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New α -galactosidase-inhibiting aminohydroxycyclopentanes†‡

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A set of cyclopentanoid α -galactosidase ligands was prepared from a partially protected ω -eno-aldose via a reliable (2 + 3)-cycloaddition protocol with slightly modified conditions. The obtained *N*-benzylisoxazolidine ring was selectively opened and the configuration of the hydroxymethyl group was inverted. Consecutive deprotection provided an aminocyclopentane, which was *N*-alkylated to furnish a set of potential α -galactosidase inhibitors. Their glycosidase inhibitory activities were screened with a panel of standard glycosidases of biological significance.

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1. Introduction

Glycoside hydrolases are a class of abundant and essential enzymes for carbohydrate catabolism, controlled trimming of oligosaccharide motifs in glycoproteins, as well as the release of stored polysaccharides in plants and animals. The Carbohydrate-Active enZYmes database^{1,2} (CAZY) currently features well over one hundred and sixty sequence-associated families of glycosidases.

α -D-Galactosides are, in a quite diverse context, an important class of oligosaccharides and carbohydrate conjugates. Their corresponding hydrolases, α -D-galactosidases, are found in glycoside hydrolase (GH) families 4, 27 (containing the Fabry disease related human lysosomal enzyme), 36, 57, 97 and 110 as well as in GH109. GH 4 and GH109 enzymes follow a mechanism requiring NAD⁺ and Mn²⁺ that starts with the oxidation at C-3 followed by β -elimination of the aglycone, with subsequent water addition and reduction,³ while the enzymes of the other five families follow standard Koshland retaining mechanisms.⁴

α -D-Galactosidases have been employed or modulated in several quite diverse applications. For example, α -galactosidases are frequently employed in combination with proteases to remove oligosaccharides from food to improve their nutritional

availability and quality.^{5,6} Improving the activity of lysosomal α -galactosidase through chaperoning is a new approach to the management of Fabry's disease, a hereditary lysosomal disorder resulting from mutations in the GLA gene that result in catalytically compromised mutants of this essential enzyme.^{7–11} In a completely different context, α -galactosidases that selectively and efficiently remove the immunodominant α -1,3-galactosyl epitope (Galili antigen) which hampers xenotransplantation from otherwise suitable mammalian sources, have been a focus of organ and tissue transplant medicine.^{12–14} Similarly, for the purpose of generating red blood cells for readily available universal donor blood, enzymatic conversion of B type red blood cells into type O, by removal of α -1,3-bound galactosides from the cell surface, has been a field of intense and sustained research.^{15–19}

Specific inhibitors of α -galactosidases have been developed as chaperones for treatment of the hereditary lysosomal disorder, Fabry's disease. A prime example is 1,5-dideoxy-1,5-imino-D-galactitol (DGJ, **1**), which enhances the residual activity of various α -galactosidase mutants, ameliorating Fabry related symptoms and providing improved quality of life for afflicted patients. α -Galactosidase inhibitors may also help the discovery, purification and characterization of enzymes for managed degradation of pathologically active α -galactosides.

Amongst competitive α -galactosidase inhibitors, compound **1** is the current bench mark (IC₅₀ 1.6 nM and 0.24 μ M with α -galactosidases from green coffee beans (gcb, GH 27) and *E. coli*, (GH 36) respectively).²⁰ Homolog **2** inhibits gcb galactosidase with IC₅₀ 0.06 μ M and *E. coli* enzyme with IC₅₀ 32 μ M.²¹ Contrasting this, structurally related 4-*epi*-isofagomine **3** exhibits β -galactosidase activity practically exclusively (IC₅₀ 200 μ M, gcb).²² Conversely, *N*-alkyl substituted analogues of the corresponding 5-fluoro derivative, such as **4**, are sub-micromolar (*K_i* 0.50 μ M) inhibitors of gcb α -galactosidase.²³ Pyranoid carbasugar 4-*epi*-

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† Dedicated to Prof. Dr Inge Lundt to the happy occasion of her 80th birthday.

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validamine (**5**) is only a weak inhibitor with IC_{50} 500 μ M (gcb) and 890 μ M (*E. coli*), respectively.²⁴ Interestingly, closely related bicyclic analogue **6** is much more potent with K_i 0.54 μ M and IC_{50} 80 μ M with these enzymes.²⁵

Turning to furanoid structures, 1,4-dideoxy-1,4-imino-D-lyxitol (**7**) was found to be an inhibitor of gcb α -galactosidase (K_i 0.13 μ M).²⁶ Its C-6-homolog, 2,5-dideoxy-2,5-imino-D-altritol (**8**), exhibited an IC_{50} of 0.78 μ M with α -galactosidase from green coffee beans without any noteworthy inhibition of a panel of other glycosidases.²⁷ Other researchers reported an IC_{50} of 5.2 μ M for compound **8** with the same enzyme.²⁸ These authors also noticed that *meso*-2,5-dideoxy-2,5-iminogalactitol (**10**) was an even better inhibitor (IC_{50} 0.19 μ M) of gcb α -galactosidase while *D*-galacto configured aminocyclopentane **12** inhibits it with K_i 0.43 μ M.²⁹ In contrast to that, aminocyclopentane **12** does not show any inhibitory activity against bovine liver α -galactosidase.³⁰ Furthermore, a study by Zheng and co-workers³¹ revealed that non-carbohydrate-based structures such as lansoprazole (IC_{50} 6.4 μ M) may also serve as gcb α -galactosidase inhibitors.

Human lysosomal α -galactosidase (Fabrazyme®, GH 27) is strongly inhibited by compounds **1** (K_i 0.23 μ M, Fabrazyme®),³² **4** (K_i 116 μ M, Fabrazyme®),²³ **8** (IC_{50} 0.69 μ M, human lysosomal),²⁷ and moderately by **11** (K_i 110 μ M, Fabrazyme®).³² Compound **9**, the 1-aminodeoxy derivative of **8**, exhibits IC_{50} 0.053 μ M (pH 7) and IC_{50} 0.67 μ M (pH 4.6) with Fabrazyme®³³ (Fig. 1). These data demonstrate that only very few small glycomimetics known thus far exhibit noteworthy activity as well as selectivity for α -galactosidases.

We have recently been interested in hydroxymethyl-branched di- and trihydroxycyclopentylamines such as compound **12**, which may be regarded as resulting from ring contraction by formal homolytic extraction of the sugar ring oxygen and bond formation between C-1 and C-5. The new carbocyclic scaffold maintains the stereochemical information of the corresponding parent free sugar or glycosylamine albeit with the characteristic conformational features of five-membered rings. There are only four leading references in which such sugar analogues are shown as proven *D*-

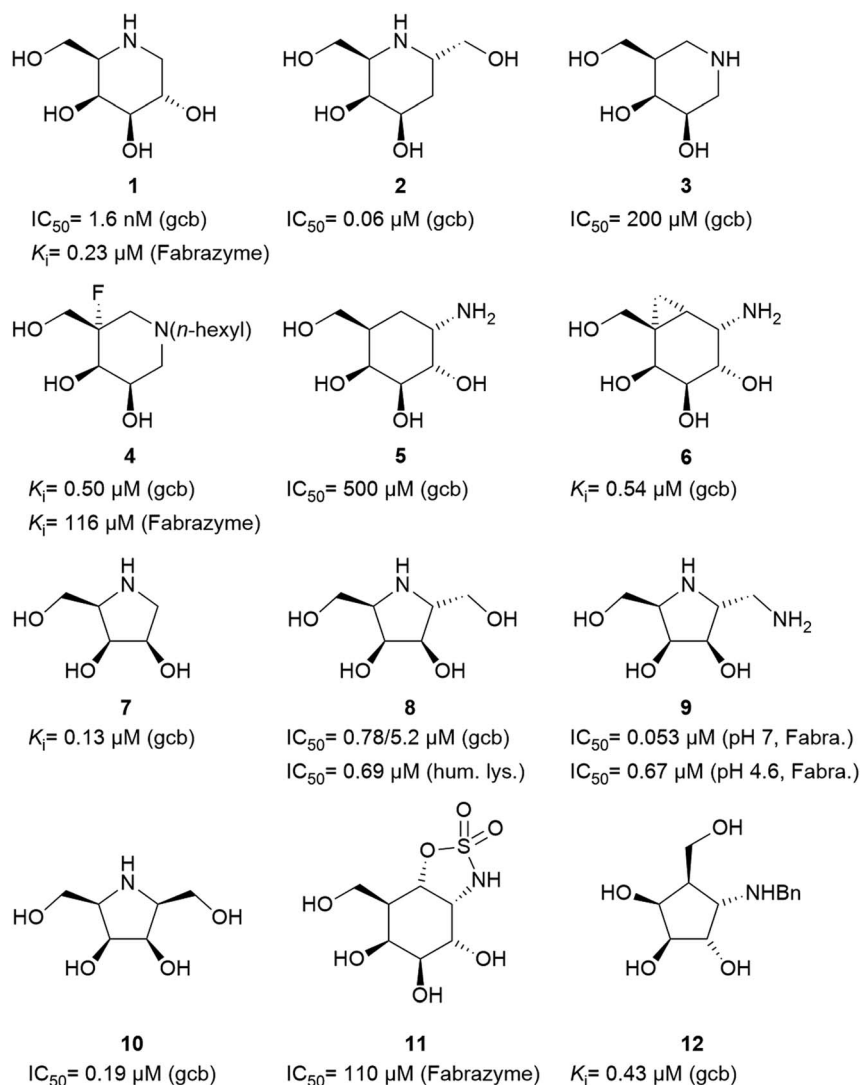
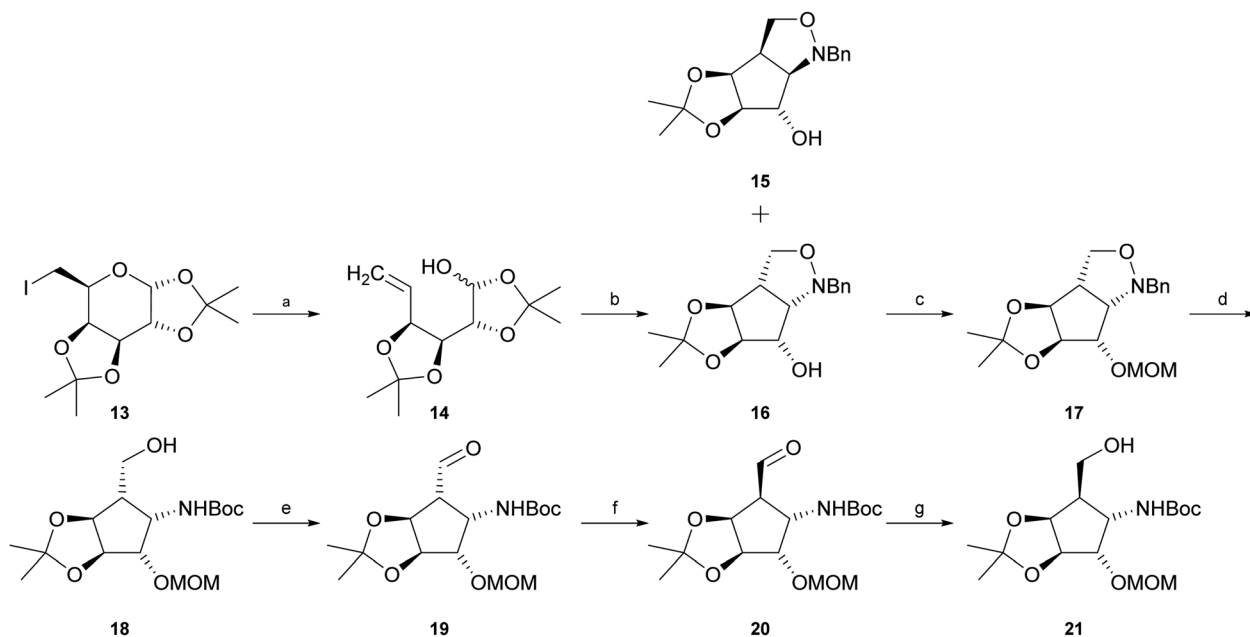


Fig. 1 Carbohydrate-related α -galactosidase inhibitors and inhibitory activities.





Scheme 1 Synthesis of intermediate **21**. (a) Zn, NH₄Cl, MeOH; (b) BnNH₂·HCl, pyridine, CH₂Cl₂, 75% over two steps, 86% by conversion; (c) MOMCl, *i*-Pr₂NEt, CH₂Cl₂, reflux, 91%; (d) H₂, 20% Pd(OH)₂/C, Boc₂O, Na₂CO₃, MeOH, 90%; (e) Dess–Martin periodinane, CH₂Cl₂ or oxalyl chloride, DMSO, *i*-Pr₂NEt, CH₂Cl₂, –60 °C; (f) pyridine or *i*-Pr₂NEt, CH₂Cl₂; (g) DIBAL-H, CH₂Cl₂, 0 °C, 47% over three steps, 62% by conversion.

galactosidase inhibitors.^{29,30,34,35} As already outlined, there is only one *N*-alkylated hydroxymethyl-trihydroxycyclopentylamine **12** reported in literature. Yet, this compound shows sufficient inhibitory activity against α -galactosidase (0.43 μ M, gbc) but also inhibitory activity against β -galactosidase (1.5 μ M, bovine liver) as well as β -glucosidase (0.51 μ M, almonds). Aim of this work, is the synthesis of new aminocyclopentanes, as powerful and selective GH27 α -galactosidase inhibitors. These structures were prepared, relying on Vasella's (2 + 3)-cycloaddition approach.³⁶

2. Results and discussion

2.1. Synthesis

Intermediate **21** was synthesised starting with reductive elimination of known iodide **13** (ref. 37) with zinc under slightly acidic conditions to give hemiacetal **14**. Its treatment with *N*-benzylhydroxylamine in CH₂Cl₂, provided a mixture of *syn*-product **15** and the desired known^{38,39} *anti*-configured *N*-benzylisoxazolidine **16** (experimental details as well as NMR-data had not been reported for this compound) in a ratio of about 1 : 7 (**15** : **16**). The stereochemical outcome of this cyclisation is highly dependent on the solvent. Jäger and co-workers³⁹ pointed out that if the reaction is carried out in a polar solvent such as methanol, the main product is compound **15**. In contrast, usage of an unpolar solvent such as CH₂Cl₂ or CHCl₃ mainly provides compound **16**. Separation of compounds **15** and **16** was easily achieved by silica gel chromatography. Protection of the free hydroxyl function in compound **16** with chloromethyl methyl ether (MOMCl) in the presence of Hünig's base (*i*-Pr₂NEt) yielded fully protected compound **17**. "One-pot" hydrogenolytic removal of the *N*-benzyl group over Pd(OH)₂/C (20%) in the

presence of Boc₂O (di-*tert*-butyl-dicarbonate) and Na₂CO₃ resulted in *N*-Boc-protected primary alcohol **18**. Subsequent Swern or Dess–Martin oxidation furnished the corresponding aldehyde **19**. Treatment of aldehyde **19** with pyridine or *i*-Pr₂NEt in methanol allowed, *via* enolization, almost complete isomerisation to epimer **20**. This reaction could easily be followed by thin-layer chromatography. The epimerization reaction was then quenched by addition of NaBH₄ at 0 °C to obtain desired primary alcohol **21**. Unexpectedly, the conditions of this attempted *in situ* reduction reaction favoured re-isomerization at C-5, now exclusively – *via* intermediate **19** – providing

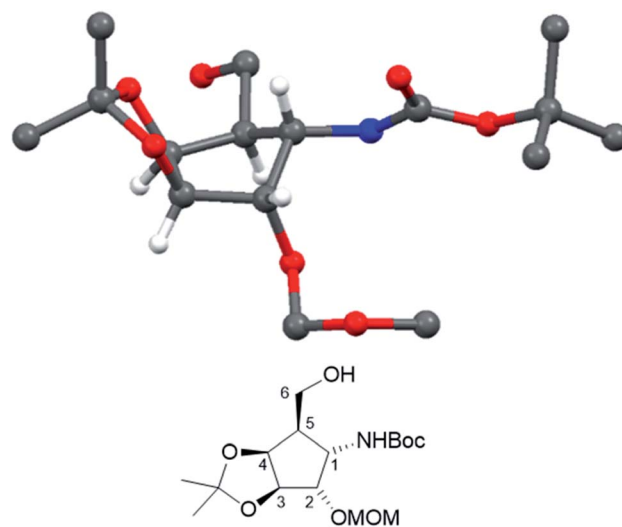
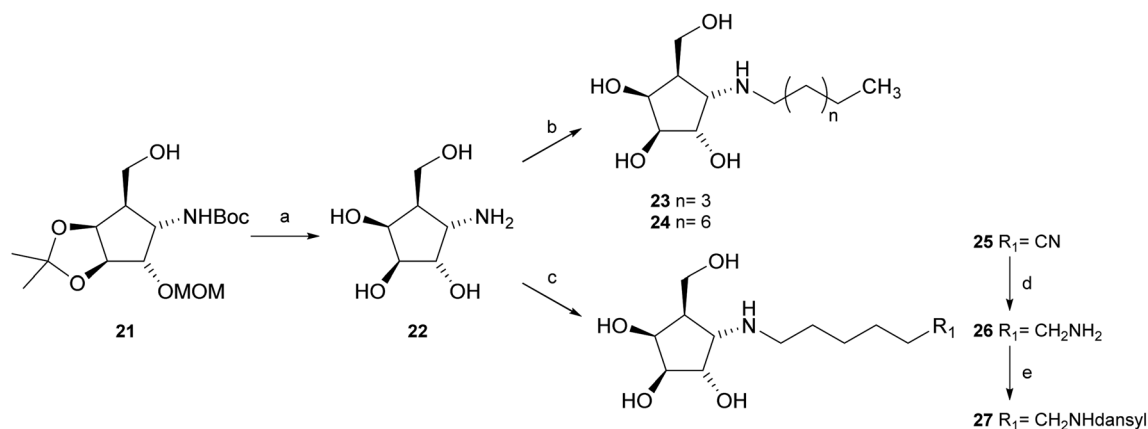


Fig. 2 Crystal structure of compound **21**. (CCDC: 2065318, for the sake of consistency, numbering follows the monosaccharide pattern starting with the amino substituted carbon (C-1) clockwise along the ring.)





Scheme 2 Synthesis of inhibitors **23**, **24** and **27**. (a) Trifluoroacetic acid, THF–MeOH–H₂O (3 : 1 : 1), 60 °C, 75%; (b) R–CHO, AcOH, H₂, 20% Pd(OH)₂/C, MeOH, 57% (**23**), 64% (**24**); (c) Br(CH₂)₅CN, NaHCO₃, DMF, 60 °C; (d) H₂, RANEY®–Ni, MeOH, 49%, two steps; (e) dansyl chloride, Na₂CO₃, MeOH, 55%.

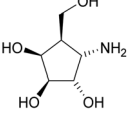
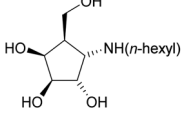
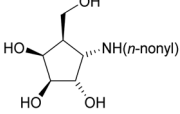
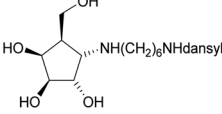
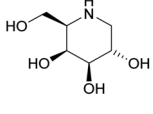
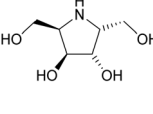
starting alcohol **18**, possibly influenced by unfavourable boronate complexation of aldehyde **19** or by easier access of the reagent *trans* to the isopropylidene group and consequently higher reactivity of the “wrong” aldehyde epimer, **19**, in the equilibrium. Improvement of experimental conditions by isomerisation of aldehyde **19** in CH₂Cl₂ in the presence of *i*-Pr₂NEt to obtain desired epimer **20**, and consecutive *in situ* reduction with diisobutylaluminium hydride (DIBAL–H) in CH₂Cl₂ resulted in a mixture of desired alcohol **21** and intermediate **18** in a ratio of about 2 : 1 (**21** : **18**). The diastereomers were separated chromatographically to obtain pure **21** (47%) and recovered starting material **18** (25%) (Scheme 1).

Crystallization from cyclohexane/ethyl acetate afforded crystals of sufficient quality to verify the structural identity of

diastereomer **21** by X-ray structure determination (Fig. 2, CCDC 2065318).

Deprotection of alcohol **21** with trifluoroacetic acid in THF–MeOH–H₂O (3 : 1 : 1) resulted in known³⁰ aminotetraol **22**. Reductive amination with the respective aldehyde in the presence of Pd/C under an atmosphere of H₂ gave chain extended inhibitors **23** and **24**, respectively. Alkylation of amine **22**, by treatment with ω-bromo hexanoic nitrile furnished compound **25**, which was subsequently reduced in the presence of RANEY®–Ni under an atmosphere of H₂ to obtain primary amine **26**. Its conventional *N*-dansylation yielded inhibitor **27** (Scheme 2).

Table 1 *K_i*-values [μM] of compounds **1**, **8**, **22**–**24**, **27**^a

Enzyme (GH family)	Compounds					
						
	22	23	24	27	1	8
Abg (β-Glc/β-Gal) (GH 1)	0.014 ± 0.003	0.013 ± 0.003	0.0037 ± 0.0016	0.005 ± 0.001	12 (ref. 40)	n.d.
<i>E. coli</i> (β-Gal) (GH 2)	200 ± 90	120 ± 20	23 ± 7	7.8 ± 4.6	13 (ref. 40)	n.d.
Bovine liver (β-Gal) (GH 35)	1.4 ± 1.0 (3.3 (ref. 30))	0.093 ± 0.054	0.19 ± 0.07	0.18 ± 0.08	n.i. ⁴¹	IC ₅₀ : 190 (ref. 27)
Fabrazyme® (α-Gal) (GH 27)	9.5 ± 1.9	4.9 ± 0.9	0.52 ± 0.21	4.4 ± 1.1	0.23 (ref. 32)	IC ₅₀ : 0.69 (ref. 27) (h. lys.)
gcb (α-Gal) (GH 27)	6.7 ± 0.9 (12 (ref. 30))	0.19 ± 0.05	0.25 ± 0.05	0.14 ± 0.04	0.0016 (ref. 40)	IC ₅₀ : 0.78 (ref. 27)
<i>S. cer.</i> (α-Glc) (GH 13)	160 ± 60	130 ± 40	73 ± 17	16 ± 4	n.i. ⁴¹ (yeast)	n.d.
GCase (β-Glc) (GH 30)	18 ± 3	40 ± 8	1.2 ± 0.5	0.63 ± 0.09	n.d.	n.d.

^a Abg = β-glucosidase/β-galactosidase from *Agrobacterium* sp.; *E. coli* = lac Z β-galactosidase from *E. coli*; bovine liver = β-galactosidase from bovine liver; Fabrazyme® = commercial recombinant lysosomal α-galactosidase; gcb = α-galactosidase from green coffee beans *S. cer.* = α-glucosidase from *S. cerevisiae*; GCase = recombinant human lysosomal β-glucocerebrosidase. h. lys. = human lysosomal α-galactosidase. n.d. = not determined, n.i. = no inhibition, with *K_i* values estimated to be >1 mM.



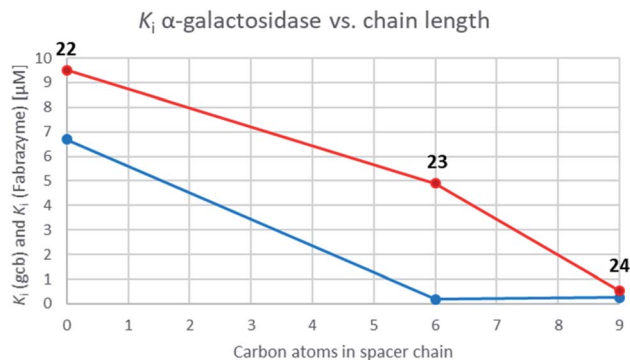


Fig. 3 Graphs correlating inhibition constants of gcb α -galactosidase (blue) as well as Fabrazyme® (red) vs. spacer chain length.

2.2. Inhibitory activities

Amines **22–24** and **27** were screened for inhibitory activities with a panel of standard glycosidases (Table 1). As can be seen the compounds act as inhibitors of both α - and β -galactosidases, though overall they perform better on α -galactosidases with inhibition constants down to the high nanomolar/low micromolar range, as discussed in more detail below.

While the parent molecule with a free amine proved to be a modest inhibitor with inhibition constants in the low to mid micromolar range, alkylation of this nitrogen improved inhibition in each case (with one exception in the case of GCCase). Further extension of the alkyl chain generally improved inhibition yet more indeed for the β -glycosidases, all of which belong to the same, structurally related clan GHA, largely parallel increases in inhibition were seen with each addition of carbon atoms. This is consistent with common interactions with closely related structures.^{42,43} For the α -galactosidases, the alkylation also generally improved inhibition, but not as predictably as with the β -galactosidases, despite the fact that both, Fabrazyme® and gcb, belong to the same sequence-related family, GH27 (Fig. 3).

Optimal affinity for the human enzyme, Fabrazyme®, was obtained with the *n*-nonyl substituted derivative **24**, with a K_i value comparable to that of galacto-isofagomine. This is the compound in current clinical use under the name Migalastat® or Galafold. The presence of the *n*-nonyl chain in **24** would likely improve its pharmacokinetic profile by keeping the compound in circulation longer, making this a candidate for testing as a chaperone. However, the fact that this compound is also a good inhibitor of other glycosidases tested here, means that its selectivity needs to be further improved.

3. Conclusions

Following a simple protocol previously outlined by Vasella's and Jäger's groups,^{35,36} a series of novel derivatives of **12**, bearing chain extensions at the nitrogen at position C-1 have been synthesized. Inhibition constants for these compounds with a range of β -galactosidases, including human lysosomal α -galactosidase, were then measured. The results of these inhibitory studies revealed that free amine **22** is the worst inhibitor within this series and the

activity increases with the introduction of an alkyl chain as seen with compounds **23**, **24** as well as **27**.

Furthermore, it is both unexpected and interesting that the α -configured compounds developed here are good inhibitors of the β -glycosidases in the first place, since these have the wrong anomeric configuration for these enzymes. Further work will elucidate the reason for this unexpected finding.

4. Experimental

4.1. General methods

Optical rotations were measured at 20 °C on a Perkin Elmer 341 polarimeter at a wavelength of 589 nm and a path length of 10 cm. $[\alpha]_D^{20}$ values are given in 10⁻¹ degree cm² per g. NMR spectra were recorded on a Bruker Ultrashield spectrometer at 300.36 (¹H) and 75.53 MHz (¹³C). CDCl₃ was employed for protected compounds and CD₃OD or D₂O for unprotected inhibitors. Carbon and hydrogen numbering in NMR spectra was conducted in analogy to carbohydrate nomenclature and clockwise, starting with the amino bearing carbon as C-1 (Fig. 2). Chemical shifts are listed in delta employing residual, non-deuterated solvent as the internal standard. Signals were assigned unambiguously by COSY, HSQC as well as APT analysis. Coupling constants '*J*' for protecting groups, alkyl chains as well as the dansyl group (spacer arms) were found in the expected range and are not listed. For intermediate **21**, the structure was confirmed by XRD structural analysis: suitable single crystals of compounds were immersed in silicone oil, mounted using a glass fiber and frozen in the cold nitrogen stream (100 K). X-Ray diffraction data were collected at low temperature on a Bruker Kappa APEX II diffractometer using Mo K α radiation ($\lambda = 0.71073$ Å) generated by an INCOATEC micro-focus source. The data reduction and absorption correction was performed with the Bruker SMART and Bruker SADABS, respectively. The structures were solved with SHELXT⁴⁴ by direct methods and refined with SHELXL⁴⁵ by least-square minimization against F^2 using first isotropic and later anisotropic thermal parameters for all non-hydrogen atoms. Hydrogen atoms were added to the structure models on calculated positions using the riding model. The space group assignments and structural solutions were evaluated using PLATON.^{46,47} MALDI-TOF Mass Spectrometry was performed on a Micromass TofSpec 2E Time-of-Flight Mass Spectrometer. Analytical thin-layer chromatography (TLC) was performed on precoated aluminium plates silica gel 60 F254 (E. Merck 5554) and detected with UV light (254 nm). For staining, a solution of vanillin (9 g) in a mixture of H₂O (950 mL)/ethanol (750 mL)/H₂SO₄ (120 mL) or ceric ammonium molybdate (100 g ammonium molybdate/8 g ceric sulfate in 10% H₂SO₄ (1 L)) were employed followed by heating on a hotplate. For column chromatography, silica gel 60 (230–400 mesh, E. Merck 9385) or silica gel 60 (Acros Organics, AC 24036) were used. Reaction monitoring was done by TLC.

4.2. Kinetic studies

Kinetic studies were performed at room temperature in an appropriate buffer for each enzyme (specific conditions can be found below). All reactions were performed in half-area 96-well-



plates (Corning) and monitored with a Synergy H1 plate reader (BioTek). In each experiment, the appropriate concentration of the enzyme was incubated with different concentrations of the inhibitors for 2–5 minutes before initiating the reaction by the addition of the glycoside substrate. The initial rate was then measured by monitoring the increase in, either absorbance at 405 nm (where the aglycone is either *p*-nitrophenol (pNP) or 2,4-dinitrophenol (DNP)) or fluorescence at 365/455 nm (where the aglycone is 4-methylumbelliferone (MU)) for up to five minutes. K_i determinations were performed using two different substrate concentrations. For each substrate concentration, a range of three to six inhibitor concentrations was used. Dixon plots ($1/v$ vs. $[I]$) were constructed to validate the use of competitive inhibition model and to assess the fit of the data. The data were then fit to a competitive inhibition model using non-linear regression analysis with Grafit 7.0.0. (Erithacus Software).

Specific assay conditions for each enzyme:

Agrobacterium sp. β -glucosidase:^{48,49} 50 mM sodium phosphate buffer (pH 7) substrate: Gal- β -pNP ($K_m = 4.1$ mM).

E. coli lac z β -galactosidase: 50 mM sodium phosphate containing 1.0 mM MgCl₂ (pH 7) substrate: Gal- β -pNP ($K_m = 60$ μ M).

Bovine liver β -galactosidase: 50 mM sodium phosphate buffer (pH 7) substrate: Gal- β -pNP ($K_m = 0.65$ mM).

Green coffee bean α -galactosidase: 50 mM sodium phosphate buffer (pH 7) substrate: Gal- α -MU, $K_m = 250$ μ M.

Fabrazyme® (acid α -galactosidase): 20 mM sodium citrate, 50 mM sodium phosphate, 1.0 mM tetrasodium EDTA, 0.25% v/v Triton X-100® and 0.25% w/v taurocholic acid buffer (pH 5.5) substrate Gal- α -dNP ($K_m = 0.65$ mM).

S. cerevisiae α -glucosidase: 50 mM sodium phosphate buffer (pH 7). Substrate: Glc- α -pNP, $K_m = 0.75$ mM.

GCase (β -glucocerebrosidase): 20 mM sodium citrate, 50 mM sodium phosphate, 1.0 mM tetrasodium EDTA, 0.25% v/v Triton X-100® and 0.25% w/v taurocholic acid buffer (pH 7) substrate: Glc- β -dNP ($K_m = 2.7$ mM).

(3aS,3bS,6aS,7S,7aS)-1-Benzyl-5,5-dimethylhexahydro-1H-[1,3]dioxolo[4',5':3,4]cyclopenta[1,2-c]isoxazol-7-ol (16). To a solution of known iodose sugar³⁷ **13** (5.38 g, 14.5 mmol) in methanol (100 mL), zinc (11.4 g, 174 mmol) and NH₄Cl (2.33 g, 43.6 mmol) were added. After completed conversion (15 min, cyclohexane–EtOAc 3 : 1) the solids were removed by filtration and the filtrate was concentrated under reduced pressure. The residue containing crude **14** was taken up in CH₂Cl₂ (100 mL) and this suspension was treated with pyridine (3.45 mL, 43.6 mmol) and *N*-benzylhydroxylamine hydrochloride (2.78 g, 17.4 mmol). After consumption of the starting material (TLC, 3 h, cyclohexane–EtOAc 2 : 1) the suspension was consecutively washed with saturated aqueous NaHCO₃ solution and brine. The combined organic layers were dried (Na₂SO₄) and the solvents were removed under reduced pressure to obtain a yellow syrup. Purification on silica gel (cyclohexane–EtOAc 6 : 1 to 1 : 1) provided desired *anti*-configured *N*-benzylisoxazolidine **16** (3.19 g, 10.9 mmol, 75% over two steps, 86% by conversion, $R_f = 0.6$). The melting point as well as the optical rotation $[\alpha]_D^{20}$ were in accordance with reported³⁸ data. ¹H-NMR (300 MHz, CDCl₃) $\delta = 7.40$ – 7.24 (m, 5H, aromatics), 4.51 (bs,

2H, $J_{2,3} < 1$ Hz, $J_{3,4} < 1$ Hz, $J_{4,5} < 1$ Hz, H-3, H-4), 4.24 (dd, 1H, $J_{5,6a} = J_{6a,6b}$ 8.6 Hz, H-6a), 4.14 (d, 1H, N–CH₂–Ph), 3.98 (dd, 1H, $J_{1,2}$ 5.9 Hz, H-2), 3.94 (dd, 1H, $J_{1,5}$ 6.0 Hz, H-1), 3.86 (d, 1H, N–CH₂–Ph), 3.68 (dd, 1H, $J_{5,6b}$ 5.3 Hz, H-6b), 3.66 (bs, 1H, OH), 3.22 (dddd, 1H, H-5), 1.45, 1.31 (2 s, 3H each, C(CH₃)₂). ¹³C-NMR (75.5 MHz, CDCl₃): $\delta = 136.6$ (*ipso*), 129.1, 128.8, 128.0 (aromatics), 111.2 (C(CH₃)₂), 87.5, 84.0 (C-3, C-4), 75.6 (C-2), 71.3 (C-1), 70.2 (C-6), 61.4 (N–CH₂–Ph), 54.1 (C-5), 27.6, 25.2 (C(CH₃)₂). MS (MALDI): calculated for [C₁₆H₂₁NO₄H]: m/z 292.1549 [M + H]⁺; found [M + H]⁺ 292.1547.

The already reported³⁵ corresponding *syn*-configured *N*-benzylisoxazolidine **15** (438 mg, 1.50 mmol, 10.3%) was obtained as colourless solid.

((3aS,3bS,6aS,7S,7aS)-1-Benzyl-7-(methoxymethoxy)-5,5-dimethylhexahydro-1H-[1,3]dioxolo[4',5':3,4]cyclopenta[1,2-c]isoxazole (17). A solution of isoxazolidine **16** (3.79 g, 13.0 mmol) in CH₂Cl₂ (50 mL) was treated with *i*-Pr₂NEt (11.1 mL, 65.0 mmol) and MOMCl (2.47 mL, 32.5 mmol) and the solution was stirred under reflux. After consumption of the starting material (24 h, cyclohexane–EtOAc 2 : 1), methanol (20 mL) was added and the solution was stirred for 20 min. The reaction mixture was allowed to reach ambient temperature and was consecutively washed with HCl (2 M) and saturated aqueous NaHCO₃. The combined organic layers were dried (Na₂SO₄), filtered and concentrated under reduced pressure. The residue was purified on silica gel (cyclohexane–EtOAc 5 : 1) to provide compound **17** as a colourless oil (3.96 g, 11.8 mmol, 91%). $[\alpha]_D^{20} = -15.7$ ($c = 1.1$, CHCl₃); ¹H-NMR (300 MHz, CDCl₃) $\delta = 7.46$ – 7.24 (m, 5H, aromatics), 4.78 (dd, 1H, $J_{2,3}$ 5.1 Hz, $J_{3,4} < 1$ Hz, H-3), 4.71 (dd, 2H, O–CH₂–O–CH₃), 4.46 (dd, 1H, $J_{4,5}$ 5.9 Hz, H-4), 4.25 (dd, 1H, $J_{1,2}$ 5.5 Hz, H-2), 4.21 (dd, 1H, $J_{5,6a} = J_{6a,6b}$ 8.6 Hz, H-6a), 4.05 (d, 1H, N–CH₂–Ph), 3.73 (dd, 1H, $J_{5,6b}$ 4.5 Hz, H-6b), 3.58 (dd, 1H, $J_{1,5}$ 7.0 Hz, H-1), 3.40 (s, 3H, O–CH₂–O–CH₃), 3.16 (dddd, 1H, H-5), 1.53, 1.34 (2s, 3H each, C(CH₃)₂). ¹³C-NMR (75.5 MHz, CDCl₃): $\delta = 137.7$ (*ipso*), 129.2, 128.4, 127.4 (aromatics), 112.1 (C(CH₃)₂), 95.7 (O–CH₂–O–CH₃), 84.8 (C-3), 83.7 (C-4), 82.0 (C-2), 71.0 (C-1), 70.3 (C-6), 62.1 (N–CH₂–Ph), 55.6 (O–CH₂–O–CH₃), 50.8 (C-5), 27.9, 25.6 (C(CH₃)₂). MS (MALDI): calculated for [C₁₆H₂₁NO₄H]: m/z 336.1811 [M + H]⁺; found [M + H]⁺ 336.1818.

tert-Butyl ((3aS,4S,5S,6S,6aS)-4-(hydroxymethyl)-6-(methoxymethoxy)-2,2-dimethyltetrahydro-4H-cyclopenta[*d*][1,3]dioxol-5-yl)carbamate or 1-(tert-butylloxycarbonyl)amino-3,4-O-isopropylidene-2-O-methoxymethyl- β -1-*altro*-cyclopentane (18). Isoxazolidine **17** (3.96 g, 11.8 mmol) was dissolved in methanol (50 mL) and was subsequently treated with Na₂CO₃ (2.50 g, 23.6 mmol), Boc₂O (3.87 g, 17.7 mmol) and 20% Pd(OH)₂/C (0.8 g). The suspension was stirred under H₂ atmosphere at ambient pressure. After completed conversion (8 h, cyclohexane–EtOAc 1 : 1) the solids were removed by filtration. The solvent was removed under reduced pressure and the residue was chromatographed (cyclohexane–EtOAc 3 : 1) to furnish alcohol **18** (3.71 g, 10.7 mmol, 90%) as a colourless oil. $[\alpha]_D^{20} = -2.0$ ($c = 0.87$, CHCl₃); ¹H-NMR (300 MHz, CDCl₃) $\delta = 5.02$ (dd, 1H, $J_{1,NH}$ 7.1 Hz, NH), 4.73 (dd, 2H, O–CH₂–O–CH₃), 4.59 (dd, 1H, $J_{3,4} < 1$ Hz, $J_{4,5}$ 6.9 Hz, H-4), 4.46 (dd, 1H, $J_{2,3}$ 5.8 Hz, H-3), 4.42 (dd, 1H, $J_{1,2} < 1$ Hz, $J_{1,5}$ 7.0 Hz, H-1), 4.05 (dd, 1H, H-2),



3.72–3.59 (m, 2H, H-6), 3.39 (s, 3H, O-CH₂-O-CH₃), 3.09 (bs, 1H, OH), 2.53 (m, 1H, H-5), 1.44 (s, 12H, C(CH₃)₂, NH-CO-O-C(CH₃)₃), 1.27 (s, 3H, C(CH₃)₂). ¹³C-NMR (75.5 MHz, CDCl₃): δ = 156.1 (NH-CO-O-(C(CH₃)₃)), 111.1 (C(CH₃)₂), 96.7 (O-CH₂-O-CH₃), 82.8 (C-3), 82.3 (C-2), 80.6 (C-4), 80.1 (NH-CO-O-(C(CH₃)₃)), 59.9 (C-6), 56.4 (O-CH₂-O-CH₃), 52.9 (C-1), 49.4 (C-5), 28.5 (NH-CO-O-(C(CH₃)₃)), 26.6, 24.1 (C(CH₃)₂). MS (MALDI): calculated for [C₁₆H₂₉NO₇H]: *m/z* 348.2022 [M + H]⁺; found [M + H]⁺ 348.2021.

tert-Butyl ((3aS,4R,5S,6S,6aS)-4-(hydroxymethyl)-6-(methoxymethoxy)-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]dioxol-5-yl)carbamate or 1-(tert-butyloxycarbonyl)amino-3,4-O-isopropylidene-2-O-methoxymethyl-α-D-galacto-cyclopentane (21). A solution of oxalyl chloride (1.04 mL, 12.1 mmol) in CH₂Cl₂ (40 mL) was treated with DMSO (1.03 mL, 14.5 mmol) at -60 °C. After 15 min, a solution of alcohol **18** (1.68 g, 4.84 mmol) in CH₂Cl₂ (5 mL) was added and the reaction was stirred for 15 min when *i*-Pr₂NEt (4.93 mL, 29.0 mmol) was added. After completed oxidation (30 min, cyclohexane-EtOAc 1 : 1), the reaction mixture was consecutively washed with HCl (2 M) and saturated aqueous NaHCO₃ solution. The collected organic layers were combined, dried (Na₂SO₄), filtered and concentrated under reduced pressure. The remaining syrup was quickly passed through a pad of silica gel (CH₂Cl₂ to EtOAc) and the solvents were removed under reduced pressure. The mixture of desired aldehyde **20** (cyclohexane-EtOAc 1 : 1, *R_f* = 0.6) and precursor **19** (cyclohexane-EtOAc 1 : 1, *R_f* = 0.7) was dissolved in CH₂Cl₂ (20 mL) and treated with *i*-Pr₂NEt (1.64 mL, 9.67 mmol) at 0 °C. The reaction mixture was stirred until only the desired diastereomer **20** was observed (1 h, cyclohexane-EtOAc 1 : 1). Then DIBAL-H (9.67 mL, 1 M in CH₂Cl₂) was dropwise added to the solution. After completed reduction (30 min, cyclohexane-EtOAc 1 : 1), 20 mL of saturated aqueous potassium sodium tartrate solution were added. The mixture was vigorously stirred overnight and was consecutively washed with HCl (2 M) and saturated aqueous NaHCO₃ solution, the combined organic layers were dried (Na₂SO₄), filtered and concentrated under reduced pressure. The residue was purified on silica gel (cyclohexane-EtOAc 4 : 1) to give alcohol **21** (785 mg, 2.26 mmol, *R_f* = 0.32, 47%, 62% by conversion) as a colourless oil. Epimer **18** (418 mg, 1.20 mmol, *R_f* = 0.40, 25%) was recovered. Crystallisation from EtOAc-cyclohexane gave colourless crystals of **21** with sufficient quality for XRD. Mp = 113–115 °C; [α]_D²⁰: -56.0 (*c* = 0.77, CHCl₃); ¹H-NMR (300 MHz, CDCl₃) δ = 5.02 (bs, 1H, NH), 4.71 (dd, 1H, *J*_{3,4} = *J*_{4,5} 6.0 Hz, H-4), 4.70 (dd, 2H, O-CH₂-O-CH₃), 4.46 (dd, 1H, *J*_{2,3} < 1 Hz, H-3), 4.19–4.04 (m, 1H, H-1), 3.95 (dd, 1H, *J*_{1,2} 4.7 Hz, H-2), 3.91–3.80 (m, 2H, H-6), 3.38 (s, 3H, O-CH₂-O-CH₃), 2.33 (bs, 1H, OH), 2.12–2.01 (m, 1H, H-5), 1.44 (s, 12H, C(CH₃)₂, NH-CO-O-C(CH₃)₃), 1.28 (s, 3H, C(CH₃)₂). ¹³C-NMR (75.5 MHz, CDCl₃): δ = 156.1 (NH-CO-O-(C(CH₃)₃)), 110.8 (C(CH₃)₂), 96.5 (O-CH₂-O-CH₃), 81.3, 81.2 (C-2, C-3), 79.9 (NH-CO-O-(C(CH₃)₃)), 79.0 (C-4), 60.6 (C-6), 56.0 (O-CH₂-O-CH₃), 52.4 (C-2), 48.2 (C-5), 28.5 (NH-CO-O-(C(CH₃)₃)), 26.0, 23.9 (C(CH₃)₂). MS (MALDI): calculated for [C₁₆H₂₁NO₄H]: *m/z* 348.2022 [M + H]⁺; found [M + H]⁺ 348.2022.

(1S,2S,3S,4S,5R)-4-Amino-5-(hydroxymethyl)cyclopentane-1,2,3-triol or 1-amino-α-D-galacto-cyclopentane (22). A solution of protected compound **21** (692 mg, 1.99 mmol) was dissolved in THF-MeOH-H₂O (3 : 1 : 1, 10 mL) and treated with 5 mL trifluoroacetic acid. The solution was heated to 60 °C until completed deprotection was observed (12 h, CHCl₃-MeOH-NH₄OH (25%) 4 : 4 : 1). The solvents were removed under reduced pressure. The remaining syrup was co-evaporated with toluene (3 times) and was purified by silica gel chromatography (CHCl₃-MeOH-NH₄OH (25%) 8 : 4 : 1) to provide polyol **22** (245 mg, 1.50 mmol, 75%) as colourless solid. The corresponding hydrochloride was obtained by addition of conc. HCl to a methanolic solution of amine **22** (10 mg) adjusting the pH value to 1. Removal of the solvents under reduced pressure yielded known³⁰ **22·HCl**. The optical rotation was in accordance to the already reported.³⁰ free base: ¹H-NMR (300 MHz, D₂O) δ = 4.15 (dd, 1H, *J*_{3,4} = *J*_{4,5} 4.6 Hz, H-4), 3.99 (dd, 1H, *J*_{1,2} = *J*_{2,3} 7.4 Hz, H-2), 3.91 (dd, 1H, H-3), 3.83–3.70 (m, 2H, H-6), 3.05 (dd, 1H, *J*_{1,5} 8.1 Hz, H-1), 2.00–1.89 (m, 1H, H-5). ¹³C NMR (75.5 MHz, D₂O) δ = 78.0 (C-3), 74.8 (C-2), 70.1 (C-4), 59.9 (C-6), 51.4 (C-1), 49.4 (C-5). Hydrochloride: ¹H-NMR (300 MHz, D₂O) δ = 4.28 (dd, 1H, *J*_{1,2} = *J*_{2,3} 7.8 Hz, H-2), 4.23 (dd, 1H, *J*_{3,4} = *J*_{4,5} 4.7 Hz, H-4), 4.02 (dd, 1H, H-3), 3.87 (dd, 1H, *J*_{5,6a} 7.3 Hz, *J*_{6a,6b} 11.0 Hz, H-6a), 3.77 (dd, 1H, *J*_{5,6b} 7.1 Hz, H-6b), 3.59 (dd, 1H, *J*_{1,5} 8.3 Hz, H-1), 2.34 (dddd, 1H, H-5). ¹³C NMR (75.5 MHz, D₂O) δ = 77.9 (C-3), 72.3 (C-2), 70.1 (C-4), 59.5 (C-6), 51.9 (C-1), 45.9 (C-5). MS (MALDI): calculated for [C₆H₁₃NO₄H]: *m/z* 164.0923 [M + H]⁺; found [M + H]⁺ 164.0922.

(1S,2S,3S,4S,5R)-4-(*n*-Hexylamino)-5-(hydroxymethyl)cyclopentane-1,2,3-triol or 1-(*n*-hexyl)amino-α-D-galacto-cyclopentane (23). A solution of amine **22** (24.5 mg, 0.150 mmol) in methanol (2 mL) was stirred in the presence of AcOH (20 μL), *n*-hexanal (27.7 μL, 0.225 mmol) and 20% Pd(OH)₂/C (50 mg) under an atmosphere of H₂ at ambient pressure. The suspension was stirred until complete consumption of the starting material was overserved (24 h, CHCl₃-MeOH 3 : 1 + 1 vol% NH₄OH (25%)). The catalyst was filtered off and the solvents were removed under reduced pressure. The residue was quickly passed through a pad of silica gel (CHCl₃-MeOH 8 : 1 + 1 vol% NH₄OH (25%)) to obtain inhibitor **23** (21.3 mg, 86.1 μmol, 57%) as a colourless solid. [α]_D²⁰: -23.5 (*c* = 0.82, MeOH); ¹H-NMR (300 MHz, CD₃OD) δ = 4.25 (dd, 1H, *J*_{3,4} 4.4 Hz, *J*_{4,5} 6.8 Hz, H-4), 4.05 (dd, 1H, *J*_{1,2} 6.5 Hz, *J*_{2,3} 4.1 Hz, H-2), 3.88 (dd, 1H, H-3), 3.79 (ddd, 2H, *J*_{5,6a} = *J*_{5,6b} 5.4 Hz, *J*_{6a,6b} 12.2 Hz, H-6), 3.30 (dd, 1H, *J*_{1,5} 7.5 Hz, H-1), 2.82 (t, 2H, H-1'), 2.16–2.04 (m, 1H, H-5), 1.65–1.26 (m, 8H, H-2', H-3', H-4', H-5'), 0.91 (t, 3H, H-6'). ¹³C NMR (75.5 MHz, CD₃OD) δ = 79.7 (C-3), 74.1 (C-2), 72.2 (C-4), 61.8 (C-6), 61.7 (C-1), 48.7 (C-1'), 47.8 (C-5), 32.7, 29.1, 27.7, 23.6 (C-2', C-3', C-4', C-5'), 48.7 (C-6'). MS (MALDI): calculated for [C₁₂H₂₅NO₄H]: *m/z* 248.1862 [M + H]⁺; found [M + H]⁺ 248.1862.

(1S,2S,3S,4S,5R)-4-(*n*-Nonylamino)-5-(hydroxymethyl)cyclopentane-1,2,3-triol or 1-(*n*-nonyl)amino-α-D-galacto-cyclopentane (24). A methanolic solution (2 mL) of amine **22** (23.8 mg, 0.146 mmol) was treated with AcOH (20 μL) and *n*-nonanal (37.6 μL, 0.219 mmol) and stirred in the presence of



20% Pd(OH)₂/C (50 mg) under an atmosphere of H₂ at ambient pressure. After completed reaction (24 h, CHCl₃-MeOH 3 : 1 + 1 vol% NH₄OH (25%)), the catalyst was removed by filtration and the filtrate was concentrated under reduced pressure. Silica gel filtration (CHCl₃-MeOH 8 : 1 + 1 vol% NH₄OH (25%)) of the residue provided inhibitor **24** (26.8 mg, 92.6 μmol, 64%) as a colourless oil. [α]_D²⁰: -22.7 (c = 1.1, MeOH); ¹H-NMR (300 MHz, CD₃OD) δ = 4.22 (dd, 1H, J_{3,4} 4.8 Hz, J_{4,5} 6.2 Hz, H-4), 4.00 (dd, 1H, J_{1,2} 6.2 Hz, J_{2,3} 4.4 Hz, H-2), 3.86 (dd, 1H, H-3), 3.84-3.72 (m, 2H, H-6), 3.12 (dd, 1H, J_{1,5} 7.6 Hz, H-1), 2.68 (t, 2H, H-1'), 2.05-1.91 (m, 1H, H-5), 1.62-1.19 (m, 14H, H-2', H-3', H-4', H-5', H-6', H-7', H-8'), 0.90 (t, 3H, H-9'). ¹³C NMR (75.5 MHz, CD₃OD) δ = 79.8 (C-3), 74.6 (C-2), 72.2 (C-4), 62.1 (C-6), 61.6 (C-1), 49.2 (C-1'), 48.7 (C-5), 33.0, 30.6, 30.6, 30.4, 30.2, 28.3, 23.7 (C-2', C-3', C-4', C-5', C-6', C-7', C-8'), 14.4 (C-9'). MS (MALDI): calculated for [C₁₅H₃₁NO₄H]: m/z 290.2331 [M + H]⁺; found [M + H]⁺ 290.2335.

(1S,2S,3S,4S,5R)-4-((6-Aminohexyl)amino)-5-(hydroxymethyl)cyclopentane-1,2,3-triol or 1-(6-aminohexyl)amino-α-D-galacto-cyclopentane (26). To a solution of amine **22** (51.7 mg, 0.612 mmol) in DMF (2 mL), NaHCO₃ (154 mg, 1.84 mmol) and 6-bromohexanenitrile (106 μL, 0.796 mmol) were added at 60 °C. The suspension was stirred until complete conversion of the starting material was observed (24 h, CHCl₃-MeOH 3 : 1 + 1 vol% NH₄OH (25%)) and thereafter the solvent was removed under reduced pressure. The residue was chromatographically purified (CHCl₃-MeOH 14 : 1 + 1 vol% NH₄OH (25%)) to obtain crude nitrile **25** as a colourless oil. ¹H-NMR (300 MHz, CD₃OD) δ = 4.21 (dd, 1H, J_{3,4} 4.6 Hz, J_{4,5} 6.4 Hz, H-4), 3.98 (dd, 1H, J_{1,2} 6.5 Hz, J_{2,3} 4.2 Hz, H-2), 3.86 (dd, 1H, H-3), 3.84-3.76 (m, 2H, H-6), 3.08 (dd, 1H, J_{1,5} 8.1 Hz, H-1), 2.72-2.61 (m, 2H, H-1'), 2.47 (t, 1H, H-5'), 2.00-1.89 (m, 1H, H-5), 1.75-1.42 (m, 6H, H-2', H-3', H-4'). ¹³C NMR (75.5 MHz, CD₃OD) δ = 121.2 (C-6'), 79.9 (C-3), 74.8 (C-2), 72.3 (C-4), 62.2 (C-6), 61.6 (C-1), 49.1 (C-5), 49.0 (C-1'), 29.8 (C-2'), 27.4, 26.3 (C-3', C-4'), 17.2 (C-5').

A solution of crude nitrile **25** in methanol (2 mL) was stirred in the presence of RANEY®-Ni under an atmosphere of hydrogen gas at ambient pressure. After completed reduction (30 min, CHCl₃-MeOH 3 : 1 + 1 vol% NH₄OH (25%)), the catalyst was filtered off and the filtrate was concentrated under reduced pressure. The residual oil was purified on silica gel (CHCl₃-MeOH-NH₄OH (25%) 8 : 4 : 1) to yield amine **26** (40.5 mg, 0.154 mmol, 49%, 2 steps) as a colourless oil. [α]_D²⁰: -30.2 (c = 1.2, MeOH); ¹H-NMR (300 MHz, D₂O) δ = 4.19 (dd, 1H, J_{3,4} 4.8 Hz, J_{4,5} 5.4 Hz, H-4), 4.09 (dd, 1H, J_{1,2} = J_{2,3} 7.0 Hz, H-2), 3.92 (dd, 1H, H-3), 3.82-3.68 (m, 2H, H-6), 2.89 (dd, 1H, J_{1,5} 7.8 Hz, H-1), 2.85 (t, 2H, H-6'), 2.58 (t, 2H, H-1'), 2.07-1.95 (m, 1H, H-5), 1.65-1.26 (m, 8H, H-2', H-3', H-4', H-5'). ¹³C NMR (75.5 MHz, D₂O) δ = 78.0 (C-3), 73.6 (C-2), 70.1 (C-4), 60.1 (C-6), 58.0 (C-1), 47.5, 47.5 (C-5, C-1'), 39.8 (C-6'), 28.3, 28.2, 26.0, 25.5 (C-2', C-3', C-4', C-5'). MS (MALDI): calculated for [C₁₂H₂₆N₂O₄H]: m/z 263.1971 [M + H]⁺; found [M + H]⁺ 263.1971.

5-(Dimethylamino)-N-(6-(((1S,2S,3S,4S,5R)-2,3,4-trihydroxy-5-(hydroxymethyl)cyclopentyl)amino)hexyl)naphthalene-1-sulfonamide or 1-(6-dansylaminoethyl)amino-α-D-galacto-cyclopentane (27). Amine **26** (38.7 mg, 0.115 mmol) was dissolved in methanol and Na₂CO₃ (24.5 mg, 0.231 mmol) and

dansyl chloride (34.2 mg, 127 μmol) were added. After completed reaction (15 min, CHCl₃-MeOH 3 : 1 + 1 vol% NH₄OH (25%)) the suspension was concentrated under reduced pressure. The residue was purified on silica gel (CHCl₃-MeOH 8 : 1 + 1 vol% NH₄OH (25%)) to provide dansyl amide **27** (31.2 mg, 62.9 μmol, 55%) as a yellow syrup. [α]_D²⁰: -6.7 (c = 0.92, MeOH); ¹H-NMR (300 MHz, CD₃OD) δ = 8.56 (d, 1H, dansyl), 8.36 (d, 1H, dansyl), 8.19 (d, 1H, dansyl), 7.63-7.54 (m, 2H, dansyl), 7.28 (d, 1H, dansyl), 4.32 (dd, 1H, J_{3,4} 4.3 Hz, J_{4,5} 7.1 Hz, H-4), 4.16 (dd, 1H, J_{1,2} = 6.5 Hz, J_{2,3} 3.8 Hz, H-2), 3.94 (dd, 1H, H-3), 3.86 (dd, 1H, J_{5,6a} 5.6 Hz, J_{6a,6b} 10.8 Hz, H-6a), 3.78 (dd, 1H, J_{5,6a} 7.0 Hz, H-6b), 3.57 (dd, 1H, J_{1,5} 7.7 Hz, H-1), 2.98 (t, 2H, H-1'), 2.89 (s, 6H, dansyl), 2.84 (t, 2H, H-6'), 2.32 (dddd, 1H, H-5), 1.65-1.14 (m, 8H, H-2', H-3', H-4', H-5'). ¹³C NMR (75.5 MHz, CD₃OD) δ = 153.2, 137.2 (ipso), 131.3, 131.1, 131.0, 130.1, 129.1, 124.3, 120.6, 119.5 (dansyl), 79.3 (C-3), 73.3 (C-2), 72.1 (C-4), 61.9 (C-1), 61.4 (C-6), 47.8 (C-1'), 46.4 (C-5), 45.8 (dansyl), 43.6 (C-6'), 30.4, 27.0, 26.9, 26.9 (C-2', C-3', C-4', C-5'). MS (MALDI): calculated for [C₂₄H₃₇N₃O₆SH]: m/z 496.2481 [M + H]⁺; found [M + H]⁺ 496.2481.

Author contributions

P. W. performed the synthesis, M. T. and A. W. assisted with the development and the syntheses. R. F. provided crystal structure for compound **21**. S. A. N. performed biochemical experiments and S. G. W. supervised and evaluated biochemical studies. P. W. and A. E. S. conceived and designed the synthetic experiments. P. W., S. A. N., A. E. S., S. G. W. and T. M. W. prepared and reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

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