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Synthesis of new β-1-C-alkylated imino-L-iditols: A comparative study of their activity as β-glucocerebrosidase inhibitors

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ABSTRACT

A short synthesis of new β-1-*C*-alkyl-1,5-dideoxy-1,5-imino-L-iditols by means of the diastereoselective addition of Grignard reagents onto a glucopyranosylamine is described. These compounds were evaluated as β-glucocerebrosidase inhibitors and their activity was compared with that of related iminosugar derivatives in the D-*gluco* and D-*xylo* series. The results allowed us to conclude on the influence of the hydroxymethyl moiety and of the piperidine-ring conformation on the inhibitory activity.

1. Introduction

Iminosugars, which are sugar mimetics with a nitrogen atom replacing the endocyclic oxygen,[1] are known as potent glycosidase inhibitors since the 70s.[2] Their activity as glycosyltransferase,[3,4] glycogen phosphorylase,[5,6] nucleoside-processing enzyme[7] or metalloproteinase[8] inhibitors has been uncovered more recently. As a consequence, a wide number of iminosugar derivatives are now lead compounds for the treatment of a variety of diseases including diabetes,[9] cancer,[10] viral infections,[11,12] cystic fibrosis,[13] and lysosomal diseases (LD).[14,15] LD are rare genetic disorders among which Gaucher disease is the most prevalent one. It is caused by a deficiency of β-glucocerebrosidase (GCase), the enzyme responsible for glucosylceramide hydrolysis. Certain mutations of this protein induce its misfolding and lead to its partial or complete destruction by the endoplasmic reticulum 'quality control' mechanism. This results in the accumulation of glucosylceramide in lysosomes, leading to severe symptoms such as bone pains, skeletal lesions, anemia, and liver or spleen damage. Three therapeutic strategies for Gaucher disease have been developed in the last years. The first one, enzyme replacement therapy (ERT), consists in the administration of a recombinant form of GCase.[16] However, ERT is very costly and only available for the non-neuronopathic form of Gaucher disease, as proteins do not cross the blood–brain barrier.[17] In another strategy, known as substrate reduction therapy (SRT), glucosylceramide synthase inhibitors are used to reduce glycosphingolipids biosynthesis and to decrease accordingly the concentration of their degradation product, glucosylceramide, in tissues.[14,18] Thus *Zavesca*®, the first orally administered treatment for a lysosomal disease (Gaucher disease), is an implementation of this therapy, as its active substance *N*-butyl-1-deoxynojirimycin is a good inhibitor of glucosylceramide synthase.[19,20] Unfortunately large doses are required, which leads to side-effects due to the concomitant inhibition of digestive glucosidases. The third strategy, so called pharmacological chaperone therapy (PCT) or active-site-specific chaperone (ASSC) therapy, is based on the concept that the activity of the misfolded GCase can be restored by the administration in sub-inhibitory concentration of a competitive inhibitor, which will stabilize the enzyme structure and prevent it from being eliminated by the organism.[21,22] This will finally allow its normal trafficking and restore its hydrolyzing activity in the lysosome, thus processing the excess glucosylceramide.

As part of our research program aimed at developing new potent and selective GCase inhibitors having chaperone effect, we reported that α-1-C-alkylated derivatives of 1-deoxynojirimycin (DNJ) **1** are more active and selective towards GCase than DNJ or its N-alkylated derivatives.[23] In addition, removal of the hydroxymethyl moiety in these structures led to a dramatic improvement of the activity. Indeed α-1-*C*-nonyl-1-deoxyimino-D-xylitol (α-1-C_9-DIX) **2** was found to strongly inhibit GCase (K_i = 2.2 nM) and an increase of the enzyme activity in Gaucher cells up to 2× was

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observed at a concentration of only 10 nM. Moreover, this compound showed an exceptional selectivity, as it had no activity on lysosomal or digestive α-glucosidases.[24]

This remarkable activity might be related to the inverted $^1C_4$ conformation of **2** (Fig. 1). Considering that the change from the D-*gluco* to the D-*xylo* series enhanced the activity by a factor of 100,[24] we envisaged that a further improvement of the activity might be gained for structures having an L-*ido* configuration, as the inverted $^1C_4$ conformation is even more strongly favored in this series. To test this hypothesis, a series of β-1-C-alkylated imino-L-iditols (β-1-*C*-alkyl-DIJ) **3** was then prepared. We present in this article the synthesis and the evaluation on GCase of β-1-*C*-propyl-, β-1-*C*-butyl-, β-1-*C*-hexyl-, and β-1-*C*-dihydroxypropyl-1-deoxyimino-L-iditols.

2. Results and discussion

To prepare stereoselectively these β-1-C-alkylated imino-L-iditol derivatives, our first approach consisted in using the chain-extension methodology that we developed for the synthesis of UDP-Gal*f* mimics based on an imino-galactofuranose skeleton (Scheme 1).[25] Thus we investigated the addition of allyltrimethylsilane onto *N*-benzyloxycarbonyl-2,3,4,6-tetra-*O*-benzyl-D-glucopyranosylamine **5**[26] under various Lewis acidic conditions ($Zn(OTf)_2$, $Bi(OTf)_3$, TMSOTf, TIPSOTf, and $TiCl_2(OiPr)_2$). Best results were obtained with the last reagent (52%), but were not easily reproducible. We therefore decided to use the addition of Grignard reagents, as originally reported by Nicotra and co-workers.[27,28] The first step was the synthesis of *N*-benzyl-2,3,4,6-tetra-*O*-benzyl-D-glucopyranosylamine **6** from commercial 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose **4** using an excess of benzylamine in the presence of PTSA. Nicotra's procedure was improved by increasing the concentration of **4** in the reaction mixture, which allowed us to reduce the quantity of benzylamine, the excess of which is quite difficult to remove. Then the addition of allylmagnesium bromide gave **7a** in a good yield (72%) with good diastereoselectivity (4:1). The two diastereoisomers were not easily separable by flash chromatography on silica gel. Traces of the second diastereoisomer could still be detected by NMR after purification, but they could be removed after cyclization.

Addition of butylmagnesium and hexylmagnesium chloride gave compounds **7b** and **7c** in lower yields but high selectivity. The presence of the other diastereoisomer could not be detected by NMR of the crude reaction mixture. The configuration of the newly formed asymmetric center was determined at a later stage of the synthesis on the cyclized compounds on the basis of $J_{H,H}$ NMR coupling constants. The major diastereoisomer **7a** was then submitted to Nicotra's cyclization conditions (triflic anhydride in pyridine). This procedure led however to a ~1:1 mixture of the expected cyclic amine **8a** and the cyclic ether **9** (Scheme 2).

Indeed our group had shown in 1995 that internal displacement of triflates by a benzyloxy group in 1,4-relationship led spontaneously to 1-*C*-alkyl furanosides.[29] In order to avoid the formation of this by-product, we chose the conditions used by Dondoni and coworkers for the synthesis of iminosugar *C*-glycosides via nucleophilic addition to chiral nitrones.[30] Compounds **7a**–**c** were thus efficiently cyclized using mesyl chloride in pyridine at 100 °C. This cyclization occurs via a S_N2 mechanism with an inversion of configuration at C5 leading to the L-*ido* isomer. $J_{H,H}$ coupling constants indicated clearly that the major stereoisomer had a 1,2-*cis* configuration (for **8c**, $J_{1,2}$ = 5.6 Hz, axial–equatorial coupling constant) and that the 'β-L-*ido*'-configured piperidine derivatives adopted predominantly the $^4C_1$ conformation (for **8c**, $J_{2,3}$ = 8.8 Hz, axial–axial coupling constant). The last step of the synthesis was the cleavage of *O*- and *N*-benzyl groups by hydrogenolysis. After extensive investigations, the best results were obtained using a 1:1 mixture

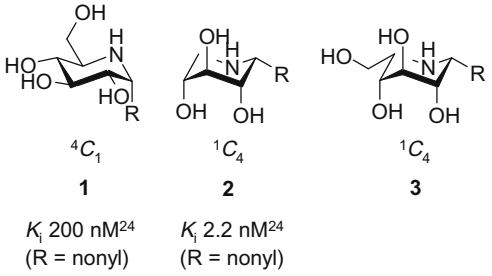
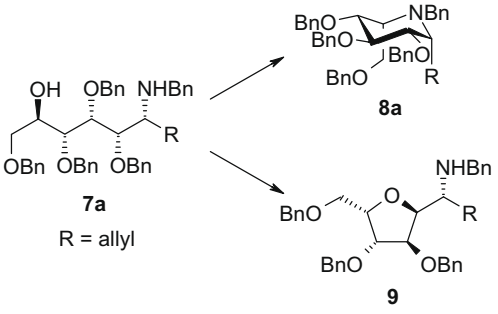
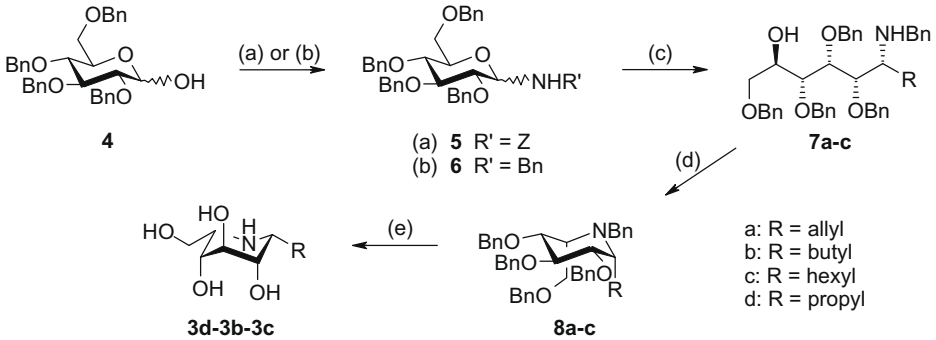


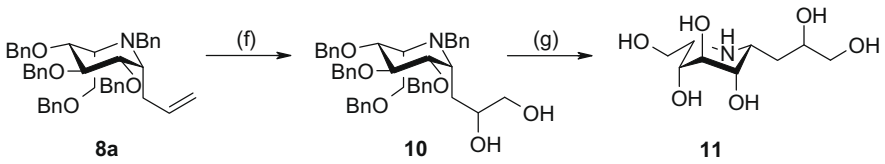
Figure 1. α-1-*C*-Alkyl-DNJ **1**, α-1-*C*-alkyl-DIX **2**, β-1-*C*-alkyl-DIJ **3**. Inhibition of GCase.



Scheme 2. Cyclization of **7a** using Tf_2O in pyridine gave a ~1:1 mixture of compounds **8a** and **9**.



Scheme 1. Synthesis of β-1-*C*-alkyl-1-deoxyimino-L-iditol **3**. Reagents and conditions: (a) NH_2CO_2Bn, TMSOTf, 4 Å MS, CH_2Cl_2, rt, 80%;[26] (b) $BnNH_2$, PTSA, 4 Å MS, CH_2Cl_2, rt, crude 100%; (c) RMgBr or RMgCl, THF, rt, **7a** 72%, **7b** 67%, **7c** 53%; (d) MsCl, 4 Å MS, pyridine, 100 °C, **8a** 72%, **8b** 46%, **8c** 71%; (e) 1 M HCl, EtOH, H_2, Pd/C, Pd black, rt, **3d** 100%, **3b** 76%, **3c** 88%.



Scheme 3. Dihydroxylation and deprotection of **8a**. Reagents and conditions: (f) OsO_4, NMO, THF, *t*-BuOH, H_2O, rt, 84%; (g) 1 M HCl, MeOH, H_2, Pd/C, Pd black, rt, 100%.

of Pd/C and Pd black under an H_2 atmosphere. This final step led to the known compound **3d**[27] and two new β-1-*C*-alkyl-1-deoxyimino-L-iditols **3b** and **3c**. NMR studies indicated clearly that these deprotected compounds adopted in solution (CD_3OD) the inverted $^1C_4$ conformation (broad singlets for H2, H3, and H4 in **3d**).

In order to check if the presence of supplementary hydroxyl groups in the 'aglycone' could increase the activity towards GCase, our 1-*C*-allyl intermediate **8a** was submitted to a dihydroxylation reaction using the OsO_4/NMO system (Scheme 3). Dihydroxylation under these conditions, followed by hydrogenolysis provided compound **11** in good yield. Dihydroxylation occurred without stereoselectivity and the resulting diastereoisomers were not separable by chromatography on silica gel. As a first indication, these compounds were assayed on GCase as a mixture of diastereoisomers.

Biological evaluation of compounds **3b**, **3c**, **3d**, and **11** was performed on human GCase and the results are reported in Table 1.

This study showed that the L-*ido* epimers were by far not as good inhibitors as the iminoxylitols and were even weaker inhibitors than the parent D-*gluco* isomers. For example, compound **3c** with a hexyl chain is 10× less active on GCase than the corresponding α-1-*C*-hexyl-DNJ **1c** and 2000× less active than α-1-*C*-hexyl-DIX **2c**, which is the best inhibitor with an IC_{50} of 19 nM. Despite this modest activity, some important features appear.

Indeed the hydroxymethyl chain has generally a detrimental effect on the affinity for GCase as the α-1-*C*-alkyl-DNJ **1** and the β-1-*C*-alkyl-DIJ **3** are both significantly less active than the α-1-*C*-alkyl-DIX **2**. The hydroxymethyl group may be essential for the recognition of α-glucosidases, but much less important for β-glucocerebrosidase. Consequently its removal improves the selectivity of our inhibitors. Moreover, the inverted $^1C_4$ conformation is not the key factor responsible for inhibition as the β-1-C-alkylated L-*ido* iminosugars **3** reported herein, which exist only in this conformation, are modest inhibitors of GCase. The ability of iminosugars derived from DIX to adopt more easily the $^4C_1$ conformation because of their greater flexibility is probably an important point for their affinity and selectivity for GCase. Finally the addition of polar substituents in the aglycone as in compound **11** abolishes completely the activity.

In conclusion, four 1-C-alkylated iminosugars having a pseudo β-L-*ido* configuration were efficiently synthesized and their inhibitory activity was evaluated on β-glucocerebrosidase. Despite their modest activity, these investigations allowed us to draw important conclusions on structure–activity relationship in GCase inhibition by DNJ-related iminosugars: although it is present in the natural substrate, the CH_2OH group is clearly not favorable in these structures; in addition, conformational flexibility appears to be a key feature for selectivity. This new information should help design more active and selective inhibitors of GCase and thus future novel therapeutic agents for Gaucher disease. Moreover, these compounds have potential as anti-cancer and anti-inflammatory agents: the related *N*-pentyl-1-deoxyimino-L-iditol, a known inhibitor of glucosylceramide synthase, is indeed a good candidate for these therapeutic applications.[32,33]

Table 1
IC_{50} values (μM) for compounds **3b**, **3c**, **3d**, and **11**, compared with different α-1-*C*-alkyl-DNJ **1** and -DIX **2**

Compounds	β-Glucocerebrosidase
β-1-*C*-Propyl-DIJ **3d**	487
α-1-*C*-Propyl-DNJ **1d**[23]	400
α-1-*C*-Propyl-DIX **2d**[31]	0.56
β-1-*C*-Butyl-DIJ **3b**	147
α-1-*C*-Butyl-DNJ **1b**[23]	100
β-1-*C*-Hexyl-DIJ **3c**	43
α-1-*C*-Hexyl-DNJ **1c**[23]	4.2
α-1-*C*-Hexyl-DIX **2c**[31]	0.019
11	NI[a]

[a] Less than 50% inhibition at 1000 μM.

3. Experimental

3.1. Chemistry

All reactions requiring anhydrous conditions were carried out using oven-dried glassware under an atmosphere of dry Ar. THF was distilled from sodium/benzophenone. Dichloromethane was distilled from calcium hydride. All reagent-grade chemicals were obtained from commercial suppliers and were used as received. Infrared spectra were recorded on a Nicolet iS10 FT-IR spectrometer. Low-resolution mass spectra were recorded on a Perkin–Elmer Sciex API 3000. High-resolution mass spectra were recorded on a Waters Q-TOF micro spectrometer by the *Centre Régional de Mesures Physiques* in Clermont-Ferrand. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer. Chemical shifts are given in ppm and are referenced to the solvent signal or to TMS as internal standard. Carbon multiplicities were assigned by distortionless enhancement by polarization transfer (DEPT) experiments. ^1H and ^{13}C signals were attributed with the help of H–H and C–H correlations. Specific rotations were measured at 20 °C in a 1 dm cell with a Perkin–Elmer 341 polarimeter. Analytical thin layer chromatography was performed using Silica Gel 60 F_{254} precoated plates (Merck) with visualization by ultraviolet light and phosphomolybdate solution (2% in H_2SO_4/EtOH 1:7). Flash chromatography was performed on Silica Gel 60 (230–400 mesh) with petroleum ether (PE) and EtOAc (EA) as eluants, unless otherwise stated.

3.1.1. *N*-Benzyl-2,3,4,6-tetra-*O*-benzyl-D-glucopyranosylamine (6)

2,3,4,6-Tetra-*O*-benzyl-D-glucopyranose **4** (317 mg, 0.586 mmol) was dissolved in dry CH_2Cl_2 (1.5 mL) in the presence of 4 Å activated molecular sieves. *p*-Toluenesulfonic acid (111 mg, 0.586 mmol, 1 equiv) was added, followed after 10 min of stirring by benzylamine (260 μL, 2.38 mmol, 4 equiv). The reaction mixture was stirred for 5 days at room temperature, and then filtered through Celite. CH_2Cl_2 (20 mL) was added. The organic phase was washed twice with saturated Na_2CO_3 (50 mL) and dried over $MgSO_4$. The solid was filtered and the filtrate was concentrated under vacuum. Most of the residual amine was co-evaporated with toluene affording crude compound **6** as a colorless and homogeneous solid (368 mg, 100%), which was submitted directly in the next step.

3.1.2. General procedure for the addition of Grignard reagents

Crude **6** was dissolved in dry THF (0.1 M) and treated with a solution of Grignard reagent (5 or 10 equiv). After 2 days of stirring at room temperature, the reaction was quenched with saturated NH_4Cl. The organic layer was diluted with CH_2Cl_2, washed with 5% aqueous HCl, saturated $NaHCO_3$, and water. The organic phase was dried over $MgSO_4$. The solid was filtered and the filtrate was concentrated under vacuum. The crude product was purified by column chromatography (PE/EA 4:1).

3.1.2.1. (1*R*)-1-*C*-Allyl-2,3,4,6-tetra-*O*-benzyl-1-benzylamino-1-deoxy-D-glucitol (7a). The reaction was carried out on 3.31 g (5.25 mmol) of **6** and commercial allylmagnesium bromide (1 M in Et_2O, 5 equiv) was used. Compound **7a** (2.54 g, 72%) was obtained as a colorless oil. Analytical data are in accordance with the literature data.[27]

3.1.2.2. (1*R*)-2,3,4,6-Tetra-*O*-benzyl-1-benzylamino-1-*C*-butyl-1-deoxy-D-glucitol (7b). The reaction was carried out on 196 mg (0.312 mmol) of **6** and commercial butylmagnesium chloride (20% (wt) in THF/toluene, 10 equiv) was used. Compound **7b** (143 mg, 67%) was obtained as a colorless oil. $[\alpha]_D^{20}$ −5.7 (*c* 0.92, $CHCl_3$). ^1H NMR ($CDCl_3$) δ 7.32–7.18 (m, 25H, H_{Ar}), 4.85, 4.81, 4.70 (3d, *J* = 11.4 Hz, 3H, PhC*H*$_2$O), 4.57–4.50 (m, 4H, PhC*H*$_2$O), 4.37 (d, *J* = 11.4 Hz, 1H, PhC*H*$_2$O), 4.29 (dd, *J* = 2.9, 7.6 Hz, 1H, H3), 4.10–4.06 (m, 1H, H5), 3.87 (d, *J* = 13.0 Hz, 1H, PhC*H*$_2$N), 3.83 (dd, *J* = 2.4, 7.6 Hz, 1H, H2), 3.65–3.55 (m, 3H, H6a, H6b, PhC*H*$_2$N), 3.50 (dd, *J* = 2.9, 6.7 Hz, 1H, H4), 2.49–2.45 (m, 1H, H1), 1.65–1.56 (m, 1H, H7b), 1.46–1.37 (m, 1H, H7a), 1.26–1.04 (m, 4H, H8, H9), 0.81 (t, *J* = 7.2 Hz, 3H, H10). ^{13}C NMR ($CDCl_3$) δ 141.1, 139.0, 138.6, 138.3 (C_{Ar}), 128.6–126.9 (CH_{Ar}), 80.4 (C2), 79.9 (C3), 78.0 (C4), 74.9, 74.7, 73.6, 72.8 (PhCH$_2$O), 71.8 (C6), 70.8 (C5), 57.3 (C1), 51.2 (PhCH$_2$N), 30.2 (C7), 29.0 (C8), 23.2 (C9), 14.3 (C10). IR (neat) ν 3029, 2940, 2866, 1495, 1453, 1085, 1067 cm^{-1}. MS (ESI+) *m/z* = 688.5 $[M+H]^+$. HRMS (ESI) $[M+H]^+$ calcd for $C_{45}H_{54}NO_5$ *m/z* = 688.4002; found *m/z* = 688.4012.

3.1.2.3. (1*R*)-2,3,4,6-Tetra-*O*-benzyl-1-benzylamino-1-deoxy-1-*C*-hexyl-D-glucitol (7c). The reaction was carried out on 689 mg (1.09 mmol) of **6** and commercial hexylmagnesium chloride (2 M in THF, 10 equiv) was used. Compound **7c** (416 mg, 53%) was obtained as a colorless oil. $[\alpha]_D^{20}$ −10.6 (*c* 1.21, $CHCl_3$). ^1H NMR ($CDCl_3$) δ 7.33–7.19 (m, 25H, H_{Ar}), 4.85, 4.80, 4.70 (3d, *J* = 11.4 Hz, 3H, PhC*H*$_2$O), 4.56–4.50 (m, 4H, PhC*H*$_2$O), 4.37 (d, *J* = 11.4 Hz, 1H, PhC*H*$_2$O), 4.28 (dd, *J* = 2.9, 7.6 Hz, 1H, H3), 4.10–4.06 (m, 1H, H5), 3.86 (d, *J* = 13.2 Hz, 1H, PhC*H*$_2$N), 3.83 (dd, *J* = 2.2, 7.6 Hz, 1H, H2), 3.65–3.55 (m, 3H, H6a, H6b, PhC*H*$_2$N), 3.50 (dd, *J* = 2.9, 6.7 Hz, 1H, H4), 2.49–2.45 (m, 1H, H1), 1.64–1.55 (m, 1H, H7b), 1.46–1.36 (m, 1H, H7a), 1.26–1.11 (m, 8H, H8, H9, H10, H11), 0.86 (t, *J* = 7.1 Hz, 3H, H12). ^{13}C NMR ($CDCl_3$) δ 141.2, 139.0, 138.6, 138.3, 138.3 (C_{Ar}), 128.6–127.0 (CH_{Ar}), 80.5 (C2), 79.9 (C3), 77.9 (C4), 74.9, 74.8, 73.6, 72.9 (PhCH$_2$O), 71.8 (C6), 70.8 (C5), 57.4 (C1), 51.3 (PhCH$_2$N), 32.0, 29.9, 26.8, 22.8 (C8, C9, C10, C11), 30.6 (C7), 14.3 (C12). IR (neat) ν 3029, 2925, 2855, 1495, 1453, 1080, 1068 cm^{-1}. HRMS (ESI) $[M+H]^+$ calcd for $C_{47}H_{58}NO_5$ *m/z* = 716.4315; found *m/z* = 716.4310.

3.1.3. General procedure for the cyclization

Open-chain compounds **7a–c** were dissolved in pyridine (0.1 M) in the presence of activated 4 Å molecular sieves. After 10 min of stirring at room temperature, methanesulfonyl chloride (2.5 equiv) was added. The mixture was stirred at 100 °C for 3–6 h, then filtered through Celite and concentrated under vacuum. The crude product was diluted with ethyl acetate. The organic phase was washed with water and dried over $MgSO_4$. The solid was filtered and the filtrate was concentrated under vacuum and co-evaporated with toluene.

3.1.3.1. β-1-*C*-Allyl-*N*-benzyl-2,3,4,6-tetra-*O*-benzyl-1,5-dideoxy-1,5-imino-L-iditol (8a). The reaction was carried out on 520 mg (0.774 mmol) of **7a**. Purification was performed by column chromatography (PE/EA 9:1) affording **8a** (362 mg, 72%). Analytical data are in accordance with the literature data.[27]

3.1.3.2. *N*-Benzyl-2,3,4,6-tetra-*O*-benzyl-β-1-*C*-butyl-1,5-dideoxy-1,5-imino-L-iditol (8b). The reaction was carried out on 207 mg (0.301 mmol) of **7b**. Purification was performed by column chromatography (PE/EA 95:5) affording **8b** (93 mg, 46%) as a colorless oil. $[\alpha]_D^{20}$ −7.8 (*c* 1.10, $CHCl_3$). ^1H NMR ($CDCl_3$) δ 7.34–7.21 (m, 25H, H_{Ar}), 4.81, 4.78 (2d, *J* = 10.9 Hz, 2H, PhC*H*$_2$O), 4.61, 4.60, 4.55, 4.54 (4d, *J* = 11.6 Hz, 4H, PhC*H*$_2$O), 4.45 (s, 2H, PhC*H*$_2$O), 4.09, 4.00 (2d, *J* = 14.9 Hz, 2H, PhC*H*$_2$N), 3.81 (dd, *J* = 5.3, 9.6 Hz, 1H, H6b), 3.73–3.67 (m, 3H, H3, H4, H6a), 3.65–3.61 (m, 1H, H2), 3.50–3.45 (m, 1H, H5), 3.01 (q, *J* = 6.2 Hz, 1H, H1), 1.73–1.64 (m, 1H, H7b), 1.58–1.49 (m, 1H, H7a), 1.39–1.17 (m, 4H, H8, H9), 0.83 (t, *J* = 6.8 Hz, 3H, H10). ^{13}C NMR ($CDCl_3$) δ 141.2, 138.2, 138.8, 138.8, 138.7 (C_{Ar}), 128.4–126.8 (CH_{Ar}), 80.9 (C2), 80.1, 78.9 (C3, C4), 75.2, 73.3, 72.9, 72.7 (PhCH$_2$O), 71.1 (C6), 59.3, 59.2 (C1, C5), 58.5 (PhCH$_2$N), 31.2 (C8), 29.1 (C7), 23.3 (C9), 14.3 (C10). IR (neat) ν 3029, 2927, 2867, 1495, 1453, 1089, 1071 cm^{-1}. MS (ESI+) *m/z* = 670.5 $[M+H]^+$. HRMS (ESI) $[M+H]^+$ calcd for $C_{45}H_{52}NO_4$ *m/z* = 670.3896; found *m/z* = 670.3879.

3.1.3.3. *N*-Benzyl-2,3,4,6-tetra-*O*-benzyl-β-1-*C*-hexyl-1,5-dideoxy-1,5-imino-L-iditol (7c). The reaction was carried out on 261 mg (0.365 mmol) of **6c**. Purification was performed by column chromatography (PE/EA 95:5) affording **8c** (181 mg, 71%) as a yellowish oil. $[\alpha]_D^{20}$ −9.4 (*c* 1.18, $CHCl_3$). ^1H NMR ($CDCl_3$) δ 7.34–7.21 (m, 25H, H_{Ar}), 4.82, 4.77 (2d, *J* = 10.9 Hz, 2H, PhC*H*$_2$O), 4.61, 4.60, 4.55, 4.54 (4d, *J* = 11.6 Hz, 4H, PhC*H*$_2$O), 4.45 (s, 2H, PhC*H*$_2$O), 4.09, 4.00 (2d, *J* = 14.8 Hz, 2H, PhC*H*$_2$N), 3.81 (dd, *J* = 5.3, 9.7 Hz, 1H, H6b), 3.75–3.67 (m, 3H, H3, H4, H6a), 3.62 (dd, *J* = 5.9, 8.8 Hz, 1H, H2), 3.49–3.45 (m, 1H, H5), 3.00 (q, *J* = 5.9 Hz, 1H, H1), 1.72–1.63 (m, 1H, H7b), 1.57–1.48 (m, 1H, H7a), 1.37–1.15 (m, 8H, H8, H9, H10, H11), 0.85 (t, *J* = 6.8 Hz, 3H, H12). ^{13}C NMR ($CDCl_3$) δ 141.2, 139.2, 138.8, 138.8, 138.7 (C_{Ar}), 128.4–126.8 (CH_{Ar}), 80.9 (C2), 80.1, 78.9 (C3, C4), 75.1, 73.2, 72.9, 72.6 (PhCH$_2$O), 70.9 (C6), 59.2, 59.0 (C1, C5), 58.5 (PhCH$_2$N), 31.9, 29.8, 29.0, 22.8 (C8, C9, C10, C11), 29.3 (C7), 14.2 (C12). IR (neat) ν 3029, 2922, 2855, 1495, 1453, 1089, 1071 cm^{-1}. MS (ESI+) *m/z* = 698.5 $[M+H]^+$. HRMS (ESI) $[M+H]^+$ calcd for $C_{47}H_{56}NO_4$ *m/z* = 698.4209; found *m/z* = 698.4195.

3.1.4. General procedure for hydrogenolysis

Protected compounds **8a–c** were dissolved in ethanol (0.1 M) in the presence of 1 M HCl (5 equiv). After few minutes of stirring Pd/C (10%) (125 g/mol) and Pd black (125 g/mol) were added. The reaction was carried out under a hydrogen atmosphere at room temperature for 16 h. The mixture was filtered through membrane, and the residual solid was then washed with methanol. The crude solution was introduced on a DOWEX 50W-X8-100 (H^+) ion exchange column. The column was washed with methanol and water. The product was then eluted with 0.5 M NH_4OH and the eluted fractions were concentrated under reduced pressure affording final pure compounds.

3.1.4.1. β-1-*C*-Propyl-1,5-dideoxy-1,5-imino-L-iditol (3d). The reaction was carried out on 128 mg (0.196 mmol) of **8a**, affording **3d** (43 mg, 100%) as a yellow oil. $[\alpha]_D^{20}$ −16.8 (*c* 1.20, MeOH). ^1H NMR (D_2O) δ 4.07 (br s, 1H, H3), 3.86 (br s, 1H, H4), 3.79 (br s, 1H, H2), 3.76–3.70 (m, 2H, H6), 3.22 (t, *J* = 5.8 Hz, 1H, H5), 3.13–3.10 (m, 1H, H1), 1.70–1.59 (m, 1H, H7b), 1.50–1.47 (m, 1H,

H7a), 1.43–1.34 (m, 2H, H8), 0.94 (t, J = 7.2 Hz, 3H, H9). ^{13}C NMR (D_2O) δ 68.4, 68.3 (C2, C3), 67.8 (C4), 60.9 (C6), 55.5 (C5), 53.6 (C1), 31.6 (C7), 18.0 (C8), 13.2 (C9). IR (neat) ν 3403, 3364, 3240, 3157, 2953, 2929, 2892, 2869, 1453, 1065, 1033 cm^{-1}. MS (ESI+) m/z = 206.5 [M+H]$^+$. HRMS (ESI) [M+H]$^+$ calcd for $C_9H_{20}NO_4$ m/z = 206.1392; found m/z = 206.1386.

3.1.4.2. β-1-*C*-Butyl-1,5-dideoxy-1,5-imino-L-iditol (3b). The reaction was carried out on 77 mg (0.115 mmol) of **8b**, affording **3b** (19 mg, 76%) as a colorless solid. $[\alpha]_D^{20}$ −11.8 (c 1.13, MeOH). ^{1}H NMR (MeOD) δ 3.92 (br s, 1H, H3), 3.67–3.65 (m, 3H, H4, H6), 3.56 (br s, 1H, H2), 3.09–3.06 (m, 1H, H5), 2.94 (t, J = 5.8 Hz, 1H, H1), 1.65–1.57 (m, 1H, H7b), 1.52–1.47 (m, 1H, H7a), 1.40–1.31 (m, 4H, H8, H9), 0.94 (t, J = 6.6 Hz, 3H, H10). ^{13}C NMR (MeOD) δ 71.2 (C2), 70.6 (C3, C4), 63.7 (C6), 57.3 (C5), 55.4 (C1), 32.0 (C7), 29.1, 23.9 (C8, C9), 14.4 (C10). IR (neat) ν 3423, 3375, 3241, 3193, 2964, 2929, 2912, 1453, 1063, 1034 cm^{-1}. MS (ESI+) m/z = 220.0 [M+H]$^+$. HRMS (ESI) [M+H]$^+$ calcd for $C_{10}H_{22}NO_4$ m/z = 220.1549; found m/z = 220.1540.

3.1.4.3. β-1-*C*-Hexyl-1,5-dideoxy-1,5-imino-L-iditol (3c). The reaction was carried out on 61 mg (0.087 mmol) of **8c**, affording **3c** (19 mg, 88%) as a colorless solid. $[\alpha]_D^{20}$ −16.7 (c 1.00, MeOH). ^{1}H NMR (MeOD) δ 3.99 (t, J = 3.4 Hz, 1H, H3), 3.84–3.83 (m, 1H, H4), 3.79–3.76 (m, 3H, H2, H6), 3.36 (t, J = 6.0 Hz, 1H, H5), 3.23 (ddd, J = 1.5, 4.8, 9.9 Hz, 1H, H1), 1.87–1.77 (m, 1H, H7b), 1.66–1.57 (m, 1H, H7a), 1.45–1.29 (m, 8H, H8, H9, H10, H11), 0.91 (t, J = 6.7 Hz, 3H, H12). ^{13}C NMR (MeOD) δ 69.7, 69.6 (C2, C4), 69.0 (C3), 61.7 (C6), 58.1 (C5), 56.3 (C1), 32.8, 30.3, 26.2, 23.6 (C8, C9, C10, C11), 30.1 (C7), 14.4 (C12). IR (neat) ν 3300, 3074, 2926, 2855, 1423, 1047, 1008 cm^{-1}. MS (ESI+) m/z = 248.0 [M+H]$^+$. HRMS (ESI) [M+H]$^+$ calcd for $C_{12}H_{26}NO_4$ m/z = 248.1862; found m/z = 248.1851.

3.1.5. *N*-Benzyl-2,3,4,6-tetra-*O*-benzyl-β-1-*C*-(2,3-dihydroxypropyl)-1,5-dideoxy-1,5-imino-L-iditol (10)

Compound **8a** (440 mg, 0.673 mmol) was dissolved in a mixture of THF (3 mL), *t*-BuOH (9 mL), and water (1.6 mL). Subsequently, *N*-methylmorpholine-*N*-oxide (95 mg, 0.808 mmol, 1.2 equiv) was added. After 5 min of stirring, a 2.5% solution of osmium tetroxide in *t*-BuOH (0.55 mL, 0.045 mmol) was added. The reaction was stirred at room temperature for 2 days. The mixture was treated with a 0.1 M $Na_2S_2O_5$ solution (5 mL) and stirred for 30 min. Thereupon, it was diluted with CH_2Cl_2 (20 mL) and water (10 mL). The aqueous phase was separated and extracted with CH_2Cl_2 (20 mL). The combined organic phases were dried over $MgSO_4$. The solid was filtered and the filtrate was concentrated under vacuum. The crude product was purified by column chromatography (PE/EA, 5:5) affording compound **10** as a mixture of diastereoisomers (1:1) (391 mg, 84%). ^{1}H NMR ($CDCl_3$) δ 7.34–7.14 (m, 25H, H_{Ar}), 4.87–4.36 (m, 8H, PhCH_2O), 3.97–3.82 (m, 2H, PhCH_2N), 3.78–3.49 (m, 6H, H2, H3, H4, H6b, H6a, H8), 3.45 (dd, J = 3.5, 11.0 Hz, 1H, H5′, H9b′), 3.39 (dd, J = 3.7, 11.0 Hz, 0.5H, H9b), 3.35–3.23 (m, 2H, H5, H9a, H9a′, H1′), 3.16 (q, J = 6.0 Hz, 0.5H, H1), 1.89–1.75 (m, 1H, H7b), 1.74–1.64 (m, 1H, H7a). ^{13}C NMR ($CDCl_3$) δ 140.0–137.7 (C_{Ar}), 129.3–127.2 (CH_{Ar}), 80.0, 79.7, 78.7, 78.2 (C2, C3, C4), 75.2, 74.8, 74.0, 73.4, 73.2, 73.0, 73.0, 72.8 (PhCH_2O), 72.7, 71.4 (C8), 70.4, 69.4 (C6), 67.1, 67.0 (C9), 60.9 (PhCH_2N), 59.6, 59.2, 58.8, 57.2 (C1, C5), 32.1, 29.9 (C7).

3.1.6. β-1-*C*-(2,3-Dihydroxypropyl)-1,5-dideoxy-1,5-imino-L-iditol (11)

Compound **10** (138 mg, 0.201 mmol) was dissolved in methanol (2 mL) in the presence of 1 M HCl (1 mL). After few minutes of stirring, Pd/C (10%) (30 mg) and Pd black (30 mg) were added. The reaction was carried out under a hydrogen atmosphere at room temperature for 18 h. The mixture was filtered through a membrane, and the residual solid was then washed with methanol. The crude solution was introduced on a DOWEX 50W-X8-100 (H^+) ion exchange column. The column was washed with methanol and water. The product was then eluted with 0.5 M NH_4OH and the eluted fractions were concentrated under vacuum affording **11** (48 mg, 100%) as a yellow oil. ^{1}H NMR (D_2O) δ 4.08–4.05 (m, 1H, H3), 3.90–3.67 (m, 5H, H2, H4, H6, H8), 3.61 (dd, J = 4.0, 11.6 Hz, 1H, H9b), 3.56–3.49 (m, 1H, H9a), 3.39–3.35 (m, 0.5H, H1′), 3.29–3.22 (m, 1H, H5′, H1), 3.18 (t, J = 6.2 Hz, 0.5H, H5), 1.89–1.64 (m, 2H, H7). ^{13}C NMR (D_2O) δ 70.4, 69.2, 68.8, 68.8, 68.7, 68.5, 67.7, 67.6 (C2, C3, C4, C8), 65.6, 65.5 (C9), 61.3, 60.9 (C6), 55.8, 55.5 (C5), 52.1, 51.2 (C1), 34.2, 32.7 (C7). MS (ESI+) m/z = 238.0 [M+H]$^+$. HRMS (ESI) [M+H]$^+$ calcd for $C_9H_{20}NO_6$ m/z = 238.1291; found m/z = 238.1287.

3.2. Biochemical assays

The inhibitory activity of compounds **2c–d** and **3b–d** was measured with recombinant human β-glucocerebrosidase (Ceredase) from Genzyme (Boston, MA) as the enzyme source and *p*-nitrophenyl-β-D-glucopyranoside (Sigma Chemical Co.) as the substrate. The reaction mixture consists of 75 μL of 0.15 M citrate-phosphate buffer (pH 5.5), 50 μL of 1% Na taurocholate/Triton X-100 (Sigma Chemical Co.), 50 μL of the enzyme solution, and 25 μL of an inhibitor solution or H_2O. The reaction mixture was pre-incubated at 0 °C for 10 min and the reaction was started by the addition of 50 μL of a 10 mM substrate solution, followed by incubation at 37 °C for 10 min. The reaction was stopped by the addition of 2 mL of a 0.4 M Na_2CO_3 solution. Liberated *p*-nitrophenolate was measured (400 nm) with a F-4500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan). Kinetic parameters were determined by the double-reciprocal-plot method of Lineweaver–Burk at increasing concentrations of substrate. Compounds **1b–d** were evaluated using 4-methylumbelliferyl-β-D-glucopyranoside as substrate.[23]

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