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Design and synthesis of 2-acetamidomethyl derivatives of isofagomine as potential inhibitors of human lysosomal β-hexosaminidases

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Abstract—As part of a program towards the development of specific inhibitors of human lysosomal β-hexosaminidase for use as chemical chaperones in therapy of G_{M2} gangliosidosis related diseases, the synthesis of 2-acetamidomethyl derivatives of isofagomine has been undertaken. Key event in this synthesis is the conversion of a C-2 substituted gluconolactone derivative into the corresponding lactam, followed by reduction to the corresponding amine. The 1-*N*-imino-2 acetamidomethyl derivative **5** proved to be a rather selective inhibitor with a K_i of 2.4 μM for homogenate of human spleen lysosomal β-hexosaminidase.

1. Introduction

In eukaryotic cells the catabolism of oligosaccharides, glycolipids, glycoproteins, and glycosaminoglycans is predominantly carried out in the lysosome by soluble hydrolases, e.g., glycosidases. Precursors of these lysosomal enzymes are synthesized on ribosomes in the endoplasmic reticulum (ER). During transport via the ER and the Golgi compartment, these precursors are further processed (e.g., by proteolysis, glycosylation and phosphorylation) to their near mature form as present in the lysosomes.[1] Genetic defects may cause incorrect folding of a lysosomal enzyme, resulting in the retention of the enzyme in the ER, and premature degradation.[2,3] The resulting low activity of the lysosomal enzyme causes accumulation of corresponding substrates within the lysosomes, which may result in a specific pathology.[4] A specific type of lysosomal storage disease is G_{M2} gangliosidosis that results in the degeneration of the central nervous system. G_{M2} gangliosidosis is caused by a deficiency of lysosomal β-hexosaminidase (E.C 3.2.1.52) that cleaves terminal β-*N*-acetyl hexosamine residues.

A potential therapy towards G_{M2} gangliosidosis might be derived from the recently postulated 'chemical chaperone' approach for Fabry disease,[5] a related lysosomal storage disorder which is caused by a deficiency of α-galactosidase A (α-Gal A). The new therapeutic intervention is based on administrating competitive inhibitors, so-called 'chemical chaperones', at sub-inhibitory intracellular concentrations. These inhibitors should help mutant enzyme precursors to fold properly, avoiding their excessive pre-lysosomal degradation. Intralysosomally, α-Gal A is not effectively reduced in activity due to sub-inhibitory concentrations of the competitive inhibitors. Thus, 1-deoxygalactonojirimycin, a competitive inhibitor of α-Gal A, increased the intracellular α-Gal A activity by 14-fold in Fabry lymphoblasts.[5b] To explore whether the concept of 'chemical chaperone' is also applicable to G_{M2} ganglioside related diseases, such as Tay-Sachs and Sandhoff disease, specific inhibitors of human lysosomal β-hexosaminidase are needed.

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An important class of reversible competitive inhibitors of glycosidases consists of polyhydroxylated piperidines (iminosugars) which are widespread found in plants and micro-organisms. In the ongoing process of unraveling the mechanism of glycosidases it has been established for β-glycosidases that a positive charge is generated at the anomeric position rather than at the position of the ring oxygen.[6] Pioneering work of Bols[7] and Ichikawa[8] on the syntheses of iminosugars in which the anomeric carbon atom was replaced by a nitrogen atom, resulted in various powerful and selective inhibitors of β-glycosidases. For example, isofagomine **1** (Fig. 1) is a very potent inhibitor of β-glucosidase ($K_i = 0.11$ μM, sweet almond), but only a poor inhibitor of α-glucosidase (yeast, 780 times less strong).[9] A discrete class of glycosidase inhibitors, the *gem*-diamine 1-*N*-iminosugars, based on natural Siastatin B,[10] proved to be powerful although less selective inhibitors of glycosidases. For example, gluco-type 1-*N*-iminosugar **2**[11] was as active towards α-($IC_{50} = 0.19$ μM, yeast) as β-glucosidase ($IC_{50} = 0.42$ μM, almond). Interestingly, Schuster[12] showed that introduction of a 2-hydroxymethylene substituent at the C-2 position of **1**, leading to homoisofagomine **3**, had no dramatic effect on the inhibitory activity towards β-glucosidase ($K_i = 6.6$ μM, sweet almond). In contrast, for α-glucosidase (yeast) the inhibitory effect was absent. Moreover, Takaoka et al. reported[13] that 2-acetamidomethyl derivatives **4** of 2,5-dihydroxymethyl-3,4-dihydroxypyrolidine were potent inhibitors of *N*-acetylglucosaminidase. In view of the above-mentioned findings we reasoned that the 1-*N*-imino-2-acetamidomethyl isofagomine derivative **5**, which contains a 2-acetamidomethyl moiety as a stable mimic of the *N*-acetyl function, might be a potent inhibitor of human lysosomal β-hexosaminidase. In this paper the synthesis of **5** is described. In addition, since it has been shown that various sugar lactams are potent glycosidase inhibitors,[6c] the closely related sugar lactam **6** was synthesized. Although the lactam function is orientated more or less reversely as compared to 'normal' sugar lactams, this compound has a highly polarized bond as well as a half-chair conformation and can therefore be regarded as a transition state analogue for the hydrolysis of a glycosidic linkage. The inhibitory effect on human lysosomal β-hexosaminidase is also reported.

2. Results and discussion

The retro-synthetic analysis of compounds **5** and **6** is outlined in Scheme 1. It was envisaged that the 1-*N*-imino moiety of **5** and the lactam function of **6** should be introduced through conversion of a lactone, carrying a protected 2-hydroxymethyl moiety at C-2 (**8**), into the corresponding lactam **7**. Reduction of the latter will give the precursor of amine **5**. Virtual rotation of the required lactone synthon reveals that it should be readily accessible from Cerny epoxide **9**.[14]

The assembly of the 2-substituted gluco-δ-lactone derivative commences with the introduction of a benzyloxymethyl moiety at C-2 of Cerny epoxide **9** via the reaction sequence depicted in Scheme 2. Regio- and stereoselective opening of the oxirane functionality of **9** with vinylmagnesium bromide in refluxing tetrahydrofuran led to the exclusive isolation of alkene **10** in an excellent yield of 91%. Subsequent oxidation of the double bond of **10** with sodium periodate in the presence of osmium tetroxide[15] gave the labile aldehyde, which was directly converted into hydroxymethyl derivative **11** by reduction with sodium borohydride in a mixture of tetrahydrofuran and isopropyl alcohol (64% over 2 steps). The hydroxyl groups of **11** were masked with a benzyl group to give compound **12** in a yield of 90%.

Surprisingly, cleavage of the 1,6-anhydro bond of **12** under the conditions reported by Jespersen et al.[7b] for opening the 1,6-anhydro bond in **11**, led to an intractable mixture of products. When slightly modified conditions were used (dioxane/1 M H_2SO_4, 2/1, v/v, 72 h under reflux), lactol **14** was isolated in a disappointing yield of

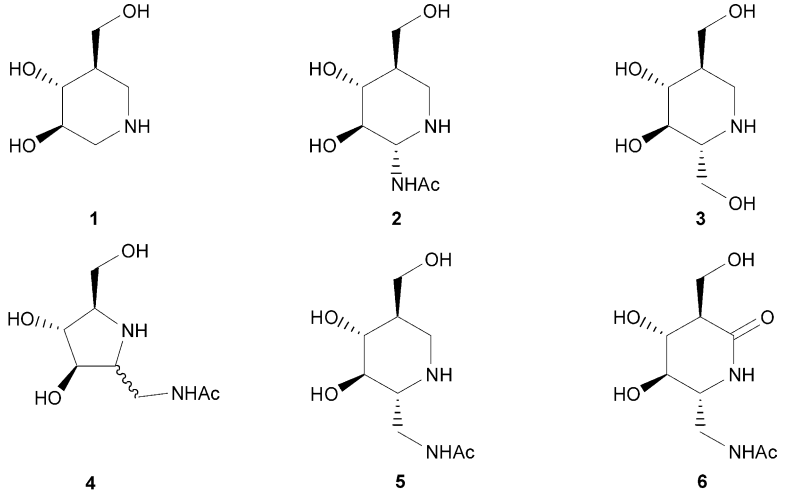
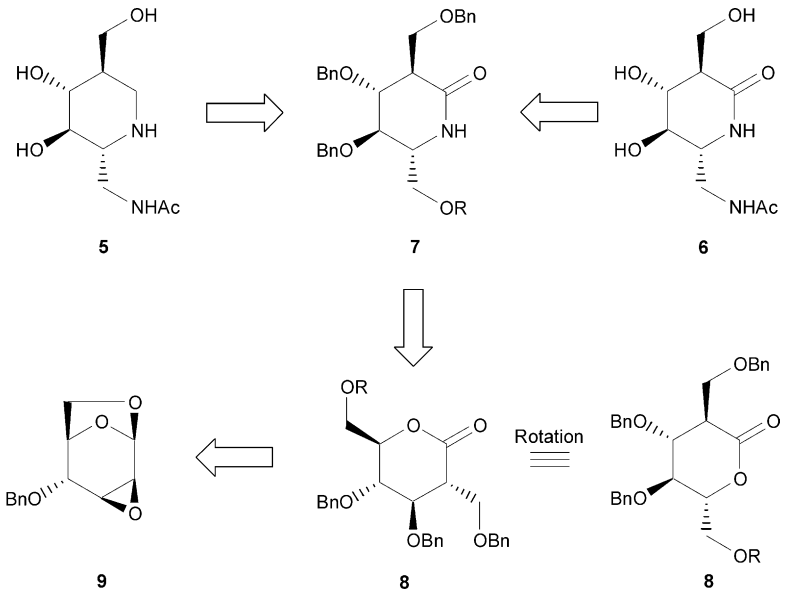
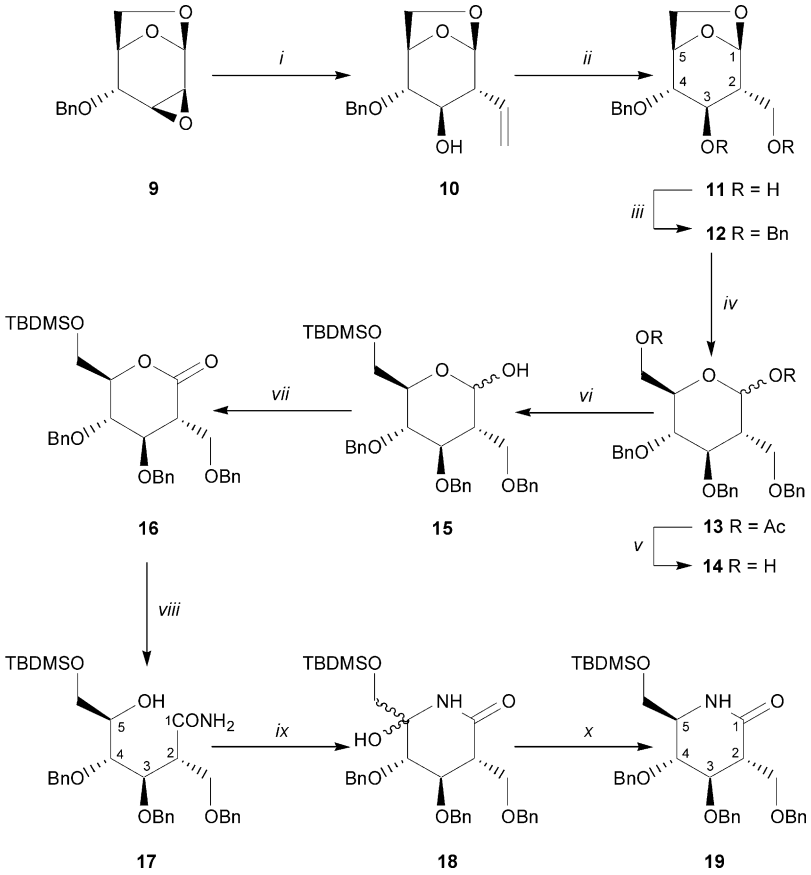


Figure 1.



Scheme 1. Retro-synthetic analysis of compounds **5** and **6**.



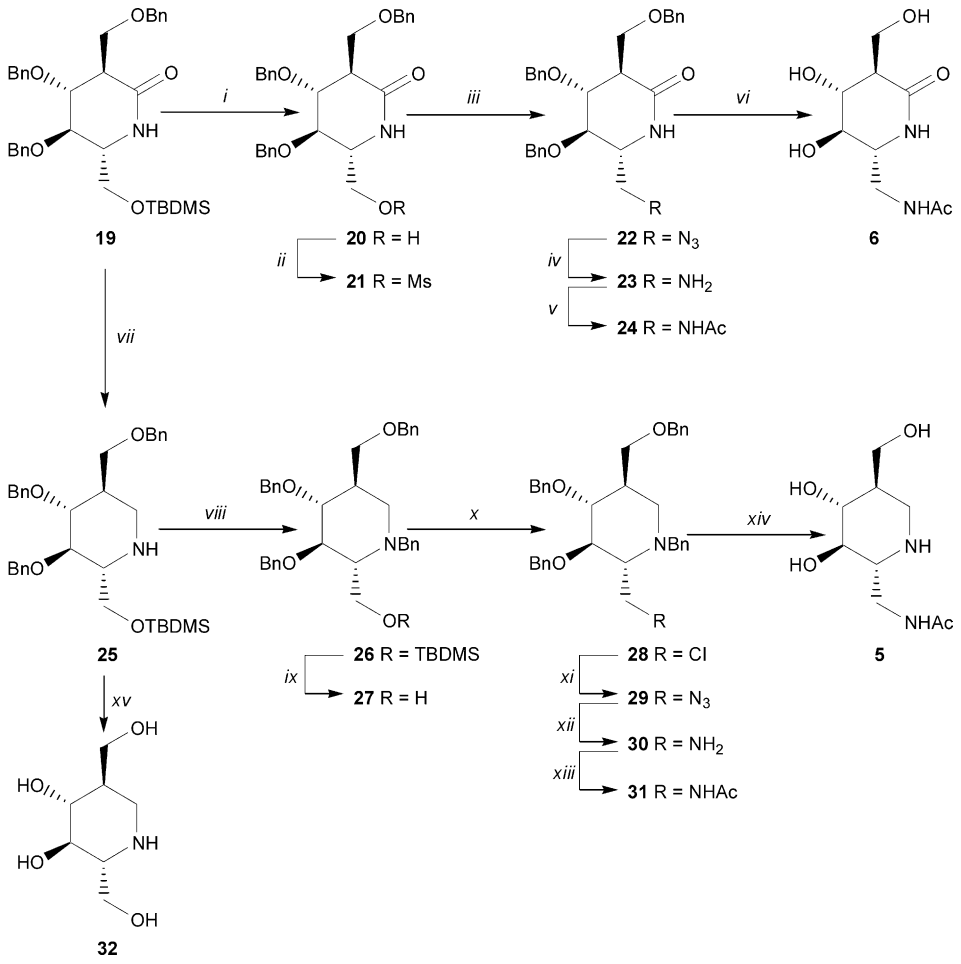
Scheme 2. Conditions: (i) $CH_2=CHMgBr$, THF, 91%; (ii) (a) OsO_4, $NaIO_4$, dioxane, H_2O; (b) $NaBH_4$, isopropanol, THF, 64%; (iii) BnBr, NaH, DMF, 90%; (iv) TFA, Ac_2O, 78%; (v) NaOMe, MeOH, 96%; (vi) TBDMSCl, imidazole, DMF, 82%; (vii) DMSO, Ac_2O, 92%; (viii) NH_3/MeOH, 53%; (ix) DMSO, Ac_2O, 81%; (x) $NaCNBH_3$, HCO_2H, CH_3CN, 63%.

32%. In contrast, cleavage of the 1,6-anhydro bond of **12** was readily accomplished using trifluoroacetic acid in acetic anhydride,[16] affording diacetate **13** as a mixture of anomers ($\alpha/\beta = 3:1$). Zemplén deacetylation of **13** gave lactol **14** in an overall yield of 75%. The primary hydroxyl function of **14** was protected with a *tert*-butyldimethylsilyl group to afford silyl-ether **15**. Oxidation of **15** under Albright–Goldman conditions[17] provided lactone **16** in a yield of 75% over the two steps. Introduction of the endocyclic imino function was accomplished according to the method originally described by the groups of Vasella[18] and Pandit,[19] and more recently by our groups.[20] Thus, reaction of **16** with methanolic ammonia resulted in the isolation of hydroxy-amide **17** in a moderate yield of 53%, together with the C-2 epimer of **17** (15%). The C-2 stereoisomer of **17** is presumably formed by base-promoted racemization. The gluco-configuration of **17** was irrefutably ascertained by ^{1}H NMR spectroscopy. The large coupling constants between H2 and H3, and H4 and H5 ($J_{2,3} = 6.8$ Hz and $J_{4,5} = 7.6$ Hz) and the small coupling constant between H3 and H4 ($J_{3,4} = 3.0$ Hz) are in full accordance with the proposed identity of compound **17**.[21] Dimethyl sulfoxide-acetic anhydride mediated oxidation[17] of **17** resulted in a diastereomeric mixture of **18** in good yield (81%). The 5-*R*-isomer of **18** could be separated from the 5-*S*-isomer of **18** by silica gel column chromatography (ratio *R*:*S* = 78:22), enabling the firm establishment of their respective chemical structures. Coupling constants of the 5-*R*-isomer of **18** ($J_{2,3} = 9.6$ Hz and $J_{3,4} = 9.1$ Hz) indicate a $^4H_3$ conformation; coupling constants of the 5-*S*-isomer of **18** ($J_{2,3} = 5.0$ Hz and $J_{3,4} = 6.3$ Hz) suggest a $^3H_4$ conformation which is in accordance with published data for analogous compounds.[22] Reduction of the diastereomeric mixture of **18** with sodium cyanoborohydride under acidic conditions afforded a mixture of the D-gluco isomer (**19**; 63%) and the L-ido isomer (17%), which were separated by silica gel column chromatography. When the *R*- and *S*-isomers of **18** were reduced separately under identical conditions, diastereomeric **19** was obtained in the same ratio of isomers and yield as obtained after reduction of the diastereomeric mixture of **18**. This indicates that in both cases reduction proceeds via the same intermediate *N*-acyliminium ion. Evidently, hydride attack occurs preferentially from the Si-face, affording the D-gluco derivative as the major product.[23] The stereochemistry of **19** was firmly established by NMR spectroscopy, the di-axial coupling constants of the ring protons ($J_{2,3} = 9.6$ Hz, $J_{3,4} = 9.1$ Hz, $J_{4,5} = 9.0$ Hz), together with NOE's observed between H2 and H4 and between H3 and H5, clearly show the D-gluco-configuration and a $^4C_1$ conformation of **19**. In addition to its use for the preparation of isofagomine derivative **5**, it was used for the preparation of lactam **6** (Scheme 3). Desilylation of **19** with TBAF gave the hydroxy-amide **20**. Subsequent reaction of **20** with methanesulfonyl chloride in pyridine afforded methylsulfonyl derivative **21**. Treatment of **21** with sodium azide in DMF at elevated temperature gave the azido derivative **22** in a yield of 80% over the three steps. Reduction of the azido function in **22** under the agency of triphenylphospine in aqueous tetrahydrofuran[24] gave the amino compound **23**. Subsequent acetylation of the amino function in **23** with acetyl chloride gave acetamido derivative **24** in 67% yield. The identity of **24** was unambiguously corroborated by mass spectrometry, and ^{1}H- and ^{13}C NMR spectroscopy. Unmasking of compound **24** by hydrogenation over palladium on carbon in dimethyl formamide afforded the requisite lactam **6** which was evaluated as an inhibitor of various hexosaminidases (*vide infra*).

The synthesis of isofagomine derivative **5** was continued with the reduction of the lactam function of compound **19** as is depicted in Scheme 3. Thus, treatment of lactam **19** with borane in tetrahydrofuran[25] provided the desired iminosugar **25** in reasonable yield (59%). Alternatively, reduction of **19** with borane-methyl sulfide complex[26] gave **25** in 61% yield together with a considerable amount of starting material **19** (32%). The configuration and conformation of **25** was ascertained by NMR spectroscopy. The di-axial proton coupling constants of the ring sugar moiety ($J_{1ax,2} = 11.6$ Hz, $J_{2,3} = 10.6$ Hz, $J_{3,4} = 9.0$ Hz and $J_{4,5} = 9.6$ Hz) are characteristic for the gluco-configuration and the $^4C_1$ conformation.[7b] Subsequently the endocyclic nitrogen atom of compound **25** was protected with a benzyl group, giving imino-glucitol **26** in 49% yield. TBAF-mediated desilylation of **26** afforded **27** in quantitative yield. Surprisingly, reaction of **27** with mesyl chloride did not afford the *O*-methanesulfonyl derivative. Instead the corresponding chloride **28** was isolated in almost quantitative yield (97%). Compound **28** could be smoothly converted by treatment with sodium azide into the desired azido derivative **29** in a yield of 91%. The high reactivity of the chloro-derivative **28** towards nucleophilic substitution suggests that the endocyclic nitrogen atom might participate in the displacement reaction, by forming an aziridinium intermediate. Reduction of the azido function in **29** was accomplished with triphenylphosphine in aqueous tetrahydrofuran[24] to give the amine **30** in 71% yield. Subsequent acylation with acetyl chloride furnished imino-glucitol **31** in 85% yield. Deprotection of **31** by catalytic hydrogenation over palladium on carbon in acidic aqueous ethanol afforded the desired homoisofagomine derivative **5** in good yield.

It is interesting to note that compound **25** could also be converted into the known[12] β-glycosidase inhibitor homoisofagomine **32** in two steps (see Scheme 3). The originally reported synthesis of **32** was based on a chemical-enzymatic approach and afforded **32** as C-5 epimeric mixture in a ratio of 1:1.2 (5-*S*:5-*R*). In the first step, the TBDMS group of **25** was removed by treatment with TBAF. Subsequent catalytic hydrogenation over palladium on carbon in aqueous ethanol containing hydrochloric acid afforded **32** in an overall yield of 70%.

The inhibitory potency of iminosugar **5** and lactam **6** was tested towards homogenate of human spleen lysosomal β-hexosaminidase. For comparison, the earlier reported 2-acetamido-2-deoxy-D-glucono-δ-lactam[20,22] (**33**) and 2-acetamido-2-deoxy-D-glucono-deoxynojirimycin[20,22] (**34**) were evaluated for their inhibitory potency. The



Scheme 3. Conditions: (i) TBAF, THF, 91%; (ii) MsCl, pyridine, 99%; (iii) NaN_3, DMF, 89%; (iv) triphenylphosphine, H_2O, THF, 25%; (v) AcCl, TEA, DCM, 67%; (vi) DMF, 10% Pd/C, H_2, 69%; (vii) BH_3Me_2S, DCM, 61%; (viii) BnBr, TEA, CH_3CN, 49%; (ix) TBAF, THF, 100%; (x) MsCl, TEA, DCM, 97%; (xi) NaN_3, DMF, 91%; (xii) triphenylphosphine, H_2O, THF, 71%; (xiii) AcCl, TEA, DCM, 85%; (xiv) HCl, EtOH, 10% Pd/C, H_2, 94% (xv) (a) TBAF, THF (b) HCl, EtOH, H_2O, 10% Pd/C, H_2, 70%.

results show that 2-acetamidomethyl-isofagomine (**5**) is a relatively strong inhibitor of human lysosomal β-hexosaminidase. Furthermore, the high inhibitory effect of **33** towards human lysosomal β-hexosaminidase (see Table 1) is striking. Apparently, the half chair conformation of **33** contributes strongly to the inhibitory activity of this compound. This is in full agreement with a recent study on conformation and inhibitory potency of various D-glycono-δ-lactams.[6c] In order to determine the specificity of the inhibitors, two other β-*N*-acetylglucosaminidases were included in the enzymatic assays, that is, β-hexosaminidase of *Aspergillus niger* and purified human chitotriosidase, an endo-*N*-acetylglucosaminidase.[27] No inhibition of human chitotriosidase was observed. The most powerful inhibitors (**5** and lactam **33**) were only poor inhibitors (K_i 612 and 34.5 μM, respectively). These results indicate that these compounds are fairly selective inhibitors of human lysosomal β-hexosaminidases. Further studies are required to determine whether these iminosugars are actually useful as enhancers of human lysosomal β-hexosaminidase activity in cultured cell lines from patients suffering from Tay-Sachs or Sandhoff disease.

3. Experimental

3.1. General methods and materials

Column chromatography was performed on Silica gel 60 (220–440 mesh ASTM, Fluka). TLC analysis was performed with silica gel TLC plates (Fluka) with detection by UV absorption (254 nm) where applicable and charring with 20% H_2SO_4 in MeOH or ammonium molybdate (25 g/L) and ceric ammonium sulfate (10 g/L) in 20% H_2SO_4. ^1H NMR and ^{13}C NMR were recorded with a Varian VXR-400S (399.9/100.6 MHz). ^1H and ^{13}C chemical shifts are given in ppm (δ) relative to tetramethylsilane (δ = 0.00), DMSO-d_5 (δ = 2.525), DMSO-d_6 (δ = 39.6) and $CDCl_3$ (δ = 77.10) as internal standard. The purity of the compounds was established by ^1H NMR spectroscopy: >95% in all cases. Mass spectra were recorded with a VG Quattro II triple quadropole mass spectrometer (Fisons Instruments, Altrincham, UK). High resolution mass spectra and tandem MS spectra were recorded on a Q-TOF mass spectrometer (Micromass, Manchester, UK). The Q-TOF instrument was calibrated; electrospray MS and electrospray MS-MS spectra were recorded with resolution 8000 (Full Width

Table 1. Apparent IC_{50}[a] values and K_i values of compounds **5**, **6**, **33**[20,22] and **34**[20,22] for human lysosomal β-hexosaminidase and corresponding isoenzymes

Enzyme	Compd **5**	Compd **6**	Compd **33**	Compd **34**	
Homogenate of human spleen lysosomal β-hexosaminidase	5 μM $K_i = 2.4$ μM	20%[b]	2.5 μM	16 μM	0.85[d]
Human spleen lysosomal β-hexosaminidase A $P_i = 5.0$	4.5 μM $K_i = 2.8$ μM	N.D.[c]	5.0 μM $K_i = 3.1$ μM	20 μM $K_i = 16$ μM	0.87[d]
Human spleen lysosomal β-hexosaminidase B $P_i = 7.2$	3.0 μM $K_i = 1.55$ μM	N.D.[c]	2.5 μM $K_i = 1.8$ μM	16 μM $K_i = 16$ μM	2.33[d]

[a] β-Hexosaminidase activities were determined with 4-methylumbelliferyl-*N*-acetyl-β-D-glucosaminide as substrate.
[b] Inhibition at a concentration of 1200 μM.
[c] Not determined.
[d] K_M value (mM) of the specific enzyme for used substrate, that is, 4-methylumbelliferyl-*N*-acetyl-β-D-glucosaminide.

Half Mass). Positive ion electrospray MS spectra were recorded at a cone voltage of 15 V. Argon gas pressure was 10^{-4} mBar. Product ion spectra (MS-MS) were recorded at a collision energy between 15 and 28 eV. Prior to reactions that require anhydrous conditions, traces of water were removed by coevaporation with dry toluene. These reactions were conducted under dry argon atmosphere. Hydrogenations were executed at atmospheric pressure under an atmosphere of hydrogen gas maintained by an inflated balloon.

3.1.1. 1,6-Anhydro-4-*O*-benzyl-2-deoxy-2-*C*-vinyl-β-D-glucose (10). Vinylmagnesium bromide in THF (1.0 M, 100 mL, 100 mmol) was added to **9** (3.20 g, 13.7 mmol) in THF (70 mL). The mixture was refluxed for 4 h, cooled to 0 °C and poured into aqueous NH_4Cl (2 M, 100 mL). The aqueous layer was extracted with ethyl acetate (3×100 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. Column chromatography of the residue over silica gel with hexane/ethyl acetate (4:1→7:3) gave **10** as a colorless oil. Yield 3.28 g (91%). TLC (silica gel, ethyl acetate/hexane, 1:1) $R_f = 0.40$. ^{1}H NMR ($CDCl_3$): δ = 2.42 (m, 1H, H-2), 2.81 (d, 1H, OH-3, $J = 5.4$ Hz), 3.41 (m, 1H, H-4), 3.68 (dd, 1H, H-6a, $J_{6a,6b} = 7.4$ Hz, $J_{5,6a} = 5.5$ Hz), 3.76 (br. s, 1H, H-3), 4.05 (dd, 1H, H-6b, $J_{6a,6b} = 7.4$ Hz, $J_{5,6b} = 0.8$ Hz), 4.57–4.68 (m, 3H, H-5, CH_2 Bn), 5.16 (m, 2H, H_2-3′), 5.39 (s, 1H, H-1), 5.96 (m, 1H, H-2′), 7.29–7.36 (m, 5H, CH-arom Bn). ^{3}C{^{1}H}-NMR ($CDCl_3$): δ = 51.99 (C-2), 65.64 (C-6), 70.72 (C-3), 71.49 (CH_2 Bn), 74.87 (C-5), 79.06 (C-4), 103.71 (C-1), 117.79 (C-3′), 127.69; 127.84; 128.51 (CH-arom Bn), 135.74 (C-2′), 137.42 (Cq Bn).

3.1.2. 1,6-Anhydro-4-*O*-benzyl-2-deoxy-2-*C*-hydroxymethyl-β-D-glucose (11). Osmium tetroxide (0.1 M in dioxane/water, 5:2, 0.4 mL, 0.04 mmol) was added to a stirred mixture of **10** (0.88 g, 3.35 mmol) in dioxane/water (70 mL, 5:2). Neat $NaIO_4$ (1.58 g, 7.4 mmol) was added and the mixture was stirred for 1 h, subsequently diluted with water (50 mL) and extracted with ethyl acetate (5×50 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was dissolved in a mixture of THF/isopropyl alcohol (50 mL, 9:1). The solution was cooled (0 °C) and $NaBH_4$ (0.14 g, 3.7 mmol) was added. After 1 h, the reaction mixture was poured into aqueous NH_4Cl (2 M, 50 mL) and extracted with ethyl acetate (5×50 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated. Column chromatography of the residue over silica gel with DCM/MeOH (100:0→95:5) gave **11** as a colorless oil. Yield 0.57 g (64%). TLC (silica gel, MeOH/DCM, 6:94) $R_f = 0.54$. ^{1}H NMR ($CDCl_3$): δ = 1.92 (m, 1H, H-2), 2.55 (br. s, 1H, OH), 2.94 (br. s, 1H, OH), 3.41 (m, 1H, H-4), 3.68 (dd, 1H, H-6a, $J_{6a,6b} = 7.4$ Hz, $J_{5,6a} = 5.6$ Hz), 3.71–3.83 (m, 3H, H_2-2′, H-3), 4.04 (dd, 1H, H-6b, $J_{6a,6b} = 7.4$ Hz, $J_{5,6b} = 0.8$ Hz), 4.56 (d, 1H, H-5), 4.64 (AB, 2H, CH_2 Bn), 5.53 (s, 1H, H-1), 7.25–7.36 (m, 5H, CH-arom Bn). ^{13}C{^{1}H}-NMR ($CDCl_3$): δ = 48.95 (C-2), 62.19 (C-2′), 65.62 (C-6), 69.04 (C-3), 71.78 (CH_2 Bn), 74.95 (C-5), 79.61 (C-4), 102.06 (C-1), 127.84-128.91 (CH-arom Bn), 137.78 (Cq Bn).

3.1.3. 1,6-Anhydro-3,4-di-*O*-benzyl-2-*C*-benzyloxymethyl-2-deoxy-β-D-glucose (12). Sodium hydride (55%, 0.85 g, 19.5 mmol) was added to a cooled (0 °C) and stirred solution of **11** (0.95 g, 3.55 mmol) in anhydrous DMF (50 mL). Benzyl bromide (0.84 mL, 7.1 mmol) was added to the mixture. After 4 h, MeOH (2 mL) was added and the reaction mixture was diluted with ethyl acetate (100 mL). The organic layer was washed with water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography of the residue over silica gel with DCM/MeOH (100:0→99:1) gave **12** as a yellow oil. Yield 1.42 g (90%). TLC (silica gel, MeOH/DCM, 0.5:99.5) $R_f = 0.48$. ^{1}H NMR ($CDCl_3$): δ = 2.23 (t, 1H,

H-2), 3.36 (br.s, 1H, H-4), 3.48 (dd, 1H, H-2′a), 3.51 (m, 1H, H-3), 3.61 (dd, 1H, H-2′b), 3.74 (t, 1H, H-6), 4.11 (dd, 1H, H-6′), 4.41–4.54 (m, 7H, H-5, 3×CH_2 Bn), 5.53 (d, 1H, H-1), 7.24–7.34 (m, 15H, CH-arom Bn). $^{13}C\{^1H\}$-NMR ($CDCl_3$): δ = 43.65 (C-2), 64.73 (C-6), 69.13 (C-2′), 70.80, 71.22, 73.16 (3×CH_2 Bn), 73.30 (C-3), 74.38 (C-5), 76.78 (C-4), 101.08 (C-1), 127.60-128.49 (CH-arom Bn), 137.85, 138.16, 138.24 (3×Cq Bn).

3.1.4. 1,6-Di-*O*-acetyl-3,4-di-*O*-benzyl-2-*C*-benzyloxymethyl-2-deoxy-α,β-D-glucose (13). TFA (2.70 mL, 35.3 mmol) was added to a cooled (0 °C) and stirred solution of **12** (2.98 g, 6.39 mmol) in acetic anhydride (90 mL, 0.95 mol). After 45 min, the mixture was concentrated and the residue was coevaporated with toluene (3×50 mL). Column chromatography of the residue over silica gel with hexane/ethyl acetate (80:20→50:50) gave **13** as a colorless oil. Yield 2.37 g (78%) TLC (silica gel, ethyl acetate/hexane, 1:2) R_f = 0.51. ^1H NMR [α-anomer] ($CDCl_3$): δ = 1.92 (s, 3H, CH_3 acetyl), 2.00 (s, 3H, CH_3 acetyl), 2.31 (m, 1H, H-2), 3.38 (t, 1H, H-2′a), 3.61 (dd, 1H, H-4, $J_{3,4}$ = 8.8 Hz, $J_{4,5}$ = 10.0 Hz), 3.65 (m, 1H, H-2′b), 3.81 (dd, 1H, H-3, $J_{2,3}$ = 11.2 Hz, $J_{3,4}$ = 8.8 Hz), 3.91 (dt, H-5, $J_{4,5}$ = 10.0 Hz, $J_{5,6a}$ = $J_{5,6b}$ = 3.2 Hz), 4.27 (d, 2H, H_2-6), 4.29–4.88 (3×AB, 6H, 3×CH_2 Bn), 6.34 (d, 1H, H-1, $J_{1,2}$ = 3.3 Hz), 7.23–7.32 (m, 15H, CH-arom Bn). $^{13}C\{^1H\}$-NMR [α-anomer] ($CDCl_3$): δ = 20.64, 20.69 (2×CH_3 acetyl), 45.56 (C-2), 62.77 (C-6), 66.37 (C-2′), 71.31 (C-5), 73.00, 74.97, 74.99 (3×CH_2 Bn), 78.15 (C-3), 78.70 (C-4), 91.67 (C-1), 127.51–128.41 (CH-arom Bn), 137.55, 137.85, 137.95 (3×Cq Bn), 168.76, 170.43 (2×C=O). ^1H NMR [β-anomer] ($CDCl_3$): δ = 1.88 (m, 1H, H-2), 1.96 (s, 3H, CH_3 acetyl), 1.99 (s, 3H, CH_3 acetyl), 3.41 (m, 1H, H-2′a), 3.55 (t, 1H, H-4, $J_{3,4}$ = 8.8 Hz, $J_{4,5}$ = 9.7 Hz), 3.63 (m, 1H, H-5) 3.67 (m, 1H, H-2′b), 3.95 (dd, 1H, H-3, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 10.8 Hz), 4.27 (d, 2H, H_2-6), 4.29–4.88 (m, 6H, 3×CH_2 Bn), 5.81 (d, H-1, $J_{1,2}$ = 9.2 Hz), 7.23–7.32 (m, 15H, CH-arom Bn). $^{13}C\{^1H\}$-NMR [β-anomer] ($CDCl_3$): δ = 20.69, 20.71 (2×CH_3 acetyl), 47.71 (C-2), 62.90 (C-6), 63.65 (C-2′), 73.53 (C-5), 72.92, 74.66, 75.16 (3×CH_2 Bn), 78.75 (C-4), 78.98 (C-3), 91.97 (C-1), 127.51-128.41 (CH-arom Bn), 137.66, 137.82, 138.18 (3×Cq Bn), 168.76, 170.43 (2×C=O acetyl).

3.1.5. 3,4-Di-*O*-benzyl-2-*C*-benzyloxymethyl-2-deoxy-α,β-D-glucose (14). Sodium methoxide (100 mg, 1.85 mmol) was added to a solution of **13** (2.73, 4.97 mmol) in MeOH (100 mL). After 45 min, the reaction mixture was neutralized with Dowex 50 W×8 (H^+), filtered and concentrated. Yield 2.21 g of a colorless oil (96%). TLC (silica gel, ethyl acetate/hexane, 1:1) R_f = 0.52. ^1H NMR [α-anomer] ($CDCl_3$): δ = 2.06 (m, 1H, H-2), 3.57 (m, 1H, H-4), 3.65 (m, 2H, H_2-2′), 3.85 (m, 2H, H_2-6), 3.91 (dd, 1H, H-3), 3.98 (m, 1H, H-5), 4.30–4.90 (3×AB, 6H, 3×CH_2 Bn), 5.37 (d, 1H, H-1), 7.15–7.40 (m, 15H, CH-arom Bn). ^1H NMR [β-anomer] ($CDCl_3$): δ = 1.72 (m, 1H, H-2), 3.39 (m, 1H, H-5), 3.57 (m, 1H, H-4), 3.78 (dd, 1H, H-3), 3.69 (m, 2H, H_2-2′), 3.85 (m, 2H, H_2-6), 4.30–4.90 (3×AB, 6H, 3×CH_2 Bn), 4.88 (d, 1H, H-1), 7.15–7.40 (m, 15H, CH-arom Bn). $^{13}C\{^1H\}$-NMR [α,β-anomer] ($CDCl_3$): δ = 46.76, 49.72 (C-2 (α,β)), 61.94, 62.01 (C-6 (α,β)), 65.78, 67.18 (C-2′ (α,β)), 71.68, 76.78 (C-5 (α,β)), 75.57–73.35 (CH_2 Bn), 77.44, 79.20 (C-3 (α,β)), 79.44, 79.79 (C-4 (α,β)), 93.70, 95.68 (C-1 (α,β)), 128.57–127.69 (CH-arom Bn), 137.48–138.40 (Cq Bn).

3.1.6. 3,4-Di-*O*-benzyl-2-*C*-benzyloxymethyl-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-α,β-D-glucose (15). To a stirred solution of **14** (2.21 g, 4.76 mmol) in DMF (47 mL) were added imidazole (0.68 g, 10.0 mmol) and *tert*-butyldimethylchlorosilane (0.97 g, 6.43 mmol). After 6 h, the reaction mixture was diluted with HCl (1M, 100 mL). The aqueous layer was separated and extracted with ethyl acetate (3×100 mL). The combined organic layers were washed with aqueous $NaHCO_3$ (1%, 100 mL), water (100 mL), dried ($MgSO_4$), filtered and concentrated. Column chromatography of the residue over silica gel with hexane/ethyl acetate (80:20→70:30) gave **15** as a colorless oil. Yield 2.28 g (82%). TLC (silica gel, ethyl acetate/hexane, 1:1) R_f = 0.88. $^{13}C\{^1H\}$-NMR ($CDCl_3$): δ = −5.26, −4.87 ($Si(CH_3)_2$), 14.20, 18.45 (Cq *t*Bu (α,β)), 25.21 (CH_3 *t*Bu (α,β)), 46.77, 49.86 (C-2 (α,β)), 62.32, 62.56 (C-6 (α,β)), 65.99, 67.17 (C-2′ (α,β)), 72.33, 72,79 (C-5 (α,β)), 73.38–75.39 (CH_2 Bn), 77.31, 77.36 (C-3 (α,β)), 79.32, 79.71 (C-4 (α,β)), 94.06, 95.67 (C-1 (α,β)).

3.1.7. 3,4-Di-*O*-benzyl-2-*C*-benzyloxymethyl-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-L-gluco-δ-lactone (16). Acetic anhydride (3.0 mL) was added to a solution of **15** (0.44 g, 0.76 mmol) in DMSO (5.0 mL). The mixture was stirred for 16 h, after which it was diluted with diethyl ether (100 mL), washed thoroughly with aqueous $NaHCO_3$ (10%, 3×10 mL) and brine (30 mL). The organic layer was dried (Na_2SO_4) and concentrated. Column chromatography of the residue over silica gel with hexane/ethyl acetate (100:0→85:15) gave **16** as a colorless oil. Yield 0.40 g (92%). TLC (silica gel, ethyl acetate/hexane, 1:5) R_f = 0.84. ^1H NMR ($CDCl_3$): δ = 0.08 (d, 6H, 2×CH_3, TBDMS), 0.89 (t, 9H, 3×CH_3, *t*Bu), 2.64 (m, 1H, H-2), 3.69 (dd, 1H, H-2′a), 3.89 (d, 2H, H_2-6), 3.98 (t, 1H, H-4), 4.00 (dd, 1H, H-2′b), 4.09 (t, 1H, H-3), 4.14 (m, 1H, H-5), 4.37–4.89 (3×AB, 6H, 3×CH_2 Bn), 7.23–7.35 (m, 15H, CH-arom Bn). $^{13}C\{^1H\}$-NMR ($CDCl_3$): δ = −5.37, −5.13 (2×CH_3 TBDMS), 18.31 (Cq *t*Bu), 25.93 (CH_3 *t*Bu), 48.79 (C-2), 61.43 (C-6), 66.67 (C-2′), 73.41, 74.81, 74.85 (3 CH_2 Bn), 76.78 (C-3/C-4), 79.27 (C-5), 127.74–128.62 (CH-arom Bn), 137.99, 138.02, 138.06 (3×Cq Bn), 170.45 (C=O lactone).

3.1.8. 3,4-Di-*O*-benzyl-2-*C*-benzyloxymethyl-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-D-gluco-amide (17). Lactone **16** (1.52 g, 2.63 mmol) was dissolved in methanolic ammonia (4 M, 50 mL). After stirring for 2 h, the mixture was concentrated and traces of ammonia were removed by coevaporation with toluene (1×10 mL). Column chromatography of the residue over silica gel with hexane/ethyl acetate (70:30→50:50) gave **17** as a colorless oil. Yield 0.83 g (53%). TLC (silica gel, ethyl acetate/hexane, 1:1) R_f = 0.41 and R_f = 0.28. The compound with R_f = 0.41 turned out to be the desired gluco-derivative. ^1H NMR ($CDCl_3$): δ = 0.05 (d, 6H, 2×CH_3, TBDMS), 0.90 (s, 9H, *t*Bu), 2.75 (br. s, 1H, OH-5), 3.63 (m, 1H, H-2, $J_{2,3}$ = 6.8 Hz, $J_{2,2'a}$ = 5.4 Hz, $J_{2,2'b}$ = 6.5 Hz), 3.62 (dd, 1H, H-4, $J_{3,4}$ = 3.0 Hz, $J_{4,5}$ = 7.6 Hz), 3.69

(dd, 1H, H-6a, $J_{5,6a}=5.4$ Hz, $J_{6a,6b}=10.2$ Hz), 3.77 (dd, 1H, H-6b, $J_{5,6b}=3.6$ Hz, $J_{6a,6b}=10.2$ Hz), 3.87 (m, 3H, H_2-2′, H-5, $J_{4,5}=8.2$ Hz, $J_{5,6a}=5.4$ Hz, $J_{5,6b}=3.6$ Hz), 4.37 (dd, 1H, H-3, $J_{2,3}=6.8$ Hz, $J_{3,4}=3.1$ Hz), 4.35–4.72 (3×AB, 6H, 3×CH_2 Bn), 5.36 (s, 1H, NH), 6.42 (s, 1H, NH), 7.24–7.36 (m, 15H, CH-arom Bn). $^{13}C\{^1H\}$-NMR ($CDCl_3$): δ = −5.3 ($Si(CH_3)_2$), 18.33 (Cq *t*Bu), 25.98 (CH_3 *t*Bu), 47.71 (C-2), 64.14 (C-6), 68.35 (C-2′), 71.46 (C-5), 73.49, 73.92, 74.32 (3×CH_2 Bn), 77.58 (C-3), 79.21 (C-4), 127.73–128.53 (CH-arom Bn), 137.81, 138.14, 138.36 (3×Cq Bn), 175.24 (C=O amide). ES–MS; *m*/*z*: 594.42, $[M+H]^+$; mono-isotopic MW calculated for $C_{34}H_{47}N_1O_6Si_1=593.32$.

3.1.9. 5-(R/S)-3,4-Di-*O*-benzyl-2-*C*-benzyloxymethyl-6-*O*-*tert*-butyldimethylsilyl-5-dehydro-2-deoxy-5-hydroxy-D-gluco-δ-lactam (18). Acetic anhydride (6.0 mL) was added to a solution of **17** (0.91 g, 1.54 mmol) in DMSO (10 mL). The mixture was stirred for 16 h, after which it was diluted with diethyl ether (100 mL), washed thoroughly with aqueous $NaHCO_3$ (10%, 3×20 mL) and brine (50 mL). The organic layer was dried (Na_2SO_4) and concentrated. Column chromatography of the residue over silica gel with hexane/ethyl acetate (80:20→40:60) gave **18** as a colorless oil. Yield 0.74 g (81%), mixture of isomers (S-configuration 78%, R-configuration 22%). TLC (silica gel, ethyl acetate/hexane, 1:1) $R_f=0.73$ and 0.37. ^1H NMR [S-configuration] ($CDCl_3$): δ = 0.03 (d, 6H, 2×CH_3, $Si(CH_3)_2$), 0.87 (s, 9H, *t*Bu), 2.51 (m, 1H, H-2, $J_{2,3}=8.7$ Hz, $J_{2,2'a}=2.9$ Hz, $J_{2,2'b}=5.8$ Hz), 3.27 (s, 1H, OH-5), 3.28 (d, 1H, H-6a, $J_{6a,6b}=10.2$ Hz), 3.52 (d, 1H, H-6b, $J_{6a,6b}=10.2$ Hz), 3.65 (dd, 1H, H-2′a, $J_{2'a,2'b}=9.0$ Hz, $J_{2,2'a}=2.9$ Hz), 3.72 (d, 1H, H-4, $J_{3,4}=9.6$ Hz), 4.07 (dd, 1H, H-2′b, $J_{2'a2'b}=9.0$ Hz), 4.39 (d, 1H, CH Bn, $J=12.1$ Hz), 4.42 (dd, 1H, H-3, $J_{2,3}=8.7$ Hz, $J_{3,4}=9.6$ Hz), 4.56 (d, 1H, CH Bn, $J=12.1$ Hz), 4.57 (d, 1H, CH Bn, $J=11.0$ Hz), 4.68 (d, 1H, CH Bn, $J=11.4$ Hz), 4.82 (d, 1H, CH Bn, $J=11.0$ Hz), 4.97 (d, 1H, CH Bn, $J=11.4$ Hz), 6.32 (s, 1H, NH amide), 7.22–7.38 (m, 15H, CH-arom Bn). $^{13}C\{^1H\}$-NMR [S-configuration] ($CDCl_3$): δ = −5.39 ($SiCH_3$), −5.31 ($SiCH_3$), 18.31 (Cq *t*Bu), 25.92 (CH_3 *t*Bu), 49.47 (C-2), 66.57 (C-2′), 67.06 (C-6), 73.36, 75.04, 75.24 (3×CH_2 Bn), 75.11 (C-3), 79.45 (C-4), 81.99 (C-5), 127.73-128.70 (CH-arom Bn), 137.54, 138.04, 138.22 (3×Cq Bn), 170.57 (C=O amide). ES–MS; *m*/*z*: 592.28, $[M+H]^+$; monoisotopic M_w calculated for $C_{34}H_{45}N_1O_6Si_1=591.30$. ^1H NMR [R-configuration] ($CDCl_3$): δ = 0.06 (d, 6H, $Si(CH_3)_2$), 0.90 (s, 9H, *t*Bu), 2.83 (m, 1H, H-2, $J_{2,3}=5.0$ Hz, $J_{2,2'a}=4.2$ Hz, $J_{2,2'b}=6.9$ Hz), 3.74 (dd, 2H, H_2-6, $J_{6a,6b}=9.7$ Hz), 3.82 (m, 2H, H-2′a, H-4, $J_{3,4}=6.7$ Hz, $J_{2'a,2'b}=9.0$ Hz, $J_{2,2'a}=4.2$ Hz), 3.87 (dd, 1H, H-2′b, $J_{2'a,2'b}=9.0$ Hz, $J_{2,2'b}=6.9$ Hz), 4.16 (dd, 1H, H-3, $J_{2,3}=5.1$ Hz, $J_{3,4}=6.3$ Hz), 4.24 (s, 1H, OH-5), 4.45–4.67 (3×AB, 6H, 3×CH_2 Bn), 6.28 (s, 1H, NH), 7.20–7.33 (m, 15H, CH-arom Bn). $^{13}C\{^1H\}$-NMR [R-configuration] ($CDCl_3$): δ-5.33 ($SiCH_3$),-5.29 ($SiCH_3$), 18.46 (Cq *t*Bu), 25.99 (CH_3 *t*Bu), 47.31 (C-2), 67.17 (C-2′), 68.01 (C-6), 73.19, 73.31, 73.85 (3×CH_2 Bn), 73.39 (C-3), 78.53 (C-4), 83.97 (C-5), 127.70–128.59 (CH-arom Bn), 137.48, 137.51, 138.30 (3×Cq Bn), 168.94 (C=O amide). ES–MS; *m*/*z*: 592.28, $[M+H]^+$; mono-isotopic MW calculated for $C_{34}H_{45}N_1O_6Si_1=$ 591.30.

3.1.10. 3,4-Di-*O*-benzyl-2-*C*-benzyloxymethyl-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-D-gluco-δ-lactam (19). $NaCNBH_3$ (0.078 g, 1.24 mmol) was added to mixture of **18** (0.74 g, 1.24 mmol; mixture of isomers) in acetonitrile (13 mL) and formic acid (1.40 mL). The reaction mixture was refluxed for 2 h, cooled to 0 °C, and quenched with aqueous HCl (0.1 N, 12.4 mL). After 15 min, the solution was poured into a mixture of ethyl acetate and aqueous $NaHCO_3$ (10%, 200 mL, 1:1). The aqueous layer was separated and extracted with ethyl acetate (3×50 mL). The combined organic layers were washed with brine (50 mL). The organic phase was dried ($MgSO_4$) and concentrated. Column chromatography of the residue over silica gel with hexane/ethyl acetate (80:20→55:45) gave **19** as a colorless oil. Yield *R*-isomer 0.45 g (63%), yield *S*-isomer 0.12 g (17%). ^1H NMR [R-isomer] ($CDCl_3$): δ = 0.02 (d, 6H, 2×CH_3 $Si(CH_3)_2$), 0.86 (s, 9H, CH_3 *t*Bu), 2.44 (m, 1H, H-2, $J_{2,3}=9.6$ Hz, $J_{2,2'a}=5.4$ Hz, $J_{2,2'b}=2.8$ Hz), 3.29 (dd, 1H, H-6a, $J_{6a,6b}=9.8$ Hz), 3.39 (m, 1H, H-5, $J_{4,5}=8.9$ Hz, $J_{5,6a}=8.4$ Hz, $J_{5,6b}=3.0$ Hz), 3.48 (t, 1H, H-4, $J_{3,4}=J_{4,5}=9.1$ Hz), 3.71 (dd, 1H, H-2′a), 3.75 (dd, 1H, H-6b), 4.09 (dd, 1H, H-2′b), 4.11 (t, 1H, H-3), 4.38–4.94 (3×AB, 6H, 3×CH_2 Bn), 6.06 (s, 1H, NH), 7.21–7.35 (m, 15H, CH-arom Bn). $^{13}C\{^1H\}$-NMR [R-isomer] ($CDCl_3$): δ = −5.39 ($SiCH_3$), −5.35 ($SiCH_3$), 18.26 (Cq *t*Bu), 25.91 (CH_3 *t*Bu), 48.56 (C-2), 55.69 (C-5), 64.14 (C-6), 65.79 (C-2′), 73.33, 74.89, 75.10 (3×CH_2 Bn), 77.71 (C-3), 78.68 (C-4), 127.63-128.64 (CH-arom Bn), 137.82, 138.34, 138.21 (3×Cq Bn), 170.23 (C=O amide). ES–MS; *m*/*z*: 576.34, $[M+H]^+$; mono-isotopic MW calculated for $C_{34}H_{45}N_1O_5Si_1=575.31$. ^1H NMR [S-isomer] ($CDCl_3$): δ = 0.02 (d, 6H, 2×CH_3 $Si(CH_3)_2$), 0.88 (s, 9H, CH_3 *t*Bu), 2.78 (m, 1H, H-2), 3.62–3.76 (m, 4H, H-4, H-5, H_2-6), 3.85 (m, 2H, H_2-2), 4.16 (dd, 1H, H-3), 4.35–4.79 (3×AB, 6H, 3×CH_2 Bn), 6.01 (s, 1H, NH), 7.20–7.32 (m, 15H, CH-arom Bn). $^{13}C\{^1H\}$-NMR [S-isomer] ($CDCl_3$): δ = −5.39 ($SiCH_3$), −5.34 ($SiCH_3$), 18.30 (Cq *t*Bu), 25.95 (CH_3 *t*Bu), 47.24 (C-2), 53.69 (C-5), 63.69 (C-6), 68.53 (C-2′), 72.15, 72.43, 73.07 (3×CH_2 Bn), 71.89 (C-3), 74.78 (C-4), 127.63–128.72 (CH-arom Bn), 137.50, 138.14, 138.34 (3×Cq Bn), 170.25 (C=O amide). ES–MS; *m*/*z*: 576.34, $[M+H]^+$; HR-MS 575.314; mono-isotopic MW calculated for $C_{34}H_{45}N_1O_5Si_1=$ 575.3067. Tandem MS data: 468.27 (MH^+−benzyl alcohol), 444.22 (MH^+−*tert*-butyldimethylsilyl alcohol), 91.06 (benzyl cation).

3.1.11. 3,4-Di-*O*-benzyl-2-*C*-benzyloxymethyl-2-deoxy-D-gluco-δ-lactam (20). TBAF (1 M in THF, 1.0 mL) was added to a cooled (0 °C) solution of **19** (0.30 g, 0.52 mmol) in THF (8 mL). After 30 min, the reaction was quenched with aqueous sodium acetate (2 M, 1.5 mL). Ethyl acetate (20 mL) was added and the organic layer was washed with water (10 mL), dried ($MgSO_4$), and concentrated. Column chromatography of the residue over silica gel with MeOH/DCM (0:100→6:94) gave **20** as a colorless oil. Yield 0.22 g (91%). TLC (silica gel, ethyl acetate/hexane, 4:1) $R_f=0.20$. ^1H NMR ($CDCl_3$): δ = 2.44 (m, 1H, H-2, $J_{2,3}=9.3$ Hz, $J_{2,2'a}=2.8$ Hz, $J_{2,2'b}=5.6$ Hz), 3.21 (br. s, 1H, OH-6), 3.38 (m, 1H, H-5, $J_{4,5}=9.0$ Hz, $J_{5,6a}=3.0$ Hz, $J_{5,6b}=5.8$ Hz), 3.56 (dd, 1H, H-6a, $J_{5,6a}=5.9$ Hz, $J_{6a,6b}=11.6$ Hz), 3.61 (t, 1H,

H-4, $J_{3,4}=9.3$ Hz, $J_{4,5}=9.1$ Hz), 3.67 (dd, 1H, H-2′b, $J_{2'a,2'b}=8.9$ Hz), 3.79 (dd, 1H, H-6b, $J_{5,6b}=2.8$ Hz, $J_{6a,6b}=11.6$ Hz), 4.01 (dd, 1H, H-2′a, $J_{2,2'a}=2.8$ Hz, $J_{2'a,2'b}=8.9$ Hz), 4.08 (t, 1H, H-3, $J_{2,3}=J_{3,4}=9.4$ Hz), 4.34–4.91 (3 AB, 6H, 3 CH_2 Bn), 7.19–7.36 (m, 15H, CH-arom Bn). $^{13}C\{^1H\}$-NMR ($CDCl_3$): δ = 48.70 (C-2), 56.26 (C-5), 62.19 (C-6), 65.94 (C-2′), 73.28, 74.93, (CH_2 Bn), 77.46 (C-3), 78.18 (C-4), 127.69–128.63 (CH-arom Bn), 137.88, 138.21, 138.23 (3×Cq Bn), 171.99 (C=O amide). ES–MS; *m*/*z*: 461.23, $[M+H]^+$; mono-isotopic MW calculated for $C_{28}H_{31}N_1O_5=461.22$.

3.1.12. 3,4-Di-*O*-benzyl-2-*C*-benzyloxymethyl-2-deoxy-6-*O*-mesyl-D-gluco-δ-lactam (21). Mesyl chloride (8.0 μL, 0.10 mmol) was added to a cooled (0 °C) solution of **20** (26 mg, 56.4 μmol) in pyridine (5 mL). After 18 h, methanol (1 mL) was added to the mixture and subsequently concentrated. The residue was dissolved in diethyl ether, the organic layer washed with water (3×10 mL), brine (10 mL), dried (Na_2SO_4), and concentrated. Column chromatography of the residue over silica gel with DCM/MeOH (100:0→96:4) gave **21** as a colorless oil. Yield 30 mg (99%). TLC (silica gel, hexane/ethyl acetate 1:4) $R_f=0.32$. ^1H NMR ($CDCl_3$): δ = 2.50 (m, 1H, H-2, $J_{2,3}=8.9$ Hz), 2.96 (s, 3H, CH_3 Ms), 3.63 (m, 2H, H-4, H-5), 3.71 (dd, 1H, H-2′a, $J_{2.2'a}=3.0$ Hz, $J_{2'a,2'b}=8.9$ Hz), 4.03 (dd, 1H, H-2′b, $J_{2,2'b}=3.3$ Hz, $J_{2'a,2'b}=8.9$ Hz), 4.13 (m, 2H, H-3, H-6a), 4.29 (dd, 1H, H-6b, $J_{5,6b}=2.4$ Hz, $J_{6a,6b}=10.4$ Hz), 4.37–4.93 (3×AB, 6H, 3×CH_2 Bn), 7.21–7.37 (m, 15H, CH-arom Bn). $^{13}C\{^1H\}$-NMR ($CDCl_3$): δ = 37.55 (CH_3 Ms), 48.41 (C-2), 53.74 (C-5), 66.02 (C-2′), 68.20 (C-6), 73.33, 74.75 (CH_2 Bn), 76.90 (C-3, C-4), 127.74-128.79 (CH-arom Bn), 137.38, 137.94, 138.16 (3×Cq Bn), 170.81 (C=O lactam). ES–MS; *m*/*z*: 540.18, $[M+H]^+$; mono-isotopic MW calculated for $C_{29}H_{33}N_1O_7S_1=539.19$.

3.1.13. 6-Azido-3,4-di-*O*-benzyl-2-*C*-benzyloxymethyl-2,6-dideoxy-D-gluco-δ-lactam (22). Sodium azide (0.13 g, 2.0 mmol) was added to a solution of **21** (0.36 g, 0.67 mmol) in DMF (10 mL) and stirred for 4 h at 70 °C. The mixture was concentrated, and the residue was dissolved in DCM (50 mL). The organic layer was washed with water (20 mL), dried ($MgSO_4$), and concentrated. Column chromatography of the residue over silica gel with DCM/MeOH (100:0→97:3) gave **22** as a colorless oil. Yield 0.29 g (89%). TLC (silica gel, hexane/ethyl acetate, 1:4) $R_f=0.76$. ^1H NMR ($CDCl_3$): δ = 2.48 (m, 1H, H-2, $J_{2,3}=9.4$ Hz, $J_{2,2'a}=2.8$ Hz, $J_{2,2'b}=6.6$ Hz), 3.31 (dd, 1H, H-6a, $J_{5,6a}=5.4$ Hz, $J_{6a,6b}=12.5$ Hz), 3.41 (m, 1H, H-5, $J_{4,5}=8.3$ Hz, $J_{5,6a}=5.2$ Hz, $J_{5,6b}=3.0$ Hz), 3.58 (dd, 1H, H-6b, $J_{5,6b}=3.0$ Hz, $J_{6a,6b}=12.5$ Hz), 3.63 (t, 1H, H-4, $J_{4,5}=8.9$ Hz, $J_{3,4}=9.2$ Hz), 3.70 (dd, 1H, H-2′a, $J_{2,2'a}=2.9$ Hz, $J_{2'a,2'b}=8.9$ Hz), 4.08 (m, 2H, H-2′b, H-3, $J_{2'a,2'b}=8.8$ Hz, $J_{2,2'b}=6.5$ Hz, $J_{2,3}=9.3$ Hz, $J_{3,4}=9.1$ Hz), 4.35–4.93 (3×AB, 6H, 3×CH_2 Bn), 7.18–7.36 (m, 15H, CH-arom Bn), 7.53 (s, 1H, NH lactam). $^{13}C\{^1H\}$-NMR ($CDCl_3$): δ = 48.46 (C-2), 51.88 (C-6), 53.96 (C-5), 65.79 (C-2′), 73.19, 74.74, 74.81 (3×CH_2 Bn), 77.24 (C-3), 78.11 (C-4), 127.56-128.52 (CH-arom Bn), 137.68, 138.07, 138.19 (3×Cq Bn), 171.47 (C=O lactam). ES–MS; *m*/*z*: 487.18, $[M+H]^+$; mono-isotopic M_W calculated for $C_{28}H_{30}N_4O_4=486.22$.

3.1.14. 6-Amino-3,4-di-*O*-benzyl-2-*C*-benzyloxymethyl-2,6-dideoxy-D-gluco-δ-lactam (23). Water (0.5 mL) and triphenylphosphine (72 mg, 0.27 mmol) were added to a solution of **22** (84 mg, 0.18 mmol) in THF (5 mL). After refluxing for 6 h, the reaction mixture was concentrated. Column chromatography of the residue over silica gel with $CHCl_3$/MeOH/acetic acid/H_2O, (94:6:1.75:0.75→88:12:3.5:1.5) gave **23** as a colorless oil. Yield 21 mg (25%). TLC (silica gel, $CHCl_3$/MeOH/acetic acid/H_2O, 88:12: 3.5:1.5) $R_f=0.38$. ^1H NMR ($CDCl_3$): δ = 2.50 (m, 1H, H-2, $J_{2,3}=9.0$ Hz, $J_{2,2'a}=2.9$ Hz, $J_{2,2'b}=3.1$ Hz), 2.75 (br. s, 1H, H-6a), 3.13 (br. s, 1H, H-6b), 3.40 (br. t, 1H, H-5), 3.57 (t, 1H, H-4, $J_{3,4}=J_{4,5}=8.7$ Hz), 3.70 (dd, 1H, H-2′a, $J_{2,2'a}=2.9$ Hz, $J_{2'a,2'b}=8.9$ Hz), 4.02 (dd, 1H, H-2′b, $J_{2'a,2'b}=8.9$ Hz, $J_{2,2'b}=3.1$ Hz), 4.08 (t, 1H, H-3, $J_{2,3}=9.1$ Hz, $J_{3,4}=8.9$ Hz), 4.34–4.91 (3×AB, 6H, 3×CH_2 Bn), 7.19–7.36 (m, 15H, CH-arom Bn), 7.55 (s, 1H, NH lactam). $^{13}C\{^1H\}$-NMR ($CDCl_3$): δ = 42.17 (C-6), 48.43 (C-2), 54.88 (C-5), 65.99 (C-2′), 73.28, 74.65, 74.76 (3×CH_2 Bn), 77.46 (C-3), 78.50 (C-4), 127.67–128.66 (CH-arom Bn), 137.76, 138.15, 138.28 (3×Cq Bn), 171.76 (C=O lactam). ES–MS; *m*/*z*: 461.17, $[M+H]^+$; HR-MS: 460.239; mono-isotopic M_W calculated for $C_{28}H_{32}N_2O_4=460.2362$. Tandem MS data: *m*/*z* 353.18 (MH^+ − benzyl alcohol), 245.12 (MH^+ − 2×benzyl alcohol).

3.1.15. 6-Acetamido-3,4-di-*O*-benzyl-2-*C*-benzyloxymethyl-2,6-dideoxy-D-gluco-δ-lactam (24). Acetyl chloride (1.6 μL, 23 μmol) and TEA (6.4 μL, 46 μmol) were added to a cooled (0 °C) solution of **23** (5.3 mg, 11.5 μmol) in DCM (1 mL). After 15 min, the reaction mixture was concentrated. Column chromatography of the residue over silica gel with DCM/MeOH (100:0→94:6) gave **24** as a colorless oil. Yield 3.9 mg (67%). TLC (silica gel, DCM/MeOH, 95:5) $R_f=0.56$. ^1H NMR ($CDCl_3$): δ = 1.78 (s, 3H, CH_3 acetamido), 2.47 (m, 1H, H-2, $J_{2,3}=8.8$ Hz, $J_{2,2'a}=3.0$ Hz, $J_{2,2'b}=3.1$ Hz), 3.11 (m, 1H, H-6a, $J_{6a,6b}=14.3$ Hz, $J_{5,6a}=4.8$ Hz, $J_{NH,6a}=6.1$ Hz), 3.36 (m, 1H, H-5, $J_{4,5}=9.1$ Hz, $J_{5,6a}=4.3$ Hz, $J_{5,6b}=3.9$ Hz), 3.43 (t, 1H, H-4, $J_{3,4}=8.8$ Hz, $J_{4,5}=9.1$ Hz), 3.55 (m, 1H, H-6b, $J_{6a,6b}=14.3$ Hz, $J_{5,6b}=3.4$ Hz, $J_{NH,6b}=6.7$ Hz), 3.66 (dd, 1H, H-2′a, $J_{2'a,2'b}=8.9$ Hz, $J_{2,2'a}=3.0$ Hz), 4.01 (dd, 1H, H-2′b, $J_{2'a,2'b}=8.9$ Hz, $J_{2,2'b}=3.2$ Hz), 4.08 (t, 1H, H-3, $J_{2,3}=8.8$ Hz, $J_{3,4}=8.7$ Hz), 4.35–4.92 (3×AB, 6H, 3×CH_2 Bn), 5.13 (br. t, 1H, NH amide, $J_{NH,6a}=J_{NH,6b}=6.5$ Hz), 6.63 (s, 1H, NH lactam), 7.22–7.44 (m, 15H, CH-arom Bn). $^{13}C\{^1H\}$-NMR ($CDCl_3$): δ = 23.03 (CH_3 acetamido) 39.94 (C-6), 49.08 (C-2), 54.88 (C-5), 66.50 (C-2′), 73.33, 74.13, 74.78 (3×CH_2 Bn), 77.04 (C-3), 78.28 (C-4), 127.74–129.15 (CH-arom Bn), 137.83, 138.11, 138.22 (3×Cq Bn), 170.99, 171.76 (C=O amide, lactam). ES–MS; *m*/*z*: 503.26, $[M+H]^+$; mono-isotopic M_W calculated for $C_{30}H_{34}N_2O_5=502.25$.

3.1.16. 6-Acetamido-2,6-dideoxy-2-*C*-hydroxymethyl-D-gluco-δ-lactam (6). Pd/C (10%, 5 mg) was added to a solution of **24** (2.5 mg, 5.0 μmol) in DMF (0.5 mL). Hydrogen was passed through the stirred mixture for 16 h. The reaction mixture was filtered and concentrated. Yield 0.8 mg of a colorless oil (69%). ^1H NMR (D_2O): δ = 2.09 (s, 3H, CH_3 acetamido), 2.46 (m, 1H, H-2,

$J_{2,3}$ = 10.1 Hz, $J_{2,2'a}$ = $J_{2,2'b}$ = 2.8 Hz), 3.46 (m, 1H, H-5, $J_{4,5}$ = 9.4 Hz, $J_{5,6a}$ = 3.4 Hz, $J_{5,6b}$ = 5.0 Hz), 3.57 (dd, 1H, H-6a, $J_{5,6a}$ = 3.4 Hz, $J_{6a,6b}$ = 14.5 Hz), 3.62 (t, 1H, H-4, $J_{3,4}$ = 9.7 Hz, $J_{4,5}$ = 9.4 Hz), 3.63 (dd, 1H, H-6b, $J_{5,6b}$ = 5.0 Hz, $J_{6a,6b}$ = 14.5 Hz), 3.95 (t, 1H, H-3, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 9.9 Hz) 3.96 (dd, 1H, H-2′a, $J_{2'a,2'b}$ = 11.4 Hz, $J_{2,2'a}$ = 2.8 Hz), 4.16 (dd, 1H, H-2′b, $J_{2'a,2'b}$ = 11.4 Hz, $J_{2,2'b}$ = 2.8 Hz). $^{13}C\{^1H\}$-NMR (D_2O): δ = 22.67 (CH_3 acetyl) 40.76 (C-6), 49.89 (C-2), 55.54 (C-5), 58.23 (C-2′), 69.03 (C-4), 70.75 (C-3). ES–MS; *m*/*z*: 233.1, $[M+H]^+$; HR-MS 232.104. mono-isotopic MW calculated for $C_9H_{16}N_2O_5$ = 232.1059. Tandem MS data: 215.10 (MH^+- H_2O), 197.10 (MH^+-2×H_2O), 179.07 (MH^+- 3×H_2O).

3.1.17. 3,4-Di-*O*-benzyl-2-*C*-benzyloxymethyl-6-*O*-*tert*-butyldimethylsilyl-1,2,5-trideoxy-1,5-imino-D-glucitol (25). BH_3Me_2S complex (2 M, 0.65 mL, 1.3 mmol) was added to a cooled (0 °C) solution of **19** (94 mg, 0.16 mmol) in DCM (5 mL). The reaction mixture was stirred for 16 h at ambient temperature. EtOH (4 mL) was added and the mixture was concentrated. Column chromatography of the residue over silica gel with hexane/ethyl acetate (80:20→60:40) gave **25** as a colorless oil. Yield 56 mg (61%). TLC (silica gel, ethyl acetate/hexane, 1:4) R_f = 0.14. ^1H NMR ($CDCl_3$): δ = 0.04 (d, 6H, 2×CH_3, TBDMS), 0.89 (t, 9H, 3×CH_3, *t*Bu), 1.64 (br. s, 1H, NH), 1.83 (m, 1H, H-2), 2.60 (m, H-5, $J_{4,5}$ = 9.7 Hz, $J_{5,6a}$ = 4.8 Hz, $J_{5,6b}$ = 2.8 Hz), 2.64 (dd, 1H, H-1ax, $J_{1ax,1eq}$ = 12.8 Hz, $J_{1ax,2}$ = 11.6 Hz), 3.16 (dd, 1H, H-1eq, $J_{1ax,1eq}$ = 13.0 Hz, $J_{1eq,2}$ = 4.2 Hz), 3.40 (t, 1H, H-4, $J_{3,4}$ = 9.0 Hz, $J_{4,5}$ = 9.5 Hz), 3.52 (dd, 1H, H-3, $J_{2,3}$ = 10.6 Hz, $J_{3,4}$ = 9.0 Hz), 3.57 (m, 2H, H_2-2′, $J_{2'a,2'b}$ = 9.1 Hz, $J_{2,2'a}$ = 3.1 Hz, $J_{2,2'b}$ = 5.3 Hz), 3.74 (dd, 1H, H-6a, $J_{6a,6b}$ = 9.8 Hz, $J_{5,6a}$ = 4.7 Hz), 3.78 (dd, 1H, H-6b, $J_{6a,6b}$ = 9.7 Hz, $J_{5,6b}$ = 2.7 Hz), 4.41–4.95 (3×AB, 6H, 3×CH_2 Bn), 7.24–7.35 (m, 15H, CH-arom Bn). $^{13}C\{^1H\}$-NMR ($CDCl_3$): δ = −5.35 ($SiCH_3$), −5.29 ($SiCH_3$), 18.32 (Cq *t*Bu), 26.01 (CH_3 *t*Bu), 45.62 (C-2), 47.66 (C-1), 61.82 (C-5), 63.12 (C-6), 68.98 (C-2′), 73.17, 75.06, 75.31 (3×CH_2 Bn), 82.13 (C-4), 82.92 (C-3), 127.61–128.54 (CH-arom Bn), 138.57, 138.76, 138.80 (3×Cq Bn). ES–MS; *m*/*z*: 562.35, $[M+H]^+$; mono-isotopic M_W calculated for $C_{34}H_{47}N_1O_4Si_1$ = 561.33.

3.1.18. *N*-Benzyl-3,4-di-*O*-benzyl-2-*C*-benzyloxymethyl-6-*O*-*tert*-butyldimethylsilyl-1,2,5-trideoxy-1,5-imino-D-glucitol (26). TEA (10 μL, 72 μmol) and benzyl bromide (3.2 μL, 27 μmol) were added to a solution of **25** (10.2 mg, 18.1 μmol) in acetonitrile (250 μL). After 16 h, the mixture was diluted with DCM (2 mL) and washed with aqueous $NaHCO_3$ (1%, 2 mL). The aqueous phase was extracted with DCM (3×2 mL). The combined organic layers were dried ($MgSO_4$) and concentrated. Column chromatography of the residue over silica gel with hexane/ethyl acetate (100:0→70:30) gave **26** as a colorless oil. Yield 5.8 mg (49%). TLC (silica gel, ethyl acetate/hexane, 4:1) R_f = 0.70. ^1H NMR ($CDCl_3$): δ = 0.01 (d, 6H, 2×CH_3, TBDMS), 0.89 (s, 9H, 3×CH_3, *t*Bu), 1.94 (m, 1H, H-2, $J_{1ax,2}$ = 11.3 Hz, $J_{1eq,2}$ = 3.7 Hz, $J_{2,3}$ = 10.4 Hz, $J_{2,2'a}$ = 2.8 Hz, $J_{2,2'b}$ = 5.9 Hz), 2.09 (t, 1H, H-1ax, $J_{1ax,1eq}$ = 11.6 Hz, $J_{1ax,2}$ = 11.3 Hz), 2.38 (m, 1H, H-5, $J_{4,5}$ = 9.1 Hz, $J_{5,6a}$ = 3.6 Hz, $J_{5,6b}$ = 2.1 Hz), 2.83 (dd, 1H, H-1eq, $J_{1ax,1eq}$ = 11.7 Hz, $J_{1eq,2}$ = 3.7 Hz), 3.25 (d, 1H, NCHa Bn, $J_{Ha,Hb}$ = 13.6 Hz), 3.41 (dd, 1H, H-2′a, $J_{2'a,2'b}$ = 9.3 Hz, $J_{2,2'a}$ = 2.8 Hz), 3.54 (m, 2H, H-3, H-2′b, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 8.5 Hz, $J_{2'a,2'b}$ = 9.1 Hz, $J_{2,2'b}$ = 5.9 Hz), 3.67 (t, 1H, H-4, $J_{3,4}$ = 8.8 Hz, $J_{4,5}$ = 8.9 Hz), 3.98 (dd, 1H, H-6a, $J_{6a,6b}$ = 11.3 Hz, $J_{5,6a}$ = 3.6 Hz), 4.07 (dd, 1H, H-6b, $J_{6a,6b}$ = 11.3 Hz, $J_{5,6b}$ = 2.1 Hz), 4.24 (d, 1H, NCHb Bn, $J_{Ha,Hb}$ = 13.6 Hz), 4.30–4.94 (3×AB, 6H, 3×CH_2 Bn), 7.18–7.38 (m, 20H, CH-arom Bn). $^{13}C\{^1H\}$-NMR ($CDCl_3$): δ = −5.31 ($SiCH_3$), 18.25 (Cq *t*Bu), 26.02 (CH_3 *t*Bu), 41.43 (C-2), 53.28 (C-1), 56.99 (NCH_2 Bn), 60.28 (C-6), 67.77 (C-5), 69.13 (C-2′), 72.94, 74.79, 74.81 (3×CH_2 Bn), 80.59 (C-4), 82.83 (C-3), 127.48-128.86 (CH-arom Bn), 138.60, 138.90, 138.99, 139.97 (4×Cq Bn). ES–MS; *m*/*z*: 652.4, $[M+H]^+$; HR-MS: 651.365; mono-isotopic MW calculated for $C_{41}H_{53}N_1O_4Si_1$ = 651.3744. Tandem MS data: *m*/*z* 544.32 (MH^+- benzyl alcohol), 520.29 (MH^+- *tert*-butyldimethylsilyl alcohol).

3.1.19. *N*-Benzyl-3,4-di-*O*-benzyl-2-*C*-benzyloxymethyl-1,2,5-trideoxy-1,5-imino-D-glucitol (27). TBAF (1M in THF, 300 μL) was added to a cooled (0 °C) solution of **26** (28 mg, 43 μmol) in THF (250 μL). After 6 h, the mixture was quenched with aqueous sodium acetate (2 M, 500 μL). The water layer was separated and extracted with DCM (3×2 mL). The combined organic layers were dried ($MgSO_4$) and concentrated. Column chromatography of the residue over silica gel with ethyl acetate/hexane, (0:100→40:60) gave **27** as a colorless oil. Yield 23 mg (100%). TLC (silica gel, ethyl acetate/hexane, 1:4) R_f = 0.25. ^1H NMR ($CDCl_3$): δ = 1.91 (m, 1H, H-2), 2.23 (t, 1H, H-1ax, $J_{1ax,1eq}$ = 11.7 Hz), 2.32 (br. s, 1H, OH-6), 2.36 (m, 1H, H-5, $J_{4,5}$ = 9.3 Hz, $J_{5,6a}$ = 1.5 Hz, $J_{5,6b}$ = 2.7 Hz), 2.97 (dd, 1H, H-1eq, $J_{1ax,1eq}$ = 11.8 Hz, $J_{1eq,2}$ = 3.8 Hz), 3.28 (d, 1H, NCHa Bn, $J_{Ha,Hb}$ = 13.7 Hz), 3.44 (dd, 1H, H-2′a, $J_{2'a,2'b}$ = 9.3 Hz, $J_{2,2'a}$ = 2.8 Hz), 3.52 (m, 2H, H-3, H-2′b, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 9.1 Hz, $J_{2'a,2'b}$ = 9.2 Hz, $J_{2,2'b}$ = 5.7 Hz), 3.72 (t, 1H, H-4, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz), 3.88 (dd, 1H, H-6a, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6a}$ = 1.5 Hz), 4.01 (dd, 1H, H-6b, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6b}$ = 3.0 Hz), 4.08 (d, 1H, NCHb Bn, $J_{Ha,Hb}$ = 13.5 Hz), 4.34–4.98 (3×AB, 6H, 3×CH_2 Bn), 7.19–7.39 (m, 20H, CH-arom Bn). $^{13}C\{^1H\}$-NMR ($CDCl_3$): δ = 41.23 (C-2), 54.02 (C-1), 57.08 (NCH_2 Bn), 58.02 (C-6), 66.66 (C-5), 68.77 (C-2′), 73.03, 74.86, 75.44 (3×CH_2 Bn), 80.34 (C-4), 82.28 (C-3), 127.31–128.85 (CH-arom Bn), 138.19, 138.44, 138.59, 138.69 (4×Cq Bn). ES–MS; *m*/*z*: 538.3, $[M+H]^+$; mono-isotopic M_W calculated for $C_{35}H_{39}N_1O_4$ = 537.29.

3.1.20. *N*-benzyl-3,4-di-*O*-benzyl-2-*C*-benzyloxymethyl-6-chloro-1,2,5,6-tetradeoxy-1,5-imino-D-glucitol (28). Mesyl chloride (5 μL, 64 μmol) and TEA (18 μL, 129 μmol) were added to a cooled (0 °C) solution of **27** (11.6 mg, 21.5 μmol) in DCM (1 mL). After 16 h, methanol (50 μL) was added and the reaction mixture was concentrated. Column chromatography of the residue over silica gel with hexane/ethyl acetate (100:0→70:30) gave **28** as a colorless oil. Yield 11.6 mg (97%). TLC (silica gel, hexane/ethyl acetate, 4:1) R_f = 0.71. ^1H NMR ($CDCl_3$): δ = 1.93 (m, 1H, H-2), 2.15 (t, 1H, H-1ax, $J_{1ax,1eq}$ = 11.6 Hz, $J_{1ax,2}$ = 11.4 Hz), 2.52 (m, 1H, H-5, $J_{4,5}$ = 8.9 Hz,

$J_{5,6a}$ = 3.0 Hz, $J_{5,6b}$ = 1.8 Hz), 2.88 (dd, 1H, H-1eq, $J_{1ax,1eq}$ = 11.8 Hz, $J_{1eq,2}$ = 3.9 Hz), 3.16 (d, 1H, NCHa Bn, $J_{Ha,Hb}$ = 13.0 Hz), 3.40 (dd, 1H, H-2′a, $J_{2'a,2'b}$ = 9.4 Hz, $J_{2,2'a}$ = 2.7 Hz), 3.54 (m, 2H, H-3, H-2′b, $J_{2,3}$ = 10.1 Hz, $J_{3,4}$ = 8.9 Hz, $J_{2'a,2'b}$ = 9.4 Hz, $J_{2,2'b}$ = 5.5 Hz), 3.77 (t, 1H, H-4, $J_{3,4}$ = $J_{4,5}$ = 8.8 Hz), 3.97 (dd, 1H, H-6a, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6a}$ = 3.2 Hz), 4.10 (dd, 1H, H-6b, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6b}$ = 1.8 Hz), 4.11 (d, 1H, NCHb Bn, $J_{Ha,Hb}$ = 13.0 Hz), 4.31–4.98 (3×AB, 6H, 3×CH_2 Bn), 7.19–7.39 (m, 20H, CH-arom Bn). $^{13}C\{^1H\}$-NMR ($CDCl_3$): δ = 41.38 (C-2), 42.16 (C-6), 52.83 (C-1), 56.62 (NCH_2 Bn), 65.40 (C-5), 68.80 (C-2′), 72.95, 74.73, 75.28 (3×CH_2 Bn), 80.44 (C-4), 82.24 (C-3), 127.16–129.20 (CH-arom Bn), 138.48, 138.50, 138.58, 138.74 (4×Cq Bn). ES–MS; *m*/*z*: 556.3, $[M+H]^+$; mono-isotopic MW calculated for $C_{35}H_{38}Cl_1N_1O_3$ = 555.25.

3.1.21. 6-Azido-*N*-benzyl-3,4-di-*O*-benzyl-2-*C*-benzyloxymethyl-1,2,5,6-tetradeoxy-1,5-imino-D-glucitol (29). Sodium azide (7.0 mg, 107 μmol) was added to a solution of **28** (5.8 mg, 10.4 μmol) in DMF (250 μL) and the mixture was stirred for 1 h at 100 °C. The mixture was concentrated. Column chromatography of the residue over silica gel with hexane/ethyl acetate (100:0→70:30) gave **29** as a colorless oil. Yield 5.4 mg (91%). TLC (silica gel, hexane/ethyl acetate, 4:1) R_f = 0.65. ^1H NMR ($CDCl_3$): δ = 1.95 (m, 1H, H-2), 2.12 (t, 1H, H-1ax, $J_{1ax,1eq}$ = 11.6 Hz, $J_{1ax,2}$ = 11.4 Hz), 2.52 (m, 1H, H-5, $J_{4,5}$ = 9.0 Hz, $J_{5,6a}$ = 5.9 Hz, $J_{5,6b}$ = 3.0 Hz), 2.89 (dd, 1H, H-1eq, $J_{1ax,1eq}$ = 11.8 Hz, $J_{1eq,2}$ = 3.8 Hz), 3.25 (d, 1H, NCHa Bn, $J_{Ha,Hb}$ = 13.4 Hz), 3.41 (dd, 1H, H-2′a, $J_{2'a,2'b}$ = 9.3 Hz, $J_{2,2'a}$ = 2.8 Hz), 3.51 (dd, 1H, H-3, $J_{2,3}$ = 10.3 Hz, $J_{3,4}$ = 8.8 Hz), 3.55 (dd, 1H, H-2′b, $J_{2'a,2'b}$ = 9.4 Hz, $J_{2,2'b}$ = 5.5 Hz), 3.61 (dd, 1H, H-6a, $J_{6a,6b}$ = 12.6 Hz, $J_{5,6a}$ = 2.9 Hz), 3.65 (t, 1H, H-4, $J_{3,4}$ = $J_{4,5}$ = 8.8 Hz), 3.87 (dd, 1H, H-6b, $J_{6a,6b}$ = 12.6 Hz, $J_{5,6b}$ = 2.9 Hz), 4.11 (d, 1H, NCHb Bn, $J_{Ha,Hb}$ = 13.4 Hz), 4.32–4.98 (3×AB, 6H, 3×CH_2 Bn), 7.18–7.37 (m, 20H, CH-arom Bn). $^{13}C\{^1H\}$-NMR ($CDCl_3$): δ = 41.20 (C-2), 48.45 (C-6), 53.49 (C-1), 57.48 (NCH_2 Bn), 65.37 (C-5), 68.81 (C-2′), 72.98, 74.71, 75.13 (3×CH_2 Bn), 80.79 (C-4), 82.35 (C-3), 127.19–128.97 (CH-arom Bn), 138.29, 138.50, 138.68 (Cq Bn). ES–MS; *m*/*z*: 563.3, $[M+H]^+$; mono-isotopic MW calculated for $C_{35}H_{38}N_4O_3$ = 562.29.

3.1.22. 6-Amino-*N*-benzyl-3,4-di-*O*-benzyl-2-*C*-benzyloxymethyl-1,2,5,6-tetradeoxy-1,5-imino-D-glucitol (30). Triphenylphosphine (5.0 mg, 19 μmol) and water (10 μL) were added to a solution of **29** (5.4 mg, 9.5 μmol) in THF (0.5 mL). The reaction mixture was stirred for 2 h at 60 °C, after which the mixture was concentrated. Column chromatography of the residue over silica gel with $CHCl_3$/MeOH (100:0→90:10) gave **30** as a colorless oil. Yield 3.6 mg (71%). TLC (silica gel, DCM/MeOH, 95:5) R_f = 0.32. ^1H NMR ($CDCl_3$): δ = 1.48 (s, 3H, NH_3), 1.93 (m, 1H, H-2), 2.15 (t, 1H, H-1ax, $J_{1ax,1eq}$ = $J_{1ax,2}$ = 11.7 Hz), 2.30 (m, 1H, H-5, $J_{4,5}$ = 9.3 Hz, $J_{5,6a}$ = 2.5 Hz, $J_{5,6b}$ = 3.3 Hz), 2.91 (dd, 1H, H-1eq, $J_{1ax,1eq}$ = 11.9 Hz, $J_{1eq,2}$ = 3.9 Hz), 3.03 (dd, 1H, H-6a, $J_{6a,6b}$ = 14.0 Hz, $J_{5,6a}$ = 2.5 Hz), 3.17 (dd, 1H, H-6b, $J_{6a,6b}$ = 13.8 Hz, $J_{5,6b}$ = 3.6 Hz), 3.20 (d, 1H, NCHa Bn, $J_{Ha,Hb}$ = 13.6 Hz), 3.43 (dd, 1H, H-2′a, $J_{2'a,2'b}$ = 9.3 Hz, $J_{2,2'a}$ = 2.7 Hz), 3.53 (m, 2H, H-2′b, H-3, $J_{2'a,2'b}$ = 9.3 Hz, $J_{2,2'b}$ = 9.4 Hz, $J_{2,3}$ = 10.7 Hz, $J_{3,4}$ = 9.3 Hz), 3.69 (t, 1H, H-4, $J_{3,4}$ = 9.1 Hz, $J_{4,5}$ = 9.3 Hz), 4.05 (d, 1H, NCHb Bn, $J_{Ha,Hb}$ = 13.7 Hz), 4.33–4.97 (3×AB, 6H, 3×CH_2 Bn), 7.18–7.40 (m, 20H, CH-arom Bn). $^{13}C\{^1H\}$-NMR ($CDCl_3$): δ = 38.39 (C-6), 41.25 (C-2), 53.92 (C-1), 56.48 (NCH_2 Bn), 66.99 (C-5), 68.96 (C-2′), 72.98, 74.83, 75.02 (3×CH_2 Bn), 79.78 (C-4), 82.96 (C-3), 127.03-129.02 (CH-arom Bn), 138.53, 138.66, 138.84, 139.02 (4×Cq Bn). ES–MS; *m*/*z*: 537.3, $[M+H]^+$; mono-isotopic M_W calculated for $C_{35}H_{40}N_2O_3$ = 536.30.

3.1.23. 6-Acetamido-*N*-benzyl-3,4-di-*O*-benzyl-2-*C*-benzyloxymethyl-1,2,5,6-tetradeoxy-1,5-imino-D-glucitol (31). TEA (5 μL, 36 μmol) and acetyl chloride (1.5 μL, 21 μmol) were added to a cooled (0 °C) solution of **30** (2.5 mg, 4.7 μmol) in DCM (0.5 mL). After 15 min, MeOH (100 μL) was added and the reaction mixture was concentrated. Column chromatography of the residue over silica gel with DCM/EtOH (100:0→95:5) gave **31** as a colorless oil. Yield 2.4 mg (85%). TLC (silica gel, MeOH/DCM, 5:95) R_f = 0.42. ^1H NMR ($CDCl_3$): δ = 1.93 (br. s, 4H, H-2, CH_3 acetamido), 2.18 (t, 1H, H-1ax, $J_{1ax,1eq}$ = $J_{1ax,2}$ = 11.7 Hz), 2.56 (m, 1H, H-5, $J_{4,5}$ = 9.0 Hz, $J_{5,6a}$ = 4.1 Hz, $J_{5,6b}$ = 2.0 Hz), 2.95 (dd, 1H, H-1eq, $J_{1ax,1eq}$ = 12.0 Hz, $J_{1eq,2}$ = 3.7 Hz), 3.26 (d, 1H, NCHa Bn, $J_{Ha,Hb}$ = 13.8 Hz), 3.37–3.56 (m, 5H, H_2-2′, H-3, H-4, H-6a), 3.98 (d, 1H, NCHb Bn, $J_{Ha,Hb}$ = 13.9 Hz), 4.01 (m, 1H, H-6b, $J_{6a,6b}$ = 14.3 Hz, $J_{5,6b}$ = 2.0 Hz, $J_{6b,NH}$ = 7.1 Hz), 4.33–4.95 (3×AB, 6H, 3×CH_2 Bn), 7.21–7.37 (m, 20H, CH-arom Bn). $^{13}C\{^1H\}$-NMR ($CDCl_3$): δ = 23.34 (CH_3 acetamido), 36.70 (C-6), 41.41 (C-2), 54.02 (C-1), 56.86 (NCH_2 Bn), 63.91 (C-5), 68.72 (C-2′), 73.06, 75.01, 75.54 (3×CH_2 Bn), 80.74 (C-4), 82.35 (C-3), 127.34-128.99 (CH-arom Bn), 138.13, 138.26, 138.40, 138.55 (4×Cq Bn), 170.20 (C=O acetamido). ES–MS; *m*/*z*: 579.3, $[M+H]^+$; HR-MS 578.299 mono-isotopic MW calculated for $C_{37}H_{42}N_2O_4$ = 578.3145. Tandem MS data: 471.26 (MH^+-benzyl alcohol), 412.22 (MH^+-benzyl alcohol–$CH_3C(O)NH_2$).

3.1.24. 6-Acetamido-2-*C*-hydroxymethyl-1,2,5,6-tetradeoxy-1,5-imino-D-glucitol (5). Compound **31** (1.8 mg, 3.1 μmol) was treated as described for the preparation of compound **6**. Yield 0.7 mg of a colorless oil (94%). ^1H NMR (D_2O): δ = 2.07 (m, 1H, H-2), 2.12 (s, 3H, CH_3 acetamido), 3.05 (t, 1H, H-1ax, $J_{1ax,1eq}$ = $J_{1ax,2}$ = 13.0 Hz), 3.29 (m, 1H, H-5, $J_{4,5}$ = 10.2 Hz, $J_{5,6a}$ = 6.6 Hz, $J_{5,6b}$ = 3.2 Hz), 3.57 (t, 1H, H-4, $J_{3,4}$ = 8.8 Hz, $J_{4,5}$ = 9.1 Hz), 3.58 (dd, 1H, H-1eq, $J_{1ax,1eq}$ = 13.0 Hz, $J_{1eq,2}$ = 4.3 Hz), 3.62 (dd, 1H, H-3, $J_{2,3}$ = 9.9 Hz, $J_{3,4}$ = 9.0 Hz), 3.64 (dd, 1H, H-6a, $J_{6a,6b}$ = 15.3 Hz, $J_{5,6a}$ = 6.6 Hz), 3.80 (dd, 1H, H-2′a, $J_{2'a,2'b}$ = 11.7 Hz, $J_{2,2'a}$ = 6.0 Hz), 3.85 (dd, 1H, H-6b, $J_{6a,6b}$ = 15.3 Hz, $J_{5,6b}$ = 3.1 Hz), 3.89 (dd, 1H, H-2′b, $J_{2'a,2'b}$ = 11.7 Hz, $J_{2,2'b}$ = 3.3 Hz). $^{13}C\{^1H\}$-NMR (D_2O): δ = 22.71 (CH_3 acetamido), 38.82 (C-6), 41.57 (C-2), 45.65 (C-1), 59.88 (C-2′), 60.34 (C-5), 71.34 (C-4), 71.87 (C-3). ES–MS; *m*/*z*: 219.2, $[M+H]^+$; HR-MS 218.119; mono-isotopic MW calculated for $C_9H_{18}N_2O_4$ = 218.1267. Tandem MS data: *m*/*z* 201.13 (MH^+-H_2O), 183.11 ($MH^+-2\times H_2O$), 165.11 ($MH^+-3\times H_2O$), 142.08 (MH^+-H_2O–$CH_3C(O)NH_2$).

3.1.25. 1,2,5-Trideoxy-2-*C*-hydroxymethyl-1,5-imino-D-glucitol (32). Compound **25** (3.3 mg, 5.8 μmol) was

treated with TBAF as described for the preparation of compound **20**. Subsequently, the residue was dissolved in aqueous EtOH (90%, 200 μL) and hydrochloric acid (1N, 5 μL) and Pd/C (10%, 5 mg) were added. Hydrogen was passed through the stirred mixture for 1 h. The reaction mixture was filtered and concentrated. Yield 1.2 mg of a colorless oil (70%). ^{1}H NMR (D_2O): δ = 2.07 (m, 1H, H-2), 3.01 (t, 1H, H-1ax, $J_{1ax,1eq} = J_{1ax,2} = 13.0$ Hz), 3.24 (m, 1H, H-5, $J_{4,5} = 9.8$ Hz, $J_{5,6a} = 5.7$ Hz, $J_{5,6b} = 3.0$ Hz), 3.60 (dd, 1H, H-1eq, $J_{1ax,1eq} = 12.9$ Hz, $J_{1eq,2} = 4.2$ Hz), 3.63 (t, 1H, H-3, $J_{2,3} = 10.3$ Hz, $J_{3,4} = 9.5$ Hz), 3.68 (t, 1H, H-4, $J_{3,4} = 9.5$ Hz, $J_{4,5} = 9.9$ Hz), 3.81 (dd, 1H, H-2′a, $J_{2'a,2'b} = 11.8$ Hz, $J_{2,2'a} = 6.2$ Hz), 3.91 (dd, 1H, H-2′b, $J_{2'a,2'b} = 11.7$ Hz, $J_{2,2'b} = 2.9$ Hz), 3.97 (dd, 1H, H-6a, $J_{6a,6b} = 12.6$ Hz, $J_{5,6b} = 5.6$ Hz), 4.03 (dd, 1H, H-6b, $J_{6a,6b} = 12.6$ Hz, $J_{5,6b} = 3.0$ Hz). ES–MS; *m*/*z*: 178.11, $[M+H]^+$; HR-MS: 177.097 mono-isotopic MW calculated for $C_7H_{15}N_1O_4 = 177.1001$. Tandem MS data: *m*/*z* 160.10 ($MH^+ - H_2O$), 142.08 ($MH^+ - 2\times H_2O$), 124.08 ($MH^+ - 3\times H_2O$).

3.1.26. Purification of homogenate of human spleen lysosomal β-hexosaminidase and isolation of isoenzymes. Spleen from a control subject was homogenized in 50 mM potassium phosphate buffer (pH 6.5) and centrifuged for 1 h at 60,000*g*. The supernatant was collected and used to measure total β-hexosaminidase activity.

Preparative isoelectric focussing of the supernatant fraction in a granulated flat bed gel was used to purify β-hexosaminidase A (P_i 5.0) and β-hexosaminidase B (P_i 7.5). Immunoprecipitation with specific antisera revealed that the β-hexosaminidase A and B fractions contained no other β-hexosaminidases.

3.2. Enzyme assays

IC_{50} values were determined by variation of inhibitory concentrations in the substrate mixtures. β-Hexosaminidase activities were determined with the fluorogenic substrate 4-methylumbelliferyl-*N*-acetyl-β-D-glucosaminide. Substrate mixtures contained 1.58 mM substrate in 75 mM McIlvain buffer (50 mM citric acid/100 mM sodium phosphate), pH = 4.0. The assays were performed at 37 °C and stopped by the addition of excess glycine-NaOH (0.3 M, pH = 10.6). Liberated 4-methylumbelliferone was fluorometrically measured using excitation and emission wavelengths of 366 and 445 nm, respectively.[27]

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