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Design and synthesis of iminosugar-based inhibitors of glucosylceramide synthase: the search for new therapeutic agents against Gaucher disease

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Abstract—A series of iminosugars bearing two or three alkyl chains ('iminoglycolipids') were designed as ceramide mimics and analogues of *N*-butyl 1-deoxynojirimycin (*N*-Bu DNJ, Zavesca®). This orally active iminosugar inhibits the biosynthesis of glucosylceramides, which accumulate pathologically in macrophages of patients with Gaucher disease (substrate reduction therapy, SRT). Molecular modeling and kinetic experiments have suggested that *N*-Bu DNJ is a competitive inhibitor that mimics the ceramide acceptor but not the donor substrate (UDP-glucose) in the glucosylceramide synthase-catalyzed process. Kinetic measurements were made with the glucosyltransferase to assess the selectivity of the new iminoglycolipids with respect to the length (C_4 or C_8) and the position of the second alkyl chain (C-1, O-2 and/or O-4). This structure–activity relationship study showed that the addition of a second alkyl chain, to obtain better ceramide mimics, led to less potent inhibitors. Moreover, the synthase active site did not discriminate inhibitors differing by the position of the second alkyl chain (C-1, O-2 or O-4). Best inhibition was found for 1,5-dideoxy-1,5-imino-*N*-octyl-4-*O*-octyl-D-glucitol (IC_{50} 134 μM).

1. Introduction

Gaucher disease,[1] the most common glycolipid storage disease, is a relatively rare hereditary disorder due to a deficiency in a specific β-glucosidase (β-glucocerebrosidase) involved in the catabolism of glycosphingolipids in lysosomes.[2] Defects in the catalytic activity of this enzyme leads to the accumulation of undegraded glucosylceramide (GlcCer) in macrophages and to severe symptoms. In type 1, the mildest and most common of the three clinical forms of Gaucher disease, patients suffer from bone pains, skeletal lesions, anaemia, thrombocytopenia and liver or spleen damage. Type 1 Gaucher disease occurs worldwide in all population (1 in 40,000–60,000) but it is the most common genetically-based disease affecting Jews (1 in 500–1000 among Ashkenazi Jews).[3]

There are currently two therapies available for Gaucher disease, which are both aimed at reducing GlcCer storage.[4] The first-line treatment is based on the administration of Cerezyme®, a recombinant form of β-glucocerebrosidase, to supplement the defective hydrolytic enzyme (enzyme replacement therapy, ERT). A second strategy uses a small molecule, *N*-butyl 1-deoxynojirimycin **1** (*N*-Bu DNJ, Zavesca®, Scheme 1), as a potent inhibitor of glucosylceramide synthase, the glucosyltransferase involved in the biosynthesis of glycosphingolipids and responsible for the glucosylation of ceramide (substrate reduction therapy, SRT).[5] More than 3000 patients have been treated worldwide with Cerezyme® since the 90s and Zavesca® is the first orally administered treatment for lysosomal diseases. Despite these therapeutic breakthroughs, both approaches have various drawbacks.[4] ERT is possible only for non-neuronopathic Gaucher patients since the enzyme does not cross the blood–brain barrier. Moreover, Cerezyme® named the 'world's most expensive drug' is extremely costly ($150,000 per year). Zavesca® has been

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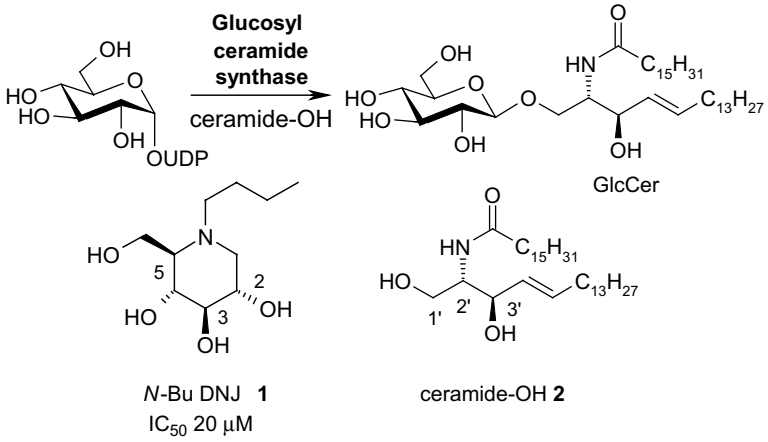


Scheme 1.

recommended only for adults with mild-to-moderate type 1 Gaucher disease for whom ERT is not an option. Large doses are required (~300 mg daily) but can lead to serious side-effects including abdominal pains and loss of weight arising mainly from the inhibition of digestive glucosidases. Moreover, the fact that this compound is also a strong inhibitor of glucosidase I, a cellular glycosidase involved in the elaboration of the glycan structure of glycoproteins, is a major concern, particularly for long-term treatments.[6] Thus, whereas the validity of the substrate deprivation approach has been clearly demonstrated, there is a need for more selective new therapeutic agents.

UDP-glucose:ceramide glucosyltransferase also known as glucosylceramide synthase catalyzes the transfer of a glucose moiety from UDP-glucose to the primary hydroxyl group of ceramide to yield GlcCer with inversion of the anomeric configuration (Scheme 1).

The study of *N*-butyl DNJ **1** and its derivatives has indicated that this compound is, somewhat unexpectedly, a mimic of ceramide **2** and not of the glucose moiety;[7,8] this is shown in particular by the fact that the inhibition of glucosylceramide synthase by this compound is competitive with respect to ceramide and not to the UDP-glucose. Molecular modeling has revealed a strong structural homology between **1** and the ceramide structure. The *N*-alkyl chain and three stereogenic centres (C-2, C-3 and C-5) of iminosugar **1** show structural similarities with the *N*-acyl chain and the C-1′–C-3′ backbone of ceramide, respectively. Remarkably, the O-3 hydroxyl group of **1** overlays with the O-3′ hydroxyl of ceramide **2** (Fig. 1).[7]

This rational model suggests very clearly that the iminosugar would be a better ceramide mimic if a second alkyl chain was present at O-2 or C-1 to simulate the second hydrophobic chain of the ceramide. The iminoglycolipids thus designed were expected to be more potent and more selective inhibitors than *N*-Bu DNJ **1**. To assess this working hypothesis, various di- or tri-alkylated iminosugars were prepared by taking advantage of a general strategy we recently developed for the practical synthesis of nojirimycin *C*-glycosides.[9] Experiments with glucosylceramide synthase were made to assess their specificity with respect to the length (C_4 or C_8) and the position of the second alkyl chain (C-1, O-2 and/or O-4).

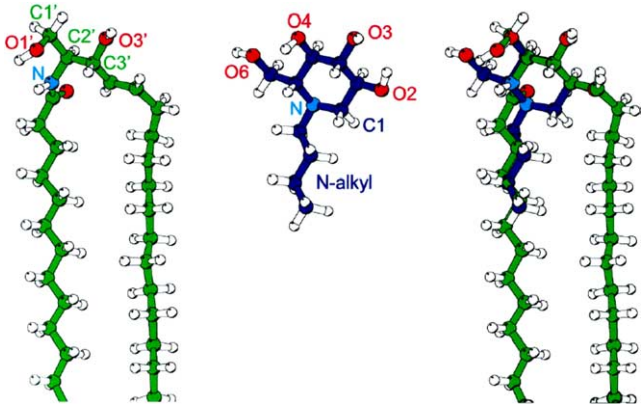
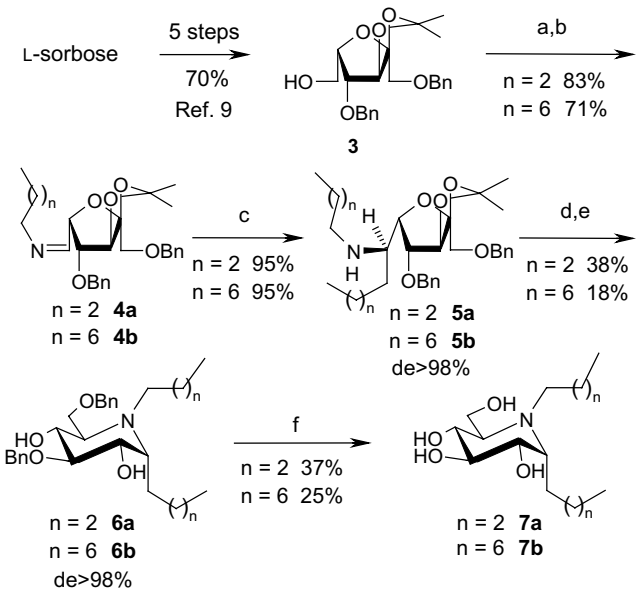


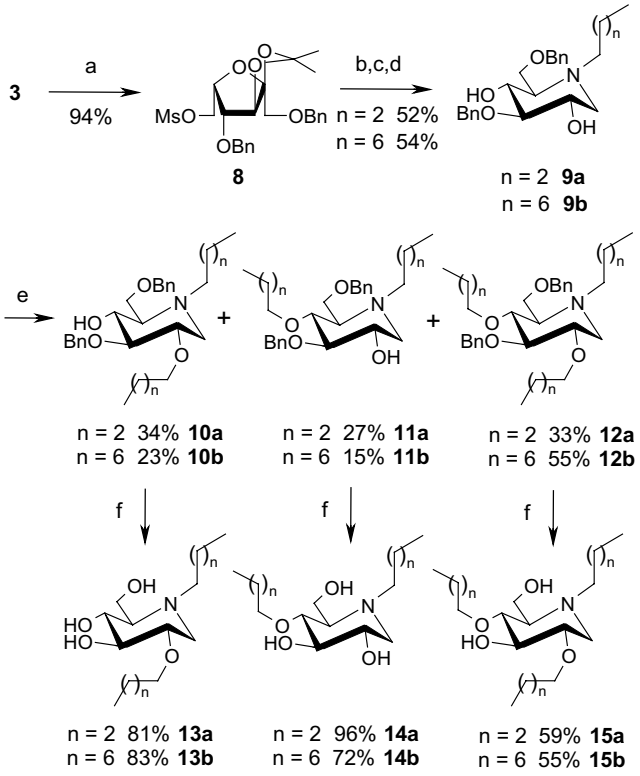
Figure 1. Possible overlay of ceramide and *N*-butyl DNJ highlighting structural mimicry.[7]

2. Results and discussion

To prepare iminosugar *C*-glycosides **7**, we applied directly our initial strategy to *N*-alkylated imines **4a** and **4b** instead of the corresponding *N*-benzyl derivatives (Scheme 2). We were pleased to find that chain extension of these imines with butylmagnesium chloride or octylmagnesium bromide proceeded in high diastereoselectivity and in almost quantitative yield as judged by proton NMR spectroscopy. Amines **5a** and **5b** were directly engaged in the next step without purification since partial decomposition occurred on silica gel.[10] The deprotection of the acetal function in aqueous trifluoroacetic acid, followed by intramolecular reductive amination afforded the expected piperidines **6a** and **6b** in high diastereoselectivity but poor yield. In sharp contrast with findings in the *N*-benzyl series, degradation occurred during the reductive amination step. Removal of the benzyl protecting groups by hydrogenolysis provided the desired iminosugar *C*-glycosides **7a** and **7b**.[11]



Scheme 2. Reagents and conditions: (a) PCC (1.8 equiv), CH_2Cl_2, 16 h; (b) butyl- or octylamine (1.1 equiv), molecular sieves 4 Å, 4 °C, 16 h; (c) butylMgCl (4 equiv) or octylMgBr (3 equiv), Et_2O, 0–20 °C, 16 h; (d) TFA/H_2O (9:1), 36 h; (e) $NaBH_3CN$ (3 equiv), AcOH (1 equiv), MeOH, 16 h; (f) H_2, Pd/C, HCl 4 M cat., MeOH ($n = 2$) or THF/H_2O ($n = 6$), 24 h.



Scheme 3. Reagents and conditions: (a) MsCl (1.2 equiv), Et_3N (1.2 equiv), CH_2Cl_2, 4 h; (b) neat butyl- or octylamine, 85 °C, 16 h; (c) TFA/H_2O (9:1), 0–20 °C, 28 h; (d) $NaBH_3CN$ (4.1 equiv for $n = 2$ or 5.2 equiv for $n = 6$), AcOH (1 equiv), MeOH, 48 h; (e) NaH (6 equiv), butyl iodide (7 equiv) or octyl iodide (8 equiv), n-Bu_4NI (25 mol %), THF, Δ, 72 h; (f) H_2, Pd/C, MeOH/HCl 5 N (10:1), 48 h ($n = 2$) or 70 h ($n = 6$).

To prepare the analogues of **1** bearing alkyl chains at O-2 and/or O-4, we took advantage of the key step of our initial strategy.[9] The one-pot sequence of acetal deprotection and intramolecular reductive amination was of particular interest since this process provides a properly protected piperidine with two free hydroxyl groups tactically positioned at C-2 and C-4. Mesylate derivative **8** was obtained efficiently under classical conditions from alcohol **3**. Nucleophilic substitution by alkyl amines under neat conditions[12] afforded the corresponding aminosorbofuranose derivatives, which were directly engaged in the next step without purification (Scheme 3). The intramolecular reductive amination of the unmasked aminosorbose hemiketal proceeded in good yields to provide the expected key intermediates **9a** and **9b**. At this stage, our aim was to functionalize or protect regioselectively the OH-2 since it is less hindered when compared to OH-4. After various attempts, no satisfactory solutions could be found in terms of reproducibility or practicability.[13] From the finding that the two regioisomers **10a** and **10b** and **11a** and **11b** could be easily separated on silica gel, we intentionally performed a non-regioselective alkylation of diols **9a** and **9b** using large amounts of NaH and alkyl iodide. This method afforded after purification three iminoglycolipids of interest including the precursors of target compounds **13a** and **13b**. Finally, removal of benzyl groups by hydrogenolysis gave, after purification on silica gel, the expected piperidinols in 11 steps with 4–10% overall yield from L-sorbose.

NMR analysis of iminoglycolipids **13b** (Table 1) and **14b** (Table 2), indicated that the piperidine ring in CD_3OD adopts a classical non-distorted $^4C_1$ (D) conformation. The $^3J_{H5,H6(R)}$ and $^3J_{H5,H6(S)}$ coupling constants are very small (2.6 Hz), indicating that there is little or no rotation around the C5–C6 bond: this pattern of coupling constants is only consistent with an N5–C5–C6–O6 torsion angle of either −60° (staggered, *gg*) or +120° (fully eclipsed), and only the former is sterically allowed. The equatorial position of the octyl group at nitrogen is confirmed by the pattern of NOE interactions between the alkyl chain protons and H5, H6 and both H1 protons.[14] The *N*- and *O*-octyl groups adopt a conformation that is typical of extended alkyl chains.

With this series of iminoglycolipids, we investigated the influence of the position and length of alkyl chains on glucosylceramide synthase inhibition (Fig. 2). For the purpose of comparison, the IC_{50} values for *N*-butyl DNJ **1**, and its *N*-nonyl analogue **16** are also included in Figure 2. This biological evaluation indicated clearly that the addition of a second or third lipophilic chain does not provide better affinity for the enzyme. The alkyl chain-length of these iminosugars plays a decisive role in their inhibition profile. The best inhibitions were found for iminosugars bearing two octyl chains, **13b**, **14b** and **7b** whereas those bearing butyl chains displayed no significant inhibition towards glucosylceramide synthase (**13a**, **14a** and **15a**) with the exception of compound **7a** (IC_{50} 609 μM). The addition of a third lipophilic chain is detrimental to the inhibitory activity of

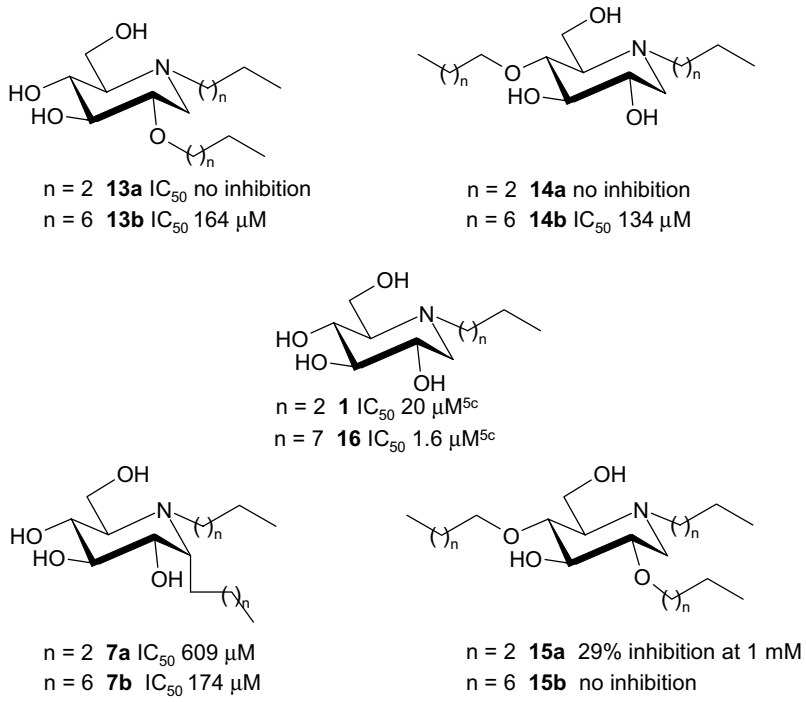


Figure 2. Inhibition of glucosylceramide synthase.

iminosugars **13b** and **14b** but has a slightly positive effect for the corresponding analogues bearing shorter butyl chains. Interestingly, comparison of the IC_{50} values between di-alkylated iminosugars **13b**, **14b** and **7b** showed that the position of the second alkyl chain (α-C-1, O-2 or O-4) has almost no influence on their inhibitory profile.

According to our initial hypothesis, compounds having a closer structural homology to ceramide than *N*-butyl DNJ **1** were expected to be better inhibitors of the synthase. Inhibition results obtained for iminoglycolipids **13a** and **13b**, **14a** and **14b** and **7a** and **7b** indicated however that this hypothesis does not hold since the best ceramide mimics were sevenfold less potent than *N*-butyl DNJ. This can be understood in terms of the relative orientation of the alkyl chains on the piperidine scaffold: in their most favourable conformation in solution, as established by NMR for **13b** (Table 1) and **14b** (Table 2), the alkyl chains are oriented in remote directions (Fig. 3), unlike the nearly parallel alkyl chains of ceramide in the solid state (Fig. 1). It appears therefore that in spite of the flexibility of the alkyl substituents, these chains do not fold easily to adopt a conformation resembling that of ceramide.

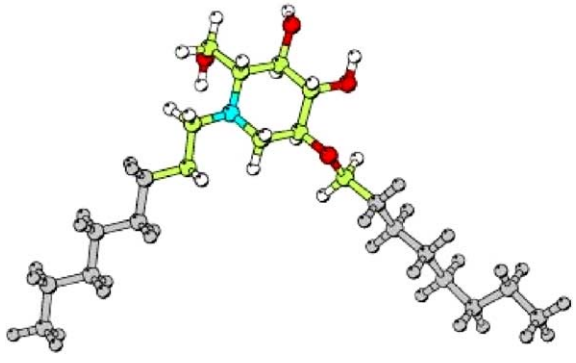


Figure 3. Solution conformation of **13b**.

Further studies are still yet to be performed to evaluate the specificity of di-alkyl iminoglycolipids **13b** and **14b** as GlcCer synthase inhibitors, since these compounds are expected to be weaker inhibitors of glucosidases than **1**.

3. Conclusion

In conclusion, we have achieved the synthesis of original iminosugars carrying two or more lipophilic chains ('iminoglycolipids') from L-sorbose. These analogues of *N*-butyl DNJ **1** were designed as ceramide mimetics and evaluated as inhibitors of glucosylceramide synthase. This study showed however that the addition of a second alkyl chain does not improve their activity. Moreover, the glucosylceramide synthase active site does not discriminate inhibitors differing by the position of the second alkyl chain (α-C-1, O-2 or O-4). Further work will focus on the confirmation and consequence of these findings for the design of new iminosugar-based glucosylceramide synthase inhibitors as potential therapeutic agents for Gaucher disease.[15]

4. Experimental

4.1. General

Unless otherwise stated, all reactions requiring anhydrous conditions were carried out under Argon. THF

and diethyl ether were freshly distilled from sodium/benzophenone under Argon prior to use. Dichloromethane was distilled from calcium hydride. Infrared spectra were recorded using films on NaCl windows or KBr pellets. Low-resolution mass spectra (MS) were recorded with a Perkin–Elmer Sciex API 3000 in the ion spray (IS) mode. High-resolution mass spectra (HRMS) were recorded with a Micromass ZABSpec TOF in the electrospray ionization (ESI) mode and with a Finnigan MAT 95 XL in the chemical ionization (CI) mode. Optical rotations were measured at room temperature (20 °C) in a 1 dm cell with a Perkin–Elmer 241 polarimeter. Analytical thin layer chromatography was performed using silica gel $60F_{254}$ precoated plates (Merck) with visualization by ultraviolet light, phosphomolybdate solution (2% in H_2SO_4/EtOH 1:7) and/or exposure to I_2 vapour. Flash chromatography was performed on silica gel 60 (230–400 mesh) with ethyl acetate (AcOEt) and petroleum ether (PE) as eluants unless indicated otherwise. ^1H and ^{13}C NMR spectra were recorded at 25 °C on a Bruker DPX 250 Advance (250 MHz) spectrometer with Me_4Si as internal reference unless otherwise stated, and J values are quoted in hertz. Carbon multiplicities were assigned by distortionless enhancement by polarization transfer (DEPT) experiments.

4.2. 3,6-Di-*O*-benzyl-4,5-*O*-isopropylidene-D-*xylo*-aldehydo-hexos-5-ulo-2,5-furanose *N*-butylimine 4a

To a stirred solution of 3,6-di-*O*-benzyl-4,5-*O*-isopropylidene-D-*xylo*-*aldehydo*-hexos-5-ulo-2,5-furanose[9] (200 mg, 0.5 mmol) in CH_2Cl_2 (4.5 mL) were added powdered 4 Å molecular sieves (50 mg) and butylamine (64.5 μL, 0.65 mmol, 1.3 equiv) at 0 °C. Stirring was stopped and the mixture allowed to stand for 16 h at 0 °C. The solids were removed by filtration and washed with anhydrous CH_2Cl_2 (10 mL). The filtrate was concentrated in vacuo to afford homogeneous **4a** (233 mg, quant.) as a yellow oil. This compound being very moisture sensitive, was used without further purification. IR (NaCl, neat) 1673 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.92 (m, 4H), 1.35 (m, 3H), 1.44 (s, 3H), 1.53 (s, 3H), 1.58 (m, 2H), 3.45 (t, 1H, J = 6.9 Hz), 3.75 (d, 1H, J = 11.0 Hz), 3.84 (d, 1H, J = 11.0 Hz), 4.18 (d, 1H, J = 3.4 Hz), 4.43 (d, 1H, J = 12.0 Hz), 4.61 (d, 2H, J = 12.0 Hz), 4.74 (d, 1H, J = 12.0 Hz), 4.81 (m, 1H), 7.21–7.37 (m, 10H), 7.68 (d, 1H, J = 5.4 Hz); MS-IS *m*/*z*: 454.0 $[MH]^+$.

4.3. 3,6-Di-*O*-benzyl-4,5-*O*-isopropylidene-D-*xylo*-aldehydo-hexos-5-ulo-2,5-furanose *N*-octylimine 4b

3,6-Di-*O*-benzyl-4,5-*O*-isopropylidene-D-*xylo*-*aldehydo*-hexos-5-ulo-2,5-furanose[9] (200 mg, 0.5 mmol) was treated with octylamine (107 μL, 0.64 mmol) as described for **4a**. The crude product was obtained as a yellow oil and used without further purification. Compound **4b** (268 mg, quant.): IR (NaCl, neat) 1674 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.87 (m, 4H), 1.26 (m, 10H), 1.42 (s, 3H), 1.51 (s, 3H), 1.58 (m, 2H), 3.41 (t, 2H, J = 6.9 Hz), 3.73 (d, 1H, J = 11.0 Hz), 3.82 (d, 1H, J = 11.0 Hz), 4.15 (d, 1H, J = 3.4 Hz), 4.42 (d, 1H, J = 12.0 Hz), 4.57 (d, 2H, J = 12.0 Hz), 4.71 (d, 1H, J = 12.0 Hz), 4.79 (m, 1H), 7.18–7.35 (m, 10H), 7.68 (d, 1H, J = 5.4 Hz); ^{13}C NMR ($CDCl_3$): δ 14.1, 26.6, 26.9, 27.5, 29.3, 29.5, 30.5, 61.2, 70.1, 72.0, 73.7, 82.2, 82.3, 84.6, 97.4, 112.6, 114.6, 127.3, 127.6, 127.8, 128.3, 128.4, 137.4, 138.1, 161.7; MS-IS *m*/*z*: 510.0 $[MH]^+$.

4.4. (6*R*)-1,4-Di-*O*-benzyl-6-butylamino-6-*C*-butyl-6-deoxy-2,3-*O*-isopropylidene-α-L-sorbofuranose 5a

To a solution of 2.0 M butylmagnesium chloride (661 μL, 1.32 mmol) in anhydrous Et_2O at 0 °C, was added slowly a 0.15 M solution of **4a** (150 mg, 0.33 mmol) in anhydrous Et_2O. The mixture was slowly warmed up to room temperature and stirred overnight (16 h). The reaction was quenched by the slow addition of saturated aqueous NH_4Cl at room temperature. The layers were separated, the aqueous phase extracted with Et_2O and the organic layer dried over Na_2SO_4 and concentrated under reduced pressure. The colourless oily product **5a** (160 mg, 95%) was used without further purification. $[\alpha]_D^{20} = +76.0$ (c 1.0, $CHCl_3$); IR (NaCl, neat) 1610, 1205, 1085, 733, 697 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.89 (m, 7H), 1.33 (m, 7H), 1.43 (s, 3H), 1.52 (s, 3H), 2.58 (m, 2H), 2.97 (m, 1H), 3.37 (br s, 1H), 3.63 (d, 1H, J = 11.0 Hz), 3.76 (d, 1H, J = 11.0 Hz), 3.88 (m, 1H), 4.18 (dd, 1H, J = 9.0, 2.7 Hz), 4.37 (d, 1H, J = 12.0 Hz), 4.62 (m, 4H), 7.26–7.31 (m, 10H); ^{13}C NMR ($CDCl_3$): δ 14.09, 14.13, 20.6, 23.2, 26.8, 27.7, 27.8, 29.8, 32.5, 46.4, 56.2, 70.5, 71.6, 73.7, 81.5, 81.9, 82.9, 112.2, 113.5, 127.7, 128.0, 128.1, 128.4, 128.5, 137.4, 138.4; MS-IS *m*/*z*: 512.0 $[MH]^+$.

4.5. (6*R*)-1,4-Di-*O*-benzyl-6-deoxy-2,3-*O*-isopropylidene-6-*C*-octyl-6-octylamino-α-L-sorbofuranose 5b

To a solution of 2.0 M octylmagnesium bromide (750 μL, 1.47 mmol) in anhydrous Et_2O at 0 °C was slowly added a 0.15 M solution of **4b** (249 mg, 0.49 mmol) in anhydrous Et_2O. The mixture was then warmed up slowly to room temperature, and stirred overnight (16 h). Work-up as described above afforded **5b** as a colourless oil (290 mg, 95%). IR (NaCl, neat) 1612, 1209, 1085, 738, 692 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.88 (m, 10H), 1.26 (m, 20H), 1.42 (s, 3H), 1.51 (s, 3H), 2.54 (m, 2H), 2.93 (m, 1H), 3.63 (m, 2H), 3.74 (d, 1H, J = 11.0 Hz), 3.86 (d, 1H, J = 3.0 Hz), 4.15 (dd, 1H, J = 9.0, 2.9 Hz), 4.35 (d, 1H, J = 12.0 Hz), 4.61 (m, 4H), 7.26–7.32 (m, 10H); ^{13}C NMR ($CDCl_3$): δ 14.1, 22.6, 25.4, 25.7, 26.7, 27.4, 27.6, 29.2, 29.4, 29.7, 30.1, 30.5, 31.8, 32.8, 46.7, 56.0, 62.9, 70.4, 71.5, 73.6, 81.4, 81.8, 83.0, 112.0, 113.4, 127.5, 127.6, 127.8, 128.3, 128.4, 137.3, 138.3; MS-IS *m*/*z*: 625.0 $[MH]^+$.

4.6. (1*R*)-3,6-Di-*O*-benzyl-1-*C*-butyl-*N*-butyl-1,5-dideoxy-1,5-imino-D-glucitol 6a

A ~0.15 M solution of precursor **5a** (47 mg, 0.09 mmol) in trifluoroacetic acid was prepared at 0 °C; H_2O was then added to form a 9:1 (v/v) TFA/H_2O mixture. The reaction mixture was warmed up to room temperature and stirred for 36 h. The solvents were removed by three

co-evaporations with toluene and the residual product taken up in MeOH to form a ~0.05 M solution. Acetic acid (1 equiv) and $NaBH_3CN$ (3 equiv) were added at 0 °C. The reaction mixture was stirred for 24 h at room temperature. The solvents were evaporated under reduced pressure. The crude product was taken up in CH_2Cl_2 and the solution was washed with saturated aqueous $NaHCO_3$ then with water. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The resulting crude product was purified by silica gel chromatography (PE/AcOEt 8:2–7:3) to provide **6a** (16 mg, 38%) as a colourless oil. $[\alpha]_D^{20} = +38.5$ (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.91 (m, 7H), 1.42 (m, 11H), 2.54 (m, 2H), 2.94 (m, 2H), 3.47 (t, 1H, $J = 8.5$ Hz), 3.79 (m, 4H), 4.54 (s, 2H), 4.70 (d, 1H, $J = 11.5$ Hz), 4.98 (d, 1H, $J = 11.5$ Hz), 7.27–7.37 (m, 10H); ^{13}C NMR ($CDCl_3$): δ 14.0, 14.1, 21.1, 22.8, 24.3, 29.5, 32.4, 48.5, 57.1, 59.3, 69.2, 71.5, 73.3, 73.5, 74.6, 84.0, 127.70, 127.73, 127.80, 127.84, 128.4, 128.5, 137.5, 138.8; HRMS-CI *m/z* 456.31102 $[M+H]^+$ ($C_{28}H_{42}NO_4$ required 456.3113).

4.7. (1*R*)-3,6-Di-*O*-benzyl-1-*C*-octyl-*N*-octyl-1,5-dideoxy-1,5-imino-D-glucitol 6b

Compound **5b** (60 mg, 0.08 mmol) was treated as described for **5a**. The resulting crude product was purified by silica gel chromatography (PE/AcOEt 8:2) to provide **6b** (8 mg, 18%) as a colourless oil. $[\alpha]_D^{20} = +23.5$ (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.86 (m, 7H), 1.28 (m, 27H), 2.53 (t, 2H, $J \sim 7.0$ Hz), 2.93 (m, 2H), 3.45 (t, 1H, $J = 8.5$ Hz), 3.76 (m, 4H), 4.51 (s, 2H), 4.67 (d, 1H, $J = 11.5$ Hz), 4.98 (d, 1H, $J = 11.5$ Hz), 7.27–7.35 (m, 10H); ^{13}C NMR ($CDCl_3$): δ 14.1, 22.6, 24.5, 27.0, 27.3, 29.2, 29.3, 29.4, 29.5, 29.6, 29.8, 30.0, 31.8, 31.9, 48.9, 57.1, 59.2, 69.1, 71.5, 73.2, 73.5, 74.6, 83.9, 127.7, 127.8, 127.9, 128.4, 128.5, 137.5, 138.8; HRMS-CI *m/z* 568.43663 $[M+H]^+$ ($C_{36}H_{58}NO_4$ required 568.4365).

4.8. (1*R*)-*N*-Butyl-1-*C*-butyl-1,5-dideoxy-1,5-imino-D-glucitol 7a

To a solution of **6a** (37 mg, 0.08 mmol) in MeOH (4 mL) was added 10% Pd/C (0.2 equiv) and four drops of 4 M aqueous HCl. The flask was purged three times with Ar and then filled with H_2. After 24 h, the solids were removed by filtration and the filtrate concentrated under reduced pressure. The crude product was filtered through Amberlyst ion-exchange resin IRA-400(OH^-) (elution with H_2O) and the filtrate concentrated under reduced pressure. The residual product was purified on an Amberlyst ion-exchange resin IR-120(H^+) (washing with H_2O, elution with 5% aqueous ammonia); the fractions containing the product were pooled and concentrated under reduced pressure to afford **7a** (8 mg, 37%) as a colourless oil. $[\alpha]_D^{20} = +27.5$ (*c* 0.7, $CHCl_3$); ^1H NMR (CD_3OD): δ 0.96 (m, 6H), 1.41 (m, 10H), 1.71 (m, 1H), 2.90 (m, 3H), 3.18 (m, 1H), 3.32 (m, 3H), 3.49 (m, 2H), 3.73 (m, 1H), 3.88 (d, 2H, $J = 4.4$ Hz); ^{13}C NMR (CD_3OD): δ 14.5, 21.5, 24.0, 24.7, 31.7, 33.1, 48.9, 60.7, 61.4, 61.8, 71.8, 72.9, 77.0; HRMS-CI *m/z* 276.2178 $[M+H]^+$ ($C_{14}H_{29}NO_4$ required 276.2176).

4.9. (1*R*)-1-*C*-Octyl-*N*-octyl-1,5-dideoxy-1,5-imino-D-glucitol 7b

To a solution of **6b** (40 mg, 0.07 mmol) in THF/H_2O 4:1 (2 mL) was added 10% Pd/C (0.2 equiv) and four drops of 4 M aqueous HCl. Work-up and purification procedure as described for **7a** provided **7b** (7 mg, 25%) as a colourless oil. ^1H NMR (CD_3OD): δ 0.89 (m, 5H), 1.31–1.42 (m, 28H), 2.63 (m, 3H), 2.95 (m, 1H), 3.39 (m, 4H), 3.75 (m, 4H); ^{13}C NMR (CD_3OD): δ 20.7, 29.9, 30.9, 31.0, 34.6, 35.6, 36.7, 36.9, 37.0, 37.2, 37.9, 39.2, 39.3, 55.0, 55.2, 66.9, 67.5, 68.0, 78.0, 79.0, 83.2; HRMS-CI *m/z* 388.3424 $[M+H]^+$ ($C_{22}H_{45}NO_4$ required 388.3427).

4.10. 1,4-Di-*O*-benzyl-2,3-*O*-isopropylidene-6-*O*-methanesulfonyl-α-L-sorbofuranose 8

To a solution of **3** (4.32 g, 10.8 mmol) in CH_2Cl_2 (32 mL) was added Et_3N (1.8 mL, 12.9 mmol) and MsCl (1 mL, 12.9 mmol). The mixture was then stirred at room temperature. After 4 h, the reaction mixture was washed with water (40 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The resulting crude product was purified by silica gel chromatography (PE/AcOEt 3:1) to provide **8** (4.87 g, 94%) as a colourless oil. $[\alpha]_D^{20} = +10.0$ (*c* 1.2, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): δ 1.42 (s, 3H), 1.51 (s, 3H), 2.95 (s, 3H), 3.62 (d, 1H, $J = 11.0$ Hz), 3.74 (d, 1H, $J = 11.0$ Hz), 4.01 (d, 1H, $J = 3.2$ Hz), 4.35–4.41 (m, 3H), 4.51–4.58 (m, 3H), 4.62–4.70 (m, 2H), 7.22–7.30 (m, 10H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 26.6, 27.6, 37.5, 67.8, 70.0, 71.9, 73.7, 78.5, 81.4, 81.5, 112.7, 114.3, 127.6, 127.7, 127.8, 128.2, 128.4, 128.6, 136.9, 138.0; MS-IS *m/z*: 496.5 $[M+NH_4]^+$.

4.11. 3,6-Di-*O*-benzyl-*N*-butyl-1,5-dideoxy-1,5-imino-D-glucitol 9a

A solution of **8** (4.7 g, 9.82 mmol) in *n*-butylamine (50 mL, 506 mmol) was stirred overnight (16 h) at 85 °C. The reaction mixture was concentrated in vacuo taken up in AcOEt (150 mL) and the solution washed with water (100 mL) and saturated aqueous NaCl (100 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The resulting product was directly used in the next step since partial decomposition of the intermediate amine occurred on silica gel. A ~0.15 M solution of the aminosorbofuranose obtained (4.3 g, 9.5 mmol) in trifluoroacetic acid was prepared at 0 °C; H_2O was then added to form a 9:1 (v/v) TFA/H_2O mixture. The reaction mixture was warmed up to room temperature and stirred for 28 h. The solvents were removed by co-evaporation with toluene and the residual product taken up in MeOH to form a ~0.05 M solution. Acetic acid (0.55 mL, 9.6 mmol) and $NaBH_3CN$ (2.45 g, 39.1 mmol) were then added at 0 °C. The reaction mixture was stirred for 48 h at room temperature. The mixture was then concentrated under reduced pressure. The crude product was taken up in CH_2Cl_2 (500 mL) and the solution was washed with saturated aqueous $NaHCO_3$ (2 × 250 mL) then with water (250 mL). The organic layer was dried

over $MgSO_4$ and concentrated under reduced pressure. The resulting crude product was purified by silica gel chromatography (PE/AcOEt 1:4) to provide **9a** (2.23 g, 52%) as a yellowish solid. $[\alpha]_D^{20} = -8.0$ (*c* 3.3, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): δ 0.86 (t, 3H, $J = 7.2$ Hz), 1.20 (m, 2H), 1.36 (m, 2H), 2.24 (t, 1H, $J = 10.3$ Hz), 2.37 (m, 1H), 2.50 (m, 1H), 2.65 (m, 1H), 3.00 (dd, 1H, $J = 4.7$, 11.3 Hz), 3.16 (t, 1H, $J = 8.5$ Hz), 3.60–3.80 (m, 4H), 4.53 (s, 2H), 4.79 (d, 1H, $J = 11.6$ Hz), 4.88 (d, 1H, $J = 11.6$ Hz), 7.26–7.38 (m, 10H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 14.1, 20.7, 26.6, 52.5, 56.1, 63.8, 67.7, 69.6, 72.1, 73.6, 74.5, 87.2, 128.0, 128.1, 128.5, 128.8, 137.9, 138.9; HRMS-ESI *m/z* 400.2480 $[M+H]^+$ ($C_{24}H_{34}NO_4$ required 400.2488), *m/z* 422.2293 $[M+Na]^+$ ($C_{24}H_{33}NO_4Na$ required 422.2307).

4.12. 3,6-Di-*O*-benzyl-*N*-octyl-1,5-dideoxy-1,5-imino-D-glucitol 9b

The *n*-octyl analogue of **9a**, compound **9b**, was obtained from **8** (2.7 g, 5.64 mmol) by the same sequence of reaction as described above, *n*-butylamine being replaced by *n*-octylamine (20 mL, 121 mmol), with the difference that octylamine could only be partially removed from the intermediate aminosorbofuranose by co-evaporation with toluene. The reductive amination process was performed in the presence of residual octylamine, and the resulting product was purified by silica gel chromatography (PE/AcOEt 2:1–1:1) to provide **9b** (1.38 g, 54%) as a yellowish solid. $[\alpha]_D^{20} = -4.5$ (*c* 1.4, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): δ 0.88 (t, 3H, $J = 6.9$ Hz), 1.23–1.39 (m, 12H), 2.24 (t, 1H, $J = 10.3$ Hz), 2.37 (m, 1H), 2.49 (m, 1H), 2.63 (m, 1H), 3.01 (dd, 1H, $J = 4.4$, 11.0 Hz), 3.17 (t, 1H, $J = 8.5$ Hz), 3.59–3.77 (m, 4H), 4.53 (s, 2H), 4.79 (d, 1H, $J = 11.9$ Hz), 4.88 (d, 1H, $J = 11.6$ Hz), 7.25–7.37 (m, 10H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 14.2, 22.8, 24.5, 27.6, 29.4, 29.6, 31.9, 52.8, 56.1, 63.8, 67.8, 69.7, 72.2, 73.7, 74.6, 87.2, 128.0, 128.1, 128.5, 128.8, 137.9, 138.9; HRMS-ESI *m/z* 456.3111 $[M+H]^+$ ($C_{28}H_{42}NO_4$ required 456.3114), *m/z* 478.2937 $[M+Na]^+$ ($C_{28}H_{41}NO_4Na$ required 478.2933).

4.13. Preparation of protected di- and tri-butyl iminosugars 10a, 11a and 12a

To a solution of **9a** (3 g, 7.5 mmol) in THF (135 mL) at 0 °C was added slowly NaH (60% dispersion in oil, 1.85 g, 46.2 mmol). After 30 min at room temperature, 1-iodobutane (6 mL, 52.7 mmol) and tetrabutylammonium iodide (0.59 g, 1.59 mmol) were added and the reaction mixture heated under reflux for 3 days. The reaction was quenched by the slow addition of MeOH at 0 °C and the mixture concentrated in vacuo. The crude mixture was taken up in CH_2Cl_2 (300 mL) and the solution washed with water (2 × 150 mL) and saturated aqueous NaCl (150 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The resulting products were separated by silica gel chromatography (PE/AcOEt 5:1–1:1) to provide in the following order of elution: **12a** (1.25 g, 33%), **10a** (1.16 g, 34%) and **11a** (0.91 g, 27%) as slightly yellow oils.

4.13.1. 3,6-Di-*O*-benzyl-*N*-butyl-2-*O*-butyl-1,5-dideoxy-1,5-imino-D-glucitol 10a.

R_f 0.3 (PE/AcOEt 4:1); $[\alpha]_D^{20} = -15.0$ (*c* 0.9, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): δ 0.89 (m, 6H), 1.15–1.28 (m, 2H), 1.33–1.42 (m, 4H), 1.50–1.61 (m, 2H), 2.20 (t, 1H, $J = 10.7$ Hz), 2.31 (m, 1H), 2.56–2.74 (m, 3H), 3.09 (dd, 1H, $J = 4.4$, 11.3 Hz), 3.20 (t, 1H, $J = 8.8$ Hz), 3.43–3.69 (m, 6H), 4.52 (s, 2H), 4.69 (d, 1H, $J = 11.6$ Hz), 4.97 (d, 1H, $J = 11.6$ Hz), 7.27–7.36 (m, 10H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 14.0, 14.1, 19.4, 20.7, 26.2, 32.5, 52.5, 54.1, 63.5, 66.7, 70.1, 70.5, 73.5, 74.6, 78.6, 85.7, 127.7, 127.8, 128.0, 128.1, 128.4, 128.6, 138.2, 139.1; HRMS-ESI *m/z* 456.3114 $[M+H]^+$ ($C_{28}H_{42}NO_4$ required 456.3114), *m/z* 478.2931 $[M+Na]^+$ ($C_{28}H_{41}NO_4Na$ required 478.2933).

4.13.2. 3,6-Di-*O*-benzyl-*N*-butyl-4-*O*-butyl-1,5-dideoxy-1,5-imino-D-glucitol 11a.

R_f 0.4 (PE/AcOEt 1:1); $[\alpha]_D^{20} = +18.0$ (*c* 1.1, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): δ 0.88 (m, 6H), 1.21–1.50 (m, 8H), 2.23 (t, 1H, $J = 10.9$ Hz), 2.39 (m, 1H), 2.48–2.71 (m, 3H), 3.00 (dd, 1H, $J = 4.4$, 11.3 Hz), 3.24 (t, 1H, $J = 8.1$ Hz), 3.37–3.46 (m, 2H), 3.55–3.77 (m, 4H), 4.52 (s, 2H), 4.66 (d, 1H, $J = 11.3$ Hz), 4.86 (d, 1H, $J = 11.6$ Hz), 7.29–7.34 (m, 10H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 14.1, 14.2, 19.6, 20.7, 26.8, 32.6, 52.5, 54.9, 63.6, 65.2, 69.3, 72.5, 73.6, 74.5, 78.5, 86.2, 127.8, 127.9, 128.4, 128.7, 138.0, 138.9; HRMS-ESI *m/z* 456.3104 $[M+H]^+$ ($C_{28}H_{42}NO_4$ required 456.3114), *m/z* 478.2924 $[M+Na]^+$ ($C_{28}H_{41}NO_4Na$ required 478.2933).

4.13.3. 3,6-Di-*O*-benzyl-*N*-butyl-2,4-di-*O*-butyl-1,5-dideoxy-1,5-imino-D-glucitol 12a.

R_f 0.75 (PE/AcOEt 4:1); $[\alpha]_D^{20} = +2.0$ (*c* 2.4, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): δ 0.88 (m, 9H), 1.19–1.57 (m, 12H), 2.14 (t, 1H, $J = 10.9$ Hz), 2.20 (m, 1H), 2.52–2.70 (m, 2H), 3.06 (dd, 1H, $J = 4.7$, 11.0 Hz), 3.22–3.38 (m, 3H), 3.39–3.47 (m, 1H), 3.51–3.60 (m, 4H), 3.76–3.85 (m, 1H), 4.52 (s, 2H), 4.74 (d, 1H, $J = 11.3$ Hz), 4.89 (d, 1H, $J = 11.0$ Hz), 7.25–7.35 (m, 10H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 14.0, 14.1, 14.2, 19.4, 19.5, 20.8, 25.7, 32.5, 32.7, 52.3, 54.6, 63.9, 65.5, 70.6, 73.2, 73.5, 75.2, 78.6, 78.9, 87.3, 127.4, 127.8, 127.9, 128.3, 128.4, 128.5, 138.0, 139.4; HRMS-ESI *m/z* 512.3740 $[M+H]^+$ ($C_{32}H_{50}NO_4$ required 512.3740).

4.14. Preparation of protected di- and tri-octyl iminosugars 10b, 11b and 12b

Compound **9b** (1.72 g, 3.78 mmol) was treated as described for **9a**. The resulting products were separated by silica gel chromatography (PE/AcOEt 8:1–6:1) to provide in the following order of elution: **12b** (1.40 g, 55%), **10b** (0.48 g, 23%) and **11b** (0.32 g, 15%) as yellowish oils.

4.14.1. 3,6-Di-*O*-benzyl-*N*-octyl-2-*O*-octyl-1,5-dideoxy-1,5-imino-D-glucitol 10b.

R_f 0.2 (PE/AcOEt 6:1); $[\alpha]_D^{20} = -8.5$ (*c* 0.6, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): δ 0.87 (m, 6H), 1.15–1.39 (m, 22H), 1.51–1.59

(m, 2H), 2.21 (t, 1H, J = 10.7 Hz), 2.31 (m, 1H), 2.53–2.68 (m, 3H), 3.09 (dd, 1H, J = 4.4, 11.3 Hz), 3.20 (t, 1H, J = 8.7 Hz), 3.43–3.69 (m, 6H), 4.53 (s, 2H), 4.69 (d, 1H, J = 11.6 Hz), 4.97 (d, 1H, J = 11.6 Hz), 7.25–7.33 (m, 10H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 14.2, 22.8, 24.1, 26.3, 27.6, 29.3, 29.4, 29.5, 29.6, 30.4, 31.9, 52.8, 54.1, 63.6, 66.7, 70.5, 70.56, 73.6, 74.7, 78.6, 85.7, 127.7, 127.8, 128.0, 128.1, 128.4, 128.6, 138.2, 139.1; MS-IS m/z 568.5 $[M+H]^+$.

4.14.2. 3,6-Di-*O*-benzyl-*N*-octyl-4-*O*-octyl-1,5-dideoxy-1,5-imino-D-glucitol 11b.

R_f 0.1 (PE/AcOEt 6:1); $[\alpha]_D^{20} = +12.0$ (c 1.1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.88 (t, 6H, J = 6.9 Hz), 1.25–1.51 (m, 24H), 2.23 (dd, 1H, J = 9.4, 11.0 Hz), 2.39 (m, 1H), 2.47–2.67 (m, 2H), 3.01 (dd, 1H, J = 4.4, 11.1 Hz), 3.24 (t, 1H, J = 8.2 Hz), 3.35–3.46 (m, 2H), 3.53–3.73 (m, 4H), 4.52 (s, 2H), 4.66 (d, 1H, J = 11.6 Hz), 4.87 (d, 1H, J = 11.6 Hz), 7.25–7.34 (m, 10H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 14.2, 22.8, 24.6, 26.4, 27.6, 29.4, 29.6, 29.7, 30.5, 31.9, 52.8, 54.9, 63.6, 65.2, 69.4, 72.8, 73.6, 74.5, 78.6, 86.2, 127.8, 127.9, 128.4, 128.7, 138.1, 138.9; MS-IS m/z 568.5 $[M+H]^+$.

4.14.3. 3,6-Di-*O*-benzyl-*N*-octyl-2,4-di-*O*-octyl-1,5-dideoxy-1,5-imino-D-glucitol 12b.

R_f 0.6 (PE/AcOEt 7:1); $[\alpha]_D^{20} = +4.0$ (c 0.8, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.88 (m, 9H), 1.24–1.57 (m, 36H), 2.13 (t, 1H, J = 10.7 Hz), 2.20 (m, 1H), 2.50–2.73 (m, 2H), 3.05 (dd, 1H, J = 4.7, 11.3 Hz), 3.25 (t, 1H, J = 9.1 Hz), 3.27–3.37 (m, 2H), 3.39–3.46 (m, 1H), 3.51–3.63 (m, 4H), 3.75–3.84 (m, 1H), 4.52 (s, 2H), 4.74 (d, 1H, J = 11.0 Hz), 4.88 (d, 1H, J = 11.3 Hz), 7.25–7.38 (m, 10H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 14.2, 22.8, 23.6, 26.3, 26.4, 27.7, 29.3, 29.4, 29.6, 29.7, 30.5, 30.6, 31.9, 32.0, 52.6, 54.7, 63.9, 65.6, 71.0, 73.6, 75.2, 78.7, 78.9, 87.3, 127.4, 127.8, 127.9, 128.3, 128.4, 128.6, 138.1, 139.4; MS-IS m/z: 681.0 $[M+H]^+$.

4.15. General procedure A for the synthesis of *N*-alkyl-1,5-dideoxy-1,5-imino-D-glucitol derivatives 13, 14 and 15

To a ~0.04 M (butyl chains) or ~0.02 M (octyl chains) solution of precursor **10**, **11** or **12** in a 10:1 (v/v) MeOH/HCl 5 M mixture was added 10% Pd/C (~0.2 equiv). The flask was purged three times with Ar then filled with H_2. The reaction mixture was stirred at room temperature. After 48 h (butyl chains) or 70 h (octyl chains), the solids were removed by filtration and the filtrate concentrated under reduced pressure. The resulting crude product was purified by silica gel chromatography.

4.16. *N*-Butyl-2-*O*-butyl-1,5-dideoxy-1,5-imino-D-glucitol 13a

Compound **10a** (284 mg, 0.624 mmol) was submitted to general procedure A, which provided **13a** (140 mg, 81%) as a colourless oil after purification by silica gel chromatography (AcOEt/MeOH 5:1). $[\alpha]_D^{20} = +12.0$ (c 0.8, MeOH); ^{1}H NMR (250 MHz, CD_3OD): δ 0.92 (m, 6H), 1.23–1.60 (m, 8H), 2.12–2.18 (m, 2H), 2.66 (m, 1H), 2.80 (m, 1H), 3.12 (dd, 1H, J = 4.4, 11.2 Hz), 3.22 (m, 2H), 3.37 (t, 1H, J = 8.8 Hz), 3.59 (t, 2H, J = 6.8 Hz), 3.84 (d, 2H, J = 2.2 Hz); ^{13}C NMR (62.9 MHz, CD_3OD): δ 14.2, 14.3, 20.2, 21.6, 27.0, 33.3, 53.5, 54.9, 58.9, 66.9, 71.4, 71.7, 78.7, 79.3; HRMS-ESI m/z 276.2172 $[M+H]^+$ ($C_{14}H_{30}NO_4$ required 276.2175), m/z 298.1998 $[M+Na]^+$ ($C_{14}H_{29}NO_4Na$ required 298.1994).

4.17. *N*-Butyl-4-*O*-butyl-1,5-dideoxy-1,5-imino-D-glucitol 14a

Compound **11a** (232 mg, 0.51 mmol) was submitted to general procedure A, which provided **14a** (134.5 mg, 96%) as a colourless oil after purification by silica gel chromatography (AcOEt/MeOH 5:1). $[\alpha]_D^{20} = -4.5$ (c 0.9, MeOH); ^{1}H NMR (250 MHz, CD_3OD): δ 0.89 (m, 6H), 1.24–1.40 (m, 4H), 1.41–1.57 (m, 4H), 2.27 (m, 2H), 2.67 (m, 1H), 2.87 (m, 1H), 3.03 (dd, 1H, J = 4.7, 11.3 Hz), 3.22 (m, 2H), 3.46 (m, 1H), 3.58 (m, 1H), 3.73 (dd, 1H, J = 1.9, ~11.9 Hz), 3.82 (overlapping signals, m, 1H), 3.86 (m, 1H); ^{13}C NMR (62.9 MHz, CD_3OD): δ 14.2, 14.3, 20.3, 21.5, 27.0, 33.5, 53.4, 56.7, 57.7, 66.6, 70.2, 73.9, 79.1, 80.1; HRMS-ESI m/z 276.2174 $[M+H]^+$ ($C_{14}H_{30}NO_4$ required 276.2175).

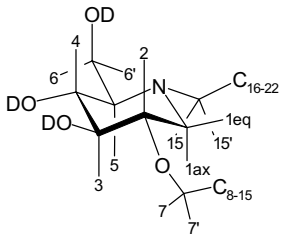
4.18. *N*-Butyl-2,4-di-*O*-butyl-1,5-dideoxy-1,5-imino-D-glucitol 15a

Compound **12a** (292 mg, 0.57 mmol) was submitted to general procedure A, which provided **15a** (111 mg, 59%) as a yellowish solid after purification by silica gel chromatography (AcOEt/MeOH 9:1). $[\alpha]_D^{20} = +15.0$ (c 1.1, MeOH); ^{1}H NMR (250 MHz, CD_3OD): δ 0.96 (m, 9H), 1.29–1.64 (m, 12H), 2.04–2.12 (m, 2H), 2.63 (m, 1H), 2.81 (m, 1H), 3.10 (dd, 1H, J = 4.1, 10.7 Hz), 3.17–3.35 (m, 3H), 3.63 (t, 3H, J = 6.6 Hz), 3.76 (dd, 1H, J = 1.9, 11.6 Hz), 3.86 (dd, 1H, J = 1.9, 11.9 Hz), 3.95 (m, 1H); ^{13}C NMR (62.9 MHz, CD_3OD): δ 14.3, 14.4, 20.2, 20.3, 21.7, 27.1, 33.4, 33.6, 53.4, 55.0, 58.5, 66.4, 71.5, 73.9, 79.4, 79.8, 79.9; HRMS-ESI m/z 332.2797 $[M+H]^+$ ($C_{18}H_{38}NO_4$ required 332.2801), m/z 354.2609 $[M+Na]^+$ ($C_{18}H_{37}NO_4Na$ required 354.2620).

4.19. *N*-Octyl-2-*O*-octyl-1,5-dideoxy-1,5-imino-D-glucitol 13b

Compound **10b** (64 mg, 0.112 mmol) was submitted to general procedure A, which provided **13b** (36 mg, 83%) as a white solid after purification by silica gel chromatography (AcOEt/MeOH/NH_4OH 10:2:1.5). $[\alpha]_D^{20} = +5.5$ (c 0.6, MeOH); ^{1}H NMR see Table 1; ^{13}C NMR (62.9 MHz, CD_3OD): δ 14.4, 23.7, 25.0, 27.1, 28.6, 30.4, 30.5, 30.6, 31.2, 32.9, 33.0, 53.8, 55.1, 59.3, 67.1, 71.7, 72.0, 79.1, 79.5; HRMS-ESI m/z 388.3425 $[M+H]^+$ ($C_{22}H_{46}NO_4$ required 388.3427).

Table 1. NMR assignments for **13b**[a]



Position[b]	13b	
	δ (ppm)[c]	$^3J_{HH}$ (Hz)
1_{eq}	3.001	−11.0/4.3
1_{ax}	2.002	−11.0/9.9
2	3.117	4.3/9.9/8.9
3	3.085	8.9/9.0
4	3.258	9.0/9.3
5	2.002	9.3/2.6/2.6
6	3.748	−12.0/2.6
6′	3.734	−12.0/2.6
7	3.52	o/l[d]
7′	3.49	o/l
8	1.476	o/l
9–13	1.22	o/l
14	0.805	7.1
15	2.692	13.5/9.0/7.3
15′	2.494	13.5/8.9/6.5
16	1.388	o/l
17–21	1.22	o/l
22	0.805	7.1

[a] ^{1}H NMR spectra were recorded in CD_3OD at 30 °C (500 MHz).
[b] C1–C6 is DNJ; C7–C14 is the octyl group attached to oxygen; C15–C22 is the octyl group attached to the ring nitrogen.
[c] Chemical shifts are measured relative to the methyl peak of trimethylsilylpropanesulfonic acid at 0.0 ppm.
[d] o/l indicates difficulty in assignment due to overlapping peaks.

4.20. *N*-Octyl-4-*O*-octyl-1,5-dideoxy-1,5-imino-D-glucitol 14b

Compound **11b** (49 mg, 0.086 mmol) was submitted to general procedure A, which provided **14b** (24 mg, 72%) as a yellowish solid after purification by silica gel chromatography (AcOEt/MeOH/NH_4OH 10:2:1.5). $[\alpha]_D^{20} = -3.5$ (*c* 0.4, MeOH); ^{1}H NMR, see Table 2; ^{13}C NMR (62.9 MHz, CD_3OD): δ 14.4, 23.7, 25.1, 27.3, 28.6, 30.4, 30.6, 30.7, 31.4, 33.0, 53.6, 57.5, 58.4, 66.7, 70.9, 74.2, 79.9, 80.7; HRMS-ESI *m*/*z* 388.3427 $[M+H]^+$ ($C_{22}H_{46}NO_4$ required 388.3427).

4.21. *N*-Octyl-2,4-di-*O*-octyl-1,5-dideoxy-1,5-imino-D-glucitol 15b

Compound **12b** (62 mg, 0.0908 mmol) was submitted to general procedure A, which provided **15b** (25 mg, 55%) as a colourless oil after purification by silica gel chromatography (PE/AcOEt/NH_4OH 4:1:0.5). $[\alpha]_D^{20} = +7.5$ (*c* 0.6, MeOH); ^{1}H NMR (250 MHz, CD_3OD): δ 0.90 (m, 9H), 1.31–1.59 (m, 36H), 2.01–2.09 (m, 2H), 2.61 (m, 1H), 2.76 (m, 1H), 3.07 (dd, 1H, *J* = 4.1, 10.7 Hz), 3.15–3.26 (m, 3H), 3.60 (t, 3H, *J* = 6.6 Hz), 3.74 (dd, 1H, *J* = 1.9, 11.6 Hz), 3.84 (dd, 1H, *J* = 1.9, 11.9 Hz), 3.91 (m, 1H); ^{13}C NMR (62.9 MHz, CD_3OD): δ 14.5, 23.7, 24.9, 27.2, 27.3, 28.7, 30.4, 30.5, 30.6, 30.7, 31.2, 31.5, 33.0, 53.7, 55.1, 58.5, 66.5, 71.8, 74.3, 79.4, 79.9, 80.0; HRMS-ESI *m*/*z* 500.4678 $[M+H]^+$ ($C_{30}H_{62}NO_4$ required 500.4679), *m*/*z* 522.4504 $[M+Na]^+$ ($C_{30}H_{61}NO_4$-Na required 522.4498).

Table 2. NMR assignments for **14b** (see footnotes to Table 1)

Position	14b	
	δ (ppm)	$^3J_{HH}$ (Hz)
1_{eq}	2.881	−11.2/5.0
1_{ax}	2.042	−11.2/10.4
2	3.355	10.4/5.0/8.6
3	3.127	8.6/8.9
4	3.098	8.9/8.9
5	2.022	8.9/2.3/2.4
6	3.744	−11.8/2.3
6′	3.646	−11.8/2.4
7	3.810	8.9/6.5
7′	3.514	8.9/7.0
8	1.494	o/l
9–13	1.22	o/l
14	0.803	7.0
15	2.689	13.4/8.1
15′	2.478	13.4/7.6
16	1.382	o/l
17–21	1.22	o/l
22	0.803	7.0

4.22. Conformational analysis and modelization

Samples for NMR analysis were prepared by dissolving approximately 6 mg of solid compound in CD_3OD. One and two-dimensional ^{1}H NMR spectra were run at 500 MHz on a Varian Unity Inova spectrometer and an additional two-dimensional NOESY spectrum at 750 MHz on a home-built/GE Omega spectrometer. All spectra were recorded at 30 °C. The mixing time used for the 2D NOESY spectra was 200 ms. Molecular modeling was performed on a Silicon Graphics Fuel workstation, using the programs Insight II and Discover (Accelrys Inc., San Diego, USA). Accurate $^3J_{HH}$ coupling constants were obtained by fitting a simulated 1D ^{1}H NMR spectrum to the experimental spectrum using the program gNMR (Cherwell Scientific, Oxford, UK). Figures were produced using the program Molscript.[16] The X-ray crystal structure of glucosylceramide[17] (Fig. 1) was obtained from the Cambridge Crystallographic Database[18] at the Chemical Database Service at Daresbury.[19]

4.23. Biochemical assays

Biochemical assays were conducted according to the procedure previously described.[7]

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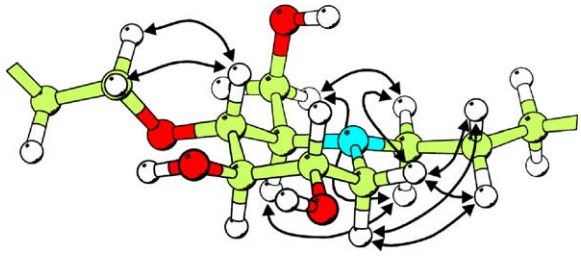
14. Main NOE interactions observed in **14b**:



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