Synthesis & biological applications of glycosylated iminosugars

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Design and Synthesis of Chitobiose Based Prodrugs for Gaucher Disease

4.1 Introduction

GBA1 (β-glucocerebrosidase) is a retaining glycosidase (family 30), which plays an essential role in the catabolisme of glycosphingolipids (GSL). GBA1 hydrolyses the β-glycosidic bond in glucosylceramide (GC), to give D-glucose and ceramide. Inefficient degradation of GC occurs when GBA1 is mutated resulting in accumulation of GC in the lysosomes and is the cause of Gaucher disease, a rare lysosomal storage disorder (LSD).[1] Accumulation of GC leads to lipid-laden macrophages, which causes enlargement of organs (spleen and liver) and inflammations. Currently two therapies for the treatment of Gaucher patients are applied, namely enzyme replacement therapy (ERT) and substrate reduction therapy (SRT) (see also chapter 1).[2–7] In ERT a recombinant GBA1 (called Cerezyme) is intravenously administrated to patients.[8] Some of this functional GBA1 enzyme ends up in the Gaucher cells where it temporarily restores the degradation of GC. A drawback of ERT is the intravenous delivery and the high costs of enzyme production. Substrate reduction therapy offers a useful alternative, the inhibition of GCS alters the influx of GC thereby restoring the influx/efflux balance of GC in Gaucher cells.[9–11] However, monitoring of the respective effect (i.e. optimal dosage and treatment regimen) of both therapies is needed. This can be done by measuring of serum chitotriosidase activity, which was found to be directly correlated to the progression of the disease.[10,12] Chitotriosidase (CHIT1) is the first identified human chitinase and is strongly expressed and secreted by the lipid-laden macrophages found

in patients suffering from Gaucher disease.[10,13,14] Chitinases are capable to cleave natural chitin (a linear polymer of β-1,4-linked-*N*-acetylglucosamine) and a wide variety of artificial chitin-like substrates such as 4'-methylumbelliferyl chitobiose (**Chapter 2**), which is nowadays used to measure CHIT1 activity.[15]

The main side effects of SRT using NB-DNJ **16** as inhibitor are associated with the inhibition of glycosidases in the intestines, resulting in diarrhea, flatulence and abnormal bloating.[16] By locally activating the GCS inhibitors, these side effects can be diminished. This can be achieved by the use of a so called prodrug. The concept of a prodrug was introduced by Albert,[17] who describes it as a substance that has to be broken down or altered to give the true/active drug. The locally elevated activity of CHIT1 in Gaucher patients and its direct correlation with the progression of the disease, makes CHIT1 a perfect target for site-specific drug delivery *via* the prodrug approach.

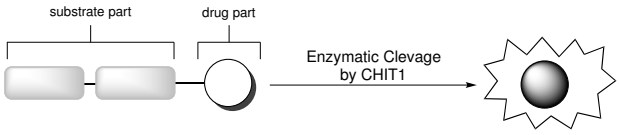


Figure 4.1: Schematic representation of an enzymatic prodrug cleavage. ∘: inactive inhibitor ; •: active inhibitor.

The designed prodrugs for Gaucher disease will consist of a substrate part, chitobiose (for prodrugs **174** and **172**) or 4'-deoxy chitobiose (for prodrug **173**) which are both known to be cleaved by CHIT1 (Figure 4.2).[12,15] As drug part of the prodrugs NB-DNJ (**16**) and AMP-DNJ (**17**) will be used, which are both known inhibitors of GCS.[18]

Recent studies showed that substitution on the 4-OH of AMP-DNJ **17** gives rise to less active GCS inhibitors, thereby making this position the perfect site for linkage with the chitobiose core (CHIT1 substrate).[19] Figure 4.1 shows the *in vivo* mode of action of the Gaucher prodrug. Upon enzymatic cleavage of the glycosidic bond, by CHIT1, the active GCS inhibitor will be liberated.

4.2 Results and Discussion

For the synthesis of chitobiose based prodrugs **172**, **173** and **174** a sequential glycosylation strategy was selected (Figure 4.2). In the first glycosylation event the chitobiose core will be formed (**175** or **176**) which will be used as CHIT1 substrate part of the prodrugs. Next the iminosugar (**177**) will be condensed with the chitobiose core, which will later be decorated with a butyl or AMP chain to form the GCS inhibitor part (drug part) of the Gaucher prodrugs.

For the synthesis of the chitobiose core **176**, imidate **179** was used as donor and thioglycoside **180**[20] as acceptor (Scheme 4.1). The phthaloyl group at the 2-

positions of both donor and acceptor ensure the formation of 1,2-trans glycosidic bonds in this and the next coupling of the resulting disaccharide **176** with DNJ acceptor **177** (Scheme 4.3). Chitobiose donor **175** was synthesized under similar conditions and used to explore the most productive coupling conditions for the next glycosylation with DNJ acceptor **177**.[21]

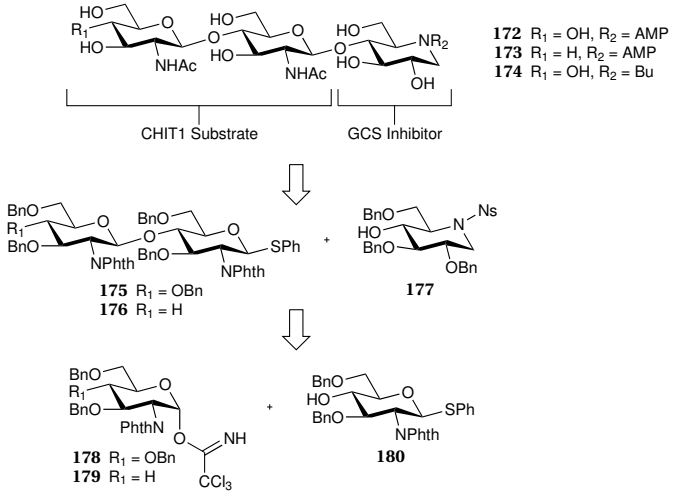
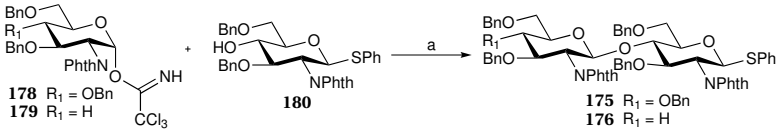


Figure 4.2: Retrosynthetic analysis of potential Gaucher prodrugs **172**, **173** and **174**.

Scheme 4.1: Synthesis of the chitobiose donors **175** and **176**.

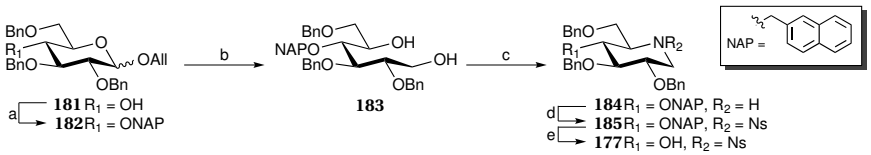


Reagents and conditions: a) TMSOTf, DCM, 0 °C, (**175**, 73%; **176**, 41%.)

DNJ acceptor **177** was synthesized from known benzylated allyl glucopyranoside **181**.[22] First, the free hydroxyl function in **181** was protected with the 2'-naphthylmethylether (NAP) group (Scheme 4.2). This relatively new protective group can be cleaved under oxidative conditions (*e.g.* DDQ or CAN) and is more acid stable than the more commonly used *p*-methoxybenzyl group.[23–25]

Next, the anomeric allyl group in **182** was isomerized using KO*t*Bu in hot DMSO. The formed vinyl-ether was hydrolyzed using molecular iodine in THF:H_2O, directly followed by $LiAlH_4$ mediated reduction to yield glucitol **183**. Cyclization to iminosugar **184** was effected by a two step sequence. First lacitol **183** was oxidized under Swern conditions. Subsequently, the crude di-carbonyl was subjected to a double reductive amination using an excess of ammonium formate

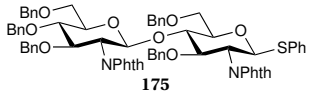
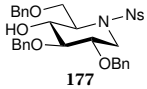
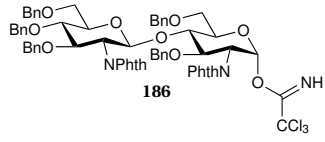
Scheme 4.2: Synthesis of DNJ acceptor **177**.



Reagents and conditions: a) NAP-Br, NaH, DMF, 0 °C, 80%; b) (1) KO*t*Bu, DMSO, 100 °C, (2) I_2, THF:H_2O, (3) $LiAlH_4$, THF, 71% over three steps; c) (1) DMSO, $(COCl)_2$, DCM, -75 °C, (2) Et_3N, -75 °C to rT, (3) $NaCNBH_3$, $HCOONH_4$, Na_2SO_4, MeOH, 0 °C, 63%; d) NsCl, pyridine, DCM, 87%, e) DDQ, DCM:MeOH, 90%.

in MeOH at 0 °C under the agency of $NaCNBH_3$ and Na_2SO_4 yielding DNJ derivative **184**.[26] After protection of the endocyclic nitrogen with 2-nitrobenzenesulfonyl (nosyl, Ns), the 4-OH was liberated by deprotection of the NAP-group using DDQ[23] yielding acceptor **177**.

Table 4.1: Optimization of coupling conditions for the synthesis of trimer **187**.

Entry	Donor	Acceptor	Activation conditions	Temp.	Yield
1	 **175**	 **177**	NIS, TMSOTf, DCM	0ºC	60%
2	**175**	**177**	Me_2S_2-Tf_2O, Et_2O, DCM	-30ºC	40%
3	**175**	**177**	Ph_2SO, Tf_2O, DCM	A	24%
4	**175**	**177**	Ph_2SO, Tf_2O, DCM	B	0%*
5	**175**	**177**	Ph_2SO, Tf_2O, TTBP, DCM	A	0%**
6	**175**	**177**	Ph_2SO, Tf_2O, TTBP, DCM	B	0%*
7	 **186**	**177**	TMSOTf, DCM	0ºC	34%

Coupling conditions **A**: Activation at -60 °C, addition of acceptor at -60 °C, quenching of the reaction by addition of Et_3N at -30 °C; **B**: Activation at -60 °C, addition of acceptor at -30 °C, quenching of the reaction by addition of Et_3N at 0 °C; * : Hydrolyzed donor and acceptor recovered; **: Thiodonor and acceptor recovered.

To investigate an effective glycosylation method for DNJ acceptor **177**, several coupling procedures were explored, using chitobiose donor **175** (Table 4.1). Activation of **175** with NIS with a catalytic amount of TMSOTf at 0 °C, gave a yield of 60% of the desired trimer **187**. The activator dimethyl disulfide-triflic anhydride ($Me_2S_2-Tf_2O$), developed by the group of Fügedi,[27] gave a lower yield (40%). In entries **3** to **6** a preactivation procedure, using the Ph_2SO/Tf_2O,[28,29] reagent sys-

tem was explored. This however, gave disappointing results with a maximum yield of 24%. Varying the temperature or addition of the non-nucleophilic base 2,4,6-tri-*tert*-butylpyrimidine (TTBP) gave either hydrolysed donors (entry **4** and **6**) or recovery of unreacted starting material (entry **5**).

Chitobiose imidate donor **186** used in entry **7** was synthesized by first hydrolysis of chitobiose thio donor **175** using NBS in wet acetone at -20 °C, after which the hemiacetal was converted into imidate **186** using CCl_3CN and DBU in dry DCM. The chitobiose imidate donor **186** was coupled to DNJ acceptor **177** using TMSOTf yielding target trimer **187** in an unsatisfactory yield of 34%.

Because of the structure of trimer **187** also allows a sequential glycosylation strategy it was of special interest to find out whether the assembly of trisaccharide **187** could also be attained in a one-pot procedure (Figure 4.3). To this end, imidate **178** was activated using TMSOTf in the presence of acceptor **180** giving rise to dimer **175**. Subsequent coupling of DNJ acceptor **177** under influence of NIS afforded trimer **187** in a disappointing yield of 12%.

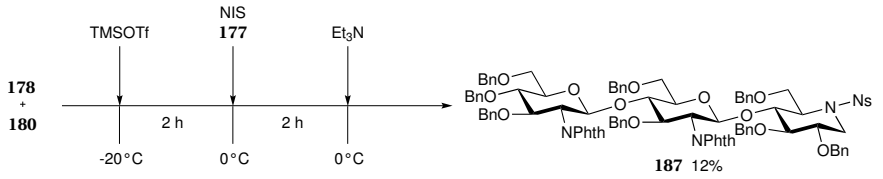


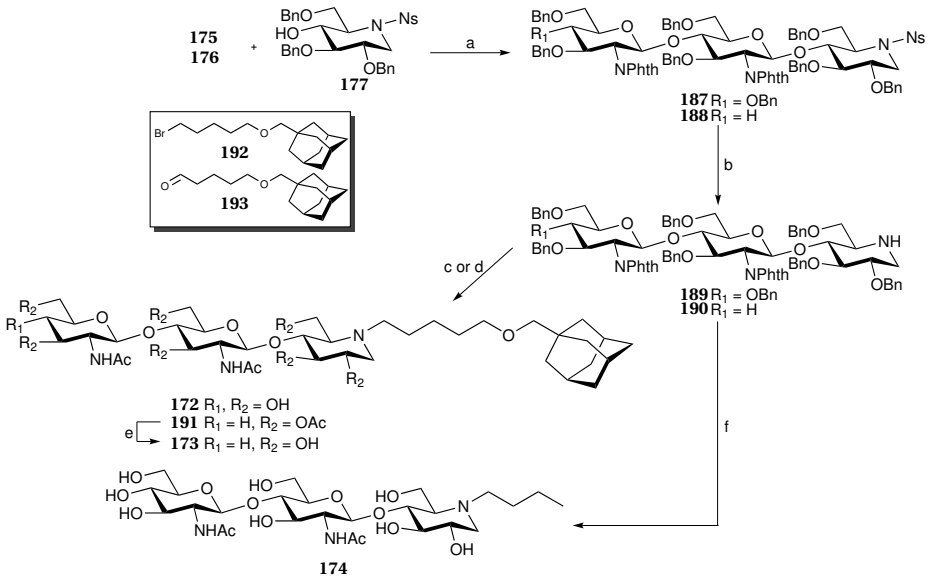
Figure 4.3: One-pot procedure for the synthesis of trimer **187**.

To obtain compound **188**, disaccharide **176** was condensed with protected DNJ **177** (Scheme 4.2) using the most productive conditions that were found for the corresponding glycosylation to give **187** (Table 4.1, entry **1**).

In the next event, removal of the nosyl protective group from the endocyclic nitrogen was accomplished by an aromatic nucleophilic substitution using thiophenol and K_2CO_3 (Scheme 4.3). The liberated secondary amine in **189** was alkylated using 1-bromobutane or 5-(adamantan-1-yl-methanol)-1-bromo-pentane **192** under mild basic conditions (K_2CO_3, DMF). The crude compounds were deprotected, by removal of the phthalimide with ethylenediamine in refluxing *n*-butanol followed by acetylation of the free amines. Hydrogenation of the benzyl groups and purification by HPLC gave target prodrugs **172** and **174**. Unfortunately, the products were obtained in low yields (3-4%).

Because of the poor yields in the alkylation reaction, attention was directed to reductive amination using Pd/C (20%), H_2 and aldehyde **193** for the synthesis of prodrug **173**.[26] Apart from successful alkylation of the endocyclic nitrogen, this reduction also conveniently removed all the benzyl-groups. The crude compound was further deprotected, by removal of the phthalimide with ethylenediamine in refluxing *n*-butanol. To facilitate the ensuing purification the resulting product was fully acetylated to give **191**. Saponification of the acetyl esters with NaOMe in

Scheme 4.3: Synthesis of prodrug **172**, **173** and **174**.



Reagents and conditions: a) NIS, TMSOTf, DCM, 0 °C, **187** 73%; **188** 70%; b) HSPh, K_2CO_3, DMF, **189** 75%; **190** 85%; c) (1) **192**, K_2CO_3, DMF, 85 °C; (2) $(H_2NCH_2)_2$, *n*BuOH, Δ, (3) Ac_2O, pyridine; (4) Pd/C, H_2, EtOH:AcOH, **172** 3%; d) (1) **193**, Pd/C, H_2, dioxane:AcOH, (2) $(H_2NCH_2)_2$, *n*BuOH, Δ, (3) Ac_2O, pyridine, **191**, 36%; e) NaOMe, MeOH, **173** 30%; f) (1) 1-bromobutane, K_2CO_3, DMF, 85 °C; (2) $(H_2NCH_2)_2$, *n*-Bu, Δ, (3) Ac_2O, pyridine; (4) Pd/C, H_2, EtOH:AcOH, **174** 4%.

MeOH and purification by Dowex™ H^+ column and HPLC completed the synthesis of target prodrug **173** 30% yield. It must be mentioned that the use of freshly oxidized aldehyde **193**, in the reductive amination, is crucial to gain a high yield, which corroborates with the results reported by Wennekes *et al.*[26]

4.3 Conclusion

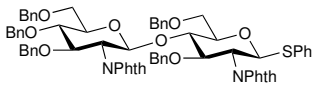
This chapter describes the synthesis of three Gaucher prodrugs **172**, **173** and **174** in which the chitobiose core is used as CHIT1 substrate and NB-DNJ or AMP-DNJ is used as GCS inhibitor or drug part. The chitobiose core was synthesized *via* an imidate coupling with acceptor **180** bearing a thiophenol group on the anomeric position, which could be immediately used in the next glycosylation step with a DNJ acceptor **177**. It was found that the NIS/TMSOTf activation method, in dry DCM, gave the highest yield and the most reproducible results (Table 4.1 entry **1**). After poor yields for the alkylation of the endocyclic nitrogen of the iminosugar in **189**. A different route was used for the synthesis of **173**. By reductive amination, using 5-(adamantan-1-yl-methoxy)-1-pentanal, compound **190** was converted into prodrug **173** in an improved yield. Key in this reductive amination of the ring nitrogen is the use of freshly oxidized aldehyde **193**.

The synthesized Gaucher prodrugs will be biologically evaluated to gain insights in the ability of CHIT1 to cleave the chitobiose core from the iminosugar, resulting in the liberation of the active GCS inhibitors **16** or **17**.

4.4 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}.

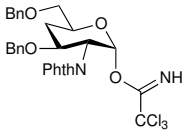
Phenyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-4-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-1-thio-D-glucopyranoside(175):



Known imidate **178**[21] (2.98 g, 4.22 mmol, 1.1 equiv) and acceptor **180**[20] (2.23 g, 3.84 mmol) were coevaporated thrice with toluene and dissolved in dry DCM (40 mL). Molecular sieves 3Å were added and the reaction was cooled to -20 °C. After 10 minutes the reaction was activated by addition of TMSOTf (76 μL, 0.42 mmol, 0.1 equiv) and was stirred for 3 h allowing the mixture to warm to 0 °C. Subsequently, the reaction mixture was quenched with TEA (0.2 mL), filtered and concentrated *in vacuo*. Purification using a short silica column (EtOAc/PE 22.5%) gave **175** in 73% yield as a colorless oil (3.20 g, 2.80 mmol). TLC: EtOAc/PE 40%; ^1H NMR (400 MHz, $CDCl_3$) δ 7.87 - 7.41 (m, 7H), 7.36 - 7.19 (m, 17H), 7.16 - 6.67 (m, 13H), 5.39 - 5.33 (d, $J = 9.9$ Hz, 1H), 5.32 - 5.28 (d, $J = 8.3$ Hz, 1H), 4.92 - 4.86 (d, $J = 12.7$ Hz, 1H), 4.84 - 4.74 (m, 2H), 4.73 - 4.62 (d, $J = 11.0$ Hz, 1H), 4.55 - 4.35 (m,

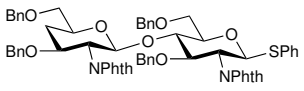
8H), 4.27 - 4.15 (m, 4H), 3.91 - 3.79 (dd, $J = 9.9$, 8.6 Hz, 1H), 3.79 - 3.60 (m, 2H), 3.57 - 3.30 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.5 - 167.3, 138.6- 133.9, 133.7, 132.2, 131.7, 131.5, 128.6 - 127.4, 97.1, 83.4, 79.7, 79.1, 78.9, 77.9, 75.6, 75.2, 74.9, 74.8, 74.7, 73.3, 72.7, 68.4, 68.0, 56.8, 54.8; IR (neat) ν 1774, 1710, 1385, 1070, 1025, 737, 719, 696, 612; HRMS: $C_{69}H_{62}N_2O_{12}S+Na^+$ requires 1165.39157, found 1165.39209; $[\alpha]_D^{23} + 33.2$ °(c = 1, $CHCl_3$).

3,6-Di-*O*-benzyl-2,4-di-deoxy-2-phthalimido-1-*O*-trichloroacetimidoyl)-β-D-glucopyranoside(179):



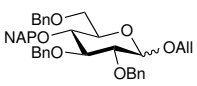
3,6-Di-*O*-benzyl-2,4-di-deoxy-2-phthalimido-D-xylo-hexapyranose[30] (3.37 g, 7,12 mmol) was coevaporated thrice with toluene after which it was dissolved in dry DCM (50 mL). The solution was cooled to 0 °C and stirred for 10 minutes followed by addition of CCl_3CN (7.14 mL, 71.2 mmol, 10 equiv) and DBU (0.27 mL, 1.78 mmol, 0.25 equiv). The reaction mixture was stirred overnight at 4 °C, after which TLC-analysis showed complete conversion of the starting material in a higher running product. The reaction mixture was concentrated *in vacuo*. Purification using a short silica column (EtOAc/PE 30% + 2.5% TEA) gave **179** in 71% yield (3.12 g, 5.05 mmol). TLC: EtOAc/PE 50%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 - 8.55 (s, 1H), 7.78 - 7.60 (m, 5H), 7.40 - 7.20 (m, 6H), 7.15 - 6.89 (m, 6H), 6.54 - 6.31 (d, $J = 8.3$ Hz, 1H), 4.64 - 4.22 (m, 7H), 4.04 - 3.91 (dd, $J = 8.2$, 3.6 Hz, 1H), 3.71 - 3.53 (m, 2H), 2.37 - 2.27 (m, 1H), 1.76 - 1.55 (td, $J = 12.6$, 10.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.7, 137.8, 137.7, 134.0, 131.3, 127.6, 127.5, 123.2, 94.5, 73.2, 72.3, 72.3, 71.6, 70.9, 55.8, 33.8.

Phenyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-4-*O*-(3,6-di-*O*-benzyl-2,4-di-deoxy-2-phthalimido-β-D-glucopyranosyl)-1-thio-D-glucopyranoside(176):



Imidate **179** (3.09 g, 5.00 mmol, 1.1 equiv) and acceptor **180**[20] (2.64 g, 4.55 mmol) were coevaporated thrice with toluene and dissolved in dry DCM (50 mL). Molecular sieves 3Å were added and the reaction was cooled to -20 °C. After 15 minutes the reaction was activated by addition of TMSOTf (90 μl, 0.50 mmol, 0.1 equiv) and was stirred for 3 h allowing the mixture to warm to 0 °C. Subsequently, the reaction mixture was quenched with Et_3N (0.2 mL), filtered and concentrated *in vacuo*. Purification using a short silica column (EtOAc/PE 22.5%) gave **176** in 70% yield as a colorless oil (3.3 g, 3.185 mmol). TLC: EtOAc/PE 40%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 - 7.45 (m, 8H), 7.39 - 7.18 (m, 16H), 7.14 - 6.94 (m, 13H), 6.87 - 6.76 (m, 4H), 5.49 - 5.39 (d, $J = 9.7$ Hz, 1H), 5.37 - 5.25 (d, $J = 8.2$ Hz, 1H), 4.94 - 4.83 (d, $J = 12.3$ Hz, 1H), 4.65 - 4.09 (m, 14H), 3.63 - 3.34 (m, 6H), 2.36 - 2.18 (m, 1H), 1.58 - 1.41 (q, $J = 11.8$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.2, 167.7, 167.6, 167.0, 138.2 - 137.8, 133.7, 133.6, 132.2, 131.5, 131.3, 128.1 - 126.9, 126.7, 122.9, 74.2, 73.1, 72.4, 71.8, 70.5, 68.2, 34.1; IR (neat) ν 2344, 1709, 1683, 1385, 1274, 1066, 1025, 764, 749, 696, 661, 461; HRMS: $C_{62}H_{56}N_2O_{11}S+Na^+$ requires 1059.34970, found 1059.35039; $[\alpha]_D^{23} + 44.8$ °(c = 1, $CHCl_3$).

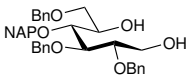
Ally 2,3,4-tri-*O*-benzyl-4-*O*-(2-naphthylmethyl)-α/β-D-glucopyranoside)(182):



Compound **181**[22] (19 g, 38.9 mmol) was coevaporated thrice using toluene, after which it was dissolved in DMF (175 mL) and cooled to 0 °C. Sodium hydride (60% in mineral oil) (2.9 g, 76.9 mmol, 2 equiv) was added portion wise. After 15 minutes the NAP-Br (17 g, 76.9

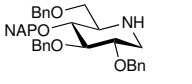
mmol, 2 equiv) was added and the reaction was stirred overnight allowing the reaction mixture to warm to rT. Subsequently, the reaction mixture was cooled to 0 °C, quenched using little MeOH, diluted with Et_2O and washed twice with 1M HCl and H_2O. The organic layer was dried using $MgSO_4$ and concentrated under reduced pressure. Purification using a short silica column (EtOAc/PE 10%) gave **182** in 89% yield (21.56 g, 34.18 mmol). TLC: EtOAc/PE 30%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 - 7.70 (m, 3H), 7.59 - 7.42 (m, 3H), 7.38 - 7.22 (m, 19H), 6.05 - 5.84 (m, 1H), 5.41 - 5.25 (m, 1H), 5.24 - 5.15 (m, 1H), 5.06 - 4.91 (m, 3H), 4.87 - 4.58 (m, 5H), 4.56 - 4.39 (m, 2H), 4.21 - 4.10 (dd,J = 12.9, 5.9 Hz, 1H), 3.90 - 3.45 (m, 5H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.84, 138.62, 138.37, 135.75, 134.23, 133.93, 128.57, 128.51, 128.40, 127.70, 126.78, 126.03, 117.39, 102.89, 84.90, 82.46, 78.04, 75.86, 75.19, 73.67, 70.48, 70.43, 69.14 ; IR (neat) ν 2918, 2864, 1454, 1361,1122, 1070, 1028, 929, 856, 748, 736, 698; HRMS: $C_{41}H_{42}O_6 + Na^+$ requires 653.28736, found 653.28750; $[\alpha]_D^{23}$ + 3.6 °(c = 0.5, $CHCl_3$).

2,3,4-Tri-*O*-benzyl-4-*O*-(2-naphthylmethyl)-D-glucitol(183):



A dry solution of **182** (10.72 g, 70 mmol) in DMSO (8.5mL) was charged with KO*t*Bu (0.95 g, 8.5 mmol, 0.5 equiv) and heated to 100 °C for 3 h, after which the reaction was quenched by addition of H_2O (5 mL). The reaction mixture was poured in H_2O and extracted twice with Et_2O. The organic layers were combined and washed with 1M HCl. The ether fraction was dried using $MgSO_4$ and concentrated *in vacuo*. The residue was redissolved in THF:H_2O (70:15 mL), followed by addition of molecular iodine (8.63 g, 34 mmol, 2 equiv). The mixture was stirred overnight after which the reaction was quenched by addition of $Na_2S_2O_3$ and washed with EtOAc and brine. The organic layer was dried and concentrated *in vacuo* resulting in a yellow solid. The solid was again redissolved in dry THF (120 mL) and cooled to 0 °C followed addition of $LiAlH_4$ (2.26 g, 59.5 mmol, 3.5 equiv) and stirred for 20 h allowing to warm to rT. The excess of $LiAlH_4$ was quenched with water. The mixture was diluted with EtOAc and washed thrice with NH_4Cl. The organic layer was dried and concentrated *in vacuo*. Purification using a short silica column (EtOAc/PE 30%) gave **183** in 71% yield (6.96 g, 12.0 mmol). TLC: EtOAc/PE 50%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 - 7.48 (m, 4H), 7.60 - 6.96 (m, 18H), 4.75 - 4.60 (m, 4H), 4.59 - 4.54 (s, 2H), 4.49 - 4.33 (q,J = 11.9 Hz, 2H), 4.11 - 4.05 (m, 1H), 3.95 - 3.90 (dd,J = 6.2, 3.7 Hz, 1H), 3.85 - 3.76 (m, 2H), 3.74 - 3.67 (dd,J = 11.8, 4.2 Hz, 1H), 3.65 - 3.54 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.1 - 132.7, 128.2 - 125.7, 79.50, 78.89, 77.56, 74.35, 73.14, 72.73, 71.09, 70.59, 61.46; IR (neat) ν 3433, 3032, 2924, 2870, 1713, 1612, 1512, 1458, 1358, 1288, 1250, 1065, 1034, 918, 818, 733; HRMS: $C_{38}H_{40}O_6 + Na^+$ requires 615.27171, found 615.27168; $[\alpha]_D^{23}$ + 3.2 °(c = 0.5, $CHCl_3$).

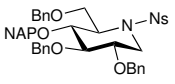
2,3,4-Tri-*O*-benzyl-4-*O*-(2-naphthylmethyl)-1-deoxynojirimycin(184):



A solution of oxalylchloride (4.1 mL, 47.68 mmol, 4 equiv) in dry DCM (40 mL) was cooled to -78 °C and stirred for 15 minutes. After dropwise addition of DMSO (4.23 mL, 59.6 mmol, 5 equiv) in dry DCM (20 mL) over 10 minutes, the reaction was stirred for 40 minutes at -70 °C. Subsequently, a dry solution of **183** (6.90 g, 11.92 mmol) in dry DCM (15 mL) was added dropwise in 15 minutes, while keeping the reaction temperature at -70 °C. The reaction mixture was stirred for 2 h after which Et_3N (20 mL, 143 mmol, 12 equiv) was dropwise added and the mixture was allowed to warm to -5 °C in 1 h. This reaction mixture was added to a cooled (0 °C) solution of $NaCNBH_3$ (2.79 g, 47.68 mmol, 4 equiv), NH_4CO_3

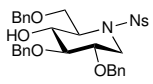
(18.84 g, 238.4 mmol, 20 equiv) and Na_2SO_4 (6.77 g, 47.68 mmol, 4 equiv) in 300 mL MeOH. The reaction was stirred overnight allowing the mixture to warm to room temperature. After TLC-analysis showed full conversion into a lower running product, the reaction mixture was filtered and concentrated under reduced pressure. The oily residue was redissolved in EtOAc and washed with $NaHCO_3$, after which the organic layer was dried using Na_2SO_4, filtered and concentrated *in vacuo*. Purification using a short silica column (EtOAc/PE 15%) gave compound **184** in 63% yield (4.32 g, 7.55 mmol). TLC: EtOAc/PE 30%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 - 7.70 (m, 3H), 7.64 - 7.59 (s, 1H), 7.48 - 7.40 (m, 2H), 7.39 - 7.16 (m, 17H), 5.06 - 4.93 (d,J = 11.0 Hz, 2H), 4.92 - 4.81 (d,J = 11.0 Hz, 1H), 4.74 - 4.58 (m, 3H), 4.50 - 4.24 (m, 2H), 3.70 - 3.46 (m, 4H), 3.46 - 3.36 (t,J = 9.2 Hz, 1H), 3.30 - 3.17 (dd,J = 12.3, 5.0 Hz, 1H), 2.78 - 2.68 (m, 1H), 2.56 - 2.45 (dd,J = 12.4, 10.3 Hz, 1H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.0 - 133.0, 128.1 - 127.6, 87.45, 80.73, 80.08, 75.75, 75.27, 73.44, 72.84, 70.27, 59.82, 48.17; IR (neat) ν 2864, 2800, 1770, 1724, 1496, 1454, 1361, 1166, 1093, 1064, 817, 734, 689, 624; HRMS: $C_{38}H_{39}NO_4 + Na^+$ requires 596.27713, found 596.27715; $[\alpha]_D^{23}$ - 8.4 °(c = 0.6, $CHCl_3$).

2,3,4-Tri-*O*-benzyl-4-*O*-(2-naphthylmethyl)-*N*-(2-nitrobenzenesulfonyl)-1-deoxynojirimycin(185):



Compound **184** was dissolved in DCM (35 mL) and Ns-Cl (8.37 g, 37.75 mmol, 5 equiv.) and pyridine (1.21 mL, 15.1 mmol, 2 equiv) were added. The reaction was stirred overnight after which TLC analysis showed incomplete conversions. An additional 2 equivalents of pyridine (1.21 mL) was added and stirring was continued for 5 h. The mixture was diluted with DCM and washed with H_2O and $NaHCO_3$. The DCM layer was dried with $MgSO_4$, filtered and concentrated *in vacuo*. Purification using a short silica column (EtOAc/PE 40%) gave compound **185** as a yellow oil in 87% yield (4.98 g, 6.56 mmol). TLC:EtOAc/PE 50% ; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 - 8.05 (m, 1H), 7.81 - 7.75 (m, 1H), 7.73 - 7.60 (m, 2H), 7.57 - 7.53 (d,J = 1.6 Hz, 1H), 7.46 - 7.37 (m, 2H), 7.29 - 7.08 (m, 15H), 7.01 - 6.93 (m, 1H), 6.79 - 6.71 (m, 1H), 4.65 - 4.33 (m, 7H), 4.33 - 4.29 (s, 2H), 4.02 - 3.97 (t,J = 3.4 Hz, 1H), 3.89 - 3.67 (m, 4H), 3.66 - 3.54 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.0, 137.9 - 137.6, 135.09, 133.0 - 122.9, 76.19, 75.28, 72.90, 72.87, 72.33, 71.89, 71.03, 68.68, 56.20, 42.29; IR (neat) ν 2858, 2349, 2310, 1541, 1456, 1354, 1338, 1163, 1089, 1074, 1028, 748, 698; HRMS: $C_{44}H_{42}N_2O_8S + Na^+$ requires 781.25541, found 781.25540; $[\alpha]_D^{23}$ + 33.2 °(c = 0.5, $CHCl_3$).

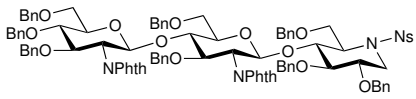
2,3,4-Tri-*O*-benzyl-4-*O*-(2-naphthylmethyl)-*N*-(2-nitrobenzenesulfonyl)-1-deoxynojirimycin(177):



To a dry solution of **185** (4.98 g, 6.56 mmol) in DCM/MeOH (300/80 mL), DDQ (4.47 g, 19.68 mmol, 3 equiv) was added portion wise. The reaction mixture turned dark instantly and was stirred for 20 h. Next the reaction mixture was diluted with DCM and extracted thrice with $NaHCO_3$ and twice with brine. The organic layer was dried using $MgSO_4$, filtered and concentrated *in vacuo*. Purification using a short silica column (EtOAc/PE 30%) gave compound **177** in 72% yield (2.92 g, 4.72 mmol). TLC: EtOAc/PE 50%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 - 8.02 (m, 1H), 7.46 - 7.19 (m, 14H), 7.19 - 7.11 (dd,J = 6.7, 2.8 Hz, 2H), 7.06 - 6.95 (dd,J = 6.6, 3.0 Hz, 2H), 4.81 - 4.74 (d,J = 11.5 Hz, 1H), 4.52 - 4.32 (m, 4H), 4.28 - 4.21 (m, 1H), 4.20 - 4.05 (m, 2H), 3.93 - 3.79 (m, 1H), 3.74 - 3.59 (m, 2H), 3.55 - 3.43 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.4, 137.6 - 134.2, 132.9 - 127.4, 123.63, 74.02, 72.82, 72.54,

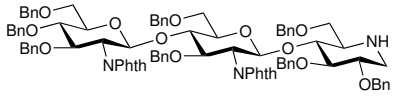
72.27, 71.16, 66.99, 66.00, 60.02, 39.43; IR (neat) ν 3522, 3500, 3487, 3086, 2922, 2866, 1541, 1496, 1371, 1357, 1174, 1089, 1076, 972, 852, 744, 698; HRMS: $C_{33}H_{34}N_2O_8S+Na^+$ requires 641.19281, found 641.19281; $[\alpha]_D^{23}$ -50 °(c = 1, $CHCl_3$).

_N_-(2-nitrobenzenesulfonyl)-2,3,6-tri-_O_-benzyl-4-_O_-[3,6-di-_O_-benzyl-2-deoxy-2-phthalimido-4-_O_-(3,4,6-tri-_O_-benzyl-2-phthalimido-β-D-glucopyranosyl)-β-D-glucopyranosyl]-1-deoxynojirimycin(187):



Dimer **175** (652 mg, 571 μmol, 1.1 equiv) and acceptor **177** (298 mg, 519 μmol) were coevaporated thrice with toluene and dissolved in dry DCM (3 mL). Molecular sieves 3Å were added and the reaction was cooled to 0 °C. After 10 minutes NIS (140 mg, 0.623 mmol, 1.2 equiv) was added and the reaction was activated by addition of TMSOTf (5 μl, cat.). The reaction mixture turned deep purple and was stirred for 2 h at 0 °C. After 2 h TLC-analysis showed complete conversion and the reaction was quenched by addition of Et_3N (0.2 mL). The reaction mixture was diluted with DCM and washed with $Na_2S_2O_3$, $NaHCO_3$ and H_2O. The organic layer was dried using $MgSO_4$, filtered and concentrated under reduced pressure. The crude oil was dissolved in an Ac_2O-pyridine cocktail (1 mL/3 mL) to acetylate the unreacted acceptor. After 3 h the reaction was quenched using a little MeOH and concentrated *in vacuo*. Purification using a short silica column (MeOH/DCM 5%) gave **187** in 60% yield as a yellow foam (507 mg, 307 μmol). TLC: MeOH/DCM 7%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 - 6.49 (m, 61H), 5.31 - 5.25 (d, J = 8.2 Hz, 1H), 5.20 - 5.15 (m, 1H), 4.95 - 2.99 (m, 37H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4, 168.2, 167.5, 147.8, 138.3 - 137.5, 132.5, 131.7, 131.3, 128.4 - 127.2, 123.0, 74.9, 74.7, 74.5, 73.1, 72.5, 72.3, 71.8, 70.5, 68.2, 67.9, 67.8; IR (neat) ν 1710, 1387, 1357, 1070, 1027, 737, 720, 697, 586; HRMS: $C_{96}H_{90}N_4O_{20}S+Na^+$ requires 1674.57949, found 1674.58146; $[\alpha]_D^{23}$ - 29.6 °(c = 1, $CHCl_3$).

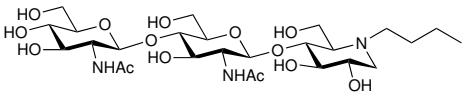
2,3,6-Tri-_O_-benzyl-4-_O_-[3,6-di-_O_-benzyl-2-deoxy-2-phthalimido-4-_O_-(3,4,6-tri-_O_-benzyl-2-phthalimido-β-D-glucopyranosyl)-β-D-glucopyranosyl]-1-deoxynojirimycin (189):



Fully protected trimer **187** (129 mg, 80 μmol) was dissolved in DMF (1 mL), followed by addition of HSPh (17 μL, 160 μmol, 2 equiv) and K_2CO_3 (33 mg, 240 μmol, 3 equiv). The reaction mixture was stirred for 20 h at rT after which TLC-analysis showed conversion into a lower running product. The reaction mixture was diluted with EtOAc and washed twice with $NaHCO_3$ and once with H_2O. The organic layer was dried using $Na_2S_2O_3$, filtered and concentrated under reduced pressure. Purification using a short silica column (EtOAc/PE 80%) gave **189** in 75% yield (88 mg, 60 μmol). TLC: EtOAc 100%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 - 7.53 (m, 7H), 7.41 - 7.07 (m, 29H), 7.04 - 6.89 (m, 9H), 6.85 - 6.75 (m, 4H), 5.34 - 5.27 (d, J = 8.1 Hz, 1H), 5.29 - 5.21 (d, J = 8.3 Hz, 1H), 4.98 - 4.87 (m, 2H), 4.85 - 4.80 (d, J = 11.3 Hz, 2H), 4.77 - 4.31 (m, 11H), 4.31 - 4.04 (m, 5H), 3.98 - 3.66 (m, 4H), 3.45 - 3.30 (m, 4H), 3.19 - 2.81 (m, 5H), 2.62 - 2.54 (m, 1H), 2.42 - 2.32 (t, J = 11.2 Hz, 1H).; ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.3, 167.7, 139.7, 138.9, 138.7 - 138.0, 132.0, 131.6, 128.5 - 127.3, 74.91, 74.86, 74.54, 74.06, 73.28, 72.92, 72.67, 72.35, 70.71, 68.01, 67.24, 59.03, 56.82; IR (neat) ν 1710, 1387, 1070, 1027, 910, 734, 721, 696, 530, 356;

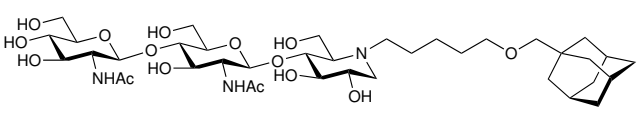
HRMS: $C_{96}H_{90}N_4O_{20}S + Na^+$ requires 1466.61591, found 1466.61775;

4-*O*-[2-deoxy-2-*N*-acetyl-4-*O*-(2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranosyl]-*N*-butyl)-1-deoxynojirimycin(174):



Trimer **189** (120 mg, 82μmol, 1 equiv), 1-bromobutane (17 mg, 123 μmol, 1.5 equiv) and K_2CO_3 (34 mg, 246 μmol, 3 equiv) were dissolved in DMF and stirred overnight at 85 °C. TLC analysis showed incomplete conversion of the starting material, so an additional 3 equivalents of 1-bromobutane (40 mg) were added and stirring was continued for 18 h. Subsequently, the mixture was filtered and concentrated *in vacuo*. The resulting oil was taken up in *n*-butanol (2 mL) and ethylenediamine (27μL) was added. The reaction mixture was refluxed for 4 h, after which it was diluted with toluene, concentrated and coevaporated twice with toluene. The resulting yellow oil was taken up in an Ac_2O-pyridine cocktail (0.5 mL/1.5 mL) and stirred overnight. The reaction was stopped by quenching with a little MeOH and concentrated *in vacuo*. The crude oil was dissolved in EtOH:HCl (1:0.1 mL), purged thrice with argon and charged with Pd/C (20%) and purged thrice again with argon, followed by purging with H_2. The mixture was stirred overnight at rT and under atmospheric pressure. HPLC-MS showed full deprotection of all the benzyl groups. Purification by HPLC (gradient H_2O-MeOH + 0.1% TFA) evaporation of MeOH and lyophilizing H_2O yielded **174** (2.08 mg, 2.9 μmol, 4%). ^{1}H NMR (600 MHz, D_2O) δ 4.50 - 4.40 (m, 1H), 3.92 - 3.87 (d,$J = 11.9$ Hz, 1H), 3.84 - 2.78 (m, 25H), 1.99 - 1.87 (m, 7H), 1.88 - 1.82 (m, 1H), 1.61 - 1.54 (s, 2H), 1.30 - 1.22 (m, 2H), 1.18 - 1.12 (m, 2H), 0.86 - 0.75 (m, 3H).

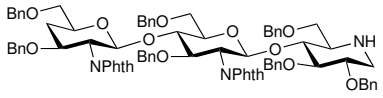
4-*O*-[2-deoxy-2-*N*-acetyl-4-*O*-(2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranosyl]-*N*-[5-(adamantan-1-yl-methoxy)-pentyl]-1-deoxynojirimycin(172):



Trimer **189** (120 mg, 82 μmol, 1 equiv), 5-(adamantan1-yl-methoxy)-1-bromopentane[19] (39 mg, 123 μmol, 1.5 equiv) and K_2CO_3 (34 mg, 246 μmol, 3 equiv) were dissolved in DMF and stirred overnight at 85 °C. TLC analysis showed incomplete conversion of the starting material, so an additional 3 equivalents of 5-(adamantan-1-yl-methoxy)-1-bromo-pentane (80 mg) were added and stirring was continued for 18 h. Subsequently, the mixture was filtered and concentrated *in vacuo*. The resulting oil was taken up in *n*-butanol (2 mL) and ethylenediamine (36 μL) was added. The reaction mixture was refluxed for 4 h, after which it was diluted with toluene, concentrated and coevaporated twice with toluene. The resulting yellow oil was taken up in an Ac_2O-pyridine cocktail (0.5 mL/1.5 mL) and stirred overnight. The reaction was stopped by quenching with a little MeOH and concentrated *in vacuo*. The crude oil was dissolved in EtOH:HCl (1:0.1 mL) and charged with Pd/C (20%) and purged thrice with argon, followed by purging with H_2. The mixture was stirred over night at rT and under atmospheric pressure. HPLC-MS showed full deprotection of all the benzyl groups. Purification by HPLC (gradient H_2O-MeOH + 0.1% TFA) evaporation of MeOH and lyophilizing H_2O yielded **172** (1.61 mg, 2.0 μmol, 3%). ^{1}H NMR (600 MHz, D_2O) δ 4.55 - 4.41 (m, 1H), 4.02 - 3.92 (1H), 3.84 - 3.48 (m, 13H), 3.48 - 3.33 (m, 7H), 3.01 - 2.94 (s, 1H), 2.84 - 2.78 (s, 1H), 2.61 - 2.50

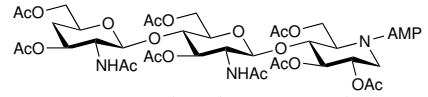
(1H), 2.33 - 2.13 (m, 1H), 2.10 - 2.01 (m, 2H), 1.99 - 1.89 (3H), 1.86 - 1.71 (s, 7H), 1.64 - 1.56 (3H), 1.54 - 1.33 (m, 11H), 1.24 - 1.11 (m, 6H).

2,3,6-Tri-*O*-benzyl-4-*O*-[3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-4-*O*-(3,4,6-tri-*O*-benzyl-2,4-di-deoxy-2-phthalimido-β-D-glucopyranosyl)-β-D-glucopyranosyl]-1-deoxy-nojirimycin(190):



Fully protected trimer **188** (153 mg, 99 μmol) was dissolved in DMF (1 mL), followed by addition of HSPh (20 μL, 200 μmol, 2 equiv) and K_2CO_3 (42 mg, 300 μmol, 3 equiv). The reaction mixture was stirred for 20 h at rT after which TLC-analysis showed conversion into a lower running product. The reaction mixture was diluted with EtOAc and washed twice with $NaHCO_3$ and once with H_2O and brine. The organic layer was dried using $Na_2S_2O_3$, filtered and concentrated under reduced pressure. Purification using a short silica column (EtOAc/PE 80%) gave **190** in 85% yield (114 mg, 83 μmol). TLC: EtOAc 100%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 - 7.90 (m, 1H), 7.83 - 7.70 (m, 1H), 7.65 - 7.54 (m, 2H), 7.36 - 6.89 (m, 40H), 6.85 - 6.79 (dd, $J = 5.3$, 1.9 Hz, 3H), 5.33 - 5.22 (d, $J = 8.1$ Hz, 1H), 5.21 - 5.10 (d, $J = 8.3$ Hz, 1H), 4.89 - 4.74 (m, 3H), 4.60 - 4.40 (m, 10H), 4.35 - 3.98 (m, 8H), 3.95 - 3.83 (d, $J = 11.4$ Hz, 1H), 3.56 - 3.46 (m, 2H), 3.44 - 3.24 (m, 5H), 3.14 - 2.89 (m, 4H), 2.87 - 2.79 (m, 1H), 2.55 - 2.47 (d, $J = 4.4$ Hz, 1H), 2.34 - 2.20 (m, 2H), 1.30 - 1.21 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4, 167.9, 139.7, 138.8, - 138.0, 134.11, 133.91, 133.87, 133.80, 131.95, 131.77, 129.7 - 126.9, 123.68, 123.33, 123.15, 98.26, 97.10, 85.29, 80.32, 78.87, 75.13, 74.66, 74.31, 74.08, 73.46, 72.87, 72.65, 72.52, 72.28, 72.10, 71.10, 70.73, 70.62, 67.22, 59.00, 57.82, 56.76, 47.92, 34.41; IR (neat) ν 2866, 2355, 1775, 1710, 1453, 1387, 1363, 1068, 1027, 911, 697, 720, 530, 352; HRMS: $C_{83}H_{81}N_3O_{15} + H^+$ requires 1360.57405 , found 1360.57852; $[\alpha]_D^{23}$ +38 °(c = 0.5, $CHCl_3$).

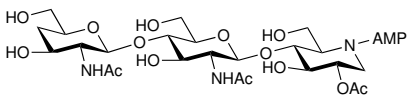
2,3,6-Tri-*O*-acetyl-4-*O*-[3,6-di-*O*-acetyl-2-deoxy-2-*N*-acetyl-4-*O*-(3,4,6-tri-*O*-acetyl-2,4-di-deoxy-2-*N*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranosyl]-*N*-[5-(adamantan-1-yl-methoxy)-pentyl]-1-deoxynojirimycin(191):



Compound **190** was coevaporated thrice with toluene and dissolved in dioxane/AcOH (1:0.1 mL). After addition of freshly prepared **193**[26] in 0.2 mL dioxane the mixture was purged with argon. Subsequently, the mixture was charged with Pd/C (20%) and purged thrice with argon, followed by purging with H_2. The mixture was stirred over night at rT and under atmospheric pressure. HPLC-MS showed full coupling of aldehyde **193** with the starting material and simultaneously cleavage of all the benzyl groups. The mixture was filtered over Celite® and concentrated *in vacuo* resulting in a white solid. The solid was taken up in *n*-butanol 5 mL and ethylenediamine (23 μL) was added. The reaction mixture was refluxed for 4 h, after which it was diluted with toluene, concentrated and coevaporated twice with toluene. The resulting yellow oil was taken up in an Ac_2O-pyridine cocktail (0.5 mL/1.5 mL) and stirred overnight. The reaction was stopped by quenching with a little MeOH and concentrated *in vacuo*. The resulting oil was applied to a Sephadex® size exclusion column (50 mmD x 1500mmL) and eluted with DCM/MeOH (1:1) yielding **191** as an amorphous solid in 36% yield over 4 steps. (9 mg, 8.34 μmol). TLC: EtOAc/Tol 80%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.08 - 4.84 (m, 2H), 4.50 - 4.14 (m, 4H), 4.10 -

3.93 (m, 2H), 3.85 - 3.60 (m, 4H), 3.43 - 3.32 (m, 2H), 3.28 - 3.15 (m, 3H), 3.02 - 2.88 (s, 2H), 2.76 - 2.62 (m, 1H), 2.61 - 2.47 (m, 1H), 2.44 - 2.24 (s, 1H), 2.21 - 1.86 (m, 32H), 1.76 - 1.16 (m, 22H), 1.06 - 0.71 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.6, 172.1 - 171.01, 102.7, 102.7, 82.9, 78.5, 76.3, 75.5, 74.1, 73.6, 72.5, 72.4, 71.6, 71.5, 71.5, 71.2, 70.9, 70.6, 66.3, 63.8, 63.3, 60.0, 55.9, 54.5, 53.9, 52.9, 40.8, 38.3, 35.1, 33.8, 32.9, 31.3, 30.7, 30.4, 30.4, 29.3, 24.9, 24.3, 24.2, 23.7, 22.0, 21.9, 21.9, 21.9, 21.8, 21.8, 15.1; IR (neat) ν 2925, 2366, 2184, 2017, 1977, 1958, 1744, 1654, 1368, 1235, 1047, 576, 464, 350, 313; HRMS: $C_{52}H_{79}N_3O_{21} + H^+$ requires 1082.63165, found 1082.63156; $[\alpha]_D^{23}$ -16 °(c = 0.2, $CHCl_3$)

4-*O*-[2-deoxy-2-*N*-acetyl-4-*O*-(2,4-di-deoxy-2-*N*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranosyl]-*N*-[5-(adamantan-1-yl-methoxy)-pentyl]-1-deoxynojirimycin(173):



Protected **191** 9 mg (8.34 μmol) was dissolved in MeOH (0.5 mL), followed by addition of a catalytic amount of NaOMe (30% in MeOH). The reaction mixture was stirred for 30 minutes after with LCMS showed conversion towards the deprotected product. The reaction mixture was quenched with 4 drops of AcOH and diluted with toluene and coevaporated twice with toluene. The oily residue was loaded as a 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 thrice 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 the target compound with some small impurities that were removed by HPLC (gradient H_2O-MeOH + 0.1% TFA). Evaporation of MeOH and lyophilizing H_2O yielded **173** in 30% (1.85 mg, 2.36 μmol). ^{1}H NMR (600 MHz, DMSO d_6) δ 7.93 - 7.78, 7.75 - 7.58, 5.76 - 5.44, 4.99 - 4.70, 4.56 - 4.46, 4.29 - 4.24, 3.79 - 3.14, 3.13 - 2.84, 1.95 - 1.40, 1.42 - 0.76; ^{13}C NMR (150 MHz, DMSO d_6) δ 169.0, 116.3, 102.5, 101.1, 81.7, 81.0, 77.9, 74.8, 74.4, 72.8, 72.4, 70.3, 68.1, 65.7, 63.4, 63.3, 60.2, 56.9, 54.6, 36.7, 33.6, 31.3, 27.6, 23.1.

Coupling conditions entry 2, Table 4.1

Dimer **175** (228 mg, 200 μmol, 1.25 equiv) and acceptor **177** (91 mg, 160 μmol) were coevaporated thrice with toluene and dissolved in 5 mL dry DCM:Et_2O (1:4). Molecular sieves 3Å were added and the reaction was cooled to -30 °C. After 1 h a 1M solution of Me_2S_2−TF_2O (300 μL, 300 μmol, 1,5 equiv relative to donor). The reaction mixture was stirred for 10 min at -30 °C, subsequently quenched by the addition of excess triethylamine (0.5 mL, 3.6 mmol) and diluted with DCM (50 mL). The mixture was washed with 2 M aqueous HCl, saturated aqueous $NaHCO_3$, and water. The organic layer was dried using $MgSO_4$, filtered and concentrated under reduced pressure. Purification using a short silica column (EtOAc/PE 30%) gave **187** in 41% yield (108 mg, 65 μmol). For analytical data see compound **187**.

Coupling conditions entry 3-6, Table 4.1

Dimer **175** (228 mg, 200 μmol, 1.25 equiv) and Ph_2SO (88 mg, 440 μmol) were coevaporated thrice with toluene and dissolved in dry DCM (8 mL). Molecular sieves 3Å were added and the reaction was cooled to -60 °C. Subsequently, Tf_2O (47 μL, 280 μmol, 1.4 equiv relative to donor) was added. After 10 minutes at -60 °C acceptor **177** (91 mg, 160 μmol) in 4 mL dry DCM was added an the reaction mixture was stirred for 1.5 h allowing

the mixture to warm to -30 °C. The reaction mixture was quenched at -30 °C by addition of excess Et_3N (0.5 mL, 3.6 mmol) and concentrated *in vacuo*. Purification using a short silica column (EtOAc/PE 30%) gave **187** in 24% yield (62 mg, 38 μmol). For analytical data see compound **187**.

Coupling conditions entry 7, Table 4.1

Dimer **175** (228 mg, 200 μmol) was dissolved in acetone:H_2O (6:2 mL) and cooled to -20 °C. Next NBS (178 mg, 1 mmol) was added and the reaction was stirred for 1 h at -20 °C. After 1 h the reaction mixture was quenched by addition of a little $Na_2S_2O_3$ and subsequently carefully concentrated to remove the acetone. The resulting mixture was taken up in ethyl acetate and washed twice with H_2O. The organic layer was dried and concentrated *in vacuo*. The resulting oil was purified using a short silica column (30% EtOAc/PE) gaining the hemiacetal in quantitative yield (221 mg, 210 μmol). TLC: 50% EtOAc/PE; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 - 7.52 (m, 7H), 7.34 - 7.18 (m, 14H), 7.03 - 6.93 (m, 3H), 6.92 - 6.81 (m, 3H), 6.80 - 6.73 (m, 2H), 5.31 - 5.24 (d, $J = 8.3$ Hz, 1H), 5.21 - 5.14 (d, $J = 8.5$ Hz, 1H), 4.91 - 4.84 (d, $J = 12.7$ Hz, 1H), 4.82 - 4.75 (dd, $J = 11.4$, 4.5 Hz, 2H), 4.70 - 4.62 (d, $J = 10.8$ Hz, 1H), 4.59 - 4.33 (m, 7H), 4.26 - 4.14 (m, 2H), 4.06 - 3.99 (m, 1H), 3.91 - 3.62 (m, 3H), 3.54 - 3.32 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.14, 168.44, 167.96, 138.63, 138.28, 138.08, 138.05, 138.01, 133.71, 131.57, 131.30, 128.4-127.3, 96.91, 92.74, 79.63, 79.02, 76.49, 75.54, 75.10, 74.87, 74.80, 74.41, 74.36, 73.16, 72.70, 68.29, 67.95, 57.49, 56.59. The dimer hemiacetal was coevaporated thrice with toluene, dissolved in dry DCM (2mL) and to 0 °C. Subsequently, DBU (8 μL, 52 μmol, 0.25 equiv) and CCl_3CN (0.21 mL, 2.1 mmol, 10 equv.) were added and the mixture was stirred overnight. The reaction was concentrated *in vacuo* and purified using a short silica column (EtOAc/PE 30% + 2.5 % TEA) yielding the dimer imidate **186** in a low 15% yield (37 mg, 30 μmol). TLC: EtOAc/PE 40%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 - 7.55 (m, 8H), 7.42 - 7.19 (m, 15H), 7.04 - 6.95 (m, 4H), 6.95 - 6.84 (m, 3H), 6.83 - 6.67 (m, 3H), 6.26 - 6.17 (d, $J = 8.9$ Hz, 1H), 5.38 - 5.25 (d, $J = 8.3$ Hz, 1H), 4.93 - 4.85 (d, $J = 12.8$ Hz, 1H), 4.84 - 4.77 (dd, $J = 11.5$, 4.1 Hz, 2H), 4.72 - 4.62 (m, 1H), 4.62 - 4.32 (m, 9H), 4.30 - 4.17 (m, 2H), 3.95 - 3.80 (dd, $J = 9.9$, 8.5 Hz, 1H), 3.77 - 3.36 (m, 6H). Dimer imidate (37 mg, 30 μmol) and acceptor **177** were coevaporated thrice with toluene and dissolved in dry DCM (1 mL). Molecular sieves 3Å were added and the reaction was cooled to 0 °C. Next TMSOTf (1 μL, 3 μmol, 0.1 equiv) was added and the mixture was stirred for 2.5 h at 0 °C, after which the reaction was quenched using excess Et_3N. Purification using a short silica column (EtOAc/PE 30%) gave **186** in 34% yield (17 mg, 10 μmol). For analytical data see compound **187**.

Coupling conditions Scheme 4.2

Imidate **178** (141 mg, 200 μmol, 1.1 equiv relative to acceptor) and acceptor **177** (105 mg, 180 μmol, 1 equiv) were coevaporated thrice with toluene and dissolved in dry DCM (2 mL). Molecular sieves 3Å were added and the reaction was cooled to -20 °C. Subsequently, TMSOTf (4 μL, 20 μmol) was added. The resulting mixture was stirred for 2 h while the mixture was allowed to warm to 0 °C. Protected DNJ **177** (138mg, 216 μmol, 1.2 equiv relative to acceptor) and NIS (48 mg, 216μmol, 1.2 equiv relative to acceptor) were coevaporated thrice with toluene and added in 0.3 mL dry DCM. The reaction mixture was stirred for an additional 2 h at 0 °C. Subsequently, the reaction was quenched using excess Et_3N. Purification using a short silica column (EtOAc/PE 30%) gave **187** in 8% yield (24 mg, 14

μmol). For analytical data see compound **187**.

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