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Structure–activity relationships in aminocyclopentitol glycosidase inhibitors

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Aminocyclopentitol analogs of β-D-glucose, β-D-galactose and α-D-galactose bearing alkyl substituents as aglycon mimics on the amine function were prepared and tested for inhibition of various glycosidases. *N*-benzyl-β-D-*gluco* derivatives **1**–**4** and *N*-benzyl-β-D-*galacto* derivative **5** inhibited β-galactosidase and β-glucosidase. *N*-benzyl-α-D-*galacto* aminocyclopentitol **6** strongly inhibited α-galactosidase. The inhibitory activities observed were generally stronger compared to those of their primary amine analogs. A structure–activity relationship analysis was carried out including data from thirty-five different aminocyclopentitol glycosidase inhibitors. The strongest inhibitions reported for any enzyme were associated with a perfect stereochemical match between aminocyclopentitol and glycosidase, including the α- or β-configuration of the amino-group corresponding to the enzyme's anomeric selectivity.

Introduction

Glycosidase inhibitors[1] can be used for treating diabetes, cancer, viral (HIV, influenza) and bacterial infections, and as insecticides.[2] In conjunction with our interest in raising glycosidase catalytic antibodies,[3,4] we have been interested in aminocyclopentitol glycosidase inhibitors because these compounds also resemble the transition state of glycosidic bond hydrolysis. Aminocyclopentitols incorporate the entire stereochemical pattern of glycopyranosides, this in an elegantly compact manner by which each of the five cyclopentane substituents represents one of the five pyranoside substituents in a stereodefined manner. In particular the amino group mimics the protonated form of the leaving group oxygen atom in an α- or β-orientation. Early studies with *N*-alkyl substituted α-L-*fuco* and β-L-*fuco* configured aminocyclopentitols showed that *N*-alkyl substitution enhanced inhibitory potency and selectivity. Herein we report the synthesis and evaluation of *N*-alkyl substituted aminocyclopentitols **1**–**6** with β-*gluco*, β-*galacto* and α-*galacto* configuration (Fig. 1). A comparative analysis of known aminocyclopentitols shows that the strongest aminocyclopentitol inhibitor of any α- or β-glycosidase is always an *N*-alkyl derivative of the stereochemically corresponding aminocyclopentitol, which clearly establishes the stereochemical design principle underlying inhibition in these compounds.

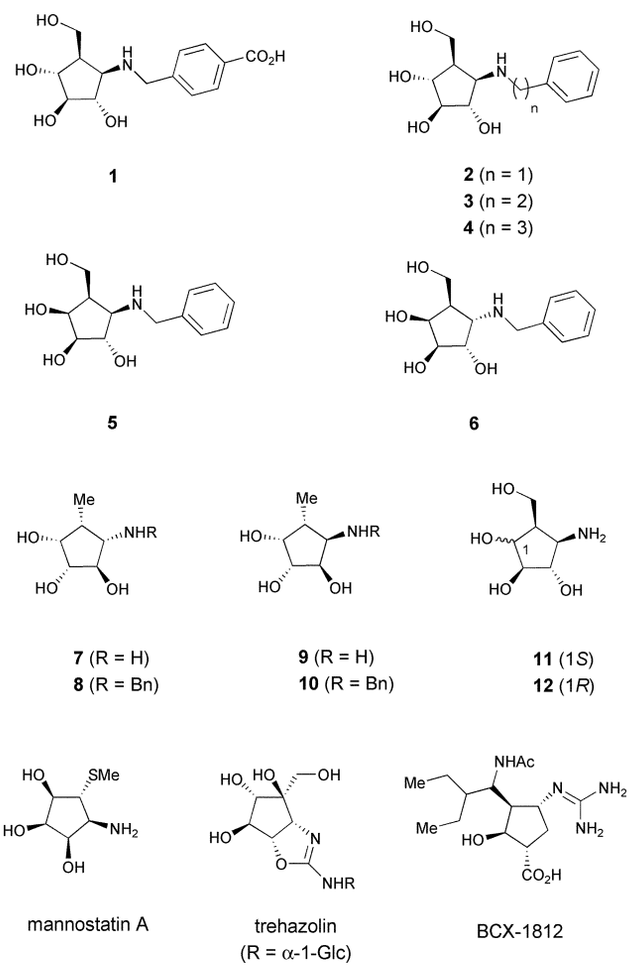

Fig. 1 Structure of aminocyclopentitols.

Results and discussion

Design

Aminocyclopentitol substructures are found in a number of natural products such as mannostatin and trehazolin.[5] The neuraminidase inhibitor BCX-1812 in clinical development to treat influenza is also an aminocyclopentitol.[6] Synthetic routes to aminocyclopentitols were developed for the total synthesis of such compounds.[5] Inspired by an early report of glycosidase inhibition,[7] we have investigated aminocyclopentitols as transition state analog inhibitors of glycosidic bond cleavage. The protonated amino group acts as a mimic of the leaving group exocyclic C(1) oxygen atom and interacts favorably with the enzyme's catalytic acidic groups by electrostatic and hydrogen bonding interactions (Fig. 2). The complete stereochemical pattern of the glycoside is encoded in the relative configuration of the cyclopentitol substituents. In particular, the anomeric β- or α-orientation of the leaving group is represented by the relative configuration of the amino group. This feature is absent from most other glycosidase inhibitor structures, in particular analogs of the intermediate oxocarbonium cation where the anomeric configuration is absent. Anomeric stereochemistry is a key feature of glycosidases if one considers that these enzymes are usually completely selective for either β- or

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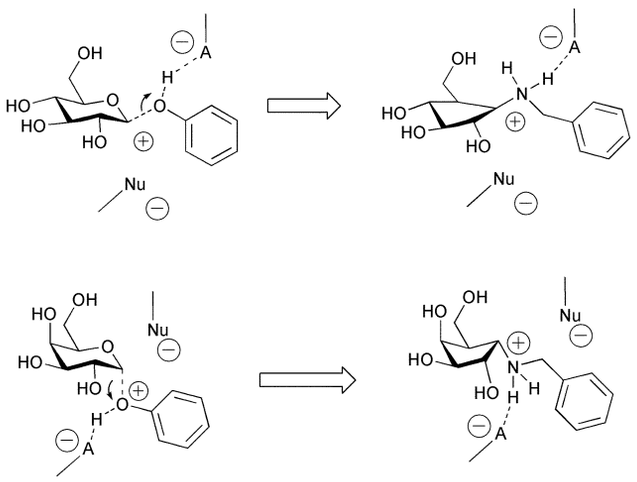


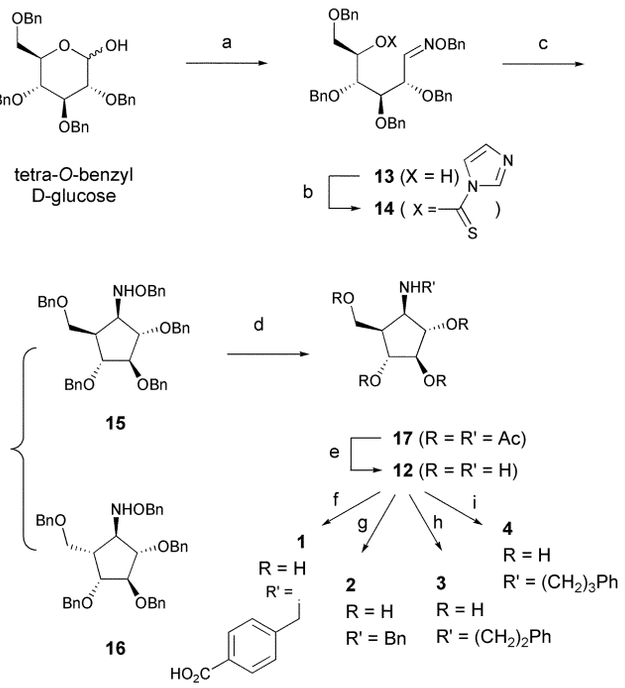
Fig. 2 Structural analogy between β- and α-glycosides undergoing enzymatic cleavage and *N*-alkyl aminocyclopentitols in the example of phenyl β-D-glucosides and α-D-galactoside and compounds **2** and **6**. The enzyme's catalytic groups AH (acidic residue) and Nu (nucleophilic residue) are usually a pair of carboxylate side chains.

α-glycosides. The cyclopentitol's amino group can be alkylated to provide a mimic of the aglycon leaving group.

Inhibition studies with aminocyclopentitols **7**–**12** bearing a primary amine with β- and α-L-*fuco*,[8] β-D-*galacto*,[9] and β-D-*gluco*[10] configuration (Fig. 1) indicated that inhibitory selectivity among the different glycosidases was governed by the stereochemistry of the cyclopentane substituents, with the hydroxyl group configuration corresponding to the carbohydrate type and the amino group configuration to the anomeric selectivity. In the case of the α-L-*fuco* aminocyclopentitol **9**, addition of a benzyl substituent at nitrogen to give **10** led to a dramatic enhancement of inhibitory potency against various α-L-fucosidases, while the stereoisomeric β-L-*fuco* aminocyclopentitol **7** lost its potency against α-L-fucosidases upon *N*-alkylation to **8**. To probe the generality of this anomer-selective inhibitor design, we set out to prepare *N*-alkylated β- and α-configured aminocyclopentitols either in the *galacto*- or *gluco*- configuration since β- and α-selective glycosidases are available in both series. During the course of this study, a series of closely related aminocyclopentitols was reported by Jäger *et al.*[11]

Synthesis

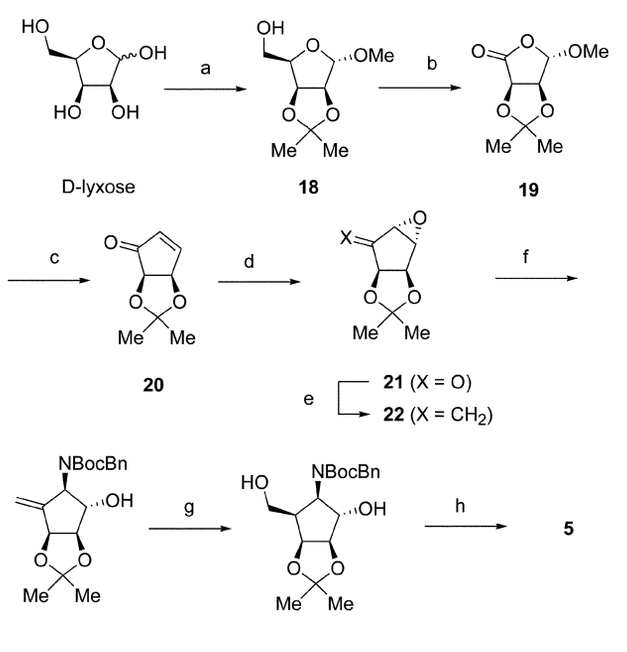
The β-D-*gluco* configured aminocyclopentitols were prepared starting from tetra-*O*-benzyl glucose using a radical cyclization procedure modified from the original report of Bartlett *et al.* (Scheme 1).[12] Reaction with *O*-benzylhydroxylamine gave oxime **13**. Reaction with thiocarbonyl diimidazole gave the thiocarbamate **14**. Reaction in refluxing benzene and slow addition of AIBN and Bu_3SnH effected radical cyclization to produce a stereoisomeric 1.6 : 1 mixture of the β-D-*gluco* and β-L-*ido* aminocyclopentitols **15** and **16**. The stereoisomers were separated by column chromatography. The major β-D-*gluco* isomer **15** was deprotected by catalytic hydrogenation in acetic acid with 12 bars of H_2 for 4 days, which resulted in cleavage of the NO bond and removal of all benzyl protecting groups. The product was isolated as the acetylated derivative **17** after acetylation of the crude product. This simple deprotection procedure avantageously replaced an earlier two-step process beginning with the rather sluggish reductive cleavage of the N–O bond with zinc–HOAc.[10] The primary aminocyclopentitol **12** was obtained quantitatively from the acetylated product **17** by acidic hydrolysis. Direct alkylation with methyl (bromomethyl)benzoate followed by hydrolysis of the ester gave the carboxybenzyl derivative **1**. On the other hand, imine formation with aldehydes and reduction following the procedure described by Jäger *et al.* for similar compounds,[11] gave the *N*-benzyl, *N*-



Scheme 1 Synthesis of *N*-alkyl β-D-*gluco* aminocyclopentitols **1**–**4**. *Reagents and conditions*: a) $BnONH_3Cl$, MeOH, 60 °C, 15 h (95%); b) Im_2CS, benzene, reflux, 4 h (96%); c) AIBN, Bu_3SnH, benzene, reflux, 4 h (55% **15**, 35% **16**); d) 12 bar H_2, Pd/C, AcOH, 25 °C, 4 d; DMAP, Ac_2O, 20 h (80%); e) 1.2 M HCl, reflux, 12 h (100%); f) $CsOH{\cdot}H_2O$, DMF, MS 4 Å, $BrCH_2C_6H_4CO_2H$, 80 °C, 12 h (16%); g) Al_2O_3, PhCHO, 50 °C, 6 h, then MS 4 Å, $NaBH_4$, 25 °C, 24 h (43%); h) as g) with $PhCH_2CHO$ (49%); i) as g) with $PhCH_2CH_2CHO$ (51%).

phenethyl and *N*-phenylpropyl derivatives **2**–**4**. All compounds were purified by preparative reverse-phase HPLC.

Our synthetic approach to *N*-benzyl-β-D-*galacto* aminocyclopentitols followed our previous synthesis of β-D-*galacto*-aminocyclopentitol **11** (Scheme 2).[9] Oxidative cleavage of the



Scheme 2 Synthesis of *N*-benzyl-β-*galacto* aminocyclopentitol **5** from D-lyxose. *Reagents and conditions*: a) 2,2-dimethoxypropane, acetone, MeOH, 0 °C, cat. $HClO_4$, 5 h (84%); b) PCC (4 equiv.), benzene, reflux, 15 h (39%); c) $LiCH_2PO(OMe)_2$, THF, −80 °C, 2 h, then −20 °C, 20 min (39%); d) H_2O_2, MeOH, NaOH, −50 °C, 1 h (79%); e) $BrCH_3PPh_3$–$NaNH_2$, THF, 25 °C, 1 h (quant.); f) benzylamine, 25 °C, 1.5 h, then Boc_2O, $NaHCO_3$, EtOAc–H_2O (24%); g) $BH_3{\cdot}$THF, THF, 0 °C, 2 h, then aq. $NaBO_3$, 25 °C, 1.5 h (75%); h) aq. 0.3 N HCl, 60 °C, 12 h (quant.).

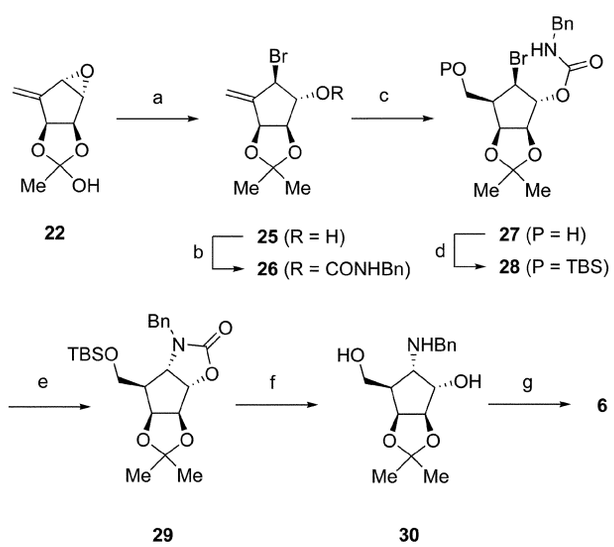
Table 1 Inhibition data on glycosidases. Inhibition data in μM for compounds **1**–**6**. IC_{50} values are marked (*) and were determined at [S] = 2.5 mM and [I] = 100, 10, 1, 0.1 and 0.01 μM. Percents (%) indicate inhibition at [I] = 100 μM and [S] = 2.5 mM. No data are given when less than 5% inhibition was observed at [I] = 100 μM and [S] = 2.5 mM. "—": not determined. Competitive inhibition constants K_i were determined from Dixon replots (see Fig. 1) at [S] = 2.5, 1.67, 1.11, 0.74 and 0.49 mM. The inhibitor concentration [I] was in the range of the previously determined IC_{50}. Hydrolysis of 4-nitrophenyl-glycosides at 30 °C was followed at 405 nm in individual wells of flat-bottomed, 96-well, half-area polystyrene microtiter plates (Costar) using a UV Spectramax 250 instrument from Molecular Devices. 100 μL assays contained both β-glucosidases and the β-galactosidase at 0.1 U mL^{-1}, the β-mannosidase at 0.75 U mL^{-1}, the α-glucosidase at 1 U mL^{-1}, the α-galactosidase at 0.1 U mL^{-1}, the α-mannosidase at 0.05 U mL^{-1} and α-L-fucosidase at 2 mU mL^{-1}. The α-glucosidase was buffered in PBS pH 7.4, all other enzymes in 0.1 M HEPES buffer at pH 6.8 and 30 °C

Enzyme	Origin	K_i (**1**) × 10^{-6} M	K_i (**2**) × 10^{-6} M	K_i (**3**) × 10^{-6} M	K_i (**4**) × 10^{-6} M	K_i (**5**) × 10^{-6} M	K_i (**6**) × 10^{-6} M
α-glucosidase	yeast	14*	14*	15*	6*		120
β-glucosidase	almonds	0.66	0.018	0.007	0.004	0.035	0.51
β-glucosidase	*C. s.*[a]	0.27	0.022	0.024	0.017	—	—
α-galactosidase	green coffee beans				7%	80	0.43
β-galactosidase	bovine liver		2	0.024	0.025	0.012	1.5
α-mannosidase	jack beans	12%	38%	50*	10*		
β-mannosidase	snail acetone powder				9%		
α-L-fucosidase	human placenta	—	—	—	—		

[a] *C. s. = Caldocellum saccharolyticum.*

protected furanoside **18** with excess PCC gave lactone **19**, which reacted with lithium dimethyl methylene phosphonate to provide enone **20** following a protocol adapted from the literature.[13] Stereoselective epoxidation with basic H_2O_2 at low temperature gave epoxide **21**. The ketone was then converted quantitatively to the volatile allylic epoxide **22** using instant ylide.[14] Aminolysis of this sensitive intermediate with benzylamine followed by Boc protection gave carbamate **23**. Hydroboration with borane–THF gave the corresponding alcohol **24** stereoselectively. Finally, acidic deprotection with aqueous HCl gave the *N*-benzyl derivative **5**.

The *N*-benzyl-α-*galacto* derivative **6** was prepared from epoxide intermediate **22** following a sequence similar to that used for the corresponding L-*fuco* compound (Scheme 3).[8] Treatment with LiBr in acetic acid resulted in regioselective opening of the epoxide at the allylic carbon to give bromohydrin **25**. The secondary alcohol function was then converted to the corresponding *N*-benzyl carbamate **26** by reaction with benzyl isocyanate. Hydroboration was then carried out with BH_3·THF, followed by a mild oxidative workup with perboric acid,[15] to give the primary alcohol **27**. The neutral oxidation conditions were chosen since the desired product was not obtained upon oxidative workup of the borane intermediate



Scheme 3 Synthesis of *N*-benzyl-α-*galacto* aminocyclopentitol **6**. *Reagents and conditions*: a) LiBr, AcOH, 25 °C, 2 h (53%); b) BnNCO, Et_3N, CH_2Cl_2, 25 °C, 15 h (69%); c) BH_3·THF, THF, 25 °C, 2 h, then aq. $NaBO_3$, 25 °C, 1.5 h (75%); d) TBSOTf, 2,6-lutidine, CH_2Cl_2, 25 °C, 2 h (84%); e) NaH, THF, 0 °C, 2 h (91%); f) NaOH, EtOH, 60 °C, 7 h (quant.); g) aq. 0.3 M HCl, 60 °C, 12 h, then prep. RP-HPLC (quant.).

with $NaOH–H_2O_2$, presumably due to the sensitivity of the bromide functional group. The primary alcohol was then protected as the TBS ether **28**. At that stage the α-configured nitrogen substituent was introduced by intramolecular displacement of the bromide by the *N*-benzyl carbamate function, which took place smoothly upon treatment with NaH in THF at 0 °C to give **29**. Deprotection was achieved by sequential treatment with aqueous NaOH to open the cyclic carbamate to **30**, followed by aqueous acidic treatment with HCl to give *N*-benzyl aminocyclopentitol **6**. Attempts to remove the *N*-benzyl group by reductive procedures (H_2 and various catalysts, or $Li–NH_3$–THF) were unsuccessful.

Glycosidase inhibition measurements

All compounds were tested for inhibition against a series of glycosidases using chromogenic nitrophenyl glycosides as substrates in aqueous buffer at pH 6.8 or pH 7.4, under which conditions all enzymes tested showed satisfactory activities. Initial inhibitor screening was done at 100 μM concentration. Compounds showing significant inhibition under these conditions were characterized more closely by determining the competitive inhibition constant (Table 1 and Fig. 3). Remarkably, most compounds inhibited at least one enzyme in the nanomolar range. The tightest inhibitions were found for the *N*-benzyl-β-D-*galacto* configured inhibitor **5** with β-galactosidase from bovine liver (K_i = 12 nM), and for the *N*-phenylpropyl-β-D-*gluco* configured inhibitor **4** with β-glucosidase from almonds (K_i = 4 nM).

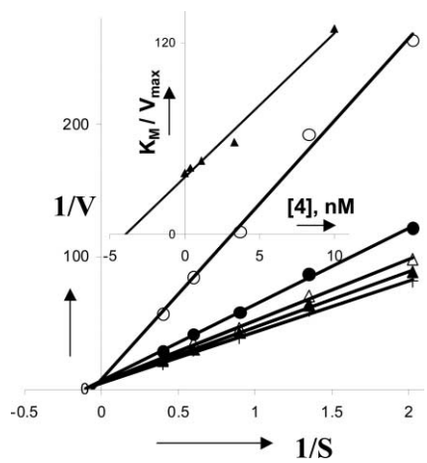


Fig. 3 Lineweaver–Burk plot and Dixon replot (insert) of almond β-glucosidase inhibition by **4**. The K_i was determined at [S] = 2.5, 1.67, 1.11, 0.74 and 0.49 mM and [I] = 10, 3.3, 1.1, 0.37 and 0 nM.

Structure–activity relationships

The structure–activity relationship of glycosidase inhibition was investigated by comparative analysis including a broad family of aminocyclopentitols reported in the literature, including data from our group together with data by Ogawa *et al.*[16] and by Jäger *et al.*[11] (Fig. 4). Inhibitory data for α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase and α-mannosidase were considered since these were available for almost all compounds. For each compound the strongest inhibitory data reported in each enzyme class was used as entry irrespective of the exact enzyme type used, and reported as pIC_{50} (= −log (IC_{50})) or pK_i (= −log (K_i)). An IC_{50} value of 10 mM was used in the case of missing data or no detected inhibition. The inhibitor series was then ordered by clustering using the multivariate analysis software Vista.[17] The inhibition pattern was visualized by a grayscale rendering of inhibition constants. In addition, the relationship among the different inhibitors was rendered by the hierarchical tree (Table 2). Inhibitors were grouped in clusters of two to eleven inhibitors displaying similar inhibition profiles, with only a few inhibitors showing unique patterns of inhibition.

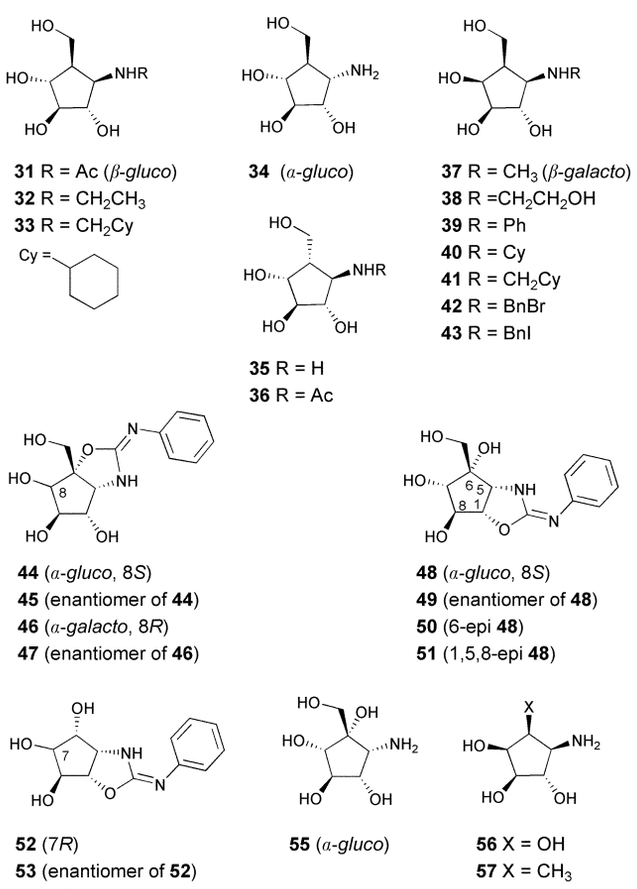


Fig. 4 Structures of glycosidase inhibitors.

Gluco-galacto selectivity

The dataset was first analyzed in terms of *gluco*/*galacto* selectivity since most inhibitors showed pronounced cross-reactivities with both enzyme types. A two-dimensional plot was constructed in which the *x*-coordinates represented the β-glucosidase/β-galactosidase selectivity and the *y*-coordinate the corresponding α-glucosidase/α-galactosidase selectivity (Fig. 5). Two groups of inhibitors were visible in this display. A first group appeared at negative *y*-coordinates representing inhibitors with α-glucosidase inhibition and comprised compounds with either α-*gluco* or β-*gluco* stereochemistry. The *N*-carboxybenzyl substituted aminocyclopentitol **1** showed the most

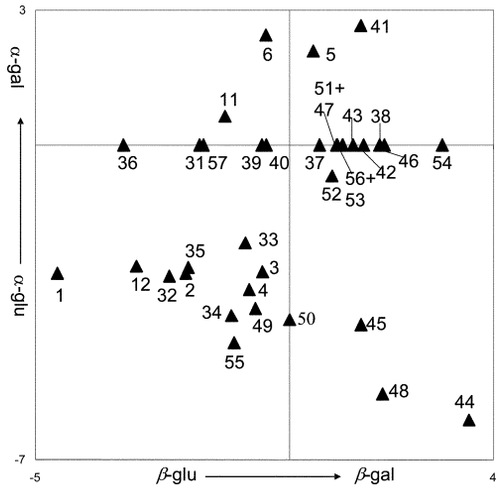


Fig. 5 XY-plot of inhibitors according to the *gluco*/*galacto*-inhibition selectivities. The coordinates for each compound are calculated as x = log K_i(β-glucosidase) − log K_i(β-galactosidase); y = log K_i(α-glucosidase) − log K_i(α-galactosidase) using the data in Tables 1 and 2. An IC_{50} value of 10 mM was assumed in the case of missing data or no detected inhibition. Compound structures in Figs. 1 and 4.

pronounced β-*gluco*/β-*galacto* selectivity and was in fact the only inhibitor showing no detectable cross-reactivity with β-galactosidase, as measured with the enzyme from bovine liver. This group of α-glucosidase reactive inhibitors also contained compound **44** reported by Ogawa, which showed the most pronounced selectivity for a β-galactosidase (enzyme from bovine liver) against a β-glucosidase (enzyme from almonds) due to an absence of cross-reactivity with the latter enzyme. A second group of inhibitors appeared at zero or positive *y*-coordinates, corresponding to either no α-type inhibition or significant α-galactosidase inhibition. This group contained mostly *galacto*-configured inhibitors. However no aminocyclopentitol showed a complete selectivity for α-galactosidase against α-glucosidase, including the α-*galacto*-configured aminocyclopentitol **6** reported here. This compound was nevertheless noteworthy since it displayed the strongest activity against α-galactosidase in the series, in agreement with its stereochemistry.

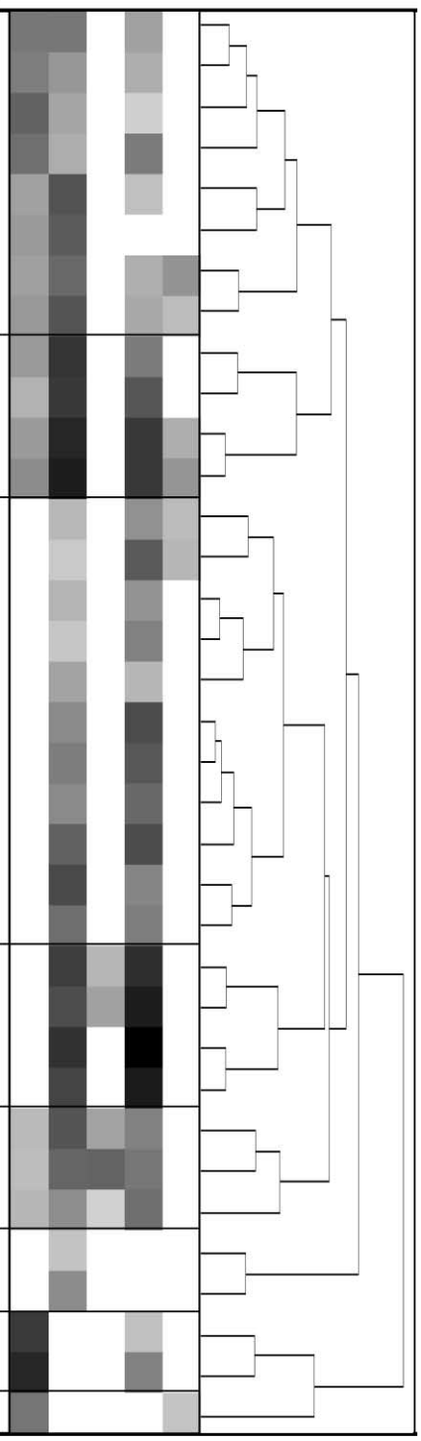
Anomer selectivity

The issue of anomer selectivity of the different inhibitors was analyzed by constructing a second plot reporting the α-/β-glucosidase selectivity on the *x*-axis and the α-/β- galactosidase selectivity on the *y*-axis (Fig. 6). The best β-selectivity in both the *gluco* and *galacto* series occurred in the *N*-bromobenzyl-β-*galacto* aminocyclopentitol **42** reported by Jäger,[11] which was also the most potent inhibitor. The strongest α-selectivities were observed with the *N*-benzyl-α-*galacto* aminocyclopentitol **6** reported here for α-galactosidase and the α-*gluco*-configured derivative **44** of Ogawa for α-glucosidase (enzyme from yeast) against β-glucosidase (enzyme from almonds).

The weak cross-reactivity with α-mannosidases reported for seven compounds in the series could not be rationalized on the basis of structural features or other inhibitory activities since it was associated with aminocyclopentitols of various stereochemistries and substitution patterns also showing other inhibition activities. Also, none of the aminocyclopentitols in the series possessed an α-*manno* stereochemistry. These weak activities (K_i = 9.1 μM in the best case of **35**) could however be compared with a competitive inhibition constant K_i = 0.062 μM reported for the *N*-methyl-α-*manno* aminocyclopentitol **58** (Fig. 7) against jack bean α-mannosidase.[7] This comparison clearly showed that the correct stereochemical pattern was also correlated with strong inhibitory activity against jack bean α-mannosidase. No data were reported for a β-mannosidase

Table 2 Glycosidase inhibition constants (μM) for aminocyclopentitol inhibitors (structures in Figs. 1 and 4). No data are given if the inhibitor was reported to be inactive for the given enzyme. "—" indicates missing data. Grayscale representation of table data at right. The inhibitors were ordered for similarity by hierarchical clustering of inhibition data (hierarchical tree at right). [a] Relative configuration of inhibitors in analogy to glycosides. Origin of enzymes: [b] yeast, [c] *Caldocellum saccharolyticum*, [d] bovine liver, [e] almonds, [f] *E. coli*, [g] jack beans, [h] green coffee beans, [i] *Aspergillus niger*, [k] *Aspergillus oryzae*

			Glycosidases				
Nr.	conf[a]	Lit	α-glu	β-glu	α-gal	β-gal	α-man
34	αglu	[11]	1.6[b]	1.5[c]	-	21[d]	-
49	-	[16]	2.3[b]	11[e]	[f]	52[d]	[g]
55	αglu	[16]	0.4[b]	29[e]	[f]	360[d]	[g]
45	-	[16]	1[b]	48[e]	[f]	1.9[d]	[g]
12	βglu		20[b]	0.14[c]	[h]	145[d]	[g]
1	βglu		14[b]	0.27[c]	[h]	[d]	[g]
35	-	[10]	19[b]	0.6[c]	[h]	59[d]	9.1[g]
32	βglu	[10]	12[b]	0.16[c]	[h]	37[d]	115[g]
2	βglu		14[b]	0.018[e]	[h]	2[d]	[g]
33	βglu	[11]	67[b]	0.024[e]	-	0.18[d]	-
3	βglu		15[b]	0.007[e]	[h]	0.024[d]	50[g]
4	βglu		6[b]	0.004[e]	[h]	0.025[d]	10[g]
56	βgal	[16]	[b]	84[e]	[f]	7.8[f]	110[g]
54	-	[16]	[b]	240[e]	[f]	0.24[f]	78[g]
51	-	[16]	[b]	72[e]	[f]	8.6[f]	[g]
46	αgal	[16]	[b]	210[e]	[f]	2.9[f]	[g]
39	βgal	[11]	-	23[c]	[h]	81[i]	-
38	βgal	[11]	-	5[e]	[h]	0.085[k]	-
53	βgal	[16]	[b]	2.2[e]	[f]	0.2[f]	[g]
47	-	[16]	[b]	4.9[e]	[f]	0.57[d]	[g]
37	βgal	[11]	-	0.35[c]	[h]	0.09[d]	-
57	βgal	[11]	-	0.08[c]	[h]	4[d]	-
40	βgal	[11]	-	0.83[e]	[h]	2.4[f]	-
5	βgal		[b]	0.035[c]	80[h]	0.012[d]	[g]
41	βgal	[11]	-	0.1[e]	22[h]	0.004[d]	-
42	βgal	[11]	-	0.017[e]	[h]	0.0006[f]	-
43	βgal	[11]	-	0.053[e]	[h]	0.003[d]	-
11	βgal	[9]	100[b]	0.16[c]	23[h]	3[d]	[g]
6	αgal		120[b]	0.51[e]	0.43[h]	1.5[d]	[g]
52	-	[16]	82[b]	6.4[e]	400[f]	0.94[f]	[g]
31	βgal	[10]	[b]	170[c]	[h]	[d]	[g]
36	-	[10]	[b]	5.4[e]	[h]	[d]	[g]
48	αglu	[16]	0.029[b]	[e]	[f]	150[d]	[g]
44	αglu	[16]	0.008[b]	[e]	[f]	3[d]	[g]
50	-	[16]	1.3[b]	[e]	[f]	[d]	190[g]



inhibition by this compound. Similarly, the possible activity of α-L-*fuco* aminocyclopentitols **9** and **10** against β-L-fucosidases has not been tested. Thus, although our *N*-benzyl-α-*galacto* aminocyclopentitol **6** was clearly the strongest and most selective aminocyclopentitol in the series against α-galactosidase, its significant cross-inhibition against bovine liver β-galactosidase and two different β-glucosidases might indicate a general limitation of *N*-alkyl aminocyclopentitols at providing α-selective inhibitors.

The relatively weaker anomer selectivity displayed by the α-*galacto* aminocyclopentitol **6** in comparison to the β-configured analogs might be caused by conformational flexibility of the *N*-alkyl group. This *N*-alkyl substituent may be considered as a mimic of the aglycon leaving group, and structural flexibility probably allows it to adopt a pseudo-equatorial conformation suitable to bind to the active site of β-selective glycosidases. Such structural flexibility might also explain the fact that monoclonal antibodies produced against the α-L-*fuco*

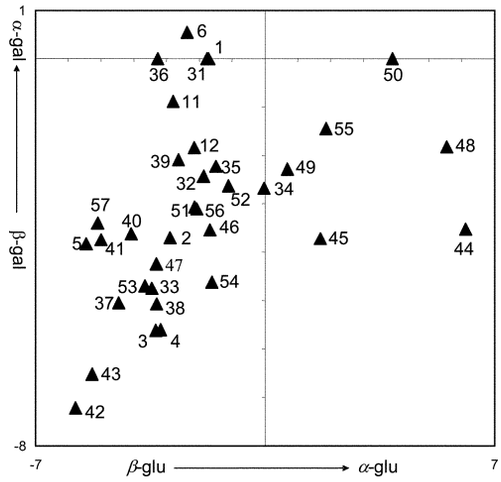


Fig. 6 XY-plot of inhibitors according to the α-/β-inhibition selectivities. The coordinates for each compound are calculated as x = log K_i(β-glucosidase) − log K_i(α-glucosidase); y = log K_i(β-galactosidase) − log K_i(α-galactosidase) using the data in Tables 1 and 2. An IC_{50} value of 10 mM was assumed in the case of missing data ("—" in Table 2) or no detected inhibition (no data in Table 2). Compound structures in Figs. 1 and 4.

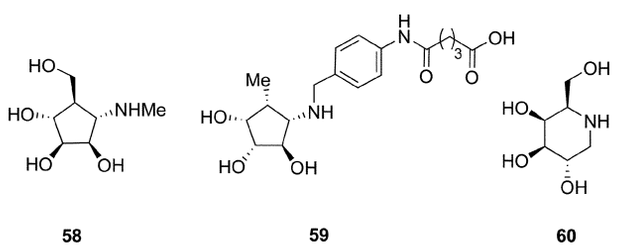


Fig. 7 Structures of *N*-methyl-α-*manno* aminocyclopentitol **58**, α-L-*fuco* hapten **59** and 1-deoxy-*galacto*-nojirimycin **60**.

aminocyclopentitol **59** are not catalytic.[18] The bicyclic derivative **44**[16] is clearly an α-selective and very potent aminocyclopentitol inhibitor. This α-selectivity might be caused by its bicyclic nature providing structural rigidity in the sense of a locked α-orientation of the leaving group mimic. However the corresponding bicyclic α-*galacto* aminocyclopentitol **46**[16] shows only weak β-glucosidase and β-galactosidase activity and no activity against α-galactosidase or α-glucosidase. The problem of selective and potent α-galactosidase inhibition is elegantly solved by the *galacto*-deoxynojirimycin **60** (K_i = 0.0016 μM for α-galactosidase from green coffee bean, K_i = 12.5 μM for β-galactosidase from *E. coli*, K_i = 540 μM for β-glucosidase from almonds).[19]

Conclusion

A series of *N*-alkyl aminocyclopentitols with β-*gluco*, β-*galacto* and α-*galacto*- stereochemistry was prepared and evaluated for glycosidase inhibition. The most potent inhibitions were observed whenever the stereochemistry was matched between aminocyclopentitol and glycosidase, including the α- or β-configuration of the *N*-alkyl-amino group mimicking the protonated leaving group at the anomeric position. This principle also emerged from a comparative study including thirty-five different aminocyclopentitols with varying stereochemistry and substitution pattern. Since the source of each enzyme also influences inhibition, the details of the classification of inhibitors produced cannot be used to predict inhibitor selectivities against any glycosidase enzyme. Most interestingly, the structure–activity relationships show that the selectivity and potency of glycosidase inhibition also depend on the nature of the aglycon mimic. Systematic variations of this group might be of interest to optimize aminocyclopentitols against enzymes of therapeutic relevance.

Experimental section

General

Enzymes and reagents were purchased from Fluka, Sigma or Aldrich. All solvents used in reactions were bought in p.a. quality or distilled and dried prior to use. Solvents for extractions were distilled from technical quality. Sensitive reactions were carried out under nitrogen or argon, the glassware was heated under high vacuum. Chromatographic purifications (flash) were performed with Silicagel 60 from Merck or Fluka (0.04–0.063 nm; 230–400 mesh ASTM). HPLC was carried out with mixtures of MilliQ deionized water containing 0.1% TFA and HPLC-grade acetonitrile. Analytical HPLC was performed in a Waters chromatography system (996 Photodiode Array Detector) using a Vydac column (218TP54 RP-C18, 300 Å pore size, 0.4 × 22 cm) with a flow rate of 1.0 mL min^{-1} (isocratic mode). Preparative HPLC was done with a Waters Delta Prep LC4000 system (Waters 486 Tunable Absorbance Detector) using a Waters prepak Cartridge 500 g column (RP-C18 20 mm, 300 Å pore size), flow rate 100 mL min^{-1} (gradients 1% min^{-1} CH_3CN). TLC controls were performed with pre-coated TLC plates Sil G-25 UV_{254} from Macherey-Nagel followed by coloration with cerium solution (10.5 g Ce(IV)-sulfate, 21 g phosphomolybdic acid, 60 mL conc. H_2SO_4 in 900 mL water) or ninhydrin (5 g in 100 mL of ethanol) followed by heating. Optical rotations were determined in a Perkin Elmer 241 polarimeter using a 10 cm cell. Infrared spectra were recorded in a Perkin Elmer 1600 series FTIR. MS and HRMS analyses were provided by the "Service of Mass Spectrometry" of the Department of Chemistry and Biochemistry. ^{1}H- and ^{13}C-NMR spectra were registered on Bruker AC-300 (300 MHz) and DRX 500 instruments. Chemical shifts δ are given in ppm, coupling constants J in hertz (Hz).

(1*R*,2*S*,3*S*,4*R*,5*R*)-4-[(2,3,4-Trihydroxy-5-hydroxymethyl-cyclopentylamino)methyl]benzoic acid (1). A solution of **12** (10.9 mg, 0.06 mmol) in DMF (0.3 mL) with CsOH monohydrate (18.9 mg, 0.11 mmol) and 4 Å molecular sieve (30 mg) was heated to 80 °C for 20 min. At 25 °C 4-(bromomethyl)-benzoic acid (13.1 mg, 0.6 mmol) was added. The mixture was heated to 80 °C for 12 hours, concentrated under vacuum, taken up in water, acidified with TFA, filtrated and purified by RP-HPLC. The compound **1** was obtained (4.5 mg, 16%) after lyophilization as a white solid; $[a]_D^{20}$ = +11.2 (c = 0.42, H_2O); ^{1}H NMR (300 MHz, MeOD): δ = 8.06 (d, 2H, J = 8.5), 7.59 (d, 2H, J = 8.5), 4.43 (dd, 2H, J = 37.7, 13.4), 4.00 (t, 1H, J = 8.1), 3.88 (dd, 1H, J = 11.6, 4.2), 3.8–3.55 (m, 4H), 2.32–2.21 (m, 1H); ^{13}C NMR (75 MHz, MeOD): δ = 169.0, 137.6, 131.4, 130.8, 82.3, 77.9, 75.4, 62.8, 60.0, 51.3, 45.6; HR-ESI-MS calcd for $C_{14}H_{20}NO_6$ [M + 1]: 298.1291; found 298.1303.

General procedure for the synthesis of 2–4 by reductive alkylation of 12. A solution of **12** (7.8 mg, 0.04 mmol) in MeOH (0.5 mL) was added to Al_2O_3 (32.9 mg) and concentrated under vacuum. Then an excess of the corresponding aldehyde (0.4 mmol) was added. The mixture was stirred at 50 °C for 6 hours. MeOH (1 mL), dried 4 Å molecular sieve (45 mg) followed by small portions of $NaBH_4$ (38 mg, 1 mmol) were added. After stirring at 25 °C for 12 hours the mixture was concentrated under vacuum, taken up in water, acidified with TFA, filtrated and purified by RP-HPLC. Lyophilisation gave the desired compounds as white TFA salts.

(1*R*,2*S*,3*S*,4*R*,5*R*)-4-Benzylamino-5-hydroxymethyl-cyclopentane-1,2,3-triol (2). Compound **2** was obtained as a white TFA salt in 43% yield; $[a]_D^{20}$ = +13.6 (c = 0.58, H_2O); ^{1}H NMR (300 MHz, MeOD): δ = 7.55–7.42 (m, 5H), 4.38 (dd, 2H, J = 41.2, 13.2), 4.02 (t, 1H, J = 8.1), 3.92 (dd, 1H, J = 11.4, 4.1), 3.82–3.76 (m, 4H), 2.34–2.23 (m, 1H); ^{13}C NMR (75 MHz, MeOD): δ = 137.6, 131.4, 130.8, 82.3, 77.9, 75.4, 62.8, 60.0,

51.3, 45.6; MS (FAB$^+$): *m*/*z* (%) = 254 (M^+, 94), 176 (8), 154 (100), 149 (14), 107 (30); HR-ESI-MS calcd for $C_{13}H_{20}NO_4$ [M^+1]: 254.1392; found 254.1387.

(1*S*,2*S*,3*R*,4*R*,5*R*)-4-Hydroxymethyl-5-(2-phenylethylamino)-cyclopentane-1,2,3-triol (3). Compound **3** was obtained as a white TFA salt in 49% yield; $[\alpha]_D^{20}$ = +10.2 (*c* = 0.52, H_2O); ^{1}H NMR (300 MHz, MeOD): δ = 7.37–7.23 (m, 5H), 3.97–3.44 (m, 8H), 3.12–2.96 (m, 2H), 2.31–2.22 (m, 1H); ^{13}C NMR (75 MHz, MeOD): δ = 137.77, 130.03, 129.79, 128.33, 82.10, 77.81, 74.98, 62.73, 59.56, 49.75, 45.47, 33.25; HR-ESI-MS calcd for $C_{14}H_{22}NO_4$ [M^+1]: 268.1549; found 268.1543.

(1*S*,2*S*,3*R*,4*R*,5*R*)-4-Hydroxymethyl-5-(3-phenylpropyl-amino)cyclopentane-1,2,3-triol (4). Compound **4** was obtained as a white TFA salt in 51% yield; $[\alpha]_D^{20}$ = +12.4 (*c* = 0.51, H_2O); ^{1}H NMR (300 MHz, MeOD): δ = 7.35–7.15 (m, 5H), 3.93–3.68 (m, 4H), 3.63–3.57 (t, 1H, *J* = 7.7), 3.56–3.46 (t, 1H, *J* = 9.0), 3.12–3.0 (m, 1H), 2.76–2.66 (t, 2H, *J* = 7.5), 2.28–2.18 (m, 1H), 2.09–1.96 (m, 2H); ^{13}C NMR (75 MHz, MeOD): δ = 141.6, 129.7, 129.4, 129.3, 127.4, 82.2, 77.9, 75.1, 62.9, 59.6, 48.3, 45.6, 33.6, 28.9. HR-ESI-MS calcd for $C_{15}H_{24}NO_4$ [M^+1]: 282.1705; found 282.1694.

(1*S*,2*S*,3*S*,4*R*,5*R*)-4-Benzylamino-5-(hydroxymethyl)cyclo-pentane-1,2,3-triol (5). A solution of **24** (5 mg, 13 μmol) in water (2 mL) and 0.3 M aq. HCl (0.25 mL) was heated at 60 °C for 12 hours and lyophilized to give **5** (3.2 mg, 100%) as a colorless solid. $[\alpha]_D^{20}$ = +9.54 (*c* = 1.23, $CHCl_3$); ^{1}H NMR (300 MHz, D_2O): 7.45 (m, 5H), 4.24 (m, 4H), 3.97 (dd, 1H, *J* = 5.88, 4.41), 3.76 (dd, 1H, *J* = 11.40, 6.99), 3.64 (dd, 1H, *J* = 11.37, 6.96), 3.54 (dd, 1H, *J* = 7.74, 7.71), 2.37 (m, 1H); ^{13}C NMR (75 MHz, D_2O + 5% DMSO-d_6): 132.2, 131.8, 80.2, 74.1, 72.5, 62.0, 61.1, 52.8, 47.2; MS: 253, 91, 75.

(1*S*,2*S*,3*S*,4*S*,5*R*)-4-Benzylamino-5-(hydroxymethyl)cyclo-pentane-1,2,3-triol (6). A solution of amine **30** (1 mg; 3.4 μmol) in water (2 mL) and aq. HCl (0.3 M, 250 μl) was heated at 60 °C for 12 hours and evaporated. The residue was purified by RP-HPLC. Lyophilization gave **6** (0.9 mg, 100%) as a colorless solid. ^{1}H NMR (300 MHz, D_2O): 7.42 (m, 5H), 4.24 (m, 4H), 3.97 (dd, 1H, *J* = 5.9, 4.4), 3.76 (dd, 1H, *J* = 11.0, 7.0), 3.64 (dd, 1H, *J* = 11.0, 7.0), 3.55 (dd, 1H, *J* = 8.1, 7.7), 2.37 (m, 1H); ^{13}C NMR (75 MHz, D_2O + 5% DMSO-d_6): 132.2, 131.2, 131.1, 130.8, 79.2, 73.1, 71.5, 61.0, 60.0, 52.0, 46.2.

(1*R*,2*S*,3*S*,4*R*,5*R*)-4-Amino-5-hydroxymethyl-cyclopentane-1,2,3-triol hydrochloride (12). Pentaacetate **17** (132.5 mg, 0.36 mmol) was dissolved in 1.2 M HCl (4 mL) and heated to reflux for 15 hours. Evaporation gave **12** (66.2 mg, 100%) as a colorless solid. ^{1}H NMR (300 MHz, MeOD): δ = 3.88–3.72 (m, 4H), 3.62 (dd, 1H, *J* = 8.5, 7.7), 3.50 (t, 1H, *J* = 9.2); ^{13}C NMR (75 MHz, MeOD): δ = 81.8, 78.2, 75.7, 59.7, 56.2, 45.3; HR-ESI-MS calcd for $C_6H_{14}NO_4$ [M + 1]: 164.0923; found 164.0927.

(2*S*,3*R*,4*R*,5*R*)-2,3,4,6-Tetrakis-benzyloxy-5-hydroxyhexanal *O*-benzyloxime (13). A suspension of tetra-*O*-benzyl-gluco-pyranose (6.03 g, 9.34 mmol) and *O*-benzylhydroxylamine hydrochloride (2.13 g, 13.34 mmol) in MeOH (85 mL) and pyridine (6 mL) was stirred at 60 °C for 15 hours. After concentration under vacuum the resulting residue was dissolved in AcOEt (240 mL) and washed with 1 M HCl, saturated $NaHCO_3$ and brine (240 mL each). The aqueous layers were washed with AcOEt (2 × 240 mL). The organic layers were dried over $MgSO_4$ and concentrated under vacuum. FC (hexane–AcOEt, 4 : 1) gave a 5 : 1 mixture of *E*/*Z* isomers of oxime ether **13** (6.84 g, 95%) as a colorless oil. R_f = 0.45 (hexane–AcOEt, 3 : 1); $[\alpha]_D^{27}$ = +24.3 (*c* = 0.70 in $CHCl_3$); IR (neat): *v* = 3499*w br*, 3090*w*, 3064*m*, 3032*m*, 2867*m*, 1497*s*, 1455*s*; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.50 (dd, 1H, *J* = 8.1, 1.8; H-1 *E* isomer, integration = 0.5), 7.4–7.2 (m, 25H), 6.95 (H-1 *Z* isomer, integration = 0.1), 5.11 (s, 1H), 4.82–4.35 (m, 10H), 3.95 (m, 1H), 3.85–3.72 (m, 2H), 3.65–3.55 (m, 2H); ^{13}C NMR (75 MHz, MeOD): δ = 129.39, 129.33, 129.31, 129.19, 128.98, 128.94, 128.82, 128.78, 128.64, 128.57, 91.79, 82.93, 81.71, 79.23, 76.54, 75.92, 74.35, 73.71, 71.15, 70.04. MS (FAB$^+$): *m*/*z* (%) = 646 (M^+, 100), 538 (20), 181 (70), 154 (37), 136 (33); HR-LSIMS calcd for $C_{41}H_{44}NO_5$ [M^+1]: 646.3138; found 646.3167.

Imidazole-1-carbothioic acid *O*-[(1*R*,2*R*,3*R*,4*S*)-2,3,4-tris-benzyloxy-5-benzyloxyimino-1-benzyloxymethyl]pentyl ester (14). Oxime ether **13** (6.6 g, 10.22 mmol) and 1,1′-thiocarbonyldiimidazole (2.8 g, 15.73 mmol) were dissolved in benzene (102 mL). The mixture was heated to reflux under an atmosphere of nitrogen for 4 hours. After evaporation of the solvent FC (hexane–AcOEt, 2 : 1) gave a 5 : 1 mixture of *E*/*Z* isomers of imidazolide **14** (7.4 g, 96%) as a colorless oil. R_f = 0.42 (hexane–AcOEt, 2 : 1); $[\alpha]_D^{27}$ = +30.5 (*c* = 0.96 in $CHCl_3$); IR (neat): *v* = 3090*w*, 3065*m*, 3032*m*, 2926*m*, 2870*m*, 1737*s*, 1497*s*, 1455*s*, 1391*s*, 1328*s*, 1287*s*, 1244*s*, 1232*s*; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.17 (s, 1H, *E* isomer integration = 0.84 and *Z* isomer integration = 0.17), 7.53 (d, 1H, *J* = 8.5), 7.47 (s, 1H), 7.4–7.1 (m, 25H), 5.75 (m, 1H), 5.10 (s, 2H), 4.73–4.32 (m, 10H), 4.18 (t, 1H, *J* = 4.2), 4.01 (dd, 1H, *J* = 2.6, 11.4), 3.82 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 181.2, 151.6, 149.19, 137.52, 131.40, 129.20, 129.12, 129.08, 129.04, 129.03, 129.01, 128.99, 128.96, 128.87, 128.78, 128.68, 128.58, 128.56, 128.51, 128.48, 128.47, 128.38, 128.36, 128.31, 118.71, 83.77, 79.97, 78.10, 77.10, 76.70, 75.15, 75.06, 73.83, 72.17, 71.84, 67.96; HR-LSIMS calcd for $C_{45}H_{45}N_3O_6S$ [M^+1]: 756.3107; found 756.3114.

***O*-Benzyl-*N*-[(1*R*,2*S*,3*S*,4*R*,5*R*)-2,3,4-tris-benzyloxy-5-benzyloxymethylcyclopentyl]-*N*-hydroxylamine (15) and *O*-benzyl-*N*-[(1*R*,2*S*,3*S*,4*R*,5*S*)-2,3,4-tris-benzyloxy-5-benzyloxy-methylcyclopentyl]-*N*-hydroxylamine (16).** A solution of imidazolide **14** (7.13 g, 9.43 mmol) in benzene (500 mL) was heated under an atmosphere of nitrogen to reflux. A solution of AIBN (778 mg, 4.74 mmol) and Bu_3SnH (5.4 mL, 20.38 mmol) in benzene (23 mL) was added dropwise over 30 min. After 4 h the solvent was evaporated. In a first FC (hexane–EtOAc 4 : 1) the two isomers (5.88 g, 99%) were separated from impurities and then separated by FC to give **15** (3.2 g, 55%) and **16** (2.06 g, 35%) as colorless oils. Data for **15**: R_f = 0.55 (hexane–EtOAc 4 : 1); $[\alpha]_D^{27}$ = −15.6 (*c* = 1.04 in $CHCl_3$); IR (neat): *v* = 3089*w*, 3065*m*, 3032*m*, 2866*m*, 1737*m*, 1497*m*, 1455*s*, 1363*s*; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.4–7.2 (m, 25H), 6.0 (s, 1H), 4.60–4.40 (m, 10H), 3.98 (m, 2H), 3.80 (m, 1H), 3.61 (m, 3H), 2.53 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 138.8, 138.4, 138.0, 128.6, 128.54, 128.5, 128.1, 128.0, 127.9, 127.7, 89.9, 84.2, 83.2, 76.5, 73.4, 72.2, 72.1, 71.1, 68.0, 63.9, 44.1; MS (FAB+): *m*/*z* (%) = 630 (M^+, 87), 522 (16), 503 (18), 463 (24), 445 (35), 387 (30), 329 (22); HR-ESI-TOF-MS calcd for $C_{41}H_{44}NO_6$ [M^+1]: 630.3246; found 630.3254.

Data for **16**: R_f = 0.50 (hexane–EtOAc 4 : 1); $[\alpha]_D^{27}$ = +8.7 (*c* = 1.01 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.4–7.2 (m, 25 H), 4.70–4.59 (m, 10H), 4.04–3.95 (m, 3H), 3.81 (dd, 1H, *J* = 9.2, 7.4), 3.68 (dd, 1H, *J* = 9.2, 6.6), 3.45 (dd, 1H, *J* = 8.8, 5.9), 2.61–2.50 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 139.33, 139.20, 139.02, 138.58, 129.05, 129.03, 129.01, 128.99, 128.98, 128.96, 128.45, 128.40, 128.36, 128.30, 128.27, 128.19, 128.15, 87.19, 85.63, 81.68, 77.13, 73.89, 72.59, 72.44, 72.39, 69.24, 68.13, 43.28.

Acetic acid *O*-(1*R*,2*R*,3*S*,4*S*,5*R*)-2,3,4-triacetoxy-5-acetyl-amino-cyclopentylmethyl ester (17). Hydrogenation of D-amino-cyclopentitol hydroxylamine **15** (1.08 g, 1.72 mmol) with 10% Pd/C (249.7 mg) in acetic acid (22 mL) in a 500 mL autoclave under 12 bar of hydrogen at 25 °C during 4 days resulted in complete debenzylation. The mixture was filtrated over Celite

and concentrated under vacuum. After adding DMAP (9.1 mg, 0.07 mmol) and pyridine (5 mL) the solution was cooled to 0 °C and acetic anhydride (5 mL) was slowly added. After 20 hours stirring at 25 °C the mixture was concentrated under vacuum. FC (hexane–EtOAc 1 : 4) gave the pentaacetate **17** (514 mg, 80%) as a yellow oil. R_f = 0.22 (hexane–EtOAc 1 : 4); $[a]_D^{27}$ = −31.3 (c = 0.88 in $CHCl_3$); IR (neat): ν = 3500–3400*w*, 3300*m br*, 3074*w*, 2966*w*, 1745*s*, 1661*m*; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.67 (d, 1H, J = 6.7), 5.35–5.25 (m, 2H), 5.10 (t, 1H, J = 4.8), 4.55 (dd, 1H, J = 16.6, 6.6), 4.25 (dd, 1H, J = 11.6, 4.4), 4.08 (dd, 1H, J = 11.8, 4.4), 2.65 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ =172.12, 171.16, 171.13, 170.87, 170.57, 78.72, 78.27, 76.79, 62.21, 53.34, 43.72, 23.56, 21.60, 21.47, 21.43, 21.39; MS: *m*/*z* (%) = 372 (0.1), 538 (0.4), 330 (0.5), 314 (0.8), 193 (45), 151 (100); HR-ESI-TOF-MS calcd for $C_{16}H_{23}NO_9$ [M^+1]: 374.1451; found 374.1438.

Methyl 2,3-*O*-isopropylidene-β-D-lyxofuranoside (18). To a solution of D-(−)-lyxose (10.0 g, 66.6 mmol) in acetone (80 mL) and 2,2-dimethoxypropane (20.0 mL, 266 mmol) 70% aq. $HClO_4$ (4 mL) was added at 0 °C. After stirring for 2 hours at 25 °C methanol (14 mL) was added. The mixture was stirred for another 3 hours at 25 °C. The reaction was cooled to 0 °C and quenched with aq. Na_2CO_3 (9.6 g in 30 mL H_2O). The precipitate was filtered and the filtrate was concentrated. Extraction (Et_2O–brine) followed by vacuum distillation gave **18** (10.4 g, 84%) as a pale yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$): 4.94 (s, 1H), 4.78 (dd, 1H, J = 5.88, 3.68), 4.58 (d, 1H, J = 5.88), 4.00 (m, 3H), 3.34 (s, 3H), 1.54 (s, 3H), 1.31 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 113.3, 107.7, 85.8, 80.9, 80.0, 61.7, 55.3, 26.6, 25.2; MS: 204, 187, 173, 171, 123, 85, 59, 43.

(3*S*,4*S*,8*R*)-6,6-Dimethyl-3-methoxy-2,5,7-trioxabicyclo-[3.3.0]octanone (19). Pyridinium chlorochromate (52.7 g, 308 mmol) was added to a stirred solution of **18** (12.6 g; 615 mmol) in benzene (500 mL). The reaction mixture was heated to reflux in a Dean–Stark apparatus overnight. The organic phase was decanted and recovered. The residues were stirred in EtOAc (100 mL) several times. The combined organic phases were filtrated over Celite and evaporated. The crude product was purified by FC (hexane–EtOAc 7 : 1, R_f = 0.2) to give **19** (4.52 g, 39%) as a colorless solid; $[a]_D^{20}$ = +47.52 (c = 0.52, $CHCl_3$); IR (KBr): 2996*w*, 2942*w*, 1809*m*, 1455*w*, 1376*w*, 1359*w*, 1210*w*, 1156*w*, 1122*m*, 1105*m*, 1048*w*, 973*w*, 931*w*, 797*s*, 774*m*, 755*m*; ^{1}H NMR (300 MHz, $CDCl_3$): 5.18 (s, 1H), 4.65 (d, 1H, J = 5.52 Hz), 4.41 (d, 1H, J = 5.52), 3.35 (s, 3H), 1.25 (s, 3H), 1.19 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 171.2, 111.8, 102.8, 76.9, 72.0, 54.5, 24.1, 23.1; HR-MS calcd for $C_8H_{13}O_5$ [M^+1]: 189.0762; found 189.0769.

(4*R*,8*R*)-6,6-Dimethyl-5,7-dioxabicyclo[3.3.0]oct-2-en-1-one (20). A solution of dimethyl methylphosphonate (0.28 mL, 1 equiv.) in THF (20 mL) at −78 °C was treated with n-BuLi (1.73 mL, 1.52 M in hexane). Then a solution of lactone **19** (493 mg, 2.62 mmol) in THF was added. The reaction mixture was stirred 150 min at −78 °C, 20 min at −20 °C and then quenched with EtOAc (50 mL). Extraction (AcOEt–water) and purification by FC (hexane–AcOEt 2 : 1, R_f = 0.46) gave **20** (158 mg, 39%) as a colorless crystalline solid, mp: 69–70 °C; IR: 2993*w*, 2937*w*, 1735*s*, 1550*m*, 1382*m*, 1374*m*, 1210*m*, 1156*w*, 1096*m*, 1068*w*, 1007*w*; ^{1}H NMR (300 MHz, $CDCl_3$): 7.61 (dd, 1H, J = 5.88, 2.22), 6.22 (d, 1H, J = 5.88), 5.27 (ddd, 1H, J = 5.52, 2.22, 0.75), 4.46 (d, 1H, J = 5.52), 1.42 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): 203.0, 159.6, 134.3, 115.5, 78.6, 76.6, 27.4, 26.1; MS: 154, 129, 96, 85, 68, 43.

(2*R*,3*R*,4*S*,5*R*)-2,3-*O*-Isopropylidene-2,3-dihydroxy-4,5-epoxycyclopentanone (21). A solution of **20** (97 mg, 6.3 mmol) in methanol (5 mL) cooled to −50 °C was treated with 30% aq. H_2O_2 (143 µl, 2 equiv.) and 2 M NaOH (133 µl). The reaction was stirred at −50 °C during 1 hour and quenched by addition of dichloromethane (10 mL) at −50 °C. The mixture was poured onto sat. aq. NH_4Cl. Extraction (aq. NH_4Cl–CH_2Cl_2) followed by FC (hexane–AcOEt 2 : 1, R_f = 0.63) gave **21** (84 mg, 79%) as a colorless oil, $[a]_D^{20}$ = −84.92 (c = 0.455, $CHCl_3$); IR (KBr): 2990*m*, 2939*m*, 2361*m*, 2343*m*, 1763*s*, 1376*s*, 1213*s*, 1156*m*, 1086*s*, 969*w*, 865*m*; MS: 170, 155, 143, 129, 113, 85, 71, 59, 43; ^{1}H NMR (300 MHz, $CDCl_3$): 4.90 (br d, 1H, J = 5.52), 4.42 (br d, 1H, J = 5.52), 4.02 (br d, 1H, J = 2.19), 3.58 (br d, 1H, J = 2.19 Hz), 1.41 (s, 3H), 1.37 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 204.2, 115.0, 78.1, 76.1, 58.7, 55.1, 27.7, 25.7.

(2*S*,3*S*,4*S*,5*R*)-1-Methylene-2,3-*O*-isopropylidene-2,3-dihydroxy-4,5-epoxycyclopentane (22). A solution of epoxyketone **21** (62.1 mg, 0.365 mmol) in THF (1 mL) was added to a solution of "instant ylide" (PPh_3CH_2Br–$NaNH_2$, 304.0 mg, 0.365 mmol, 1 equiv.) in THF (1.5 mL). The reaction mixture was stirred at 25 °C for 1 hour, diluted in diethyl ether (10 mL), and filtered over Celite. Evaporation and purification by FC (Et_2O–pentane) gave **22** (61.4 mg, 100%) as a colorless oil, $[a]_D^{20}$ = −132.2 (c = 1.495, $CHCl_3$); IR (KBr): 3049*w*, 2952*m*, 2924*m*, 1735*w*, 1585*w*, 1486*w*, 1376*w*, 1270*w*; ^{1}H NMR (300 MHz, $CDCl_3$): 5.62 (d, 1H, J = 1.86 Hz), 5.53 (d, 1H, J = 1.86), 4.73 (d, 1H, J = 5.88), 4.67 (d, 1H, J = 5.88), 3.84 (d, 1H, J = 1.83), 3.80 (d, 1H, J = 2.2), 1.43 (s, 3H), 1.37 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 147.4, 118.0, 113.5, 81.1, 79.8, 60.0, 59.8, 28.2, 26.2.

(1*R*,2*S*,3*R*,4*S*)-1-(*N*-Benzyl)-(*N*-*tert*-butoxycarbonyl)amino-3,4-*O*-isopropylidene-5-methylenecyclopentane-2,3,4-triol (23). Epoxyalkene **22** (67 mg, 0.4 mmol) was stirred in neat benzylamine (2.5 mL) for 90 min at 25 °C. The benzylamine was evaporated under vacuum. After FC (hexane–AcOEt 1 : 1, R_f = 0.67) the product still was contaminated with benzylamine. ^{1}H NMR (300 MHz, $CDCl_3$): 7.32 (m, 5H), 5.52, 5.35 (2s, 1H), 4.91 (d, 1H, J = 6.60), 4.38 (ddd, 1H, J = 6.24, 1.83, 1.08), 4.04 (dd, 1H, J = 3.66, 1.83), 3.94, 3.74 (2d, 1H, J = 12.87), 3.39 (d, 1H, J = 2.58), 1.41 (s, 3H), 1.33 (s, 3H); HR-MS calcd for $C_{16}H_{21}NO_3$: 275.1521; found: 275.1524. This intermediate was dissolved in water (2 mL) and AcOEt (2 mL) and treated with di-*tert*-butyl dicarbonate (58 mg, 0.27 mmol) and $NaHCO_3$ (40 mg). The mixture was stirred vigorously overnight at 25 °C. Extraction (brine–AcOEt) followed by FC (hexane–AcOEt, R_f = 0.25) gave **23** (36 mg, 24%) as a colorless oil, $[a]_D^{20}$ = +6.94 (c = 1.56, $CHCl_3$); IR (KBr): 3684*w*, 3618*w*, 3444*w br*, 3019*s*, 2978*m*, 2935*w*, 2400*w*, 1686*m*, 1522*w*, 1418*m*, 1368*m*, 1214*s*, 1163*m*, 1071*m*, 928*m*; ^{1}H NMR (300 MHz, $CDCl_3$): 7.31 (m, 5H), 5.43 (1s, 1H), 5.10 (br s, 2H), 4.85 (d, 1H, J = 6.99), 4.34 (s, 1H), 4.06 (m, 2H), 3.92 (m, 1H), 1.43 (s, 12H), 1.33 (s, 3H); HR-MS calcd for $C_{21}H_{29}NO_5$: 375.2046; found 375.2048.

(1*S*,2*R*,3*S*,4*R*,5*R*)-1,2-*O*-Isopropylidene-4-(*N*-benzyl)-(*N*-*tert*-butoxycarbonyl)-amino-5-(hydroxymethyl)-cyclopentane-1,2,3-triol (24). BH_3·THF (0.4 ml, 0.4 mmol) was added to a solution of **23** (15.4 mg, 40.3 µmol) in THF (0.5 mL) at 0 °C and the solution stirred for 2 hours at 25 °C. Water (5 mL) and $NaBO_3$ (186 mg, 30 equiv.) were added and the reaction was stirred for 90 minutes at 25 °C. Extraction (AcOEt–water) and purification by FC (hexane–AcOEt 2 : 1, R_f = 0.28) gave **24** (12.1 mg, 75%) as a colorless oil. $[a]_D^{20}$ = +13.6 (c = 1.84, $CHCl_3$); ^{1}H NMR (300 MHz in $CDCl_3$): 7.33 (m, 5H), 4.72 (dd, 1H, J = 7.74, 7.35), 4.46 (br s, 2H), 4.35 (dd, 1H, J = 7.31, 4.8), 4.20 (m, 1H), 3.74 (m, 3H), 1.56 (s, 3H), 1.46 (s, 9H), 1.32 (s, 3H), 1.45 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): 139.9, 129.6, 128.1, 127.3, 113.3, 84.6, 81.4, 77.9, 64.8, 60.4, 29.0, 27.1, 24.7; HR-MS calcd for $C_{21}H_{31}NO_6$: 393.2151; found 393.2170.

(1*R*,2*R*,3*R*,4*S*)-1-Bromo-3,4-*O*-isopropylidene-5-methylene-cyclopentane-2,3,4-triol (25). To a solution of epoxyolefin **22** (83 mg, 0.50 mmol) in acetic acid (1.67 mL) was added LiBr (215 mg, 5 equiv.) and the mixture was stirred at room temperature

during 2 hours. Co evaporation with toluene and purification by FC (hexane–EtOAc 2 : 1, $R_f = 0.53$) gave **25** (65.4 mg, 53%) as a colorless oil. $[\alpha]_D^{20} = +43.20$ ($c = 1.32$, $CHCl_3$); IR (KBr): 2958*s*, 2928*s*, 2873*m*, 2854*m*, 1451*w*, 1381*w*, 1209*w*, 1158*w*, 1077*w*, 868*w*; ^{1}H NMR (300 MHz, $CDCl_3$): 5.63 (ddd, 2H, J = 4.0, 1.8, 1.5 Hz), 5.00 (dd, 1H, J = 1.5, 1.1), 4.50 (m, 2H), 4.39 (dd, 1H, J = 4.4, 2.9), 1.56 (s, 3H), 1.36 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 147.6, 119.6, 113.5, 85.5, 83.3, 80.4, 53.0, 27.2, 25.6; MS: 251, 249 ([M^+1]), 155.

(1*R*,2*R*,3*S*,4*S*)-1-Bromo-2-*O*-(*N*-benzylcarbamoyl)-3,4-*O*-isopropylidene-5-methylenecyclopentane-2,3,4-triol (26). To a solution of bromoolefin **25** (15.7 mg, 63.0 μmol) in dry dichloromethane (0.5 mL) were added at 0 °C, benzyl isocyanate (8.9 μl, 100 μmol) and dry triethylamine (3.5 μl, 25.2 μmol). After stirring overnight at 25 °C, the mixture was diluted in diethyl ether. The organic phase was washed with water (5 mL) and the aqueous phase was extracted with diethyl ether (2 × 10 mL). The recombined organic phases were dried (Na_2SO_4) and evaporated. Purification by FC (hexane–AcOEt, $R_f = 0.62$) gave **26** (16.6 mg, 69%) as a colorless oil. $[\alpha]_D^{20} = +2.3$ (c = 0.82, $CHCl_3$); IR (KBr): 3453*w*, 3307*w br*, 2959*m*, 2932*m*, 2870*m*, 1734*s*, 1725*s*, 1540*m*, 1522*m*, 1508*m*, 1455*m*, 1381*m*, 1256*s*, 1034*s*, 795*s*; ^{1}H NMR (300 MHz, $CDCl_3$): 7.32 (m, 5H), 5.61 (br d, 2H, J = 4.41), 5.30 (dd, 1H, J = 2.19, 1.11), 5.07 (br s, 1H), 4.99 (br d, 1H, J = 6.63), 4.60 (br d, 2H, J = 4.41), 4.38 (d, 2H, J = 5.88), 1.61 (s, 3H), 1.34 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 155.3, 148.6, 129.4, 128.4, 128.3, 120.2, 119.7, 113.5, 84.7, 84.1, 81.4, 48.9, 46.0, 26.8, 25.2; MS: 384, 382 ([M^+1]).

(1*R*,2*R*,3*S*,4*S*,5*R*)-1-Bromo-2-*O*-(*N*-benzylcarbamoyl)-5-*O*-(hydroxymethyl)-3,4-*O*-isopropylidenecyclopentane-2,3,4-triol (27). BH_3·THF (403 μL, 10 equiv.) was added to a solution of bromoolefin **26** (15.4 mg, 40.3 μmoL) in dry THF (0.5 mL) at 0 °C and the reaction was stirred for 2 hours at 25 °C. Water (5 mL) and $NaBO_3$ (186 mg, 30 equiv.) were then added and the reaction was stirred for another 90 minutes at 25 °C. Aqueous workup (H_2O–AcOEt) and column chromatography gave **27** (12.1 mg, 75%) as a colorless oil, $[\alpha]_D^{20} = -16.48$ (c = 0.3, $CHCl_3$); IR (KBr): 3362*w*, 2934*w*, 2340*w*, 1730*m*, 1546*m*, 1510*m*, 1454*m*, 1382*m*, 1245*m*, 1208*s*, 1004*m*; ^{1}H NMR (300 MHz, $CDCl_3$): 7.34 (m, 5H), 5.37 (s, 1H), 5.03 (br s, 1H), 4.80 (dd, 1H, J = 5.88, 5.52), 4.59 (d, 1H, J = 5.88), 4.38 (s, 1H), 4.28 (d, 1H, J = 5.88), 4.01 (m, 2H), 2.55 (m, 1H), 1.58 (s, 3H), 1.29 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 129.5, 128.4, 128.3, 112.9, 85.3, 84.2, 80.9, 62.0, 51.6, 48.1, 45.9, 26.0, 24.3; MS: 402, 400 ([M^+1]), 386, 384, 320, 91.

(1*R*,2*R*,3*S*,4*S*,5*R*)-2-*O*-(*N*-Benzylcarbamoyl)-1-bromo-5-(*O*-*tert*-butyldimethylsilyl)-hydroxymethyl-3,4-isopropylidenecyclopentane-1,2,3-triol (28). To a solution of bromoalcohol **27** (25.4 mg, 63.5 μmol) in dry dichloromethane (0.5 mL), 2,6-lutidine (15 μl, 127 μmol) and *tert*-butyldimethylsilyl triflate (22 μl, 95 μmol) were added at 0 °C. After 2 hours at 25 °C, the reaction mixture was evaporated. Aqueous workup (H_2O–AcOEt) and purification by FC (hexane–AcOEt = 8 : 1, $R_f = 0.14$) gave **28** (27 mg, 84%) as a colorless solid; mp 106 °C; $[\alpha]_D^{20} = -17.7$ (c = 0.26, $CHCl_3$); IR (KBr): 3446*m*, 3019*m*, 2929*w*, 2400*w*, 1729*s*, 1511*s*, 1472*w*, 1383*w*, 1214*s*, 1124*w*, 1080*s*; ^{1}H NMR (300 MHz, $CDCl_3$): 7.33 (m, 5H), 5.37 (s, 1H), 4.98 (br d, 1H, J = 5.16), 4.75 (dd, 1H, J = 5.52, 5.49), 4.56 (d, 1H, J = 5.88), 4.39 (m, 1H), 4.38 (d, 2H, J = 5.88), 4.27 (d, 1H, J = 5.52 Hz), 3.97 (m, 2H), 2.45 (m, 1H), 1.57 (s, 3H), 1.27 (s, 3H), 0.91 (s, 9H), 0.10 (s, 3H), 0.09 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 155.3, 138.7, 129.5, 128.4, 112.6, 85.6, 84.6, 81.2, 62.1, 52.0, 49.2, 45.9, 26.7, 26.0, 24.2, 12.1, −4.6, −4.7; HR-MS calcd for $C_{23}H_{37}BrNO_5Si$ [M^+1]: 514.1624; found 514.1628.

(1*S*,2*S*,3*S*,4*S*,5*R*)-4-Benzylamino-3-*O*-4-*N*-(oxomethylidene)-1,2-*O*-isopropylidene-5-((*tert*-butyl-dimethylsilyloxy)methyl)-cyclopentane-1,2,3-triol (29). A solution of intermediate **28** (14.8 mg, 28.8 μmol) in dry THF (2 mL) at 0 °C was treated with NaH (4.3 mg, 0.12 mmol). The reaction mixture was stirred during 2 hours at 0 °C. Evaporation of the solvent and purification by FC (hexane–AcOEt 8 : 1, $R_f = 0.14$) gave **29** (11.4 mg, 91%) as a colorless oil; $[\alpha]_D^{20} = +17.38$ (c = 0.1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): 7.33 (m, 5H), 4.79 (d, 1H, J = 15.1), 4.31 (d, 1H, J = 15.1), 2.36 (m, 1H), 1.57 (s, 3H), 1.36 (s, 1H), 1.28 (s, 1H), 1.26 (s, 3H), 0.91 (s, 9H), 0.09 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): 157.7, 137.0, 129.5, 128.7, 128.6, 112.3, 85.4, 84.6, 81.8, 62.5, 62.4, 52.9, 46.9, 27.6, 25.2, 26.7, 14.8, −4.6, −4.7; HR-MS calcd for $C_{23}H_{36}BrNO_5Si$ [M^+1]: 434.2362; found 434.2365.

(1*S*,2*R*,3*S*,4*S*,5*R*)-4-Benzylamino-5-(hydroxymethyl)-1,2-isopropylidenecyclopentane-1,2,3-triol (30). A solution of **29** (2 mg, 4.6 μmol) in EtOH (0.3 mL), and 2 M NaOH (0.35 mL) was heated at 60 °C for 7 hours. The reaction was evaporated under vacuum and the residue was purified by FC (AcOEt–MeOH 5 : 1, $R_f = 0.61$) to give **30** (1.4 mg, 100%) as a colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$): 7.33 (m, 5H), 4.65 (dd, 1H, J = 5.52, 5.52), 4.49 (d, 1H, J = 5.88), 4.03 (d, 1H, J = 4.02), 3.90 (m, 4H), 3.37 (dd, 1H, J = 11.4, 4.05), 2.19 (m, 1H), 1.40 (s, 3H), 1.27 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 129.4, 129.2, 128.5, 111.0, 84.1, 80.6, 73.0, 62.2, 62.1, 52.9, 46.3, 26.6, 24.2; HR-MS calcd for $C_{16}H_{23}BrNO_4$: 293.1627; found: 293.1634.

Enzyme measurements

Enzymes were purchased from Fluka and Sigma. The α-glucosidase (yeast, EC 3.2.1.20) was buffered in PBS at pH 7.4; all other glycosidases were buffered in 0.1 M HEPES at pH 6.8: β-glucosidase (almonds and *Caldocellum saccharolyticum*, EC 3.2.1.21), α-galactosidase (green coffee beans, EC 3.2.1.22), β-galactosidase (*E. coli*, EC 3.2.1.23), α-mannosidase (jack beans, EC 3.2.1.24), β-mannosidase (snail acetone powder, EC 3.2.1.25) and α-L-fucosidase (human placenta, EC 3.2.1.51). All buffers and solutions were prepared using MilliQ deionized water. Substrates were used as 25 mM stock solutions in buffer and inhibitors as 10 mM stock solutions in water.

Enzymes and inhibitors were mixed. After 30 minutes these solutions were added to the substrates. The 100 μl assays were followed in individual wells of flat-bottomed 96-well half-area polystyrene cell culture plates (Costar) using a UV Spectramax 250 instrument from Molecular Devices. The release of 4-nitrophenol was followed at 405 nm over 30 min at pH 7.4 or pH 6.8 and 30 °C. The concentration of each enzyme in the assays was adjusted so as to give an approximately 0.2 to 1 OD increase as given by the instrument. The initial rate of reaction of the first 10 minutes was linear and was used to calculate the rate. Rates were expressed in relative units as milliOD min^{-1}. The competitive inhibition constants K_i were determined by Dixon replot of inhibition data.

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References

1 (*a*) T. D. Heightman and A. T. Vasella, *Angew. Chem.*, 1999, **111**, 795; T. D. Heightman and A. T. Vasella, *Angew. Chem., Int. Ed.*, 1999, **38**, 750; (*b*) Y. Bourne and B. Henrissat, *Curr. Opin. Struct. Biol.*, 2001, **11**, 593; (*c*) G. J. Davies and B. Henrissat, *Biochem. Soc. Trans.*, 2002, **30**, 291.

2 (*a*) P. Compain and O. R. Martin, *Curr. Top. Med. Chem.*, 2003, **3**, 541; (*b*) N. Asano, *Glycobiology*, 2003, **13**, 93R; (*c*) N. Asano, R. J. Nash, R. J. Molyneux and G. W. J. Fleet, *Tetrahedron:*

Asymmetry, 2000, **11**, 1645; (*d*) P. Sears and C.-H. Wong, *Angew. Chem., Int. Ed.*, 1999, **38**, 2300; (*e*) V. H. Lillelund, H. H. Jensen, X. Liang and M. Bols, *Chem. Rev.*, 2002, **102**, 515; (*f*) M. Bols, *Acc. Chem. Res.*, 1998, **31**, 1; (*g*) G. Davies, M. L. Sinnott, S. G. Withers, pp. 119–208 in *Comprehensive Biological Catalysis*, Ed. M. Sinnott, Academic Press, London, 1998.
3 (*a*) J.-L. Reymond, K. D. Janda and R. A. Lerner, *Angew. Chem., Int. Ed. Engl.*, 1991, **30**, 1711; (*b*) D. Shabat, S. C. Sinha, J.-L. Reymond and E. Keinan, *Angew. Chem.*, 1996, **35**, 2628.
4 (*a*) P. G. Schultz and R. A. Lerner, *Science*, 1995, **269**, 1835; (*b*) R. A. Lerner, S. J. Benkovic and P. G. Schultz, *Science*, 1991, **252**, 659; (*c*) N. R. Thomas, *Nat. Prod. Rep.*, 1996, 479; (*d*) J.-L. Reymond, *Top. Curr. Chem.*, 1999, **200**, 59.
5 A. Berecibar, C. Grandjean and A. Siriwardena, *Chem. Rev.*, 1999, **99**, 779.
6 B. J. Smith, J. L. McKimm-Breshkin, M. McDonald, R. T. Fernley, J. N. Varghese and P. M. Colman, *J. Med. Chem.*, 2002, **45**, 2207.
7 R. A. Farr, N. P. Peet and M. S. Kand, *Tetrahedron Lett.*, 1990, **31**, 7109.
8 (*a*) A. Blaser and J.-L. Reymond, *Helv. Chim. Acta*, 1999, **82**, 760; (*b*) A. Blaser and J.-L. Reymond, *Org. Lett.*, 2000, **2**, 1733; (*c*) A. Blaser and J.-L. Reymond, *Helv. Chim. Acta*, 2001, **84**, 2119.
9 E. Leroy and J.-L. Reymond, *Org. Lett.*, 1999, **1**, 775.
10 O. Boss, E. Leroy, A. Blaser and J.-L. Reymond, *Org. Lett.*, 2000, **2**, 151.
11 (*a*) M. Kleban, P. Hilgers, J. N. Greul, R. D. Kugler, S. Picasso, P. Vogel and V. Jäger, *Chem. Bio. Chem.*, 2001, **2**, 365; (*b*) J. N. Greul, M. Kleban, B. Schneider, S. Picasso and V. Jäger, *Chem. Bio. Chem.*, 2001, **2**, 368.
12 P. A. Bartlett, K. L. McLaren and P. C. Ting, *J. Am. Chem. Soc.*, 1988, **110**, 1633.
13 S. M. Ali, K. Ramesh and R. T. Borchardt, *Tetrahedron Lett.*, 1990, **31**, 1509.
14 M. Schlosser and B. Schaub, *Chimia*, 1982, **10**, 396.
15 G. W. Kabalka, T. M. Shoup and N. M. Goudgaon, *Tetrahedron Lett.*, 1989, **30**, 1483.
16 C. Uchida, H. Kimura and S. Ogawa, *Bioorg. Med. Chem.*, 1997, **5**, 921.
17 http://www.visualstats.org/.
18 None of more than five hundred hybridoma cell lines generated from an immunization against a protein conjugate of **59** and producing strong hapten-binding antibodies showed any α-L-fucosidase activity. L. Gartenmann Dickson, Ph.D. thesis, Bern, 2004.
19 G. Legeler, *Adv. Carbohydr. Chem. Biochem.*, 1990, **48**, 319.