Synthesis of modified 1,5-imino-D-xylitols as ligands for lysosomal β-glucocerebrosidase

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Abstract
Modified 1,5-dideoxy-1,5-imino-D-xylitol analogues with different substitution patterns involving position C-1 and/or the ring nitrogen were prepared, which were designed to serve as precursors for the preparation of iminoxylitol-based ligands and tools for the elucidation and modulation of human lysosomal β-glucocerebrosidase. Biological evaluation of the synthesized glycomimetics with a series of glycoside hydrolases revealed that these substitution patterns elicit excellent β-glucosidase selectivities.

Graphical abstract

Keywords Carbohydrates · Conformation · Enzymes · β-Glucosidase ligands · Iminoxylitol · β-Glucocerebrosidase

Introduction
Iminoalditols, also termed iminosugars, are natural occurring glycomimetics, in which the ring oxygen of the carbohydrate moiety is replaced by a trivalent basic nitrogen. Paradigmatic structural scaffolds are polyhydroxylated piperidines 1, pyrrolidines 2, indolizidines 3, and pyrrolizidines 4 (Fig. 1) [1–5]. The nitrogen in the endocyclic position is responsible for the unique biological behavior of this compound class to interact and modulate active site specifically glycoside-processing enzymes. Since the last decades, such compounds have been of great interest for an interdisciplinary scientific community, including chemists, biochemists, as well as physicians.

Many different naturally occurring structures are known, exceeded by the number of synthetic derivatives, with manifold different modification patterns concerning the carbohydrate scaffold as well as customized derivatisations for different applications. This substance class has been implicated as potential therapeutic agents [6], for example, as immunomodulators [7, 8], as antibacterial [9, 10], antiviral [11, 12], anti-cancer [13], and anti-fungal [14] agents. In addition, iminoalditol-based glycomimetics have been

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Dedicated to Professor Dr. Heinz Falk on the happy occasion of his 80th birthday anniversary.
identified as plant growth inhibitors [15]. An interesting field of application has emerged when iminoalditols have been applied at sub-inhibitory concentrations to act as protein-folding templates [16, 17] for mutant lysosomal enzymes, thus becoming candidates for the management of lysosomal storage disorders in the pharmacological chaperone therapy [18]. Moreover, this compound class has received great attention as probes for activity-based profiling of glycoside-processing enzymes [19–21].

The d-xylo configuration in the dideoxy iminoalditol scaffold has been shown to have very interesting ligand properties for glycoside-processing enzymes in terms of activity as well as selectivity [22]. Various modifications with respect to substituents as well as positions on the iminoxylitol scaffold have been synthesized and biologically investigated. Basically all of these compounds have been shown to be highly selective ligands for β-glucosidases. For example, fluorinated iminoxylitols carrying an N-alkyl group [23] (Fig. 2), such as compound 5, have been found to exhibit immunosuppressive as well as glycosidase inhibitory activities. Based on Lehmann’s early finding [24], iminoxylitols bearing a guanidino or urea function at the ring nitrogen [25, 26], for example compound 6, were synthesized and found to be selective inhibitors of human lysosomal β-glucocerebrosidase (GCase) with IC₅₀ values in the low nm range. A deficiency of this enzyme causes Gaucher disease [27]. We have synthesized iminoxylitols modified at the endocyclic ring nitrogen with functionalized alkyl groups, such as compound structure 7 [28], as well as featuring more sophisticated substituents, including structure 8 [29]. These compounds exhibited inhibitory properties against β-xylosidase from *Thermoanaerobacterium saccharolyticum* (Xyl Therm. sac.), with *Kᵢ* values in the lower µm range (Table 1).

Martin and co-workers have developed elegant synthetic routes towards 1-C-alkyl imino-d-xylitols 9 (Fig. 3), and showed that the introduction of the substituent at position C-1 improved the ligand properties as well as the
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selectivity for GCase significantly [30–32]. In addition, in a structure–activity study, the influence of the position of an alkyl chain has been investigated, showing that a 1–2 shift of the alkyl substituent from C-1 to O-2 (compounds 10a–10b) increased the inhibitory property of the respective compound against GCase by a factor of 2 [33]. The same group has also synthesized 1,5-dideoxy-1,5-imino-α-xylitol (DIX) derivatives with alkyl substituents similar to ceramide at position C-1, for example compound 11, and obtained highly potent GCase inhibitors which also showed selective chaperone properties for mutations associated to Types 1 and 2 Gaucher Disease [34].

Compain and co-workers synthesized a library of 1-C-alkyl DIX derivatives (Fig. 4), including compounds 12, by a click chemistry approach, and found that some of these are GCase enhancers for selected Gaucher disease genotype mutants [35, 36].

Withers and co-workers developed a thiol-ene reaction sequence for rapid assembly of 1-C-alkyl DIX derivatives containing a sulfur atom between the DIX scaffold and the lipophilic substituent (Fig. 5), such as compounds 13, and also found excellent ligand properties in terms of activity as well as selectivity for GCase furnishing promising potent and selective pharmacological chaperones for GCase mutants [37].

Overkleeft and co-workers included into their structure–activity relationship study of lipophilic glycomimetics various α-xylo configured 1-C-iminosugar glycosides, for example compounds 14 (Fig. 6), and could demonstrate that these glycomimetics significantly exceed in terms of inhibitory activity as well as selectivity for GCase compared to the corresponding of α-gluco as well as α-ido configured analogues [38].

We have developed a convenient synthetic protocol for the modification of the DIX scaffold at position C-1 taking
advantage of the Staudinger/aza-Wittig/nucleophile reaction sequence [39, 40]. By this method, we have synthesized a range of simple C-1 alkyl modified DIX analogues (Fig. 7) and have found the same trend for these compounds, which are highly selective ligands for GCase.

All DIX derivatives carrying a substituent at position C-1, 9–15, have been found to be locked in the 1C4 conformation when the alkyl substituent is introduced from the β-face at the pseudoanomeric center (Fig. 8). The hydroxyl groups at positions O-2, O-3, and O-4 are in an axial orientation and the substituent at position C-1 is equatorial due to a piperidine ring inversion under acidic conditions such as in the lysosomal environment. In contrast, ring nitrogen substituted DIX derivatives, 5–8, are found in the typical 4C1 conformation (A, Fig. 8). This might be an explanation for the exceptional ligand properties as well as the selectivity of C-1-substituted DIX derivatives, which has been observed previously by others and us for similar alkyl-iminoxyitolos [30, 34, 36–40].

We are interested in the synthesis of iminosugar-based glycomimetics as tools for profiling and as ligands for modulating GCase activity. Consequently, we want to develop a simple and convenient approach towards N-modified DIX-based building blocks locked in the 1C4 conformation which carry a substituent suitable for further modifications for
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Results and discussion

For this study, we had to take two considerations into account. We wanted to investigate which modification pattern is best for ligand properties, modification at position C-1 or at the ring nitrogen. In addition, we were looking for a suitable functional group at the terminus of the handle which would allow further functionalisation for different applications, including introduction of reporter groups such as fluorescent dyes or click chemistry features. We decided to introduce either an ester group or an imidazole residue. Both functional groups have been found to be suitable for ligand properties of GCase \([34, 36]\).

For the synthesis of the C-1-modified DIX compounds, 1-C-ethenyliminoxylitol derivative 16, which has been synthesized previously by a Staudinger/aza-Wittig/Grignard reaction sequence \([40]\), served as suitable starting material (Scheme 1). Ozonolysis of compound 16 followed by a Horner–Wadsworth–Emmons reaction employing triethyl phosphonoacetate gave 1-C-(ethyloxycarbonyl-2-ethenyl) iminoxylitol derivative 17 in 78% over two steps. The double bond was reduced employing Pd/BaSO\(_4\) as catalyst under hydrogen atmosphere to provide compound 18 in 45% yield, which, after the final deprotection under hydrogenolytical conditions, furnished (1R)-1-C-ethyloxycarbonyl-1,5-dideoxy-1,5-imino-\(\alpha\)-xylitol (19) in 72%. As expected, this compound exhibits the \(1\text{C}_4\) conformation according to the NMR analysis, coupling constants of protons along the sugar ring exhibit characteristic values in the range of 3–5 Hz as are typical for this conformation.

For the introduction of the histamine moiety (Scheme 2), imine 18 was protected with a carboxybenzyl group (Cbz) at the ring nitrogen to give compound 20. The terminal ester group was saponified employing NaOH to furnish 1-C-propionic acid derivative 21, which was used without purification for the coupling step employing histamine dihydrochloride, (1-cyano-2-ethoxy-2-oxoethylidenaminoxy) dimethylaminomorpholinocarbenium hexafluorophosphate (COMU) and N,N-diisopropylethylamine (DIEA) as coupling cocktail to give protected (1R)-1-C-(imidazo-4-yl) ethyloxycarbonyl-1,5-imino-\(\alpha\)-xylitol 22 in 75% yield. Final deprotection under hydrogenolytical conditions gave the imidazole-modified iminoxylitol 23 in a yield of 78%. As expected, also this compound features the \(1\text{C}_4\) conformation according to the NMR analysis, coupling constants of protons along the sugar ring exhibit characteristic values in the range of 3–5 Hz.

To install the same modification patterns, an ester as well as the imidazole group, at the ring nitrogen, the double bond in protected 1-C-ethenyliminoxylitol 16 was reduced employing Pd/BaSO\(_4\) as catalyst under hydrogen atmosphere (Scheme 3). Under the same reaction conditions, the N-Cbz protecting group was cleaved off to give benzyl-protected (1R)-1-C-ethyloxycarbonyl-1,5-imino-\(\alpha\)-xylitol 24 in 81% yield. Introduction of the methoxycarbonylpentyl group at the ring nitrogen was achieved by employing methyl 6-iodohexanoate and sodium carbonate as base in DMF to give N-alkylated iminoxylitol 25 in 67% yield. No formation of a quaternary...
ammonium ion by double alkylation of the ring nitrogen has been observed during this reaction. Final deprotection of the benzyl groups under hydrogenolytical conditions gave (1R)-1-C-ethyl-N-methoxycarbonylpentyliminoxylitol (26) in 88% yield. Also compounds 24–26 were found in the 1C4 conformation exclusively, due to NMR analysis. Likely, the ethyl group at the position C-1 is being responsible for this finding.

The introduction of the imidazole moiety was conducted accordingly to the synthesis of compound 23 (Scheme 4). Saponification of the methyl ester of compound 25 followed by coupling of the histamine moiety led to protected imidazole-modified iminosugar derivative 27. Final debenzylation by hydrogenolysis gave (1R)-1-C-ethyl-N-(imidazol-4-yl)ethylethylaminocarbonylpentyliminoxylitol (28) in 86% yield. Accordingly, all compounds in this series were also found to adopt the 1C4 conformation by NMR analysis. The coupling constants of protons along the sugar ring exhibit characteristic values in the range of 3–5 Hz as are typical for this conformation.

For the biological evaluation of the synthesized DIX derivatives 19, 23, 26, and 28, we have probed a series of standard glycoside hydrolases, including β-glucosidase from Agrobacterium sp. (ABG), β-galactosidase from E. coli, Fabrazyme (commercial recombinant human lysosomal α-galactosidase), α-glucosidase S. cerevisiae, and human β-glucocerebrosidase GCase, to investigate ligand activity as well as selectivity. All compounds were found highly selective inhibitors of β-glucosidases and showed practically no detectable interaction with α-glucosidase (S. cere., β-Gal (E. coli), as well as human α-Gal (Fabrazyme), respectively, confirming the findings of other groups mentioned above. Both imidazole-modified compounds, 23 (Kᵢ value 1.1 µM) as well as 28 (4.1 µM), showed better inhibitory activity for
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Concerning our question regarding the modification pattern, we have obtained a very clear picture: 1-C-modified iminoxylitols 19 and 23 did not distinguish in their ligand properties between β-Glu from ABG and GCase with Kᵢ values in the same low µM range. In contrast, the ring nitrogen-modified compounds 26 and 28 showed excellent selectivity, with Kᵢ values of 57 and 4.1 µM, respectively, for GCase. No detectable inhibition of 26 as well as 28 was found with the other enzymes investigated, including β-Glu ABG. This increase in selectivity might be explained by the fact that compounds 26 and 28 combine the advantages of both features, the ethyl group at position C-1 locking the structure in the 1C4 conformation as well as the lipophilic substituents at the ring nitrogen. The former has been implied for favorable ligand properties and the fitting into the active site of GCase. The latter interacts with the lipophilic entrance to the active site of GCase mimicking the ceramide residue of the natural substrate glucosyl ceramide. Compounds 26 and 28 will serve as building blocks for further functionalisation as proposed.

Table 1 Kᵢ values [µM] of compounds with ABG = β-glucosidase, β-galactosidase from Agrobacterium sp.; β-galactosidase from E. coli; Fabrazyme = commercial recombinant human lysosomal α-galactosidase; α-glucosidase S. cerevisiae; human β-glucocerebrosidase Gaucher; N.I. = no inhibition or weak inhibition with estimated Kᵢ values higher than 1 mM

| Enzyme        | Compound | β-Glu, ABG | β-Gal, E. coli | α-Gal, Fabrazyme | α-Glu, S. cerevisiae | β-Glu, Gaucher |
|---------------|----------|------------|---------------|-----------------|----------------------|---------------|
|               | 19       | 2.1        | N.I.          | N.I.            | N.I.                 | N.I.          |
|               | 23       | 1.6        | N.I.          | N.I.            | N.I.                 | N.I.          |
|               | 26       | N.I.       | N.I.          | N.I.            | N.I.                 | 57            |
|               | 28       | N.I.       | N.I.          | N.I.            | N.I.                 | 4.1           |

Conclusion

We have investigated which position of substitution at the iminoxylitol scaffold for the introduction of further modifications is favorable, the ring nitrogen or position C-1. Therefore, we have synthesized two compounds in both patterns, one carrying a terminal ester group, compounds 19 and 26, and the other presenting an imidazole motif, compounds 23 and 28, for further modification. All four compounds were biologically evaluated with a series of standard glycosidases including human lysosomal β-glucocerebrosidase (GCase). Compounds 19 and 23, with the modification at position C-1 of the DIX scaffold, showed excellent selectivity towards β-glucosidases; however, both did not discriminate β-Glu from ABG and human lysosomal GCase, with Kᵢ values found in the low µM range. Compounds 26 and 28, carrying the modifications at the ring nitrogen and additionally an ethyl group at position C-1, turned out to interact exclusively with human lysosomal GCase with Kᵢ values of 57 and 4.1 µM, respectively. No detectable inhibition for any other enzyme included in this study has been observed. Thus, DIX-based scaffolds 26 and 28 are excellent building blocks for further functionalisation as proposed.
blocks for further modifications customized for different applications, such as for ligands to modulate and tools for profiling GCase activity.

**Experimental**

Optical rotations were measured at 20 °C on a Perkin Elmer 341 polarimeter at a wavelength of 589 nm and a path length of 10 cm. NMR spectra were recorded on a Varian INOVA 500 operating at 499.82 MHz (1H), and at 125.894 MHz (13C) or on a Bruker Ultra-shield spectrometer at 300.36 and 75.53 MHz, respectively. CDCls was employed for protected compounds and methanol-d4 or D2O for unprotected iminosylitols. Carbon and hydrogen numbering in NMR spectra was conducted in analogy to carbohydrate nomenclature and clockwise, starting with the pseudo anomeric position carbon as C-1. Chemical shifts are listed in delta notation and clockwise, starting with the pseudo anomeric position carbon as C-1. Signals were assigned unambiguously by COSY, HSQC, as well as APT analysis. The signals of the protecting groups as well as of the N-substituents were found in the expected regions and are only listed explicitly when overlapping with important spectral features of the respective compound. MALDI-TOF mass spectrometry was performed on a Micromass TofSpec 2E Time-of-Flight Mass Spectrometer. Analytical TLC was performed on precoated aluminum plates silica gel 60 F254 (E. Merck 5554) and detected with UV light (254 nm). For staining, a solution of 9 g vanillin in plates silica gel 60 F254 (E. Merck 5554) and detected with ozone traces and 200 mm3 dimethylsulfide was added to the reaction mixture, which was stirred for 45 min, followed by concentration under reduced pressure. The resulting colorless oil was added dropwise to a prepared solution of 330 mg KOtBu (2.90 mmol, 3 eq) and 580 mm3 triethylphosphonoacetate (2.90 mmol, 3 eq) in 50 cm3 THF. Upon consumption of the starting material (detected by TLC: cyclohexane/EtOAc = 2/1, v/v), CH2Cl2 was added and extracted with 2 N HCl and satd. NaHCO3 solution. The organic phase was dried over Na2SO4 and concentrated under reduced pressure.

Kinetic studies were performed at 37 °C in an appropriate buffer using a known concentration of enzyme (specific conditions depicted below). K_i determinations were performed using the corresponding 4-nitrophenyl α- or β-D-glycopyranoside as substrate. In a typical assay, the enzyme was incubated with different inhibitor concentrations for up to 5 min before initiating the reaction by the addition of substrate. The initial reaction rate was measured by monitoring the increase in absorbance at 400 nm for up to 10 min. K_i determinations were performed using at least two different substrate concentrations. For each inhibitor, a range of four-to-six inhibitor concentrations bracketing the K_i value ultimately determined was used for each substrate concentration. Dixon plots (1/v vs. [I]) were constructed to validate the use of the competitive inhibition model. The data were then fitted using non-linear regression analysis with Grafit 7.0. Specific assay conditions for each enzyme: *Agrobacterium sp.* β-glucosidase was expressed and purified recombinantly in *E. coli* as previously described [43]; 50 mM sodium phosphate buffer (pH 7) using 1.85 × 10^{-4} mg/cm3 of enzyme (K_m = 4.1 mM) [41, 42]; *E. coli* lac z β-galactosidase (Sigma-Aldrich): 50 mM sodium phosphate, 1.0 mM MgCl_2 (pH 7) using 6.4 × 10^{-4} mg/cm3 of enzyme (K_m = 60 μM); Fabrazyme (acid α-galactosidase, generously gifted by Dr Lorne Clarke, Department of Medical Genetics, University of British Columbia): 20 mM sodium citrate, 50 mM sodium phosphate, 1.0 mM tetrasodium EDTA, 0.25% v/v Triton X-100, and 0.25% w/v taurocholic acid buffer (pH 7.0) (K_m = 1.1 mM).
by TLC (cyclohexane/EtOAc = 2/1, v/v). The reaction mixture was filtered and concentrated under reduced pressure. Compound 18 (150 mg, 0.30 mmol) was dissolved in MeOH/H2O (1/1, v/v) and Pd(OH)2 on activated charcoal was added to the solution. The reaction mixture was stirred under hydrogen atmosphere until the starting material was consumed TLC (cyclohexane/EtOAc = 1/2, v/v). The reaction mixture was filtered, concentrated under reduced pressure and the obtained oil was purified using silica gel chromatography (CHCl3/Methanol/conc. NH3OH = 3/1/0.01, v/v/v). Compound 19 (50 mg) was obtained in 72% yield as colorless oil. Rf = 0.80 (CHCl3/Methanol/conc. NH3OH = 1/1/0.25, v/v/v); MS: m/z calcld. for C10H19NO2Na 256.1161, found 256.1188; [α]D21 = −13.8 (c = 1.2, H2O); 1H NMR (300 MHz, D2O): δ = 4.10 (q, 2H, H-9), 3.99–3.94 (m, 2H, H-3, H-4), 3.88 (dd, J1,12 = 3.6 Hz, J2,3 = 4.6 Hz, 1H, H-2), 3.46 (ddd, J1,12 = 1.3 Hz, 1H, H-1), 3.36 (dd, J4,5,5a = 2.2 Hz, J5a,5e = 13.8 Hz, 1H, H-5a), 3.24 (dd, J6,7 = 1.6 Hz, 1H, H-5e), 2.58–2.40 (m, 2H, H-7), 2.08–1.91 (m, 2H, H-6), 1.18 (t, 3H, H-10) ppm; 13C NMR (75.5 MHz, D2O): δ = 174.9 (C-8), 67.5 (C-2), 67.0 (C-3), 66.1 (C-4), 62.1 (C-9), 54.3 (C-11), 45.5 (C-5), 29.4 (C-7), 23.0 (C-6), 13.4 (C-10) ppm.

(1R)-1-C-(Ethoxy carbonyl ethyl)-1,5-dideoxy-1,5-imino-o-xylitol (19, C19H19NO2) Compound 18 (150 mg, 0.30 mmol) was dissolved in MeOH/H2O (1/1, v/v) and concentrated under reduced pressure. Compound 19 (50 mg) was obtained in 72% yield as colorless oil. Rf = 0.80 (CHCl3/Methanol/conc. NH3OH = 1/1/0.25, v/v/v); MS: m/z calcld. for C10H19NO2Na 256.1161, found 256.1188; [α]D21 = −13.8 (c = 1.2, H2O); 1H NMR (300 MHz, D2O): δ = 4.10 (q, 2H, H-9), 3.99–3.94 (m, 2H, H-3, H-4), 3.88 (dd, J1,12 = 3.6 Hz, J2,3 = 4.6 Hz, 1H, H-2), 3.46 (ddd, J1,12 = 1.3 Hz, 1H, H-1), 3.36 (dd, J4,5,5a = 2.2 Hz, J5a,5e = 13.8 Hz, 1H, H-5a), 3.24 (dd, J6,7 = 1.6 Hz, 1H, H-5e), 2.58–2.40 (m, 2H, H-7), 2.08–1.91 (m, 2H, H-6), 1.18 (t, 3H, H-10) ppm; 13C NMR (75.5 MHz, D2O): δ = 174.9 (C-8), 67.5 (C-2), 67.0 (C-3), 66.1 (C-4), 62.1 (C-9), 54.3 (C-11), 45.5 (C-5), 29.4 (C-7), 23.0 (C-6), 13.4 (C-10) ppm.

(1R)-2,3,4-Tri-O-benzyl-N-(benzyloxycarbonyl)-1-C-(ethoxy carbonyl ethyl)-1,5-dideoxy-1,5-imino-o-xylitol (20, C39H43NO7) Compound 18 (750 mg, 1.40 mmol) was dissolved in 20 cm3 MeOH and 480 mm3 Et3N (3.40 mmol, 2.4 eq). CbzlCl (250 mm3, 1.70 mmol, 1.2 eq) was added and the reaction mixture was stirred at ambient temperature. Upon consumption of the starting material (detected by TLC: cyclohexane/EtOAc = 1/1, v/v), the reaction mixture was concentrated under reduced pressure, dissolved in CH2Cl2, and extracted with 2 N HCl and sat. NaHCO3 solution. The organic layer was dried over Na2SO4 and concentrated under reduced pressure. Compound 20 (270 mg) was obtained after purification utilizing silica gel chromatography (cyclohexane/EtOAc = 1/1, v/v) in 24% yield as colorless oil. Rf = 0.45 (cyclohexane/EtOAc = 3/1, v/v); 1H NMR (300 MHz, CDCl3): δ = 7.29–7.12 (m, 20H, Ph), 5.03–4.94 (m, 2H, CH2Cbz), 4.80–4.74 (m, 2H, CH2Ph), 4.65–4.50 (m, 5H, 2xC2H5Ph, H-1), 4.36–4.26 (m, 1H, H-1, H-5e), 4.09–3.90 (m, 2H, H-9, H-5e), 3.58 (dd, J5,5a = 9.0 Hz, J3,4 = 9.2 Hz, 1H, H-3), 3.42 (dd, J2,1 = 6.1 Hz, 1H, H-2), 3.32 (dd, J4,5 = 5.5 Hz, 1H, H-4), 2.65 (dd, J5a,5e = 13.1 Hz, 1H, H-5a), 2.22–2.07 (m, 2H, H-7), 1.93–1.72 (m, 2H, H-6), 1.12 (t, 3H, H-10) ppm; 13C NMR (75.5 MHz, CDCl3): δ = 173.1 (d, C-8), 156.5 (d, Cbz), 138.9, 138.2, 136.4 (C2Ph), 128.7–127.0 (Ph), 82.0 (d, C-3), 79.6 (d, C-2), 78.2 (C-4), 75.8, 73.2, 72.8 (d, CH2Ph), 67.7 (d, CH2Cbz), 60.5 (d, C-9), 52.7 (d, C-1), 40.9 (d, C-5), 30.7 (C-7), 19.9 (d, C-6), 14.3 (C-10) ppm. Due to two pronounced rotameric populations (20) of the N-Cbz group, signal splitting in the respective NMR spectra has been observed leading to somehow poor resolution of NMR spectra.
0.86 mmol, 1.5 eq) was added and the reaction mixture was stirred until the starting material was consumed, TLC (EtOAc/MeOH = 10/1, v/v). The reaction mixture was concentrated under reduced pressure and purified by silica gel chromatography (EtOAc/MeOH = 10/1, v/v) to give compound 22 (300 mg) in 75% yield. \( R_f = 0.60 \) (CHCl\(_3\)/MeOH/concd NH\(_4\)OH = 6/1/0.01, v/v/v); MS: \( m/z \) calcd. for C\(_{42}\)H\(_{46}\)N\(_2\)O\(_{13}\)Na = 725.3315, found 725.3347. Due to two pronounced rotomeric populations (22) of the N-Cbz group, signal splitting in the respective NMR spectra has been observed leading to poor resolution of the NMR spectra.

(1R)-1-C[(imidazo-4-yl)ethylaminophenyl]-1,5-dideoxy-1,5-imino-\( \alpha \)-xylitol (23, C\(_{11}\)H\(_{22}\)N\(_4\)O\(_4\)) Compound 22 (300 mg, 0.43 mmol) was dissolved in 15 cm\(^3\) MeOH/H\(_2\)O (1/1, v/v), Pd(OH)\(_2\) on activated charcoal was added and the reaction mixture was stirred under hydrogen atmosphere. Upon consumption of the starting material (detected by TLC: CHCl\(_3\)/MeOH/concd NH\(_4\)OH = 6/1/0.01, v/v/v), the reaction mixture was filtered, concentrated under reduced pressure. After purification by silica gel chromatography (CHCl\(_3\)/MeOH/concd NH\(_4\)OH = 3/1/0.01, v/v/v), the reaction mixture was filtered, concentrated under reduced pressure. The reaction mixture was stirred under hydrogen atmosphere at ambient pressure. Upon consumption of the starting material (detected by TLC: cyclohexane/EtOAc = 2/1, v/v), the reaction mixture was filtered, concentrated under reduced pressure, dissolved in CH\(_2\)Cl\(_2\), and extracted with 2 N HCl and satd. NaHCO\(_3\) solution. The organic layer was dried over Na\(_2\)SO\(_4\) and concentrated under reduced pressure. Purification by silica gel chromatography gave compound 25 (640 mg) in a yield of 67% as colorless oil. \( R_f = 0.55 \) (cyclohexane/EtOAc = 2/1, v/v); \( [\alpha]_D^{20} = +12.7 \) (c = 1.1, CHCl\(_3\)); MS: \( m/z \) calcd. for C\(_{32}\)H\(_{46}\)N\(_4\)O\(_{12}\)Na = 582.3195, found 582.3217; \( ^1\)H NMR (300 MHz, CDCl\(_3\)): \( \delta = 7.29-7.16 \) (m, 15H, Ph), 4.80, 4.74 (2xd, 2H, C\(_2\)H\(_2\)Ph). 4.66–4.53 (m, 4H, C\(_2\)H\(_2\)Ph), 53.9 (s, 3H, H-14), 3.56–3.43 (m, 3H, H-2, H-3, H-4), 2.78–2.61 (m, J\(_{1,2} = 3.6\) Hz, J\(_{5e,5a} = 4.4\) Hz, J\(_{5e,5a} = 12.9\) Hz, H-1, H-5e), 2.52–2.34 (m, J\(_{5a,5e} = 4.4\) Hz, J\(_{5e,5a} = 12.9\) Hz, H-1, H-5e), 2.25 (t, 2H-11), 1.77 ppm; \( ^{13}\)C NMR (75.5 MHz, CDCl\(_3\)): \( \delta = 175.1 (C-8), 139.2, 138.8, 138.7 (3C, 3x Cq), 128.4–127.4 (Ph), 83.2 (C-4), 80.6 (C-2), 78.3 (C-3), 75.5, 73.1, 72.8 (3x C\(_2\)H\(_2\)Ph), 61.6 (C-1), 54.2 (C-8), 51.5 (C-14), 48.5 (C-5), 34.2 (C-12), 28.4 (C-9), 26.7 (C-10), 24.9 (C-11), 16.5 (C-6), 13.5 (C-7) ppm.

(1R)-2,3,4-Tri-O-benzyl-1-C-ethyl-1,5-dideoxy-1,5-imino-\( \alpha \)-xylitol (24, C\(_{28}\)H\(_{33}\)NO\(_3\)) Compound 24 (740 mg) was obtained with a yield of 81% as colorless oil. \( R_f = 0.66 \) (CHCl\(_3\)/MeOH/concd NH\(_4\)OH = 3/1/0.01, v/v/v); MS: \( m/z \) calcd. for C\(_{42}\)H\(_{46}\)N\(_2\)O\(_{13}\)Na = 725.3315, found 725.3347. Due to two pronounced rotomeric populations (22) of the N-Cbz group, signal splitting in the respective NMR spectra has been observed leading to poor resolution of the NMR spectra.
3H, H-7) ppm; $^{13}$C NMR (75.5 MHz, MeOH-$d_{4}$): $\delta$ = 175.7 (C-13), 69.4 (C-4), 69.2 (C-3, C-2), 63.7 (C-1), 54.3 (C-5), 53.7 (C-8), 52.1 (C-14), 34.5 (C-12), 27.2 (C-10), 25.5 (C-11), 23.4 (C-9), 19.8 (C-6), 10.3 (C-7) ppm.

(1R)-2,3,4-Tri-O-benzyl-N-(carboxypentyl)-1-C-ethyl-1,5-dideoxy-1,5-imino-$\alpha$-xylitol (25a, C$_{39}$H$_{50}$N$_{4}$O$_{4}$) Compound 25a (60 mg, 0.11 mmol) was dissolved in 5 cm$^{3}$ dioxane/H$_{2}$O (1/1, v/v). NaOH solution (3 M, 10 drops) was added and the reaction mixture was stirred until the starting material was consumed (TLC cyclohexane/EtOAc = 2/1, v/v). The reaction mixture was filtered, concentrated under reduced pressure, and purified by silica gel chromatography (CHCl$_{3}$/MeOH/conc. NH$_{4}$OH = 3/1/0.25, v/v/v), the reaction mixture was filtered, concentrated under reduced pressure, and purified by silica gel chromatography (CHCl$_{3}$/MeOH/conc. NH$_{4}$OH = 3/1/0.25, v/v/v), which gave compound 27 (70 mg) in a yield of 86% as colorless oil. $R_{f}$ = 0.60 (CHCl$_{3}$/MeOH/conc. NH$_{4}$OH = 2/1/0.25, v/v/v); MS: m/z calc. for C$_{18}$H$_{32}$N$_{4}$O$_{4}$Na 391.2321, found 391.2384; [a]$_{D}^{20}$ = +5.8 (c = 1.04, H$_{2}$O). $^{1}$H NMR (300 MHz, D$_{2}$O): $\delta$ = 7.95 (s, 1H, H-18), 6.96 (s, 1H, H-17), 3.82 (dd, J$_{4,5a}$ = 3.5 Hz, J$_{2,3}$ = 5.8 Hz, 1H, H-2, H-3), 3.75 (dd, J$_{4,3}$ = 5.6 Hz, 1H, H-4), 3.28 (dd, J$_{4,5a}$ = 3.1 Hz, J$_{4,3}$ = 5.6 Hz, 1H, H-4), 3.87 (dd, J$_{4,5a}$ = 3.1 Hz, J$_{4,5a}$ = 6.4 Hz, 1H, H-5a), 2.93 (t, 2H, H-12), 2.76 (t, 2H, H-15), 2.13 (t, 2H, H-8), 1.79–1.42 (m, 6H, H-6, H-9, H-10, H-11), 0.89 (t, 3H, H-7) ppm; $^{13}$C NMR (75.5 MHz, MeOH-$d_{4}$): $\delta$ = 178.2 (C=O), 139.1, 138.7, 138.6 (3x C$_{q}$), 128.6–127.7 (Ph), 81.0, 79.3, 77.3 (C-2, C-3, C-4), 75.2, 73.2, 73.0 (3x CH$_{2}$Ph), 61.0 (C-1), 53.4 (C-8), 48.3 (C-5), 34.6 (C-12), 27.0, 26.6, 24.9 (C-9, C-10, C-11), 16.4 (C-6), 13.4 (C-7) ppm.

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