA2), are implicated in several pathological events. An abnormal α-L-fucosidase distribution, both extracellular and intracellular, is found in inflammatory responses,[4a] cancer[6] and cystic fibrosis.[7] Human α-L-fucosidases are of substantial interest as diagnostic markers of early colorectal[1b,6b] and hepatocellular cancers,[6a,8] and as modulators of metastasis in breast cancer cells.[9] Furthermore, α-L-fucosidases have been found in human seminal plasma and in the membranes of human sperm cells and facilitate sperm transport and sperm-egg interactions.[10]

The inhibition of α-L-fucosidases may be clinically important for a variety of reasons. α-L-Fucosidase may be a target for small molecule chaperone therapy for fucosidosis.[11] This approach has been applied successfully to other lysosomal storage diseases.[12,13] *Helicobacter pylori* infection has been shown to have a correlation with increased expression of α-L-fucosidase in the stomach. The introduction of inhibitory compounds against α-L-fucosidase has been shown to reduce *H. pylori* virulence in vitro.[14] Because L-fucose and/or L-fucose-containing molecules have been shown to inhibit sperm–egg interactions in humans, specific α-L-fucosidase inhibitors are expected to be powerful tools in elucidating the biological role of α-L-fucosidase in spermiogenesis and sperm maturation.[15]

For these reasons, a number of structural studies of α-L-fucosidases have been reported in the last decade. In this context, the focus has been on CAZY (www.cazy.org[16]) family GH29 fucosidases into which the relevant mammalian enzymes are classified on the basis of amino-acid sequence similarities. In 2004 a small angle X-ray scattering model of the GH29 α-L-fucosidase from *Sulfolobus Solfataricus* was proposed.[17] Since then, crystallographic structures have been determined for α-L-fucosidases from *Thermotoga maritima*,[18] *Bifidobacterium bifidum*[19] and *Bacteroides thetaiotaomicron*.[20] All these structures allow, to differing extents, the study of enzyme inhibition by small molecule sugar mimics providing important information about the mechanism of action and chemical topography of the active site of the enzyme. Thus, crystal structures of a number of complexes of α-L-fucosidases from both *T. mari-*

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tima and *Bacteroides thetaiotaomicron* with inhibitors displaying both pyranose and iminocyclitol configurations have been reported.[18–21]

In the last several years efforts have been devoted to the synthesis of α-L-fucosidase inhibitors based on monosaccharides reaching the nano- and pico-molar range.[22] Recently, fucosidase-targeted ligands have shown strong antiproliferative effects on MDA-MB-231 cancer cells.[23] These types of compounds have been shown to be useful for the treatment of liver disorders and liver tumors,[24] such as hepatocellular carcinoma and also for treatment and diagnosis of *H. pylori* infection.[25]

Our group is striving to find new ways to extend these successful studies with "monovalent" sugar mimics to multivalent systems. In carbohydrate binding to receptors, multivalency effects may increase the affinity of sugar for a given target as well as potentially improving their solubility in aqueous media.[26] The recognition of clustered sugars by proteins has been broadly studied in the case of lectins; however, the effect of multivalency on glycosidase inhibition has been only scarcely probed. It is generally believed that multivalency may provide an enhancement to binding affinity in enzymatic systems by one of two processes.[26] One process involves the simultaneous binding or chelation of multiple (sub)ligands to different binding sites of a protein – the classical avidity mechanism with polyvalent receptors and multivalent ligands. The second process, named the "statistical rebinding" or "proximity effect", reflects the increased propensity for ligand rebinding when two relevant coupled ligands are in close proximity (Figure 1).[27] Thus, even in the absence of appropriately situated sites for increased binding through avidity, there may be virtue in enzyme inhibitors wherein multiple copies of the inhibitory warhead are coupled reflecting the slower off-rate of the multivalent glycomimetic moiety relative to its monovalent counterparts. Several examples of multivalent effects on glycosidases have been recently reported (i.e. for amyloglucosidases and α-mannosidases,[28] for α- and β-glucosidases[29] and β-galactosidases).[30] The concept of multivalency for a glycosidase of therapeutic interest has also been applied in the development of chaperones for the treatment of Gaucher's disease.[31] Herein, we explore such a multivalent approach on α-L-fucosidase inhibition for the first time. We describe the synthesis and biological evaluation of short and long-tethered di- and trivalent derivatives (**1**–**4**, Figure 2) incorporating different imino sugars based on *fuco*-configured 1,4-imino- and 1,4-bis(imino)- moieties; these compounds display α-L-fucosidase inhibitory activities in the μM and nM range. The 3D structures of both monomer and multivalent forms of one compound class have been studied using the *Bacteroides thetaiotaomicron* GH29 α-L-fucosidase (*Bt*Fuc2970) as a target system providing insight into both the conformational basis for enzyme inhibition and a consideration of different models for multivalency on this system.

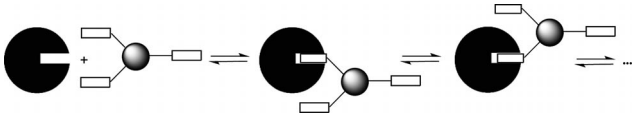


Figure 1. Multivalent ligand binding via a statistical rebinding mechanism.

Results and Discussions

Synthesis

The design of di- and trivalent-scaffolded imino sugars of interest is based on the inhibitory properties towards α-L-fucosidase of monovalent 1,4-imino- and 1,4-bis(imino)-cyclitols **5**–**9**. Compounds **7** and **8** are prepared for the first time herein and the others were previously prepared by our research group: **5**,[32] **6**[33] and **9**,[34] (Figure 3). Biphenyl derivatives **5** and **6** are valid reference compounds for the comparison with dimer **1**, when attaching another imino sugar moiety to the biphenyl moiety. In a similar way, benz-

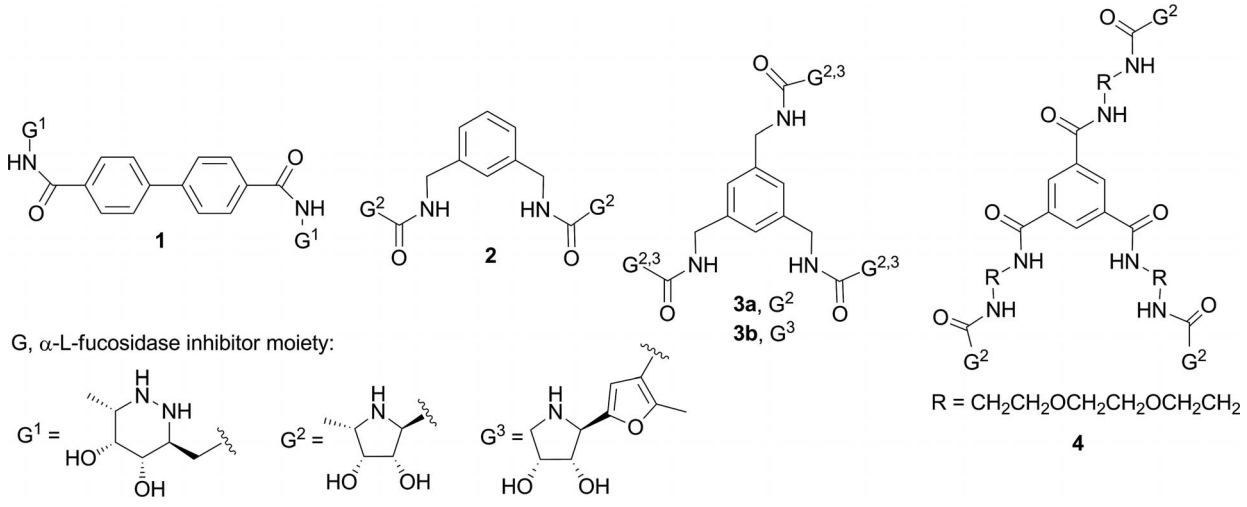


Figure 2. Structures for short-tethered bivalent glycomimetics **1** and **2**, trivalent glycomimetics **3** and long-tethered trivalent glycomimetic **4**.

ylamino derivatives **7**, **8** and **9** are monovalent compounds for the comparison with dimer **2** and trimers of type **3** and **4**. For this purpose, the preparation of new pyrrolidines **7** and **8** was accomplished (Scheme 1). The synthesis was carried out starting from diol **10** that was easily obtained from D-mannose diacetonide as previously reported by us. Glycol cleavage and oxidation gave carboxylic acid **12**.[32a] Peptide coupling with benzylamine using PyBOP as the coupling agent and DIPEA as base, gave protected amide **13**. Subsequent standard deprotection of **13** afforded pyrrolidine **7** in good yield. Amine **8** was obtained from *N*-Boc-protected diol **11** after glycol cleavage, reductive amination of the aldehyde with benzylamine and final acid hydrolysis.

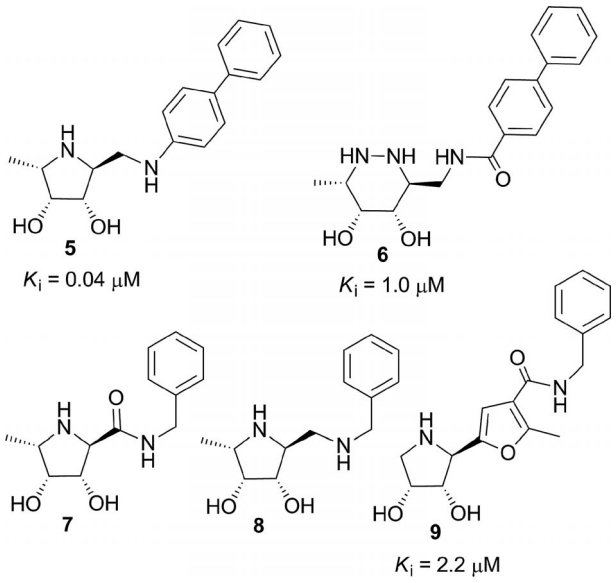
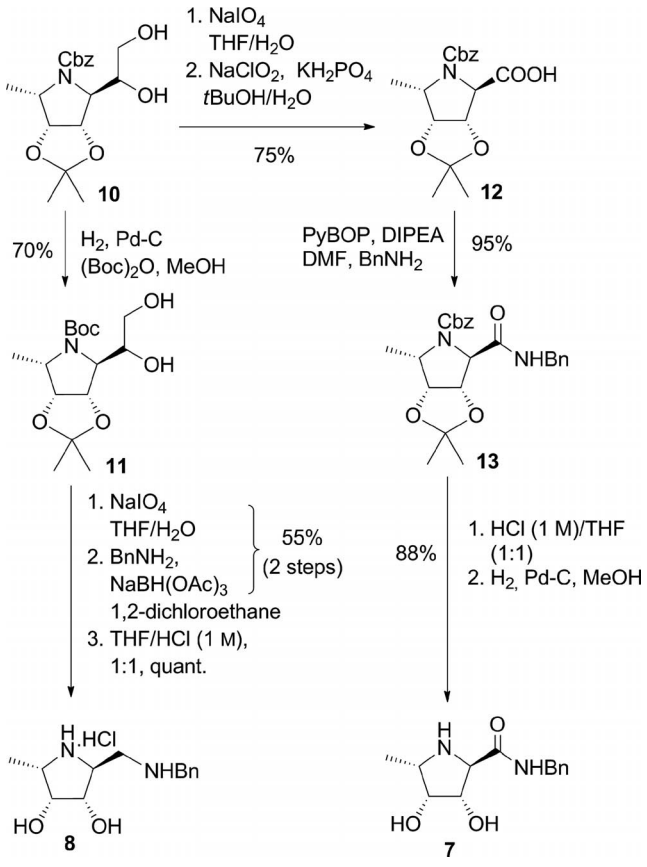


Figure 3. Monovalent α-L-fucosidase inhibitors.

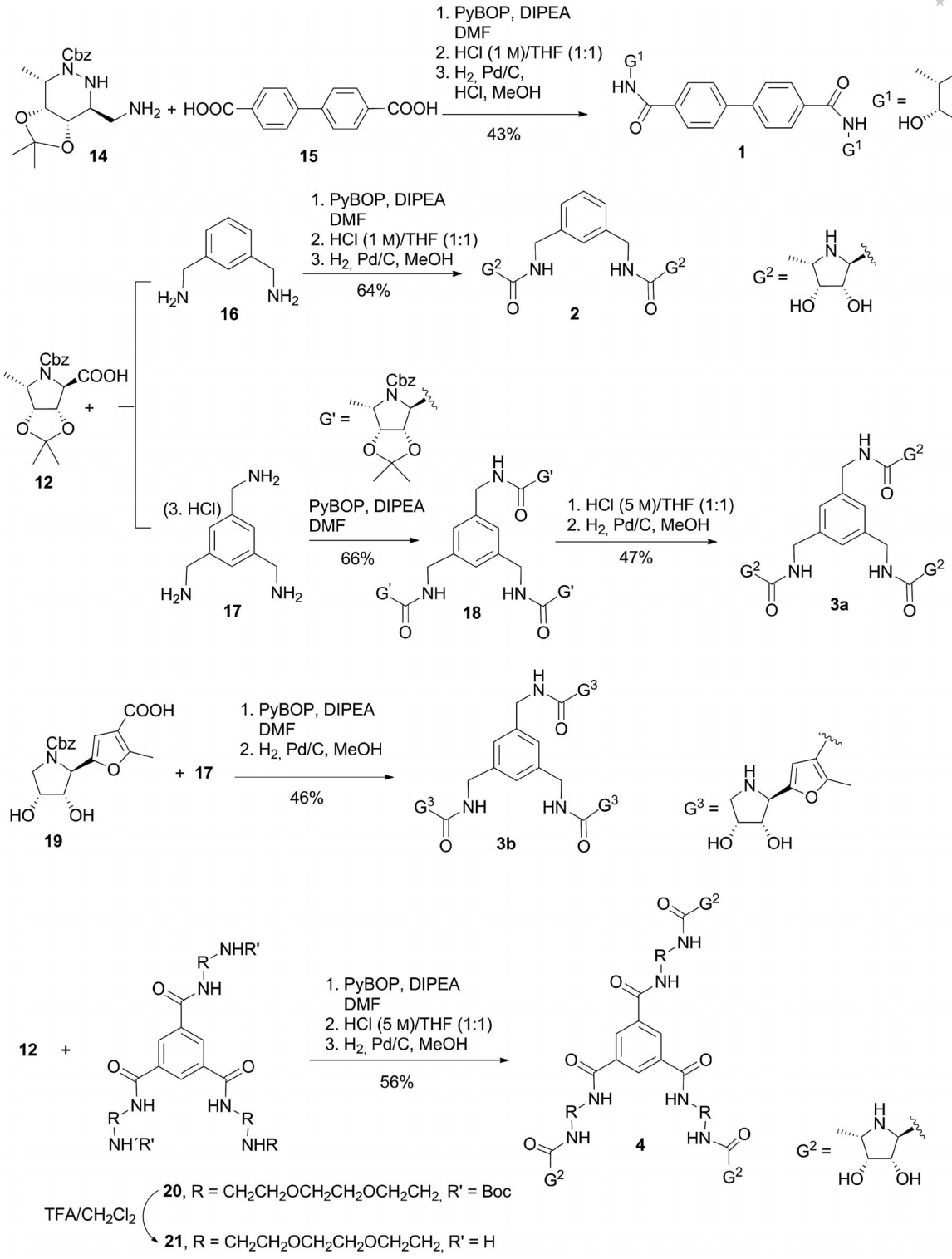


Scheme 1.

For the syntheses of short-tethered bivalent glycomimetics **1** and **2** and trivalent glycomimetics **3**, commercial dicarboxylic acid **15**, *m*-xylylenediamine **16** or synthetic triamine **17**[35] were used as templates (Scheme 2). Standard amide coupling (PyBOP as coupling agent and DIPEA as base) between **15** and aminomethyl-imino sugar **14**[33b] followed by standard deprotection gave **1** in 43% yield. Similarly, reaction of imino sugar acid derivative **12**,[32a] and amines **16** and **17** and subsequent deprotection gave dimer **2** and trimer **3a**, respectively, in moderate to good yields. Reaction of 1,4-imino-furan carboxylic acid **19**[34] with triamine **17** and deprotection, equally gave trimer **3b** in 46% yield. For the synthesis of long-tethered trivalent glycomimetic **4**, compound **20**[36] was used as a C-3 symmetric template. Thus, after removal of the Boc groups in **20**, the coupling reaction with carboxylic acid **12** was carried out under standard amide coupling conditions. Final deprotection of the corresponding adduct gave **4** in good yield (Scheme 2).

3D Structural Analysis of a Representative Mono/Trivalent Inhibitor Pair

In order to probe the structural basis for GH29 fucosidase inhibition by the iminocyclitols, crystal structures of *Bt*Fuc2970 liganded with compounds **3a** and **7** were determined, both at a resolution of ≈ 1.7 Å (see Supporting Information, Table S1). Compounds **3a** and **7** inhibit *Bt*Fuc2970 with K_i values of 0.7 ± 0.02 and 4.7 ± 0.30 μM, respectively (see Supporting Information, Figure S1). Electron density for the iminocyclitol ring for each inhibitor is clear and unambiguous. Each adopts an essentially identical ^3E envelope conformation as recently observed for other five-membered iminocyclitols on this enzyme.[37] This conformation reflects the putative $^3H_4$ transition-state for the enzyme-catalyzed reaction (discussed, for example, in Refs. 18, 20 & 37, Figure 4). Beyond the core cyclitol, the conformations observed for the amide and beyond are both dynamic (indeed occasionally disordered) and dependent on crystal packing environment resulting in a large spread of observed conformations (see Supporting Information, Figure S2). Representations of inhibitors **3a** and **7** lying in their respective crystallographic active sites are provided in Figure S3. The *Bt*Fuc2970 crystal form used conveys two independent observations of ligand binding, reflecting the two independent molecules in the crystallographic asymmetric unit. For monovalent compound **7**, clear unambiguous density is observed for one independent observation (Figure 4, a) (that with the most interactions with a crystal-packing neighboring molecule) whereas a more disordered "aglycon" is observed in the second molecule in the asym-



Scheme 2.

metric unit. Indeed in this latter case, with no packing constraints, the "aglycon" is completely disordered beyond the first methylene carbon pendant to the amide unit (see Supporting Information, Figure S2). In contrast, but likely reflecting its larger steric bulk, the trimeric counterpart **3a** is better ordered in the less tightly packed protein molecule (Figure 4, b). Despite this freedom, no electron density is observed for **3a** beyond the phenyl ring reflecting a highly disordered trimer (and the absence of appropriately placed active sites for avidity effects). That the different degrees of order observed reflects adventitious interactions provides encouragement and valuable insight for those working on inhibitor design as well as providing a rationale for the different K_i values reported on different enzyme targets. In

light of these data the full range of inhibitors was tested for fucosidase inhibition on the mammalian enzyme.

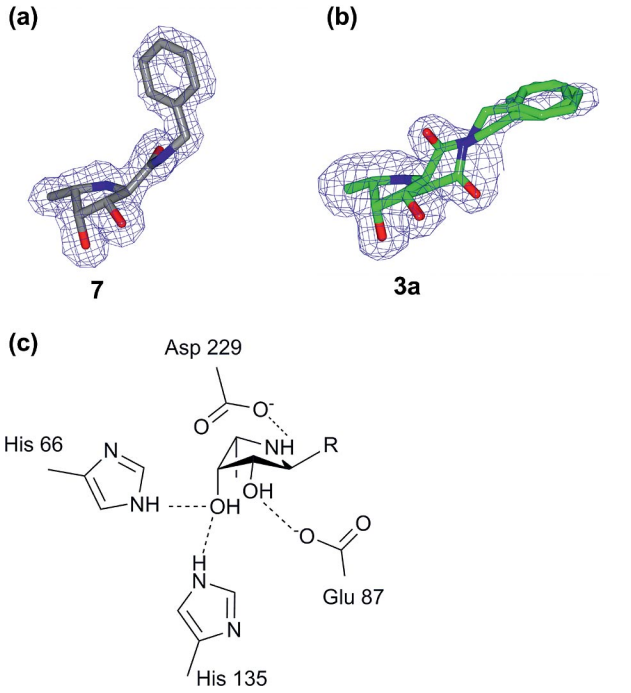


Figure 4. (a) and (b) observed electron density ($F_o - F_c$ maps, prior to the incorporation of ligand in refinement) contoured at (a) 5 σ (equates to 0.3 electrons/Å^3, compound **7**) and (b) 3 σ (equates to 0.15 electrons/Å^3, compound **3a**) bound to *Bt*Fuc2970. (c) Interactions made between the iminocyclitol core of compounds **3a** and **7** and residues at or near the active site of *Bt*Fuc2970.

Biological Evaluation of Glycomimetics

Multivalent glycomimetics **1–4** and monovalent imino sugars **7** and **8** were analyzed for their inhibitory activities against a panel of twelve commercially available glycosidases.[38] Table 1 shows the results for inhibition analysis against α-L-fucosidase from bovine kidney, in comparison with previous results of imino sugars **6** and **9** (inhibition data for other glycosidases are summarized in the Supporting Information). Most of the new compounds are specific inhibitors of α-L-fucosidase; no significant inhibition was observed for any of the other enzymes assayed (α-galactosidases from coffee beans, β-galactosidases from *Escherichia coli* and *Aspergillus oryzae*, α-glucosidases from yeast and from rice, amyloglucosidases from *Aspergillus niger*, β-glucosidases from almonds, α-mannosidases from Jack beans, β-mannosidases from snail, β-xylosidases from *Aspergillus niger*, and β-*N*-acetylglucosaminidases from Jack beans).

The monovalent compounds, along with all new di- and trivalent glycomimetics inhibited α-L-fucosidases in the low μM range. Upon comparing each dimeric and trimeric inhibitor with its corresponding monovalent analogue (**1** vs. **6**, **2** vs. **7**, **3a** vs. **7**, **3b** vs. **9**, **4** vs. **7**), enhanced inhibition was only found to occur in the case of trivalent imino sugar **3a** (K_i = 0.3 μM). Compound **3a** is a seven-fold more potent inhibitor than the monovalent analogue **7** (K_i = 2.1 μM) of both bovine kidney and *Bacteroides* enzymes. Furthermore, bivalent inhibitor **2** was less potent an inhibitor than both its monovalent parent **7** and trivalent offspring **3a**. An increase in the length of the spacers in the scaffold (**3a** vs. **4**), did not improve the inhibitory activity; both compounds displayed practically equivalent activities.

Table 1. Inhibitory activities of mono- and multi-valent imino sugars towards bovine kidney α-L-fucosidase.

Compound		% Inhibition at 1 mM, IC_{50} and K_i in μM optimal pH = 6, 37 °C.[a],[b]
Monomers	**6**[c]	85%, (IC_{50} = 8.6 μM), K_i = 1.0 μM
	7	99%, (IC_{50} = 15 μM), K_i = 2.1 μM
	8	89%, (IC_{50} = 100 μM), K_i = 11.7 μM
	9[d]	91%, (IC_{50} = 38 μM), K_i = 3.8 μM
Dimers	**1**	98%, (IC_{50} = 12.8 μM), K_i = 1.3 μM
	2	99%, (IC_{50} = 6.5 μM), K_i = 4.0 μM
Trimers	**3a**	99%, (IC_{50} = 1.6 μM), K_i = 0.3 μM
	3b	98%, (IC_{50} = 17 μM), K_i = 2.1 μM
	4	96%, (IC_{50} = 3.8 μM), K_i = 0.4 μM

[a] For measurement conditions, see ref. 38. [b] Competitive mode of inhibition for given K_i. [c] Percentage of inhibition was determined at 0.1 mM and was previously reported.[33b] [d] A K_i of 2.2 μM was previously determined against human placental fucosidase.[34]

Conclusions

We have provided an efficient method for the preparation and biological evaluation of five di/tri-valent imino sugars by coupling different aromatic templates to imino sugar precursors. It is worth noting that amide **7** is a six-fold more potent inhibitor than amine **8**. This observation provides justification for the use of amide linkages to attach monovalent imino sugars to their corresponding templates. Although much more research is clearly needed to understand multivalency and its utility in the area of glycosidase inhibition, the synthetic approach reported is certainly suitable for the development of such multivalent glycosidase inhibitors. As expected, most derivatives proved to be specific inhibitors of α-L-fucosidases with inhibition constants in the μM range. Data on enhanced inhibition are less convincing. Only trivalent imino sugar **3a** proved to be a more potent inhibitor of α-L-fucosidases than its monovalent; the trivalent inhibitor was seven-fold more active than its monovalent analogue **7** against both bovine kidney fucosidase and the *Bacteroides* enzyme. The 3D structures of mammalian GH29 enzymes are not known, but it is unlikely from any of the reported 3D structures of bacterial homologs that the multi-valent inhibitors could span different active centres and gain the massive affinity increase through avidity effects that is possible in lectin-like systems. Any increase observed may reflect subtle differences in (*de*)solvation and entropic effects or may indeed represent a statistical rebinding phenomenon that is more likely in trimeric over di- or monomeric compounds. Across the series, the ΔΔG values for binding cover just a small range. However, given the structural data which highlight the role of adventitious in-

teractions beyond the imino-cyclitol core, there is clearly room to consider expansion of our current inventory of fucosidase inhibitors so as to generate therapeutically significant and specific inhibitors.

Experimental Section

General Methods: Optical rotations were measured with a 1.0 cm or 1.0 dm tube with a Jasco P-2000 spectropolarimeter. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AV300, AMX300 and AV500 for solutions in D_2O, CD_3OD and [D$_6$]DMSO. All assignments were confirmed by two-dimensional NMR experiments (COSY and HSQC). Infrared spectra were recorded with a Jasco FTIR-410 spectrophotometer. Mass spectra (CI, LSI and ESI) were recorded using Micromass AutoSpeQ and QTRAP spectrometers. The LSI was performed using thioglycerol as the matrix. TLC was performed on silica gel HF_{254} (Merck), with detection by UV light charring with H_2SO_4, vanillin, ninhydrin or with Pancaldi reagent [$(NH_4)_6MoO_4$, $Ce(SO_4)_2$, H_2SO_4, H_2O]. Silica gel 60 (Merck, 230 mesh) was used for preparative chromatography.

Glycosidase Inhibition Assays: Glycosidases and the corresponding *p*-nitrophenyl-*O*-glycoside substrates for inhibition assays were purchased from Sigma–Aldrich. The experiments were performed essentially as described previously.[38] Briefly, 0.01–0.5 units/mL of enzyme and inhibitor were pre-incubated for 5 min at room temp., and the reaction started by addition of the substrate, buffered to the optimal pH of the enzyme. After 20 min of incubation at 37 °C, the reaction was stopped by addition of sodium borate buffer pH 9.8. The *p*-nitrophenolate formed was measured by visible absorption spectroscopy at 405 nm.

(2*S*,3*S*,4*R*,5*S*)-*N*-(*tert*-Butoxycarbonyl)-2(1′,2′-dihydroxyethyl)-3,4-*O*-isopropylidene-5-methylpyrrolidine-3,4-diol (11): To a solution of *N*-(Benzyloxycarbonyl-2(1′,2′-dihydroxyethyl)-3,4-*O*-isopropylidene-5-methylpyrrolidine-3,4-diol **10**[32a] (86.3 mg, 0.246 mmol) in MeOH (2.5 mL), Pd/C (10%) and $(Boc)_2O$ (81 mg, 0.37 mmol) were added. The mixture was hydrogenated for 48 h. After filtration through celite, the filtrate was purified by column chromatography (CH_2Cl_2/MeOH, 30:1) to give **11** (54.5 mg, 0.172 mmol, 70%). $[\alpha]_D^{24}$ = +76.4 (*c* = 1.3 in MeOH). ^{1}H NMR (300 MHz, [D$_6$]DMSO, 363 K): δ = 4.73 (d, $J_{3,4}$ = 6.3 Hz, 1 H, 3-H), 4.57 (br. s, 1 H, OH), 4.56 (t, $J_{4,5}$ = 6.2 Hz, 1 H, 4-H), 4.16 (br. s, 1 H, OH), 3.99 (d, $J_{2,1'}$ = 4.5 Hz, 1 H, 2-H), 3.89–3.81 (m, 1 H, 1′-H), 3.78 (q, $J_{5,Me}$ = 6.5 Hz, 1 H, 5-H), 3.37 (dd, $J_{2'a,1'}$ = 4.5, $^2J_{2'a,2'b}$ = 11.2 Hz, 1 H, 2′-Ha), 3.27 (dd, $J_{2'b,1'}$ = 6.3 Hz, 1 H, 2′-Hb), 1.42 (s, 9 H, $(CH_3)_3C$), 1.38 (s, 3 H, $C(CH_3)_2$), 1.28–1.26 (m, 6 H, $C(CH_3)_2$, Me-5) ppm. ^{13}C NMR (75.4 MHz, [D$_6$]DMSO, 363 K): δ = 153.7 (C=O of Boc), 109.5 ($(CH_3)_2C$), 80.3 (C-4), 79.6 (C-3), 78.2 ($(CH_3)_3C$), 70.6 (C-1′), 64.6 (C-2), 62.2 (C-2′), 56.7 (C-5), 27.7 ($CH_3)_3C$), 25.5, 24.5 ($C(CH_3)_2$), 14.8 (Me-5). CIMS: *m*/*z* (%) = 318 (20) [M + H]$^+$, 218 (100) [M – Boc + H]$^+$. CIHRMS *m*/*z* found 318.1923, calcd. for $C_{15}H_{28}NO_6$ [M + H]$^+$: 318.1917.

(2*R*,3*S*,4*R*,5*S*)-*N*-Benzyloxycarbonyl-2-benzylcarbamoyl-3,4-*O*-isopropylidene-5-methylpyrrolidine-3,4-diol (13): Acid **12**[32a] (82 mg, 0.245 mmol) was dissolved in DMF and benzylamine (27 μL, 0.247 mmol), DIPEA (85 μL, 0.49 mmol) and PyBOP (130 mg, 0.249 mmol) were added. The mixture was stirred overnight at room temp. Then, the solvent was evaporated and the residue dissolved in CH_2Cl_2 (10 mL) and washed with satd. aq. sol. of citric acid (2× 10 mL) and brine (10 mL). The organic phase was dried (Na_2SO_4), filtered and evaporated in vacuo. The resulting crude mixture was purified by column chromatography (CH_2Cl_2/MeOH, 40:1) affording **13** (98.6 mg, 0.232 mmol, 95%). $[\alpha]_D^{24}$ = +28.0 (*c* = 0.89 in CH_2Cl_2). ^{1}H NMR (300 MHz, [D$_6$]DMSO, 363 K): δ = 8.58–8.48 (m, 1 H, CON*H*), 7.34–7.25 (m, 10 H, H-arom.), 5.06 (d, $^2J_{H,H'}$ = 12.7 Hz, 1 H, CH_2 of Cbz), 4.97 (d, 1 H, CH_2 of Cbz), 4.68 (t, $J_{4,5}$ = $J_{4,3}$ = 6.0 Hz, 1 H, 4-H), 4.59 (d, 1 H, 3-H), 4.43 (s, 1 H, 2-H), 4.29 (dd, $^2J_{1'a,1'b}$ = 15.2, $J_{1'a,\ NH}$ = 5.8 Hz, 1 H, 1′-Ha), 4.22 (dd, $J_{1'b,\ NH}$ = 6.0 Hz, 1 H, 1′-Hb), 4.08 (q, $J_{5,Me-5}$ = 6.3 Hz, 1 H, 5-H), 1.43, 1.30 (2s, 3H each, $C(CH_3)_2$), 1.34 (d, $J_{Me-5,5}$ = 6.6 Hz, 3 H, Me-5) ppm. ^{13}C NMR (75.4 MHz, [D$_6$]DMSO, 363 K): δ = 169.6 (*C*ONH), 154.6 (C=O of Cbz), 138.5, 136.2 (C$_q$-arom.), 127.7, 127.6, 127.1, 126.9, 126.8, 126.2 (C-arom.), 110.5 ($C(CH_3)_2$), 81.2, 80.1 (C-3, C-4), 66.5 (C-2), 65.5 (*C*H$_2$ of Cbz), 57.3 (C-5), 42.0 (C-1′), 25.6, 24.5 ($C(CH_3)_2$), 14.5 (Me-5) ppm. IR: $\tilde{\nu}$ = 3344, 2983, 2936, 1708, 1653, 1539, 1397, 1310, 1233, 1213, 1027, 875 cm^{-1}. CIMS: *m*/*z* (%)= 425 (10) [M + H]$^+$. CIHRMS *m*/*z* found 425.2062, calcd. for $C_{24}H_{29}N_2O_5$ [M + H]$^+$: 425.2076.

(2*R*,3*S*,4*R*,5*S*)-2-Benzylcarbamoyl-5-methylpyrrolidine-3,4-diol (7): A solution of **13** (66 mg, 0.156 mmol) in HCl (1 M)/THF, 1:1 (3.6 mL) was stirred overnight at room temp. The solvent was then evaporated and the resulting residue was purified by column chromatography (CH_2Cl_2/MeOH, 20:1). The obtained product (58.7 mg, 0.153 mmol) was dissolved in MeOH (3 mL) and hydrogenated with Pd/C (10%) as catalyst. After 1.5 h, the catalyst was removed by filtration through celite and the solution concentrated. The residue was purified by column chromatography (CH_2Cl_2/MeOH, 10:1) affording **7** (34.4 mg, 0.137 mmol, 88%, 2 steps). $[\alpha]_D^{24}$ = +18.3 (*c* = 0.89 in MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.31–7.20 (m, 5 H, H-arom.), 4.44 (d, $^2J_{H,H}$ = 15.2 Hz, 1 H, C*H*-Ph), 4.39 (d, 1 H, C*H*-Ph), 4.15 (dd, $J_{3,2}$ = 7.4, $J_{3,4}$ = 4.2 Hz, 1 H, 3-H), 3.85–3.82 (m, 1 H, 4-H), 3.58 (d, 1 H, 2-H), 3.23 (qd, $J_{5,Me-5}$ = 6.6, $J_{5,4}$ = 3.2 Hz, 1 H, 5-H), 1.16 (d, 3 H, Me-5) ppm. ^{13}C NMR (75.4 MHz, CD_3OD): δ = 176.0 (*C*ONH), 139.9 (C$_q$-arom.), 129.6, 128.5, 128.2 (C-arom.), 79.2 (C-3), 75.9 (C-4), 66.2 (C-2), 57.2 (C-5), 43.9 (*C*H$_2$-Ph), 15.0 (Me-5) ppm. CIMS: *m*/*z* (%) = 251 [(100) [M + H]$^+$]. CIHRMS *m*/*z* found 251.1289, calcd. for $C_{13}H_{19}N_2O_3$ [M + H]$^+$: 251.1396.

(2*S*,3*S*,4*R*,5*S*)-2-Benzylaminomethyl-5-methylpyrrolidine-3,4-diol Hydrochloride (8): A solution of $NaIO_4$ (440.6 mg, 2.06 mmol) in water (4 mL) was added dropwise to a solution of **11** (326.5 mg, 1.03 mmol) in THF (4 mL) cooled to 0 °C. After stirring for 1 h at room temp., THF was evaporated and the residue dissolved in CH_2Cl_2 and washed successively with water, saturated $NaHCO_3$ (aq.) and brine. The organic phase was dried, filtered and concentrated. To a solution of the corresponding aldehyde in dry 1,2-dichloroethane (9 mL), benzylamine (337 μL, 3.09 mmol) and $NaBH(OAc)_3$ (343.3 mg, 1.54 mmol) were added. The reaction mixture was stirred overnight at room temp. under N_2. Then, saturated $NaHCO_3$(aq.) was added and the mixture extracted with AcOEt, dried (Na_2SO_4), filtered and evaporated in vacuo. The residue was purified by column chromatography (AcOEt/petroleum ether, 1:3) affording the corresponding amino pyrrolidine (214 mg, 0.569 mmol, 55%, 2 steps). A solution of the protected pyrrolidine (75.2 mg, 0.2 mmol) in THF/HCl (1 M) (1:1, 5 mL) was stirred at room temp. for 3 h. The solvent was evaporated affording **8** (56.5 mg, 0.2 mmol, quant.). $[\alpha]_D^{24}$ = –16.4 (*c* = 0.88 in MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.62–7.58 (m, 2 H, H-arom.), 7.51–7.45 (m, 3 H, H-arom.), 4.37 (d, ^2J = 13.1 Hz, 1 H, CH_2 of Bn), 4.33 (d, 1 H, CH_2 of Bn), 4.24 (dd, $J_{3,2}$ = 7.3, $J_{3,4}$ = 4.8 Hz, 1 H, 3-H), 3.96 (t, $J_{4,5}$ = 4.8 Hz, 1 H, 4-H), 3.86 (td, $J_{2,1'a}$ = 7.4, $J_{2,1'b}$ = 5.9 Hz, 1 H, 2-H), 3.75–3.67 (m, 1 H, 5-H), 3.69 (dd, $^2J_{1'a,1'b}$ = 13.6 Hz, 1 H, 1′-Ha), 3.60 (dd, 1 H, 1′-Hb), 1.49 (d, $J_{Me,5}$ = 7.1 Hz, 3 H, Me-5) ppm. ^{13}C NMR (75.4 MHz, CD_3OD): δ = 131.9 (C$_q$-arom.), 131.3, 131.0, 130.4 (C-arom.), 75.3 (C-4), 73.9 (C-3), 62.6

(C-5), 59.9 (C-2), 53.1 (CH_2 of Bn), 47.8 (C-1′), 16.1 (Me-5) ppm. IR: $\tilde{\nu}$ = 3389, 3164–3155, 3073, 2941, 2927, 2845–2610, 1536, 1457, 1129, 1086, 1004, 787, 745, 692 cm^{-1}. CIMS: *m*/*z* (%) = 237 [(43) $[M + H]^+$]. CIHRMS *m*/*z* found 237.1598, calcd. for $C_{13}H_{21}N_2O_2$ $[M + H]^+$: 237.1603.

4,4′-Bis((3*S*,4*S*,5*R*,6*S*)-3-carbonylaminomethyl-6-methyl-hexahydropyridazine-4,5-diol)biphenyl Dihydrochloride (1): To a solution of **14**[33b] (163 mg, 0.486 mmol) and commercial dicarboxylic acid **15** (59 mg, 0.243 mmol) in DMF (3 mL), DIPEA (166 μL, 0.953 mmol) and PyBOP (252 mg, 0.484 mmol) were added. The mixture was stirred overnight at room temp. Then, the solvent was evaporated and the residue dissolved in CH_2Cl_2 and washed with satd. aq. sol. of citric acid and brine. The organic phase was dried (Na_2SO_4), filtered and evaporated in vacuo. The crude product thus obtained was treated with HCl (1 M)/THF, 1:1 (10 mL) and stirred 5 h at room temp. The solvent was then evaporated and the resulting residue was purified by column chromatography (CH_2Cl_2/MeOH, 20:1). The obtained product (83.4 mg, 0.105 mmol) was dissolved in MeOH (15 mL), and Pd/C (10%) and HCl (5 M, 100 μL) were added. The mixture was hydrogenated at 1 atm for 3 h, then diluted with MeOH, filtered through celite, and evaporated, affording corresponding unprotected derivative **1** in 43% overall yield. $[\alpha]_D^{25}$ = –20.9 (*c* = 0.6 in MeOH). ^{1}H NMR (300 MHz, D_2O): δ = 7.79 (d, *J* = 8.3 Hz, 4 H, H-arom.), 7.68 (d, *J* = 8.3 Hz, 4 H, H-arom.), 4.04 (m, 2 H, 4-H or 5-H), 3.78 (dd, $^2J_{1'a,1'b}$ = 14.2, $J_{1'a,3}$ = 2.5 Hz, 2 H, 1′-Ha), 3.69 (dd, *J* = 10.0, *J* = 2.8 Hz, 2 H, 4-H or 5-H), 3.60–3.45 (m, 6 H, 1′-Hb, 3-H, 6-H), 1.31 (d, $J_{Me,6}$ = 6.8 Hz, 6 H, Me-6) ppm. ^{13}C NMR (75.4 MHz, D_2O): δ = 170.6 (*C*ONH), 142.7, 132.4 (C_q-arom.), 127.8, 127.2 (C-arom.), 68.1, 67.9 (C-4, C-5), 57.1, 54.6 (C-6, C-3), 38.9 (C-1′), 12.6 (Me-6) ppm. IR: $\tilde{\nu}$ = 3552–3046, 1635, 1550, 1532, 1492, 1308, 1165, 1115, 1024, 1006, 840 cm^{-1}. LSIHRMS *m*/*z* found 551.2587, calcd. for $C_{26}H_{36}N_6O_6Na$ $[M + Na]^+$: 551.2594.

1,3-Bis((2*R*,3*S*,4*R*,5*S*)-2-carbonylaminomethyl-5-methyl-pyrrolidine-3,4-diol)benzene (2): Acid **12**[32a] (150 mg, 0.448 mmol) was dissolved in DMF and commercial *m*-xylylenediamine (23 μL, 0.174 mmol), DIPEA (122 μL, 0.71 mmol) and PyBOP (233 mg, 0.448 mmol) were added. The mixture was stirred overnight at room temp. Then, the solvent was evaporated and the residue dissolved in CH_2Cl_2 (10 mL) and washed with satd. aq. sol. of citric acid (2 × 10 mL) and brine (10 mL). The organic phase was dried (Na_2SO_4), filtered and evaporated in vacuo. The resulting crude product was purified by column chromatography (CH_2Cl_2/MeOH, 50:1) affording the corresponding protected diamide (134.3 mg, 0.174 mmol, quant.), whose solution (60 mg, 0.08 mmol) in HCl (1 M)/THF, 1:1 (1.4 mL) was stirred overnight at room temp. The solvent was then evaporated and the resulting residue was purified by column chromatography (CH_2Cl_2/MeOH, 10:1). The obtained product (35 mg, 0.05 mmol) was dissolved in MeOH (3 mL), and hydrogenated with Pd/C (10%) as catalyst. After 2 h, the catalyst was removed by filtration through celite and the solution concentrated affording **2** (22.9 mg, 0.05 mmol, 64%, 2 steps). $[\alpha]_D^{25}$ = –15.6 (*c* = 0.43 in MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.32–7.09 (m, 4 H, H-arom.), 4.41 (br. s, 4 H, CH_2-Ph), 4.16 (dd, $J_{3,2}$ = 7.5, $J_{3,4}$ = 4.2 Hz, 2 H, 3-H), 3.86–3.84 (m, 2 H, 4-H), 3.58 (d, 2 H, 2-H), 3.25 (qd, $J_{5,Me-5}$ = 6.6, $J_{5,4}$ = 3.3 Hz, 2 H, 5-H), 1.22 (d, 6 H, Me-5) ppm. ^{13}C NMR (75.4 MHz, CD_3OD): δ = 175.9 (*C*ONH), 140.3 (C_q-arom.), 129.9, 129.2, 127.3 (C-arom.), 79.1 (C-3), 75.9 (C-4), 66.1 (C-2), 57.2 (C-5), 43.8 (*C*H_2-Ph), 15.0 (Me-5) ppm. IR: $\tilde{\nu}$ = 3626–3105, 2920, 1650, 1541, 1347, 1269, 1123, 1283, 990, 750 cm^{-1}. CIMS: *m*/*z* (%) = 423 [(100) $[M + H]^+$]. CIHRMS *m*/*z* found 423.2238, calcd. for $C_{20}H_{31}N_4O_6$ $[M + H]^+$: 423.2244.

1,3,5-Tris((2*R*,3*S*,4*R*,5*S*)-*N*-benzyloxycarbonyl-2-carbonylaminomethyl-3,4-*O*-isopropylidene-5-methylpyrrolidine-3,4-diol)benzene (18): The acid **12**[32a] (176 mg, 0.525 mmol) was dissolved in DMF and 1,3,5-tris(aminomethyl)benzene trihydrochloride[35] (43.5 mg, 0.159 mmol), DIPEA (1.6 mL, 9.45 mmol) and PyBOP (278.6 mg, 0.535 mmol) were added. The mixture was stirred overnight at room temp. Then, the solvent was evaporated and the residue dissolved in CH_2Cl_2 (30 mL) and washed with satd. aq. sol. of citric acid (2 × 30 mL) and brine (30 mL). The organic phase was dried (Na_2SO_4), filtered and evaporated in vacuo. The resulting crude mixture was purified by column chromatography (CH_2Cl_2/MeOH, 30:1) affording **18** (117 mg, 0.105 mmol, 66%). $[\alpha]_D^{24}$ = –34.3 (*c* = 0.96 in CH_2Cl_2). ^{1}H NMR (500 MHz, [D_6]DMSO, 363 K): δ = 8.53 (t, $J_{NH,1'a}$ = $J_{NH,1'b}$ = 5.9 Hz, 3 H, CO*NH*), 7.34–7.27 (m, 15 H, H-arom.), 7.04 (s, 3 H, H-arom.), 5.02 (s, 6 H, CH_2 of Cbz), 4.67 (t, $J_{4,5}$ = $J_{4,3}$ = 6.1 Hz, 3 H, 4-H), 4.59 (dd, $J_{3,2}$ = 1.0 Hz, 3 H, 3-H), 4.42 (br. s, 3 H, 2-H), 4.30 (dd, $^2J_{1'a,1'b}$ = 15.1 Hz, 3 H, 1′-Ha), 4.09–4.04 (m, 6 H, 1′-Hb, 5-H), 1.43, 1.30 (2s, 9H each, C(CH_3)$_2$), 1.32 (d, $J_{Me-5,5}$ = 6.5 Hz, 9 H, Me-5) ppm. ^{13}C NMR (125.7 MHz, [D_6]DMSO, 363 K): δ = 169.6 (*C*ONH), 154.7 (CO of Cbz), 138.8, 136.3 (C_q-arom.), 127.8, 127.2, 126.9, 124.6 (C-arom.), 110.6 (*C*(CH$_3$)$_2$), 81.2 (C-3), 80.1 (C-4), 66.5 (C-2), 65.6 (CH_2 of Cbz), 57.3 (C-5), 41.9 (C-1′), 25.7, 24.6 (C(*C*H$_3$)$_2$), 14.6 (Me-5) ppm. IR: $\tilde{\nu}$ = 2988, 2937, 1751–1587, 1454, 1405, 1352, 1306, 1209, 1140, 1027, 867 697 cm^{-1}. LSIMS: *m*/*z* (%) = 1139 [(5) $[M + Na]^+$]. LSIHRMS *m*/*z* found 1139.4910, calcd. for $C_{60}H_{72}N_6O_{15}Na$ $[M + Na]^+$: 1139.4953.

1,3,5-Tris((2*R*,3*S*,4*R*,5*S*)-2-carbonylaminomethyl-5-methylpyrrolidine-3,4-diol)benzene (3a): A solution of **18** (78.8 mg, 0.07 mmol) in HCl (5 M)/THF, 1:1 (1.2 mL) was stirred overnight at room temp. The solvent was then evaporated and the resulting residue was purified by column chromatography (CH_2Cl_2/MeOH, 10:1). The obtained product (57.7 mg, 0.058 mmol) was dissolved in MeOH (2 mL), and hydrogenated with Pd/C (10%) as catalyst. After 2 h the catalyst was removed by filtration through celite and the solution concentrated affording **3a** (19.5 mg, 0.033 mmol, 47%, 2 steps). $[\alpha]_D^{24}$ = –15.4 (*c* = 0.99 in MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.13 (s, 3 H, H-arom.), 4.41 (d, $^2J_{1'a,1'b}$ = 15.6 Hz, 3 H, 1′-Ha), 4.36 (d, 3 H, 1′-Hb), 4.15 (dd, $J_{3,2}$ = 7.5, $J_{3,4}$ = 4.2 Hz, 3 H, 3-H), 3.86–3.83 (m, 3 H, 4-H), 3.58 (d, 3 H, 2-H), 3.24 (qd, $J_{5,Me-5}$ = 6.6, $J_{5,4}$ = 3.2 Hz, 3 H, 5-H), 1.16 (d, 9 H, Me-5) ppm. ^{13}C NMR (75.4 MHz, CD_3OD): δ = 176.1 (*C*ONH), 140.8 (C_q-arom.), 126.2 (C-arom.), 76.1 (C-3), 75.9 (C-4), 66.1 (C-2), 57.1 (C-5), 43.7 (C-1′), 15.1 (Me-5) ppm. LSIMS: *m*/*z* (%) = 617 [(10) $[M + Na]^+$]. LSIHRMS *m*/*z* found 617.2911, calcd. for $C_{27}H_{42}N_6O_9Na$ $[M + Na]^+$: 617.2911.

***N*,*N*′,*N*′′-(1,3,5-Phenylenetris(methylene))tris-[5-((2*R*,3*S*,4*R*)-3,4-dihydroxypyrrolidin-2-yl)-2-methylfuran-3-carboxamide](3b):** Acid **19**[34b] (88.9 mg, 0.246 mmol) was dissolved in DMF and 1,3,5-tris(aminomethyl)benzene[35] (11.5 mg, 0.07 mmol), DIPEA (43 μL, 0.246 mmol) and PyBOP (143.6 mg, 0.27 mmol) were added. The mixture was stirred overnight at room temp. Then, the solvent was evaporated and the residue dissolved in AcOEt (50 mL) and washed with HCl (1 M) (3 × 20 mL) and brine (30 mL). The organic phase was dried (Na_2SO_4), filtered and evaporated in vacuo. The resulting crude mixture was purified by column chromatography (CH_2Cl_2/MeOH, 10:1) affording the corresponding protected triamide (41.2 mg, 0.034 mmol, 49%), whose solution (39.6 mg, 0.033 mmol) in MeOH (2 mL) was hydrogenated with Pd/C (10%) as catalyst. After 1.5 h the catalyst was removed by filtration through celite and the solution concentrated affording **3b** (24.5 mg, 0.031 mmol, 94%). $[\alpha]_D^{25}$ = –68.6 (*c* = 0.23 in H_2O). ^{1}H NMR (500 MHz, D_2O): δ = 7.23 (s, 3 H, H-arom.), 6.83 (s, 3 H, H-furan),

4.59–4.50 (m, 15 H, 2-H, 3-H, 4-H, 1′-Ha, 1′-Hb), 3.64 (dd, $J_{5a,5b}$ = 12.9, $J_{5a,4}$ = 4.5 Hz, 3 H, 5-Ha), 3.35 (dd, $J_{5b,4}$ = 2.0 Hz, 3 H, 5-Hb), 2.48 (s, 9 H, Me) ppm. ^{13}C NMR (125.7 MHz, D_2O): δ = 166.1 (C=O), 158.2, 146.1 (C_q-arom.), 139.2 (C-arom.), 124.0 (C_q-arom.), 116.2, 109.8 (C-arom.), 74.1, 69.7, 56.7 (C-2, C-3, C-4), 49.9 (C-5), 42.7 (C-1′), 13.0 (Me-5) ppm. IR: $\tilde{\nu}$ = 3596–300, 2923, 1636, 1579, 1534, 1421, 1401, 1340, 1228, 1119–1066 cm^{-1}. ESI MS: *m*/*z* (%) = 793 [(64) [M + H]$^+$], 815 [(37) [M + Na]$^+$].

N^1,N^3,N^5-Tris(2-(2-(2-((2*R*,3*S*,4*R*,5*S*)-3,4-dihydroxy-5-methylpyrrolidine-2-carboxamido)ethoxy)ethoxy)ethyl)benzene-1,3,5-tricarboxamide (4): A solution of **20**[36] (57 mg, 0.063 mmol) in 20% TFA/CH_2Cl_2 (2 mL) was stirred at room temp. for 30 min. Evaporation afforded crude **21**. To a solution of **21** (0.063 mmol) and **12** (69.6 mg, 0.208 mmol) in DMF (3 mL), DIPEA (137 μL, 0.794 mmol) and PyBOP (110 mg, 0.212 mmol) were added. The mixture was stirred overnight at room temp. Then, the solvent was evaporated and the residue dissolved in CH_2Cl_2 and washed with satd. aq. sol. of citric acid and brine. The organic phase was dried (Na_2SO_4), filtered and evaporated in vacuo. The resulting crude reaction was purified by column chromatography (CH_2Cl_2/MeOH, 30:1→15:1) affording the corresponding protected triamide (68.6 mg, 70%). This derivative (62.0 mg, 0.040 mmol) was stirred in HCl (5 M)/THF, 1:1 (2 mL) at room temp. for 5 h. Solvent was then evaporated and the residue was purified by column chromatography (CH_2Cl_2/MeOH, 10:1→6:1). A solution of the obtained product (45.7 mg, 0.032 mmol) in MeOH (5 mL) was hydrogenated with Pd/C (10%) as catalyst. After 4 h the catalyst was removed by filtration through celite and the solution concentrated affording **4** in quantitative yield. ^{1}H NMR (300 MHz, CD_3OD): δ = 8.46 (s, 3 H, H-arom.), 4.13 (dd, *J* = 7.5, *J* = 4.1 Hz, 3 H, 3-H), 3.85–3.82 (m, 3 H, 4-H), 3.71–3.55 (m, 33 H, 2-H, CH_2), 3.41–3.37 (m, 6 H, CH_2), 3.22 (qd, $J_{5,Me-5}$ = 6.6, $J_{5,4}$ = 3.0 Hz, 3 H, 5-H), 1.17 (d, 9 H, Me-5) ppm. ^{13}C NMR (75.4 MHz, CD_3OD): δ = 168.7 (*C*ONH), 136.6 (C_q-arom.), 130.1 (C-arom.), 78.8 (C-3), 75.5 (C-4), 71.4, 70.5, 65.5 (C-2, *C*H$_2$), 57.6 (C-5), 41.1, 40.2 (*C*H$_2$), 14.4 (Me-5) ppm. IR: $\tilde{\nu}$ = 3655–3080, 1645, 1541, 1449–1437, 1292, 1122–1093, 995 cm^{-1}. LSIMS: *m*/*z* (%) = 1052 [(5) [M + Na]$^+$]. LSIHRMS *m*/*z* found 1052.5142, calcd. for $C_{45}H_{75}N_9O_{18}Na$ [M + Na]$^+$: 1052.5128.

Supporting Information (see footnote on the first page of this article): Supplemental crystallographic data, ^{1}H- and ^{13}C-NMR spectra for all new compounds and complete glycosidase inhibition data. PDB files, and observed structure factor data for complexes **3a** and **7** bound to *Bt*Fuc2970, have been deposited in the PDB with accession codes 2JL1 and 2JL2, respectively. See DOI: 10.1039/b000000x/.

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