Synthetic deoxynojirimycin derivatives bearing a thiolated, fluorinated or unsaturated *N*-alkyl chain: identification of potent α-glucosidase and trehalase inhibitors as well as F508del-CFTR correctors

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Electronic Supplementary Information (ESI) available: Copies of ^{1}H, ^{19}F and ^{13}C spectra of all new compounds

The synthesis of eleven 1-deoxynojirimycin (DNJ) derivatives presenting either a monofluoro, difluoro, thiolated or unsaturated *N*-alkyl chain of various length is described. Exploiting the unsaturated moiety on the nitrogen, fluorine has been introduced through a HF/SbF_5 superacid catalysed hydrofluorination and thiol-ene click chemistry allowed introduction of sulfur. The synthetic derivatives have been tested for their ability to inhibit glycosidases and correct F508del-CFTR. Two of the unsaturated iminosugars exhibited potency similar to Miglustat as F508del-CFTR correctors. The thioalkyl iminosugars as well as the corresponding alkyl iminosugars demonstrated low micromolar α-glucosidases and trehalases inhibition. Introduction of fluorine abolished F508del-CFTR correction and trehalase inhibition.

Introduction

The single replacement of the endocyclic oxygen by a nitrogen atom in monosaccharides leads to iminosugars, a class of sugar mimics with high therapeutic potential.[i] Its most famous representative, 1-deoxynojirimycin (DNJ), is a natural product[ii] that exhibits potent α- and β-glucosidases inhibition. This molecule has been since converted into two approved medicines, Zavesca® and Miglitol® targeting Gaucher's disease[iii] and type II diabetes[iv] respectively. A rather simple structural modification, the alkylation of the endocyclic nitrogen with either a butyl or a hydroxyethyl chain, has been necessary to convert DNJ into these two therapeutics (Figure 1). Such a dramatic effect has prompted many research groups to introduce a vast array of functional groups at the endocyclic nitrogen of DNJ including alkyl chains,[v] elaborated substituents[vi] and fluorescent moieties[vii] to inactivate specific glycosyl processing enzymes or act as probes to label these latter (Figure 1). Functionalized alkyl chains have been also introduced to allow further chemical derivatisation including the popular copper-catalyzed azide-alkyne cycloaddition (CuAAC).[viii] As a part of our continuous efforts in the area of iminosugars,[ix] we were interested in evaluating the impact of the introduction of an unsaturated N-alkyl chain on DNJ on its glycosidase inhibitory profile. Such modification might positively modify the inhibition profile of this α-glucosidase inhibitor. This insaturation was further used as a handle to incorporate a sulfur atom or fluorine atoms in the alkyl chain.

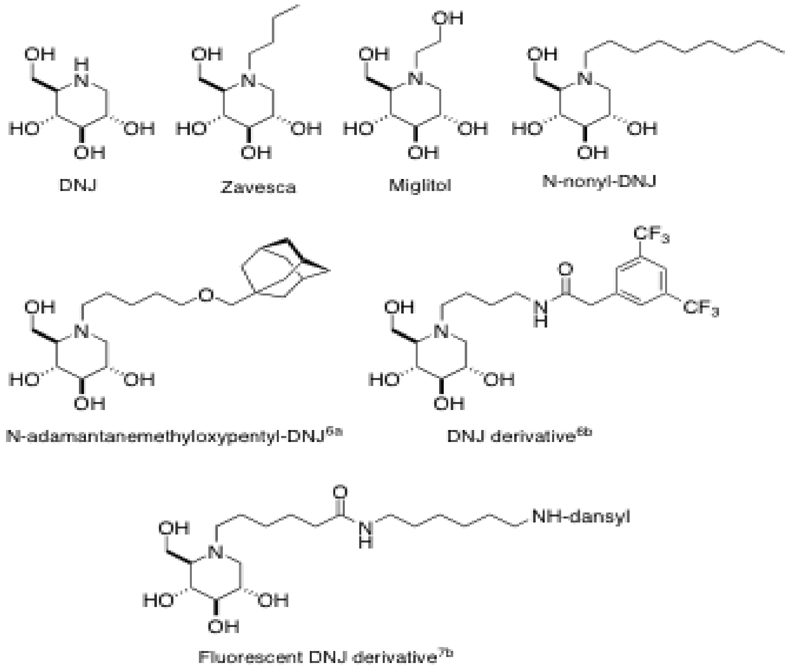


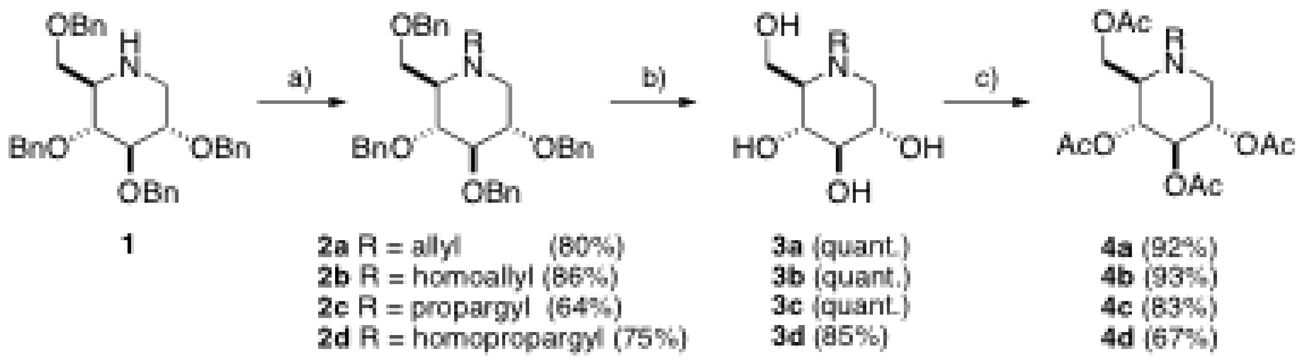
Figure 1: Structure of DNJ and representative derivatives

Results and discussion

Synthesis

Synthesis of unsaturated derivatives

The synthesis of the DNJ derivatives displaying an unsaturated *N*-alkyl chain started from the easily available tetra-*O*-benzyl DNJ **1**.[x] *N*-Alkylation of **1** with the allyl, homoallyl, propargyl and homopropargyl bromides in acetonitrile or ethyl acetate/water in the presence of K_2CO_3 under reflux yielded the corresponding *N*-alkyl derivatives **2a-2d** in good yields (64-86%). In order to preserve the insaturation, removal of the benzyl groups was achieved with BCl_3 at -78°C in dry CH_2Cl_2 to yield the target new **3a** and **3b** and known **3c**[11a] and **3d**[xib] iminosugars in high yield. These iminosugars were also peracetylated to provide the tetraacetylated iminosugars **4a-d** (67-93% yield) available for further chemical transformation performed on the *N*-alkyl chain (Scheme 1).

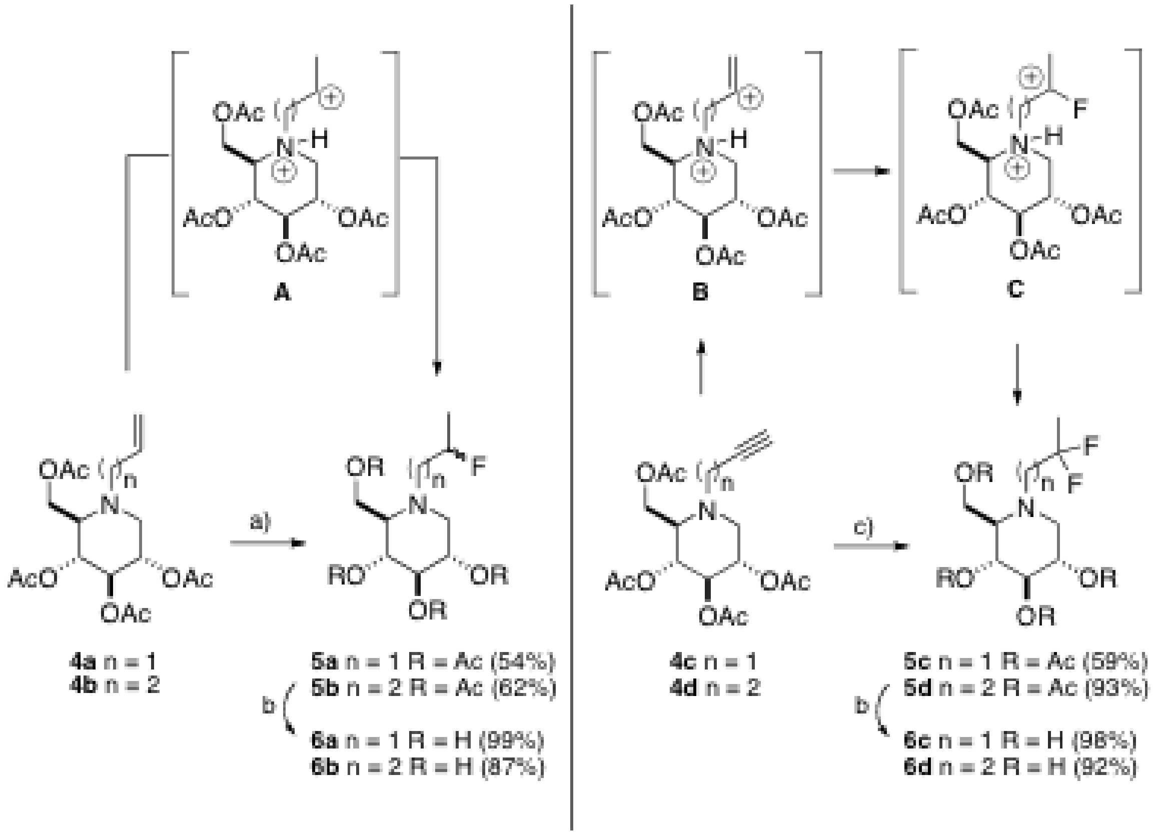


Reagents and conditions: a) RBr, K_2CO_3, CH_3CN or EtOAc/H_2O; b) BCl_3, CH_2Cl_2, - 78°C; c) Ac_2O, pyridine.

Scheme 1: Synthesis of unsaturated *N*-alkyl iminosugars **3a-d** and peracetylated derivatives **4a-d**

Synthesis of fluorinated derivatives

The role of fluorine in medicinal chemistry is well recognized.[xii] For nitrogen containing compounds, the modification of the basicity of the nitrogen containing proximal functions, by using fluorine strong electron withdrawing effect,[xiii] has been largely used in medicinal chemistry SAR studies.[xiv] It is also accepted that through a fluorine gauche effect and through electrostatic interactions, fluorine atoms can strongly modify the preferred conformation of nitrogen containing biomolecules.[xv] Regarding iminosugars, introduction of fluorinated alkyl substituents on the endocyclic nitrogen is scarce but yielded compounds with promising biological activities.[xvi] We reasoned that the unsaturation on the alkyl chain could be used to perform a regioselective hydrofluorination[xvii] using an in house expertise in superacid chemistry.[xviii] Such methodology generates harsh conditions that are not compatible so far with most of the sugar protecting groups except the acetate.[xix] Satisfyingly, treatment of iminosugars **4a** and **4b** in neat HF/SbF_5 (7:1 ratio v:v) at -20°C for 10 min furnished the corresponding monofluorinated iminosugars **5a** and **5b** as inseparable diastereomeric mixtures in satisfactory yield (52-62%) in which the fluorine atom is attached at the last but one carbon of the alkyl chain. Subsequent peracetylation of the crude product was necessary to obtain the target sugar analogues in decent yield as some deacetylation was observed during the process. The regioselectivity of the fluorination can be tentatively explained by the formation of a transient superelectrophilic dication[xx] **A**, the ammonium cation activating the nearby electrophilic carbon cation allowing its fluorination, and thus despite the very low nucleophilic character of the fluoride ions in their antimony complexed forms.[xxi] Introduction of a gem-difluoromethylene group on the alkynes **4c** and **4d** was next examined and requires stronger fluorinating conditions that can be achieved by increasing the SbF_5/HF ratio and the temperature. Treatment of alkynes **4c** and **4d** in neat HF/SbF_5 (3:1 ratio) at 0°C for 10 min furnished the corresponding gem-difluorinated iminosugars **5c** and **5d** in satisfactory yield (59-93%). In this case, two successive regioselective superacid-catalyzed hydrofluorinations, involving the superelectrophilic dications **B** and **C**, accounts for the formation of the desired difluorinated product.[xxii] Final deprotection of the fluorinated derivatives **5a-d** required some tuning of the conditions as these compounds are rather sensitive to base. While use of Zemplen conditions (Na, MeOH) provided the starting alkenes and alkynes through HF elimination, milder conditions (triethylamine, methanol) furnished the desired fluorinated iminosugars **6a-d** in excellent yield (87-99%) (Scheme 2).

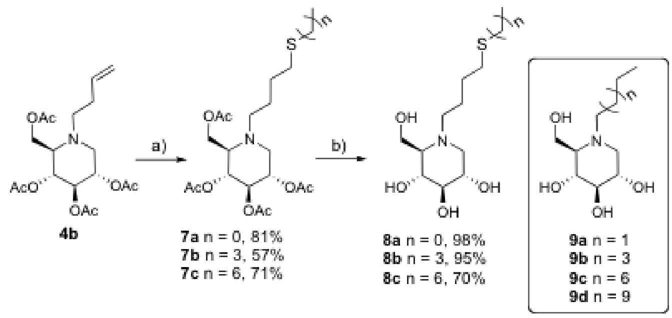


Reagents and conditions: a) HF/SbF_5 (7:1 v:v), - 20°C, 10 min then Ac_2O, pyridine, rt, overnight; b) Et_3N (4 eq.), MeOH, rt, 1-3 days; c) HF/SbF_5 (3:1 v:v), 0°C, 10 min then Ac_2O, pyridine, rt, overnight.

Scheme 2: Synthesis of fluorinated *N*-alkyl DNJ **6a-d**

Synthesis of thiolated derivatives

Terminal alkenes are useful appendages to introduce additional functional groups exploiting the thiol-ene click chemistry.[xxiii] This strategy has been extensively used in carbohydrate chemistry[xxiv] but there is only one example involving iminosugars to the best of our knowledge.[xxv] We thought this methodology could be helpful to study the influence of the introduction of a sulfur atom in the alkyl chain. Previous studies emphasized the positive role played by a single oxygen atom introduced in the chain regarding glucosylceramide metabolism inhibition.[xxvi] To this end, homoallyl derivative **4b** was treated with 3 equiv. of alkyl thiol in the presence of catalytic 2,2-dimethoxy-2-phenylacetophenone (DPAP) under UV irradiation to generate the expected thioalkyl DNJ derivatives **7a-c** in 57-81% yield. Final deprotection (Et_3N, MeOH) furnished the desired iminosugars **8a-c** in 70-98% yield. For comparison purposes, the known N-butyl **9a** (Zavesca®)[3] N-hexyl **9b**,[5b] N-nonyl **9c**[xxvii] and N-dodecyl **9d**[5b] DNJ derivatives (Scheme 3) were also synthesized according to literature procedures.[xxviii]



Reagents and conditions: a) alkyl thiol, DPAP, MeOH, hν, rt, 30-60 min; b) Et_3N (7 eq.), MeOH, rt, 1-3 days.

Scheme 3: Synthesis of thiolated iminosugars **8a-c** and structure of *N*-alkyl DNJ **9a-d**

Biological activity

Inhibition of glycosidases

Polyhydroxylated piperidines **3a-d, 6a-d, 8a-c, 9a-d** were assayed as inhibitors of a collection of sixteen glycosidases, including glucosidases, galactosidases, mannosidases, fucosidases, glucuronidase, trehalase, amyloglucosidase and rhamnosidase. All compounds proved to retain the α-glucosidase inhibition of DNJ, being submicromolar inhibitors of rice α-glucosidase and low micromolar inhibitors of rat intestinal maltase. Most of them also demonstrated low micromolar inhibition of HL60 glucosyl transferase (Table 1, see ESI). Interestingly, while unsaturated and fluorinated derivatives **3a-c** and **6a-d** poorly inhibited α,α-trehalase, *Aspergillus niger* and *Rhizopus* amyloglucosidases, almond β-glucosidase and α-L-rhamnosidase, the *N*-thioalkyl derivatives **8a-c** showed some inhibition toward these enzymes and were especially potent toward porcine kidney α,α-trehalase. Similar potency was observed for the corresponding C-alkyl derivatives **9b-d** (Table 2, see ESI). This result forced us to explore this trehalase inactivation further.

Trehalase inhibition

Trehalase is an inverting glycosidase[xxix] belonging to the GH37 family of the carbohydrate-active enzyme (CAZy) classification[xxx] that catalyses trehalose hydrolysis, a reaction fundamental for insect flight[xxxi] and spore germination of fungi. Due to the biological relevance of trehalase inhibitors as fungicides, insecticides or antibiotics, several trehalose mimics have been synthesized.[xxxii] In this context, and to confirm the potency and evaluate the selectivity of compounds **8a-c** and **9b-d** toward α,α-trehalases, these molecules were tested for their inhibitory activity against insect trehalase of midge larvae of C. riparius,[xxxiii] a good model for biochemical studies and porcine kidney trehalase as the mammalian counterpart. As shown from the IC_{50} values, compounds **8a-c** and **9b-d** potently inhibit insect and mammalian trehalases with inhibition in the low micromolar range. Unfortunately, no selectivity toward insect trehalases could be observed.

compound	IC_{50} *C. riparius* trehalase (μM)	IC_{50} porcine trehalase (μM)
8a	2.19 ± 0.32	1.21 ± 0.30
8b	1.46 ± 0.04	0.46 ± 0.05
8c	3.75 ± 0.28	1.66 ± 0.25
9b	5.87 ± 0.31	2.22 ± 0.04
9c	6.94 ± 0.36	0.94 ± 0.06
9d	1.99 ± 0.15	0.27 ± 0.06

IC_{50} values are given in μM ± S.D.

Table 3. Effect of compounds **8a-c** and **9b-d** on insect and mammalian trehalases.

Correction of F508del-CFTR function

Iminosugars have been identified as pharmacological chaperones that can stabilize or correct the structure of misfolded proteins. The Cystic Fibrosis Transmembrane conductance Regulator (CFTR) protein is glycosylated, even though it does not involve any sugar metabolism; CFTR is an ABC transporter-class protein and ion channel that transports chloride ions across the apical membrane of epithelial cells. Mutations of the *CFTR* gene affect folding and/or functioning of the CFTR chloride channels in these cell membranes, causing Cystic Fibrosis (CF). The most common CF mutation F508del causes misfolding of the protein and intracellular retention by the endoplasmic reticulum quality control and premature degradation; iminosugars may help in the trafficking of the misfolded protein. Miglustat, its multivalent derivatives[xxxiv] and branched pyrrolidine isoLAB[xxxv] have been found to show significant rescue of the defective F508del-CFTR function as assessed by single-cell fluorescence imaging and/or iodide effluxes.[xxxvi] *N*-alkyl DNJ derivatives **3a-d**, **6a-d** and **9b-d**[xxxvii] were compared to miglustat for their corrector effect on CFTR function in CF-KM4 cells[xxxviii] using iodide effluxes (Figure 2).[xxxix] Results obtained with **9b-d** indicate that a four carbon butyl chain is optimal to provide good correctors, higher chains being detrimental to correction. Introduction of fluorine on the chain as in **6a-d** also abolishes F508del-CFTR correction. Interestingly, the unsaturated derivatives **3a-d** proved the most promising derivatives amongst which the *N*-homoallyl DNJ **3b** and the *N*-propargyl DNJ **3c** showed similar activity to Miglustat.

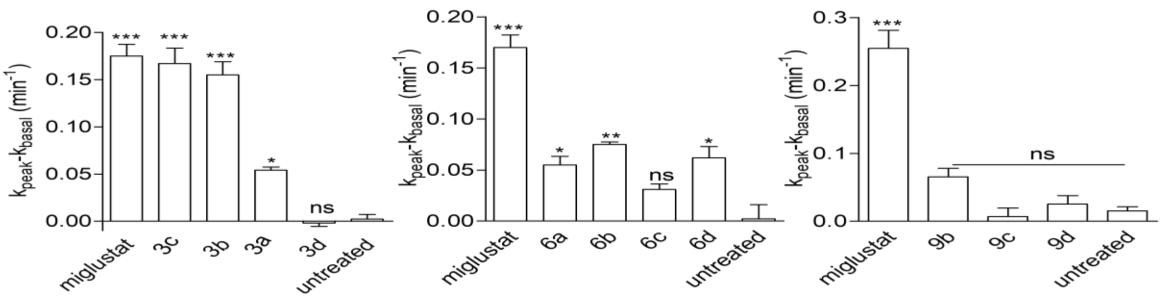


Figure 2: F508del-CFTR correction by DNJ derivatives **3a-d**, **6a-d** and **9b-d**

Experimental

General methods: All commercial reagents were used as supplied. TLC plates were visualized under 254 nm UV light and/or by dipping the TLC plate into a solution of phosphomolybdic acid in ethanol (3 g/100 mL) followed by heating with a heat gun. Flash columns chromatographies were performed using silica gel 60 (15-40 µm). NMR experiments were recorded with a 400 Bruker spectrometer at 400 MHz for ^{1}H, 376 MHz for ^{19}F and 100 MHz for ^{13}C nuclei. The chemical shifts are expressed in part per million (ppm) relative to TMS (δ = 0 ppm) and the coupling constant J in hertz (Hz). NMR multiplicities are reported using the following abbreviations: b = broad, s = singulet, d = doublet, t = triplet, q = quadruplet, m = multiplet. HRMS were obtained with a Q-TOF spectrometer. The melting points were recorded with a SMP3 Stuart Scientific melting point apparatus. Optical rotations were measured using a Perkin-Elmer 341 polarimeter.
The authors draw the reader's attention to the dangerous features of superacidic chemistry. Handling of hydrogen fluoride and antimony pentafluoride must be done by experienced chemists with all the necessary safety arrangements in place. Experiments performed in superacid were carried out in a sealed Teflon® flask with a magnetic stirrer. No further precautions have to be taken to prevent mixture from moisture (test reaction worked out in anhydrous conditions leads to the same results as expected).
General procedure A for TEC reaction: To a solution of *N*-butenyl-2,3,4,6-tetra-*O*-acetyl-1-deoxynojirimycin in degassed (10 min under Ar atmosphere) MeOH (C = 6.10^{-2} mol/L) were added alkylthiol (3.0 eq) and DPAP (0.5 eq). The reaction mixture was then irradiated for 30 min with a UV facial tanner (12 lamps x 15 W) under Ar atmosphere. After concentration, the crude was purified by silica gel column chromatography.
General procedure B for deprotection of acetylated compounds obtained after TEC reactions: Et_3N (7.2 eq) was added to a suspension of the acetylated compound in MeOH (C = 10.10^{-2} mol/L) and the mixture was stirred for three to four days at room temperature. The solvent was then evaporated under reduced pressure, co-evaporated with toluene, then freeze dried to provide the deprotected compound.

2,3,4,6-tetra-*O*-benzyl-*N*-(3-prop-1-enyl)-1-deoxynojirimycin 2a: K_2CO_3 (5 eq, 17.67 mmol, 2.44 g) was added to a stirred solution of **1** (3.53 mmol, 1.85 g) and allylbromide (2.5 eq, 883 mmol, 769 µL) in a mixture EtOAc:H_2O (88/11 mL). The reaction mixture was refluxed for 24h and the aqueous layer was extracted then back-extracted with EtOAc (2 x 20 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The resulting residue was purified by silica gel column chromatography (9:1 to 8:2, PE:EtOAc) to provide **2a** (1.60 g, 80%). *Rf* = 0.48 (85:15, PE:EtOAc); $[\alpha]^{20}_{D}$ = +1.0 (c = 0.30, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$), δ: 7.39-7.28 (m, 18H, H_{Ar} Bn), 7.17-7.15 (m, 2H, H_{Ar} Bn), 5.95-5.85 (m, 1H, H-2'), 5.16 (dd, 2H, *J* = 10.1 Hz, *J* = 17.2 Hz, H-3'), 5.00 (d, 1H, *J* = 11.1 Hz, C*H*HPh), 4.91 (d, 1H, *J* = 10.8 Hz, C*H*HPh), 4.85 (d, 1H, *J* = 11.1 Hz, CH*H*Ph), 4.70 (dd, 2H, *J* = 11.6 Hz, CH_2Ph), 4.52 (bs, 2H, CH_2Ph), 4.44 (d, 1H, *J* = 10.8 Hz, CH*H*Ph), 3.76-3.61 (m, 4H, H-2, H-4, H-6a, H-6b), 3.50 (t, 1H, $J_{3\text{-}2} = J_{3\text{-}4}$ = 9.1 Hz, H-3), 3.43 (dd, 1H, $J_{1'a\text{-}2}$ = 5.4 Hz , $J_{1'a\text{-}1'b}$ = 14.3 Hz, H-1'a), 3.25-3.20 (m, 1H, H-1'b), 3.16 (dd, 1H, $J_{1a\text{-}2}$ = 4.9 Hz , $J_{1a\text{-}1b}$ = 11.4 Hz, H-1a), 2.38-2.31 (m, 1H, H-5), 2.23 (t, 1H, $J_{1b\text{-}1a} = J_{1b\text{-}2}$ = 10.9 Hz, H-1b); ^{13}C NMR (100 MHz, $CDCl_3$), δ: 139.1, 138.6, 137.9 (4 x C_q Bn), 133.4 (C-2'), 128.53, 128.50, 128.45, 128.41, 128.40, 127.98, 127.96, 127.90, 127.71, 127.62, 127.53 (CH_{Ar} Bn), 118.7 (C-3'), 87.4 (C-3), 78.6, 78.5 (C-2, C-4), 75.4, 75.3, 73.6, 72.8 (4 x CH_2Ph), 65.3 (C-6), 64.0 (C-5), 55.7 (C-1'), 54.6 (C-1); HRMS (ESI^{+}): *m/z* $[M+H]^{+}$ calculated for $C_{37}H_{42}NO_4$ 564.3108, found 564.3131.

***N*-(3-prop-1-enyl)-1-deoxynojirimycin 3a**: A solution of BCl_3 (1M in DCM) (20 eq, 43.17 mmol, 43.17 mL) was added slowly to a stirred solution of compound **2a** (2.70 mmol, 1.52 g) in dry DCM (47 mL) at -78°C. The reaction mixture was stirred overnight at -78°C then quenched by addition of MeOH. The solvent was then evaporated. The residue was taken up in water (15 mL) and extracted with DCM (2 x 30 mL). The aqueous phase was then evaporated to afford **3a** (550 mg) in quantitative yield as a colourless oil. $[\alpha]^{20}_{D}$ = +8.7 (c = 0.54, MeOH); ^{1}H NMR (400 MHz, MeOD), δ: 6.08-5.97 (m, 1H, H-2'), 5.69-5.63 (m, 2H, H-3'a, H-3'b), 4.13 (d, 1H, $J_{6a\text{-}6b}$ = 12.3 Hz, H-6a), 4.07 (dd, 1H, $J_{1'a\text{-}2'}$ = 5.3 Hz, $J_{1'a\text{-}1'b}$ = 12.6 Hz, H-1'a), 3.96 (d, 1H, $J_{6b\text{-}6a}$ = 12.3 Hz, H-6b), 3.85 (dd, 1H, $J_{1'b\text{-}2'}$ = 7.7 Hz, $J_{1'b\text{-}1'a}$ = 11.5 Hz, H-1'b), 3.72-3.66 (m, 1H, H-2), 3.61 (t, 1H, $J_{4\text{-}3}$ = $J_{4\text{-}5}$ = 9.5 Hz, H-4), 3.41 (dd, 1H, $J_{1a\text{-}2}$ = 4.5 Hz, $J_{1a\text{-}1b}$= 11.8 Hz, H-1a), 3.38-3.33 (m, 1H, H-3), 3.06-3.01 (m, 1H, H-5), 2.93 (t, 1H, *J* = 11.7 Hz, H-1b); ^{13}C NMR (100 MHz, MeOD), δ: 127.5 (C-3'), 127.0 (C-2'), 78.1 (C-3), 68.8 (C-4), 67.7 (C-2), 67.1 (C-5), 56.6 (C-1'), 54.8 (C-6), 54.7 (C-1); HRMS (ESI^{+}): *m/z* $[M+H]^{+}$ calculated for $C_9H_{18}NO_4$ 204.1230, found 204.1242.

2,3,4,6-tetra-*O*-acetyl-*N*-(3-prop-1-enyl)-1-deoxynojirimycin 4a: Acetic anhydride (10 eq., 10.8 mmol, 1.01 mL) was added dropwise to a solution of compound **3a** (1.07 mmol, 219 mg) in pyridine (15 mL) at 0°C. The mixture was warmed up to room temperature and after 15h the reaction was quenched by the addition of MeOH at 0°C and evaporated. The residue was dissolved in EtOAc (30 mL) then successively washed with saturated aq. NH_4Cl (20 mL), $NaHCO_3$ (20 mL) and water (20 mL), dried over sodium sulfate, filtered and concentrated. The resulting residue was purified by silica gel column chromatography (Combiflash 100%PE to 100% EtOAc) to give **4a** (368 mg, 92%). *Rf* = 0.68 (6:4, PE:EtOAc); $[\alpha]^{20}_{D}$ = +19.2 (c = 0.38, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$), δ: 5.86-5.76 (m, 1H, H-2'), 5.23-5.19 (m, 2H, H-3'a, H-3'b), 5.09-5.05 (m, 1H, H-4), 5.05-4.93 (m, 2H, H-3, H-2), 4.21 (dd, 1H, $J_{6a\text{-}5}$ = 2.2 Hz, H-6a), 4.12 (dd, 1H, $J_{6b\text{-}6a}$ = 22.9 Hz, $J_{6b\text{-}5}$ = 3.1 Hz, H-6b), 3.40 (ddt, 1H, $J_{1'a\text{-}1'b}$ = 14.6 Hz, $J_{1'a\text{-}2'}$ = 5.8 Hz, $J_{1'a\text{-}3'}$ = 1.3 Hz, H-1'a), 3.21-3.15 (m, 2H, H-1'b, H-1a), 2.64-2.60 (m, 1H, H-5), 2.29 (dd, 1H, $J_{1b\text{-}1a}$ = 10.1 Hz, $J_{1b\text{-}2}$ = 9.8 Hz, H-1b), 2.07, 2.01, 2.0, 1.99 (CH_3COO); ^{13}C NMR (100 MHz, $CDCl_3$), δ: 171.0, 170.5, 170.1, 169.8 (4xCH_3COO), 132.7 (C-2'), 119.4 (C-3'), 74.8 (C-3), 69.5 (C-4), 69.4 (C-2), 61.5 (C-5), 59.4 (C-6), 55.3 (C-1'), 53.1 (C-1), 20.9, 20.9, 20.8, 20.7 (4xCH_3COO); HRMS (ESI^{+}): *m/z* $[M+H]^{+}$ calculated for $C_{17}H_{26}NO_8$ 372.1653, found 372.1655.

2,3,4,6-tetra-*O*-benzyl-*N*-(4-but-1-enyl)-1-deoxynojirimycin 2b: To a solution of 2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimyin **1** (2.73 mmol, 1.43 g) and 4-bromobutene (3 eq, 3.32 mmol, 832 µL) in dry acetonitrile (13.5 mL) was added potassium carbonate (2.1 eq, 5.74 mmol, 793 mg). The reaction mixture was stirred at 82°C for 12h under argon then cooled. Most of the acetonitrile was evaporated under reduced pressure. Water and dichloromethane were added to the residue and the whole mixture was stirred for 10 min and then portioned. The aqueous layer was extracted with dichloromethane (3 times). The combined organic extracts were dried over sodium sulfate, filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (100% PE to 8:2, PE:EtOAc) to provide **2b** (1.35 g, 86%). *Rf* = 0.85 (8:2, PE:EtOAc); $[\alpha]^{20}_{D}$ -2.3 (c = 0.30, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$), δ: 7.37-7.28 (m, 18H, H_{Ar} Bn), 7.16 (dd, 2H, *J* = 2.1 HZ, *J* = 7.8 Hz, H_{Ar} Bn), 5.76-5.66 (m, 1H, H-3'), 5.06-4.98 (m, 3H, H-4'a, H-4'b, C*H*HPh), 4.88 (dd, 2H, *J* = 10.8 Hz, *J* = 11.1 Hz, CH_2Ph), 4.70 (dd, 2H, *J* = 11.3, CH_2Ph), 4.51 (bs, 2H, CH_2Ph), 4.44 (d, 1H, *J* = 10.6 Hz, CH*H*Ph), 3.71-3.47 (m, 4H, H-2, H-4, H-6a, H-6b), 3.49 (t, 1H, $J_{3\text{-}2}$ = $J_{3\text{-}4}$ = 8.9 Hz, H-3), 3.13 (dd, 1H, $J_{1a\text{-}1b}$ = 11.3 Hz, $J_{1a\text{-}2}$ = 4.5 Hz, H-1a), 2.84-2.71 (m, 2H, H-1'a, H-1'b), 2.39-2.35 (m, 1H, H-1b), 2.26-2.10 (m, 2H, H-2'a, H-2'b); ^{13}C NMR (100 MHz, $CDCl_3$), δ: 139.1, 138.6, 137.5 (4 x C_q Bn), 136.3 (C-3'), 128..5, 128.5, 128.4, 128.4, 128.0, 127.9, 127.9, 127.7, 127.6, 127.5 (CH_{Ar} Bn), 116.0 (C-4'), 87.4 (C-3), 78.7, 78.6 (C-2, C-4), 75.4, 75.3, 73.6, 75.3 (4 x CH_2Ph), 65.5 (C-6), 63.5 (C-5), 54.5 (C-1), 51.9 (C-1'), 28.3 (C-2'); HRMS (ESI^{+}): *m/z* $[M+H]^{+}$ calculated for $C_{38}H_{44}NO_4$ 578.3264, found 578.3266.

***N*-(4-but-1-enyl)-1-deoxynojirimycin 3b**: A solution of BCl_3 (1M in DCM) (20 eq, 26.11 mmol, 26.11 mL) was added slowly to a stirred solution of **2b** (1.3 mmol, 754 mg) in dry DCM (20 mL) at -78°C. The reaction mixture was stirred overnight at -78°C then quenched by addition of MeOH. The solvent was then evaporated. The residue was taken up in water (15 mL) and extracted with DCM (2x 15 mL). The aqueous phase was then evaporated to afford **3b** (285 mg) in quantitative yield as a white solid. $[\alpha]^{20}_{D}$ = -1.2 (c = 0.81, MeOH); ^{1}H NMR (400 MHz, D_2O), δ: 5.77-5.67 (m, 1H, H-3'), 5.14 (dd, 2H, $J_{4'a\text{-}4'b}$ = 1.5 Hz, $J_{4'b\text{-}3'}$ = 17.2 Hz, $J_{4'a\text{-}3'}$ = 10.3 Hz, H-4'a, H-4'b), 4.01 (d, 1H, $J_{6a\text{-}6b}$ = 13.7 Hz, H-6a), 3.91 (dd, 1H, $J_{6b\text{-}6a}$ = 13.4 Hz, $J_{6b\text{-}5}$ = 2.5 Hz, H-6b), 3.75-3.69 (m, 1H, H-2), 3.61 -3.51 (m, 2H, H-4, H-1a), 3.47-3.38 (m, 2H, H-1'a, H-3), 3.23-3.17 (m, 1H, H-1'b), 3.15-3.12 (m, 1H, H-5), 3.03 (t, 1H, $J_{1b\text{-}1a}$ = $J_{1b\text{-}2}$ = 11.9 Hz, H-1b), 2.54-2.39 (m, 2H, H-2'a, H-2'b); ^{13}C NMR (100 MHz, D_2O), δ: 133.4 (C-3'), 119.1 (C-4'), 77.6 (C-3), 68.5 (C-4), 67.5, 67.3 (C-2, C-5), 55.0 (C-6), 54.6 (C-1), 53.3 (C-1'), 28.4 (C-2'); HRMS (ESI^{+}): *m/z* $[M+H]^{+}$ calculated for $C_{10}H_{20}NO_4$ 218.1386, found 218.1390.

2,3,4,6-tetra-*O*-acetyl-*N*-(4-but-1-enyl)-1-deoxynojirimycin 4b: Acetic anhydride (10 eq, 14.04 mmol, 1.32 mL) was added dropwise to a solution of *N*-butenyl-1-deoxynojorimycin **3b** (1.4 mmol, 305 mg) in pyridine (25 mL) at 0°C. The mixture was warmed up to room temperature and after 15h the reaction was quenched by the addition of MeOH at 0°C and then evaporated. The residue was dissolved in EtOAc (30 mL) then successfully washed with saturated aq. NH_4Cl (20 mL), $NaHCO_3$ (20 mL) and water (20 mL), dried over sodium sulfate, filtered and concentrated. The resulting residue was purified by silica gel column chromatography (7:3, PE:EtOAc) to provide **4b** (507 mg, 93%). *Rf* = 0.34 (7:3, PE:EtOAc); $[\alpha]^{20}_{D}$ = +10.9 (c = 0.34, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$), δ: 5.76-5.66 (m, 1H, H-3'), 5.11-4.94 (m, 4H, H-4'a, H-4'b, H-3, H-4), 4.89-4.82 (m, 1H, H-2), 4.21 (dd, 1H, $J_{6a\text{-}6b}$ = 13.1 Hz, $J_{6a\text{-}5}$ = 2.1 Hz, H-6a), 4.13 (dd, 1H, $J_{6b\text{-}6a}$ = 13.1 Hz, $J_{6b\text{-}5}$ = 3.5 Hz, H-6b), 3.17 (dd, 1H, $J_{1a\text{-}1b}$ = 11.3 Hz, $J_{1a\text{-}2}$ = 5.3 Hz, H-1a), 2.90-2.77 (m, 2H, H-1'a, H-1'b), 2.75-2.70 (m, 1H, H-5), 2.48 (dd, 1H, $J_{1b\text{-}1a}$ = 10.3 Hz, $J_{1b\text{-}2}$ = 1.1 Hz, H-1b), 2.28-2.22 (m, 2H, H-2'a, H-2'b), 2.00, 1.98, 1.97, 1.94 (4s, 4x3H, CH_3COO); ^{13}C NMR (100 MHz, $CDCl_3$), δ: 171, 170.4, 170.1, 169.8 (4 x CH_3COO), 135.6 (C-3'), 116.6 (C-4'), 74.7 (C-3 or C-4), 69.5, 69.4 (C-2, C-3 or C-4), 61.2 (C-5), 59.7 (C-6), 53.0 (C-1'), 51.3 (C-1), 29.3 (C-2'), 21.0, 20.9, 20.8, 20.8 (4 x CH_3COO); $[\alpha]^{20}_{D}$ = +10.8° (c = 0.34, $CHCl_3$); HRMS (ESI^{+}): *m/z* $[M+H]^{+}$ calculated for $C_{18}H_{28}NO_8$ 386.1809, found 386.1811.

2,3,4,6-tetra-*O*-benzyl-*N*-(3-prop-1-ynyl)-1-deoxynojirimycin 2c: To a solution of 2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimyin **1** (3.43 mmol, 1.80 g) and propargyl bromide (5.16 mmol, 575 µL) in dry acetonitrile (17.2 mL) was added potassium carbonate (7.2 mmol, 998 mg). The reaction mixture was stirred at 82°C for 24h under argon then cooled. Most of the acetonitrile was evaporated under reduced pressure. Water (50 mL) and dichloromethane (50 mL) were added to the residue and the whole mixture was stirred for 10 min and then portioned. The aqueous layer was extracted with dichloromethane (3 times). The combined organic extracts were dried over sodium sulfate, filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (Combiflash 100%PE to

100% EtOAC) to provide **2c** (1.23 g, 64%). *Rf* = 0.24 (85:15, PE:EtOAc); $[\alpha]^{20}_D$ = -13.0 (c = 0.33, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$), δ: 7.36-7.26 (m, 18H, H_{Ar} Bn), 7.12-7.10 (m, 2H, H_{Ar} Bn), 4.97 (d, 1H, *J* = 10.7 Hz, CH*H*Ph), 4.88 (d, 1H, *J* = 10.6 Hz, CH*H*Ph), 4.83 (d, 1H, *J* = 10.9 Hz, C*H*HPh), 4.68 (d, 1H, *J* = 11.6 Hz, CH*H*Ph), 4.64 (d, 1H, *J* = 11.5 Hz, C*H*HPh), 4.55 (d, 1H, *J* = 12.1 Hz, C*H*HPh), 4.43 (d, 1H, *J* = 12.2 Hz, CH*H*Ph), 4.35 (d, 1H, *J* = 10.8 Hz, C*H*HPh), 3.77-3.70 (m, 3H, H-2, H-1'a, H-6a), 3.65 (t, 1H, $J_{4\text{-}3}$ = $J_{4\text{-}5}$ = 9.2 Hz, H-4), 3.58 (dd, 1H, $J_{6b\text{-}6a}$ = 10.6 Hz, $J_{6b\text{-}5}$ = 2.6 Hz, H-6b), 3.49 (t, 1H, $J_{3\text{-}2}$ = $J_{3\text{-}4}$ = 9.2 Hz, H-3), 3.40 (dd, 1H, $J_{1'a\text{-}1'b}$ = 17.5 Hz, $J_{1'b\text{-}3'}$ = 1.9 Hz, H-1'b), 2.98 (dd, 1H, $J_{1ax\text{-}1eq}$ = 11 Hz, $J_{1ax\text{-}2}$ = 5 Hz, H-1ax), 2.55 (dd, 1H, $J_{1eq\text{-}ax}$ = 11 Hz, $J_{1eq\text{-}2}$ = 10.6 Hz, H-1eq), 2.43 (ddd, 1H, $J_{5\text{-}4}$ = 9.2 Hz, $J_{5\text{-}6a}$ = 2.2 Hz, $J_{5\text{-}6b}$ = 1.8 Hz, H-5), 2.22 (t, 1H, $J_{3'\text{-}1'}$ = 2.2 Hz, H-3'); ^{13}C NMR (100 MHz, $CDCl_3$), δ: 139.0, 138.6, 138.5, 137.7 (4 x Cq Bn), 128.6, 128.6, 128.5, 128.5, 128.4, 128.1, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6 (CH_{Ar}Bn), 87.2 (C-3), 78.2 (C-2), 75.6, 75.3 (2xCH_2Ph), 74.3 (C-2'), 73.8, 72.9 (2xCH_2Ph), 64.8 (C-6), 62.2 (C-5), 55.1 (C-1), 42.4 (C-1'); HRMS (ESI$^+$): *m/z* $[M+H]^+$ calculated for $C_{37}H_{40}NO_4$ 562.2952, found 562.2969.

***N*-(3-prop-1-ynyl)-1-deoxynojirimycin 3c**: A solution of BCl_3 (1M in DCM) (20 eq, 31.6 mmol, 31.6 mL) was added slowly to a stirred solution of compound **2c** (1.97 mmol, 1.11 g) in dry DCM (34 mL) at -78°C. The reaction mixture was stirred overnight at -78°C then quenched by addition of MeOH. The solvent was then evaporated. The residue was taken up in water (15 mL) and extracted with DCM (2 x 30 mL). The aqueous phase was then evaporated to give **3c** (400 mg) as a brown solid in quantitative yield. $[\alpha]^{20}_D$ = +3.0 (c = 0.54, MeOH); ^1H NMR (400 MHz, MeOD), δ: 4.31 (d, 1H, $J_{1'a\text{-}3'}$ = 2.8 Hz, $J_{1'a\text{-}1'b}$ = 17 Hz, H-1'a), 4.25 (d, 1H, $J_{1'b\text{-}3'}$ = 2.5 Hz, $J_{1'b\text{-}1'a}$ = 17 Hz, H-1'b), 4.14 (bd, 1H, $J_{6a\text{-}6b}$ = 12.5 Hz, H-6a), 3.88 (dd, 1H, $J_{6b\text{-}6a}$ = 12.5 Hz, $J_{6b\text{-}5}$ = 2.8 Hz, H-6b), 3.73-3.67 (m, 1H, H-2 or H-4), 3.64-3.57 (m, 2H, H-1a, H-2 or H-4), 3.45 (t, 1H, $J_{3'\text{-}1'}$ = 2.5 Hz, H-3'), 3.38-3.34 (m, 1H, H-3), 3.18-3.13 (m, 2H, H-1b, H-5); ^{13}C NMR (100 MHz, MeOD), δ: 82.2 (C-2'), 78.2 (C-3), 72.1 (C-3'), 68.5 (C-2 or C-4), 67.7 (C-2 or C-4), 66.8 (C-5), 55.4 (C-1), 54.7 (C-6), 43.9 (C-1'); HRMS (ESI$^+$): *m/z* $[M+H]^+$ calculated for $C_9H_{16}NO_4$ 202.1074, found 202.1082.

2,3,4,6-tetra-*O*-acetyl-*N*-(3-prop-1-ynyl)-1-deoxynojirimycin 4c:
Acetic anhydride (10 eq., 7.45 mmol, 705 μL) was added dropwise to a solution of **3c** (0.745 mmol, 150 mg) in pyridine (10 mL) at 0°C. The mixture was warmed up to room temperature and after 15h the reaction was quenched by the addition of MeOH at 0°C and evaporated. The residue was dissolved in EtOAc (30 mL) then successively washed with saturated aq. NH_4Cl (20 mL), $NaHCO_3$ (20 mL) and water (20 mL), dried over sodium sulfate, filtered and concentrated. The resulting residue was purified by silica gel column chromatography (Combiflash 100%PE to 100% EtOAC) to provide **4c** (228 mg, 83%). *Rf* = 0.66 (6:4, PE:EtOAc); $[\alpha]^{20}_D$ = +5.7 (c = 0.3, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$), δ: 5.11-4.95 (m, 3H, H-4, H-3, H-2), 4.21-4.11 (m, 2H, H-6a, H-6b), 3.74 (bd, 1H, $J_{1'a\text{-}1'b}$ = 17.8 Hz, H-1'a), 3.40 (dd, 1H, $J_{1'b\text{-}1'a}$ = 18.1 Hz, $J_{1'b\text{-}3'}$ = 2.3 Hz, H-1'b), 3.02 (dd, 1H, $J_{1a\text{-}1b}$ = 11.2 Hz, $J_{1a\text{-}2}$ = 5 Hz, H-1a), 2.73-2.71 (m, 1H, H-5), 2.63-2.58 (m, 1H, H-1b), 2.29 (t, 1H, $J_{3'\text{-}1'}$ = 2.3 Hz, H-3'), 2.07, 2.02, 2.01, 2.00 (CH_3COO); ^{13}C NMR (100 MHz, $CDCl_3$), δ: 171.0, 170.4, 170.1, 169.8 (4xCH_3*C*OO), 76.1 (C-3'), 75.1 (C-2'), 74.4 (C-3 or C-4), 69.4 (C-2), 69.1 (C-3 or C-4), 60.0 (C-5), 58.8 (C-6), 53.9 (C-1), 42.5 (C-1'), 20.9, 20.9, 20.8, 20.8 (4x*C*H_3COO); HRMS (ESI$^+$): *m/z* $[M+H]^+$ calculated for $C_{17}H_{24}NO_8$ 370.1496, found 370.1501.

2,3,4,6-tetra-*O*-benzyl-*N*-(4-but-1-ynyl)-1-deoxynojirimycin 2d: To a solution of 2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin **1** (400 mg, 0.764 mmol) and 3-butynyl *p*-toluenesulfonate (514 mg, 2.29 mmol) in acetonitrile (4 mL) was added potassium carbonate (317 mg, 2.29 mmol). The reaction mixture was stirred at 85°C for 48h and then cooled. Acetonitrile was evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 (50 mL) and water (50 mL). The aqueous layer was extracted three times with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtrated and concentrated under reduced pressure. Purification by flash chromatography (PE/EtOAc 95:5 to 90:10) afforded compound **2d** (330 mg, 75%) as a brown solid. Mp: 69°C; $[\alpha]_D$ = +9.1 (*c* = 1.16, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$), δ: 7.37-7.24 (m, 18H, ArH), 7.13-7.11 (m, 2H, ArH), 4.96 (d, 1H, *J* = 11.1 Hz, C*H*$_2$Ph), 4.87 (d, 1H, *J* = 10.8 Hz, C*H*$_2$Ph), 4.82 (d, 1H, *J* = 11.0 Hz, C*H*$_2$Ph), 4.71-4.64 (m, 2H, C*H*$_2$Ph), 4.50 (dd, 2H, *J* = 11.9 Hz, *J* = 16.4 Hz, C*H*$_2$Ph), 4.38 (d, 1H, *J* = 10.8 Hz, C*H*$_2$Ph), 3.72-3.61 (m, 3H, H-2, H-6), 3.55 (t, 1H, *J* = 9.0 Hz, H-4), 3.46 (t, 1H, *J* = 9.0 Hz, H-3), 3.11-2.92 (m, 3H, 2H-a, 1H-1), 2.43-2.30 (m, 4H, 2H-b, 1H-1, 1H-5), 1.97 (t, 1H, *J* = 2.6 Hz, H-d); ^{13}C NMR (100 MHz, $CDCl_3$), δ: 139.0, 138.5 (2C), 137.8 (4ArC), 128.6-127.6 (20ArCH), 87.3 (C-3), 82.7 (C-3'), 78.5, 78.4 (C-2, C-4), 75.5, 75.3, 73.6, 72.9 (4*C*H_2Ph), 69.6 (C-4'), 65.8 (C-6), 63.1 (C-5), 54.5 (C-1), 51.2 (C-1'), 14.1 (C-2'); HRMS (ESI$^+$): *m/z* $[M+Na]^+$ calculated for $C_{38}H_{41}NNaO_4$ 598.2928, found 598.2930.

***N*-(4-but-1-ynyl)-1-deoxynojirimycin 3d**: A solution of BCl_3 (1M in CH_2Cl_2) (5.28 mL, 5.28 mmol) was added slowly to a stirred solution of compound **2d** (152 mg, 0.264 mmol) in dry CH_2Cl_2 (3.9 mL) at -78°C. The reaction mixture was stirred for 40 hours at -78°C then quenched by addition of MeOH. The solvent was evaporated, the residue was taken up in water (15 mL) and extracted extensively with EtOAc (10 x 15 mL). The aqueous phase was then evaporated under reduced pressure to afford compound **3d** (48 mg, 85%) as a brown oil. $[\alpha]_D$ = +6.2 (*c* = 0.96, MeOH); ^1H NMR (400 MHz, MeOD), δ: 4.11 (d, 1H, *J* = 12.5 Hz, 1H-6), 3.96 (dd, 1H, *J* = 2.4 Hz, *J* = 12.5 Hz, 1H-6), 3.75 (m, 1H, H-2), 3.64-3.51 (m, 3H, H-1', H-1, H-4), 3.48-3.38 (m, 2H, H-1', H-3), 3.19 (m, 1H, H-5), 3.10 (t, 1H, *J* = 11.6 Hz, H-1), 2.86-2.73 (m, 2H, H-2'), 2.59 (t, 1H, *J* = 2.6 Hz, H-4'); ^{13}C NMR (100 MHz, MeOD), δ: 79.7 (C-3'), 77.8 (C-3), 73.2 (C-4'), 68.8 (C-4), 67.7 (2C, C-2, C-5), 55.4 (C-6), 55.0 (C-1), 52.5 (C-1'), 14.7 (C-2'); HRMS (ESI$^+$): *m/z* $[M+H]^+$ calculated for $C_{10}H_{18}NO_4$ 216.1230, found 216.1233.

2,3,4,6-tetra-*O*-acetyl-*N*-(4-but-1-ynyl)-1-deoxynojirimycin 4d:
Acetic anhydride (542 μL, 5.73 mmol) was added dropwise to a solution of compound **3d** (123 mg, 0.571 mmol) in pyridine (10.2 mL) at 0°C. The mixture was stirred at room temperature for 18h, quenched by the addition of MeOH at 0°C and evaporated. The residue was dissolved in EtOAc and water, the aqueous layer was extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered and concentrated. Purification by flash chromatography (PE/EtOAc 95:5 to 90:10) afforded compound **4d** (148 mg, 67%) as a white solid. Mp: 82°C; $[\alpha]_D$ = +21.1 (*c* = 1.42, MeOH);. ^1H NMR (400 MHz, $CDCl_3$), δ: 5.00-4.93 (m, 2H, H-3, H-4), 4.92-4.86 (m, 1H, H-2), 4.18-4.10 (m, 2H, H-6), 3.13 (dd, *J* = 5.1 Hz, *J* = 11.5 Hz, H-1), 2.97-2.86 (m, 2H, H-1'), 2.77-2.73 (m, 1H, H-5), 2.43 (dd, 1H, *J* = 10.1 Hz, *J* = 11.5 Hz, H-1), 2.29-2.24 (m, 2H, H-2'), 2.02 (s, 3H, H-OAc), 1.96 (s, 6H, H-OAc), 1.95 (s, 4H, H-OAc, H-4'); ^{13}C NMR (100 MHz, $CDCl_3$), δ: 170.8, 170.3, 170.0, 169.7

(4C-OAc), 81.9 (C-3'), 74.4 (C-3 or C-4), 70.1 (C-4'), 69.3, 69.2 (C-3 or C-4, C-2), 60.7 (C-5), 59.9 (C-6), 52.9 (C-1), 50.4 (C-1'), 20.8, 20.7 (4 CH_3), 15.2 (C-2'); HRMS (ESI$^+$): *m/z* [M+Na]$^+$ calculated for $C_{18}H_{25}NNaO_8$ 406.1472, found 406.1489.

2,3,4,6-tetra-*O*-acetyl-1,5-dideoxy-*N*-(2-fluoropropyl)-1,5-imino-D-glucitol 5a: A mixture of HF/SbF_5 (7/1, v:v, 5 mL) was added to compound **4a** (0.259 mmol, 96.5 mg) at -20°C. The reaction mixture was stirred at -20°C for 10 min then neutralized with aqueous Na_2CO_3 and ice until pH reached 7. The aqueous layer was then extracted with CH_2Cl_2 (3x20 mL) and the organic layers were combined, dried over $MgSO_4$, filtered and evaporated. The crude was acetylated with Ac_20 (8 eq, 2.07 mmol, 196 µL) and pyridine (400 µL). The reaction mixture was stirred overnight at RT then evaporated. The resulting residue was purified by silica gel column chromatography (Combiflash 100%PE to 100% EtOAc) to provide **5a** (55.1 mg, 54%) as a mixture of diastereosiomers. *Rf* = 0.62 (6:4, PE:EtOAc); $[\alpha]^{20}_D$ = +18.8 (c = 0.91, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$), δ: 5.09-4.72 (m, 8H, H-3, H-3*, H-4, H-4*, H-2, H-2*, H-2', H-2'*), 4.28 (dd, 1H, J_{6a-6b} = 13.1 Hz, J_{6a-5} = 5.2 Hz, H-6a), 4.20 (dd, 1H, J_{6b-6a} = 13.1 Hz, J_{6b-5} = 2.9 Hz, H-6b), 4.14 (d, 2H, J_{6*-5*} = 3.1 Hz, H-6a*, H-6b*), 3.30 (dd, 1H, J_{1a-1b} = 12.1 Hz, J_{1a-2} = 5.1 Hz, H-1a), 3.23 (dd, 1H, $J_{1a*-1b*}$ = 11.5 Hz, J_{1a*-2*} = 5.1 Hz, H-1a*), 3.02-2.72 (m, 6H, H-1'a, H-1'b, H-1'a*, H-1'b*, H-5, H-5*), 2.62 (ddd, 1H, J_{1b-1a} = 12.1 Hz, J_{1b-2} = 2 Hz, H-1b), 2.57 (dd, 1H, $J_{1b*-1a*}$ = 11.3 Hz, J_{1b-2} = 1.9 Hz, H-1b*), 2.10, 2.08, 2.07, 2.03, 2.02, 2.01 (s, 24H, CH_3COO, CH_3COO*), 1.32 (dd, 3H, $J_{3'-F}$ = 23.5 Hz, $J_{3'-2'}$ = 6.3 Hz, H-3'), 1.26 (dd, 3H, $J_{3*'-F}$ = 23.5 Hz, $J_{3'-2'}$ = 6.3 Hz, H-3'*); ^{13}C NMR (100 MHz, $CDCl_3$), δ: 171.1, 170.8, 170.5, 170.5, 170.2, 170.1, 169.9, 169.9 (CH_3COO, CH_3COO*), 88.8 (d, $J_{C2'-F}$ = 168 Hz, C-2'), 88.7 (d, $J_{C2'*-F}$ = 167 Hz, C-2'*), 74.6 (C-3 or C-3*), 74.5 (C-3 or C-3*), 69.4, 69.3, 69.3, 69.1 (C-2, C-2*, C-4, C-4*), 61.7 (C-5*), 61.2 (C-5), 60.2 (C-6), 59.3 (C-6*), 56.8 (d, $J_{C-1'-F}$ = 20.8 Hz, C-1'), 56.4 (d, $J_{C1'*-F}$ = 20.8 Hz, C-1'*), 54.5 (C-1*), 53.3 (C-1), 21.0, 21.0, 20.9, 20.9, 20.9, 20.8, 20.8 (CH_3COO, CH_3COO*), 19.2 (d, $J_{C3'-F}$ = 22.3 Hz, C-3' or C-3'*), 18.9 (d, $J_{C3'-F}$ = 22.4 Hz, C-3' or C-3'*); ^{19}F RMN {^1H} (376 MHz, $CDCl_3$), δ: - 174.1, -174.2; HRMS (ESI$^+$): *m/z* [M+H]$^+$ calculated for $C_{17}H_{27}FNO_8$ 392.1715, found 392.1726.

2,3,4,6-tetra-*O*-acetyl-1,5-dideoxy-*N*-(3-fluorobutyl)-1,5-imino-D-glucitol 5b: A mixture of HF/SbF_5 (7/1, v:v, 5 mL) was added to compound **4b** (0.469 mmol, 180.7 mg) at -20°C. The reaction mixture was stirred at -20°C during 10 min. then neutralized with aqueous Na_2CO_3 and ice until pH reached 7. The aqueous layer was then extracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$, filtered and evaporated. The crude was acetylated with Ac_20 (8 eq, 3.73 mmol, 353 µL) and pyridine (600 µL). The reaction mixture was stirred overnight then evaporated. The resulting residue was purified by silica gel column chromatography (Combiflash 100%PE to 100% EtOAc) to provide **5b** (mixture of diastereosiomers (1:0.8*), 117.5 mg, 62%). *Rf* = 0.64 (6:4, PE:EtOAc); $[\alpha]^{20}_D$ = +9.6° (c = 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$), δ: 5.05-4.95 (m, 4H, H-3, H-3*, H-4, H-4*), 4.91-4.63 (m, 4H, H-2, H-2*, H-3', H-3'*), 4.28-4.08 (m, 4H, H-6a, H-6b, H-6a*, H-6b*), 3.19-3.14 (m, 2H, H-1a, H-1a*), 3.00-2.91 (m, 2H, H-1'a, H-1'a*), 2.79-2.67 (m, 4H, H-1'b, H-1'b*, H-5, H-5*), 2.49-2.38 (m, 2H, H-1b, H-1b*), 2.00-1.94 (8s, 24H, CH_3COO), 1.85-1.67 (m, 4H, H-2'a, H-2'b, H-2'a*, H-2'b*), 1.35 (dd, 3H, $J_{4'*-3'*}$ = 6.2 Hz, $J_{4'*-F}$ = 23.8 Hz, H-4'*), 1.29 (dd, 3H, $J_{4'-3'}$ = 6.1 Hz, $J_{4'-F}$ = 23.8 Hz, H-4'); ^{13}C NMR (100 MHz, $CDCl_3$), δ: 170.8, 170.8, 170.3, 170.1, 170.1, 170.1 (CH_3COO), 89.8 (d, $J_{C-3'-F}$ = 164.1 Hz, C-3'), 89.8 (d, $J_{C-3'*-F}$ = 163.5 Hz, C-3'*), 75.2 (C-3), 75. 1 (C-3*), 70.4 (C-2* or C-4*), 70.3 (C-2 or C-4), 70.0 (C-2* or C-4*), 70.0 (C-2 or C-4), 62.2 (C-5*), 62.1 (C-5), 60.4 (C-6*), 60.3 (C-6), 53.5 (C-1), 53.4 (C-1*), 48.4 (d, $J_{C-1'-F}$ = 11.6 Hz, C-1'), 48.2 (d, $J_{C-1'*-F}$ = 10.5 Hz, C-1'*), 33.3 (d, $J_{C-2'*-F}$ = 20.9 Hz, C-2'*), 33.2 (d, $J_{C-2'-F}$ = 20.6 Hz, C-2'), 21.5 (d, $J_{C-4'*-F}$ = 22.5 Hz, C-4'*), 21.2 (d, $J_{C-4'-F}$ = 22.7 Hz, C-4'), 20.7, 20.6, 20.6 (CH_3COO); ^{19}F RMN {^1H} (376 MHz, $CDCl_3$), δ: - 174.4, -174.9; HRMS (ESI$^+$): *m/z* [M+Na]$^+$ calculated for $C_{18}H_{28}FNNaO_8$ 428.1691, found 428.1696.

2,3,4,6-tetra-*O*-acetyl-1,5-dideoxy-*N*-(2,2-difluoropropyl)-1,5-imino-D-glucitol 5c: A mixture of HF/SbF_5 (3/1, v:v, 1 mL) was added to compound **4c** (0.503 mmol, 185.7 mg) at 0°C. The reaction mixture was stirred at 0°C during 10 min then neutralized with aqueous Na_2CO_3 and ice until pH reached 7. The aqueous layer was then extracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$, filtered and evaporated. The crude was acetylated with Ac_20 (8 eq, 4 mmol, 378 µL) and pyridine (800 µL). The reaction mixture was stirred overnight then evaporated. The resulting residue was then purified by silica gel column chromatography (Combiflash 100%PE to 100% EtOAc) to provide **5c** (122.8 mg, 59%). *Rf* = 0.64 (6:4, PE:EtOAc); $[\alpha]^{20}_D$ = +15° (c = 0.24, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$), δ: 5.09-4.99 (m, 2H, H-3, H-4), 4.96-4.90 (m, 1H, H-2), 4.25 (dd, 1H, J_{6a-5} = 3.7 Hz, J_{6a-6b} = 13.1 Hz, H-6a), 4.18 (dd, 1H, J_{6b-5} = 2.2 Hz, J_{6b-6a} = 13.1 Hz, H-6b), 3.33 (dd, 1H, J_{1a-2} = 5.1 Hz, J_{1a-1b} = 12.5 Hz, H-1a), 3.09-2.98 (m, 3H, H-1'a, H-1'b, H-5), 2.74-2.68 (m, 1H, H-1b), 2.07, 2.03, 2.01 (3s, 12H, 4 CH_3COO), 1.59 (t, 3H, $J_{3'-F}$ = 18.3 Hz, H-3'); ^{13}C NMR (100 MHz, $CDCl_3$), δ: 170.8; 170.3, 170.1, 169.9 (CH_3COO), 124.9 (t, $J_{C2'-F}$ = 240 Hz, C-2'), 74.2 (C-3), 69.1 (C-4), 68.9 (C-2), 61.5 (C-5), 59.9 (C-6), 55.6 (t, $J_{C1'-F}$ = 26.3 Hz, C-1'), 53.8 (C-1), 22.1 (t, $J_{C3'-F}$ = 26.7 Hz, C-3'), 21.0, 20.9, 20.9, 20.8 (CH_3COO); ^{19}F RMN {^1H} (376 MHz, $CDCl_3$), δ: - 91.8 (d, *J* = 244 Hz), -93.8 (d, *J* = 241 Hz); HRMS (ESI$^+$): *m/z* [M+H]$^+$ calculated for $C_{17}H_{26}F_2NO_8$ 410.1621, found 410.1628.

2,3,4,6-tetra-*O*-acetyl-1,5-dideoxy-*N*-(3,3-difluorobutyl)-1,5-imino-D-glucitol 5d: A mixture of HF/SbF_5 (7/1, v:v, 2 mL) was added to compound **4d** (50 mg, 0.130 mmol) at 0°C. The reaction mixture was stirred at 0°C during 10 minutes and then neutralized with solid Na_2CO_3 and ice until pH reached 7. The aqueous layer was then extracted with EtOAc (3x20 mL) and the organic layers were dried over $MgSO_4$, filtered and evaporated. The crude was acetylated with Ac_2O (1.04 mmol, 98 µL) and pyridine (200 µL). The reaction mixture was stirred overnight then evaporated. The residue was then purified by flash chromatography (PE/EtOAc 85:15 to 70:30) to afford compound **5d** (51 mg, 93%) as colorless oil. $[\alpha]_D$ = +12.5 (c = 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$), δ: 5.04-5.00 (m, 2H, H-3, H-4), 4.98-4.90 (m, 1H, H-2), 4.16 (d, 2H, *J* = 2.7 Hz, H-6), 3.16 (dd, *J* = 5.0 Hz, *J* = 11.3 Hz, H-1), 3.04-2.96 (m, 1H, H-1'), 2.84-2.77 (m, 1H, 1H-1'), 2.66-2.62 (m, 1H, H-5), 2.32 (dd, 1H, *J* = 10.4 Hz, *J* = 11.3 Hz, H-1), 2.06 (s, 3H, H-OAc), 2.03-1.94 (m, 11H, 2H-2', 9H-OAc), 1.61 (t, 3H, *J* = 18.6 Hz, H-4'); ^{13}C NMR (100 MHz, $CDCl_3$), δ: 170.9, 170.4, 170.1, 169.8 (4C-OAc), 123.5 (t, *J* = 239.1 Hz, C-3'), 74.4 (C-3 or C-4), 69.4, 69.3 (C-2, C-3 or C-4), 61.5 (C-5), 59.6 (C-6), 53.0 (C-1), 45.5 (t, *J* = 5.3 Hz, C-1'), 33.5 (t, *J* = 25.1 Hz, C-2'), 23.8 (t, *J* = 27.6 Hz, C-4'), 20.9, 20.8 (2C), 20.7 (4 CH_3); ^{19}F NMR {^1H} (376 MHz, $CDCl_3$), δ: -89.5 (d, *J* = 241 Hz), -91.4 (d, *J* = 241 Hz); HRMS (ESI): *m/z* [M+Na]$^+$ calculated for $C_{18}H_{27}F_2NNaO_8$ 446.1587, found 446.1611.

1,5-dideoxy-*N*-(2-fluoropropyl)-1,5-imino-D-glucitol 6a: Et_3N (4 eq, 0.512 mmol, 69 µL) was added to a solution of **5a** (0.128 mmol, 50.2 mg) in MeOH (5.5 mL) and the reaction mixture was stirred for 3 days at room temperature then evaporated under reduce pressure to provide **6a** (28.4 mg, 99%) after lyophilisation. $[\alpha]^{20}_D$ = +4.8° (c = 0.25, MeOH); ^1H NMR (400 MHz, MeOD), δ: 5.04-4.98 (m, 2H, H-2', H-2'*), 3.95-3.74 (m, 4H, H-6a, H-6b, H-6a*, H-6b*), 3.51-3.41 (m, 2H, H-2, H-2*), 3.35-3.30 (m, 1H, H-4*), 3.27 (1H, t, J_{4-3} = J_{4-5} = 9.2 Hz, H-4), 3.18-3.12 (m, 2H, H-3, H-3*), 3.10-3.04 (m, 2H, H-1a, H-1a*), 3.02-2.98 (m, 2H, H-1'a, H-1'a*), 2.90-2.82 (m, 1H, H-1'b*), 2.79-2.70 (m, 1H, H-1'b), 2.43-2.28 (m, 2H, H-1b, H-1b*), 2.30-2.21 (m, 2H, H-5, H-5*), 1.28 (dd, 3H, *J* = 23.6 Hz, *J* = 5.4 Hz, H-3), 1.27 (dd, 3H, *J* = 23.4 Hz, *J* = 5.2 Hz, H-3*); ^{13}C NMR (100 MHz, MeOD), δ: 90.8 (d, $J_{C2'-F}$ = 130.7 Hz, C-2'), 89.1 (d, $J_{C2'*-F}$ = 133.8 Hz, C-2'*), 80.5 (C-3, C-3*), 72.1, 72.0 (C-4, C-4*), 70.7 (C-2, C-2*), 68.1, 67.3 (C-5, C-5*), 60.5 (C-6, C-6*), 59.5, 59.2, 59.2, 58.7 (C-1, C-1*, C-1', C-1'*), 19.7 (d, $J_{C3'-F}$ = 22.5 Hz, C-3'), 19.3 (d, $J_{C3'*-F}$ = 22.0 Hz, C-3'*); ^{19}F RMN {^1H} (376 MHz, MeOD), δ: - 174.3, - 175.3; HRMS (ESI+): *m/z* $[M+H]^+$ calculated for $C_9H_{19}FNO_4$ 224.1293, found 224.1292.

1,5-dideoxy-*N*-(3-fluorobutyl)-1,5-imino-D-glucitol 6b: Et_3N (4 eq, 0.624 mmol, 84.4 µL) was added to a solution of **5b** (0.156 mmol, 63.3 mg) in MeOH (6.5 mL) and the reaction mixture was stirred for 3 days at room temperature then evaporated under reduce pressure to provide **6b** (32.7 mg, 87%) after lyophilisation. $[\alpha]^{20}_D$ = -15.9° (c = 0.32, MeOH); ^1H NMR (400 MHz, MeOD), δ: 4.70 (dm, 2H, *J* = 47.6 Hz, H-3', H-3'*), 4.64-4.56 (m, 1H, H-3'), 3.90-3.81 (m, 4H, H-6a, H-6b, H-6a*, H-6b*), 3.49-3.43 (m, 2H, H-2, H-2*), 3.36-3.30 (m, 2H, H-4, H-4*), 3.15-3.09 (m, 2H, H-3, H-3*), 3.02-2.89 (m, 4H, H-1'a, H-1'a*, H-1a, H-1a*), 2.78-2.66 (m, 2H, H-1'b, H-1'b*), 2.22-2.16 (m, 2H, H-1b, H-1b*), 2.13-2.08 (m, 2H, H-5, H-5*), 1.84-1.69 (m, 4H, H-2'a, H-2'b, H-2'a*, H-2'b*), 1.33 (d, 3H, $J_{4'*-F}$ = 23.7 Hz, H-4'*), 1.32 (d, $J_{4'-F}$ = 23.8 Hz, H-4'); ^{13}C NMR (100 MHz, MeOD), δ: 90.6 (d, $J_{C3'-F}$ = 164.8 Hz, C-3'), 90.6 (d, $J_{C3'*-F}$ = 164.1 Hz, C-3'*), 80.5 (C-3*), 80.5 (C-3), 72.0 (C-4, C-4*), 70.7 (C-2*), 70.7 (C-2), 67.2 (C-5, C-5*), 59.5 (C-6), 59.3 (C-6*), 57.8 (C-1), 57.7 (C-1*), 32.7 (d, $J_{C2'*-F}$ = 20.5 Hz, C-2'*), 32.7 (d, $J_{C2'-F}$ = 20.7 Hz, C-2'), 21.5 (d, $J_{C4'*-F}$ = 22.7 Hz, C-4'*), 21.4 (d, $J_{C4'-F}$ = 22.6 Hz, C-4'); ^{19}F RMN {^1H} (376 MHz, MeOD), δ: - 174.7, - 175.4; HRMS (ESI+): *m/z* $[M+H]^+$ calculated for $C_{10}H_{21}FNO_4$ 238.1449, found 238.1442.

1,5-dideoxy-*N*-(2,2-difluoropropyl)-1,5-imino-D-glucitol 6c: Et_3N (2 eq, 0.148 mmol, 20 µL) was added to a solution of compound **5c** (30.3 mg, 0.074 mmol) in MeOH (3.1 mL) and the reaction mixture was stirred for 3 days at room temperature then evaporated under reduce pressure to provide **6c** (17.6 mg, 98%) after lyophilisation. $[\alpha]^{20}_D$ = -7.2 (c = 0.18, MeOH); ^1H NMR (400 MHz, MeOD), δ: 3.97 (d, 1H, J_{6a-6b} = 12.1 Hz, J_{6a-5} = 2.6 Hz, H-6a), 3.76 (d, 1H, J_{6b-6a} = 11.6 Hz, J_{6b-5} = 4.1 Hz, H-6b), 3.48-3.38 (m, 1H, H-2), 3.36-3.34 (m, 1H, H-1'a), 3.20 (t, 1H, J_{4-3} = J_{4-5} = 9.1 Hz, H-4), 3.16-3.11 (m, 2H, H-1a, H-3), 2.87-2.77 (m,1H, H-1'b), 2.39-2.31 (m, 2H, H-1b, H-5), 1.61 (t, 3H, $J_{3'-F}$ = 18.7 Hz, H-3'); ^{13}C NMR (100 MHz, MeOD), δ = 126.3 (t, $J_{C2'-F}$ = 239.6 Hz, C-2'), 80.4 (C-3), 72.3 (C-4), 70.6 (C-2), 68.1 (C-5), 61.2 (C-6), 59.6 (C-1), 57.2 (t, $J_{C1'-F}$ = 26.6 Hz, C-1'), 22.4 (t, $J_{C3'-F}$ = 25.6 Hz, C-3'); ^{19}F RMN {^1H} (376 MHz, MeOD), δ: - 92.6 (d, *J* = 241 Hz), - 94.2 (d, *J* = 244 Hz; HRMS (ESI+): *m/z* $[M+Na]^+$ calculated for $C_9H_{18}F_2NO_4$ 242.1198, found 242.1197.

1,5-dideoxy-*N*-(3,3-difluorobutyl)-1,5-imino-D-glucitol 6d: Et_3N (4 eq, 0.424 mmol, 58 µL) was added to a solution of **5d** (45 mg, 0.106 mmol) in MeOH (4.6 mL) and the reaction mixture was stirred for 3 days at room temperature then evaporated under reduced pressure and freeze dried to provide **6d** (25 mg, 92%) as a white foam. $[\alpha]^{20}_D$ = -14.0 (c = 0.14, MeOH); ^1H NMR (400 MHz, MeOD), δ: 3.90 (dd, 1H, J_{6a-6b} = 11.9 Hz, J_{6a-5} = 2.5 Hz, H-6a), 3.85 (dd, 1H, J_{6b-6a} = 11.9, J_{6b-5} = 2.9 Hz, H-6b), 3.48 (m, 1H, H-2), 3.34 (m, 1H, H-4), 3.14 (t, 1H, $J_{3,4}$ = $J_{3,2}$ = 9.1 Hz, H-3), 3.06-2.85 (m, 3H, H-1a, 2 x H-1'), 2.23 (t, 1H, $J_{1b,2}$ = $J_{1b,1a}$ = 10.8 Hz, H-1b), 2.17-2.05 (m, 3H, H-5, 2 x H-2'), 1.62 (t, 3H, $J_{4'-F}$ = 18.6 Hz, 3 x H-4'); ^{19}F NMR (376 MHz, $CDCl_3$) δ: -91.5 (s); ^{13}C NMR (100 MHz, MeOD), δ: 125.3 (t, $J_{C2'-F}$ = 237.3 Hz, C-3'), 80.5 (C-3), 71.9 (C-4), 70.7 (C-2), 66.8 (C-5), 59.3 (C-6), 57.7 (C-1), 47.1 (t, $J_{C1'-F}$ = 5.3 Hz, C-1'), 33.2 (t, $J_{C2'-F}$ = 24.7 Hz, C-2'), 23.7 (t, $J_{C4'-F}$ = 27.8 Hz, C-4'); ^{19}F RMN {^1H} (376 MHz, MeOD), δ: - 91.5; HRMS (ESI+): *m/z* $[M+H]^+$ calculated for $C_{10}H_{20}F_2NO_4$ 256.1355, found 256.1362.

***N*-methylsulfanyl-butyl-2,3,4,6-tetra-*O*-acetyl-1-deoxynojirimycin 7a:** Procedure A was applied at 0°C to **4b** (0.305 mmol, 117.9 mg). The solvent was then evaporated and the resulting residue was purified by silica gel column chromatography (7:3, PE:EtOAc) to provide **7a** (107 mg, 81%). *Rf* = 0.37 (7:3, PE:EtOAc); $[\alpha]^{20}_D$ = +5.7 (c = 0.14, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$), δ: 4.76-4.67 (m, 2H, H-3, H-4), 4.60-4.54 (m, 1H, H-2), 3.93 (dd, 1H, J_{6a-6b} = 12.9 Hz, J_{6a-5} = 2.5 Hz, H-6a), 3.83 (dd, 1H, J_{6b-6a} = 12.9 Hz, J_{6b-5} = 3.4 Hz, H-6b), 2.89 (dd, 1H, J_{1a-1b} = 11.3 Hz, J_{1a-2} = 5.2 Hz, H-1a), 2.60-2.49 (m, 1H, H-1'a), 2.48-2.39 (m, 1H, H-5), 2.36-2.26 (m, 1H, H-1'b), 2.24-2.20 (m, 2H, H-4'a, H-4'b), 2.09 (d, 1H, J_{1b-1a} = 11.3 Hz, H-1b), 1.76, 1.71, 1.68, 1.65 (4s, 15H, H-5', 4xCH_3COO); ^{13}C NMR (100 MHz, $CDCl_3$), δ: 170.8, 170.3, 170.2, 170.1 (4xCH_3*C*OO), 75.2, 70.3 (C-3, C-4), 70.1 (C-2), 62.5 (C-5), 60.2 (C-6), 53.3 (C-1), 51.7 (C-1'), 34.3 (C-4), 27.2, 24.8 (C-2', C-3'), 20.7, 20.7, 20.6, 20.6 (4x*C*H_3COO), 15.2 (C-5'); HRMS (ESI+): *m/z* $[M+H]^+$ calculated for $C_{19}H_{32}NO_8S$ 434.1843, found 434.1845.

***N*-butylsulfanyl-butyl-2,3,4,6-tetra-*O*-acetyl-1-deoxynojirimycin 7b:** Procedure A was applied to **4b** (0.298 mmol, 114.9 mg). The solvent was then evaporated and the resulting residue was purified by silica gel column chromatography (9:1 to 7:3, PE:EtOAc) to provide **7b** (80.6 mg, 57%). *Rf* = 0.81 (7:3, PE:EtOAc); $[\alpha]^{20}_D$ = +15.6 (c = 0.16, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$), δ: 5.07-4.99 (m, 2H, H-3, H-4), 4.97-4.91 (m, 1H, H-2), 4.17-4.11 (m, 2H, H-6a, H-6b), 3.18 (dd, 1H, J_{1a-1b} = 11.6 Hz, J_{1a-2} = 5.1 Hz, H-1a), 2.82-2.55 (m, 3H, H-1'a, H-5, H-1'b), 2.52-2.41 (m, 4H, H-5'a, H-5'b, 2H'), 2.34-2.28 (m, 1H, H-1b), 2.06, 2.00, 1.98 (3s, 12H, CH_3COO), 1.57-1.49 (m, 6H, H-6'a, H-6'b, 4H'), 1.42-1.33 (m, 2H, H-7'a, H-7'b,), 0.89 (t, 2H, *J* = 7.5 Hz, H-8'a, H-8'b); ^{13}C NMR (100 MHz, $CDCl_3$), δ: 171.0, 170.4, 170.1, 169.8 (4xCH_3*C*OO), 74.7 (C-3), 69.5, 69.4 (C-2, C-4), 61.6 (C-5), 59.5 (C-6), 52.9 (C-1), 51.3 (C-1'), 31.9, 31.9, 27.1, 24.0, 22.1 (C-2', C-3', C-4', C-5', C-6'), 22.1 (C-7'), 20.9, 20.9, 20.8, 20.8 (4x*C*H_3COO), 13.8 (C-8'); HRMS (ESI+): *m/z* $[M+H]^+$ calculated for $C_{22}H_{38}NO_8S$ 476.2321, found 476.2314.

***N*-heptylsulfanyl-butyl-2,3,4,6-tetra-*O*-acetyl-1-deoxynojirimycin 7c:** Procedure A was applied to **4b** (0.263 mmol, 101.3 mg). The solvent was then evaporated and the resulting residue was purified by silica gel column chromatography (8:2, PE:EtOAc) to provide **7c** (97.1 mg, 71%). *Rf* = 0.26 (8:2, PE:EtOAc); $[\alpha]^{20}_D$ = +11.2 (c = 0.17, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$), δ: 5.07-4.98 (m, 2H, H-3, H-4), 4.96-4.90 (m, 1H, H-2), 4.17-4.14 (m, 2H, H-6a, H-6b), 3.17 (dd, 1H, J_{1a-1b} = 11.4 Hz, J_{1a-2} = 5.5 Hz, H-1a), 2.77-2.52 (m, 3H, H-1'a, H-5, H-1'b), 2.30 (t, 1H, J_{1b-1a} = J_{1b-2} = 11.4 Hz, H-1b), 2.05, 1.99, 1.98 (3s, 12H, CH_3COO), 1.57-1.50 (m, 6H, H-2'a, H-2'b, 4H'), 1.39-1.24 (m, 6H, H-10'a, H-10'b, 4H'), 0.87-

0.83 (m, 2H, H-11'a, H-11'b); ^{13}C NMR (100 MHz, $CDCl_3$), δ: 170.9, 170.4, 170.1, 169.8 (CH_3COO), 74.7 (C-3), 69.5, 69.4 (C-2, C-4), 61.6 (C-5), 59.5 (C-6), 52.9 (C-1), 51.3 (C-1'), 32.9, 31.9, 31.8, 29.8, 29.0, 29.0, 27.1, 24.0, 22.7 (C-2', C-3', C-4', C-5', C-6', C-7', C-8', C-9', C-10'), 14.2 (C-11'); HRMS (ESI$^+$): *m/z* $[M+H]^+$ calculated for $C_{25}H_{44}NO_8S$ 518.2782, found 518.2781.

***N*-methylsulfanyl-butyl-1-deoxynojirimycin 8a:** Procedure B was applied to compound **7a** (0.233 mmol, 97 mg) to provide after freeze drying **8a** (52.3 mg, 98%). $[\alpha]^{20}_D$ = -16.7 (c = 0.73, MeOH); ^{1}H NMR (400 MHz, D_2O), δ: 3.89-3.78 (m, 2H, H-6a, H-6b), 3.54-3.48 (m, 1H, H-2), 3.36-3.31 (m, 1H, H-4), 3.24-3.19 (m, 1H, H-3), 2.99 (dd, 1H, $J_{1a\text{-}1b}$ = 11.4 Hz, $J_{1a\text{-}2}$ = 4.9 Hz, H-1a), 2.75-2.70 (m, 1H, H-1'a), 2.66-2.61 (m, 1H, H-1'b), 2.55-2.51 (m, 2H, H-4'a, H-4'b), 2.32-2.29 (m, 1H, H-1b), 2.26-2.22 (m, 1H, H-5), 2.06 (s, 3H, H-5'), 1.54 (bs, 4H, H-2'a, H-2'b, H-3'a, H-3'b); ^{13}C NMR (100 MHz, D_2O), δ: 79.0 (C-3), 70.7 (C-4), 69.6 (C-2), 65.7 (C-5), 58.2 (C-6), 56.0 (C-1), 52.3 (C-1'), 33.8 (C-4'), 26.9, 22.7 (C-2', C-3'), 14.8 (C-5'). HRMS (ESI$^+$): *m/z* $[M+H]^+$ calculated for $C_{11}H_{24}NO_4S$ 266.1420, found 266.1423.

***N*-butylsulfanyl-butyl-1-deoxynojirimycin 8b:** Procedure B was applied to compound **7b** (0.146 mmol, 69.7 mg) to provide **8b** (42.9 mg, 95%) after lyophylisation. $[\alpha]^{20}_D$ = - 15.2 (c = 0.60, MeOH); ^{1}H NMR (400 MHz, D_2O), δ: 3.90-3.78 (m, 2H, H-6a, H-6b), 3.54-3.48 (m, 1H, H-2), 3.37-3.31 (m, 1H, H-4), 3.24-3.19 (m, 1H, H-3), 3.03-2.96 (m, 1H, H-1a), 2.83-2.60 (m, 2H, H-1'a, H-1'b), 2.57-2.52 (m, 4H), 2.34-2.21 (m, 2H, H-1b, H-5), 1.57-1.50 (m, 6H, H-6'a, H-6'b, 4H), 1.40-1.30 (m, 2H, H-7'a, H-7'b), 0.87 (t, 2H, *J* = 7.5 Hz, H-8'a, H-8'b); ^{13}C NMR (100 MHz, D_2O), δ: 79.3 (C-3), 70.9 (C-4), 69.8 (C-2), 65.9 (C-5), 58.4 (C-6), 56.4 (C-1), 52.6 (C-1'), 32.1, 32.1, 32.0, 27.6, 23.1 (C-2', C-3', C-4', C-5', C-6'), 22.5 (C-7'), 14.1 (C-8'); HRMS (ESI$^+$): *m/z* $[M+H]^+$ calculated for $C_{14}H_{30}NO_4S$ 308.1890, found 308.1893.

***N*-heptylsulfanyl-butyl-1-deoxynojirimycin 8c:** Procedure B was applied to compound **7c** (0.167 mmol, 86.5 mg) to provide **8c** (41 mg, 70%) after lyophylisation. $[\alpha]^{20}_D$ = - 14 (c = 0.50, MeOH); ^{1}H NMR (400 MHz, D_2O), δ: 3.78 (2dd, 2H, $J_{6a\text{-}6b}$ = 12.1 Hz, $J_{6a\text{-}5}$ = 2.81 Hz, $J_{6b\text{-}5}$ = 3.0 Hz, H-6a, H-6b), 3.42-3.36 (m, 1H, H-2), 3.26 (t, 1H, $J_{4\text{-}3}$ = $J_{4\text{-}5}$ = 9.1 Hz, H-4), 3.05 (t, 1H, $J_{3\text{-}4}$ = $J_{3\text{-}2}$ = 9.1 Hz, H-3), 2.92 (dd, 1H, $J_{1a\text{-}1b}$ = 11.1 Hz, $J_{1a\text{-}2}$ = 4.8 Hz, H-1a), 2.78-2.71 (m, 1H, H-1'a), 2.56-2.49 (m, 1H, H-1'b), 2.48-2.40 (m, 4H'), 2.11 (t, 1H, *J* = 10.7 Hz, H-1b), 2.07-2.03 (m, 1H, H-5), 1.52-1.45 (m, 4H, H-2'a, H-2'b, 2H'), 1.33-1.17 (m, 8H, H-10'a, H-10'b, 6H'), 0.84-0.81 (m, 2H, H-11'a, H-11'b); ^{13}C NMR (100 MHz, D_2O), δ: 80.5 (C-3), 72.0 (C-4), 70.7 (C-2), 67.4 (C-5), 59.4 (C-6), 57.6 (C-1), 53.3 (C-1'), 32.9, 32.8, 32.7, 30.8, 30.0, 29.9, 28.6, 24.4, 23.7 (C-2', C-3', C-4', C-5', C-6', C-7', C-8', C-9', C-10'), 14.4 (C-11'); HRMS (ESI$^+$): *m/z* $[M+H]^+$ calculated for $C_{17}H_{36}NO_4S$ 350.2359, found 350.2362.

Biological assays:

- Glycosidase inhibition profiling

The glycosidase activities were determined using appropriate *p*-nitrophenyl glycosides as substrates 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.

- Trehalase inhibition

Compounds were tested for their inhibitory activity against insect trehalase of midge larvae of *C. riparius*, a good model for biochemical studies, and porcine kidney trehalase (purchased from Sigma-Aldrich) as the mammalian counterpart. Proteins were measured according to Bradford using bovine serum albumin as standard.[xl] Trehalase activity was measured through a coupled assay with glucose-6-phosphate dehydrogenase and hexokinase according to Wegener et al..[41] To examine the potential of each compound as a trehalase inhibitor, dose-response curves were established to determine the IC_{50} values. Experiments were performed at fixed substrate concentration close to the K_m value (0.5 mM for *C. riparius* and 2.5 mM for porcine trehalase), in the presence of increasing inhibitor concentrations. Initial rates as a function of inhibitor concentration were fitted to the following equation:

where v_i and v are the initial rate in the presence and in the absence of inhibitor, respectively, $[I]$ is the inhibitor concentration, IC_{50} is the inhibitor concentration producing half-maximal inhibition, and n is the Hill coefficient. All enzyme assays were performed in triplicates at 30°C by using sample volumes varying from 5 to 20 µL in 1 mL test and using a Cary3 UV/Vis Spectrophotometer. Enzyme activities were analyzed by Cary Win UV application software for Windows XP.

- F508del-CFTR restoration assay

CFTR activity was assayed by iodide (^{125}I) efflux as previously described.[39] Briefly, iodide efflux curves were constructed by plotting rate of ^{125}I, noted k and expressed in min^{-1}. All comparisons were based on maximal values for the time-dependent rates k_{peak} excluding the points used to establish the baseline k_{basal} and were expressed as k_{peak} - k_{basal} (min $^{-1}$).

Conclusions

We have synthesized a small library of DNJ derivatives bearing a thiolated, a fluorinated or an unsaturated *N*-alkyl chain. Fluorine and sulfur atoms were introduced using hydrofluorination and thiol ene click reactions respectively starting from a common unsaturated iminosugar precursor. The thiolated derivatives exhibit low micromolar trehalases inhibition while the *N*-propargyl DNJ shows potency similar to Zavesca as F508del-CFTR corrector.

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