Synthesis & biological applications of glycosylated iminosugars

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7

Summary and Future Prospects

7.1 Summary

Iminosugars are carbohydrate analogs in which the endocyclic oxygen is replaced by a nitrogen. This class of polyhydroxylated alkaloids have interesting inhibitory properties towards glycosidases and glycotransferases, due to their close resemblance of natural carbohydrates. These iminosugars are wildly distributed throughout nature. A great variety of iminosugars can be found in all parts of the Mullberry tree (*Morus* spp.) including 1-deoxynojirimycin **2** (DNJ, **2**). DNJ was earlier synthesized as stable analog of nojirimycin **1** (NJ, **1**), which in turn was the first iminosugar isolated from natural sources. Over the years several *N*-alkylated DNJ derivatives were synthesized. Two examples being; *N*-butyl- and a *N*-5-(adamantan-1-yl-methoxy)-pentyl (AMP)-DNJ (**16** and **17**, Figure 7.1) were found to inhibit glycosylceramide synthase (GCS). NB-DNJ **16** is currently used in SRT of patients suffering from Gaucher disease. 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 which results in enlargement of organs (spleen and liver) and inflammation. Inhibition of GCS restores the influx/efflux balance of GC in Gaucher cells and thereby reduces its effects. The research in this thesis describes the synthesis of several prodrugs based on NB-DNJ **16** and AMP-DNJ **17**. Furthermore several new chitotriosidase substrates are synthesized which were designed to withstand stepwise degradation of other enzymes.

By glycosylating iminosugars one can gain more selective glycosidase inhibitors, because the formed glycosylated iminosugars may better mimic the natural

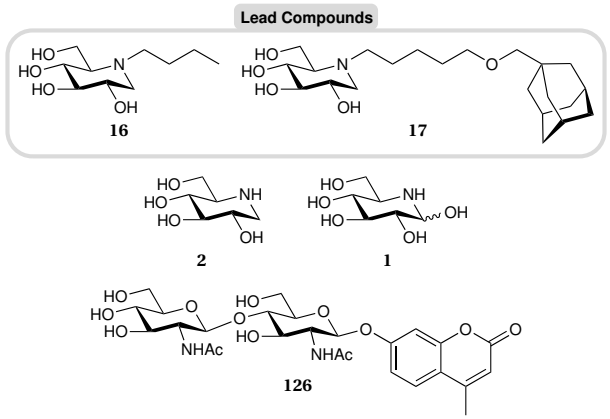


Figure 7.1: Compounds described in section 7.1.

substrate of the enzyme. The first section of **Chapter 1** summarises the synthesis of all *O*-glycosylated iminosugars from the piperidine class, reported in literature. Several syntheses are described in which iminosugars are glycosylated on various positions using a variety of chemical and enzymatic synthetic strategies. In the second part of **Chapter 1** an overview is given of glycosylated iminosugars which bear a different linkage between the carbohydrate and the iminosugar.

Chitotriosidase (CHIT1) is the first identified human chitinase and is strongly expressed and secreted by lipid-laden tissue macrophages that are found in patients suffering from Gaucher disease. CHIT1 is correlated to the progression of the disease and the effect of therapeutic intervention. Originally 4-methylumbelliferyl chitobioside **126** was used as a fluorogenic substrate in biological assays to give a fluorescent read-out. However, it was found that human CHIT1 possesses intrinsic transglycosylase activity, resulting in a suboptimal fluorescent read-out. Transglycosylase activity can be circumvented by the use of **125** in which the 4'-OH is removed. This 4'-deoxychitobiosyl methylumbelliferone **125** indeed showed to be a superior CHIT1 substrate as compared to **126**. Previously **125** was synthesized *via* a nine-step low-yielding sequence. Therefore, **Chapter 2** presents a reliable and scalable route for the synthesis of 4'-deoxychitobiosyl umbelliferone **125**. In the synthetic route one partially protected thiophenyl glucosamine is used as main building block. This building block was readily transformed into both the reducing and non-reducing end building blocks which were condensed to form the carbohydrate core. This disaccharide was in turn coupled with the fluorophore (4-MU) under phase transfer conditions.

In **Chapter 3**, three novel human CHIT1 substrates are designed, synthesized and biologically evaluated. All compounds (**136**, **137** and **138**) bear an anomeric 4-MU fluorophore for fluorometric read-out and have a different modification on the 4-hydroxyl of the non-reducing end sugar. This modification goes from the relative small *O*-methyl group (OMe) to the more sterically demanding *O*-isopropyl

(*i*OPr) and *O*-methyl cyclohexane group (OMCH). These substrates were synthesized using a 1,6-anhydro glucosamine derivative as the key building block in the synthesis of the donor and acceptor glycosides. Biological evaluation of the substrates showed that all compounds follow Michaelis-Menten kinetics like the parent 4'-deoxy substrate **125**, but proved to be more stable towards stepwise degradation by β-hexosaminidase.

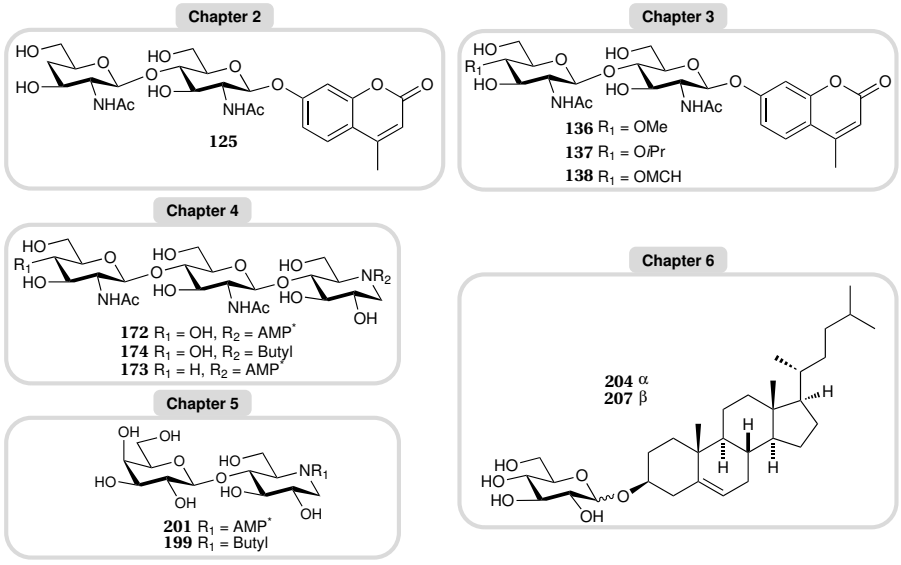


Figure 7.2: Overview of the chapters described in this thesis.
* AMP = *N*-5-(adamantan-1-yl-methoxy)-pentyl

Due to the direct correlation of CHIT1 to the progression of Gaucher disease, the locally elevated CHIT1 activity in Gaucher cells is a potential target for site-specific drug delivery. In **Chapter 4** 4'-deoxychitobiose and chitobiose are linked to **16** and **17** to form potential prodrugs for Gaucher disease (**172**, **174** and **173**). By linkage of the CHIT1 substrates to the 4-position of **16** and **17** both inhibitors become less active. Further research is needed to see if through cleavage of the substrates, by CHIT1, the free *N*-alkylated DNJ derivatives will be liberated, with restored activity. Synthesis of the chitobiose core is based on the work described in **Chapter 2**. The DNJ part of the prodrug was based on a reported synthesis in which glucose is converted into an iminosugar by an initial reduction and oxidation under Swern conditions, followed by a double reductive amination to yield a protected DNJ derivative. By orthogonal protection of the 4-position of DNJ this position could be selectively liberated, after which the DNJ derivative was used as acceptor in the glycosylation. After coupling, the endocyclic nitrogen was deprotected, followed by the introduction of a butyl or AMP chain *via* alkylation or reductive amination.

It is known that some alkaloids and derivatives thereof have a bitter taste. The human primeval aversion against bitterness is a defense mechanism to preclude digestion of potential toxic substances. Therefore, **Chapter 5** describes the synthesis of two different prodrugs (**199** and **201**, Figure 7.2) in which **16** and **17** bear a galactose moiety on the 4-position of the iminosugar, thereby gaining a lactose derivatives with a potential sweeter taste. Upon arrival of the prodrugs in the intestine the galactose moiety will be cleaved by lactase and the activity of the GCS inhibitors will be restored. For the synthesis of the prodrugs (**199** and **201**) octa-*O*-acetyl-α/β-D-lactose was used as starting material because it is cheap and saves a glycosylation step. Transformation into the iminosugar derivative was done by reduction of the reducing end sugar, followed by a oxidation under Swern conditions and a double reductive amination. Next the endocyclic nitrogen was decorated with butyl or AMP chain. Biological assays showed that lactase-phlorizin hydrolase (LPH) found in intestinal rat muscosa was able to cleave prodrug **201** and thereby liberate GCS inhibitor **17**.

During the ongoing research to find the common denominator for parkinsonism and glucosylceramide metabolism, it was shown that high levels of steryl glycosides are found in people suffering from parkinsonism. Very recently evidence was reported that not UDP-glucose but GC acts of sugar donor for the biosynthesis of glycosylated cholesterol. **Chapter 6** describes the synthesis of α- and β-cholesteryl glycoside (**204** and **207**) which can be used as internal standard.

7.2 Future Prospects

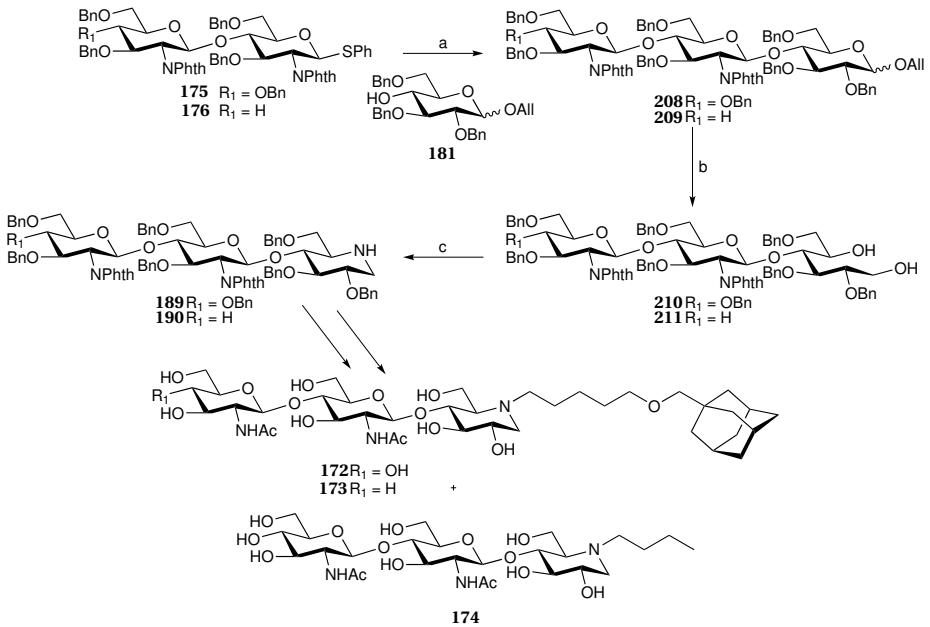
As shown in **Chapter 1** there are several synthetic strategies that can be used for the synthesis of *O*-glycosylated iminosugars. One can tackle this synthetic challenge of synthesizing *O*-glycosylated iminosugars by two different routes. The first strategy employs a carbohydrate that acts as a donor and a suitably protected iminosugar that acts as the acceptor in the key glycosylation step. This strategy was used for the synthesis of the prodrugs described in **Chapter 4**, where the chitobiose donor was first synthesized using two suitable protected glucosamine moieties. This chitobiose core was than condensed with a DNJ acceptor, in the next glycosylation event.

The second route towards glycosylated iminosugars is pursued by the synthesis of the carbohydrate core, followed by transformation of the reducing end carbohydrate into an iminosugar. This approach is used by Stütz *et al.*[1,2] as reviewed in **Chapter 1**. The synthesis of galactosylated DNJ derivatives described in **Chapter 5** is also based on this strategy, in which the use of octa-*O*-acetyl-α/β-D-lactose circumvents a glycosylation step.

The strategy used in **Chapter 5** can be applied for synthesis of compounds **172**, **173** and **174** described in **Chapter 4** and *vice versa.* For example, by using benzy-

lated allyl glucopyranoside **181** as acceptor in a glycosylation with chitobiose cores **175** or **176**, trimers **208** and **209** will be produced (Scheme 7.1). Transformation of the reducing end carbohydrate into the corresponding iminosugar gives prodrugs **172**, **173** and **174**. This can be achieved by deallylation, followed by $LiAlH_4$ mediated reduction to give glucitols **210** and **211**. Formation of the respective iminosugar derivatives can be achieved *via* oxidation of lacitols **210** and **211** using the Swern reaction followed by subsequent double reductive amination of the dicarbonyl yielding compounds **189** and **190**. The endocyclic nitrogen could in turn be decorated with a butyl or AMP chain to gain prodrugs **172**, **173** and **174**, after deprotection.

Scheme 7.1: Synthesis of prodrugs **172**, **173** and **174** *via* the strategy presented in **Chapter 5**.

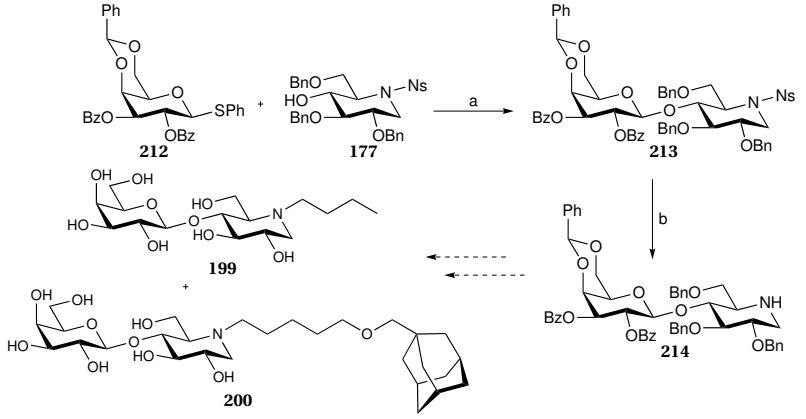


Reagents and conditions: a) NIS, TMSOTf, DCM, 0°C, b) (1) KO*t*Bu, DMSO, 100°C, (2) I_2, THF/H_2O, (3) $LiAlH_4$, THF; c) (1) DMSO, $(COCl)_2$, DCM, -75°C, (2) Et_3N, -75°C to rT, (3) $NaCNBH_3$, $HCOONH_4$, Na_2SO_4, MeOH, 0°C.

A different strategy for the synthesis of the prodrugs from **Chapter 5** is represented by the condensation of galactose donor **212** with DNJ acceptor **177** (Scheme 7.2). Galactose thio donor **212** was synthesized using a literature procedure[3] and used in the coupling with DNJ acceptor **177** (**Chapter 4**) under influence of NIS and TMSOTf in DCM at -20°C to give dimer **213** in 68% yield. Liberation of the endocyclic nitrogen was achieved under similar conditions used in **Chapter 4** (HSPh and K_2CO_3) giving **214** in 80% yield. The free secondary amine

can be linked to the desired alkyl chain (butyl or AMP), to gain prodrugs **199** and **200**, after deprotection.

Scheme 7.2: Synthesis of prodrug **199** and **200** *via* a strategy similar to **Chapter 4**.



Reagents and conditions: a) NIS, TMSOTf, DCM, -20°C, 68%; b) HSPh, K_2CO_3, DMF, 80%.

The prodrug strategy presented in **Chapter 4** can fail, when CHIT1 is unable to hydrolyze the substrate-drug bond due to the steric hindrance.[4,5] Hence, a so-called tripartite produg may be a useful alternative (Figure 7.3 B). The linker group of a tripartite prodrug creates a distance between the drug part and the enzyme substrate. In this way, the enzyme cleaves the substrate-linker bond in **217** rather than the substrate-drug bond as in **215**. However, the linker portion must be chosen wisely so that the linker-drug bond (**218**) hydrolyses under physiological conditions after hydrolysis of the substrate-linker bond, resulting in the release of the active drug **216**. These type of linkers are known as self-immolative linkers.[6]

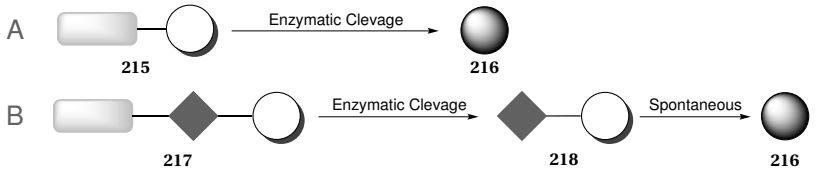


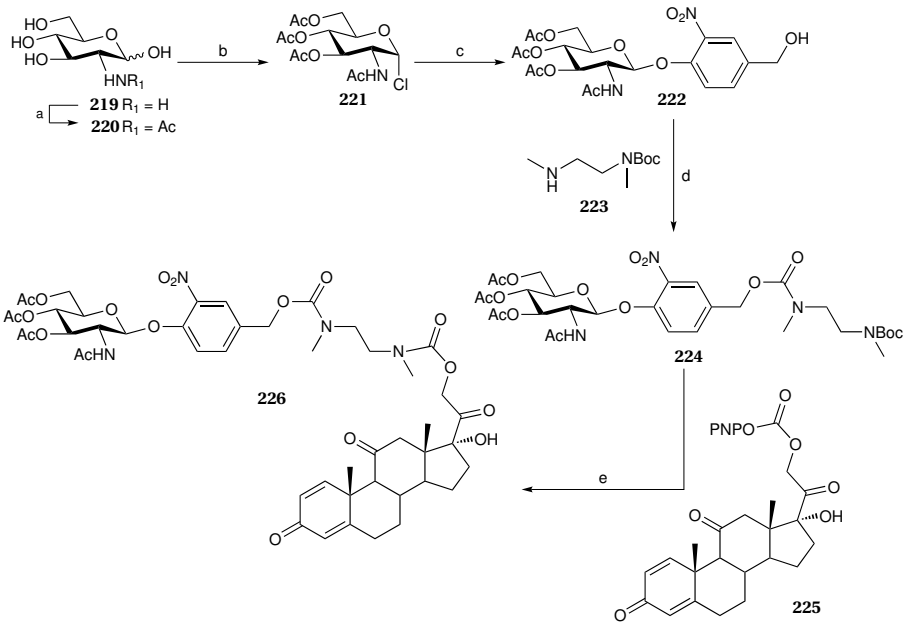
Figure 7.3: Schematic representation of enzymatic prodrug cleavage of bipartite and tripartite prodrugs. ○: inactive drug ; ●: active drug.

In a preliminary study tripartite prodrug **226** was synthesized based on the work of Monneret and co-workers, who used a two-part spacer system, linked together by carbamate functions, in the synthesis of a novel anti-cancer prodrug.[7,8] In this study GlcNAc was chosen as the enzyme substrate-part to optimize the coupling conditions with 4-hydroxy-3-nitro benzyl alcohol. Changing the substrate-part to chitobiose or 4'-deoxy chitobiose would result in more usable prodrug

model. Overdijk *et al.* found a local elevation of CHIT1 activity during inflammation.[9] Therefore, the drug-part of this tripartite prodrug is prednisone, which is a well known anti-inflammatory drug (Figure 7.4).

The chloride donor **221** used in the coupling with the phenol, could be prepared in a two-step procedure, *via* first regioselective introduction of the acetyl-group at the nitrogen of D-glucosamine **219** to give **220** which in turn was treated with anhydrous AcCl resulting in the formation of chloride donor **221** (Scheme 7.3).[10] Using a optimized phase transfer conditions (PTC), α-chloride in **221** was substituted, *via* a S_N2 displacement, with 4-hydroxy-3-nitro benzyl alcohol to give **222**.

Scheme 7.3: Synthesis of tripartite prodrug **226**.



Reagents and conditions: a) NaOMe (30% in MeOH), MeOH, Ac_2O, 0°C; b) AcCl, rT, 65% over two steps; c) $NaHCO_3$, TBABr, 4-hydroxy-3-nitrobenzyl alcohol, DCM, rT, 3.5h, 46%; d) (1) 4-nitrophenyl chloroformate, TEA, DCM, 0°C to rT, (2) **223**, DCM, 0°C, 87% over two steps; e) (1) 4M HCl in dioxane, rT, (2) **225**, DIPEA, DCM, 21% two steps.

Product **222** was used as starting material for the synthesis of the second part of the self-immolative linker. The free hydroxyl function in **222** was activated as a *para*-nitrophenylcarbamate and condensed with the mono-Boc-protected diamine **223**[11] affording benzyl carbamate **224**. A higher yield was achieved by using a one-pot procedure, thereby avoiding isolation of the intermediate carbonate. Anhydrous 4M HCl in dioxane was used to remove the Boc-group in **224** giving the corresponding free amine as a HCl salt. No attempts were made to isolate the

free amine, which would obviously cyclize very rapidly resulting in liberation of the starting benzylic alcohol. Treatment of prednisone with 4-nitrophenyl chloroformate and pyridine in anhydrous chloroform produced *para*-nitrophenylcarbonate **225**. Activated carbonate **225** was dissolved in DCM and added to a cooled solution of **224**. Dropwise addition of DIPEA gave the desired tripartite produg of prednisone **226** (21%) as a white foam.

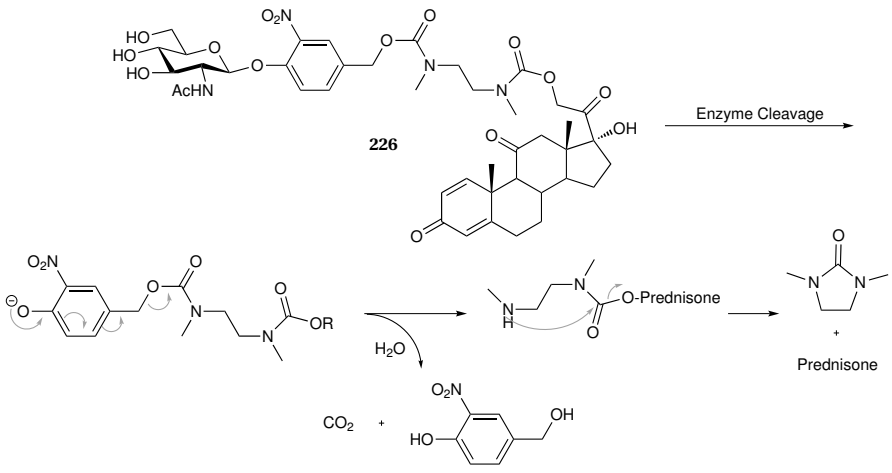


Figure 7.4: Release of prednisone upon cleavage of the substrate by chitinase.

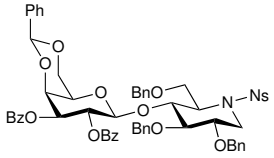
7.3 Experimental section

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}.

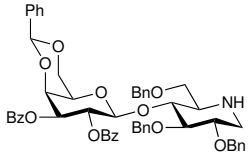
*Synthesis of Prodrug **199** and **200**. (Scheme 7.2)*

_N_-(2-nitrobenzenesulfonyl)-2,3,6-tri-_O_-benzyl-4-_O_-(-di-_O_-benzoyl-4,6-_O_-benzylidene-β-D-galactopyranosyl)-1-deoxynorjirimycin (213):



Donor **212**[3] 1 g (1.76 mmol, 1.1 equiv) and DNJ acceptor **177** 0.92 g (1.60 mmol) were coevaporated thrice with toluene and dissolved in 10 mL dry DCM. Molecular sieves 3Å were added and the reaction was cooled to -20°C. NIS 0.43 g (1.92 mmol, 1.2 equiv) was added and the mixture was stirred for 15 minutes. Next, a catalytic amount of TMSOTf (25 μL) was added and the reaction mixture was stirred for 45 minutes, after which the reaction was quenched using Et_3N (0.6 mL). The mixture was diluted with DCM and washed with NaS_2O_4 and brine. The organic layer was dried using $MgSO_4$ and concentrated *in vacuo*. Purification using a short silica column (EtOAc/PE 10%) gave **213** in 68% yield. (1.177 g, 1.09 mmol) TLC: EtOAc/Tol 20%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 - 7.95 (m, 3H), 7.84 - 7.71 (m, 2H), 7.56 - 7.04 (m), 6.96 - 6.85 (m, 1H), 5.82 - 5.72 (dd, $J = 10.3, 7.9$ Hz, 1H), 5.56 - 5.50 (s, 1H), 5.28 - 5.19 (dd, $J = 10.3, 3.4$ Hz, 1H), 4.86 - 4.75 (d, $J = 8.1$ Hz, 1H), 4.68 - 3.99 (m, 12H), 3.95 - 3.83 (t, $J = 9.5$ Hz, 1H), 3.71 - 3.63 (m, 2H), 3.59 - 3.52 (d, $J = 2.8$ Hz, 1H), 3.53 - 3.43 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.3, 165.5, 148.0, 138.6, 138.2, 137.9, 137.8, 137.6, 133.5 - 126.3, 123.03, 102.4, 100.8, 73.6, 73.7, 73.2, 73.1, 72.5, 70.9, 69.4, 69.0, 68.6, 66.8, 57.3, 42.4.

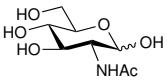
2,3,6-Tri-_O_-benzyl-4-_O_-(-di-_O_-benzoyl-4,6-_O_-benzylidene-β-D-galactopyranosyl)-1-deoxynorjirimycin (214):



Compound **213** 0.92 g (0.85 mmol) was dissolved in DMF (5 mL), followed by addition of HSPh 0.44 mL (4.26 mmol, 5 equiv) and DIPEA 0.6 mL (3.41 mmol, 4 equiv). The mixture was stirred for 18 h after which it was taken up in EtOAc and washed with $NaHCO_3$. The organic layer was dried and concentrated under reduced pressure. Purification using a short silica column (EtOAc/PE 80%) gave **214** in 80% yield. (0.58 g, 0.68 mmol) TLC: EtOAc/Tol 60%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 - 7.89 (t, $J = 6.8$ Hz, 4H), 7.56 - 7.11 (m, 27H), 5.87 - 5.74 (dd, $J = 10.4, 7.9$ Hz, 1H), 5.46 - 5.41 (s, 1H), 5.19 - 4.99 (m, 3H), 4.93 - 4.84 (d, $J = 7.9$ Hz, 1H), 4.71 - 4.52 (m, 2H), 4.47 - 4.37 (d, $J = 3.7$ Hz, 1H), 4.31 - 4.22 (d, $J = 11.6$ Hz, 1H), 4.18 - 4.09 (d, $J = 12.5$ Hz, 1H), 4.05 - 3.94 (d, $J = 11.4$ Hz, 1H), 3.86 - 3.73 (d, $J = 12.3$ Hz, 1H), 3.68 - 3.46 (m, 4H), 3.41 - 3.31 (dd, $J = 9.0, 6.1$ Hz, 1H), 3.22 - 3.08 (m, 2H), 2.69 - 2.61 (m, 1H), 2.51 - 2.37 (t, $J = 11.2$ Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.3, 165.2, 139.6, 138.5, 138.0, 137.7, 133.4, 133.4, 130.0 - 126.4, 101.8, 100.9, 85.5, 80.6, 80.3, 73.5, 73.3, 73.2, 72.8, 70.3, 70.2, 68.8, 66.6, 59.8, 47.8.

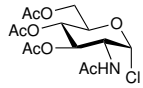
*Synthesis of Triparte Prodrug **226**. (Scheme 7.3)*

2-Acetamido-2-deoxy-D-glucopyranose (220):



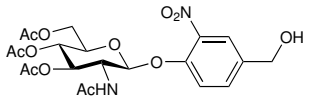
A mixture of 172 mL MeOH and 28 mL NaOMe (30% in MeOH) was added to 43.6 g (200 mmol) D-glucosamine hydrochloride **219**. The mixture was stirred at ambient temperature for 10 minutes, after which it was gently heated and filtrated. The filtrate was cooled to 0°C. Subsequently 250mL Ac_2O was added and the solution was left overnight at room temperature to crystallize. The crystals were filtered affording 30 g of **220** as an off-white solid which was used without further purification.

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (221):



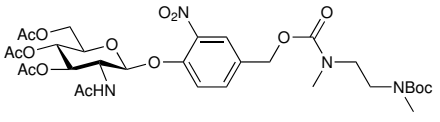
Crude compound **220** (7.5 g, 33.9 mmol) was dissolved in 25 mL distilled AcCl. The reaction mixture starts to boil spontaneously after 1 h. The reaction is left overnight yielding an amber colored clear liquid. The solution is diluted with 20 mL DCM and rapidly washed with 2x 20 mL of cold water, 2x 30 mL of Na_2CO_3 and brine. The organic layer is dried and concentrated under reduced pressure. Crystallization in EtOAc/PE afforded 8.10 g (22.20 mmol, 65%) of the title compound **221** as a beige solid. Proton and carbon NMR were similar to literature.[10]

(2-Nitro-4-hydroxymethyl)phenyl-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (222):



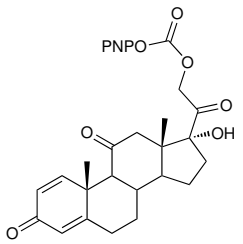
In a two phase-system of 50 mL DCM and 25 mL 1M $NaHCO_3$ were 4-hydroxy-3-nitrobenzyl alcohol (2.53 g, 15 mmol, 1.5 equiv) and tetra-butylammonium bromide (3.32 g, 10 mmol) dissolved. The mixture was vigorously stirred for 15 minutes. After which 3.7 g (10 mmol) of **221** in 5 mL DCM was added dropwise. Vigorous stirring was continued for 3.5 hours after which the organic layer was washed with 1x 35 mL H_2O and 1x 35 mL brine. The DCM layer was dried, filtered and concentrated *in vacuo*. The yellow oil was purified by silica gel chromatography. Evaporation of the eluent afforded a yellow solid which was re-crystalized (EtOAc/PE) yielding title compound **222** in 46% yied (2.3 g, 4.6 mmol) as yellow crystals. TLC: EtOAc/PE 40%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.94 (s, 3H, CH_3, NAc); 2.01 (s, 3H, CH_3, Ac); 2.02 (s, 3H, CH_3, Ac); 2.04 (s, 3H, CH_3, Ac); 3.89 (d, $J = 8.4$ Hz, 1H, CH); 4.23 (dd, $J = 12.28$, 5.17 Hz, 2H, CH_2, C'-6); 5.05-5.12 (m, 4H, CH, CH_2, C'-4, C'-5, CH_2); 5.51 (t, $J = 8.4$ Hz, 1H, CH, C'-3); 5.58 (d, $J = 8.04$ Hz, 1H, CH, C'-1β); 7.41-7.75 (m, 3H, CH, arom); ^{13}C NMR (101 MHz, $CDCl_3$) δ 20.54, 20.59, 20.64 (CH_3, 3x Ac); 23.10 (CH_3, NAc); 55.03 (CH, C'-2); 61.86 (CH_2, C'-6); 67.82 (CH_2); 68.55, 71.17, 72.06 (CH, C'-3, C'-4, C'-5); 99.46 (CH, C'-1); 120.44, 126.02, 133.54 (CH, arom); 148.24, 149.52, 154.43 (Cq, arom); 169.23, 170.27, 170.47, 171.20 (Cq, 3x Ac, NAc); IR (neat) ν 373.8, 463.9, 600.1, 762.1, 791.9, 822.7, 1032.1, 1083.7, 1111.1, 1218.1, 1374.3, 1537.9, 1625.9, 2360.1, 3284.0; ESI-MS: 499.3 ($M+H^+$)

(*N,N'*-dimethyl)-ethylenediamine-*N'*-tert-butoxycarbonyl-*N*-oxycarbonyl-(4-hydroxymethyl-2-nitro)phenyl-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (224):



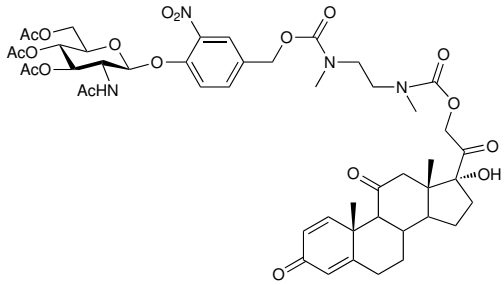
Compound **222** (0.848 g, 1.70 mmol) was dissolved in 15 mL DCM. The solution was cooled to ice bath temperature followed by dropwise addition of 0.71 mL Et_3N (5.1 mmol, 3 equiv). A solution of 0.50 g (2.55 mmol, 1.5 equiv) 4-nitrophenyl chloroformate in 2 mL DCM was added, over 15 minutes. The reaction mixture was stirred overnight at room temperature, after which is was re-cooled to icebath temperature and 0.47 g (2.55mmol, 1.5 equiv) of compound **223** in 2 mL DCM was added drop by drop. After 18 hours the reaction mixture was washed with 1x10 mL H_2O and 1x 15 mL brine. The organic layer was dried, filtrated and concentrated *in vacuo*. Silica gel purification (1% MeOH in DCM) afforded compound **224** in 87% yield. (1.05 g, 1.48 mmol) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (s, 9H, CH_3, t-Bu); 1.95, 2.05, 2.06, 2.09 (s, 12H, CH_3, Ac); 2.85 (d, $J = 22.26$ Hz, 3H, CH_3, NMe); 2.96 (s, 3H, CH_3, NMe); 3.38 (d, $J = 15.64$ Hz, 4H, CH_2, Et); 3.93 (td, $J = 10.30, 8.24, 8.24$ Hz, 1H, CH, C'-2); 4.21 (d, $J = 12.12$ Hz, 1H, CH_2, C'-6); 4.29 (dd, $J = 12.28, 5.13$ Hz, 1H, CH_2, C'-6); 5.17-5.07 (m, 1H, CH, CH_2, C'-4, C'-5, CH_2); 5.57 (d, $J = 8.55$ Hz, 1H, C'-1β); 5.64-5.58 (m, 1H, CH, C'-3); 6.31 (s, 1H, NH); 7.80-7.34 (m, 3H, CH, arom); ^{13}C NMR (150 MHz, $CDCl_3$) δ 21.05-20.22 (CH_3, 3x Ac); 23.13 (CH_3, NAc); 28.31 (CH_3, t-Bu); 34.60 (d, $J = 30.80$ Hz, CH_3, NMe); 35.28 (CH_3, NMe); 46.44 (CH_2, Et); 46.73 (CH_2, Et); 55.11 (CH, C'-2); 61.90 (CH_2, C'-6); 65.25 (dd, $J = 17.72, 4.17$ Hz, CH_2, CH_2); 68.54 (CH, C'-4, C'-5); 71.20 (CH, C'-4, C'-5); 72.11 (CH, C'-3); 99.34 (CH, C'-1); 120.47 (CH, arom); 124.35 (CH, arom); 132.86 (Cq, arom, Cq, Boc); 133.29 (CH, arom); 141.22 (Cq, arom); 148.96 (Cq, arom); 155.57 (Cq, Boc); 169.38 (Cq, NAc); 170.33 (Cq, Ac); 170.44 (Cq, Ac); 171.13 (Cq, Ac); IR (neat) ν 332.0, 356.3, 374.0, 430.0, 597.7, 1038.0, 1224.2, 1366.0, 1537.8, 1699.8, 1747.1; ESI-MS: 713.4 ($M+H^+$)

Prednisone 21-(*para*-nitrophenyl carbonate) (225):



Anhydrous prednisone (1.8 g, 5 mmol) was dissolved in 25 mL $CHCl_3$. The solution was cooled to ice bath temperature, after which a solution of 1.5 g (6 mmol, 1.2 equiv) 4-nitrophenyl chloroformate in 4 mL $CHCl_3$ was slowly added. The suspension was stirred for 1 h, followed by addition of pyridine (1.2 mL, 15 mmol, 3 equiv). When the reaction turned clear the mixture was coevaporated thrice with 20 mL toluene, yielding a off white solid **225** which was used without any further purification.

17-α-hydroxy-3,11,20-trioxo-pregnadien-(1,4)-yl-(21)-oxycarbonyl-*N*-(*N,N'*-dimethyl)-ethylenediamine-*N'*-tert-butoxycarbonyl-*N*-oxycarbonyl-(4-hydroxymethyl-2-nitro)phenyl-(4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (226):



Compound **224** (0.98 g, 1.4 mmol) was dissolved in cooled 4M HCl in dioxane (7 mL). After 45 minutes, TLC-analysis showed tcomplete deprotection of the starting material. The reaction mixture was was coevaporated twice with toluene yielding a white foam. This foam was dissolved in dry DCM 5 mL and cooled with a ice bath. A slurry of prednisone derivative **225** in 8 mL of dry DCM was added dropwise, followed by addition of DIPEA (0.2 mL, 1.38 mmol, 1 equiv). The suspension was stirred for 3 h, allowing the mixture to warm to room temperature. The clear oker colored solution was concentrated *in vacuo* and applied to a Sephadex® size exclusion column (50 mmD x 1500 mmL) and eluted with MeOH yielding a off-white solid. Silica gel purification (2.5% EtOH in $CHCl_3$) afforded title compound **226** as off-white solid in 21% yield (292 mg, 0.29 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.66 (s, 3H, CH_3, C'-18); 1.43 (s, 3H, CH_3, C'-19); 1.93, 2.04, 2.08 (s, 12H, CH_3, 4x Ac); 2.25-2.70 (m, 5H); 2.86-2.95 (m, 6H, CH_3, 2x Me); 3.44 (s, 4H, 2x CH_2); 3.94 (s, 1H, CH, C-2); 4.18-4.29 (m, 2H, CH_2, C-6); 4.57 (m, 3H); 4.91-4.96 (m, 1H); 5.04-5.17 (m, 2H, CH, C-5, C-4, CH); 5.57-5.59 (m, 2H, CH, C-3, C-1); 6.06 (s, 1H); 6.18 (d, $J = 10.4$ Hz, 1H); 6.80 (s, 1H, NH); 7.32-7.83 (m, 4H) IR (neat) ν 312.1, 326.0, 340.0, 376.1, 435.8, 507.9, 602.0, 668.1, 765.6, 822.9, 889.9, 1040.2, 1218.0, 1366.7, 1537.8, 1660.9, 1699.8, 2360.2, 2945.7

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