Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Research 342 (2007) 1960–1965
Carbohydrate RESEARCH

Note

Synthesis of 4-O-glycosylated 1-deoxynojirimycin derivatives as disaccharide mimics-based inhibitors of human β-glucocerebrosidase

Charlotte Boucheron,[a] Sylvestre Toumieux,[a] Philippe Compain,[a,*] Olivier R. Martin,[a,*] Kyoko Ikeda[b] and Naoki Asano[b]

[a]*Institut de Chimie Organique et Analytique, CNRS—Université d'Orléans, rue de Chartres, BP 6759, 45067 Orléans, France*
[b]*Faculty of Pharmaceutical Sciences, Hokuriku University, Kanazawa 920-1181, Japan*

Received 29 January 2007; received in revised form 9 March 2007; accepted 11 March 2007
Available online 16 March 2007

Abstract—Examples of a new type of inhibitor of human β-glucocerebrosidase based on imino-disaccharides as glycosylceramide mimetics have been synthesized by way of the glycosylation of 1-deoxynojirimycin derivatives with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide.

Keywords: Glycosylation; Glycosidase inhibitors; Iminosugars; Cellobiose mimics; Lysosomal diseases; Gaucher disease

Gaucher disease[1] is one of the most prevalent lysosomal storage disorders. This relatively rare inherited disease is due to the deficiency of β-glucocerebrosidase, also named glucosylceramide β-glucosidase (GCase), an enzyme involved in the catabolism of glycosphingolipids in lysosomes.[2] Defects in the catalytic activity of GCase lead to the accumulation of un-degraded glucosylceramides (GlcCer) mainly in macrophages and to severe symptoms including skeletal lesions, anemia and liver damage. Two pharmacological approaches[3] have recently emerged: Substrate Reduction Therapy (SRT),[4] which is based on the limitation of the GCase substrate by inhibiting GlcCer biosynthesis, and Pharmacological Chaperone Therapy (PCT),[5] which is based on the use of competitive inhibitors of GCase capable of enhancing its residual hydrolytic activity at sub-inhibitory concentrations. The SRT approach has led to Zavesca® (*N*-Bu-DNJ, **1**),[4] the first marketed drug for Gaucher disease (Fig. 1). This iminosugar acts as a potent inhibitor of glucosylceramide synthase, the enzyme that catalyses the transfer of a glucose moiety from UDP-glucose to the primary hydroxyl group of ceramide to yield β-GlcCer.

The PCT approach may be explained by the fact that, even though the defective enzyme is predisposed to misfolding and/or instability, it is still catalytically active.[5b]

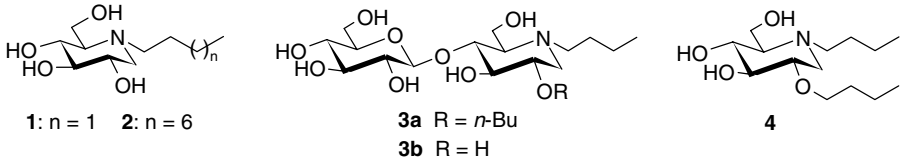


Figure 1.

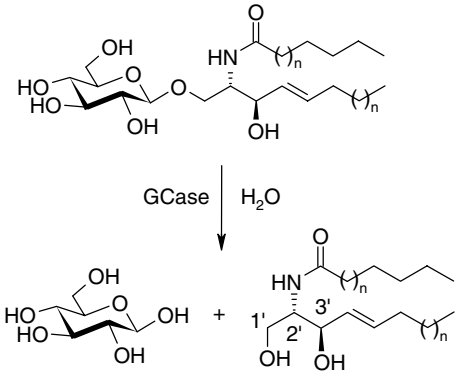
* Corresponding authors. Tel.: +33 0 2 38 49 48 55; fax: +33 0 2 38 41 72 81 (P.C.); e-mail addresses: philippe.compain@univ-orleans.fr; olivier.martin@univ-orleans.fr

0008-6215/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2007.03.015

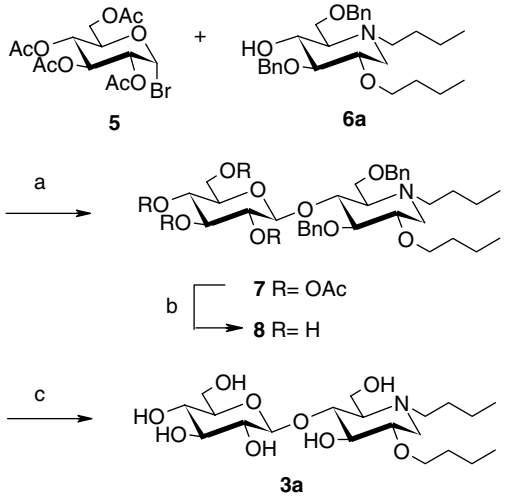
Reversible competitive inhibitors positively influence the folding state of the abnormal enzyme, thus preventing its degradation by quality control in the endoplasmic reticulum (ER) and ER-associated degradation before trafficking to lysosomes.[6] In 2002, Kelly et al. disclosed that the addition of sub-inhibitory concentrations (10 μM) of *N*-nonyl-1-deoxynojirimycin (NN-DNJ, **2**) to the culture medium leads to a two-fold increase in the activity of N370S fibroblast GCase, the most common mutation causing Gaucher disease.[7] This result demonstrated that GCase inhibitors may constitute new targets for drug design. However, because NN-DNJ and related analogs are known to be potent glycosidase inhibitors, serious side effects similar to those observed for Zavesca® may be expected.[3a] We recently reported that α-1-C-alkyl derivatives of DNJ and of 1,5-dideoxy-1,5-imino-D-xylitol (DIX) were promising candidates for the treatment of Gaucher disease without the side-effects associated with α-glucosidase inhibition.[8,9] In connection with these studies and our recent work on the SRT approach,[10] we turned our attention to the design of selective inhibitors of GCase as potential active-site-specific chaperones based on imino-disaccharides. It is known that GCase cleaves the β-glycosidic bond of GlcCer to release the ceramide and glucose with retention of configuration (Scheme 1).[11]

Based on previous SRT studies using Zavesca® (**1**), we designed the pseudo β-linked disaccharides **3** as substrate mimics of GCase based on the replacement of the ceramide moiety with DNJ derivatives (Fig. 1). Indeed, Butters et al. showed that the inhibition of glucosylceramide synthase by **1** is competitive with respect to ceramide and not to UDP-glucose and that this compound could, somewhat unexpectedly, be a mimic of ceramide and not of the glucose moiety.[12,4b] Molecular modelling has revealed a strong structural homology between **1** and the ceramide structure. The *N*-alkyl chain and three chiral centres (C-2, C-3 and C-5) of iminosugar **1** show structural similarity with the *N*-acyl chain and the C-1′–C-3′ backbone of ceramide respectively. This model suggests that **1** would be a better ceramide mimic if a second alkyl chain was present at O-2 to simulate the second hydrophobic chain of the ceramide. However, quite surprisingly, *N*-alkyl DNJ derivatives bearing a second alkyl chain at O-2 were found to display much lower inhibition than *N*-butyl DNJ (**1**) towards glucosylceramide synthase.[10] We further challenged the hypothesis of NB-DNJ being a ceramide mimic by designing glycosylated derivatives **3a** and **3b** as substrate-like inhibitors of GCase. In this paper, we report the synthesis of two iminodisaccharides **3a** and **3b** and their properties as GCase inhibitors.

Beyond the biological interest of pseudo-disaccharides **3**, the synthetic challenge was to devise a direct glycosylation strategy to obtain glycosides of iminosugars **6**, which contain a reactive endocyclic amino function. Few examples have been published dealing with the coupling of iminosugars with sugar derivatives.[13,14] In those studies, the endocyclic amino function of the iminosugar acceptors were always replaced by a less reactive carbamate group except in a reaction reported by Banwell et al., which involved the glycosylation of the primary 6-OH group of 1-deoxymannojirimycin derivatives.[13h] We found that coupling of iminosugar **6a**[10] and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**5**) could be achieved in the presence of a stoichiometric amount of silver triflate (Scheme 2). As expected under Koenigs–Knorr conditions, the β-glucoside **7** was obtained in 55% yield with a very high diastereoselectivity. The stereochemistry and purity of the anomeric centre was established by ^{1}H, ^{13}C NMR and 2D NMR experiments. In particular, characteristic signals for the β-linkage were observed (C-1′ δ 100.1; $J_{1',2'} = 7.8$ Hz). Removal of the acetate groups with sodium methoxide



Scheme 1.

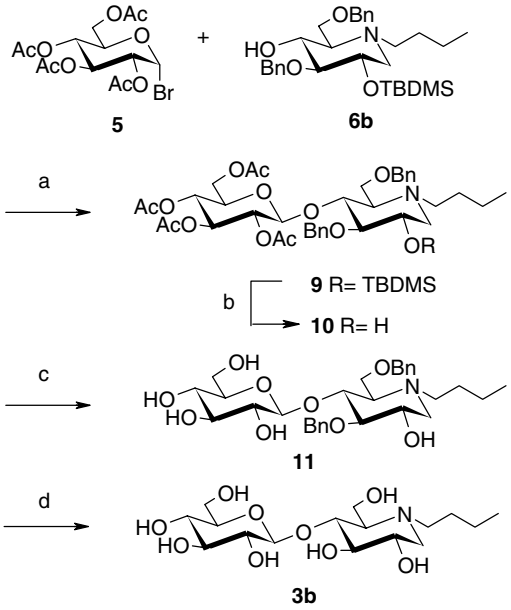


Scheme 2. Reagents and conditions: (a) AgOTf (2.2 equiv), 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (1.5 equiv), molecular sieves 4 Å, CH_2Cl_2, −78 °C to rt, 24 h, 55%. (b) MeONa, MeOH, 2 h, quant. (c) (i) H_2, Pd/C, *i*PrOH/AcOH (20/1), 5 h, (ii) Dowex 1-X2 (OH^- form).

to afford **8** and subsequent hydrogenolysis of the benzyl groups gave the expected pseudo-disaccharide **3a**. It is important to note that the order of deprotection steps matters as hydrogenolysis of benzyl groups in **7** under slightly acidic conditions (MeOH/AcOH 10/1) afforded a mixture of partially deprotected pseudo-disaccharides along with small amounts of monosaccharide derivatives.

Following the same synthetic strategy, we prepared pseudo-disaccharide **3b** from suitably protected iminosugar **6b**[15] and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**5**). The glycosylation reaction afforded β-glucoside **9** in 34% yield (22% of starting material **6b** was recovered after purification on silica gel). Sequential removal of the protecting groups provided the expected pseudo-disaccharide **3b**,[16,17] a cellobiose mimic (Scheme 3).

The inhibitory effect of pseudo-disaccharides **3** on GCase was then examined (Fig. 2). For the purpose of comparison, iminoglycolipid **4**[10] was also evaluated along with *N*-butyl DNJ (**1**). Iminoglycolipid **4** displayed no inhibitory activity (less than 50% of inhibition at 1 mM). The addition of a glucosyl moiety at C-4 to give **3a** resulted in a marked improvement of affinity for GCase (IC_{50} 56 μM). In contrast, despite the fact that *N*-butyl DNJ (**1**) displayed better inhibition than **4**,[8b] pseudo-disaccharide **3b** showed no inhibitory activity at 100 μM against GCase. TLC experiments performed with GCase indicated that **3b** is not a substrate of the enzyme at concentrations in the mM range. In addition, **3b** did not inhibit almond and *Caldocellum saccharolyticum* β-glucosidase.



Scheme 3. Reagents and conditions: (a) AgOTf (2.2 equiv), 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (1.5 equiv), molecular sieves 4 Å, CH_2Cl_2, −78°C to rt, 24 h, 34%. (b) *n*-Bu_4NF (1.5 equiv), THF, 0 °C to rt, 18 h, 70%. (c) MeONa, MeOH, 2 h, 86%. (d) (i) H_2, Pd/C, *i*PrOH/AcOH (20/1), (ii) Dowex 1-X2 (OH^- form), 44%.

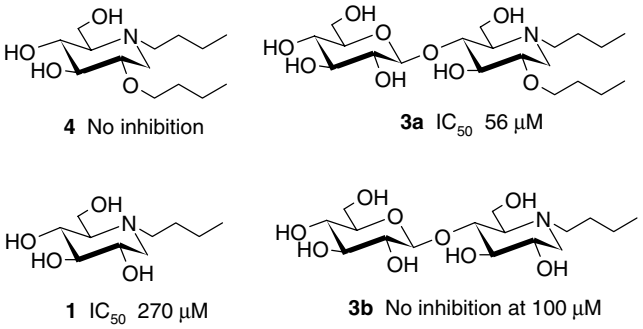


Figure 2. Inhibition of β-glucocerebrosidase (GCase).

In conclusion, we have synthesized a new type of GCase inhibitor based on iminodisaccharides as glycosylceramide mimetics. Our strategy takes advantage of a direct glycosylation reaction of protected *N*-butyl-DNJ **6** with α-D-glucopyranosyl bromide **5**. The biological results obtained with compounds **4** and **3a** partly validate our initial design hypothesis and demonstrate that the conjugation of a D-glucose moiety with an iminosugar mimetic of ceramide could enhance the binding towards GCase. Following these lines, further research towards the discovery of potent iminosugar-based inhibitors of human GCase are underway in our laboratory.[18]

1. Experimental

1.1. General methods

Unless otherwise stated, all reactions requiring anhydrous conditions were carried out under Argon. Dichloromethane was distilled from calcium hydride. Optical rotations were measured with a Jasco DIP-370 digital polarimeter. ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded at 25 °C on a JEOL ECP-500 spectrometer. ^1H NMR (250 MHz) and ^{13}C NMR (62.9 MHz) spectra were recorded at 25 °C on a Bruker DPX 250 Advance (250 MHz) spectrometer. High-resolution mass spectra (HRMS) were recorded with a Micromass ZABSpec TOF in the electrospray ionization (ESI) mode and using glycerol as a matrix on a JEOL JMS-700 spectrometer (FAB).

1.2. 4-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-di-*O*-benzyl-*N*-butyl-2-*O*-butyl-1,5-dideoxy-1,5-imino-D-glucitol (7)

To a solution of **6a**[10] (105 mg, 0.23 mmol) in CH_2Cl_2 (7 mL) at −78 °C was added activated 4 Å molecular sieves and bromide **5** (143 mg, 0.35 mmol). The reaction mixture was stirred for 15 min at −78 °C and silver triflate (130 mg, 0.51 mmol) was added. The reaction was

warmed up slowly to rt and stirred in the dark for 24 h. The reaction mixture was washed with saturated aqueous $NaHCO_3$ (2 × 10 mL) and saturated aqueous NaCl (10 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The resulting crude product was purified by silica gel chromatography (petroleum ether/AcOEt, 2/1 to 1/1) to afford **7** (99 mg, 55%) as a syrup. $[\alpha]_D$ −11.0 (*c* 0.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.84 (t, 3H, $J = 7.4$ Hz), 0.89 (t, 3H, $J = 7.3$ Hz), 1.26 (m, 4H), 1.47 (m, 4H), 1.96 (s, 3H), 1.99 (s, 6H), 2.00 (s, 3H), 2.10 (t, 1H, $J = \sim 11.0$ Hz, H-1ax), 2.23 (br d, 1H, H-5), 2.55 (m, 1H, CHN), 2.68 (m, 1H, CHN), 3.07 (dd, 1H, $J = 4.7$, ~11.0 Hz, H-1eq), 3.26 (t, 1H, $J = 8.8$ Hz, H-3), 3.31 (m, 1H, H-5′), 3.42 (m, 1H, H-2), 3.53 (t, 2H, $J = 6.7$ Hz, CH_2O), 3.53 (br d masked by the signal of CH_2O, 1H, H-6A), 3.66 (br d, 1H, $J = 9.9$ Hz, H-6B), 3.85 (t, 1H, $J = 9.1$ Hz, H-4), 3.87 (dd, 1H, $J = 2.2$, 12.1 Hz, H-6A′), 4.03 (dd, 1H, $J = 4.8$, 12.2 Hz, H-6B′), 4.48 (d, 1H, $J = 12.1$ Hz, CH benzyl), 4.51 (d, 1H, $J = 7.8$ Hz, H-1′), 4.64 (d, 1H, $J = 12.1$ Hz, CH benzyl), 4.72 (d, 1H, $J = 11.7$ Hz, CH benzyl), 4.88–4.98 (m, 4H, CH benzyl, H-2′,3′,4′), 7.22–7.39 (m, 10H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 14.0, 14.1, 19.4, 20.7, 20.8, 20.9, 26.2, 29.8, 32.4, 52.1 (CH_2N), 54.6 (C-1), 62.1 (C-6′), 64.0 (C-5), 64.2 (C-6), 68.6 (C-3′), 70.9 (CH_2O), 71.6 (C-5′), 72.3 (C-2′), 73.5 (C-4′), 73.6, 74.5, 78.3 (C-4), 78.4 (C-2), 84.8 (C-3), 100.1 (C-1′), 127.1, 127.3, 128.1, 128.5, 128.8, 137.6, 139.9, 169.2, 169.6, 170.4, 170.8; HRMS (ESI): *m*/*z* 786.4073 $[M+H]^+$ ($C_{42}H_{60}NO_{13}$ requires 786.4065).

1.3. 4-*O*-(β-D-Glucopyranosyl)-3,6-di-*O*-benzyl-*N*-butyl-2-*O*-butyl-1,5-dideoxy-1,5-imino-D-glucitol (8)

Sodium (4 mg, 0.17 mmol) was added to anhydrous MeOH (2 mL) at rt. After 0.5 h, a solution of **7** (32 mg, 0.041 mmol) in anhydrous MeOH (2 mL) was added. The reaction mixture was stirred for 2 h, filtered through a pad of Celite® and concentrated under reduced pressure to afford **8** (100% conversion as judged by NMR spectroscopy). The yellowish solid obtained was used without further purification in the next step. ^{1}H NMR (250 MHz, CD_3OD): δ 0.84 (t, 3H, $J = 7.2$ Hz), 0.89 (t, 3H, $J = 7.2$ Hz), 1.25–1.55 (m, 8H), 2.12 (t, 1H, $J = 9.7$ Hz, H-1ax), 2.32 (br d, 1H, H-5), 2.52 (m, 2H, CH_2N), 3.04 (dd, 1H, $J = 4.7$, 11.6 Hz, H-1eq), 3.15–3.90 (several m, 13H), 4.41 (d, 1H, $J = 11.6$ Hz, CH benzyl), 4.42 (d, 1H, $J = 6.9$ Hz, H-1′), 4.57 (d, 1H, $J = 11.6$ Hz, CH benzyl), 4.68 (d, 1H, $J = 10.4$ Hz, CH benzyl), 4.93 (d, 1H, $J = 10.6$ Hz, CH benzyl), 7.23–7.40 (m, 10H); ^{13}C NMR (62.9 MHz, CD_3OD): δ 14.3, 20.4, 21.6, 26.8, 33.5, 52.8 (CH_2N), 55.5 (C-1), 63.2 (C-6′), 65.0 (C-6), 65.4 (C-5), 71.7 (CH_2O), 72.2 (C-3′), 73.9, 75.8 (C-5′), 77.0, 77.2 (C-2′), 78.1 (C-4′), 78.9 (C-4), 79.0 (C-2), 86.6 (C-3), 103.8 (C-1′), 128.9, 129.0, 129.2, 129.5, 129.6, 129.9, 139.1, 139.7; MS (IS): *m*/*z* 618.5 $[M+H]^+$, 640.5 $[M+Na]^+$.

1.4. 4-*O*-(β-D-Glucopyranosyl)-*N*-butyl-2-*O*-butyl-1,5-dideoxy-1,5-imino-D-glucitol (3a)

To a ~0.1 M solution of precursor **8** (22.8 mg) in a 20:1 (v/v) *i*-PrOH/AcOH mixture was added 10% Pd/C (~0.2 equiv). The flask was purged 3× with Ar then filled with H_2. The reaction mixture was stirred at rt. After 6 h, the solids were removed by filtration and washed with *i*-PrOH. The filtrate was concentrated under reduced pressure to afford **3a** (100% conversion as judged by NMR spectroscopy). A pure analytical sample of **3a** was obtained after purification by filtration on Dowex 1-X2 (OH^-) ion-exchange resin (elution with 1/1 (v/v) MeOH/H_2O mixture). The fractions containing the product were concentrated under reduced pressure and lyophilized to afford **3a** as a colourless powder. $[\alpha]_D$ +7.5 (*c* 0.3, H_2O); ^{1}H NMR (500 MHz, D_2O): δ (ppm from TSP) 0.93 (t, 3H, $J = 7.3$ Hz), 0.94 (t, 3H, $J = 7.3$ Hz), 1.31 (m, 2H), 1.39 (m, 2H), 1.49 (m, 2H), 1.59 (m, 2H), 2.28 (t, 1H, $J = 11.0$ Hz, H-1ax), 2.43 (m, 1H, H-5), 2.68 (m, 1H, CHN), 2.77 (m, 1H, CHN), 3.21 (dd, 1H, $J = 4.6$, 11.9 Hz, H-1eq), 3.37 (dd, 1H, $J = 7.8$, 9.6 Hz, H-2′), 3.41 (ddd, 1H, $J = 4.6$, 10.1, 10.5 Hz, H-2), 3.47 (t, 1H, $J = 10.5$ Hz, H-3), 3.47 (dd, 1H, $J = 9.0$, 9.6 Hz, H-4′), 3.55 (dd, 1H, $J = 9.0$, 9.6 Hz, H-3′), 3.55 (m, 1H, H-5′), 3.64 (dd, 1H, $J = 10.1$, 10.5 Hz, H-4), 3.66 (m, 1H, CHO), 3.71 (m, 1H, CHO), 3.78 (dd, 1H, $J = 5.5$, 12.4 Hz, H-6A′), 3.94 (dd, 1H, $J = 2.3$, 12.4 Hz, H-6B′), 3.97 (m, 2H, H-6), 4.63 (d, 1H, $J = 7.8$ Hz, H-1′); ^{13}C NMR (125 MHz, D_2O): δ 15.9, 16.0, 21.4, 23.0, 27.9, 34.0, 54.3 (CH_2N), 55.2 (C-1), 59.3 (C-6), 63.3 (C-6′), 66.7 (C-5), 72.2 (C-4′), 73.4 (CH_2O), 76.1 (C-2′), 78.4 (C-3′), 78.6 (C-3), 78.8 (C-5′), 79.6 (C-2), 83.3 (C-4), 105.6 (C-1′); HRMS (FAB): *m*/*z* 438.2702 $[M+H]^+$ ($C_{20}H_{40}NO_9$ requires 438.2703).

1.5. 4-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-di-*O*-benzyl-*N*-butyl-2-*O*-(*tert*-butyldimethylsilyl)-1,5-dideoxy-1,5-imino-D-glucitol (9)

To a solution of **6b** (99.4 mg, 0.19 mmol) in CH_2Cl_2 (8 mL) at −78 °C was added activated 4 Å molecular sieves and bromide **5** (118 mg, 0.29 mmol). The reaction mixture was stirred for 15 min at −78 °C and silver triflate (107 mg, 0.42 mmol) was added. The reaction was warmed up slowly to rt and stirred in the dark for 24 h. The reaction mixture was washed with saturated aqueous $NaHCO_3$ (2 × 10 mL) and saturated aqueous NaCl (10 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The resulting crude product was purified by silica gel chromatography

(petroleum ether/AcOEt, 7/3) to afford **9** (53.8 mg, 34%) as a colourless oil. $[\alpha]_D$ −20.0 (*c* 1.0, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): δ −0.1 (s, 3H), 0.01 (s, 3H), 0.81 (s, 9H), 0.90 (t, 3H, $J = 7.3$ Hz), 1.14–1.52 (m, 4H), 1.92 (s, 3H), 1.97 (s, 3H), 1.98 (s, 3H), 1.99 (s, 3H), 2.15–2.23 (m, 2H, H-1ax, H-5), 2.46–2.73 (m, 4H), 2.90 (dd, 1H, $J = 5.2$ Hz, 11.2 Hz, H-1eq), 3.15 (t, 1H, $J = 8.7$ Hz, H-3), 3.31 (dt, 1H, $J = 3.4$ Hz, $J = 9.8$ Hz, H-5′), 3.52 (br d, 1H, H-6A), 3.66 (br d, 1H, H-6B), 3.75 (m, 1H, H-4), 3.83–3.90 (m, 2H, 2 H-6′), 4.45–4.50 (m, 2H, H-3′, CH benzyl), 4.61–4.69 (m, 2H, H-2′, CH benzyl), 4.81–4.98 (m, 4H, H-4′, H-1′, CH_2 benzyl), 7.16–7.46 (m, 10H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ −4.7, −4.6, 14.1, 18.1, 20.7, 20.8, 20.9, 21.2, 25.9, 26.3, 51.8 (CH_2N), 57.6 (C-1), 62.3, 63.9, 64.3, 68.8, 71.2, 71.5, 72.3, 73.5, 73.7 (CH_2 benzyl), 74.6 (CH_2 benzyl), 78.4, 85.7, 100.1 (C-1′), 126.7, 126.8, 127.8, 128.3, 128.6, 128.8, 128.9, 129.1, 137.4, 139.9, 169.2, 169.5, 170.3, 170.8; MS (IS): *m*/*z* 844.5 $[M+H]^+$.

1.6. 4-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-di-*O*-benzyl-*N*-butyl-1,5-dideoxy-1,5-imino-D-glucitol (10)

To a solution of **9** (217 mg, 0.26 mmol) in THF (10 mL) was added *n*-Bu_4NF (401 μL, 0.4 mmol) at 0 °C and the reaction was warmed up slowly to rt and stirred for 18 h. The reaction mixture was extracted with AcOEt (20 mL) and washed with water (3 × 20 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The resulting crude product was purified by silica gel chromatography (petroleum ether/AcOEt, 4/6) to afford **10** (120 mg, 70%) as a colourless oil. $[\alpha]_D$ −4.5 (*c* 0.85, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): δ 0.89 (t, 3H, $J = 6.9$ Hz), 1.14–1.48 (m, 4H), 1.97 (s, 3H), 1.99 (s, 3H), 2.00 (s, 3H), 2.02 (s, 3H), 2.26 (dd, 1H, $J = 8.5$, 11.0 Hz, H-1ax), 2.40–2.65 (m, 3H, H-5, CH_2N), 3.01 (dd, 1H, $J = 4.2$, 11.2 Hz, H-1eq), 3.26 (t, 1H, $J = 7.7$ Hz, H-3), 3.47 (m, 1H, H-5′), 3.55–3.71 (m, 3H, H-2, 2 H-6), 3.96 (t, 1H, $J = 7.5$ Hz, H-4), 4.00 (dd, 1H, $J = 2.2$, 12.5 Hz, H-6′A), 4.17 (dd, 1H, $J = 4.7$, 12.3 Hz, H-6′B), 4.44–4.54 (m, 3H, H-1′, 2CH benzyl), 4.64 (d, 1H, $J = 12.0$ Hz, CH benzyl), 4.91–5.08 (m, 4H, CH benzyl, H-2′,3′,4′), 7.22–7.46 (m, 10H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 14.1, 20.6, 20.70, 20.73, 20.8, 27.2, 52.4 (CH_2N), 53.9 (C-1), 62.0 (C-6′), 63.5 (C-5), 63.6 (C-6), 68.6, 71.7 (C-5′), 71.9, 73.1 , 73.6 (CH_2 benzyl), 74.0 (CH_2 benzyl), 77.13 (C-4), 83.6 (C-3), 100.0 (C-1′), 127.7, 128.5, 128.6, 128.8, 137.5, 139.1, 169.1, 169.5, 170.3, 170.7; MS (IS): *m*/*z* 730.5 $[M+H]^+$.

1.7. 4-*O*-(β-D-Glucopyranosyl)-3,6-di-*O*-benzyl-*N*-butyl-1,5-dideoxy-1,5-imino-D-glucitol (11)

Sodium (6 mg, 0.26 mmol) was added to anhydrous MeOH (2 mL) at rt. After 15 min, a solution of **10** (50 mg, 0.068 mmol) in anhydrous MeOH (2 mL) was added. The reaction mixture was stirred for 2 h, filtered through a pad of Celite® and concentrated under reduced pressure. The mixture was filtered through a pad of C_{18}-silica gel (Water 100% to MeOH 100%) and the resulting crude product was purified by silica gel chromatography (CH_2Cl_2/AcOEt/MeOH, 6/6/1) to afford **11** (33 mg, 86%) as a colourless oil. $[\alpha]_D$ −10.5 (*c* 1.1, $CHCl_3$); ^1H NMR (250 MHz, MeOD): δ 0.88 (t, 3H, $J = 6.9$ Hz), 1.10–1.50 (m, 4H), 2.27 (t, 1H, $J = 10.8$ Hz, H-1ax), 2.45 (m, 1H, H-5), 2.61 (m, 2H, CH_2N), 2.99 (dd, 1H, $J = 5.0$, 11.2 Hz, H-1eq), 3.10–3.22 (m, 2H, H-3′, H-5′), 3.23–3.39 (m, 3H, H-2′, H-3, H-4′), 3.45 (dd, 1H, $J = 6.2$ Hz, 11.7 Hz, H-6′A), 3.60–3.82 (m, 3H, H-6′B, H-6A, H-2), 3.90–4.05 (m, 2H, H-6B, H-4), 4.40–4.53 (m, 2H, H-1′, CH benzyl), 4.61 (d, 1H, $J = 11.7$ Hz, CH benzyl), 4.76 (d, 1H, $J = 10.6$ Hz, CH benzyl), 4.98 (d, 1H, $J = 10.6$ Hz, CH benzyl), 7.21–7.58 (m, 10H); ^{13}C NMR (62.9 MHz, MeOD): δ14.3, 21.6, 27.04, 52.9 (CH_2N), 57.7 (C-1), 63.1 (C-6′), 64.9 (C-6), 65.5 (C-5), 70.2 (C-2), 72.2 (C-5′), 73.9 (CH_2 benzyl), 75.8 (C-2′ or C-4′), 76.7 (CH_2 benzyl), 77.0 (C-4), 78.1 (C-2′ or C-4′), 78.9 (C-3′), 87.1 (C-3), 103.7 (C-1′), 128.8, 129.0, 129.1, 129.6, 129.8, 139.1, 139.8.

1.8. 4-*O*-(β-D-Glucopyranosyl)-*N*-butyl-1,5-dideoxy-1,5-imino-D-glucitol (3b)

To a ~0.1 M solution of precursor **11** (30 mg) in a 20:1 (v/v) *i*-PrOH/AcOH mixture was added 10% Pd/C (0.2 equiv). The flask was purged 3× with Ar then filled with H_2. The reaction mixture was stirred at rt until complete conversion of starting material. The solids were removed by filtration and washed with *i*-PrOH. The filtrate was concentrated under reduced pressure and the resulting crude product was purified by filtration on Dowex 1-X2 (OH^-) ion exchange resin (elution with 1/1 (v/v) MeOH/H_2O mixture). The fractions containing the product were concentrated under reduced pressure and lyophilized to afforded **3b** as a colourless powder (9 mg, 44%). $[\alpha]_D$ +9.5 (*c* 0.4, H_2O); ^1H NMR (500 MHz, D_2O): δ (ppm from TSP) 0.99 (3H, t, $J = 7.3$ Hz), 1.36 (2H, m), 1.55 (2H, m), 2.40 (1H, t, $J = 11.0$ Hz, H-1ax), 2.51 (1H, dt, $J = 2.7$, 9.0 Hz, H-5), 2.72 (1H, m, CHN), 2.83 (1H, m, CHN), 3.11 (1H, dd, $J = 5.0$, 11.0 Hz, H-1eq), 3.43 (1H, dd, $J = 7.8$, 9.6 Hz, H-2′), 3.49 (1H, m, H-2), 3.49 (1H, t, $J = 9.0$ Hz, H-3), 3.52 (1H, dd, $J = 9.2$, 9.6 Hz, H-4′), 3.61 (1H, t, $J = 9.6$ Hz, H-3′), 3.60 (1H, m, H-5′), 3.70 (1H, t, $J = 9.0$ Hz, H-4), 3.83 (1H, dd, $J = 5.9$, 12.4 Hz, H-6A′), 3.91 (1H, dd, $J = 2.7$, 12.8 Hz, H-6A), 3.99 (1H, dd, $J = 2.7$, 12.8 Hz, H-6B), 4.00 (1H, dd, $J = 2.3$, 12.4 Hz, H-6B′), 4.68 (1H, d, $J = 7.8$ Hz, H-1′); ^{13}C NMR (125 MHz, D_2O): δ 16.1, 23.0, 28.0, 54.3 (CH_2N), 58.2 (C-1), 59.5 (C-6), 63.4 (C-6′), 66.9

(C-5), 71.5 (C-2); 71.5 (C-4′), 72.3 (C-2′), 78.5 (C-3′), 79.7 (C-3), 78.9 (C-5′), 83.3 (C-4), 105.7 (C-1′); HRMS: *m*/*z* 382.2076 [M+H] ($C_{16}H_{32}NO_9$ requires 382.2077).

1.9. Inhibitory activity

Human β-glucocerebrosidase (glucosylceramide β-glucosidase, Ceredase) was purchased from Genzyme (Boston, MA) and assayed at pH 5.2. The reaction mixture consists of 50 μL of 0.15 M sodium phosphate-citrate buffer, 50 μL of 2% Triton X-100 (Sigma Chemical Co.), 30 μL of the enzyme solution, and 20 μL of an inhibitor solution or H_2O. The reaction mixture was pre-incubated at 0 °C for 10 min and started by the addition of 50 μL of 6 mM 4-methylumbelliferyl glycoside (Sigma Chemical Co.), followed by incubation at 37 °C. The reaction was stopped by the addition of 2 mL of 0.1 M glycine buffer (pH 10.6). Liberated 4-methylumbelliferone was measured (excitation 362 nm, emission 450 nm) with a F-4500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan).

Acknowledgments

Financial support of this study by Grants from CNRS, the French Department of Research, the associations 'Vaincre les Maladies Lysosomales' and 'AFM' (Association Française contre les Myopathies) is gratefully acknowledged. The authors express their gratitude to Charlotte Vibert and Jérôme Blu for assistance with synthetic work.

References

1. Zimran, A. *Gaucher's Disease*; Balliere Tindall, 1997.
2. A special issue of *Philosophical Transaction: Biological Sciences* has been devoted to the glycolipids in cell biology and medicine: *Philos. Trans. R. Soc. London B* **2003**, *358*, 845–983.
3. (a) Futerman, A. H.; Sussman, J. L.; Horowitz, M.; Silman, I.; Zimran, A. *Trends in Pharm. Sci.* **2004**, *25*, 147–151; (b) Butters, T. D.; Dwek, R. A.; Platt, F. M. *Glycobiology* **2005**, *10*, 43R–52R.
4. (a) Cox, T.; Lachmann, R.; Hollak, C.; Aerts, J.; van Weely, S.; Hrebicek, M.; Platt, F. M.; Butters, T. D.; Dwek, R. A.; Moyses, C.; Gow, I.; Elstein, D.; Zimran, A. *Lancet* **2000**, *355*, 1481–1485; (b) Butters, T. D.; Dwek, R. A.; Platt, F. M. *Curr. Top. Med. Chem.* **2003**, *3*, 561–574.
5. (a) Kolter, T.; Wendeler, M. *ChemBioChem* **2003**, *4*, 260–264; (b) Fan, J.-Q. *Trends Pharm. Sci.* **2003**, *24*, 355–360; (c) Fan, J.-Q.; Ishii, S.; Asano, N.; Suzuki, Y. *Nature Med.* **1999**, *5*, 112–115; (d) Asano, N.; Ishii, S.; Kizu, H.; Ikeda, K.; Yasuda, K.; Kato, A.; Martin, O. R.; Fan, J.-Q. *Eur. J. Biochem.* **2000**, *267*, 4179–4186; (e) Ishii, S.; Kase, R.; Sakuraba, H.; Suzuki, Y. *Biochem. Biophys. Res. Commun.* **1993**, *197*, 1585–1589.
6. (a) Yam, G. H.-F.; Zuber, C.; Roth, J. *FASEB J.* **2005**, *19*, 12–18; (b) Steet, R. A.; Chung, S.; Wustman, B.; Powe, A.; Do, H.; Kornfeldt, S. A. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 13813–13818; (c) Lieberman, R. L.; Wustman, B. A.; Huertas, P.; Powe, A. C.; Pine, C. W.; Khanna, R.; Schlossmacher, M. G.; Ringe, D.; Petsko, G. A. *Nature Chem. Biol.* **2007**, *3*, 101–107.
7. Sawkar, A. R.; Cheng, W.-C.; Beutler, E.; Wong, C.-H.; Balch, W. E.; Kelly, J. W. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 15428–15433.
8. (a) Compain, P.; Martin, O. R.; Boucheron, C.; Godin, G.; Yu, L.; Ikeda, K.; Asano, N. *ChemBioChem* **2006**, *7*, 1356–1359; (b) Yu, L.; Ikeda, K.; Kato, A.; Adachi, I.; Godin, G.; Compain, P.; Martin, O. R.; Asano, N. *Bioorg. Med. Chem.* **2006**, *14*, 7736–7744.
9. For other efforts directed towards the development of chemical chaperones against Gaucher disease see: (a) Lin, H.; Sugimoto, Y.; Ohsaki, Y.; Ninomiya, H.; Oka, A.; Taniguchi, M.; Ida, H.; Eto, Y.; Ogawa, S.; Matsuzaki, Y.; Sawa, M.; Inoue, T.; Higaki, K.; Nanba, E.; Ohno, K.; Suzuki, Y. *Biochim. Biophys. Acta* **2004**, *1689*, 219–228; (b) Lysek, R.; Schütz, C.; Vogel, P. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3071–3075; (c) Zhu, X.; Sheth, K. A.; Li, S.; Chang, H.-H.; Fan, J.-Q. *Angew. Chem., Int. Ed.* **2005**, *44*, 7450–7453; (d) Yu, Z.; Sawkar, A. R.; Whalen, L. J.; Wong, C.-H.; Kelly, J. W. *J. Med. Chem.* **2007**, *50*, 94–100; (e) Chang, H.-H.; Asano, N.; Ishii, S.; Ichikawa, Y.; Fan, J.-Q. *FEBS J.* **2006**, *273*, 4082–4092.
10. Boucheron, C.; Desvergnes, V.; Compain, P.; Martin, O. R.; Lavi, A.; Mackeen, M.; Wormald, M. R.; Dwek, R. A.; Butters, T. D. *Tetrahedron: Asymmetry* **2005**, *16*, 1747–1756.
11. (a) Fabrega, S.; Durand, P.; Codogno, P.; Bauvy, C.; Delomenie, C.; Henrissat, B.; Martin, B. M.; McKinney, C.; Ginns, E. I.; Mornon, J. P.; Lehn, P. *Glycobiology* **2000**, *10*, 1217–1224; (b) Fabrega, S.; Durand, P.; Mornon, J. P.; Lehn, P. *J. Soc. Biol.* **2002**, *196*, 151–160; (c) Davies, C.; Henrissat, B. *Structure* **1995**, *3*, 853–859.
12. Butters, T. D.; van den Broek, L. A. G. M.; Fleet, G. W. J.; Krulle, T. M.; Wormald, M. R.; Dwek, R. A.; Platt, F. M. *Tetrahedron: Asymmetry* **2000**, *11*, 113–124.
13. (a) Felpin, F. X.; Boubekeur, K.; Lebreton, J. *J. Org. Chem* **2004**, *69*, 1497–1503; (b) Sawada, D.; Takahashi, H.; Shiro, M.; Ikegami, S. *Tetrahedron Lett.* **2005**, *47*, 2399–2403; (c) Takahashi, S.; Kuzuhara, H.; Nakajima, M. *Tetrahedron* **2001**, *57*, 6915–6926; (d) Kiso, M.; Katagiri, H.; Furui, H.; Hasegawa, A. *J. Carbohydr. Chem.* **1992**, *11*, 627–644; (e) Spohr, U.; Bach, M. *Can. J. Chem.* **1993**, *71*, 1943–1954; (f) Liu, P. S. *J. Org. Chem* **1987**, *52*, 4717–4721; (g) Anzeveno, P. B.; Creemer, L. J.; Daniel, J. K.; King, C.-H. R.; Liu, P. S. *J. Org. Chem* **1989**, *54*, 2539–2542; (h) Banwell, M. G.; Ma, X.; Asano, N.; Ikeda, K.; Lambert, J. N. *Org. Biomol. Chem.* **2003**, *1*, 2035–2037.
14. For a review on the synthesis of imino-*C*-disaccharides, see: Robina, I.; Vogel, P. *Synthesis* **2005**, 675–702.
15. Boucheron, C.; Compain, P.; Martin, O. R. *Tetrahedron Lett.* **2006**, *47*, 3081–3084.
16. (a) For a chemoenzymatic synthesis of the *p*-toluenesulfonate salt of **3b**, see: Yoshikuni, Y.; Ezure, Y.; Seto, T.; Mori, K.; Watanabe, M.; Enomoto, H. *Chem. Pharm. Bull.* **1989**, *37*, 106–109, and references cited therein.
17. For syntheses of 4-O-glucosylated 1-deoxynojirimycins, see: Steiner, A. J.; Stütz, A. E. *Carbohydr. Res.* **2004**, *339*, 2615–2619.
18. A comprehensive volume devoted to the chemistry and biology of iminosugars will be published in summer 2007: Compain, P.; Martin, O. R. *Iminosugars: From Synthesis to Therapeutic Applications*; Wiley-VCH: Weinheim, in press.