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PAPER

Selection of the biological activity of DNJ neoglycoconjugates through click length variation of the side chain†

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A series of neoglycoconjugates derived from deoxynojirimycin has been prepared by click connection with functionalised adamantanes. They have been assayed as glycosidase inhibitors, as inhibitors of the glycoenzymes relevant to the treatment of Gaucher disease, as well as correctors of the defective ion-transport protein involved in cystic fibrosis. We have demonstrated that it is possible to selectively either strongly inhibit ER-α-glucosidases and ceramide glucosyltransferase or restore the activity of CFTR in CF-KM4 cells by varying the length of the alkyl chain linking DNJ and adamantane.

Introduction

Iminosugars, sugar mimics in which the endocyclic oxygen has been replaced by nitrogen, represent the most promising class of sugar analogues as therapeutic agents.[1] Since the discovery of their most famous representative, deoxynojirimycin (DNJ) **1** (Fig. 1), a vast array of derivatives, in which the nature and stereochemistry of the substituents on the piperidine ring has been modified extensively, has been synthesized and assayed on numerous pathologies.[2] Promising results have been obtained in the field of cancer[3] and viral infections[4] including HIV,[5] but more significantly, a *N*-hydroxyethyl analogue, miglitol, was approved by the FDA in 1996 for the treatment of late onset diabetes (marketed as Glyset®).[6] Additionally, a *N*-butyl derivative, NB-DNJ or miglustat **2**,[7] marketed as Zavesca®, (Fig. 1) has been launched for the treatment of Gaucher disease, a rare lysosomal disorder. The same compound is in phase II trials for the treatment of cystic fibrosis.[8]

Gaucher disease,[9] the most prevalent lysosomal storage disorder, is caused by mutations in the gene encoding for glucosylceramidase (or β-glucocerebrosidase, EC 3.2.1.45).[10] This enzyme is responsible for the cleavage of the glucosylceramide sphingolipid into glucose and ceramide. Its deficiency leads to the accumulation of glucosylceramide in the lysosome causing severe symptoms. Additionally, glucosylceramide is the precursor of complex glycosphingolipids involved in key human physiological processes such as cell–cell and cell–pathogen recognition.[11] To date, two therapies that reduce glucosylceramide storage have been approved for the treatment of Gaucher disease. Enzyme replacement therapy (ERT) is based on the administration of Cerezyme®,[12] a recombinant form of glucosylceramidase, to supplement the defective enzyme (Fig. 1). Its main drawbacks are its cost (over 150 000 $/year), as well as its limited use to non-neuronopathic patients as the enzyme is unable to cross the blood–brain barrier. The second approach, substrate reduction therapy (SRT),[13] is based on the use of a small molecule able to inhibit the biosynthesis of glucosylceramide through inactivation of a ceramide glucosyltransferase, glucosylceramide synthase (GCS), which condenses UDP-glucose and ceramide. Its major drawbacks are its limited use to patients with mild-to-moderate type 1 Gaucher's disease and its side effects related to its broad-spectrum inhibition, especially to digestive glucosidases. More recently, a third approach, the active-site-specific chaperone (ASSC) therapy,[14] has received increased attention. It is based on the use of small molecules that partially stabilise the defective and misfolded glucosylceramidase, thus avoiding its degradation in the endoplasmic reticulum by the quality control mechanism of the cell and allowing its normal trafficking to the lysosome. Administration of sub-inhibitory concentrations of potent and competitive glucosylceramidase inhibitors including iminosugars and aminocyclitols has demonstrated a chaperone-like profile for these molecules.[15]

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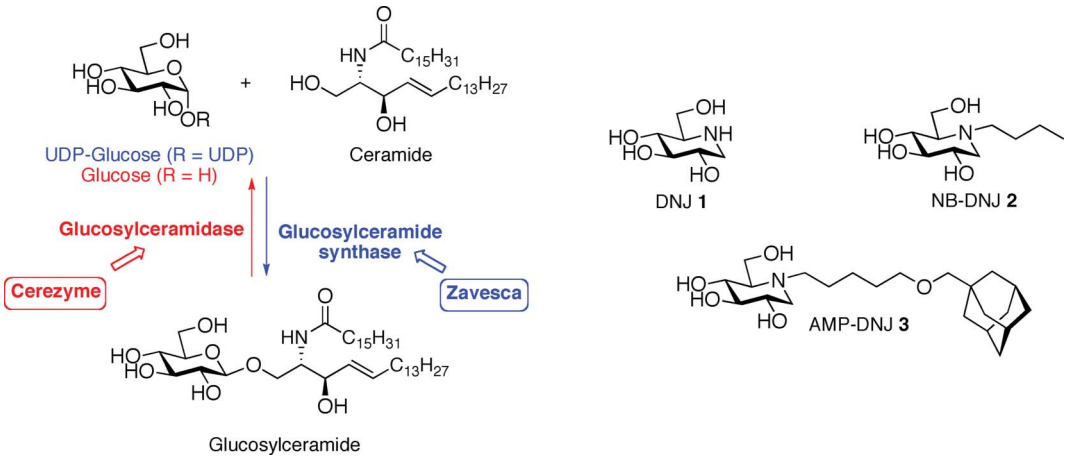

Fig. 1 Glucosyl ceramide biosynthesis and catabolism, and structure of DNJ and potent glucosylceramide synthase inhibitors.

Since SRT is a relatively general and affordable approach, glucosylceramide synthase is becoming a very attractive therapeutic target for an increasing number of pathological processes, which are being correlated with excessive glycosphingolipid levels. Therefore more potent and selective GCS inhibitors are needed and NB-DNJ is a good starting point for the design of improved structures. Counter-intuitively, it has been shown at the molecular level that NB-DNJ is a ceramide mimic rather than a glucose surrogate, molecular modelling showing good superimposition between the ceramide and NB-DNJ.[16] As a consequence, *C*, *N* or *O*-alkylation of DNJ with alkyl chains of various length has been explored to yield lipophilic 1-deoxynojirimycin-based inhibitors as ceramide surrogates which, when *C* or *O*-alkylated, were less efficient than reference NB-DNJ. This indicated that *N*-substitution seems to be the best DNJ modification to obtain potent glucosylceramide synthase inhibitors.[17,18] Amongst the great number of *N*-substituted DNJ analogues synthesized, Overkleeft demonstrated that introduction of an adamantane moiety capping of the *N*-alkyl chain significantly increased the potency of the inhibitor, and AMP-DNJ **3** (*N*-[5-(adamantan-1-yl-methoxy)-pentyl]-1-deoxynojirimycin, Fig. 1) proved to be 100-fold more potent than NB-DNJ.[19] From these results it is clear that the DNJ and adamantane moieties are amongst key motifs for strong inactivation.

Cystic fibrosis (CF) is an autosomal recessive disorder, which is caused by mutations in the gene coding for the cystic fibrosis transmembrane conductance regulator (CFTR). CFTR is an ABC transporter-class protein and ion channel that transports chloride anions across the apical membrane of epithelial cells. Mutations of the *CFTR* gene affect folding and/or functioning of the chloride channels in these cell membranes, causing CF. The most common CF mutation, F508del, observed in more than 90% of patients, causes misfolding of the protein, intracellular retention by the endoplasmic reticulum quality control and premature degradation.[20] It has been shown that NB-DNJ **2** is a partial corrector of F508del-CFTR acting as a pharmacological chaperone,[21] and that incubation with NB-DNJ **2** partially rescues the defective F508del-CFTR function.[8,21] Since, other iminosugars have shown similar potency.[22] The hypothesis to explain the mechanism of action of NB-DNJ **2** is related to its α-glucosidase inhibition, which disturbs the ER quality control, more specifically the interaction between the ER lectin calnexin and F508del-CFTR. This perturbation might allow the restoration of F508del-CFTR to the plasma membrane. However, whether the glucosidase inhibition of NB-DNJ is associated with the chaperone activity has yet to be established. A host of chaperones, enzymes and regulatory proteins control the folding, complex assembly and ultimately exit of secretory proteins, which can be viewed as the clients of this ER machinery and as potential corrector targets.[23]

As part of our ongoing program devoted to the development of new lipophilic DNJ analogues as glucosylceramide synthase inhibitors and F508del-CFTR correctors, we embarked on a click strategy starting from **3** as a lead compound to design more potent structures. Possible modifications of the structure of **3** can be as follows: (a) tuning of the length and nature of the spacer, (b) alteration of the hydrophobicity of the adamantyl-linker moiety, (c) modification of stereochemistry and substitution pattern of the iminosugar moiety, (d) variation of the attachment site of the adamantyl-spacer on the iminosugar core. As modifications (c) and (d) have already been explored and optimised,[18,24,25] we focused on the modification of the nature and the length of the tether. One efficient way to access a library of AMP-DNJ derivatives is to exploit the popular click azide–alkyne coupling methodology that has been used to connect a vast array of natural and unnatural patterns. Surprisingly, such an approach has only recently been exploited in the field of iminosugars.[26] This should allow the construction of AMP-DNJ analogues in which the key DNJ scaffold and the adamantane moiety are connected through a triazole core bearing alkyl chains of various length such as in analogues **A** and **B** (Fig. 2). The work reported herein describes the synthesis and biological evaluation of a small library of AMP-DNJ analogues in which the length of the tether and the position and orientation of the triazole moiety have been studied. Click[27] coupling conditions have also been optimised to furnish the iminosugar glycoconjugates in high yield.

Synthesis

We first investigated the direct *N*-alkylation of unprotected DNJ **1** synthesized using well established methodology.[28]

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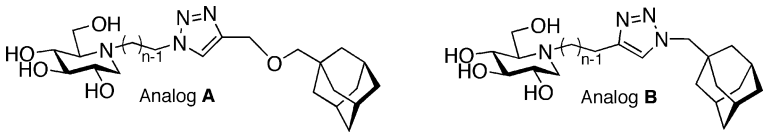


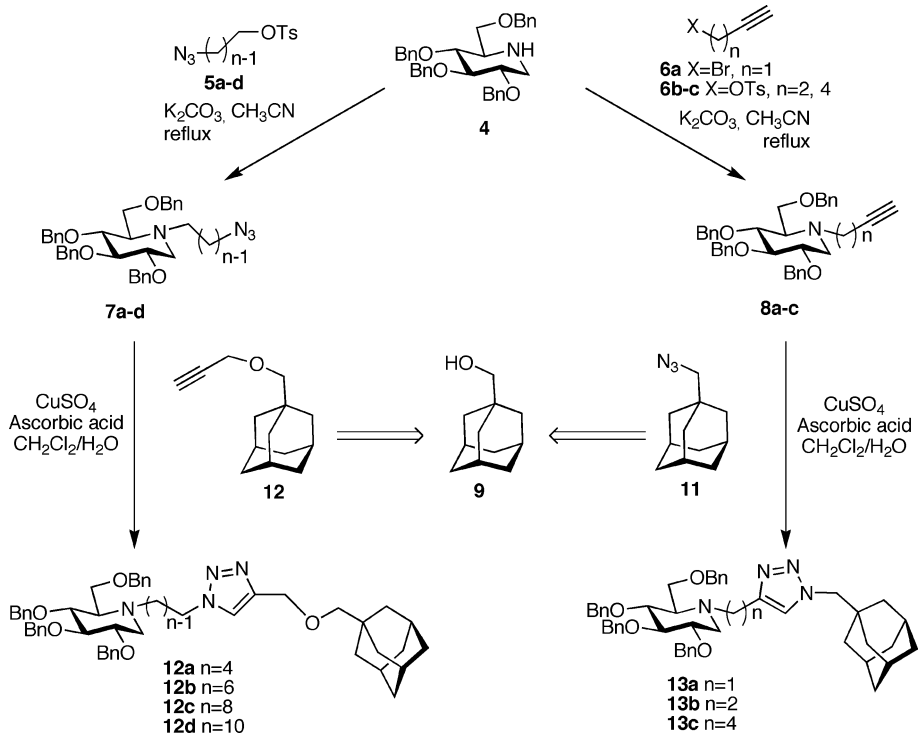
Fig. 2 General structure of the library of AMP-DNJ analogues of this study.

Disappointingly, *N*-alkylation through reductive amination with functionalised aldehydes or *via* nucleophilic displacement of alkyl tosylates or bromides[29] afforded the desired *N*-alkyl DNJ derivatives only in modest yields, especially for those with longer chains.[30] In contrast, *N*-alkylation of tetra-*O*-benzyl-deoxynojirimycin **4**, synthesized according to Overkleeft,[18] with alkyl moieties bearing an alkyne (propargyl bromide **6a** and longer chains **6b–c**) or an azido group (derivatives **5a–d**) was successful but required a fine tuning of the reaction parameters including temperature, solvent (acetonitrile, DMF) and base (NaH, Na_2CO_3, K_2CO_3) (see the ESI†). Reaction with alkyl tosylates in refluxing acetonitrile with potassium carbonate as base proved to be the best conditions to perform the *N*-alkylation and yielded the click precursors **7a–d** and **8a–c**. The copper-catalyzed azide–alkyne cycloaddition (CuAAC)[31] between these *N*-alkylated DNJ derivatives **7a–d** and **8a–c** and the adamantane partners **10**[32] and **11**,[33] obtained, respectively, through alkylation of alcohol **9** with propargyl bromide or tosylation of alcohol **9**, followed by displacement with azide, was then examined. Optimised click reaction conditions that were previously established in our laboratory regarding temperature (room temperature), substrate concentration (0.25 M), the amounts of copper sulfate (5%) and sodium ascorbate (15 mol%)[34] were used in a dichloromethane/water (1 : 1) solvent system. This mixture proved better than the extensively used aqueous alcohol systems (see the ESI†).[35] Noteworthily, we observed that the reaction performed in a screw-cap sealed tube allowed optimum conversion as undesired reoxidation of copper(I) to copper(II) was otherwise probably taking place during the reaction. These conditions were applied to the efficient 1,3-cycloaddition of all complementary adamantane and DNJ partners (Scheme 1).

All the triazole glycoconjugates were obtained in good (86%) to excellent (100%) yields independently of the chain length of the coupling partner. The DNJ-adamantane conjugates **12a–d** and **13a–c** were then deprotected by hydrogenolysis (Pd/C, EtOH, 1 M HCl) to afford the target glycoconjugates **14–20** in almost quantitative yield (Fig. 3). In summary, the whole coupling process (*N*-alkylation, 1,3-cycloaddition, deprotection) starting from protected DNJ **4** and modified adamantanes **10** and **11** is very efficient (yields ranging between 51% and 82% for the three steps) and validates the sequence, which could be applied to other key iminosugars that have demonstrated therapeutic potential.

Biological evaluation

The small library of DNJ-adamantane conjugates was submitted to a series of biological evaluations, which include glycoenzyme inhibition profiling, activity on the cellular enzymes relevant to the treatment of Gaucher disease, as well as correction of defective F508del-CFTR function. These experiments are summarised below.



Scheme 1 Synthesis of DNJ-adamantane conjugates.

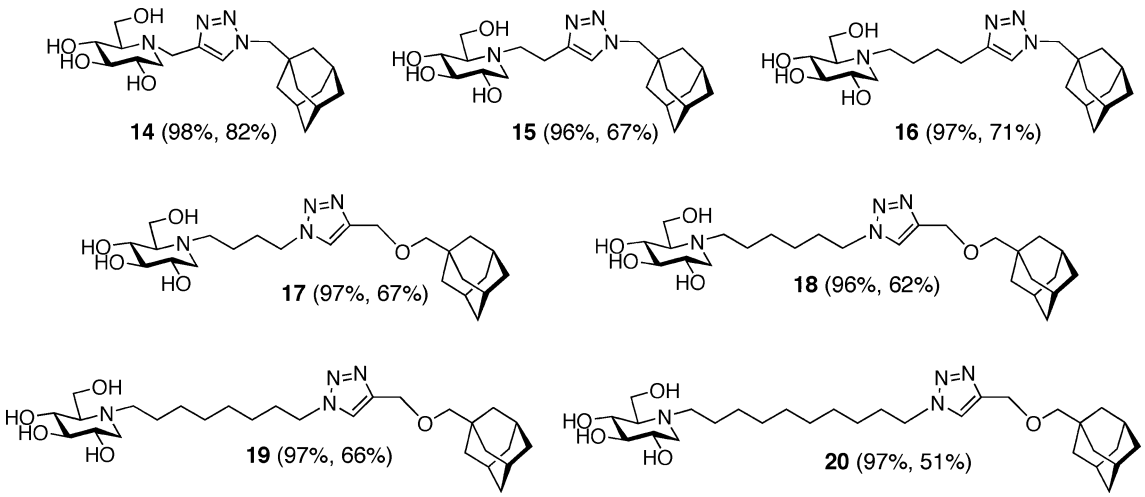


Fig. 3 Structure of the target adamantane-DNJ conjugates **14–20** synthesized (yields are indicated in brackets; left: debenzylation yield; right: overall yield, starting from tetra-*O*-benzyl-deoxynojirimycin **4**).

Glycoenzymes inhibition profiling

Compounds **14–20** were assayed as inhibitors of a collection of glycosidases, including glucosidases, galactosidases and mannosidases, in comparison with DNJ and miglitol (Table 1). They all display good inhibition towards α-glucosidases (from rice, rat intestinal maltase, rat intestinal sucrase), with a trend correlating higher inhibition with increased chain length. It is, however, interesting to note that the shorter compounds **14** and, to a lesser extent, **15** proved to be relatively poor inhibitors compared to DNJ and miglitol. The inhibition profile is notably different with respect to β-glucosidases. Whereas only compounds **17–20** inhibit almond β-glucosidase, with potencies similar to DNJ, compound **15** is the only derivative in our library showing higher activity than miglitol towards rat intestinal cellobiase. None of these derivatives showed inactivation of coffee bean α-galactosidase, but their behaviour towards β-galactosidases showed once again distinct trends depending on the specific enzyme: for bovine liver β-galactosidase, increased length of the tether led to increased inhibition, whereas a more complex picture could be observed for rat intestinal lactase. Finally, no inhibition of jack beans α-mannosidase or snail β-mannosidase was observed.

Cellular glycoenzymes inhibition

The compounds were used at the highest non-cytotoxic concentration, as determined by MTS[36] cell proliferation assay, for ER-α-glucosidase inhibition, using a free oligosaccharide (FOS) analysis, and glycosphingolipid biosynthesis (GSL) inhibition, following a 3 day incubation with compound in HL60 cells.

Table 1 Concentration of iminosugars **14–20** resulting in 50% inhibition of various glucosidases, in comparison with DNJ and miglitol

	IC_{50}/μM								
Enzyme	**14**	**15**	**16**	**17**	**18**	**19**	**20**	DNJ	miglitol
α-glucosidase									
rice	0.32	0.24	0.084	0.15	0.047	0.030	0.025	0.05	0.099
rat intestinal maltase	4.5	6.2	0.59	1.8	0.29	0.16	0.37	0.36	0.59
rat intestinal sucrase	13	7.3	1.2	2.6	0.91	0.87	3.4	16	1.0
β-glucosidase									
almond	NI[a]	NI	NI	128	62	73	118	85	378
rat intestinal cellobiase	NI	70	1000	549	152	541	NI	327	164
α-galactosidase									
coffee beans	NI	NI	NI	NI	NI	NI	NI	890	NI
β-galactosidase									
bovine liver	163	7.4	110	34	32	7.1	2.1	NI	183
rat intestinal lactase	NI	14	158	97	56	222	NI	49	41
α-mannosidase									
jack beans	NI	NI	NI	NI	NI	NI	NI	NI	NI
β-mannosidase									
snail	NI	NI	NI	NI	NI	NI	NI	NI	NI

[a] NI: No inhibition (less than 50% inhibition at 1000 μM).

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ER-α-glucosidase inhibition

Glucosidase I inhibition by NB-DNJ is a major concern regarding long term treatments as this cellular glycosidase is involved in the maturation of the glycan moiety of glycoproteins. Therefore compounds that can selectively restore F508del-CFTR activity without inhibiting α-1,2 glucosidase are highly needed.[37]

Compounds **14–18** were non-toxic at 100 μM over a 24 h incubation time. Compounds **19** (CC_{50} = 150 μM) and **20** (CC_{50} = 50 μM) were evaluated at their highest non-toxic concentrations, 50 and 5 μM, respectively.

FOS analysis in HL60 cells was used to evaluate levels of α-glucosidases I and II inhibition as determined by the amount of $Glc_3Man_5GlcNAc_1$ and $Glc_1Man_4GlcNAc_1$ oligosaccharide, respectively, produced in the cytosol as a result of endoplasmic reticulum-associated protein degradation (ERAD). These FOS species are the major tri- and mono-glucosylated FOS species produced in response to α-glucosidase I and II inhibition in the endoplasmic reticulum (ER), as a result of retrotranslocation *via* an ERAD pathway and the actions of PNGase and cytosolic α-mannosidase.[38] The FOS species $Glc_3Man_5GlcNAc_1$ eluted from the TSKgel amide-80 column at approximately 36.5 min with a glucose unit (GU) value of 8.26, consistent with our previously published data (see the ESI, Fig. S1A†).[38,39] The structure of all FOS species was confirmed by α-glucosidase digestion and mass spectrometry as reported[38] (results not shown).

In control untreated HL60 cells no $Glc_3Man_5GlcNAc_1$ was produced, and using 100 μM NB-DNJ treatment for 24 h significant tri-glucosylated FOS species was observed (Table 2). At 5 μM concentration AMP-DNJ was totally ineffective at inhibiting α-glucosidase I, whereas at 100 μM **18** provided the most inhibition (487 ± 21 pmol mg^{-1}, Table 2). Whilst **19** and **20** showed effective α-glucosidase I inhibition, lower concentrations (50 and 5 μM, respectively) had to be used due to cytotoxicity at 100 μM. To determine the relative inhibition of ER-glucosidases at the same concentration of compound, FOS analysis was repeated at a compound concentration of 5 μM and the change in the FOS profile evaluated as the result of α-glucosidase II inhibition and the appearance of $Glc_1Man_4GlcNAc_1$ (eluting at approximately 27.2 min, GU value 5.31, see the ESI, Fig. S1B†). At lower concentrations of inhibitor, α-glucosidase II activity is preferentially affected leading to the appearance of $Glc_1Man_4GlcNAc_1$ oligosaccharide.[38,39] NB-DNJ showed very little inhibition of α-glucosidase II at 5 μM when compared to inhibition by all glycoconjugates of this series except **14**. At the lower concentration of 5 μM, compounds **19** and **20** proved to be the most potent inhibitors of α-glucosidase II (Table 2).

Ceramide glucosyltransferase inhibition

To evaluate the cellular inhibition of ceramide glucosyltransferase, a key enzyme in the biosynthesis of glycosphingolipids,[16] the compounds were administered at non-toxic concentrations to HL60 cells for 3 days (see the ESI for the determination of cytotoxic concentrations over 3 day incubation times†). Following lipid extraction, enzymatic release of the oligosaccharide head group and fluorescence labelling, normal phase HPLC (NP-HPLC) was used to analyse the effects of inhibition on biosynthesis. HL60 cells have a simple repertoire of glycolipids and the dominant species is a mono-sialylated ganglioside, GM3.[40] Inhibition of ceramide glucosyltransferase by imino sugars results in the decrease in GM3 which was measured following HPLC separation (see the ESI, Fig. S2†). The amount of GM3 reduction as result of inhibition is shown in Fig. 4. At 5 μM, **19** and **20** were more inhibitory than AMP-DNJ, a potent inhibitor developed by Overkleeft and co-workers,[19] and similar to 100 μM concentrations of NB-DNJ. These compounds are therefore approximately 20 times more potent in cells than NB-DNJ.

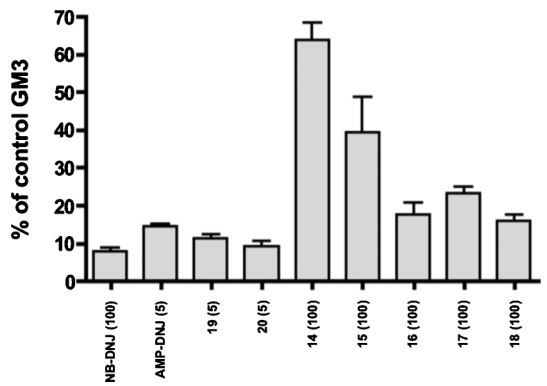

Fig. 4 Reduction in GM3 synthesis in HL60 cells following inhibitor treatment. Compounds were incubated at the highest non-cytotoxic concentration for 3 days with cells and the GSL-oligosaccharides separated by NP-HPLC following enzyme release and labelling, as described in the text.

Table 2 FOS concentrations in HL60 cells following treatment with inhibitor. Cells were treated with compounds at the concentrations shown for 24 h, FOS extracted, fluorescently labelled and separated by NP-HPLC. Peak areas corresponding to the oligosaccharide species shown (see the ESI, Fig. S1†), were measured and normalised to cell protein concentration. Experiments were performed in triplicate and standard deviations are shown

Inhibitor concentration	$[Glc_3Man_5GlcNAc_1]$/pmol mg^{-1}	Inhibitor concentration	$[Glc_1Man_4GlcNAc_1]$/pmol mg^{-1}
0.2% DMSO	0.0	0.2% DMSO	0.0
5 μM AMP-DNJ	0.0	5 μM AMP-DNJ	17.5 ± 4
100 μM NB-DNJ	172 ± 6	5 μM NB-DNJ	3.0 ± 0.5
100 μM **14**	24 ± 2	5 μM **14**	0.0
100 μM **15**	38 ± 4	5 μM **15**	5.8 ± 1
100 μM **16**	105 ± 5	5 μM **16**	133 ± 8
100 μM **17**	111 ± 8	5 μM **17**	172 ± 28
100 μM **18**	487 ± 21	5 μM **18**	358 ± 4
50 μM **19**	190 ± 13	5 μM **19**	578 ± 13
5 μM **20**	16 ± 2	5 μM **20**	476 ± 46

Table 3 Concentration of iminosugars **14–20** resulting in 50% inhibition of β-glucocerebrosidase activity, in comparison with NB-DNJ and AMP-DNJ

Compound	IC_{50}/μM
AMP-DNJ	5.06 ± 0.84
NB-DNJ	259.00 ± 22
14	2.11 ± 0.48
15	1.12 ± 0.15
16	0.60 ± 0.11
17	0.30 ± 0.04
18	0.46 ± 0.02
19	0.47 ± 0.10
20	0.81 ± 0.28

Measurement of the GM3 (see the ESI, Fig. S2†) peak area was used to determine inhibition of GSL biosynthesis. Experiments were conducted in triplicate and the error bars show standard deviations.

β-Glucocerebrosidase inhibition

All compounds showed improved inhibitory potency for human placental β-glucocerebrosidase compared to NB-DNJ, as determined by a fluorogenic assay using 4-methylumbelliferyl-β-glucoside (Table 3). In general, extending the *N*-alkyl linker chain length provided increased efficacy, where similar inhibition constants were observed for **16**, **17**, **18**, **19** and **20**. These data contrast with α-glucosidase inhibition where inhibition was maximal in **19** treated cells but correlates with cellular ceramide glucosyltransferase inhibition, showing improvement in inhibition with *N*-alkyl chain length extension.

Cystic fibrosis assay

The potential corrector effect of triazoles **14–20** on CFTR function in CF-KM4 cells[41] was assessed using single-cell fluorescence imaging. The cells were treated for 2 h with 100 μM of triazoles **14–20** and then CFTR proteins were stimulated by a cocktail of forskolin (Fsk) + genistein (Gst). As expected, no variation of the fluorescence was observed after the cocktail stimulation in untreated CF-KM4 bearing the defective F508del-CFTR protein. However, following a treatment of CF-KM4 cells with triazole **14**, a strong increase of the recorded fluorescence signal after Fsk + Gst stimulation (Fig. 5A) was observed. The signal was then fully inhibited by the CFTR inhibitor $CFTR_{inh}$-172 (see the ESI, Fig. S4†). Fig. 5B summarizes the result obtained by iodide efflux assay, after stimulation by Fsk + Gst on untreated or treated CF-KM4 cells. These results also showed no effect of triazoles **15–20**, while **14** was active. No apparent CF-KM4 cell toxicity was observed for triazole **14** up to 1 mM as measured by the MTT test.[21]

Discussion

Regarding glycosidase inhibition, the small library of deoxynojirimycin-adamantane neoglycoconjugates exhibit potent α-glucosidase inhibition, most of them being more potent than DNJ or miglitol, with the exception of the shortest conjugates **14** and **15**. Although the general trend observed is consistent with the inhibition profile of DNJ and miglitol, the results clearly show that our synthetic approach allows efficient modulation of the inhibition profile of our inhibitors. This is easily exemplified by the data obtained for the two structurally extreme members of our library, the short derivative **14** and the extended compound **20**.

This library also contains more efficient ER α-glucosidase inhibitors than NB-DNJ. Since the key oligosaccharides resulting from ER α-glucosidase inhibition are observed with all our iminosugars we can assume an intracellular and ER localisation of these compounds, but the extent may vary with structure. The length of the aliphatic linker connecting the imino sugar nitrogen and the triazole has a major influence on cellular potency (**20**, **19** > **18** > **16**, **17** > **15** > **14**). These data confirm our previous findings regarding the importance of alkyl chain length between the iminosugar and a hydrophobic group,[39] and that potency reflects enzyme affinity rather than increased cellular uptake.[30c] Insertion of an ether function between the triazole and adamantyl groups does not have a significant effect on inhibitory activity (**16** = **17**). A similar analysis can be invoked to explain the increased potency towards ceramide glucosyltransferase of compounds bearing longer chains.[42] As a consequence, **19** and **20** are superior to **18**, **16** and **14**.

Ultimately, given the higher ER-glucosidase and ceramide glucosyltransferase inhibitions obtained here over previously published iminosugar conjugates, our approach provides a useful

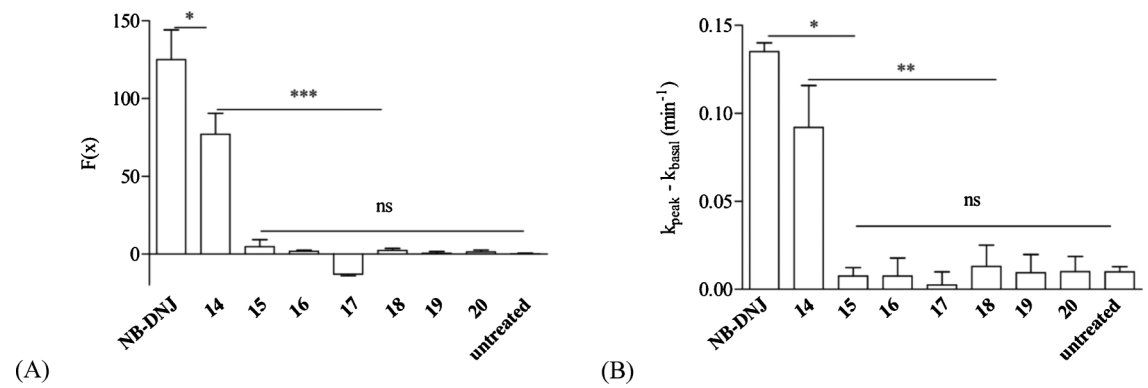

Fig. 5 **(A)** Functional evaluation of F508del-CFTR by DiSBAC2(3) assay in CFKM4 cells treated with 100 μM of compounds during 2 h. Histograms report the mean of the relative fluorescence collected from separate experiments ($N = 3$) with a total of 36 cells. **(B)** F508del-CFTR activity was assayed with the iodide efflux technique in the presence of forskolin (10 μM) + genistein (30 μM). Histograms summarizing the rescue of CFTR activity observed on CF-KM4 cells. $n = 8$ for each bar. Statistical significance is $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; ns, non significant.

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platform for the design and synthesis of ever more potent therapeutics for the treatment of lysosomal storage disorders.[43,44]

Furthermore, the library of DNJ derivatives shows high β-glucocerebrosidase inactivation, paving the way to their potential use as molecular chaperones in order to restore the cellular activity of this enzyme. Interestingly, derivatives of DNJ bearing a terminal adamantyl group have previously been described as very active compounds in this field.[45]

Concerning CF, miglustat **2**[7] has been recently shown to be a corrector of the abnormal trafficking of the mutated protein, F508del-CFTR.[46] Pre-clinical data were accumulated on various CF models on the basis of the α-glucosidase inhibitor effect of miglustat,[8,46–48] which is now evaluated in phase II trials for the treatment of cystic fibrosis.[8] The identification of **14**, which is not an α-glucosidase inhibitor, as a corrector of F508del-CFTR activity is thus very promising and suggests a different mechanism of action compared to **2**. Interestingly, other iminosugar derivatives have recently been evaluated on CF models[22,49] and isoLAB,[22] a trihydroxylated pyrrolidine, has also been found to be a CFTR corrector while not inhibiting ER α-glucosidase. Thus, the effects of iminosugars on CFTR involved in cystic fibrosis are probably more complicated than previously anticipated and certainly deserve further studies based on at least two hypotheses, one depending on α-glucosidase inhibition in the case of miglustat, and the second one being independent of glucosidase in the case of isoLAB and **14**.

Conclusion

A series of deoxynojirimycin-adamantane neoglycoconjugates has been efficiently prepared by click chemistry from protected DNJ **4** and adamantane derivatives **10** and **11**, allowing their connection through triazole-based linkers of various lengths. These DNJ derivatives showed interesting inhibition profiles towards a collection of glycosidases, their selectivities being modulated by the variation of the linker chain length. Their evaluation as correctors of defective F508del-CFTR involved in cystic fibrosis has also shown that DNJ-triazole-adamantane conjugate **14**, displaying the shortest spacer in the series, was able to rescue F508del-CFTR function. Interestingly, **14** is not an ER α-glucosidase I or α-glucosidase II inhibitor, and is a relatively poor inhibitor of intestinal glycosidases, which makes **14** a compound to explore further as it seems to act by a different mechanism compared to NB-DNJ. Regarding Gaucher disease and glucosylceramide synthase inactivation, the DNJ-triazole-adamantane conjugates **19** and **20** bearing the longest arms were approximately 20-fold more potent than model NB-DNJ **2** and even more potent than reference compound AMP-DNJ **3**. Evaluation of the ER α-glucosidase inhibition showed that DNJ-triazole-adamantanes **18–20** were potent α-glucosidase I inhibitors but cytotoxicity was detected at 100 μM for the longest compounds **19** and **20**. These two derivatives also proved to be the most potent α-glucosidase II inhibitors of this series, all AMP-DNJ triazoles demonstrating inhibition towards this enzyme, with the important exception of compound **14**. Finally, all neoglycoconjugates proved more potent β-glucocerebrosidase inhibitors than NB-DNJ and AMP-DNJ, an observation relevant to their potential use as glucocerebrosidase correctors for active-site-specific chaperone therapy. In conclusion, click coupling is therefore a powerful strategy to access new iminosugar mimics with high potential as therapeutic agents, the triazole core being well tolerated by all the enzymes assayed in this work. This strategy allows simple and rapid library generation,[50] and has been shown to enable efficient modulation of the biological activity of the conjugates.

Experimental

General

NMR spectra (^{1}H and ^{13}C) were recorded on Bruker DRX-250, AM-250, DRX-300, AV-360 or DRX-400 instruments. Chemical shifts were reported in the δ scale relative to $CHCl_3$ as an internal reference (7.26 ppm for ^{1}H and 77.0 ppm for ^{13}C). The following abbreviations have been used to describe the signal multiplicity: br (broad), s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), h (hextet), m (multiplet), dd (doublet of doublets), dt (doublet of triplets).

Electrospray ionisation mass spectra (MS) were measured on a Finnigan MAT 95 S instrument. Optical rotations were measured at 20 °C with a Perkin Elmer 341 polarimeter. Thin layer chromatography (TLC) analyses were performed on commercial glass plates bearing a 0.25 mm layer of Merck silica gel 60 F254 (Merck Art. No.5715). These were developed using standard visualising techniques: anisaldehyde/heating, 2,4-dinitrophenylhydrazine/heating, potassium permanganate/heating and UV fluorescence (254 nm). R_f values are reported to the nearest 0.01. Mixed solvent system compositions are quoted as volumetric ratios. Flash chromatography was carried out with silica gel, Merck Type 60 (70–325 mesh ASTM) or Merck Type 60 (230–400 mesh ASTM), adapting the method of Still.[51] Pet. ether refers to the petroleum fraction boiling in the range 40–60 °C. In general, reactions were carried out in dry solvents under an argon atmosphere, unless otherwise mentioned. All other reagents were purified in accordance with the recommended procedures,[52] or used as obtained from commercial sources. NMR, and MS data were obtained on all intermediates described herein using chromatographically homogeneous samples.

General procedure A – alkylation with bromide 6a or tosylates 6b–c, 5a–d. To a solution of 2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin **4** (1.0 eq.), propargyl bromide **6a**, compound **6b–c** or **5a–d** (1.5 eq.) in acetonitrile (0.2 M) was added potassium carbonate (2.1 eq.). The reaction mixture was heated to 85 °C (oil bath) for 12 h 30 under argon then cooled. Most of the acetonitrile was evaporated under reduced pressure. Water and chloroform were added to the residue and the whole mixture was stirred for 10 min, and then partitioned. The aqueous layer was extracted with chloroform (3×). The combined organic extracts were dried over sodium sulfate, filtrated and concentrated under reduced pressure. Further purification by flash chromatography gave the title compound.

General procedure B – 1,3-dipolar cycloaddition with 10 or 11. To a solution of compound **7a–d** or **8a–c** (1.0 eq.) and **10** or **11** (1.5 eq.) in dichloromethane/water (0.25 M, 1 : 1) in a screw cap tube was added an aqueous solution of copper(II) sulfate (0.2 M, 0.06 eq.), followed by an aqueous solution of sodium ascorbate (0.2 M, 0.15 eq.). The reaction mixture was purged under argon, the cap screwed on and the solution then stirred

at room temperature for 18 h. An aqueous saturated solution of sodium bicarbonate and chloroform were added. The mixture was partitioned and the aqueous layer was extracted with chloroform (3×), dried over sodium sulfate and filtrated.

General procedure C – Pd/C catalyzed hydrogenolysis of compounds 12a–b and 13a–c. A solution of compound **12a–d** or **13a–c** (1.0 eq.) in ethanol (0.03 M) was acidified to pH ~2 with 1 M aq. HCl. Argon was passed through the solution for 5 min, after which palladium 10% wt on carbon (0.6–0.9 eq.) was added. Hydrogen was passed through the reaction mixture for 5 min and the reaction was stirred for 20 h under atmospheric hydrogen pressure. Pd/C was removed by filtration over a microfilter syringe, followed by thorough rinsing with CH_3OH. The filtrate was concentrated with co-evaporation of toluene. Further purification with either a Strata C18-E column cartridge (H_2O/ACN) or by flash chromatography ($CHCl_3$/CH_3OH) gave the desired compound.

2,3,4,6-Tetra-*O*-benzyl-*N*-(3-prop-1-ynyl)-1-deoxynojirimycin 8a. General procedure A was applied to 2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin **4** (860 mg, 1.64 mmol) and **6a**. The residue was treated with water (2 mL) and extracted with EtOAc (3 × 3 mL), dried over $MgSO_4$, filtered and concentrated. Further purification by flash chromatography (silica/sample ratio 50 : 1; Pet. Ether/EtOAc 9 : 1 to 4 : 6) gave 2,3,4,6-tetra-*O*-benzyl-*N*-(2-propynyl)-1-deoxynojirimycin **8a** (845 mg, 1.50 mmol, 92% yield) as a thick oil.

R_f 0.24 (Pet. Ether/EtOAc 85 : 15). ^{1}H NMR (360 MHz, $CDCl_3$) δ 2.19 (t, 1H, *J 2.2 Hz*, H-3′), 2.43 (ddd, 1H, $J_{4,5}$*9.2*, $J_{5,6a}$*2.2*, $J_{5,6b}$*1.8 Hz*, H-5), 2.52 (dd, 1H, $J_{1ax,1eq}$*11.0*, $J_{1eq,2}$*10.6 Hz*, H-1$_{eq}$), 2.95 (dd, 1H, $J_{1ax,1eq}$*11.0*, $J_{1ax,2}$*4.8 Hz*, H-1$_{ax}$), 3.37 (dd, 1H, $J_{1'a,1'b}$ *18.0*, $J_{1a',3'}$ *2.2 Hz*, H-1′a), 3.47 (dd, 1H, $J_{2,3}$*9.2*, $J_{3,4}$*9.2 Hz*, H-3), 3.57 (dd, 1H, $J_{6a,6}$*10.5*,$J_{5,6a}$*2.2 Hz*, H-6$_a$), 3.62 (dd, 1H, $J_{3,4}$*9.2*, $J_{4,5}$*9.2 Hz*, H-4), 3.70 (ddd, 1H, $J_{1eq,2}$*10.6*, $J_{2,3}$*9.2 Hz*, $J_{1eq,2}$*4.8 Hz*, H-2), 3.72 (dd, 1H, $J_{1'a,1'b}$ *18.0*, $J_{1'b,3'}$*2.2 Hz*, H-1′b), 3.73 (dd, 1H, $J_{6a,6b}$*10.5*, $J_{5,6b}$*1.8 Hz*, H-6$_b$), 4.34 (d, 1H, *J 11.0 Hz*, C*H*H Bn), 4.42 (d, 1H, *J 12.3 Hz*, CH*H* Bn), 4.53 (d, 1H, *J 12.3 Hz*, C*H*H Bn), 4.64 (d, 1H, *J 12.3 Hz*, C*H*H Bn), 4.68 (d, 1H, *J 12.3 Hz*, CH*H* Bn), 4.81 (d, 1H, *J 11.0 Hz*, C*H*H Bn), 4.86 (d, 1H, *J 11.0 Hz*, CH*H* Bn), 4.96 (d, 1H, *J 11.0 Hz*, CH*H* Bn), 7.05–7.39 (m, 20H, CH aromatic). ^{13}C (90 MHz, $CDCl_3$) δ 42.3 (C-1′), 55.0 (C-1), 62.1 (C-5), 64.8 (C-6), 72.7 (CH_2 benzyl), 73.6 (CH_2 benzyl), 74.1 (C-3′), 75.2 (CH_2 benzyl),75.4 (CH_2 benzyl), 78.2 (C-2), 78.2 (C-4), 84.2 (C-2′), 87.1 (C-3), 127.5 (CH aromatic), 127.5 (CH aromatic), 127.6 (CH aromatic), 127.8 (CH aromatic), 127.9 (CH aromatic), 128.3 (CH aromatic), 128.4 (CH aromatic), 128.4 (CH aromatic), 137.6 (C aromatic), 138.4 (C aromatic), 138.5 (C aromatic), 138.8 (C aromatic). LRMS (ESI$^+$) 562.3 [M+H]$^+$, 100%, 584.3 [M+Na]$^+$, 40%. HRMS: Found 562.2951[M+H]$^+$. $C_{37}H_{40}NO_4$ requires 562.2957.

***N*-[1-(Adamant-1-yl-methyl)-1*H*-1,2,3-triazol-4-yl]methyl-2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin 13a.** 2,3,4,6-Tetra-*O*-benzyl-*N*-(2-propynyl)-1-deoxynojirimycin **8a** (316 mg, 0.562 mmol) was used in general procedure B. Further purification of the residue by flash silica gel column chromatography (Pet. Ether/EtOAc 8 : 2 to 4 : 6) gave *N*-[1-(adamant-1-yl-methyl)-1*H*-1,2,3-triazol-4-yl]methyl-2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin **13a** (383 mg, 0.509 mmol, 91% yield).

R_f 0.20 (Pet. Ether/EtOAc 7 : 3). ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.40–1.48 (m, 6H, 3×$CH_{2\,Ad}$), 1.52–1.73 (m, 6H, 3×$CH_{2\,Ad}$), 1.93–2.01 (m, 3H, 3×CH_{Ad}), 2.16 (dd, 1H, $J_{1ax,1eq}$*10.9*, $J_{1eq,2}$*10.9 Hz*, H-1$_{eq}$), 2.26 (ddd, 1H, $J_{4,5}$*9.5*, $J_{5,6a}$*2.8*, $J_{5,6b}$*1.8 Hz*, H-5), 3.14 (dd, 1H, $J_{1ax,1eq}$*10.9*, $J_{1ax,2}$*4.9 Hz*, H-1$_{ax}$), 3.36 (dd, 1H, $J_{2,3}$*9.1*, $J_{3,4}$*9.1 Hz*, H-3), 3.54 (dd, 1H, $J_{4,5}$*9.5*, $J_{3,4}$*9.1 Hz*, H-4), 3.65 (ddd, 1H, $J_{1eq,2}$*10.9*, $J_{2,3}$*9.1*, $J_{1ax,2}$*4.9 Hz*, H-2), 3.74 (dd, 1H, $J_{6a,6b}$*10.7*, $J_{5,6a}$*2.8 Hz*, H-6$_a$), 3.91 (s, 2H, CH_2Ad), 3.95 (d, 1H, $J_{1'a,1'b}$ *15.2 Hz*, H-1′$_a$), 3.97 (dd, 1H, $J_{6a,6b}$*10.7*, $J_{5,6b}$*1.8 Hz*, *H-6b*), 4.15 (d, 1H, $J_{1'a,1'b}$ *15.2 Hz*, H-1′$_b$), 4.37 (d, 1H, *J 10.9 Hz*, C*H*H Bn), 4.46 (d, 1H, *J 11.9 Hz*, C*H*H Bn), 4.54 (d, 1H, *J 11.9 Hz*, CH*H* Bn), 4.62 (s, 2H, OCH_2Ph), 4.75 (d, 1H, *J 11.1 Hz*, C*H*H Bn), 4.84 (d, 1H, *J 10.9 Hz*, CH*H* Bn), 4.90 (d, 1H, *J 11.1 Hz*, CH*H* Bn), 7.06–7.36 (m, 21H, CH aromatic+CH triazole). ^{13}C (90 MHz, $CDCl_3$) δ 28.1 (CH_{Ad}), 34.2 (C_{Ad}), 36.6 ($CH_{2\,Ad}$), 40.3 ($CH_{2\,Ad}$), 47.4 (C-1′), 54.5 (C-1), 62.1 (CH_2Ad), 62.9 (C-5), 66.5 (C-6), 72.6 (CH_2 benzyl), 73.5 (CH_2 benzyl), 75.2 (CH_2 benzyl), 75.3 (CH_2 benzyl), 78.5 (C-2), 78.6 (C-4), 87.1 (C-3), 124.3 (CH triazole), 127.5 (CH aromatic), 127.6 (CH aromatic), 127.8 (CH aromatic), 127.8 (CH aromatic), 127.9 (CH aromatic), 128.3 (CH aromatic), 128.3 (CH aromatic), 128.4 (CH aromatic), 128.4 (CH aromatic), 137.9 (C aromatic), 138.5 (C aromatic), 138.5 (C aromatic), 139.0 (C aromatic), 141.6 (C triazole). LRMS (ESI$^+$) 753.4 [M+H]$^+$, 60%, 775.4 [M+Na]$^+$, 100%. HRMS Found 775.4199 [M+Na]$^+$. $C_{48}H_{56}N_4O_4Na$ requires 775.4194.

***N*-[1-(Adamant-1-yl-methyl)-1*H*-1,2,3-triazol-4-yl]methyl-1-deoxynojirimycin 14.** Hydrogenolysis of *N*-[1-(adamant-1-yl-methyl)-1*H*-1,2,3-triazol-4-yl]methyl-2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin **13a** (258 mg, 343 μmol) following general procedure C gave after purification by flash silica gel column chromatography ($CHCl_3$/CH_3OH, 8 : 2) gave *N*-[1-(adamant-1-yl-methyl)-1*H*-1,2,3-triazol-4-yl]methyl-1-deoxynojirimycin **14** (132 mg, 336 μmol, 98%) as a white solid.

R_f 0.22 ($CHCl_3$/CH_3OH 80 : 20). ^{1}H NMR (300 MHz, CD_3OD) δ 1.46–1.59 (m, 6H, 3×$CH_{2\,Ad}$), 1.59–1.81 (m, 6H, 3×$CH_{2\,Ad}$), 1.90–2.07 (m, 3H, 3×CH_{Ad}), 2.47–2.80 (m, 2H, H-1$_{eq}$), 3.13–3.28 (m, 3H, H$_{1ax}$, H-3, H-5), 3.35 (s, 2H, H-1′), 3.49–3.77 (m, 2H, H-2, H-4), 4.05–4.30 (m, 2H, H-6$_a$, H-6$_b$), 4.12 (s, 2H, CH_2Ad), 4.38 (bs, OH), 8.04 (s, 1H, CH triazole). ^{13}C (90 MHz, CD_3OD) δ 29.8 (CH_{Ad}), 35.4 (C_{Ad}), 37.8 ($CH_{2\,Ad}$), 41.4 ($CH_{2\,Ad}$), 48.4, 49.4, 56.0 (C-1), 57.3, 63.2 (CH_2Ad), 67.0, 70.4, 79.0 (C-3), 128.9 (CH triazole), 139.5 (C triazole). HRMS Found 393.25051 [M+H]$^+$. $C_{20}H_{33}N_4O_4$ requires 393.2496. (δ 0.9 ppm). LRMS (ESI$^+$) 393.3 [M+H]$^+$, 93%, 415.3 [M+Na]$^+$, 100%. Found 415.2314 [M+Na]$^+$. $C_{20}H_{32}N_4O_4Na$ requires 415.2321.

2,3,4,6-Tetra-*O*-benzyl-*N*-(4-but-1-ynyl)-1-deoxynojirimycin 8b. Applying general procedure A to 2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin **4** (846 mg, 1.62 mmol) and **6b** gave after flash chromatography on silica gel (Pet. Ether/EtOAc 9 : 1 to 4 : 6) 2,3,4,6-tetra-*O*-benzyl-*N*-(3-butynyl)-1-deoxynojirimycin **8b** (659 mg, 1.15 mmol, 71%) as a white solid.

R_f 0.24 (Pet. Ether/EtOAc 85 : 15). ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.94 (t, *J 2.6 Hz*, 1H, H alkyne), 2.19 (m, 2H, H-2′), 2.33 (dd, 1H, $J_{1ax,1eq}$*11.0*, $J_{1eq,2}$*10.5 Hz*, H-1$_{eq}$), 2.39 (ddd, 1H, $J_{4,5}$*9.6*, $J_{5,6a}$*2.6*, $J_{5,6b}$*2.2 Hz*, H-5), 2.88–3.02 (m, 2H, H-1′), 3.06 (dd, 1H, $J_{1ax,1eq}$*11.0*, $J_{1ax,2}$*4.8 Hz*, H-1$_{ax}$), 3.43 (dd, 1H, $J_{2,3}$*9.2*, $J_{3,4}$*9.2 Hz*, H-3), 3.52 (dd, 1H, $J_{3,4}$*9.2*, $J_{4,5}$*9.2 Hz*, H-4), 3.60 (dd, 1H, $J_{6a,6}$*10.5*,$J_{5,6a}$*2.2 Hz*, H-6$_a$), 3.62 (ddd, 1H, $J_{1eq,2}$*10.5*, $J_{2,3}$*9.2 Hz*, $J_{1eq,2}$*4.8 Hz*, H-2), 3.67 (dd,

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1H, $J_{6a,6b}$ *10.5*, $J_{5,6b}$ *2.6 Hz*, H-6_b), 4.36 (d, 1H, *J 11.0 Hz*, C*H*H Bn), 4.44 (d, 1H, *J 12.2 Hz*, CH*H* Bn), 4.51 (d, 1H, *J 12.2 Hz*, C*H*H Bn), 4.63 (d, 1H, *J 11.8 Hz*, C*H*H Bn), 4.67 (d, 1H, *J 11.8 Hz*, CH*H* Bn), 4.79 (d, 1H, *J 11.0 Hz*, C*H*H Bn), 4.85 (d, 1H, *J 11.0 Hz*, CH*H* Bn), 4.94 (d, 1H, *J 11.0 Hz*, CH*H* Bn), 7.05–7.36 (m, 20H, CH aromatic). ^{13}C (90 MHz, $CDCl_3$) δ 14.0 (C-2′), 51.1 (C-1′), 54.4 (C-1), 63.0 (C-5), 65.8 (C-6), 69.4 (C-4′), 72.7 (CH_2 benzyl), 73.4 (CH_2 benzyl), 75.1 (CH_2 benzyl),75.3 (CH_2 benzyl), 78.3 (C-2), 78.4 (C-4), 82.6 (C-3′), 87.1 (C-3), 127.4 (CH aromatic), 127.5 (CH aromatic), 127.6 (CH aromatic), 127.8 (CH aromatic), 128.3 (CH aromatic), 128.4 (CH aromatic), 137.7 (C aromatic), 138.4 (C aromatic), 138.9 (C aromatic). LRMS (ESI$^+$) 576.3 $[M+H]^+$, 100%. HRMS Found 576.3118 $[M+H]^+$. $C_{38}H_{42}NO_4$ requires 576.3114 (δ 0.7 ppm). $[\alpha]_D^{20}$ 8.5 (*c* 1.2, CH_2Cl_2)

***N*-[1-(Adamant-1-yl-methyl)-1*H*-1,2,3-triazol-4-yl]ethyl-2,3,4, 6-tetra-*O*-benzyl-1-deoxynojirimycin 13b.** 2,3,4,6-Tetra-*O*-benzyl-*N*-(3-butynyl)-1-deoxynojirimycin **8b** (302 mg, 0.524 mmol) was subjected to general procedure B. Further purification by flash silica gel column chromatography (Pet. Ether/EtOAc 9 : 1 to 6 : 4) gave *N*-[1-(adamant-1-yl-methyl)-1*H*-1,2,3-triazol-4-yl]ethyl-2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin **13b** (402 mg, 0.524 mmol, 100%).

R_f 0.10 (Pet. Ether/EtOAc 85 : 15). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38–1.48 (m, 6H, 3×$CH_{2\,Ad}$), 1.51–1.73 (m, 6H, 3×$CH_{2\,Ad}$), 1.93–2.01 (m, 3H, 3×CH_{Ad}), 2.29 (dd, 1H, $J_{1ax,1eq}$ *10.9*, $J_{1eq,2}$ *10.6 Hz*, H-1_{eq}), 2.39–2.48 (m, 1H, H-5), 2.74–2.97 (m, 3H, H-2′, H-1′$_a$), 3.02–3.13 (m, 1H, H-1′$_b$), 3.16 (dd, 1H, $J_{1ax,1eq}$ *10.9*, $J_{1ax,2}$ *4.5 Hz*, H-1_{ax}), 3.48 (dd, 1H, $J_{3,4}$ *9.1*, $J_{2,3}$ *8.8 Hz*, H-3), 3.56 (dd, 1H, $J_{3,4}$ *9.1*, $J_{4,5}$ *8.6 Hz*, H-4), 3.58 (ddd, 1H, $J_{1eq,2}$ *10.6*, $J_{2,3}$ *8.8*, $J_{1ax,2}$ *4.5 Hz*, H-2), 3.66–3.75 (bs, 2H, H-6), 3.80 (d, 1H, *J 13.8 Hz*, NC*H*HAd), 3.91 (d, 1H, *J 13.8 Hz*, NCH*H*Ad), 4.43 (d, 1H, *J 10.9 Hz*, C*H*H Bn), 4.45 (s, 2H, OCH_2Ph), 4.63 (s, 2H, OCH_2Ph), 4.81 (d, 1H, *J 11.1 Hz*, C*H*H Bn), 4.88 (d, 1H, *J 10.9 Hz*, CH*H* Bn), 4.96 (d, 1H, *J 11.1 Hz*, CH*H* Bn), 7.05 (s, 1H, CH triazole), 7.12–7.37 (m, 20H, CH aromatic). ^{13}C (100 MHz, $CDCl_3$) δ 20.9 (C-2′), 28.0 (CH_{Ad}), 34.0 (C_{Ad}), 36.5 ($CH_{2\,Ad}$), 40.2 ($CH_{2\,Ad}$), 51.4 (C-1′), 54.0 (C-1), 61.9 (CH_2Ad), 63.7 (C-5), 66.0 (C-6), 72.6 (CH_2 benzyl), 73.4 (CH_2 benzyl), 75.2 (CH_2 benzyl), 75.3 (CH_2 benzyl), 78.4 (C-2), 78.6 (C-4), 87.2 (C-3), 122.8 (CH triazole), 127.4 (CH aromatic), 127.5 (CH aromatic), 127.6 (CH aromatic), 127.8 (CH aromatic), 128.0 (CH aromatic), 128.3 (CH aromatic), 137.8 (C aromatic), 138.3 (C aromatic), 138.4 (C aromatic), 138.9 (C aromatic), 145.0 (C triazole). LRMS (ESI$^+$) 767.5 $[M+H]^+$, 100%. HRMS Found 767.4259 $[M+H]^+$. $C_{49}H_{59}N_4O_4$ requires 767.4531. $[\alpha]_D^{20}$ 4.0 (*c* 1.210, CH_2Cl_2)

***N*-[1-(Adamant-1-yl-methyl)-1*H*-1,2,3-triazol-4-yl]ethyl-1-deoxynojirimycin 15.** Hydrogenolysis of *N*-[1-(adamant-1-yl-methyl)-1*H*-1,2,3-triazol-4-yl]ethyl-2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin **13b** (314 mg, 409 μmol), following procedure C, gave after purification by flash silica gel column chromatography ($CHCl_3$/CH_3OH 8 : 2) gave *N*-[1-(adamant-1-yl-methyl)-1*H*-1,2,3-triazol-4-yl]ethyl-1-deoxynojirimycin **15** (159 mg, 391 μmol, 96%) as a white solid.

R_f 0.28 ($CHCl_3$/CH_3OH 80 : 20). ^{1}H NMR (360 MHz, CD_3OD) δ 1.49–1.59 (m, 6H, 3×$CH_{2\,Ad}$), 1.59–1.83 (m, 6H, 3×$CH_{2\,Ad}$), 1.93–2.03 (m, 3H, 3×CH_{Ad}), 3.11 (dd, 1H, $J_{1ax,1eq}$ *12.3*, $J_{1eq,2}$ *11.4 Hz*, H-1_{eq}), 3.16–3.30 (m, 3H, H-5, H-2′$_a$, H-2′$_b$), 3.42 (dd, 1H, $J_{3,4}$ *9.1*, $J_{2,3}$ *9.1 Hz*, H-3), 3.52–3.68 (m, 1H, H-1′$_a$), 3.59 (dd, 1H, $J_{1ax,1eq}$ *12.3*, $J_{1ax,2}$ *5.0 Hz*, H-1_{ax}), 3.65 (dd, 1H, $J_{4,5}$ *9.5*, $J_{3,4}$ *9.0 Hz*, H-4), 3.69–3.81 (m, 2H, H-2, H-1′$_b$), 3.98 (dd, 1H, $J_{6a,6b}$ *12.3*, $J_{5,6a}$ *2.7 Hz*, H-6_a), 4.07 (s, 2H, CH_2Ad), 4.13 (d, 1H, $J_{6a,6b}$ *12.3 Hz*, H-6_b), 7.85 (s, 1H, CH triazole). ^{13}C (90 MHz, CD_3OD) δ 20.9 (C-2′), 29.7 (CH_{Ad}), 35.2 (C_{Ad}), 37.7 ($CH_{2\,Ad}$), 41.3 ($CH_{2\,Ad}$), 54.9 (C-1, C-6), 63.1 (CH_2Ad), 67.7 (C-5), 67.8 (C-1′, C-2), 68.9 (C-4), 77.9 (C-3), 125.8 (CH triazole), 143.1 (C triazole). LRMS (ESI$^+$) 407.3 $[M+H]^+$, 100%, 429.3 $[M+Na]^+$, 50%. HRMS Found 407.2651 $[M+H]^+$. $C_{21}H_{35}N_4O_4$ requires 407.2658 (δ –0.7 ppm)

2,3,4,6-Tetra-*O*-benzyl-*N*-(6-hex-1-ynyl)-1-deoxynojirimycin 8c. General procedure A, using 2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin **4** (728 mg, 1.39 mmol) and **6c**, gave after flash chromatography (silica/sample ratio 50 : 1; Pet. Ether/EtOAc 9 : 1 to 1 : 1) 2,3,4,6-tetra-*O*-benzyl-*N*-(5-hexynyl)-1-deoxynojirimycin **8c** (621 mg, 1.03 mmol, 74%) as a white solid.

R_f 0.18 (Pet. Ether/EtOAc 85 : 15). ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.35–1.61 (m, 4H, H-2′, H-3′), 1.93 (t, 1H, *J 2.6 Hz*, H-6′), 2.14 (dt, 2H, H-4′), 2.22 (dd, 1H, $J_{1ax,1eq}$ *10.9*, $J_{1eq,2}$ *10.4 Hz*, H-1_{eq}), 2.29–2.35 (m, 1H, H-5), 2.50–2.62 (m, 1H, H-1′$_a$), 2.62–2.76 (m, 1H, H-1′$_b$), 3.09 (dd, 1H, $J_{1ax,1eq}$ *10.9*, $J_{1ax,2}$ *5.0 Hz*, H-1_{ax}), 3.46 (dd, 1H, $J_{2,3}$ *9.1*, $J_{3,4}$ *8.6 Hz*, H-3), 3.52–3.61 (m, 1H, H-6_a), 3.58 (dd, 1H, $J_{3,4}$ *9.1*, $J_{4,5}$ *9.1 Hz*, H-4), 3.61–3.70 (m, 1H, H-6_b), 3.65 (ddd, 1H, $J_{1eq,2}$ *10.5*, $J_{2,3}$ *9.1 Hz*, $J_{1eq,2}$ *5.0 Hz*, H-2), 4.43 (d, 1H, *J 10.9 Hz*, C*H*H Bn), 4.45 (d, 1H, *J 12.3 Hz*, CH*H* Bn), 4.49 (d, 1H, *J 12.3 Hz*, C*H*H Bn), 4.65 (d, 1H, *J 11.8 Hz*, C*H*H Bn), 4.70 (d, 1H, *J 11.8 Hz*, CH*H* Bn), 4.81 (d, 1H, *J 10.9 Hz*, C*H*H Bn), 4.88 (d, 1H, *J 10.9 Hz*, CH*H* Bn), 4.95 (d, 1H, *J 10.9 Hz*, CH*H* Bn), 7.11–7.40 (m, 20H, CH aromatic). ^{13}C (90 MHz, $CDCl_3$) δ 18.2 (C-4′), 22.9, 26.3 (C-2′, C-3′), 51.7 (C-1′), 54.4 (C-1), 63.9 (C-5), 65.6 (C-6), 68.6 (C-6′), 72.8 (CH_2 benzyl), 73.4 (CH_2 benzyl), 75.2 (CH_2 benzyl),75.3 (CH_2 benzyl), 78.6 (C-2), 78.7 (C-4), 84.2 (C-5′), 87.4 (C-3), 127.4 (CH aromatic), 127.5 (CH aromatic), 127.6 (CH aromatic), 127.8 (CH aromatic), 128.3 (CH aromatic), 128.4 (CH aromatic), 137.8 (C aromatic), 138.6 (C aromatic), 139.0 (C aromatic). LRMS (ESI$^+$) 604.4 $[M+H]^+$, 100%. HRMS Found 604.3425 $[M+H]^+$. $C_{40}H_{46}NO_4$ requires 604.3427.$[\alpha]_D^{20}$ 17.2 (*c* 1.2, CH_2Cl_2).

***N*-[1-(Adamant-1-yl-methyl)-1*H*-1,2,3-triazol-4-yl]butyl-2,3,4, 6-tetra-*O*-benzyl-1-deoxynojirimycin 13c.** 2,3,4,6-Tetra-*O*-benzyl-*N*-(5-hexynyl)-1-deoxynojirimycin **8c** (303 mg, 502 μmol) was treated according general procedure B to afford after purification by flash silica gel column chromatography (Pet. Ether/EtOAc 6 : 4) *N*-[1-(adamant-1-yl-methyl)-1*H*-1,2,3-triazol-4-yl]butyl-2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin **13c** (397 mg, 499 μmol, 99% yield) as a white solid.

R_f 0.16 (Pet. Ether/EtOAC 7 : 3). ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.34–1.73 (m, 16H, 6×$CH_{2\,Ad}$, H-2′, H-3′), 1.94–2.01 (m, 3H, 3×CH_{Ad}), 2.20 (dd, 1H, $J_{1ax,1eq}$ *10.9*, $J_{1eq,2}$ *10.4 Hz*, H-1_{eq}), 2.24–2.32 (m, 1H, H-5), 2.55–2.78 (m, 2H, H-1′), 2.66 (t, 2H, *J 7.3 Hz*, H-4′), 3.06 (dd, 1H, $J_{1ax,1eq}$ *10.9*, $J_{1ax,2}$ *4.5 Hz*, H-1_{ax}), 3.43 (dd, 1H, $J_{2,3}$ *9.1*, $J_{3,4}$ *9.1 Hz*, H-3), 3.49–3.69 (m, 4H, H-6_a, H-4, H-6_b, H-2), 3.94 (s, 2H, CH_2Ad), 4.40 (d, 1H, *J 10.9 Hz*, C*H*H Bn), 4.45 (s, 2H, OCH_2Ph), 4.62 (d, 1H, *J 11.8 Hz*, C*H*H Bn), 4.67 (d, 1H, *J 11.8 Hz*, CH*H* Bn), 4.79 (d, 1H, *J 10.9 Hz*, C*H*H Bn), 4.85 (d, 1H, *J 10.9 Hz*, CH*H* Bn), 4.93 (d, 1H, *J 10.9 Hz*, CH*H* Bn), 7.08 (s, 1H, CH triazole), 7.09–7.36 (m, 20H, CH aromatic). ^{13}C (90 MHz, $CDCl_3$) δ 23.1 (C-2′), 25.4 (C-4′), 27.1 (C-3′), 28.0 (CH_{Ad}), 34.1 (C_{Ad}), 36.5 ($CH_{2\,Ad}$), 40.2 ($CH_{2\,Ad}$), 52.0 (C-1′), 54.3 (C-1), 63.0 (CH_2Ad), 63.7 (C-5), 65.4 (C-6), 72.7 (CH_2 benzyl), 73.3 (CH_2

benzyl), 75.1 (CH_2 benzyl),75.2 (CH_2 benzyl), 78.4 (C-2), 78.4 (C-4), 87.2 (C-3), 122.0 (CH triazole), 127.4 (CH aromatic), 127.4 (CH aromatic), 127.6 (CH aromatic), 128.0 (CH aromatic), 128.6 (CH aromatic), 137.7 (C aromatic), 138.4 (C aromatic), 138.9 (C aromatic), 147.0 (C triazole). LRMS (ESI^+) 795.5 $[M+H]^+$, 100%. HRMS Found 795.4854 $[M+H]^+$. $C_{51}H_{62}N_4O_4$ requires 795.4844. $[\alpha]_D^{20}$ 9.7 (*c* 1.150, CH_2Cl_2)

***N*-[1-(Adamant-1-yl-methyl)-1*H*-1,2,3-triazol-4-yl]butyl-1-deoxynojirimycin 16.** Hydrogenolysis of *N*-[1-(adamant-1-yl-methyl)-1*H*-1,2,3-triazol-4-yl]butyl-2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin **13c** (278 mg, 349 μmol) using general procedure C gave after purification by flash chromatography ($CHCl_3$/CH_3OH 8 : 2 to 7 : 3) *N*-[1-(adamant-1-yl-methyl)-1*H*-1,2,3-triazol-4-yl]butyl-1-deoxynojirimycin **16** (144 mg, 330 μmol, 95%) which was concentrated and taken up in water then lyophilized to afford a pale yellow solid.

R_f 0.16 ($CHCl_3$/CH_3OH 80 : 20). ^1H NMR (360 MHz, CD_3OD) δ 1.49–1.60 (m, 6H, 3×$CH_{2\,Ad}$), 1.60–1.80 (m, 6H, 3×$CH_{2\,Ad}$), 1.80–1.95 (m, 4H, H-2′, H-3′), 1.96–2.07 (m, 3H, 3×CH_{Ad}), 2.84–2.94 (m, 2H, H-4′), 3.00 (dd, 1H, $J_{1ax,1eq}$*11.8*, $J_{1eq,2}$*11.4 Hz*, H-1_{eq}), 3.07 (~d, 1H, H-5), 3.22–3.41 (m, 1H, H-1′$_a$), 3.38 (dd, 1H, $J_{2,3}$*10.0*, $J_{3,4}$*9.0 Hz*, H-3), 3.42–3.53 (m, 1H, H-1′$_b$), 3.47 (dd, 1H, $J_{1ax,1eq}$*11.8*, $J_{1ax,2}$*5.0 Hz*, H-1_{ax}), 3.60 (dd, 1H, $J_{4,5}$*10.0*, $J_{3,4}$*9.0 Hz*, H-4), 3.66–3.79 (m, 1H, H-2), 3.93 (~d, 1H, $J_{6a,6b}$*12.3 Hz*, H-6_a), 4.11 (~d, 1H, $J_{6a,6b}$*12.3 Hz*, H-6_b), 4.17 (s, 2H, CH_2Ad), 8.07 (s, 1H, CH triazole). ^{13}C (90 MHz, CD_3OD) δ 23.6 (C-2′), 24.7 (C-4′), 26.9 (C-3′), 29.6 (CH_{Ad}), 35.3 (C_{Ad}), 37.6 ($CH_{2\,Ad}$), 41.1 ($CH_{2\,Ad}$), 54.1 (C-1′), 55.1 (C-1), 64.1 (CH_2Ad), 67.6 (C-5), 67.8 (C-2), 68.8 (C-4), 78.1 (C-3), 127.1 (CH triazole), 146.2 (C triazole). LRMS (ESI^+) 435.3 $[M+H]^+$, 100%, 457.3 $[M+Na]^+$, 30%. HRMS Found 435.2979 $[M+H]^+$. $C_{23}H_{39}N_4O_4$ requires 435.2971.

***N*-(4-Azidobutyl)-2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin 7a.** 2,3,4,6-Tetra-*O*-benzyl-1-deoxynojirimycin **4** (188 mg, 0.359 mmol) was alkylated following general procedure A with **5a** to give after purification by flash chromatography (Pet. Ether/EtOAc 9 : 1 to 3 : 7) 2,3,4,6-tetra-*O*-benzyl-*N*-(4-azidobutyl)-1-deoxynojirimycin **7a** (179 mg, 0.289 mmol, 80% yield) as a thick colourless oil.

R_f 0.28 (Pet. Ether/EtOAc 4 : 1). ^1H NMR (360 MHz, $CDCl_3$) δ 1.34–1.57 (m, 4H, H-2′, H-3′), 2.19 (dd, 1H, $J_{1ax,1eq}$*11.2*, $J_{1eq,2}$*10.4 Hz*, H-1_{eq}), 2.28–2.36 (m, 1H, H-5), 2.50–2.61 (m, 1H, H-1′a), 2.64–2.76 (m, 1H, H-1′b), 3.08 (dd, 1H, $J_{1ax,1eq}$*11.2*, $J_{1ax,2}$*4.7 Hz*, H-1_{ax}), 3.18 (t, 2H, *J 6.4 Hz*, H-4′), 3.47 (dd, 1H, $J_{3,4}$*9.1*, $J_{2,3}$*8.6 Hz*, H-3), 3.53–3.69 (m, 2H, H-6_a, H-2), 3.57 (dd, 1H, $J_{4,5}$*9.5*, $J_{3,4}$*9.1 Hz*, H-4), 3.66 (dd, 1H, $J_{6a,6b}$*10.4*, $J_{5,6b}$*2.7 Hz*, H-6_b), 4.43 (d, 1H, *J 12.2 Hz*, C*H*H Bn), 4.45 (d, 1H, *J 11.0 Hz*, CH*H* Bn), 4.49 (d, 1H, *J 12.2 Hz*, C*H*H Bn), 4.65 (d, 1H, *J 11.8 Hz*, C*H*H Bn), 4.70 (d, 1H, *J 11.8 Hz*, CH*H* Bn), 4.82 (d, 1H, *J 11.4 Hz*, C*H*H Bn), 4.91 (d, 1H, *J 11.0 Hz*, CH*H* Bn), 4.96 (d, 1H, *J 11.4 Hz*, CH*H* Bn), 7.12–7.36 (m, 20H, CH aromatic). ^{13}C (90 MHz, $CDCl_3$) δ 21.3, 26.7 (C-2′, C-3′), 51.1 (C-4′), 551.6 (C-1′), 54.3 (C-1), 64.0 (C-5), 65.7 (C-6), 72.7 (CH_2 benzyl), 73.6 (CH_2 benzyl), 75.1 (CH_2 benzyl),75.2 (CH_2 benzyl), 78.4 (C-2), 78.6 (C-4), 87.3 (C-3), 127.4 (CH aromatic), 127.5 (CH aromatic), 127.6 (CH aromatic), 127.8 (CH aromatic), 127.8 (CH aromatic), 128.2 (CH aromatic), 128.3 (CH aromatic), 128.3 (CH aromatic), 137.7 (C aromatic), 138.5 (C aromatic), 139.0. LRMS (ESI^+) 621.3 $[M+H]^+$, 100%. HRMS Found 621.3438 $[M+H]^+$. $C_{38}H_{45}N_4O_4$ requires 621.3441.

***N*-(4-{4-[Adamant-1-yl-methoxymethyl]-1*H*-1,2,3-triazol-1-yl]}butyl)-2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin 12a.** General procedure B was applied to *N*-(4-azidobutyl)-2,3,4,6-tetra-*O*-benzyl 1-deoxynojirimycin **7a** (206 mg, 0.332 mmol) and 3-(adamant-1-yl-methoxy)-prop-1-yne **10** (99 mg, 0,48 mmol). Further purification by flash chromatography (Pet. Ether/EtOAc 8 : 2 to 4 : 6) gave *N*-(4-{4-[adamant-1-yl-methoxymethyl]-1*H*-1,2,3-triazol-1-yl]}butyl)-2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin **12a** (236 mg, 0.287 mmol, 86%).

R_f 0.22 (Pet. Ether/EtOAc 6 : 4). ^1H NMR (360 MHz, $CDCl_3$) δ 1.28–1.48 (m, 2H, H-2′), 1.49–1.55 (m, 6H, 3×$CH_{2\,Ad}$), 1.56–1.83 (m, 8H, 3×$CH_{2\,Ad}$ + H-3′), 1.89–1.99 (m, 3H, 3×CH_{Ad}), 2.12 (dd, 1H, $J_{1ax,1eq}$*10.7*, $J_{1eq,2}$*10.7 Hz*, H-1_{eq}), 2.28 (ddd, 1H, $J_{4,5}$*9.1*, $J_{5,6a}$*3.1*, $J_{5,6b}$*2.7 Hz*, H-5), 2.46–2.53 (m, 1H, NCH*H*), 2.68–2.76 (m, 1H, NC*H*H), 3.00 (dd, 1H, $J_{1ax,1eq}$*10.7*, $J_{1ax,2}$*4.5 Hz*, H-1_{ax}), 3.07 (s, 2H, OCH_2Ad), 3.44 (dd, 1H, $J_{2,3}$*9.1*, $J_{3,4}$*9.1 Hz*, H-3), 3.52 (dd, 1H, $J_{3,4} = J_{4,5}$*9.1 Hz*, H-4), 3.54 (dd, 1H, $J_{6a,6b}$*10.6*, $J_{5,6a}$*2.3 Hz*, H-6_a), 3.61 (ddd, 1H, $J_{1eq,2}$*10.7*, $J_{2,3}$*9.1*, $J_{1ax,2}$*4.5 Hz*, H-2), 3.63 (dd, 1H, $J_{6a,6b}$*10.6*, $J_{5,6b}$*2.7 Hz*, H-6_b), 4.22 (t, 2H, *J 7.3 Hz*, CH_2N-triazole), 4.43 (d, 1H, *J 10.9 Hz*, C*H*H Bn), 4.43 (d, 1H, *J 11.8 Hz*, CH*H* Bn), 4.44 (d, 1H, *J* 11.8 Hz, C*H*H Bn), 4.58 (s, 2H, OCH_2-triazole), 4.61 (d, 1H, *J 11.8 Hz*, C*H*H Bn), 4.67 (d, 1H, *J 11.8 Hz*, CH*H* Bn), 4.79 (d, 1H, *J 11.2 Hz*, C*H*H Bn), 4.87 (d, 1H, *J 10.9 Hz*, CH*H* Bn), 4.93 (d, 1H, *J 11.2 Hz*, CH*H* Bn), 7.13–7.32 (m, 20H, CH aromatic), 7.35 (s, 1H, CH triazole). ^{13}C (90 MHz, $CDCl_3$) δ 21.4 (C-2′), 28.1 (C-3′), 28.2 (CH_{Ad}), 34.0 (C_{Ad}), 37.2 ($CH_{2\,Ad}$), 39.7 ($CH_{2\,Ad}$), 49.9 (C-4′), 51.3 (C-1′), 54.3 (C-1), 64.1 (C-5), 65.3 (OCH_2-triazole), 65.9 (C-6), 72.8 (CH_2 benzyl), 73.4 (CH_2 benzyl), 75.2 (CH_2 benzyl), 75.3 (CH_2 benzyl), 78.4 (C-2), 78.6 (C-4), 81.6 (OCH_2Ad), 87.3 (C-3), 121.8 (CH triazole), 127.4 (CH aromatic), 127.5 (CH aromatic), 127.6 (CH aromatic), 127.8 (CH aromatic), 128.3 (CH aromatic), 128.4 (CH aromatic), 137.7 (C aromatic), 138.5 (C aromatic), 138.9 (C aromatic), 145.9 (C triazole). LRMS (ESI^+) 825.5 $[M+H]^+$, 100%, 847.6 $[M+Na]^+$, 20%. HRMS Found 825.4984 $[M+H]^+$. $C_{52}H_{65}N_4O_5$ requires 825.4955. $[\alpha]_D^{20}$ 2.9 (*c* 2.1, CH_2Cl_2).

***N*-(4-{4-[Adamant-1-yl-methoxymethyl]-1*H*-1,2,3-triazol-1-yl]}butyl)- 1-deoxynojirimycin 17.** Hydrogenolysis of *N*-(4-{4-[Adamant-1-yl-methoxymethyl]-1*H*-1,2,3-triazol-1-yl]}butyl)-2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin **12a** (178 mg, 216 μmol) gave after purification by flash silica gel column chromatography ($CHCl_3$/CH_3OH 8 : 2) *N*-(4-{4-[adamant-1-yl-methoxymethyl]-1*H*-1,2,3-triazol-1-yl]}butyl)-1-deoxynojirimycin **17** (97 mg, 21 μmol, 97% yield) as a pale yellow solid.

R_f 0.18 ($CHCl_3$/CH_3OH 80 : 20). ^1H NMR (360 MHz, CD_3OD) δ 1.52–1.60 (m, 6H, 3×$CH_{2\,Ad}$), 1.60–1.81 (m, 8H, 3×$CH_{2\,Ad}$ + H-2′), 1.89–2.06 (m, 5H, 3×CH_{Ad} + H-3′), 2.49–2.68 (m, 2H, H-1_{eq}, H-5), 2.86–3.00 (m, 1H, H-1′$_a$), 3.07 (s, 2H, OCH_2Ad), 3.09–3.30 (m, 3H, H-1′$_b$, H-1_{ax}), 3.24 (dd, 1H, $J_{2,3}$*9.1*, $J_{3,4}$*8.6 Hz*, H-3), 3.46 (dd, 1H, $J_{4,5}$*9.5*, $J_{3,4}$*9.1 Hz*, H-4), 3.57 (ddd, 1H, $J_{1eq,2}$*10.9*, $J_{2,3}$*9.1*, $J_{1ax,2}$*4.9 Hz*, H-2), 3.89 (dd, 1H, $J_{6a,6b}$*11.8*, $J_{5,6a}$*2.7 Hz*, H-6_a), 3.96 (dd, 1H, $J_{6a,6b}$*11.8* $J_{5,6b}$*2.3 Hz*, H-6_b), 4.48 (t, 2H, *J 6.8 Hz*, H-4′), 4.58 (s, 2H, OCH_2-triazole), 7.96 (s, 1H, CH triazole). ^{13}C (90 MHz, CD_3OD) δ 22.0 (C-2′), 28.7 (C-3′), 29.7 (CH_{Ad}), 35.1 (C_{Ad}), 38.3 ($CH_{2\,Ad}$), 40.7 ($CH_{2\,Ad}$), 50.7 (C-4′), 53.2 (C-1), 56.0 (C-1′), 57.1 (C-6), 65.4 (OCH_2-triazole), 67.6(C-5), 69.0 (C-2), 70.2 (C-4), 79.1 (C-3), 82.7 (OCH_2Ad), 124.9 (CH triazole), 146.5 (C triazole). LRMS (ESI^+) 465.4 $[M+H]^+$ 85%, 487.4 $[M+Na]^+$,

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100%. HRMS Found 487.2892 $[M+Na]^+$. $C_{24}H_{40}N_4O_5Na$ requires 487.2896.

***N*-(6-Azidohexyl)-2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin 7b.** 2,3,4,6-Tetra-*O*-benzyl-1-deoxynojirimycin **4** (835 mg, 1.60 mmol) was subjected to general procedure A with **5b** to afford after flash silica gel column chromatography (Pet. Ether/EtOAc 4 : 1) 2,3,4,6-tetra-*O*-benzyl-*N*-(6-azidohexyl)-1-deoxynojirimycin **7b** (717 mg, 1.10 mmol, 69%).

R_f 0.14 (Pet. Ether/EtOAc 85 : 15). ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.09–1.48 (m, 6H, H-2′, H-3′, H4′), 1.55 (~p, 2H, *J 7.0 Hz*, H-5′), 2.21 (dd, 1H, $J_{1ax,1eq}$*10.9*, $J_{1eq,2}$*10.9 Hz*, H-1_{eq}), 2.26–2.36 (m, 1H, H-5), 2.48–2.61 (m, 1H, H-1′$_a$), 2.61–2.75 (m, 1H, H-1′$_b$), 3.08 (dd, 1H, $J_{1ax,1eq}$*10.9*, $J_{1ax,2}$*4.8 Hz*, H-1_{ax}), 3.22 (t, 2H, *J 7.0 Hz*, H-6′), 3.46 (dd, 1H, $J_{2,3}$*9.1*, $J_{3,4}$*8.6 Hz*, H-3), 3.52–3.70 (m, 4H, H-4, H-6_a, H-2, H-6_b), 4.44 (d, 1H, *J 10.9 Hz*, C*H*H Bn), 4.44 (d, 1H, *J 12.3 Hz*, CH*H* Bn), 4.49 (d, 1H, *J 12.3 Hz*, C*H*H Bn), 4.64 (d, 1H, *J 11.6 Hz*, C*H*H Bn), 4.69 (d, 1H, *J 11.6 Hz*, CH*H* Bn), 4.81 (d, 1H, *J 10.9 Hz*, C*H*H Bn), 4.89 (d, 1H, *J 10.9 Hz*, CH*H* Bn), 4.95 (d, 1H, *J 10.9 Hz*, CH*H* Bn), 7.10–7.39 (m, 20H, CH aromatic). ^{13}C (90 MHz, $CDCl_3$) δ 23.7 (C-4′), 26.6 (C-3′), 27.0 (C-2′), 28.8 (C-5′), 51.3 (C-6′), 52.2 (C-1′), 54.4 (C-1), 63.9 (C-5), 65.6 (C-6), 72.7 (CH_2 benzyl), 73.4 (CH_2 benzyl), 75.1 (CH_2 benzyl), 75.2 (CH_2 benzyl), 78.5 (C-2), 78.6 (C-4), 87.3 (C-3), 127.4 (CH aromatic), 127.5 (CH aromatic), 127.6 (CH aromatic), 127.8 (CH aromatic), 128.3 (CH aromatic), 128.3 (CH aromatic), 137.8 (C aromatic), 138.6 (C aromatic), 139.0 (C aromatic). LRMS (ESI^+) 649.5 $[M+H]^+$, 100%. HRMS Found 649.3745 $[M+H]^+$. $C_{40}H_{49}N_4O_4$ requires 649.3754.$[\alpha]_D^{20}$ 9.0 (*c* 1.1, CH_2Cl_2)

***N*-(6-{4-[Adamant-1-yl-methoxymethyl]-1*H*-1,2,3-triazol-1-yl}hexyl)-2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin 12b.** Cycloaddition of *N*-(6-azidohexyl)-1-deoxynojirimycin **7b** (300.8 mg, 0.464 mmol) was achieved using general procedure B. Further purification by silica gel flash column chromatography (Pet. Ether/EtOAc 8 : 2 to 1 : 1) gave *N*-(6-{4-[adamant-1-yl-methoxymethyl]-1*H*-1,2,3-triazol-1-yl]}hexyl)-2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin **12b** (367 mg, 0.430 mmol, 93%).

R_f 0.38 (Pet. Ether/EtOAc 8 : 2). ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.12–1.46 (m, 6H, H-2′, H-3′, H-4′), 1.52 (m, 6H, 3×$CH_{2\,Ad}$), 1.61–1.76 (m, 6H, 3×$CH_{2\,Ad}$), 1.86 (p, 2H, *J 7.3 Hz*, H-5′), 1.93–1.99 (m, 3H, 3×CH_{Ad}), 2.20 (dd, 1H, $J_{1eq,1ax}$*10.8 Hz*, $J_{1eq,2}$*10.8 Hz*, H-1_{eq}), 2.27–2.34 (m, 1H, H-5), 2.49–2.57 (m, 1H, H-1′$_a$), 2.63–2.71 (m, 1H, H-1′$_b$), 3.07 (dd, 1H, $J_{1eq,1ax}$*10.8*, $J_{1ax,2}$*4.5 Hz*, H-1_{ax}), 3.09 (s, 2H, OCH_2Ad), 3.46 (dd, 1H, $J_{2,3}$*9.2*, $J_{3,4}$*9.1 Hz*, H-3), 3.55 (dd, 1H, $J_{6a,6b}$*10.4*, $J_{5,6a}$*1.8 Hz*, H-6_a), 3.57 (dd, 1H, $J_{3,4}$ 9.1, $J_{4,5}$*9.1 Hz*, H-4), 3.65 (ddd, 1H, $J_{1eq,2}$*10.8*, $J_{2,3}$*9.1*, $J_{1ax,2}$*4.5 Hz*, H-2), 3.66 (dd, 1H, $J_{6a,6b}$*10.4*, $J_{5,6a}$*2.7 Hz*, H-6_b), 4.30 (t, 2H, *J 7.3 Hz*, H-6′), 4.44 (d, 1H, *J 10.9 Hz*, C*H*H Bn), 4.41–4.46 (m, 1H, CH*H* Bn), 4.48 (d, 1H, *J 12.3 Hz*, C*H*H Bn), 4.62 (s, 2H, OCH_2-triazole), 4.65 (d, *J 11.8 Hz*, 1H, C*H*H Bn), 4.70 (d, 1H, *J 11.8 Hz*, CH*H* Bn), 4.82 (d, *J 11.2 Hz*, 1H, C*H*H Bn), 4.89 (d, 1H, *J 10.9 Hz*, CH*H* Bn), 4.96 (d, 1H, *J 11.2 Hz*, CH*H* Bn), 7.08–7.40 (m, 20H, CH aromatic), 7.47 (s, 1H, CH triazole). ^{13}C NMR (90 MHz, $CDCl_3$) 23.9, 26.5, 27.0 (C-2′, C-3′, C-4′), 28.4 (CH_{Ad}), 30.4 (C-5′), 34.2 (C_{Ad}), 37.3($CH_{2\,Ad}$), 39.8($CH_{2\,Ad}$), 50.3 (C-6′), 52.3 (C-1′), 54.6 (C-1), 64.0 (C-5), 65.4 (OCH_2-triazole), 65.8 (C-6), 72.9 (CH_2 benzyl), 73.5 (CH_2 benzyl), 75.3 (CH_2 benzyl), 75.4 (CH_2 benzyl), 78.7 (C-2), 78.8 (C-4), 81.7 (OCH_2Ad), 87.5 (C-3), 122.0 (CH triazole), 127.5 (CH benzyl), 127.6 (CH benzyl), 127.7 (CH benzyl), 128.0 (CH benzyl), 128.4 (CH benzyl), 128.5 (CH benzyl), 137.9 (C benzyl), 138.7 (C benzyl), 139.1 (C benzyl), 146.1 (C triazole). LRMS (ESI^+) 853.6 $[M+H]^+$, 100%. HRMS Found 853.5258 $[M+H]^+$. $C_{54}H_{69}N_4O_5$ requires 853.5268. $[\alpha]_D^{20}$ 4.2 (*c* 1.0, CH_2Cl_2).

***N*-(6-{4-[Adamant-1-yl-methoxymethyl]-1*H*-1,2,3-triazol-1-yl]}hexyl-1-deoxynojirimycin 18.** *N*-(6-{4-[Adamant-1-yl-methoxymethyl]-1*H*-1,2,3-triazol-1-yl]}hexyl)-2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin **12b** (319 mg, 373 μmol) was hydrogenated following general procedure C. Further purification by flash chromatography ($CHCl_3/CH_3OH$ 8 : 2) gave *N*-(6-{4-[adamant-1-yl-methoxymethyl]-1*H*-1,2,3-triazol-1-yl]}hexyl-1-deoxynojirimycin **18** (191 mg, 362 μmol, 97%) as a white solid.

R_f 0.22 ($CHCl_3/CH_3OH$ 80 : 20). ^{1}H NMR (360 MHz, CD_3OD) δ 1.29–1.37 (m, 4H, H-3′, H-4′), 1.54–1.55 (m, 8H, 3×$CH_{2\,Ad}$ + H-2′), 1.65–1.77 (m, 6H, 3×$CH_{2\,Ad}$), 1.91–1.94 (m, 5H, 3×CH_{Ad}, H-5′), 2.23 (m, 1H, H-5), 2.26 (dd, 1H, $J_{1eq,1ax}$*11.4 Hz*, $J_{1eq,2}$*10.4 Hz*, H-1_{eq}), 2.56–2.70 (m, 1H, H-1′$_a$), 2.80–2.93 (m, 1H, H-1′$_b$), 3.03 (dd, 1H, $J_{1eq,1ax}$*11.4*, $J_{1ax,2}$*4.8 Hz*, H-1_{ax}), 3.06 (s, 2H, OCH_2Ad), 3.15 (dd, 1H, $J_{2,3}$*9.4*, $J_{3,4}$*9.3 Hz*, H-3), 3.37 (dd, 1H, $J_{3,4}$*9.4*, $J_{4,5}$*8.9 Hz*, H-4), 3.49 (ddd, 1H, $J_{1eq,2}$*10.4*, $J_{2,3}$*9.4*, $J_{1ax,2}$*4.8 Hz*, H-2), 3.85 (s, 1H, H-6_a), 3.86 (s, 1H, H-6_b), 4.41 (t, 2H, *J 7.0 Hz*, H-6′), 4.54 (s, 2H, OCH_2-triazole), 7.94 (s, 1H, CH triazole). ^{13}C NMR (90 MHz, CD_3OD) 25.0, 27.3, 27.8, 29.7, 31.2, 35, 38.3, 40.7, 51.2, 53.6, 57.3, 59.0, 65.3, 67.5, 70.3, 71.6, 80.2, 82.5, 124.8 (CH triazole), 146.3 (C triazole). LRMS (ESI^+) 493.4 $[M+H]^+$, 100%, 515.4$[M+Na]^+$, 70%. HRMS Found 493.3386 $[M+H]^+$. $C_{26}H_{45}N_4O_5$ requires 494.3390.

***N*-(8-Azidooctyl)-2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin 7c.** 2,3,4,6-Tetra-*O*-benzyl-1-deoxynojirimycin **4** (179 mg, 0.342 mmol), and **5c** (199 mg, 0.850 mmol) were reacted according to general procedure A to give after purification by flash chromatography (Pet. Ether/EtOAc 4 : 1) 2,3,4,6-tetra-*O*-benzyl-*N*-(8-azidooctyl)-1-deoxynojirimycin **7c** (161 mg, 0.238 mmol, 70%)

R_f (Pet. Ether/EtOAc 6 : 4). ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.05–1.45 (m, 10H, H-2′, H-3′, H-4′, H-5′,H-6′), 1.58 (~p, 2H, *J 7.0 Hz*, H-7′), 2.22 (dd, 1H, $J_{1ax,1eq}$*10.9*, $J_{1eq,2}$*10.4 Hz*, H-1_{eq}), 2.26–2.35 (m, 1H, H-5), 2.48–2.61 (m, 1H, H-1′$_a$), 2.61–2.74 (m, 1H, H-1′$_b$), 3.09 (dd, 1H, $J_{1ax,1eq}$*10.9*, $J_{1ax,2}$*4.5 Hz*, H-1_{ax}), 3.25 (t, 2H, *J 7.0 Hz*, H-8′), 3.45 (dd, 1H, $J_{2,3}$*9.1*, $J_{3,4}$*9.1 Hz*, H-3), 3.51–3.72 (m, 4H, H-4, H-6_a, H-2, H-6_b), 4.42 (d, 1H, *J 10.9 Hz*, C*H*H Bn), 4.44 (d, 1H, *J 12.3 Hz*, CH*H* Bn), 4.49 (d, 1H, *J 12.3 Hz*, C*H*H Bn), 4.64 (d, 1H, *J 11.7 Hz*, C*H*H Bn), 4.69 (d, 1H, *J 11.7 Hz*, CH*H* Bn), 4.81 (d, 1H, *J 11.1 Hz*, C*H*H Bn), 4.87 (d, 1H, *J 11.1 Hz*, CH*H* Bn), 4.95 (d, 1H, *J 10.9 Hz*, CH*H* Bn), 7.10–7.38 (m, 20H, CH aromatic). ^{13}C (90 MHz, $CDCl_3$) δ 23.7, 26.6, 27.3 (C-2′, C-3′, C-4′), 28.8 (C-7′), 29.1, 29.3 (C-5′, C6′), 51.4 (C-8′), 52.4 (C-1′), 54.4 (C-1), 63.9 (C-5), 65.6 (C-6), 72.7 (CH_2 benzyl), 73.4 (CH_2 benzyl), 75.1 (CH_2 benzyl), 75.2 (CH_2 benzyl), 78.6 (C-2), 78.6 (C-4), 87.3 (C-3), 127.4 (CH aromatic), 127.5 (CH aromatic), 127.6 (CH aromatic), 127.8 (CH aromatic), 128.3 (CH aromatic), 128.3 (CH aromatic), 137.8 (C aromatic), 138.6 (C aromatic), 139.0 (C aromatic). LRMS (EI) 677.4 $[M+H]^+$, 100%. LRMS (ESI^+) 677.4 $[M+H]^+$, 100%. HRMS Found 677.4055 $[M+H]^+$. $C_{42}H_{53}N_4O_4$ requires 677.4067. $[\alpha]_D^{20}$ 6.8 (*c* 1.1, CH_2Cl_2)

***N*-(8-{4-[Adamant-1-yl-methoxymethyl]-1*H*-1,2,3-triazol-1-yl]}octyl)-2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin 12c.** General procedure B was used with *N*-(8-azidooctyl)-2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin **7c** (309 mg, 0.457 mmol) and **11** to afford after purification by flash chromatography (Pet. Ether/EtOAc 8 : 2 to 1 : 1) *N*-(8-{4-[adamant-1-yl-methoxymethyl]-1*H*-1,2,3-triazol-1-yl]}octyl)-2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin **12c** (392 mg, 0.445 mmol, 97% yield).

R_f 0.20 (Pet. Ether/EtOAc 6 : 4). ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.03–1.45 (m, 10H, H-2′, H-3′, H-4′, H-5′, H-6′), 1.48–1.56 (m, 6H, 3 × $CH_{2\,Ad}$), 1.58–1.75 (m, 6H, 3 × $CH_{2\,Ad}$), 1.83–1.91 (m, 2H, H-7′), 1.91–1.98 (M, 3H, CH_{Ad}), 2.21 (dd, 1H, $J_{1ax,1eq}$*10.9*, $J_{1eq,2}$*10.9 Hz*, H-1_{eq}), 2.26–2.33 (m, 1H, H-5), 2.46–2.59 (m, 1H, H-$1'_a$), 2.60–2.72 (m, 1H, H-$1'_b$), 3.07 (dd, 1H, $J_{1ax,1eq}$*10.9*, $J_{1ax,2}$*5.0 Hz*, H-1_{ax}), 3.07 (s, 2H, OCH_2Ad), 3.44 (dd, 1H, $J_{2,3}$*9.1*, $J_{3,4}$*9.1 Hz*, H-3), 3.50–3.59 (m, 2H, H-4, H-6_a), 3.59–3;70 (m, 2H, H-2, H-6_b), 4.32 (t, 2H, *J 7.3 Hz*, H-8′), 4.41 (d, 1H, *J 10.9 Hz*, C*H*H Bn), 4.43 (d, 1H, *J 12.3 Hz*, CH*H* Bn), 4.47 (d, 1H, *J 12.3 Hz*, C*H*H Bn), 4.60 (s, 2H, triazole-CH_2O), 4.63 (d, 1H, *J 11.6 Hz*, C*H*H Bn), 4.68 (d, 1H, *J 11.6 Hz*, CH*H* Bn), 4.80 (d, 1H, *J 10.9 Hz*, C*H*H Bn), 4.86 (d, 1H, *J 10.9 Hz*, CH*H* Bn), 4.94 (d, 1H, *J 10.9 Hz*, CH*H* Bn), 7.09–7.37 (m, 20H, CH aromatic), 7.47 (s, 1H, CH triazole). ^{13}C (90 MHz, $CDCl_3$) δ 23.4, 27.3, 28.9, 29.2 (C-2′, C-3′,C-4′, C-5′), 26.4 (C-6′), 28.2 (CH_{Ad}), 30.3 (C-7′), 33.9 (C_{Ad}), 37.1 ($CH_{2\,Ad}$), 39.6 ($CH_{2\,Ad}$), 50.2 (C-8′), 52.3 (C-1′), 54.4 (C-1), 63.6 (C-5), 65.1 (triazole-CH_2O), 65.2 (C-6), 72.7 (CH_2 benzyl), 73.4 (CH_2 benzyl), 75.1 (CH_2 benzyl), 75.2 (CH_2 benzyl), 78.5 (C-2), 78.5 (C-4), 81.5 (OCH_2Ad), 87.3 (C-3), 121.8 (CH triazole), 127.4 (CH aromatic), 127.5 (CH aromatic), 127.6 (CH aromatic), 127.8 (CH aromatic), 127.9 (CH aromatic), 128.0 (CH aromatic), 128.1 (CH aromatic), 128.2 (CH aromatic), 128.3 (CH aromatic), 137.7 (C aromatic), 138.5 (C aromatic), 138.9 (C aromatic), 145.9 (C triazole). LRMS (ESI$^+$) 881.6 [M+H]$^+$, 100%. HRMS Found 881.5587 [M+H]$^+$. $C_{56}H_{73}N_4O_5$ requires 881.5581. $[\alpha]_D^{20}$ 6.4 (*c* 1.2, CH_2Cl_2)

***N*-(8-{4-[Adamant-1-yl-methoxymethyl]-1*H*-1,2,3-triazol-1-yl]}octyl-1-deoxynojirimycin 19.** Hydrogenolysis of *N*-(8-{4-[adamant-1-yl-methoxymethyl]-1*H*-1,2,3-triazol-1-yl]}octyl)-2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin **12c** (306 mg, 347 μmol) following procedure C gave after purification by flash silica gel column chromatography ($CHCl_3/CH_3OH$ 8 : 2) *N*-(8-{4-[adamant-1-yl-methoxymethyl]-1*H*-1,2,3-triazol-1-yl]}octyl-1-deoxynojirimycin **19** (175 mg, 335 μmol, 97%) as a white solid.

R_f 0.24 ($CHCl_3/CH_3OH$ 80 : 20). ^{1}H NMR (360 MHz, CD_3OD) δ 1.25–1.45 (m, 8H, H-3′, H-4′, H-5′, H-6′), 1.50–1.59 (m, 6H, 3×$CH_{2\,Ad}$), 1.62–1.82 (m, 8H, 3×$CH_{2\,Ad}$ + H-2′), 1.86–1.98 (m, 5H, 3×CH_{Ad}, H-7′), 2.98 (dd, 1H, $J_{1ax,1eq}$*12.3*, $J_{1eq,2}$*11.4 Hz*, H-1_{eq}), 3.00 (dd, 1H, $J_{4,5}$*10.0*, $J_{5,6a}$*2.3 Hz*, H-5), 3.06 (s, 2H, OCH_2Ad), 3.12–3.23 (m, 1H, H-$1'_a$), 3.31–3.41 (m, 1H, H-$1'_b$), 3.36 (dd, 1H, $J_{2,3}$*10.0*, $J_{3,4}$*9.5 Hz*, H-3), 3.44 (dd, 1H, $J_{1eq,1ax}$*12.3*, $J_{1ax,2}$*4.5 Hz*, H-1_{ax}), 3.59 (dd, 1H, $J_{4,5}$*10.0*, $J_{3,4}$*9.5 Hz*, H-4), 3.63–3.73 (m, 1H, H-2), 3.89 (dd, 1H, $J_{6a,6b}$*12.2*, $J_{5,6a}$*2.3 Hz*, H-6_a), 4.12 (d, 1H, $J_{6a,6b}$*12.3 Hz*, H-6_b), 4.41 (t, 2H, *J 6.8 Hz*, H-8′), 4.54 (s, 2H, OCH_2-triazole), 7.93 (s, 1H, CH triazole). ^{13}C NMR (90 MHz, CD_3OD) 24.1 (C-2′), 27.2, 27.4, 29.6, 29.9 (C-3′, C-4′, C-5′, C-6′), 29.7 (CH_{Ad}), 31.1 (C-7′), 35.1 (C_{Ad}), 38.3 ($CH_{2\,Ad}$), 40.7 ($CH_{2\,Ad}$), 51.2 (C-8′), 54.4 (C-1′), 54.9 (C-1, C-6), 65.3 (OCH_2-triazole), 67.4 (C-5), 67.8 (C-2), 68.8 (C-4), 78.2 (C-3), 82.5 (OCH_2Ad), 124.8 (CH triazole), 146.4 (C triazole). LRMS (ESI$^+$) 521.4 [M+H]$^+$, 100%, 543.4 [M+Na]$^+$, 50%. HRMS Found 521.3687 4 [M+H]$^+$. $C_{28}H_{49}N_4O_5$ requires 521.3703.

***N*-(10-Azidodecyl)-2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin 7d.** 2,3,4,6-Tetra-*O*-benzyl-1-deoxynojirimycin **4** (189 mg, 0.360 mmol) and **5d** were treated following general procedure A to give after purification by flash chromatography (Pet. Ether/EtOAc 9 : 1 to 1 : 1) 2,3,4,6-tetra-*O*-benzyl-*N*-(10-azidodecyl)-1-deoxynojirimycin **7d** (140 mg, 0.199 mmol, 55%).

R_f 0.38 (Pet. Ether/EtOAc 4 : 1). ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.06–1.45 (m, 12H, H-2′, H-3′, H-4′, H-5′,H-6′,H-7′, H-8′), 1.59 (~p, 2H, *J 7.0 Hz*, H-9′), 2.21 (dd, 1H, $J_{1ax,1eq}$*10.9*, $J_{1eq,2}$*10.4 Hz*, H-1_{eq}), 2.21 (dd, 1H, $J_{4,5}$*9.5*, $J_{5,6a}$*1.8*, $J_{5,6b}$*2.7 Hz*, H-5), 2.48–2.60 (m, 1H, H-$1'_a$), 2.60–2.73 (m, 1H, H-$1'_b$), 3.08 (dd, 1H, $J_{1ax,1eq}$*10.9*, $J_{1ax,2}$*4.5 Hz*, H-1_{ax}), 3.25 (t, 2H, *J 7.0 Hz*, H-10′), 3.45 (dd, 1H, $J_{2,3}$*9.1*, $J_{3,4}$*9.1 Hz*, H-3), 3.54 (dd, 1H, $J_{6a,6b}$*10.4*, $J_{5,6a}$*1.8 Hz*, H-6_a), 3.58 (dd, 1H, $J_{3,4}$*9.1*, $J_{4,5}$*9.5 Hz*, H-4), 3.65 (dd, 1H, $J_{6a,6b}$*10.4*, $J_{5,6b}$*2.7 Hz*, H-6_b), 3.65 (ddd, 1H, $J_{1eq,2}$*10.9*, $J_{2,3}$*9.1*, $J_{1ax,2}$*4.5 Hz*, H-2), 4.42 (d, *J 10.9 Hz*, 1H, C*H*H Bn), 4.44 (d, 1H, *J 12.3 Hz*, CH*H* Bn), 4.48 (d, 1H, *J 12.3 Hz*, C*H*H Bn), 4.64 (d, 1H, *J 11.6 Hz*, C*H*H Bn), 4.68 (d, 1H, *J 11.6 Hz*, CH*H* Bn), 4.80 (d, 1H, *J 11.0 Hz*, C*H*H Bn), 4.87 (d, 1H, *J 10.9 Hz*, CH*H* Bn), 4.94 (d, 1H, *J 11.0 Hz*, CH*H* Bn), 7.10–7.38 (m, 20H, CH aromatic). ^{13}C (90 MHz, $CDCl_3$) δ 23.6, 26.7, 27.5 (C-2′, C-3′,C-4′), 28.8 (C-9′), 29.1, 29.4, 29.5 (C-5′, C6′, C-7′, C-8′), 51.5 (C-10′), 52.4 (C-1′), 54.5 (C-1), 63.8 (C-5), 65.4 (C-6), 72.7 (CH_2 benzyl), 73.4 (CH_2 benzyl), 75.1 (CH_2 benzyl), 75.3 (CH_2 benzyl), 78.6 (C-2), 78.6 (C-4), 87.4 (C-3), 127.4 (CH aromatic), 127.5 (CH aromatic), 127.6 (CH aromatic), 127.8 (CH aromatic), 128.3 (CH aromatic), 137.8 (C aromatic), 138.6 (C aromatic), 139.1 (C aromatic). LRMS (ESI$^+$) 705.4 [M+H]$^+$, 100%. HRMS Found 705.4374 [M+H]$^+$. $C_{44}H_{57}N_4O_4$ requires 705.4380. $[\alpha]_D^{20}$ 7.3 (*c* 1.1, CH_2Cl_2)

***N*-(10-{4-[Adamant-1-yl-methoxymethyl]-1*H*-1,2,3-triazol-1-yl]}decyl)-2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin 12d.** A solution of **13d** (154 mg, 0.218 mmol) and **10** (67 mg, 0,327 mmol) was subjected to general procedure B. Further purification by flash chromatography (Pet. Ether/EtOAc 8 : 2 to 1 : 1) gave **12d** (190 mg, 0.209 mmol, 96%).

R_f 0.32 (Pet. Ether/EtOAc 6 : 4). ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.04–1.44 (m, 14H, H-2′, H-3′, H-4′, H-5′,H-6′,H-7′, H-8′), 1.49–1.55 (m, 6H, 3 × $CH_{2\,Ad}$), 1.59–1.75 (m, 6H, 3 × $CH_{2\,Ad}$), 1.84–1.98 (m, 5H, H-7′, 3 × CH_{Ad}), 2.21 (dd, 1H, $J_{1ax,1eq}$*10.9*, $J_{1eq,2}$*10.4 Hz*, H-1_{eq}), 2.29 (dd, 1H, $J_{4,5}$*10.4*, $J_{5,6a}$*2.3*, $J_{5,6b}$*2.3 Hz*, H-5), 2.50–2.60 (m, 1H, H-$1'_a$), 2.61–2.71 (m, 1H, H-$1'_b$), 3.07 (s, 2H, OCH_2Ad), 3.08 (dd, 1H, $J_{1ax,1eq}$*10.9*, $J_{1ax,2}$*4.8 Hz*, H-1_{ax}), 3.45 (dd, 1H, $J_{2,3}$*9.1*, $J_{3,4}$*9.1 Hz*, H-3), 3.54 (dd, 1H, $J_{6a,6b}$*10.4*, $J_{5,6a}$*2.3 Hz*, H-6_a), 3.58 (dd, 1H, $J_{4,5}$*9.5*, $J_{3,4}$*9.1 Hz*, H-4), 3.65 (ddd, 1H, $J_{1eq,2}$*10.4*, $J_{2,3}$*9.1*, $J_{1ax,2}$*4.8 Hz*, H-2), 3.65 (dd, 1H, $J_{6a,6b}$*10.4*, $J_{5,6b}$*2.3 Hz*, H-6_b), 4.32 (t, 2H, *J 7.3 Hz*, H-10′), 4.41 (d, 1H, *J 10.9 Hz*, C*H*H Bn), 4.44 (d, 1H, *J 12.3 Hz*, CH*H* Bn), 4.48 (d, 1H, *J 12.3 Hz*, C*H*H Bn), 4.60 (s, 2H, triazole-CH_2O), 4.64 (d, 1H, *J 11.8 Hz*, C*H*H Bn), 4.68 (d, 1H, *J 11.8 Hz*, CH*H* Bn), 4.80 (d, 1H, *J 10.9 Hz*, C*H*H Bn), 4.84 (d, 1H, *J 10.9 Hz*, CH*H* Bn), 4.94 (d, 1H, *J 10.9 Hz*, CH*H* Bn), 7.09–7.35 (m, 20H, CH aromatic), 7.47 (s, 1H, CH triazole). ^{13}C (90 MHz, $CDCl_3$) δ 23.7, 27.4, 29.0, 29.3, 29.4 (C-2′, C-3′,C-4′, C-5′, C-6′, C-7′), 26.5 (C-8′), 28.2 (CH_{Ad}), 30.3 (C-9′), 34.0 (C_{Ad}), 37.2 ($CH_{2\,Ad}$), 39.6 ($CH_{2\,Ad}$), 50.3 (C-10′), 52.4 (C-1′), 54.4 (C-1), 63.8 (C-5), 65.2 (triazole-CH_2O), 65.5 (C-6), 72.7 (CH_2 benzyl), 73.4

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(CH_2 benzyl), 75.1 (CH_2 benzyl), 75.2 (CH_2 benzyl), 78.5 (C-2), 78.6 (C-4), 81.5 (OCH_2Ad), 87.3 (C-3), 121.8 (CH triazole), 127.3 (CH aromatic), 127.4 (CH aromatic), 127.5 (CH aromatic), 127.8 (CH aromatic), 128.2 (CH aromatic), 128.3 (CH aromatic), 128.5 (CH aromatic), 137.8 (C aromatic), 138.6 (C aromatic), 139.0 (C aromatic), 145.9 (C triazole). LRMS (ESI^+) 909.6 $[M+H]^+$, 100%. HRMS Found 909.5875 $[M+H]^+$. $C_{58}H_{77}N_4O_5$ requires 909.5894. $[\alpha]_D^{20}$ 7.7 (*c* 1.1, CH_2Cl_2)

***N*-(10-{4-[Adamant-1-yl-methoxymethyl]-1*H*-1,2,3-triazol-1-yl]}decyl)-1-deoxynojirimycin 20.** *N*-(10-{4-[Adamant-1-yl-methoxymethyl]-1*H*-1,2,3-triazol-1-yl]}decyl)-2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin **12d** (141 mg, 155 μmol) was subjected to general procedure C. Further purification by flash chromatography ($CHCl_3/CH_3OH$ 8 : 2) gave *N*-(10-{4-[adamant-1-yl-methoxymethyl]-1*H*-1,2,3-triazol-1-yl]}decyl)-1-deoxynojirimycin **20** (88 mg, 150 μmol, 97% yield) as a pale yellow solid.

R_f 0.26 ($CHCl_3/CH_3OH$ 80 : 20). ^1H NMR (360 MHz, CD_3OD) δ 1.22–1.41 (m, 12H, H-3′, H-4′, H-5′, H-6′), 1.44–1.61 (m, 8H, 3×$CH_{2\,Ad}$ + H-2′), 1.62–1.80 (m, 6H, 3×$CH_{2\,Ad}$), 1.85–1.98 (m, 5H, 3×CH_{Ad}, H-9′), 2.23–2.39 (m, 2H, H-1_{eq}, H-5), 2.61–2.74 (m, 1H, H-$1'_a$), 2.84–2.95 (m, 1H, H-$1'_b$), 3.05 (s, 2H, OCH_2Ad), 3.07 (dd, 1H, $J_{1eq,1ax}$*11.3*, $J_{1ax,2}$*4.8 Hz*, H-1_{ax}), 3.17 (dd, 1H, $J_{2,3}$*9.1*, $J_{3,4}$*9.1 Hz*, H-3), 3.39 (dd, 1H, $J_{4,5}$*9.5*, $J_{3,4}$*9.1 Hz*, H-4), 3.51 (ddd, 1H, $J_{1eq,2}$*10.7*, $J_{2,3}$*9.1*, $J_{1ax,2}$*4.8 Hz*, H-2), 3.85 (dd, 1H, $J_{6a,6b}$*12.1*, $J_{5,6a}$*2.8 Hz*, H-6_a), 3.89 (dd, 1H, $J_{6a,6b}$*12.1*, $J_{5,6b}$*2.3 Hz*, H-6_b), 4.40 (t, 2H, *J 6.9 Hz*, H-10′), 4.54 (s, 2H, OCH_2-triazole), 7.93 (s, 1H, CH triazole). ^{13}C NMR (90 MHz, CD_3OD) 25.1 (C-2′), 27.4 (C-8′), 28.4, 30.0, 30.5 (C-3′, C-4′, C-5′, C-6′, C-7′), 29.7 (CH_{Ad}), 31.2 (C-9′), 35.0 (C_{Ad}), 38.3 ($CH_{2\,Ad}$), 40.7 ($CH_{2\,Ad}$), 51.3 (C-10′), 53.9 (C-1′), 57.2 (C-1), 58.7 (C-6), 65.3 (OCH_2-triazole), 67.4 (C-5), 70.2 (C-2), 71.5 (C-4), 80.1 (C-3), 82.4 (OCH_2Ad), 124.9 (CH triazole), 146.4 (C triazole). LRMS (ESI^+) 549.4 $[M+H]^+$, 100%, 571.5 $[M+Na]^+$, 50%. HRMS Found 549.4003 $[M+H]^+$. $C_{30}H_{53}N_4O_5$ requires 549.4016.

Other compounds

NB-DNJ was provided by Pharmacia. AMP-DNJ was a generous gift of Prof. Robert Moriarty, University of Illinois at Chicago, USA). Compounds were greater than 95% pure when analysed by 500 MHz NMR.

Glycosydases inhibition profiling[53]

The animal experimental protocols with live animals were approved by the Animal Experiments Committee of the University of Toyama (S-2010 UH-2). Male Wistar rats with body weight of 130 g were obtained from Japan SLC, Inc. (Hamamatsu, Japan). Brush border membranes were prepared from the rat small intestine according to the method of Kessler *et al.*, and were assayed at pH 5.8 for rat intestinal maltase, isomaltase, sucrase, cellobiase, and lactase using the appropriate disaccharides as substrates. The released D-glucose was determined colorimetically using Glucose CII-test Wako (Wako Pure Chemical Ind., Osaka, Japan). Other glycosidase activities were determined using appropriate *p*-nitrophenyl glycoside as a substrate at the optimum pH of each enzyme. The reaction was stopped by adding 400 mM Na_2CO_3. The released *p*-nitrophenol was measured spectrometrically at 400 nm.

Cell culture

HL60 were cultured in RPMI media containing 10% foetal calf serum, 2 mM L-glutamine and 1% of a stock solution of penicillin-streptomycin (Invitrogen). The human airway epithelial CF-KM4 cells were used to evaluate the efficacy of compounds on F508del-CFTR function. Cells were cultured at 37 °C in 5% CO_2 under standard culture conditions as previously described.[54]

Cell proliferation assay for ER α-glucosidase assay

The effects of compounds on HL60 cell proliferation were determined using a Promega CellTiter 96® AQueous Cell Proliferation Assay as described previously.[42] Cells were seeded at a density of 5 × 105 per mL in 96 well plates with or without a range of concentrations of compound and incubated at 37 °C for 16 h. MTS reagent (20 μL) was added to each well, the plates mixed and incubated at 37 °C for 1 h. The absorbance was read at 490 nm using a UV max Kinetic microplate reader (Molecular Devices) to determine the amount of formazan product, which is directly proportional to the number of living cells in culture. The mean CC50 (concentration of compound required to reduce proliferation by 50%) and standard error was then calculated for each compound relative to untreated cells based on the results of three experiments.

Free oligosaccharides (FOS) Isolation from Cells

Cells were cultured to high density (1 × 107 cells mL^{-1}) before the medium was replaced with fresh medium containing inhibitor at varying concentrations and the cells were seeded at a lower density so as to achieve a high density at the end of the incubation period. Following cell culture the medium was removed and the cells were washed 3 times with PBS by centrifugation. Washed cells were stored at –20 °C for a short time before thawing and Dounce homogenisation in water. The conditions for extraction of FOS were optimised to maximise recovery of FOS. The homogenate was desalted and deproteinated by passage through a mixed bed ion exchange column [0.2 mL AG50W-X12 (H^+, 100–200 mesh) over 0.4 mL AG3–X4 (OH^-, 100–200 mesh)], pre-equilibrated with water (5 × 1 mL). The homogenate was added and collected with 4 × 1 mL water washes. The extracted purified FOS was dried under vacuum and dissolved in 30 μL water.

Inhibition of glycolipid biosynthesis

To determine the inhibition of ceramide glucosyltransferase activity in a cell-based assay, HL60 cells were cultured in the presence of various concentrations (0–100 μM) of compound for 3 days until confluence. Cells were harvested and washed with PBS before re-suspension in water and Dounce homogenisation. An aliquot of this homogenate was taken for protein assay. The remainder was made 4 : 8 : 3 (v/v/v) chloroform : methanol : water to extract glycolipids as described.[55] Extracted glycolipids were hydrolysed overnight at 37 °C using a preparation of ceramide glycanase (purified in house from Hirudo medicinalis) in 20 μL of 50 mM sodium acetate buffer, pH 5.0, containing 1 mg mL^{-1}

sodium taurodeoxycholate. Glycolipid-derived oligosaccharides were made to 30 μL with water and labelled with anthranilic acid (2-AA) as described below. Labelled oligosaccharides were analysed by NP-HPLC as described below.[55,56]

Carbohydrate fluorescent labelling

FOS and glycolipid derived oligosaccharides were labelled with anthranilic acid as described previously (Neville *et al.*, 2004).[55] Briefly, anthranilic acid (30 mg mL^{-1}) was dissolved in a solution of sodium acetate trihydrate (4%, w/v) and boric acid (2% w/v) in methanol. This solution was added to sodium cyanoborohydride (final concentration 45 mg mL^{-1}) and mixed to give the final labelling mixture. 2-AA labeling mixture (80 μL) was added to FOS samples (30 μL water) or glycolipid-derived oligosaccharides followed by incubation at 80 °C for 1 h. The reaction was allowed to cool to room temperature, 1 mL acetonitrile/water (97 : 3, v/v) was added, and the mixture was vortexed. Labelled oligosaccharides were purified by chromatography through Speed Amide 2 columns (Applied Separations, Allentown, USA). The columns were pre-equilibrated with 2 × 1 mL acetonitrile, 2 × 1 mL water followed by 2 × 1 mL acetonitrile. The samples were loaded using gravity flow and allowed to drip through the column. The column was washed with 2 × 1 mL acetonitrile/water (95 : 5, v/v) and labelled oligosaccharides eluted with 2 × 0.75 mL water.

Purification of fluorescently labelled FOS

Labelled FOS in 50 mM Tris/HCl buffer, pH 7.2 were purified through Concanavalin A (Con A)-Sepharose 4B column (100 μL packed resin). The column was pre-equilibrated with 2 × 1 mL water followed by 1 mL of 1 mM $MgCl_2$, 1 mM $CaCl_2$ and 1 mM $MnCl_2$ in water and finally 2 × 1 mL 50 mM TrisHCl buffer, pH 7.2. The sample was added and washed with 2 × 1 mL 50 mM TrisHCl buffer, pH 7.2. The bound FOS were then eluted with 2 × 1 mL hot (70 °C) 0.5 M methyl-α-D-mannopyranoside in 50 mM TrisHCl buffer, pH 7.2.

Carbohydrate analysis by normal-phase high performance liquid chromatography (NP-HPLC)

ConA-Sepharose purified 2-AA-labelled free oligosaccharides and glycolipid-derived oligosaccharides were separated by NP-HPLC using a 4.6 × 250 mM TSK gel Amide-80 column (Sigma, UK) according to previously published methods.[38,55] The chromatography system consisted of a Waters Alliance 2695 separations module and an in-line Waters 474 fluorescence detector set at Exλ 360 nm and Emλ 425 nm. All chromatography was performed at 30 °C. Solvent A was acetonitrile. Solvent B was Milli-Q water. Solvent C was composed of 100 mM ammonium hydroxide, titrated to pH 3.85 with acetic acid, in Milli-Q water and was prepared using a standard 5.0 N ammonium hydroxide solution (Sigma, UK). Gradient conditions were as follows: time = 0 min (t = 0), 71.6% A, 8.4% B, 20% C (0.8 mL min^{-1}); t = 6, 71.6% A, 8.4% B, 20% C (0.8 mL min^{-1}); t = 6, 71.6% A, 8.4% B, 20% C (0.8 mL min^{-1}); t = 40, 52% A, 28% B, 20% C (0.8 mL min^{-1}); t = 41, 23% A, 57% B, 20% C (1.0 mL min^{-1}); t = 43, 23% A, 57% B, 20% C (1.0 mL min^{-1}); t = 44, 71.6% A, 8.4% B, 20% C (1.2 mL min^{-1}); t = 59, 71.6% A, 8.4% B, 20% C (1.2 mL min^{-1}); t = 60, 71.6% A, 8.4% B, 20% C (0.8 mL min^{-1}). Samples (<50 μL) were injected in Milli-Q water/acetonitrile (1 : 1, v/v).

Peak areas of FOS corresponding to $Glc_1Man_4GlcNAc_1$ and $Glc_3Man_5GlcNAc_1$ species were analysed and used as a measure for α-glucosidase II and α-glucosidase I inhibition, respectively, in the presence of imino sugar.[38,39] For GSL analysis, peak areas corresponding to monosialyl-ganglioside GM3 were measured in response to inhibitor treatment to generate inhibition constants.[57]

β-Glucocerebrosidase inhibition assay

Human placental β-glucocerebrosidase was isolated and purified by a modified procedure of Furbish *et al.*[58] Enzyme activity was measured in 50 μl of 5 mM 4-methylumbelliferyl-β-glucoside (4-MU-β-glucoside) in 0.1 M citrate phosphate buffer, pH 5.2 containing 0.25% sodium taurocholate, 0.1% TX100 at 37 °C for 15–60 min. The reaction was stopped by the addition of 200 μl 0.5 M sodium carbonate and the fluorescence measured at ex 350 nm, em 460 nm. Inhibition constants (IC_{50}) were generated for placental β-glucocerebrosidase (K_m for 4-MU-β-glucoside, 1.9 ± 0.3 mM) using 0.5 mM substrate concentration. Determinations were made in triplicate. Data were fitted using Hill Slope plots (Prizm software) and symmetrical standard errors determined for each IC_{50} value.

F508del-CFTR restoration assay

CFTR Cl^- channel activity was assayed by single-cell fluorescence imaging using the potential-sensitive probe, bis-(1,3-diethylthiobarbituric acid)trimethine oxonol.[8] The results of fluorescence imaging are presented as transformed data to obtain the percentage signal variation (F_x) relative to the time of addition of the stimulus, according to the equation: $F_x = ([F_t - F_0]/F_0) \times 100$ where F_t and F_0 are the fluorescent values at the time t and at the time of addition of the stimulus, respectively. For histogram representation, the values correspond to the level of stable variation of fluorescence induced by each drug.

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