Synthesis & biological applications of glycosylated iminosugars

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5
Sweet DNJ

5.1 Introduction

Iminosugars are naturally occurring carbohydrate analogs in which the endocyclic oxygen is replaced by a nitrogen atom. Because of their structural similarities they can act as carbohydrate mimics and are therefore often found to be good inhibitors of glycosidases and glycosyltransferases.[1] The first member of the iminosugar family, nojirimycin (NJ, **1**), was isolated from *Streptomyces roseochromogenes* R-468 and *Streptomyces lavendulae* SF-425. This compound showed remarkable biological activity.[2,3] Because nojirimycin bears a hemiaminal function which renders it rather unstable under neutral and acidic conditions at room temperature, it is stored as its bisulphite adduct or reduced to the more stable 1-deoxynojirimycin (DNJ, **2**).[2,4] Later it was found that DNJ could also be isolated from bacterial cultures (*Bacillus* and *Streptomyces*)[5,6] and from white mulberry (*Morus alba*) root bark.[7] Over the years several *N*-alkylated derivatives of DNJ have been synthesized such as *N*-butyl-1-deoxynojirimycin (NB-DNJ, **16**) and *N*-[5-(adamantan-1-yl-methoxy)-pentyl-1-deoxynojirimycin (AMP-DNJ, **17**)[8] which both showed to be good inhibitors for glucosylceramide synthase (GCS), with the latter being the more active inhibitor.[9] NB-DNJ is the first orally administered drug, which is active in the treatment of type 1 Gaucher disease.[10] Gaucher disease is a rare lysosomal storage disorder in which glucosylceramide (GC) is inefficiently hydrolyzed by mutant glucocerebrosidase (GBA1). This causes accumulation of GC-laden macrophages. Inhibition of GCS restores the balance of GC in Gaucher cells.

It is known that some iminosugars and *N*-alkylated derivatives thereof have a bitter taste.[11] The daily intake of NB-DNJ, as a drug against Gaucher, will become

more convenient for the patients when the bitter taste of the drug (NB-DNJ) is masked.[11–13] To palliate the bitter taste of both NB-DNJ and AMP-DNJ, it was envisaged that appendage of a galactose moiety to the 4-position of DNJ would give an analog of lactose, which is known to have a mild sweet taste.[14] However, alternation of the 4-position of DNJ renders it inactive.[15] It is therefore anticipated that lactase-phlorizin hydrolase (LPH) would be able to cleave the terminal galactose moiety,[16] thereby releasing the active drug from prodrugs **199** and **201**.

This chapter describes the synthesis of galactosylated NB-DNJ (**199**) and AMP-DNJ (**201**) as potential prodrugs. Both compounds were accessible from octa-*O*-acetyl-α/β-D-lactose. The ability of LPH, from rat mucosa, to cleave the glycosidic bond of **201** is evaluated.

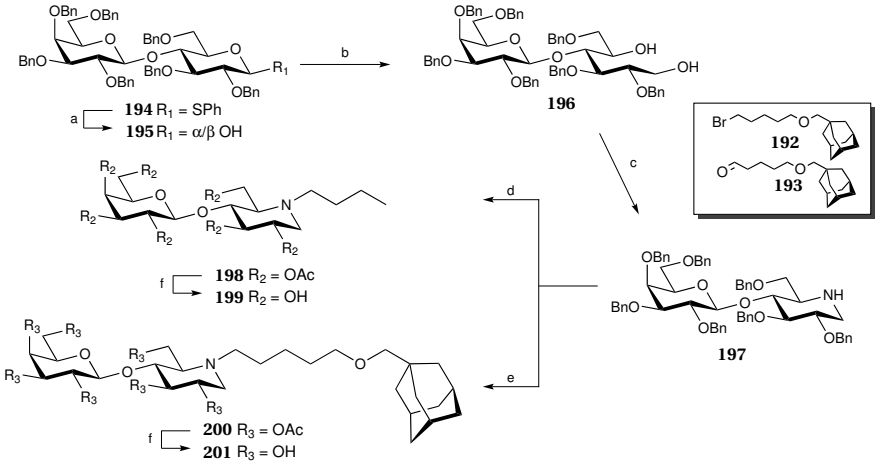
5.2 Results and Discussion

The use of octa-*O*-acetyl-α/β-D-lactose as starting material for the synthesis of compounds **199** and **201** has the following advantages; it is cheap and saves a glycosylation step. Lactose octa-acetate was converted into thio lactoside **194** according to a published procedure.[17] Subsequent treatment with NBS in aqueous acetone furnished hemi acetal **195**, which was reduced with $LiAlH_4$ in dry THF to give diol **196**. To access the iminosugar, lacitol **196** was first oxidized using the Swern procedure.[18] Next, the crude di-carbonyl was subjected to a double reductive amination using an excess of ammonium formate in methanol at 0 °C in the presence of $NaBH_3CN$ and Na_2SO_4 to give **197**.[19]

Mono-alkylation of the endocyclic nitrogen in **197** with either 5-(adamantan-1-yl-methanol)-1-bromo-pentane (**192**) or 1-bromobutane under influence of K_2CO_3 and TBAI in hot DMF proved to be difficult. Better results were obtained by performing a reductive amination, using aldehydes, 5-(adamantan-1-yl-methoxy)-1-pentanal[19] (**193**) or butanal in a dioxane:AcOH with H_2 and Pd/C (20%) as a catalyst. Because of the partial deprotection of the benzyl groups under the reaction conditions used, a second reduction was performed. Hydrogenolysis of the crude product in the presence of Pd/C (20%), HCl and 5 bar H_2 pressure gave target compounds **199** and **201**, respectively. To facilitate their purification by silica gel chromatography both compounds were acetylated in a mixture of Ac_2O-pyridine with a catalytic amount of DMAP. Deacetylation of **198** and **200** under Zemplén conditions and purification by a Dowex™ H^+ column yielded target compounds **199** and **201** as white solids.

Next, it was examined whether the inactive galactosylated GCS inhibitors (**199** and **201**) could be processed by LPH to gain access to the active GCS inhibitors. Because AMP-DNJ (**17**) was found to be a more active GCS inhibitor than NB-DNJ (**227**) the preliminary biological tests were done using prodrug **201**. Therefore, mucosa was isolated by scraping the intestine of freshly sacrificed rats. The in-

Scheme 5.1: Synthesis and deprotection of DNJ based produgs **199** and **201**.



Reagents and conditions: a) NBS, acetone/H_2O, -20 °C, 51%; b) $LiAlH_4$, THF, 0 °C, 62%; c) (1) $(COCl)_2$, DMSO, -78 °C, next Et_3N, 0 °C; (2) $NaBH_3CN$, $HCOONH_4$, Na_2SO_4, MeOH, 0 °C, 47% d) (1) butanal, Pd/C, H_2, dioxane, AcOH; (2) Pd/C, 5 bar H_2 , dioxane, HCl; (3) Ac_2O, pyr., **198** 41%; e) (1) **193**, Pd/C, H_2, dioxane, AcOH; (2) Pd/C, 5 bar H_2 , dioxane, HCl; (3) Ac_2O, pyr., **200** 34%; f) NaOMe, MeOH, **199** 68%, **201** 67%.

testinal mucosa fraction (220 μg total protein per assay) was incubated for 2 h at 37 °C in a 0.1 mM potassium phosphate buffer (pH 6.5) with either 1 mM of compound **201**, or 1 mM compound of **17** as control. In a second assay the LPH, from intestinal rat mucosa, was inactivated by boiling for 5 minutes prior to incubation of the homogenate with 1 mM compound **201**.

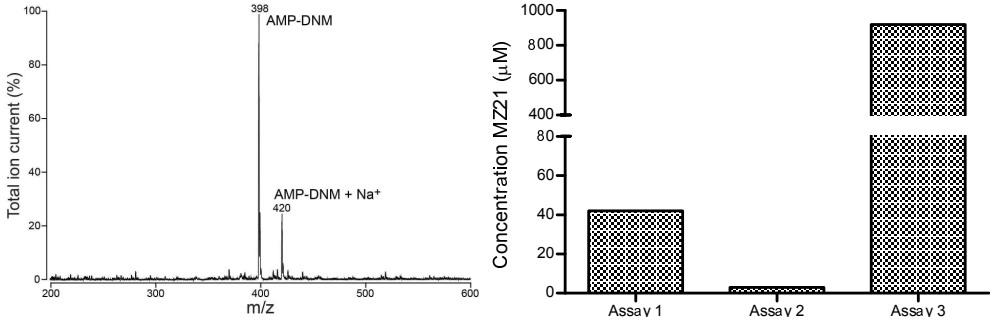


Figure 5.1: Detection of **17** by LC-MS/MS, after cleavage of prodrug **201** by LPH (Left); Recovery of AMP-DNJ (**17**) after incubation of LPH from in intestinal rats mucosa (Right); Assay 1: LPH + 1mM **201**, Assay 2: Inactive LPH + 1mM **201**; Assay 3: LPH + 1mM **17**.

During the incubation the concentration of AMP-DNJ (**17**) was determined by LC-ESI-MS/MS or by a bio-assay employing the inhibition properties of AMP-DNJ (but not prodrug **201**) to inhibit recombinant glucocerebrosidase (GBA1). Fig-

ure 5.1 (left) shows an example of AMP-DNM detection by LC-MS/MS. Figure 5.1 (right) shows that AMP-DNJ (**17**) was nicely recovered (92%) and that approximately 4% of the prodrug (**201**) was partially converted during the 2 h incubation, with LPH, to AMP-DNJ (**17**).

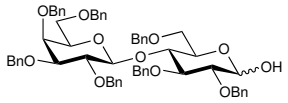
5.3 Conclusion

A convergent and scalable route of synthesis to prodrugs **199** and **201** was achieved using octa-*O*-acetyl-α/β-D-lactose as starting material. In an eight step sequence lactose was transformed into galactosylated DNJ derivative **197**. This intermediate furnished the target compounds **199** and **201** by reductive amination and hydrogenolysis. Biological evaluation of **201** showed the ability of LPH, from intestinal rat mucosa, to cleave the glycosidic bond thereby releasing AMP-DNJ (**17**).

5.4 Experimental section

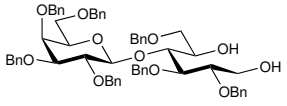
All reagent were of commercial grade and used as received (Acros, Fluka, Merck, Schleicher & Schuell) unless stated otherwise. Diethyl ether (Et_2O), light petroleum ether (PE 40-60), en toluene (Tol) were purchased from Riedel-de Haën. Dichloromethane (DCM), *N,N*-dimethylformamide (DMF), methanol (MeOH), pyridine (pyr) and tetrahydrofuran (THF) were obtained from Biosolve. THF was distilled over $LiAlH_4$ before use. Dichloromethane was boiled under reflux over P_2O_5 for 2 h and distilled prior to use. Molecular sieves 3Å were flame dried under vacuum before use. All reactions sensitive to moisture or oxygen were performed under an inert atmosphere of argon unless stated otherwise. Solvents used for flash chromatography were of pro analysis quality. Flash chromatography was performed on Screening Devices silica gel 60 (0.004 - 0.063 mm). TLC-analysis was conducted on DC-alufolien (Merck, Kieselgel60, F245) with detection by UV-absorption (254 nm) for UV-active compounds and by spraying with 20% H_2SO_4 in ethanol or with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4\,H_2O$ 25 g/L, $(NH_4)_4Ce(SO_4)_4\cdot 2\,H_2O$ 10 g/L, 10% H_2SO_4 in H_2O followed by charring at ~150 °C. ^1H and ^{13}C NMR spectra were recorded on a Bruker DMX-400 (400/100 MHz), a Bruker AV 400 (400/100 MHz), a Bruker AV 500 (500/125 MHz) or a Bruker DMX-600 (600/150 MHz) spectrometer. Chemical shifts (δ) are given in ppm relative to the chloroform residual solvent peak or tetramethylsilane as internal standard. Coupling constants are given in Hz. All given ^{13}C spectra are proton decoupled. High resolution mass spectra were recorded on a LTQ-Orbitrap (Thermo Finnigan) Mass spectrometer. LC/MS analysis was performed on a Jasco HPLC-system (detection simultaneous at 214 nm and 245 nm) equipped with an analytical Alltima C_{18} column (Alltech, 4.6 mmD x 50 mmL, 3μ particle size) in combination with buffers A: H_2O, B: MeCN and C: 0.5% aq. TFA and coupled to a Perkin Almer Sciex API 165 mass spectrometer. Optical rotations were measured on a Propol automatic polarimeter. IR spectra were recorded on a Shimadzu FTIR-8300 and are reported in cm^{-1}.

2,3,6-Tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-α/β-D-glucopyranose (195):



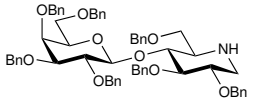
A solution of **194** (24 g, 22.55 mmol) in acetone:H_2O (350:25 mL) was cooled to -20 °C followed by portion wise addition of NBS (20 g, 112.73 mmol, 5 equiv). The reaction mixture turned into a yellow suspension which became clear in 10 minutes. After 15 minutes the reaction turned bright orange and the starting material was completely converted in a polar spot on TLC. The reaction mixture was quenched by the addition of 50 mL of $Na_2S_2O_3$ and subsequently carefully concentrated *in vacuo* to remove the acetone. The resulting mixture was taken up in EtOAc and washed twice with H_2O. The EtOAc layer was dried and concentrated *in vacuo*. The resulting oil was purified using a short silica column (EtOAc/PE 30%) yielding compound **195** in 51 %. (11.1 g, 11.4 mmol). TLC: EtOAc/PE 50%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 - 7.08 (m, 35H, CH_{arom} Bn), 5.15 (d, $J = 3.6$ Hz, 1H, H-1β), 5.11 - 5.01 (m, 2H), 4.96 (dd, $J = 11.4$, 7.1 Hz, 2H), 4.90 - 4.79 (m, 1H), 4.79 - 4.43 (m, 17H), 4.40 - 4.17 (m, 8H), 4.02 - 3.81 (m, 7H), 3.79 - 3.70 (m, 3H), 3.66 - 3.61 (m, 1H), 3.57 - 3.47 (m, 5H), 3.41 - 3.29 (m, 7H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.3-138.0 (C_q), 129.0-125.3 (C_{arom}), 102.9 (C-1'β), 102.8 (C-1'α), 97.3 (C-1α), 91.3 (C-1β), 82.8, 82.7, 82.5, 82.4, 79.9, 79.1, 77.5, 77.2, 76.7, 75.4, 75.3, 75.2, 74.7, 73.7, 73.6, 73.4, 73.4, 73.1, 73.0, 72.5, 72.5, 70.3, 68.4, 68.2, 68.1; IR (neat) ν 3063, 3028, 2914, 2866, 1497, 1452, 1362, 1090, 1059, 1026, 908, 731, 677, 615; HRMS: $C_{61}H_{64}O_{11} + Na^+$ requires 995.43408, found 995.43447.

2,3,6-Tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-D-glucitol (196):



Compound **195** (11.1 g, 11.4 mmol) was coevaporated thrice with toluene and dissolved in dry THF 60 mL. The reaction mixture was cooled with an ice-bath and $LiAlH_4$ (1.50 g, 39.9 mmol, 3.5 equiv) was added portion wise. The mixture was allowed to warm to room temperature overnight after which the reaction mixture was cooled to 0 °C and quenched with MeOH . Subsequently the mixture was diluted with EtOAc and washed with 1M HCl. The organic layer was dried with $MgSO_4$, filtered and concentrated *in vacuo*. Purification using a short silica column (EtOAc/PE 30%) gave compound **196** in 62% yield.(6.96 g, 7.14 mmol) TLC: EtOAc/PE 50%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 - 7.08 (m, 35H, CH_{arom} Bn), 4.96 - 4.50 (m, 10H), 4.43 - 4.21 (m, 5H), 4.10 - 3.95 (m, 4H), 3.84 - 3.66 (m, 6H), 3.59 - 3.45 (m, 3H), 3.41 - 3.32 (m, 2H), 2.66 (s, OH, 1H) ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.7-137.6 (C_q), 128.9-127.3 (C_{arom}), 103.5 (C-1'), 82.2, 79.8, 79.5, 79.2, 77.4, 75.1, 74.7, 74.4, 73.6, 73.3, 73.4, 72.8, 72.7, 70.6, 70.6, 68.7, 62.0; IR (neat) ν 3030, 2920, 2866, 1467, 1454, 1361, 1207, 1062, 1026, 906, 706, 692 ; HRMS: $C_{61}H_{66}O_{11} + Na^+$ requires 997.44973, found 997.44986

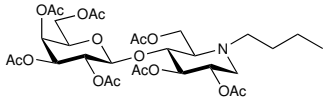
2,3,6-Tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-1-deoxynojirimycin (197):



A solution of oxalylchloride (2.5 mL, 28.56 mmol, 4 equiv) in dry DCM (30 mL) was cooled to -78 °C and stirred for 15 minutes. After the dropwise addition of a solution of DMSO (2.63 mL, 37 mmol, 5 equiv) in dry DCM (5 mL) over 10 minutes, the reaction was stirred for 40 minutes at -70 °C. Subsequently a dry solution of **196** (6.96 g, 7.14 mmol) in DCM (10 mL) was added dropwise, while keeping the reaction temperature at -70 °C. The reaction mixture was stirred for 2 h

after which Et_3N (11.91 mL, 85.68 mmol, 12 equiv) was added dropwise. The mixture was allowed to gradually warm to -5 °C. This reaction mixture was then added to a cooled (0 °C) solution of $NaBH_3CN$ (1.79 g, 28.56 mmol, 4 equiv), $HCOONH_4$ (11.28 g, 142.8 mmol, 20 equiv) and Na_2SO_4 (3.04 g, 21.42 mmol, 3 equiv) in 300 mL MeOH. The reaction was stirred overnight allowing the mixture to warm to room temperature. After TLC-analysis showed full conversion into a polar product, the reaction mixture was filtered and concentrated *in vacuo*. The oily residue was dissolved in EtOAc and washed with $NaHCO_3$, after which the organic layer was dried with Na_2SO_4, filtered and concentrated *in vacuo*. Purification using a short silica column (EtOAc/PE 20%) yield compound **197** in 47% (3.19 g, 3.33 mmol). TLC: EtOAc/PE 40%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 - 7.09 (m, 35H, CH_{arom} Bn), 5.06 (d, J = 10.7 Hz, 1H), 5.00 - 4.94 (m, 1H), 4.86 - 4.78 (m, 2H), 4.78 - 4.67 (m, 5H), 4.65 - 4.51 (m, 3H), 4.41 - 4.28 (m, 3H), 4.23 (dd, J = 11.7, 7.9 Hz, 2H), 3.93 - 3.87 (m, 1H), 3.77 (dd, J = 12.0, 4.3 Hz, 1H), 3.71 - 3.58 (m, 3H), 3.55 - 3.47 (m, 1H), 3.47 - 3.41 (m, 1H), 3.39 - 3.30 (m, 2H), 3.23 - 3.14 (m, 1H, CH_2, DNJ), 2.73 - 2.66 (m, 1H, CH, H-2), 2.50 (dd, J = 12.1, 10.1 Hz, 1H, CH_2, DNJ); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.7-138.2 (C_q), 128.4-127.0 (C^{arom}), 103.5 (C-1'), 85.47, 82.73, 80.19, 79.97, 79.73, 75.44, 75.34, 74.80, 73.75, 73.49, 73.21, 73.17, 72.98, 72.68, 70.02, 68.29, 60.57 (C-2), 48.4 (CH_2, DNJ); IR (neat) ν 3102, 2859, 2360, 1496, 1454, 1362, 1102, 1061, 1027, 730, 694, 458; HRMS: $C_{61}H_{65}NO_9 + H^+$ requires 956.47321, found 956.47468

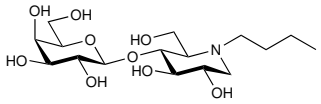
2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-*N*-butyl-1-deoxynojirimycin (198):



Compound **197** (1 g, 1.05 mmol) was dissolved in a mixture of 10 mL dioxane and 0.1 mL AcOH. Butanal (0.28 mL, 3.2 mmol, 3 equiv) was added and the mixture was purged trice with argon. Subsequently Pd/C (20%) was added and the reaction was again purged (trice) with argon followed by purging with H_2. The reaction was stirred overnight. Presence of starting material was indicated by HPLC-MS. Therefore mixture was filtered, concentrated and recharged with Pd/C in a dioxane/AcOH mixture. After overnight HPLC-MS analysis indicated product formation together with partial cleavage of the benzyl groups. The mixture was filtered, concentrated and dissolved in a mixture of 10 mL dioxane and 0.2 mL HCl (2M), followed by addition of Pd/C. The mixture was shaken overnight in a Parr apparatus® under 5 bar hydrogen pressure. The resulting mixture was filtered over Whatmann® filter paper, concentrated *in vacuo* and taken up in an Ac_2O-pyridine mixture (3 mL/9 mL). The reaction was stirred at room temperature for 18 h, after which it was cooled using an ice-bath and quenched with MeOH. The resulting mixture was concentrated *in vacuo* and purified using a short silica column (EtOAc/PE 60%) yielding compound **198** in 41% yield. (276 mg, 408 μmol) TLC: EtOAc/PE 70%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.36 - 5.29 (m, 1H), 5.10 (dd, J = 10.4, 7.9 Hz, 1H), 5.02 (t, J = 9.2 Hz, 1H), 4.96 - 4.83 (m, 2H), 4.50 (m, 2H), 4.18 - 4.02 (m, 3H), 3.84 (t, J = 6.9 Hz, 1H), 3.76 (t, J = 9.1 Hz, 1H), 3.11 (dd, J = 11.3, 5.1 Hz, 1H), 2.67 (m, 1H), 2.54 (d, J = 9.2 Hz, 1H), 2.46 (m, 1H), 2.29 (t, J = 10.8 Hz, 1H), 2.14 (s, 3H), 2.12 (s, 3H), 2.05 (s, 3H), 2.04 (m, 6H), 2.01 (s, 3H), 1.95 (s, 3H), 1.42 - 1.18 (m, 4H), 0.89 (t, J = 7.2 Hz, 3H).; ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.49, 170.33, 170.21, 170.15, 170.08, 169.94, 168.99, 101.10, 76.77, 74.10, 71.17, 70.57, 69.58, 69.26, 66.69, 62.54, 60.86, 59.00, 52.65, 51.59, 26.74, 20.97, 20.89, 20.68, 20.64, 20.50, 20.32, 13.90.; IR (neat) ν 3035, 1738, 1431, 1367, 1213, 1172, 1134, 1043, 979, 952, 912, 731;

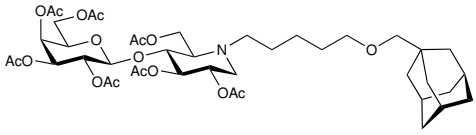
HRMS: $C_{30}H_{45}NO_{16} + H^+$ requires 676.28111, found 676.28099

4-*O*-(β-D-galactopyranosyl)-*N*-butyl-1-deoxynojirimycin (199):



To a solution of compound **198** (276 mg, 408 μmol) in MeOH a catalytic amount of NaOMe (30% in MeOH) was added. The reaction was stirred overnight after wich HPLC-MS indicated cleavage of all the acetyl groups. The mixture was quenched with five drops of AcOH (pH $\sim$ 7) and concentrated *in vacuo*. Compound **228** was purified by loading the mixture (in H_2O) on a Dowex™ H^+ cation exchange resin (type 50 WX4-200), which was stored on 2 M H_2SO_4 and flushed with H_2O and MeOH prior to use. The column was flushed trice with H_2O (30 mL) followed by twice with 1 M NH_4OH in H_2O. Concentration *in vacuo* and lyophilizing H_2O yielded **199** in 68% yield (103 mg, 270 μmol) as a white fluffy solid. ^{1}H NMR (400 MHz, MeOD) δ 4.45 (d, $J = 7.6$ Hz, 1H), 4.08 - 4.00 (m, 1H), 3.90 - 3.68 (m, 4H), 3.67 - 3.49 (m, 5H), 3.31 (m, 2H), 3.00 (dd, $J = 11.2, 4.9$ Hz, 1H), 2.87 - 2.75 (m, 1H), 2.69 - 2.57 (m, 1H), 2.30 (d, $J = 9.6$ Hz, 1H), 2.22 (t, $J = 10.9$ Hz, 1H), 154 - 1.44 (m, 2H), 1.39 - 1.28 (m, 2H), 0.98 (t, $J = 7.3$ Hz, 3H).; ^{13}C NMR (151 MHz, (D_4) MeOD) δ 103.90, 80.58, 77.38, 75.69, 73.49, 71.39, 69.08, 68.86, 64.90, 61.00, 56.65, 55.92, 51.73, 26.01, 20.34, 12.98.; HRMS: $C_{16}H_{31}NO_9 + H^+$ requires 382.19988, found 382.19975

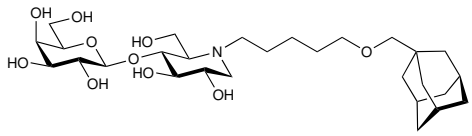
2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-*N*-[5-(adamantan-1-yl-methoxy)-pentyl]-1-deoxynojirimycin (200):



Compound **197** (1 g, 1.05 mmol) was dissolved in a mixture of 10 mL dioxane and 0.1 mL AcOH. Aldehyde **229**[19] was freshly oxidized and added to the mixture and purged trice with argon. Subsequently Pd/C was added and the reaction mixture was again purge trice with argon followed by purging with H_2 (trice). The reaction was stirred overnight. Presence of starting material was indicated by HPLC-MS. Therefore mixture was filtered, concentrated and recharged with Pd/C in a dioxane/AcOH mixture. After overnight HPLC-MS analysis indicated product formation together with partial cleavage of the benzyl groups. The mixture was filtered, concentrated and dissolved in 10 mL dioxane and 0.2 mL HCl (2 M), followed by the addition of Pd/C. The mixture was shaken overnight on a Parr apparatus® under 5 bar hydrogen pressure. The resulting mixture was filtered over Whatmann® filter paper, concentrated *in vacuo* and taken up in an Ac_2O-pyridine mixture (3 mL/9 mL). The reaction was stirred at room temperature for 18 h, after which it was cooled using an ice-bath and quenched wiotyh MeOH. The resulting mixture was concentrated *in vacuo* and purified using a short silica column (EtOAc/PE 70%) yielding compound **200** in 35% yield. (300 mg, 351 μmol) TLC: EtOAc/PE 80%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.25 (d, $J = 3.4$ Hz, 1H), 5.00 (dd, $J = 10.3, 7.9$ Hz, 1H), 4.93 (t, $J = 9.2$ Hz, 1H), 4.88 - 4.74 (m, 2H), 4.40 (d, $J = 7.9$ Hz, 2H), 4.02 (m, 3H), 3.77 (t, $J = 6.8$ Hz, 1H), 3.67 (t, $J = 9.1$ Hz, 1H), 3.25 (t, $J = 6.4$ Hz, 2H), 3.02 (dd, $J = 11.3, 5.0$ Hz, 1H), 2.84 (s, 2H), 2.66 - 2.32 (m, 3H), 2.21 (t, $J = 10.7$ Hz, 1H), 2.05 (s, 2H), 2.03 (s, 2H), 1.96 (s, 3H), 1.95 (s, 4H), 1.92 (s, 2H), 1.86 (s, 3H), 1.57 (dd, $J = 27.9, 12.0$ Hz, 7H), 1.46 - 1.13 (m, 13H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.39, 170.23, 170.06, 169.97, 169.86, 168.93, 101.03, 81.83, 76.75, 74.06, 71.28, 71.10, 70.50, 69.48, 69.20, 66.65, 62.45, 60.83, 58.99, 52.58, 51.76, 39.66, 37.15, 33.99, 29.32,

28.18, 24.45, 23.81, 20.91, 20.81, 20.61, 20.57, 20.43.; IR (neat) ν 3021, 2902, 2848, 1739, 1367,1217, 116, 1045, 908, 727, 648; HRMS: $C_{42}H_{63}NO_{17} + H^+$ requires 854.41688, found 854.41742

4-*O*-(β-D-galactopyranosyl)-*N*-[5-(adamantan-1-yl-methoxy)-pentyl]-1-deoxynojirimycin (201):



To a solution of compound **200** (300 mg, 351 μmol) in MeOH a catalytic amount of NaOMe (30% in MeOH) was added. The reaction mixture was stirred overnight after wich HPLC-MS indicated cleavage of all the acetyl groups. The mixture was quenched with five drops of AcOH (pH $\sim$ 7)and concentrated *in vacuo*. Compound **230** was purified by loading the mixture (in H_2O) on a Dowex™ H^+ cation exchange resin (type 50 WX4-200), which was stored on 2M H_2SO_4 and flushed with H_2O and MeOH prior to use. The column was flushed trice with H_2O (30 mL) followed by twice 2M NH_4OH in MeOH:H_2O (1:1). Concentration *in vacuo* and lyophilizing H_2O yielded **201** in 67% yield (133 mg, 237 μmol) as a white fluffy solid. ^{1}H NMR (600 MHz, D_2O) δ 4.54 (d, $J = 7.4$ Hz, 1H, H-1), 4.18 (d, $J = 12.5$ Hz, 1H), 4.01 (d, $J = 12.6$ Hz, 1H), 3.91 (m, 2H), 3.85 (s, 1H), 3.80 - 3.71 (m, 4H), 3.69 - 3.64 (m, 2H), 3.56 (m, 2H), 3.50 (t, $J = 6.4$ Hz, 2H), 3.38 (m, 2H), 3.24 (s, 1H), 3.14 - 3.06 (m, 4H), 1.92 (s, 4H), 1.83 - 1.74 (m, 2H), 1.71 (m, 4H), 1.61 (m, 6H), 1.49 (s, 8H), 1.46 - 1.38 (m, 2H); ^{13}C NMR (151 MHz, D_2O) δ 103.9 (C-1), 82.6 (OCH_2 Ada), 76.81, 76.47, 75.34, 73.41, 72.02, 71.89, 69.42, 65.14 (CH_2 C-6'/5'-pentyl), 61.95 (CH_2 C-6'), 47.6 (CH_2, DNJ), 40.0 (3x CH_2 Ada), 37.5 (3x CH_2 Ada), 34.4 (Cq Ada), 28.8 (CH Ada), 28.7 (CH_2 4'-pentyl), 23.4 (CH_2 3'/2'-pentyl); IR (neat) ν 3366, 2904, 1652, 1668, 1435, 1186, 1130, 841, 800, 722, 593, 448; HRMS: $C_{28}H_{49}NO_{10} + H^+$ requires 560.34292, found 560.34286

5.5 Biological Evaluation

*Detection of AMP-DNJ (**17**) in homogenate of intestinal Mucosa:* The homogenate was subjected to butanol extraction. The organic phase was desiccated in a heat block set at (37 °C) using a mild N_2 flow. The dried samples were dissolved in 100 μL MeOH, of which 10 μL was analyzed by LC-ESI-MS/MS (Waters Corp., Milford, MA, USA). Chromatographic elution of glucosylsphingosine was achieved on a BEH C_{18} Column, 1.0 x 50 mm., 1.7 μm (Waters Corp., Milford, MA, USA) using the following eluent. Eluent A: 1 mM ammonium formate in 37% MeOH, 62.5% MQ-H_2O, with 0.1% formic acid. Eluent B: 1 mM ammonium formate in 99.5% MeOH, with 0.5% formic acid. On an Acquity UPLC system, glucosylsphingosine was resolved at a flow rate of 0.25 mL/min. For this the following gradient was used: 0 -> 2.5 min. from 100% A to 100% eluent B, 2.5 -> 4 min. 100% eluent B, 4 -> 5 min. from 100% B to 100% eluent A, and from 5 -> 5.5 min. 100% eluent A to equilibrate the column. Subsequent detection was achieved on a tandem quadrupole mass spectrometer (TQD, Waters corp., Milford, MA, USA) using electrospray ionisation in pos-

itive mode. For optimization of ion source parameters and ionization conditions, direct infusion of standard (D-glucosyl-β-1-1'-D-*erythro*-sphingosine;) at a 1 μM concentration in MeOH (+ 0.5% formic acid (vol/vol)) was performed. Optimized ion source parameters: capillair voltage, 3.5 kV; cone voltage, 30 V; source and desolvation temperatures were 120 °C and 450 °C, respectively. Nitrogen gas flow in the cone was 50 L/h and desolvation gas was 500 L/h. Argon gas was used for collision-induced dissociation. Single reaction monitoring of precursor, fragment ions (m/z 398 > X) was used for quantification and data were analyzed using MassLynx software (version 4.1, Waters, Manchester, UK). Limit of detection was defined as a signal to noise ratio S/N higher than 5.

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