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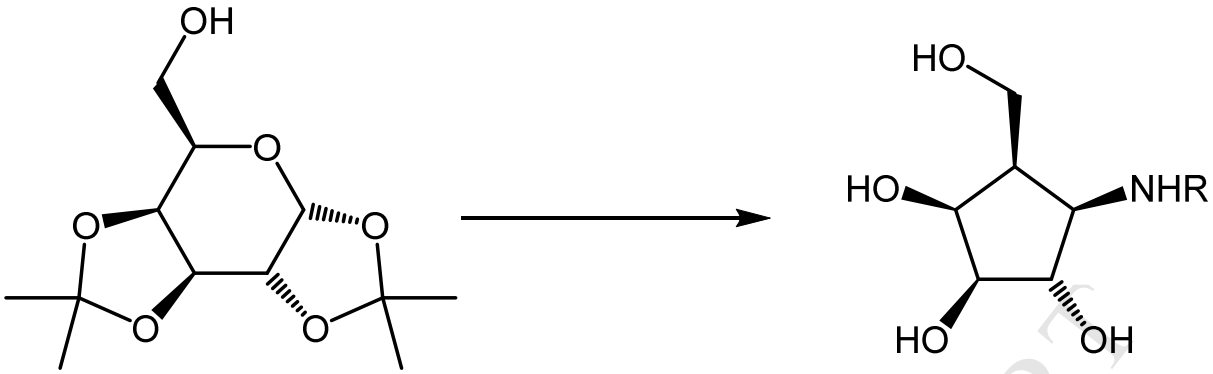


Correspondence to
Arnold E. Stütz
Glycogroup, Institut für Organische Chemie
Technische Universität Graz
Stremayrgasse 9
A-8010 Graz
Austria
Phone 0043 316 873 32430
Fax: 0043 316 873 32402

email:stuetz@tugraz.at

N-Substituted 5-amino-1-hydroxymethyl-cyclopentanetriols: A new family of activity promotors for a G_{M1}-gangliosidosis related human lysosomal β-galactosidase mutant

Michael Schalli[a], Christina Tysoe[b], Roland Fischer[c], Bettina M. Pabst[d], Martin Thonhofer[a], Eduard Paschke[d], Tanja Rappitsch[a], Arnold E. Stütz[a,*], Marion Tschernutter[d], Werner Windischhofer[d], Stephen G. Withers[b]

[a]*Glycogroup, Institute of Organic Chemistry, Graz University of Technology, Stremayrgasse 9, A-8010 Graz, Austria*

[b]*Chemistry Department, University of British Columbia, 2036 Main Mall, Vancouver, BC, Canada V6T 1Z1*

[c]*Institute of Inorganic Chemistry Graz University of Technology, Stremayrgasse 9, A-8010 Graz, Austria*

[d]*Laboratory of Metabolic Diseases, Department of Pediatrics, MedUni Graz, Auenbruggerplatz 30, A-8036 Graz,Austria*

ABSTRACT

From 1,2;3,4-di-*O*-isopropylidene-α-D-galactopyranose, a series of highly functionalized (hydroxymethyl)cyclopentanes was easily available. In line with reports by Reymond and Jäger on similar structures, these amine containing basic carbasugars are potent inhibitors of β-D-galactosidases and, for the first time, could be shown to act as pharmacological chaperones for G_{M1}-gangliosidosis-associated lysosomal acid β-galactosidase mutant R201C, thus representing a new structural type of pharmacological chaperones for this lysosomal storage disease.

Keywords: Aminocyclopentane, Carbafuranose, Galactosidase Inhibitor, Pharmacological Chaperone, G_{M1}-Gangliosidosis.

1. Introduction

Due to the vital roles of glycoside hydrolases, specific inhibitors may exert notable biological activities against various pathological processes, including certain forms of hereditary lysosomal disorders.[1,2,3,4] This latter group of about 50 metabolic diseases arises from mutations in specific genes that lead to deficiencies in enzymes involved in the lysosomal degradation of glycolipids and glycans. Considerable efforts have been made to provide novel therapeutic compounds that may relieve the various symptoms arising from the inability of lysosomal glycosidases to degrade their respective substrates. The latter consequently accumulate in the cells and lead to irreversible damage of nerve tissue, bones as well as various organs. For some of these lysosomal disorders, hematopoietic stem cell transplant,[5] gene therapy[6,7] as well as combination therapies[8] by additional administration of small molecules are envisaged to become tools of profound intervention.

Efforts of many groups have shown that imino sugar- as well as carba sugar-based glycomimetics may provide suitable therapeutic agents that function either by inhibiting upstream enzymes, thereby reducing the production of metabolites that cannot be degraded

rapidly enough by the respective mutant enzyme (substrate reduction therapy, SRT) or by assisting the folding and transport of mutant enzymes to the lysosome (chaperone mediated therapy, CMT), for which it was suggested that sub-inhibitory concentrations of active site specific molecules (pharmacological chaperones) could be exploited.[9,10,11,12,13,14,15,16,17] Mutant proteins that cannot obtain/retain their functional conformation are recognized as misfolded by the quality control machinery in the endoplasmic reticulum and are eventually targeted for degradation. These carba or imino sugars can bind to and stabilize mutant forms of enzymes such as the lysosomal β-glucosidase, β-galactosidase or β-*N*-acetylhexosaminidase in their functional folded conformations thus facilitating their exit from the endoplasmic reticulum and their subsequent transport to the lysosome.

Guiding contributions by various leaders in the field and recent reviews[18,19] have shown that non-polar *N*-substituted iminoalditols and similarly functionalized structures bind better than their more polar parent compounds through stronger interactions with the aglycon binding site or with lipophilic pockets around the enzymes' active sites. In this context, *N*-butyl-1-deoxynojirimycin is one of the best studied iminosugars, thus far.[4] Other researchers[20,21,22,23] have observed that large, lipophilic substituents, for example, adamantyl capped spacer arms, greatly improve the interaction between iminoalditol derivatives and the lysosomal β-glucosidase.

Compounds **1**-**3** have been found in nature. The so-called isoimino sugars invented by Lundt[24] and Bols[25] are not known natural products. The first such isoimino sugar, isofagomine (**4**), was found to be a potent β-glucosidase inhibitor. *C*-5a-Chain extended derivatives such as *C*-nonyl analog **5**, introduced by Fan and co-workers[26] turned out to be even more powerful. Derivatives **6** and **7** were synthesized and investigated by Withers and collaborators.[27]

G_{M1}-Gangliosidosis and Morquio B disease are two manifestations of mutations on the *GLB1* gene which encodes the vital lysosomal β-galactosidase. This enzyme is responsible for removal of a β-galactosyl residue from the non-reducing end of its substrate, converting gangliosides G_{M1}

and G_{A1} into G_{M2} and G_{A2}, respectively. Depending on the location of the mutation, the resulting lysosomal storage disorder is neurodegenerative (G_{M1}-gangliosidosis) or bone-destructive (Morquio B).[28,29]

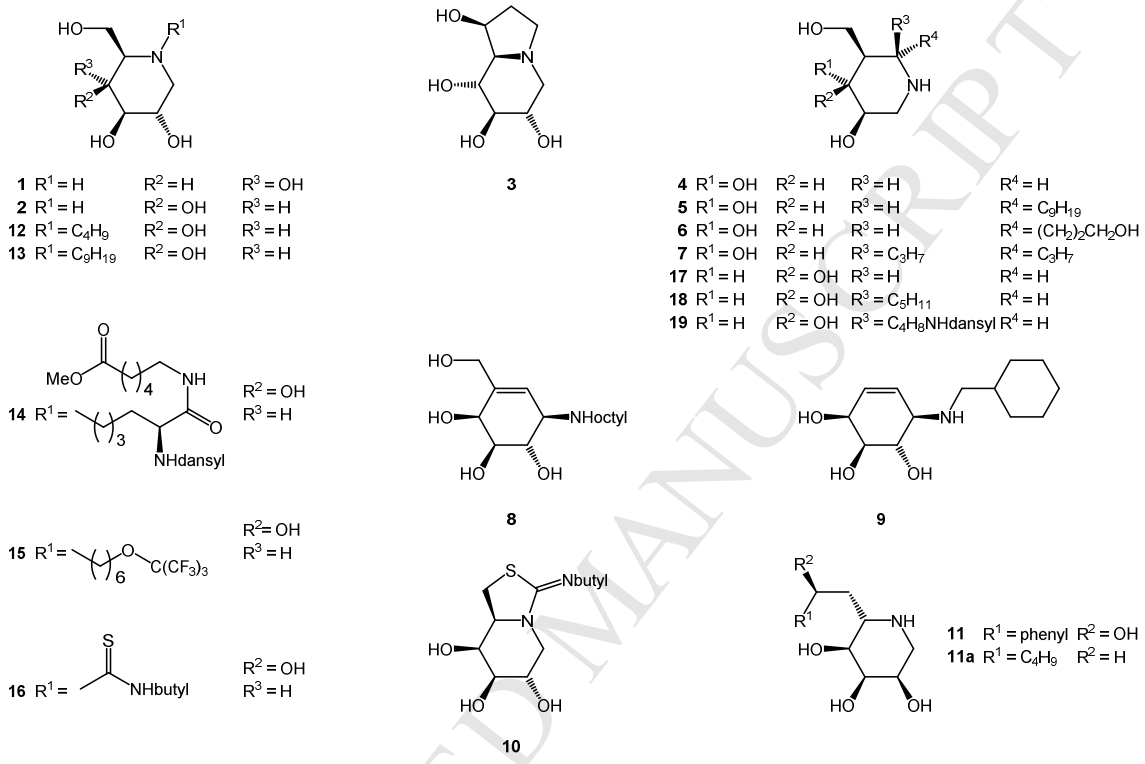


Figure 1: Glucosidase and galactosidase inhibitors as potential therapeutic principles for lysosomal deficiencies of D-glucosidase and D-galactosidase, respectively.

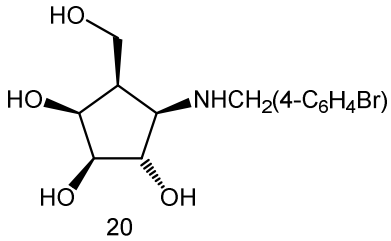
Only three distinct types of β-D-galactosidase inhibitors have been investigated as potential pharmacological chaperones for these two disorders. The first compound reported, carbasugar NOEV (*N*-octyl-*epi*-valienamine, **8**) is a powerful inhibitor[30] and reportedly even at sub-micromolar concentrations a highly potent pharmacological chaperone for a wide range of mutants of lysosomal β-galactosidase.[31] More recently, selected unbranched analogs lacking the hydroxymethyl group have been presented and their activities with patients' skin fibroblasts carrying the R201C mutation were evaluated.[32] Other potential chaperones were based on the structure of compound **2** which, by various structural modifications, could be transformed into a relatively small number of derivative classes (**10**-**16**, in the case of several *N*-substituent-

variations in the same publication, only the most active is depicted) with interesting properties as experimental pharmacological chaperones.[33,34,35,36,37,38,39,40,41]

C-5a-substituted derivatives of the isoiminosugar 4-*epi*-isofagomine[42] (**17**), such as compounds **18** and **19** have recently been reported and were found to be potent β-galactosidase inhibitors and superior pharmacological chaperones for selected G_{M1}-gangliosidosis and Morquio B disease cell lines.[43,44]

Amongst other types of potentially suitable sugar analogs in this context, carbasugars are a noteworthy and biologically powerful family, second only to the much larger group of imino and isoimino sugars. Comprehensive reviews have covered this important field recently.[45,46,47] Both pyranoid and furanoid-like carbasugars have been found in nature, with 5a-carba-α-D-galactopyranose having been the first such product discovered.[48] Examples of natural products containing cyclopentane-derived carbasugars include trehazolines,[49,50] allosamidine,[51] and the nucleoside analogs neplanocin[52] and aristeromycin.[53] Based on these and related structures, various non-natural derivatives have been prepared for several purposes. Included in these are synthetic D-*galacto*-related derivatives of valienamine and validamine, which have been investigated as pharmacological chaperones for a range of lysosomal storage disorders, including G_{M1}-gangliosidosis.[54]

We have now become interested in another substance class, the aminocyclopentane-based carbasugars (hydroxymethyl branched di- and trihydroxycyclopentylamines). There are only three leading references presenting such sugar analogs as proven D-galactosidase inhibitors.[55] Even more surprisingly, this family of compounds reportedly harbours some of the most powerful inhibitors of β-galactosidases known to date featuring inhibition constants in the sub-nanomolar range.[55] In particular, the β-D-*galacto* configured cyclopentyl amine bearing a (4-bromo)benzyl moiety at the nitrogen (**20**, figure 2), has been found to excel in terms of inhibitory activity.[55b,c]



20

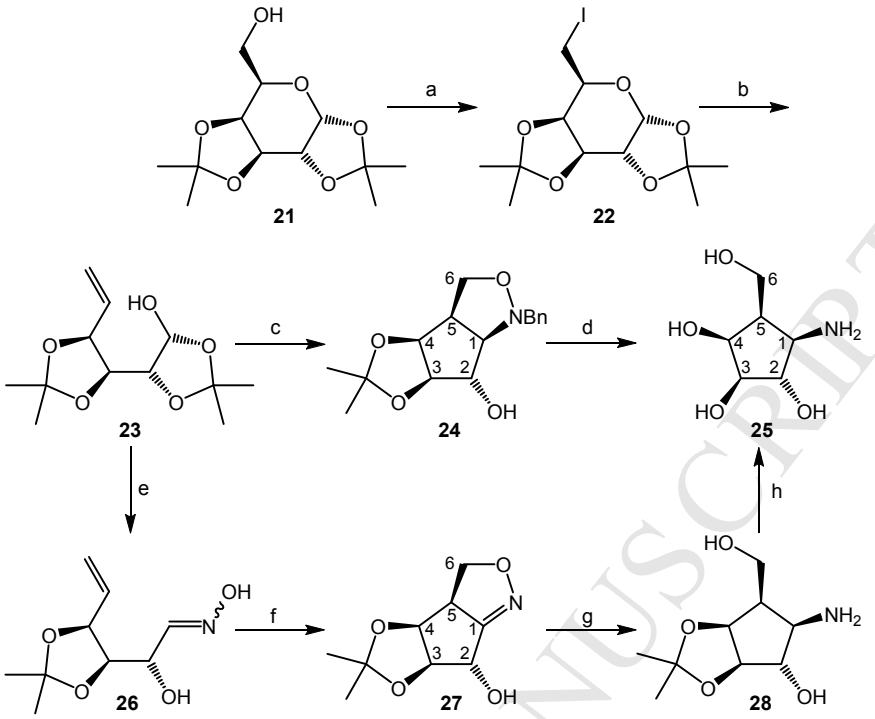
Figure 2: Inhibitor **20**

With a view to extending the selection of chaperone structures for G_{M1}-gangliosidosis and Morquio B, we wanted to explore whether such furanoid D-galactosidase inhibitors were suitable lead compounds.

2. Results and discussion

2.1. Synthesis

Our initial approach was based on Jäger's[55b] route: Starting from 1,2;4,6-di-*O*-isopropylidene-α-D-galactose (**21**), employing Garegg's conditions, known 6-deoxyiodosugar **22** was prepared. Taking advantage of the classical Vasella approach,[56] its reaction with Zn-dust under slightly acidic conditions provided open-chain intermediate **23**. Previously reported[57] vitamin B_{12} catalysis in this fragmentation reaction was found not to be necessary for satisfactory yields. By subsequent reaction with *N*-benzylhydroxylamine the initially formed oxime was smoothly converted by intramolecular 2+3 cycloaddition[57] with excellent stereoselectivity into desired known bicyclic cyclopentane derivative **24** the structure of which was unambiguously confirmed by X-ray crystallography (fig. 3). Nonetheless, despite all efforts made, we were not able to exceed an isolated yield of 40% in this cyclization step. Unexpectedly, formation of the second possible isomer could not be detected under the conditions employed. Catalytic hydrogenolysis under acidic conditions of the oxime ether bond with concomitant de-*N*-benzylation and removal of the isopropylidene moiety provided core structure **25** in only six steps from D-galactose. (26.3% overall yield from **22**)



Scheme 1: a) PPh_3, I_2, imidazole, toluene; b) Zn dust, NH_4Cl, MeOH, 81%; c) BnNHOH.HCl, $NaHCO_3$, NaOMe, MeOH, 41%; d) H_2, $Pd(OH)_2/C$ (20%), MeOH/HCl, 78%; e) H_2NOH (50wt% in H_2O), MeOH, 91% over 2 steps from **22**; f) NaOCl (10-15% active chlorine), silica gel, 2-propanol, 81%; g) $LiAlH_4$, THF, 78%; h) MeOH/H_2O 4:1, HCl conc., 87%.

Aiming to improve the yields overall and, in particular, in the carbacyclization step, we turned to a modified route based on exploiting unsubstituted hydroxylamine and subsequent oxidative conditions in the ring closing reaction[58] but employing 2-propanol in homogenous solution as solvent in the cyclization step, thus reducing the reaction times from several hours to around 60 min and improving yields by a factor of more than 2. Thus, open-chain unsaturated sugar **23** was reacted with hydroxylamine to give oxime **26**, which, in turn, smoothly reacted in the presence of NaOCl providing isoxazoline **27** in good yields. X-Ray crystallography unambiguously established the correct configuration at the branching point also nicely showing the bowl-like structure of **27** which only allows for hydride access from the exo-face. $LiAlH_4$-mediated reduction gave partially protected carbasugar **28** which was deprotected under acidic conditions to furnish free central intermediate **25** in 49.6 % overall yield from **22**.

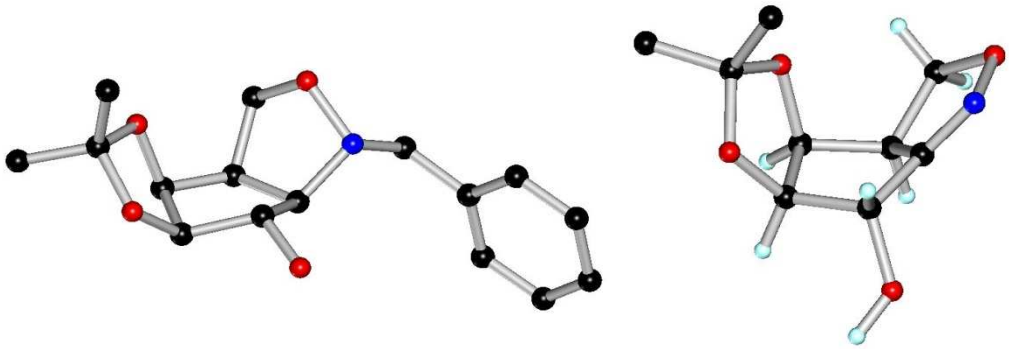
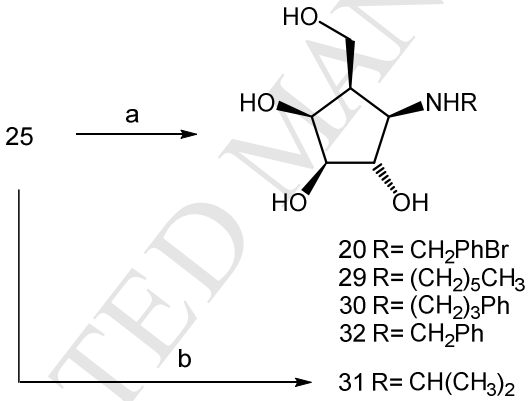


Figure 3. Carbasugar precursors **24** (CCDC 1530133; left) and **27** (CCDC 1530134).

With compound **25** readily at hand, subsequent conventional chemoselective *N*-alkylation furnished, in one single step each, a range of *N*-modified final products featuring "D-*galacto*" configuration (Scheme 2).



Scheme 2: a) RBr, $NaHCO_3$, DMF, (**20**: 80%; **29**: 83%; **30**: 87%; **32**: 72%); b) H_2, $Pd(OH)_2/C$, acetone, MeOH, 90%.

Thus, individual reaction of compound **25** with 1-bromohexane, as well as (3-phenyl)-1-bromopropane gave new products **29** and **30**, respectively. Conventional catalytic reductive amination with acetone led to new *N*-isopropyl derivative **31**. For internal biochemical comparison, known compounds **32** and **20** (the latter - as mentioned - one of the most powerful β-galactosidase inhibitors reported thus far)[55] were also prepared by reaction with benzyl bromide and (4-bromo)benzyl bromide, respectively.

2.2.Biological evaluation

Inhibitory activities of free aminocyclopentane tetraols **20**, as well as **29 - 32** in comparison with unsubstituted parent compound **25** and bench mark molecule **8** are summarized in Table 1. In all cases where inhibition was observed the pattern of inhibition was competitive. It is noteworthy that none of the compounds inhibited either of the α-glycosidases tested. Likewise, inhibition was reasonably galactosidase-specific relative to glucosidase. Nonetheless, undesired high activities with recombinant lysosomal glucocerebrosidase were noted in 75% of the compounds probed in this study and suggest structural re-iteration attempts to reduce this feature. Of the inhibitors tested, compounds **20**, **29** and **30** showed the best performance against the mammalian β-galactosidases, thus these were taken forward for cell-based testing.

Evaluation of inhibitors **20**, **29** and **30** as potential chaperones for the R201C mutant enzyme (Fig. 2) employing patients' skin fibroblasts revealed an effective concentration range of two orders of magnitude with an enhancement of 3.5- to 4-fold β-galactosidase activity at a concentration of 2-2.5 µM, 5-fold (compounds **20** and **29)** to 6-fold activity (compound **30**) at 10 µM and a maximum chaperone effect of more than 8-fold activity at 50 µM in case of compound **30**. For comparison, in-house data for compound **8** are also depicted (Fig. 4, black graph).

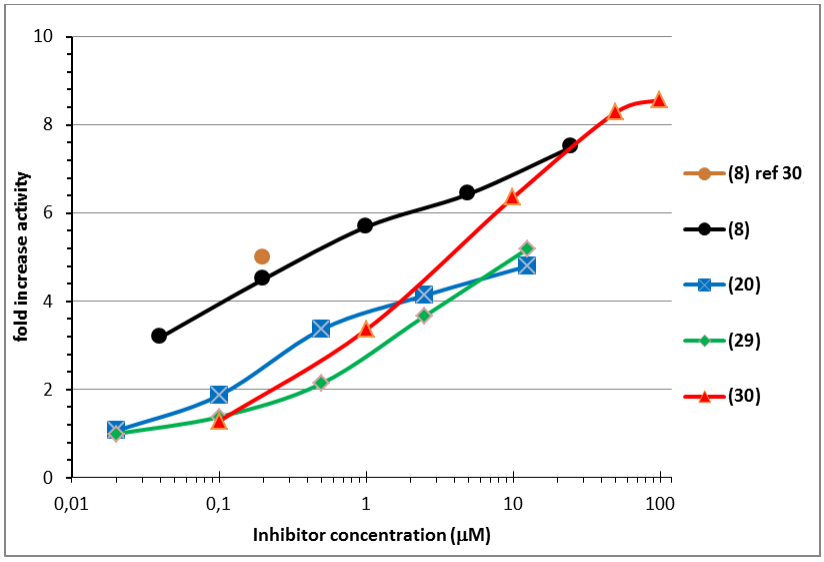
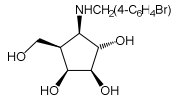
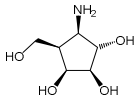
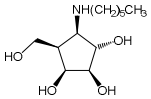
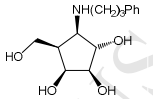
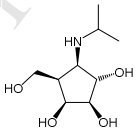
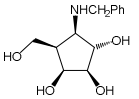
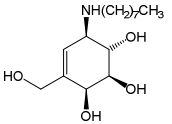


Figure 4: Activity enhancements with compounds **20**, **29** and **30** (in comparison with pyranoid carbasugar **8**) with human lysosomal β-galactosidase mutant R201C

Table 1. K_i-values [μM] of compounds with 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; *S. cer*. = α-glucosidase from *S. cerevisiae*; GCase = recombinant human lysosomal β-D-glucocerebrosidase; β-Gal. human lys. = human lysosomal acid β-galactosidase; IC_{50} [μM] with β-Gal (human lysosomal); N.I. = no inhibition, with Ki > 2 mM; n.d., not determined.

Enzyme	Compounds						
	 **20**	 **25**	 **29**	 **30**	 **31**	 **32**	 **8**
Abg (β-Glc/Gal)	0.0010	0.095	0.0046	0.0062	3.8	0.013	7.7
***E. coli* (β-Gal)**	0.0039	33.3	0.102	0.061	5.0	0.065	2.8
Bovine liver (β-Gal)	0.0030	54.3	0.049	0.085	13.0	0.14	0.87[59]
Fabrazyme (α-Gal)	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.
***S. cer* (α-Glc)**	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.
GCase (β-Glc)	0.067	78.1	0.23	0.21	N.I.	0.38	83
β-Gal hum. lys (IC50)	0.47	n.d.	1.44	1.44	n.d.	n.d.	0.51

3. Conclusions

Following a simple protocol previously outlined by Vasella's and Jäger's groups, a series of novel derivatives of **25**, bearing chain extensions at N-1 have been made available and were screened with a range of D-galactosidases including human lysosomal β-galactosidase. New inhibitors, compounds **29** and **30**, turned out to be highly potent and exhibited encouraging dose-dependent chaperoning profiles with enzyme mutant R201C. Results obviously merit screening for potential chaperone activity with other frequent mutants of this enzyme.

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. NMR spectra were recorded on a Varian INOVA 500 operating at 499.82 MHz (^{1}H), and at 125.894 MHz (^{13}C) or on a Bruker Ultrashield spectrometer at 300.36 and 75.53 MHz, respectively. $CDCl_3$ was employed for protected compounds and methanol-d_4 or D_2O 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. 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. The signals of the protecting groups as well as of the *N*-substituents were found in the expected regions and are only listed explicitly when overlapping with important spectral features of the respective compound. For crucial intermediates, structures were 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 (100K). X-ray diffraction data were collected at low temperature on a Bruker Kappa APEX II diffractometer using Mo K_a radiation (l = 0.71073 Å) generated by an INCOATEC micro-focus source. The data reduction and absorption correction was performed with the Bruker SMART[60]

and Bruker SADABS,[61] respectively. The structures were solved with SHELXT[62] by direct methods and refined with SHELXL[62] 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.[63,64]

MALDI-TOF Mass Spectrometry was performed on a Micromass TofSpec 2E Time-of-Flight Mass Spectrometer. Analytical TLC was performed on precoated aluminum 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 H_2O (950 mL)/ EtOH (750 mL)/ H_2SO_4 (120 mL) or ceric ammonium molybdate (100 g ammonium molybdate/8 g ceric sulfate in 1 L 10% H_2SO_4) 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.

4.2. Kinetic Studies

Kinetic studies were performed at 37°C in an appropriate buffer using a known concentration of enzyme (specific conditions depicted below). K_i determinations were performed using the corresponding 4-nitrophenyl α- or β-D-galactopyranoside as substrate, except for Fabrazyme and GCase, where 2,4-dinitrophenyl α-D-galactopyranoside and β-D-glucosylpyranoside[65] were employed. In a typical assay, the enzyme was incubated with different inhibitor concentrations for up to 5 minutes before initiating the reaction by the addition of substrate. The initial reaction rate was measured by monitoring the increase in absorbance at 400 nm for up to ten minutes. K_i determinations were performed using at least two different substrate concentrations. For each inhibitor, a range of four to six inhibitor concentrations bracketing the K_i value ultimately determined was used for each substrate concentration. Dixon plots (1/v vs [I]) were constructed

to validate the use of competitive inhibition model. The data were then fit using non-linear regression analysis with Grafit 7.0.

N.I. stands for no inhibition or weak inhibition with an estimated K_i value higher than 2 mM.

Specific assay conditions for each enzyme:

***Agrobacterium* sp. β-glucosidase:**[66,67] 50 mM sodium phosphate buffer (pH 7) using 1.85×10^{-4} mg/mL of enzyme (K_m = 4.1 mM).

***E.coli* lac *z* β-galactosidase:** 50 mM sodium phosphate, 1.0 mM $MgCl_2$ (pH 7) using 6.4×10^{-4} mg/mL of enzyme (K_m = 60 μM).

***Bovine liver* β-galactosidase:** 50 mM sodium phosphate buffer (pH 7) using 4.9×10^{-2} mg/mL of enzyme (K_m = 0.65 mM).

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 (pH5.5) using 5×10^{-5} mg/mL of enzyme (K_m = 0.65 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) using 4×10^{-5} mg/mL of enzyme (K_m = 2.7 mM).

Human β-galactosidase and **β-hexosaminidase** activity measurements were performed in duplicate assays, unless otherwise stated. Fibroblast cells were harvested by trypsinization in 0.9% NaCl containing 0.01% Triton, homogenized by sonication (3 x 10 sec, Sonifier Bandelin Sonopuls) and centrifuged at 13,000 rpm for 2 min in a table top centrifuge (Biofuge Pico, Heraeus). Protein amounts were determined according to the method of Lowry.[68]

For assessment of β-Gal activity, 20 µl of cell homogenate were mixed with 100 µl of 0.5 mM 4-methylumbelliferyl-β-D-galactopyranoside (Sigma-Aldrich), in 100 mM citrate buffer (pH 4.0) containing 100 mM NaCl and 0.02% NaN_3. After incubation at 37°C for 30 min, the reaction was stopped by adding 2.5 ml 400 mM glycine/NaOH (pH 10.4).

β-Hexosaminidase activity was measured by adding 10 µl homogenate to 90 µl 0.9% NaCl and 100 µl of 1 mM 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide (Sigma-Aldrich) in 100 mM citrate buffer (pH 4.6) containing 0.2% BSA and 0.04% NaN_3. The reaction was stopped after 10 min at 37°C by addition of 2.5 ml of 400 mM glycine/NaOH (pH 10.4). The amount of hydrolyzed 4-methylumbelliferone was determined with a fluorescence spectrometer (Hitachi F7000).

Modified β-Gal assays were used to estimate the half maximal inhibitory concentration (IC_{50}) of the particular chaperone. For IC_{50} determination, 0.001 to 100 µM of chaperone was added to the assay mixture. Activity was measured in normal fibroblasts. Data analysis was performed with Microcal™ Origin® v6.0 using a non-linear curve fitting module based on sigmoid curve fitting.

4.2.1. Patients and cell lines

Cell lines from one G_{M1}-Gangliosidosis-patient (R201C) and one WT, were exposed to compounds **20**, **29**, and **30**, for evaluation of their chaperone effects.

Human skin fibroblasts were grown in minimal essential medium (MEM) with Earle's Salts (SIGMA) containing 10% fetal bovine serum, 400 µM L-glutamine, and 50 µg/ml gentamycin at 37°C and 5% CO_2. All cells used in this study were between the third and nineteenth passages.

Potential chaperones were dissolved in DMSO and diluted in 10 mM phosphate buffer (pH 7.0) containing 100 mM NaCl, 0.01% NaN_3, and 0.01% Triton for the IC_{50}-measurements and in MEM for the *in-vivo* tests. The total DMSO concentration in the media was less than 1% and had no effect on β-Gal activity or cell viability.

4.2.2. Treatment of cultured fibroblasts

Fibroblasts were grown to semi-confluency in 6-well plates. Particular chaperone was added to the culture medium at following concentrations: 100µmol-0,02µmol. Cells were incubated for

four additional days at 37°C. Cells were harvested by scraping and prepared for β-galactosidase and β-hexosaminidase assays as described above.

4.3. General procedures

General procedure for *N*-alkylation: To a 10% solution of free aminocyclopentane tetraol **25** in DMF, solid $NaHCO_3$ (4 equiv.) and the respective alkyl bromide (2 equiv.) were added and the mixture was strirred at 60 °C until TLC indicated completed conversion of the starting material. The reaction was quenched with MeOH, the resulting suspension was concentrated under reduced pressure, and the remaining residue was passed over a short column of silica gel.

*4.4. (3aR,3bS,6aR,7S,7aR)-Hexahydro-5,5-dimethyl-1-(phenylmethyl)-1H-[1,3]Dioxolo[3,4]cyclopent[1,2-c]isoxazol-7-ol (**24**)*

A suspension of Zn dust (1.4 g, 21.9 mmol) and NH_4Cl (1.2 g, 21.9 mmol) in MeOH (15 mL) was stirred at ambient temp for 30 min. A solution of deoxyiodo sugar **22** (0.54 g, 1.46 mmol) in MeOH (6 mL) was added and the mixture was stirred or 60 min. After filtration over a short plug of silica gel, the filtrate was concentrated under reduced pressure, the residue was taken up in ethyl acetate and the organic layer was washed with water, dried (Na_2SO_4) and removed under reduced pressure providing compound **23** (0.29 g, 1.19 mmol, 81.4%) as a pale yellow oil. The resulting crude material was directly taken into the next step.

To a stirred solution of *N*-benzylhydroxylamine.HCl (279 mg, 1.75 mmol) in MeOH (6 mL), a methanolic solution of NaOMe (6 mL, 1 M) and solid $NaHCO_3$ (490 mg, 5.83 mmol) were added. After 15 min, a solution of crude enosugar **23** in MeOH (6 mL) was added and the mixture was stirred at 50 °C for 6 h. The resulting reaction mixture was diluted with ethyl acetate and washed with water. The organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure. The remaining residue was purified on silica gel to provide desired *N*-

benzylisoxazolidine **24** (141 mg, 0.48 mmol, 40.8%). Crystallization from MeOH/ethyl acetate provided pale yellow crystals which were suitable for X-ray structure analysis.

$[a]_D^{20}$= +62.9 (c 1.70, $CHCl_3$); m.p.: 108-110°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ =4.51 (dd, 1H, $J_{4,5}$ 7.1 Hz, H-4), 4.30 (dd, 1H, $J_{3,4}$ 6.3 Hz, H-3), 4.16 (dd, 1H, $J_{5,6a}$ 4.2 Hz, $J_{6a,6b}$ 8.8 Hz, H-6a), 3.98-3.82 (m, 3H, H-2, H-6b, C$\underline{H}_2$Ar), 3.62 (d, 1H, C$\underline{H}_2$Ar), 3.48 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{1,5}$ 7.1 Hz, H-1), 3.18 (m, 1H, H-5), 1.42, 1.22 (2s, 3H each, C(C$\underline{H}_3$)$_2$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 136.7 (ipso Ar), 129.1, 128.5, 127.5 (Ar), 112.7 (*C*(CH$_3$)$_2$), 86.4 (C-3), 79.7 (C-2), 77.5 (C-1), 77.1 (C-4), 64.9 (C-6), 59.7 (*C*H$_2$Ar), 45.6 (C-5), 27.3, 25.5 (C(*C*H$_3$)$_2$); MS: Calcd for [$C_{16}H_{21}NO_4$H]: *m/z* 292.1549 $[M+H]^+$; found $[M+H]^+$ 292.1613.

*4.5. (1S,2S,3S,4R,5R)-4-Amino-5-hydroxymethyl-1,2,3-cyclopentanetriol (**25**)*

Condition A:

To a solution of intermediate **24** (83,0 mg, 0,29 mmol) in MeOH (4 mL), HCl (conc., 100 µL) and Pd(OH)$_2$/C (20%, 50 mg) were added and the mixture was stirred under an atmosphere of H_2 at ambient pressure for 23 h. The catalyst was removed by filtration and washed with MeOH. Combined solvents were removed under reduced pressure and the remaining residue was subjected to silica gel chromatography to give free aminocyclopentane **25 (**36.2 mg, 0.22 mmol, 77.9%) as a pale yellow syrup.

Condition B:

To a solution of compound **28** (60.0 mg, 0.30 mmol) in 5 ml MeOH/H_2O 4:1, HCl conc.(50 µl) was added and the mixture was stirred for 60 minutes. After complete conversion indicated by TLC, the reaction mixture was evaporated to dryness. Purification on silica gel gave **25** (41.7 mg, 0.26 mmol, 86.6%) as a pale yellow syrup.

$[a]_D^{20}$= +24.6 (c 0.97, H_2O), ^{1}H NMR (300 MHz, D_2O) δ= 4.14 (dd, 1H, $J_{4,5}$ 3.5 Hz, H-4), 4.11 (dd, 1H, $J_{1,2}$ 4.7 Hz, $J_{2,3}$ 7.4 Hz, H-2), 3.89 (dd, 1H, $J_{5,6a}$ 4.0 Hz, $J_{6a,6b}$ 11.5 Hz, H-6a), 3.88 (m, 1H, H-3), 3.82 (dd, 1H, $J_{5,6b}$ 8.4 Hz, H-6b), 3.47 (dd, 1H, $J_{1,2}$ 4.7 Hz, $J_{1,5}$ 8.4 Hz, H-1); ^{13}C NMR

(75.5 MHz, D_2O) δ= 81.0 (C-2), 77.9 (C-3), 72.3 (C-4), 57.0 (C-6), 56.7 (C-1), 41.9 (C-5); MS: Calcd for $[C_6H_{13}NO_4H]$: *m/z* 164.0923 $[M+H]^+$; found $[M+H]^+$ 164.0929.

*4.6. (E/Z)-5,6-Dideoxy-5-eno-3,4-O-isopropylidene-L-arabinohexose oxime (**26**)*

A suspension of Zn dust (3.7 g, 55.9 mmol) and NH_4Cl (3.0 g, 55.9 mmol) in 40mL MeOH is stirred for 30 minutes at ambient temperature. A solution of **22** (1.38 g, 3.73 mmol) in 5mL MeOH is added, stirred for additional 60 minutes filtrated over silica gel concentrated under reduced pressure diluted with ethyl acetate and washed with H_2O. The combined organic layers were dried over Na_2SO_4 and evaporated. The resulting residue was diluted in 20ml MeOH and H_2NOH (50 %wt in H_2O, 685 µl, 11.2 mmol) was added and stirred for additional 60 minutes at ambient temperature. After full conversion of the starting material the reaction was evaporated to dryness. **26** (680.1 mg, 3.38 mmol, 90.7 % over 2 steps) was observed as an inseparable mixture of E/Z as a colourless syrup.

Major isomer: ^{13}C NMR (75.5 MHz $CDCl_3$) δ = 150.1 (C-1), 133.5 (C-5), 119.9 (C-6), 109.3 ($C(CH_3)_2$), 79.1 (C-3), 78.9 (C-4), 68.2 (C-2), 27.2, 25.0 ($C(CH_3)_2$);

Minor isomer: ^{13}C NMR (75.5 MHz $CDCl_3$) δ =152.5 (C-1), 133.5 (C-5), 120.1 (C-6), 109.2 ($C(CH_3)_2$), 79.1 (C-3), 78.2 (C-4), 64.5 (C-2), 26.8, 24.7 ($C(CH_3)_2$).

*4.7. (3aS,4S,5S,6S)-6-Hydroxy-4,5-isopropylidenedioxy-3a,4,5,6-tetrahydro-3H-cyclopent[c]isoxazole (**27**)*

To a stirred solution of **26** (590.7 mg, 2.94 mmol) in 20 ml 2-propanol was added silica gel (8 g) and 4.5 ml NaOCl (10-15% active chlorine) and stirred for 60 minutes at ambient temperature. After full conversion of the starting material, the reaction mixture was filtered and diluted with CH_2Cl_2 and consecutively washed with H_2O, HCl (2 *N*) and saturated $NaHCO_3$. The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. Purification on

silica gel afforded **27** (473.3 mg, 2.38 mmol, 80.9%) as a colourless syrup. Recrystallisation with ethyl acetate/cyclohexane gave colourless crystals for XRD analysis.

$[a]_D^{20}$ = -140.2 (c 1.62, $CHCl_3$); m.p.: 113-116°C; ^{1}H NMR (300 MHz) $CDCl_3$) δ = 4.70 (dd, 1H, $J_{3,4}$ 5.2 Hz, H-3), 4.61 (dd, 1H, $J_{4,5}$ 5.6 Hz, H-4), 4.47 (s, 1H, H-2), 4.41 (dd, 1H, $J_{5,6a}$ 8.2 Hz, $J_{6a,6b}$ 11.7 Hz, H-6a), 4.31 (dd,1H, $J_{5,6b}$ 8.4 Hz, H-6b), 4.04 (m, 1H, H-5), 3.39 (bs, 1H, 2-OH), 1.34, 1.24 (2s, 3H each, C(C$\underline{H}_3$)$_2$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 165.3 (C-1), 111.6 (*C*(CH$_3$)$_2$), 90.1 (C-3), 75.1 (C-4), 70.0 (C-6), 68.9 (C-2), 54.4 (C-5), 26.4, 24.7 (C(*C*H$_3$)$_2$).

MS: Calcd for [$C_9H_{13}NO_4$H]: *m/z* 200.0923 $[M+H]^+$; Found $[M+H]^+$ 200.0936.

*4.8. (1S,2S,3S,4R,5R)-1,2-O-Isopropylidene-4-amino-5-hydroxymethyl-1,2,3-cyclopentanetriol (**28**)*

To a stirred suspension of $LiAlH_4$ (902 mg 24 mmol) in THF (40 ml) a solution of compound **27** (470.2 mg, 2.4 mmol) in 15ml THF was added dropwise at 0°C. After full conversion of the starting material, H_2O and NaOH (3M) was added dropwise until the reaction mixture turned milky white. The resulting suspension was filtered and evaporated to dryness. Purification on silica gel gave **28** (374.0 mg, 1.84 mmol; 78.0%) as a colourless oil.

$[a]_D^{20}$ = +1.2 (c 1.35, MeOH); ^{1}H NMR (300 MHz, MeOH-d_4) δ = 4.80 (dd, 1H, $J_{4,5}$ 5.2 Hz, H-4), 4.47 (d, 1H, $J_{3,4}$ 5.7 Hz, H-3), 4.13 (s, 1H, H-2), 3.94 (dd, 1H, $J_{5,6a}$ 7.2 Hz, $J_{6a,6b}$ 11.1 Hz, H-6a), 3.85 (dd, 1H, $J_{5,6b}$ 8.2 Hz, H-6b), 3.35 (m, 1H, H-1), 2.59 (m, 1H, H-5), 1.47, 1.31 (2s, 3H each, C(C$\underline{H}_3$)$_2$); ^{13}C NMR (75.5 MHz, MeOH-d_4) δ= 112.1 (*C*(CH$_3$)$_2$) 87.4 (C-3), 81.7 (C-4), 79.9 (C-2), 60.7 (C-1), 58.2 (C-6), 47.0 (C-5), 26.2, 23.0 (C(*C*H$_3$)$_2$).

MS: Calcd for [$C_9H_{17}NO_4$H]: *m/z* 204.1236 $[M+H]^+$; Found $[M+H]^+$ 204.1221.

*4.9. (1S,2S,3S,4R,5R)-N-Hexyl-4-amino-5-hydroxymethyl-1,2,3-cyclopentanetriol (**29**)*

Following the general procedure, compound **25** (20.1 mg, 0.12 mmol) was treated with 1-bromohexane (19 µL, 0.14 mmol) in the presence of solid $NaHCO_3$ (40 mg, 0.48 mmol) for 90 h.

Purification on silica gel afforded *N*-hexyl derivative **29** (25.4 mg, 0.10 mmol, 83.4%) as a white wax.

$[a]_D^{20}$= +24.0 (c 0.78, MeOH), ^{1}H NMR (300 MHz, MeOH-d_4) δ= 4.22 (dd, 1H, H-2), 4.04 (dd, 1H, $J_{3,4}$ 4.2 Hz, H-4), 3.92 (m, 2H, H-6a, H-6b), 3.80 (dd, 1H, $J_{2,3}$ 6.6 Hz, H-3), 3.48 (dd, 1H, $J_{1,2}$ 8.7 Hz, $J_{1,5}$ 8.7 Hz, H-1), 3.28-3.03 (m, 2H, H-1'), 2.60 (m, 1H, H-5), 1.86-1.64 (m, 2H, H-2'), 1.47-1.28 (m, 6H, H-3', H-4', H-5'), 0.93 (t, 3H, H-6'); ^{13}C NMR (75.5 MHz, MeOH-d_4) δ= 80.2 (C-2), 79.8 (C-3), 73.3 (C-4), 64.9 (C-1), 58.6 (C-6), 48.5 (C-1') 43.7 (C-5), 32.4, 27.2, 26.7, 23.5 (C-2', C-3', C-4', C-5'), 14.3 (C-6').

MS: Calcd for $[C_{12}H_{25}NO_4H]$: *m/z* 248.1862 $[M+H]^+$; Found $[M+H]^+$ 248.1864.

*4.10. (1S,2S,3S,4R,5R)-N-(3-Phenyl)propyl-4-amino-5-hydroxymethyl-1,2,3-cyclopentanetriol (**30**)*

Following the general procedure, compound **25** (19.5 mg, 0.12 mmol) was treated with 3-phenyl-1-bromopropane (20.5 μL, 0.13 mmol) in the presence of solid $NaHCO_3$ (40 mg, 0.48 mmol) for 96 h. Purification on silica gel gave (3-phenyl)propyl derivative **30** (29.4 mg, 0.10 mmol, 87.4%) as a colorless syrup.

$[a]_D^{20}$= +26.8 (c 1.14, MeOH), ^{1}H NMR (300 MHz, MeOH-d_4) δ= 4.14 (dd, 1H, $J_{1,2}$ 5.4 Hz, H-2), 3.97 (dd, 1H, $J_{3,4}$ 4.2 Hz, H-4), 3.85 (m, 2H, H-6a, H-6b), 3.73 (dd, 1H, $J_{2,3}$ 6.7 Hz, H-3), 3.41 (dd, 1H, $J_{1,2}$ 5.4 Hz, $J_{1,5}$ 8.9 Hz, H-1), 3.23-2.99 (m, 2H, H-1'); 2.71-2.61 (t, 2H, H-3'), 2.53 (m, 1H, H-5), 2.13-1.91 (m, 2H, H-2'); ^{13}C NMR (75.5 MHz, MeOH-d_4) δ=141.6 (ipso Ar), 129.6, 129.4, 127.4 (Ar), 80.2 (C-2), 79.8 (C-3), 73.3 (C-4), 65.0 (C-1), 58.6 (C-6), 43.7 (C-1'), 33.6 (C-3'), 28.5 (C-2').

MS: Calcd for $[C_{15}H_{23}NO_4H]$: *m/z* 282.1705 $[M+H]^+$; Found $[M+H]^+$ 282.1709.

*4.11. (1S,2S,3S,4R,5R)-N-isopropyl-4-amino-5-hydroxymethyl-1,2,3-cyclopentanetriol (**31**)*

To a stirred solution of **25** (42.8 mg, 0.26 mmol) in MeOH (3 ml) and HCl conc. (10 µL), acetone (29 µl, 0.39 mmol) was added and the mixture was stirred under an atmosphere of hydrogen at ambient pressure in prescence of $Pd(OH)_2/C$ (20 %; 40 mg) for 24 hours. The catalyst was removed by filtration and washed with MeOH. The solvent was removed under reduced pressure and the remaining residue was chromatographed on silica gel to give compound **31** (48.7 mg, 0.24 mmol, 90.5%) as a colourless wax.

$[a]_D^{20}$ = +19.8 (c 1.31, H_2O); ^{1}H NMR (300 MHz, D_2O) δ= 4.25 (dd, 1H, $J_{2,3}$ 6.9 Hz, H-2), 4.11 (dd, 1H, $J_{3,4}$ 4.1 Hz, H-4), 3.93-3.79 (m, 3H, H-3, H-6a, H-6b), 3.69 (dd, 1H, $J_{1,2}$ 6.0 Hz, $J_{1,5}$ 9.1 Hz, H-1), 3.63 (m, 1H, C<u>H</u>$(CH_3)_2$), 2.65 (m, 1H, H-5), 1.32 (m, 6H, CH(C<u>H</u>$_3)_2$); ^{13}C NMR (75.5 MHz, D_2O) δ= 78.6 (C-2), 77.4 (C-3), 71.4 (C-4),59.9 (C-1), 57.1 (C-6), 50.6 (*C*H$(CH_3)_2$), 41.5 (C-5), 19.1, 17.5 (CH(*C*$H_3)_2$).

MS: Calcd for [$C_9H_{19}NO_4$H]: *m/z* 206.1392 $[M+H]^+$; Found $[M+H]^+$ 206.1381.

*4.12. (1S,2S,3S,4R,5R)-N-benzyl-4-amino-5-hydroxymethyl-1,2,3-cyclopentanetriol (**32**)*

Following the general procedure, compound **25** (19.8 mg, 0.12 mmol) was treated with benzyl bromide (16 µL, 0.13 mmol) in the presence of solid $NaHCO_3$ (40 mg, 0.48 mmol). Purification on silica gel yielded *N*-benzyl derivative **32** (22.0 mg, 0.09 mmol, 71.6%) as a colorless wax.

$[a]_D^{20}$= +39.9 (c 1.35, MeOH); ^{1}H NMR (300 MHz, D_2O) δ= 4.50 (d, 1H, C<u>H</u>$_2$Ar), 4.38 (dd, 1H, $J_{1,2}$ 5.8 Hz, $J_{2,3}$ 7.4 Hz, H-2), 4.27 (d, 1H, C<u>H</u>$_2$Ar), 4.06 (dd, 1H, $J_{3,4}$ 3.8 Hz, H-4), 3.94-3.74 (m, 3H, H-3, H-6a, H-6b), 3.51 (dd, 1H, $J_{1,5}$ 9.0 Hz, H-1), 2.61 (m, 1H, H-5); ^{13}C NMR (75.5 MHz, D_2O) δ= 130.1, 129.8, 129.8, 129.3 (Ar), 78.5 (C-2), 77.7 (C-3), 71.4 (C-4), 61.3 (C-1), 56.9 (C-6), 50.1 (*C*H_2Ar), 41.4 (C-5).

MS: Calcd for [$C_{13}H_{19}NO_4$Na]: *m/z* 276.1212 $[M+Na]^+$; Found $[M+Na]^+$ 276.1205.

*4.13. (1S,2S,3S,4R,5R)-N-(4-bromo)benzyl-4-amino-5-hydroxymethyl-1,2,3-cyclopentanetriol (**20**)*

Following the general procedure, compound **25** (19.3 mg, 0.12 mmol) was treated with (4-bromo)benzyl bromide (32.5 mg, 0.13 mmol) in the presence of solid $NaHCO_3$ (40 mg, 0.48 mmol). Purification on silica gel gave *N*-(4-bromo)benzyl derivative **20** (31.5 mg, 0.10 mmol, 80.2%) as a slightly yellow syrup.

$[a]_D^{20}$= +17.6 (c 0.48, H_2O); ^{1}H NMR **(**300 MHz, D_2O) δ= 4.16-4.06 (m, 2H, H-2, H-4), 3.98 (d, 1H, C$\underline{H}_2$Ar), 3.88-3.73 (m, 4H, H-3, H-6a, H-6b, C$\underline{H}_2$Ar), 3.11 (dd, 1H, $J_{1,2}$ 5.0 Hz, $J_{1,5}$ 8.8 Hz, H-1), 2.46 (m, 1H, H-5); ^{13}C NMR (75.5 MHz, D_2O) δ= 137.0 (ipso Ar), 131.6, 130.5 (Ar), 120.8 (ArBr), 82.1 (C-2), 78.3 (C-3), 72.0 (C-4), 61.7 (C-1), 57.4 (C-6), 50.1 (CH_2Ar), 43.1 (C-5).

MS: Calcd for [$C_{13}H_{18}BrNO_4H$]: *m/z* 332.0497 $[M+H]^+$; Found $[M+H]^+$ 332.0863.

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A simple, short and high yielding route enables access to amino(hydroxymethyl)pentanetriols.

Additional modifications are available by simple 1-*N*-alkylation.

Final products are potent inhibitors of a panel of β-galactosidases.

New compounds act as efficient experimental pharmacological chaperones for G_{M1}-gangliosidosis-related R201C mutant of human lysosomal β-galactosidase.