Synthesis & biological applications of glycosylated iminosugars

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Synthesis & Biological Applications of Glycosylated Iminosugars

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Voor de mensen waar ik van hou.

Table of Contents

List of Abbreviations

4-MU	4-methylumbelliferyl/ 7-hydroxy-4-methylcoumarin
Å	Ångstrom
Ac	acetyl
Ada	adamantane
All	allyl
ALS-PDC	amyotrophic lateral sclerosis parkinsomism dementia complex
AMCase	acidic mammalian chitinase
AMP	5-(adamantan-1-yl-methoxy)-pentyl
aq	aqueous
Ar	aromatic
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
Bu	butyl
Bz	benzoyl
C. sacc.	*Caldocellum saccharolyticum*
CAN	ceric ammonium nitrate
cat	catalytic
Cbz	benzyloxycarbonyl
CHIT1	chitotriosidase
d	doublet
Δ	heat to reflux
δ	chemical shift
DAB	1,4-dideoxy-1,4-imino-D-arbinitol
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
DCMME	α,α-2,2-dichloromethyl methyl ether
dd	double doublet
DDQ	2,3-dichloro-5,6-dicyano-benzoquinone
DIPEA	*N*,*N*-diisopropyl-*N*-ethylamine
DMAP	4-(*N*,*N*-dimethylamino)pyridine
DMDP	2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
DMTST	dimethyl(methylthio)sulfonium trifluoromethanesulfonate
DNJ	1-deoxynojirimycin
et al.	*et alii* (and others)
e.g.	*exempli gratia*
equiv	(molar) equivalents
ERT	enzyme replacement therapy
Et	ethyl
g	gram(s)
Gal	galactose
GBA1	glucocerebrosidase
GC	glucosylceramide
GCS	glucosylceramide synthase
Glc	glucose
GSL	glycosphigolipid
h	hour(s)
HNJ	homonojirimycin

HPLC	high-performance liquid chromatography
HRMS	high resolution mass spectroscopy
Hz	hertz
i.e.	*id est*
IR	infrared
J	coupling constant
L	liter(s)
LCMS	liquid chromatography mass spectrometry
LPH	lactase-phlorizin hydrolase
LSD	lysosomal storage disease
M	molar(s)
m	multiplet
m/z	mass over charge ratio
MCH	methyl cyclohexane
Me	methyl
mg	milligram(s)
MHz	mega Hertz
min	minute(s)
mL	milliliter(s)
mmol	millimol(s)
MPM	*para*-methoxybenzyl
MS	mass spectrometry
MS	molecular sieves
NAP	2-naphthylmethyl
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
NJ	nojirimycin
Ns	4-nitrobenzenesulfonyl
p	para
Pd/C	palladium on activated charcoal
PE	petroleum ether
Ph	phenyl
PTC	phase transfer conditions
pyr	pyridine
q	quartet
ref	reference
RP	reverse phase
rT	room temperature
s	singlet
sat	saturated
spp	species
SRT	substrate reduction therapy
t	triplet
TBAHS	*tert*-butylammonium hydrogen sulfate
TBDMS	*tert*-butyldimethylsilyl
TEBA	benzyl triethyl ammonium chloride
TES	triethyl silane
Tf	trifluoromethanesulfonyl (triflate)
TFA	trifluoroacetic acid
TFE	2,2,2-trifluoroethanol
THF	tetrahydrofuran
TLC	thin layer chromatography
Tol	toluene
TTBP	2,4,6-tri-*tert*-butylpyrimidine
UDP	uridine diphosphate

1

General Introduction and Outline

1.1 Iminosugars: Structures, Activities and Applications

Alkaloids are nitrogen containing molecules which are widely distributed in nature. They are produced by a wide variety of organisms such as plants, fungi, bacteria, marine animals, amphibians, some birds and a few mammals.[1–7] Over the years the group of polyhydroxylated alkaloids has gained considerable interest as potential therapeutic agents and as tools to gain a better insight in biological processes. This specific group of alkaloids can be considered as carbohydrate mimics in which the endocyclic oxygen is replaced by a nitrogen. This alteration in combination with their structural resemblance to normal sugars makes that they are often evaluated as inhibitors of glycosidases[8] and glycosyltransferases.[9] These enzymes in turn, both play an essential role in various biological processes including carbohydrate catabolism, maturation, transport and secretion of glycoproteins and cell recognition processes.[10,11] Polyhydroxylated alkaloids, often referred to as iminosugars, can be divided in several different classes depending on their ring structures (Figure 1.1).[12]

Nojirimycin **1** (NJ) is the first iminosugar isolated from natural sources (*S. roseo.* R-468 and *S. laven.* SF-425), and shows remarkable biological activity. In subsequent studies NJ was shown to be a good inhibitor of various α- and β-glycosidases.[13,14] Nojirimycin contains a hemiaminal function, which renders it rather unstable under neutral and acidic conditions at room temperature, therefore it is usually stored as bisulphite adduct or reduced to the more stable 1-deoxynojirimycin **2** (DNJ).[13,15] Over the years a wide range of iminosugar and related alkaloids were isolated from the leaves, root bark and fruits of the mulberry tree

(*Morus* spp.). Prominent examples are DNJ **2**,[16] fagomine **4**, *N*-methyl-DNJ **3** and 1,4-dideoxy-1,4-imino-D-arbinitol **7** (DAB) (Figure 1.1).[17–19]

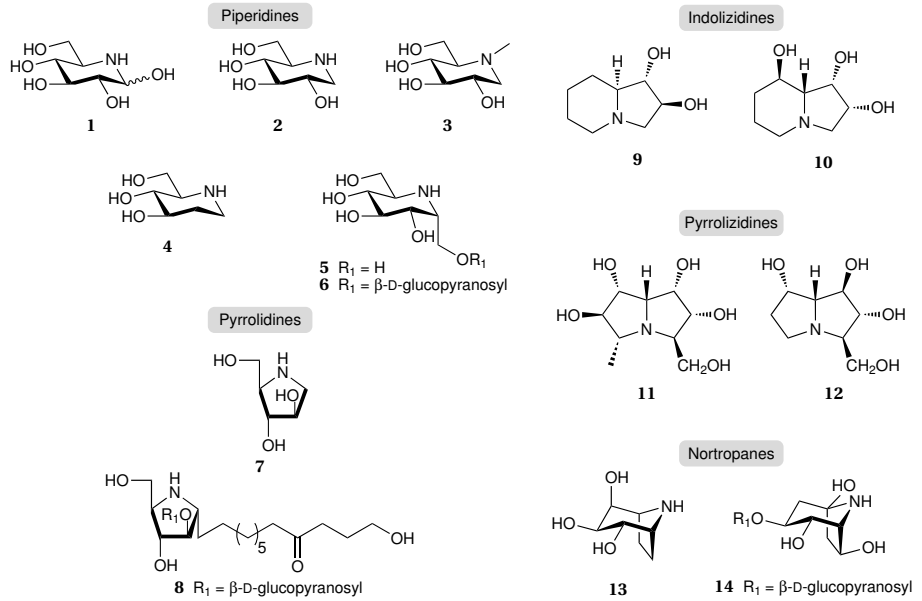


Figure 1.1: Five classes of iminosugars with some examples.
Nojirimycin (NJ, **1**); 1-Deoxynojirimycin (DNJ, **2**); *N*-methyl-DNJ (**3**); Fagomine (**4**); α-Homonojirimycin (α-HNJ, **5**); 7-*O*-β-D-glucopyranosyl-α-HNJ (**6**); 1,4-Dideoxy-1,4-imino-D-arabinitol (DAB, **7**); Broussonetin B (**8**); Lentiginosine (**9**); Swainsonine (**10**); Hyacinthacine C_1 (**11**); Australine (**12**); Calystegine B_4 (**13**); Calystegine B_1-3-*O*-β-D-glucopyranosyide (**14**).

In the field of iminosugar research many *N*-alkylated derivatives of DNJ have been synthesized. Miglitol (**15**, Figure 1.2) is the first α-glucosidase inhibitor based on DNJ **2** and is used as drug for diabetes mellitus type 2.[20,21] By inhibition of α-glucosidase, **15** slows down the rate by which large carbohydrates (poly- and oligomers) are processed in the gut.[21,22] Fleet *et al.*[23] synthesized *N*-butyl-1-deoxynojirimycin **16** (NB-DNJ or Miglustat) which was found to be an inhibitor of glucosylceramide synthase (GCS).[24,25] GCS plays an essential role in the biosynthesis of glucosylceramide, the precursor for more complex glycosphingolipids (Figure 1.2C). Inhibitory properties of NB-DNJ **16** are used to the full extent in the so called substrate reduction therapy (SRT)[26–28] to prevent the accumulation of glucosylceramide (GC) in cells (Figure 1.2B). NB-DNJ is the first orally administered drug to be active in the treatment of type 1 Gaucher disease.[29] Gaucher disease is a rare lysosomal storage disorder in which GC is inefficiently hydrolyzed by mutant glucocerebrosidase (GBA1, Figure 1.2B). This causes accumulation of GC-laden macrophages which results in enlargement of organs (spleen and liver) and inflammation. The first therapy developed for the treatment of Gaucher dis-

ease was enzyme replacement therapy (ERT), in which recombinant GBA1 (called cerezyme) is intravenously administrated to patients.[30] This functional GBA1 enzyme ends up in the Gaucher cells where it temporarily restores the efflux of GC (Figure 1.2B). The disadvantages of ERT are the intravenous administration and the high costs of enzyme production. Substrate reduction therapy offers a useful alternative. Inhibition of GCS alters the influx of GC thereby restoring the influx/-efflux balance of GC in Gaucher cells (Figure 1.2B).[31–33]

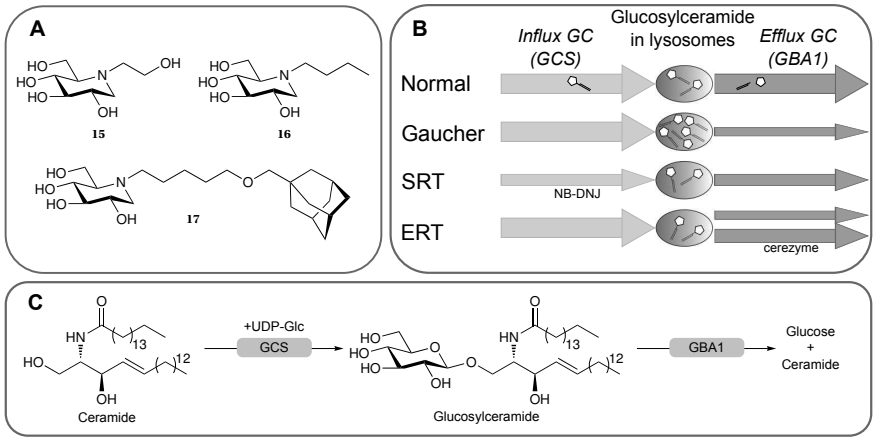


Figure 1.2: **A**: Structures of Miglitol (**15**), NB-DNJ (**16**), AMP-DNJ (**17**); **B**: Schematic overview of Gaucher disease and currently used therapies; **C** : Anabolism and catabolism of glucosylceramide.

Compound **17**, also known as AMP-DNJ, bears a *N*-5-(adamantan-1-yl-methoxy)-pentyl (AMP) chain on the ring nitrogen and has been found to be a better inhibitor of GCS as compared to NB-DNJ.[34] AMP-DNJ has great potential as a novel drug for Gaucher disease and other sphingolipidoses[25,35] and shows promising results regarding treatment of daibetes mellitus type 2,[36] hepatosteatosis and inflammatory bowel disease.[37] Oral adminstration of AMP-DNJ has also been found to result in prevention of atherosclerosis[38] and neurodegenenration in Sandhoff disease.[39]

Next to decorating iminosugars by alkylation of its endocyclic nitrogen to gain better or more selective inhibitors, iminosugars can also be glycosylated to yield a new class of potential inhibitors. Glycosylated iminosugars may be closer mimics of the natural substrates for the enzyme of interest, thereby making them potentially more selective inhibitors than the non-glycosylated iminosugars. Glycosylated iminosugars can also give a better insight in the mechanism of action of glycosidases, as well as potentially being prodrugs or slow-releasing agents that have to undergo an enzymatic transformation to liberate the active inhibitor. There are several examples of naturally occurring glycosylated iminosugars, which are mostly found in iminosugar producing plants. Isolation is often done by extrac-

tion of leaves, bark or roots with aqueous MeOH or EtOH, after which the extracts are purified by a variety of ion-exchange chromatography steps. After isolation and purification careful characterization, is done by Nuclear Magnetic Resonance Spectroscopy (NMR), High Resolution Mass Spectroscopy (HRMS) and enzymatic assays to confirm their structure. Glycosylated iminosugars have been found to contain, amongst others, α- and β-glucosides, α-galactosides, apiosides, β-xylosides, β-mannosides and β-fructofuranosyl glycosides. Some examples are given in Figure 1.1 and Figure 1.3.[8,12,17,40–44] Biological evaluation show that most glycosylated iminosugars are selective inhibitors, probably due tot their close resemblance of the enzymes natural substrates.[8,12,17,40–44]

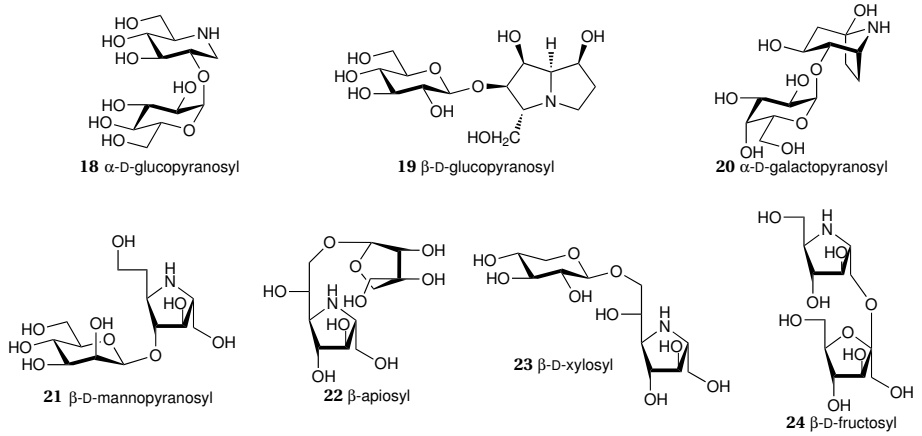


Figure 1.3: Structures of natural occuring glycosylated iminosugars.
2-*O*-α-D-glucopyranosyl-1-deoxynorjirimycin (**18**), 1-*epi*-australine-2-*O*-β-D-glucopyranoside (**19**), 4-*O*-α-D-galactopyranosyl-calystegine B_2 (**20**), 4-*O*-β-D-mannopyranosyl-6-deoxy-homoDMDP (**21**), homoDMDP-7-*O*-apioside (**22**), homoDMDP-7-*O*-β-D-xylopyranoside (**23**); DMDP-7-*O*-β-D-fructofuranoside (**24**).

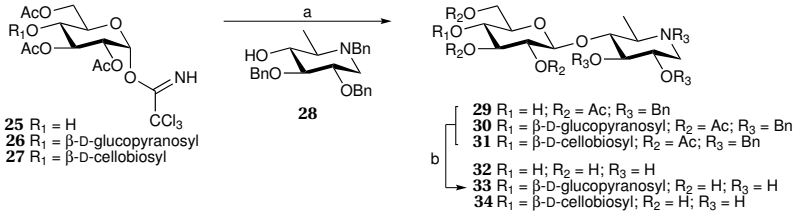
1.2 Synthesis of *O*-Glycosylated Iminosugars

1.2.1 Chemical Synthesis

The natural abundance of *O*-glycosylated iminosugars is extremely low and most of them are potent inhibitors of several glycosidases.[19] To fully explore the potential of *O*-glycosylated iminosugars larger quantities are needed. This goal can be achieved through chemical or enzymatic synthesis.

One of the first syntheses of glycosylated iminosugars was reported by Ganem *et al.*[45] who prepared a cellulase inhibitor. Using the trichloroacetimidate method[46] a glucose mono-, di- or trimer was coupled in a β-1,4 fashion to an iminosugar (Scheme 1.1). Biological evaluation of the resulting glycosylated iminosugars **32**, **33** and **34** showed potent inhibitory effects towards different endo-cellulases from *T. fusca.*[47,48]

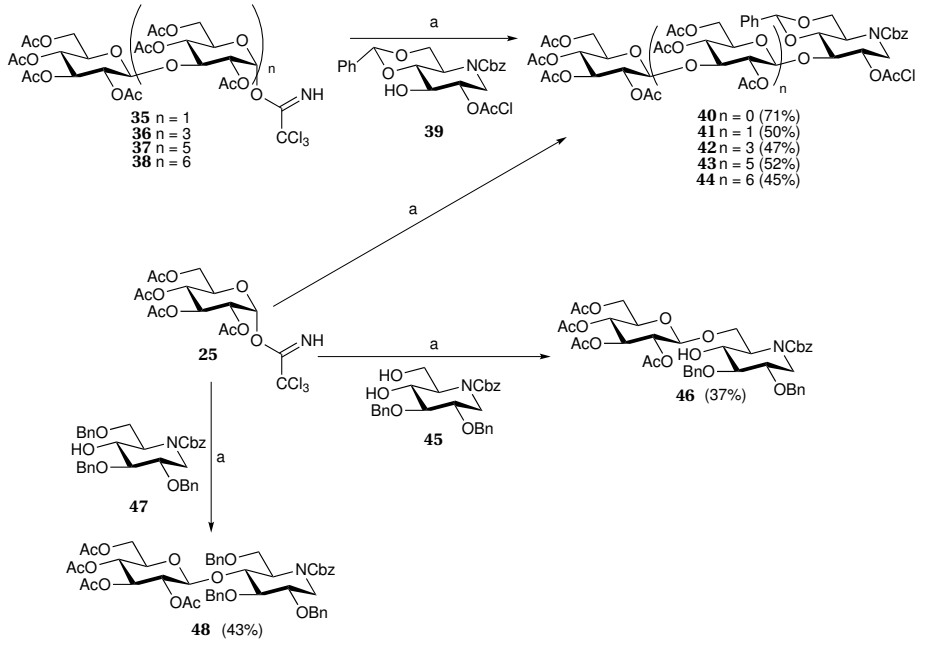
Scheme 1.1: Synthesis of cellulase inhibitors **32**, **33**, **34** as reported by Ganem.[45]



Reagents and conditions: a) $BF_3 \cdot OEt_2$, DCM, 0°C; b) (1) KOH, MeOH; (2) Pd/C, H_2, EtOH:HCl, **32** (50% overall), **33** (38% overall), **34** (40% overall).

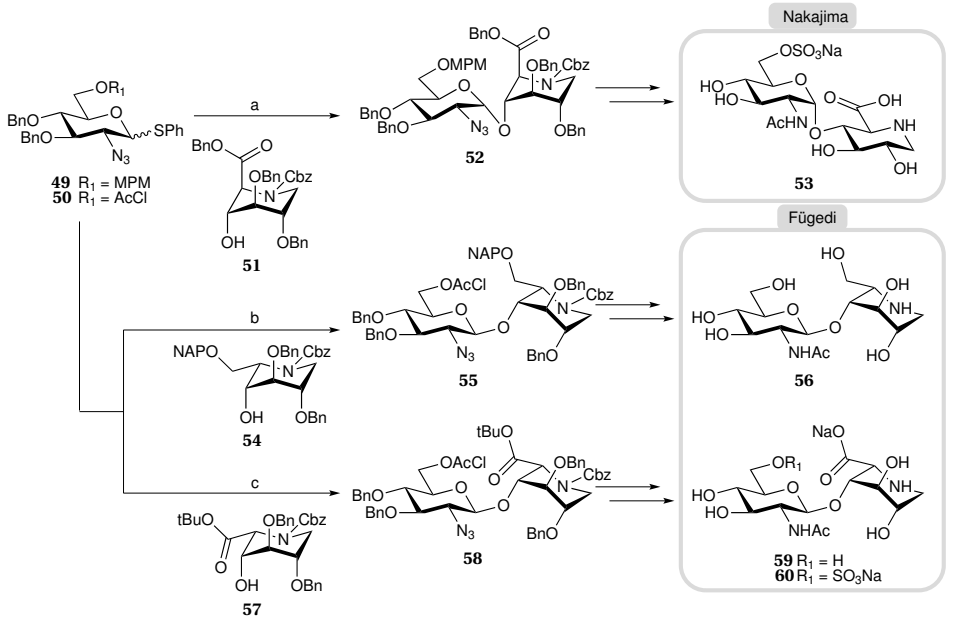
To get a better insight in the processing of cross-linked polysaccharides Blatter and co-workers[49] *O*-glycosylated DNJ at various positions (Scheme 1.2) and evaluated several β-1,3, β-1,4 and β-1,6 linked DNJ oligo-glucosides as potential fungicides. For the synthesis several *O*-acetylated glycosyl trichloroacetimidate donors were condensed with a protected DNJ derivative. A regioselective coupling was achieved in the synthesis of β-1,6 linked disaccharide **46**.[50] All compounds were tested, after deprotection, on a wide variety of fungi and small organisms of which only the brine shrimp (*Artemia salina*) showed to be vulnerable to most of compounds.

Scheme 1.2: Synthesis of fungicides, based on DNJ glucosylated at various positions.[49]



Reagents and conditions: a) TMSOTf, DCM, 0°C.

Scheme 1.3: Synthesis of heparanase inhibitors with D-Glu or L-Ido configuration.[51–53]

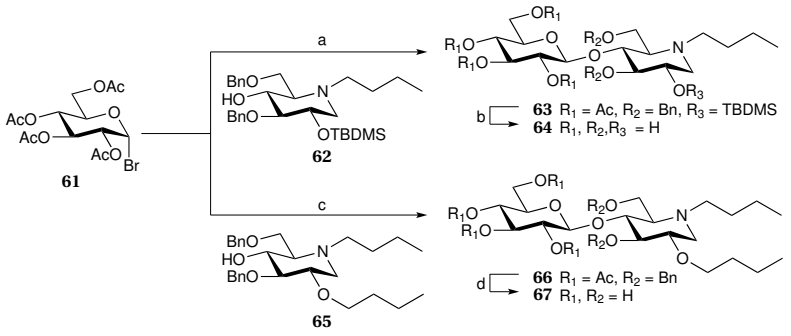


Reagents and conditions: a) NIS, TMSOTf, DCM:Et_2O, -50°C, 72%; b) DMTST, DCM:Et_2O, 59%; c) $Me_2S_2-Tf_2O$, DCM:Et_2O, 80%.

Glycosylated iminosugars have also been used as inhibitors for heparanase, as a potential antimetastatic cancer drug.[54,55] The groups of Nakajima[51] and Fügedi[52,53] independently synthesized a set of iminosugar containing heparanase inhibitors using 2-azido-2-deoxy-D-glucopyranosyl donors **49** and **50**. Condensing **49** with iminosugar **51**, having the D-glucuronic acid configuration,[51] afforded, after deprotection, inhibitor **53**. Compound **53** showed to inhibit heparanase, thereby preventing the degradation of heparan sulfate.[56,57] Fügedi and co-workers[52,53] based their design on the use of iminosugars having the L-ido configuration. Condensation of the iminosugars having a L-idose (**54**) or L-iduronic acid configuration (**57**) with donor **50** using DMTST or $Me_2S_2-Tf_2O$ led to the pseudo disaccharides **55** and **58** which were transformed into **56** and **60**. No biological data were reported on these compounds.

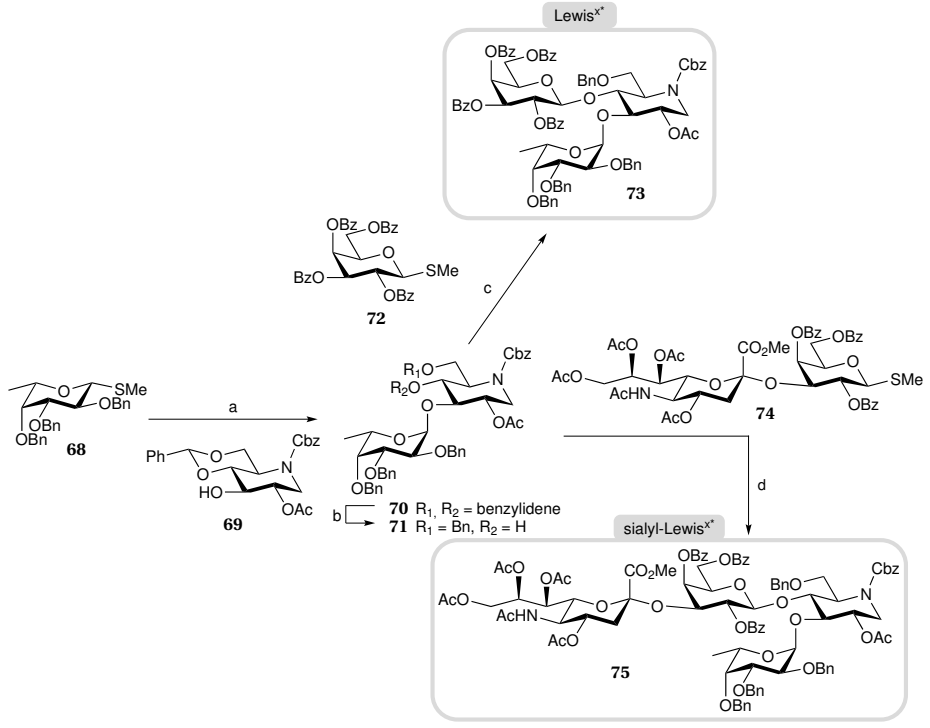
To assess if iminosugars can act as a ceramide mimic in β-glucocerebrosidase (GBA1), Martin and Compain[58] developed two GBA1 inhibitors, featuring a *N*-alkylated DNJ derivative bearing two alkyl chains (**65**) and a glucose, to fully mimic the natural substrate of GBA1. Condensation of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide donor **61** with DNJ acceptor **62** or **65** under Koenings-Knorr conditions afforded, after deprotection, inhibitors **64** and **67**. Biological results show improved affinity of **67** towards GBA1 (IC_{50} 56 μM) as compared to the monoalkylated **64** (no inhibition) and even as compared to DNJ **2** (IC_{270} 56 μM).

Scheme 1.4: Synthesis of β-glucocerebrosidase inhibitors.[58]



Reagents and conditions: a) AgOTf, DCM, -78°C, 34%; b) (1) nBu_4NF, THF, 0°C, 70%, (2) NaOMe, MeOH, 86%, (3) Pd/C, H_2, *i*PrOH/AcOH, (4) Dowex^{TM} OH^-, 44%; c) AgOTf, DCM, -78°C, 55%; d) (1) NaOMe, MeOH, quant., (2) Pd/C, H_2, *i*PrOH/AcOH, (3) Dowex^{TM} OH^-, quant.

Scheme 1.5: Synthesis of Lewis^x **73** and sialyl-Lewis^x **75**.[59]

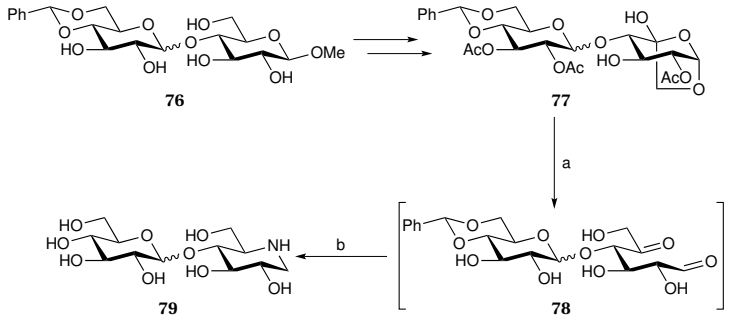


Reagents and conditions: a) DMTST, benzene, 7°C, 92%; b) $NaCNBH_4$, Et_2O, 81%; c) NIS, TfOH, DCM, 70%; d) NIS, TfOH, DCM, 61%; * protected forms of Lewis^x and sialyl-Lewis^x iminosugar analogs.

Next to iminosugars that are glycosylated on one position, various iminosugars have been synthesized that bear more than one carbohydrate. Furui and

co-workers[59] reported the synthesis of Lewis^x **73** and sialyl-Lewis^x **75** iminosugar analogs in which DNJ is di-glycosylated (Scheme 1.5). Coupling of L-fucose **68** to the 3-position of DNJ **69** followed by selective opening of the benzylidene in **70** gave acceptor **71**. Mono glycosylated DNJ acceptor **71** was then condensed with D-galactose **72** under influence of NIS and TfOH to yield trimer **73**, which after deprotection gave the DNJ derivative of Lewis^x. By coupling of thio donor **74** to DNJ acceptor **71**, using similar conditions as in the assembly of **73**, tetramer **75** was gained, which after deprotection afforded DNJ analog of sialyl-Lewis^x.

Scheme 1.6: Synthesis of glucosidase inhibitors β-**79** and α-**79** starting with cellobiose and maltose.[60]



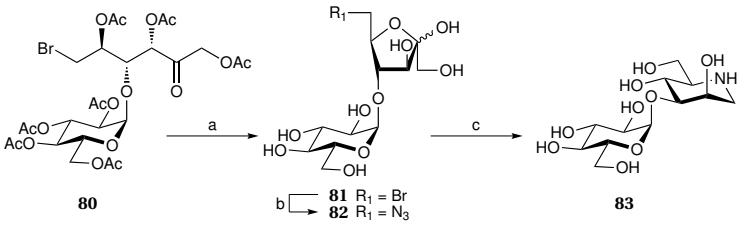
Reagents and conditions: a) NaOMe, MeOH; b) $Pd(OH)_2$, H_2, NH_4OH, H_2O, 24% over two steps.

A different approach for the synthesis of glycosylated iminosugars is to first synthesize a carbohydrate oligomer, after which the reducing end sugar is converted in the corresponding iminosugar. By using naturally occurring oligomers as starting material, this approach circumvents the use of a glycosylation steps and lengthy protective group manipulations. The group of Stütz reported three syntheses in which they use cellobiose, maltose or maltulose as starting materials for the synthesis of glucosylated iminosugars (Scheme 1.6 and Scheme 1.7).[60,61] Conversion of cellobiose and maltose into their 1,6-anhydrosugar derivatives (β-**77** and α-**77**), followed by deprotection of the acetyl functions and concomitant ring opening afforded di-carbonyl β-**78** and α-**78** (Scheme 1.6). Double reductive amination using Pearlmans catalyst in aqueous ammonia under a hydrogen atmosphere yielded target compounds β-**79** and α-**79**.

The maltulose derivative was synthesized *via* open-chain bromide **80**[62] (Scheme 1.7), which cyclized under Zemplén conditions to give **81**, which was subsequently reacted with NaN_3 in DMF to gain compound **82**. Conventional catalytic hydrogenation of azidodeoxysugar **82** in dry methanol using $Pd(OH)_2$ furnished title compound **83**.

The group of Piancatelli[63] used glycosyl glycals (D-lactal **84a**, D-cellobial **84b**, D-maltal **84c** and D-melibial **84d**) for the synthesis of glycosylated L-fagomine

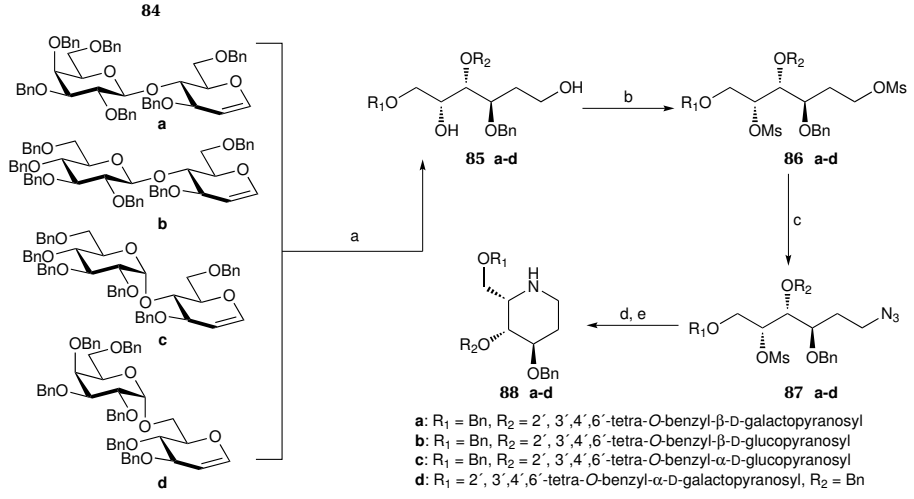
Scheme 1.7: Synthesis of glucosidase inhibitor **83**.[61,62]



Reagents and conditions: a) NaOMe, MeOH; b) NaN_3, DMF; c) $Pd(OH)_2$, H_2, MeOH, 26% over 3 steps.

derivatives. Opening of the glycals **84a-d** by mercury(II) acetate/sodium borohydride,[64,65] gave compounds **85a-d** which were converted in *N*-heterocyclized compounds **88a-d** in a three-step sequence: 1) formation of the 2,6-di-*O*-mesylates (**86a-d**), 2) regioselective azidation by treatment with NaN_3 in DMF (**87a-d**), 3) cyclization by reduction of the azide (**88a-d**).

Scheme 1.8: Synthesis of glycosylated imonosugars *via* D-lactal (**84a**), D-cellobial (**84b**), D-maltal (**84c**) and D-melibial (**84d**).[63]



Reagents and conditions: a) $Hg(OAc)_2$, $NaBH_4$, DCM, **85 a-d** ~90%; b) Et_3N, MsCl, DCM, **86 a-d** ~80%; c) NaN_3, DMF, 70°C, **87 a-d** ~80%; d) $P(Ph)_3$, THF:H_2O; e) Et_3N, 40°C **88 a-d** ~70%.

1.2.2 Enzymatic Synthesis

Enzymatic synthesis using glycosidases and glycosyltransferases can be a useful alternative for the synthesis of iminosugar containing oligomers. There are a few examples in which DNJ or *N*-protected DNJ is used as acceptor for enzymatic syntheses of glycosylated DNJs.

For the syntheses of the DNJ derivatives of sialyl Lewis^x and Lewis^a on a large scale, Kojima *et al.*[66] used a β-galactosidase to gain a large amount of the galactosylated DNJ building block. Mixing of lactose (50 kg), DNJ (5 kg) and β-galactosidase (250 mL) in H_2O (250 L) for 18 hours gave 8.2 kg of product as a mixture of galactosyl-DNJ derivatives. Purification by strong base anion-exchange resin yielded 300 grams of different galactosyl-DNJ in the following ratio; unreacted DNJ **2** (32%), 1,2-linked **89** (6%), 1,3-linked **90** (20%), 1,4-linked **91** (25%), 1,6-linked **92** (7%) and other unidentified transgalactosylated DNJs (Figure 1.4).

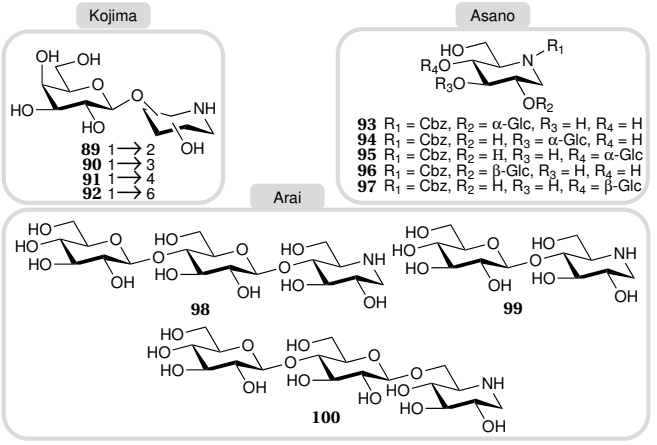


Figure 1.4: Structures of glycosylated iminosugars described in section 1.2.2.

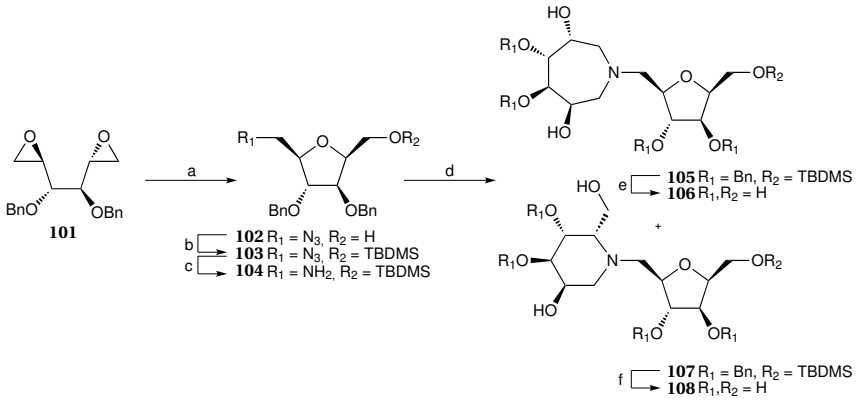
By using α- and β-glucosidases and *N*-benzyloxycarbonyl protected DNJ, Asano and co-workers[67] made a series of α- and β-linked glucosylated DNJ derivatives. Maltose and DNJ were stirred with rice α-glucosidase yielding 1,4-linked (**95**), 1,3-linked (**94**) and 1,2-linked (**93**) α-glucosyl DNJ in yields of 40, 13 and 2% respectively (Figure 1.4). No 1,6-linked coupling was observed, probably due to steric hindrance of the *N*-benzyloxycarbonyl group. Cellobiose was used as glucose donor in the coupling effected by yeast β-glucosidase to give 1,2-linked (**96**) and 1,4-linked (**97**) β-glucosylated DNJ in yields of 69% and 3% respectively (Figure 1.4). After deprotection the glucosylated iminosugars were tested for their biological activity showing that α-1,2-linked (**93**) and α-1,3-linked (**94**) were more effective than DNJ against trehalases and rice α-glucosidase, respectively.

To elucidate the mechanism of hydrolysis of cellulase, the group of Arai[68] synthesized cellulase inhibitors by condensing cellobiose and DNJ using a transglycosylase. They synthesized three inhibitors, two bearing a disaccharide either on the 4- (**98**) or the 6-position (**100**) of DNJ and one bearing a glucose on the 4-position of DNJ (**99**) (Figure 1.4). Trimer **98** (1,4) was found to be the best inhibitor for several fungal and bacterial cellulases as it best resembles natural cellulose.[69]

1.3 Synthesis of Different Linked Glycosylated Iminosugars

Aside from the *O*-glycosylated iminosugars there a few examples in which the endocyclic nitrogen of an iminosugar is linked to a carbohydrate by a non-hydrolyzable bond. The group of Merrer reported[70,71] the synthesis of DNJ which bears D-glucitol on the ring nitrogen. First bis-epoxide **101** was reacted with NaN_3 and SiO_2, directly followed by an *O*-cyclization according to a 5-*exo*-*tet* process giving D-glucitol **102**.[72 71]

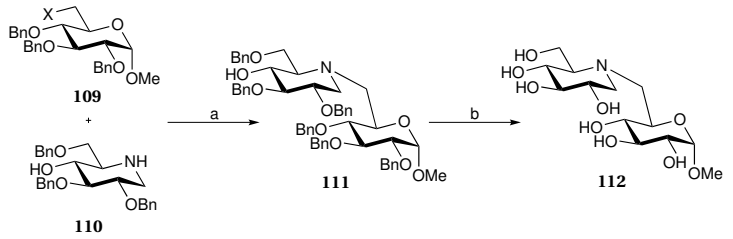
Scheme 1.9: Synthesis of *N*-glycosylated iminosugars.[71]



Reagents and conditions: a) NaN_3, SiO_2, ACN, Δ, 95%; b) TBDMSCl, imidazole, DMF, 95%; c) Pd black, H_2, EtOAc, 95%; d) **101**, EtOH, **105** 40%, **107** 30%; e) (1) $nBuN_4F$, THF, 85%, (2) Pd black, H_2, AcOH, 70%; f) (1) $nBuN_4F$, THF, 80%, (2) Pd black, H_2, AcOH, 75%.

Next the primary hydroxyl was protected to give **103**, followed by reduction of the azide moiety in **103** to give **104**. The free amine in **104** was subsequently reacted with another equivalent of bis-epoxide **101** to form azepane derivative **105** and DNJ derivative **107**, *via* an *N*-cyclyzation in 40% and 30% yield respectively.

Scheme 1.10: Synthesis of MDL 7395.[73]

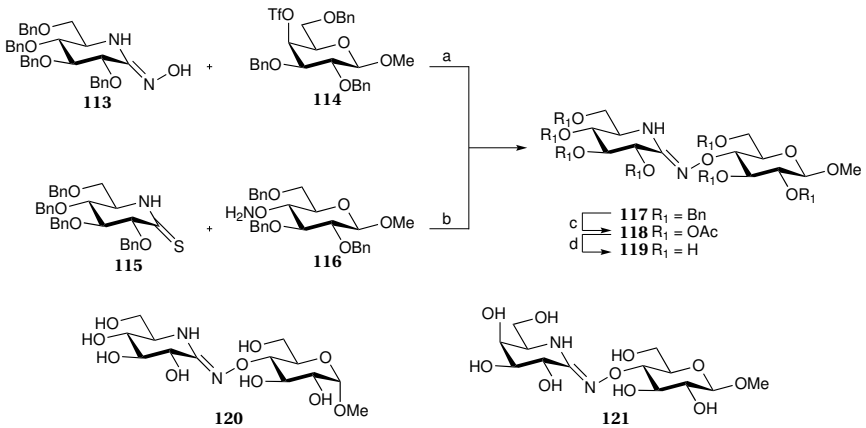


Reagents and conditions: a) DMF, Δ, 80%; b) Pd/C, H_2, EtOH, 79%.

Another example of a *N*-glycosylated iminosugar is *N*-[6-deoxy-1-*O*-methyl-6-α-glucopyranosyl]-1-deoxynojirimycin or MDL 7395 (**112** Scheme 1.10), which was synthesized by the pharmaceutical company Merrel Dow (Strasbourg, France).[73] Coupling using an excess glucosyl halide (**109**) with DNJ acceptor (**110**) yielded, after deprotection, **112**.[73] Biological evaluation of MDL 7395 (**112**) showed that it reduced the glycemic response, by inhibition of the intestinal α-glucohydrolase, which makes it a potential diabetes mellitus drug.[74]

Vasella and co-workers[75] used anomeric oximes such as **113** to link monosaccharides to iminosugars, gaining selective α- and β-glycosidase inhibitors (Scheme 1.11). They used two approaches to synthesize methyl β-cellobioside analog **119**: one by alkylation of the hydroximolactam **113**[76] with trifate **114**[77] and the other by condensation of the thiogluconolactam **115**[78] with hydroxylamine **116**. By use of the latter method compounds **120** and **121** were also synthesized. It was found that compounds **119**, **120** and **121** were strong inhibitors of several different β-glucosidases.[75]

Scheme 1.11: Synthesis of compounds **120**, **119**, **121**.[75]



Reagents and conditions: a) NaOH, Et_4NBr, Tol, 59%; b) $Hg(OAc)_2$, $Et(iPr)_2N$, THF, 72%; c) (1) Li, $EtNH_2$, THF, (2) Ac_2O, pyr., 80%; d) NH_3, MeOH, 77%.

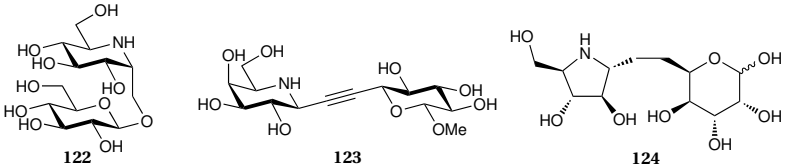


Figure 1.5: Examples of *C*-glycosylated iminosugars.**122**[79], **123**[80], **124**.[81]

A different class of iminosugars with promising biological and therapeutic properties are iminosugars bearing *C*-glycosides. An overview of the synthesis and

strategical design of this class of stable iminosugar analogs is given in several reviews.[82–84]

1.4 Thesis Outline

The ongoing research in the field of lysosomal storage diseases (LSD), and more specific Gaucher disease is the basis for the research described in this thesis. The progress of Gaucher disease and the effect of therapeutic intervention is correlated to the level of chitotriosidase (CHIT1), the first identified human chitinase. Measurement of plasma CHIT1 activity in man is done by an assays using fluorogenic substrate **125**. The ability of CHIT1 to transglycosylate can complicate the enzyme assay, however compound **125** is not prone to be transglycosylated. And gives a proportional fluorophore to active enzyme ratio read-out. Because of this umbelliferone 4'-deoxychitobioside **125** has become a popular fluorogenic substrate for the measurement of human chitinases, an improved scalable route towards **125** is described in **Chapter 2**.

Chapter 3 describes the synthesis and biological evaluation of three novel fluorogenic substrates, containing substituents of different sizes on the 4'-OH of the non-reducing sugar.

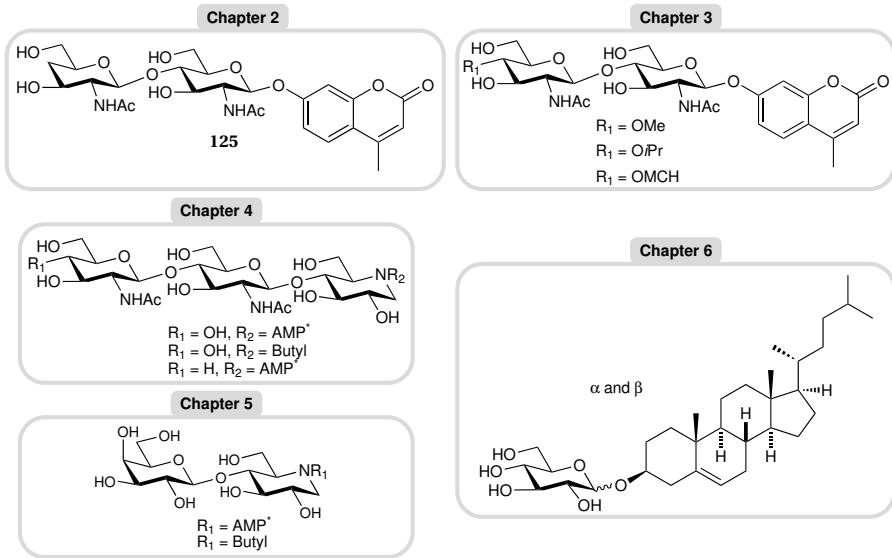


Figure 1.6: Overview of the compounds described in this thesis.
* AMP = *N*-5-(adamantan-1-yl-methoxy)-pentyl

The locally elevated activity of CHIT1 allows site-specific drug delivery *via* the prodrug approach. **Chapter 4** describes the design and synthesis of novel prodrugs in which a chitobiose core, the substrate for CHIT1, is coupled to known in-

hibitors of GCS which are able to restore the influx/efflux balance of GC in Gaucher cells.

It is known that some iminosugars and *N*-alkylated derivatives thereof have a taste bitter. In **Chapter 5** attempts are made to palliated this bitter taste by appending a galactosyl moiety to DNJ. Aside from potentially masking the bitter taste this modification will also help to direct the inhibitors to the colon were they will be processed by lactase.

Cholesteryl-α-glucoside and cholesteryl-β-glucoside, the synthesis of which is described in **Chapter 6**, will be used as as internal standards to get a better insight in the biosynthesis of the potentially neurotoxic steryl-glucosides, which are potentially linked to a high level of glycosylceramide. **Chapter 7** summarizes the research described in chapters 2 to 6 and future prospects based on these results are presented.

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2

A Preparative Synthesis of Human Chitinase Fluorogenic Substrate*

2.1 Introduction

Chitinases are a class of enzymes capable of cleaving natural chitin (a linear polymer of β-1,4-linked-*N*-acetylglucosamine) and a wide variety of artificial chitin-like substrates. The existence of endogenous chitinases in mammals was discovered at the end of the last century.[1] First to be identified was chitotriosidase (CHIT1), a human chitinase that is strongly expressed and secreted by lipid-laden tissue macrophages that are found in patients suffering from Gaucher disease.[2–4] Gaucher disease is a rare lysosomal storage disease in which the influx/efflux balance of glucosylceramide (GC) is disturbed by the inefficient hydrolysis, of GC, by mutant β-glucocerebrosidase (GBA1). A second mammalian chitinase, named AMCase (Acidic Mammalian Chitinase), was identified some years later[5,6] and its role in the etiology of asthma has been proposed.[7]

The CHIT1 activity in plasma of Gaucher patients correlates to the progression of the disease and the effect of therapeutic intervention.[1,8] Chitotriosidase activity thus is an ideal marker by which Gaucher patients are identified and by which their susceptibility towards therapeutic agents is monitored.[9] Currently two

* Duivenvoorden, B. A.; Dinkelaar, J.; Wennekes, T.; Overkleeft, H. S.; Boot, R. G.; Aerts, J. M. F. G.; Codée, J. D. C.; van der Marel, G. A. *Eur. J. Org. Chem.*, **2010**, *13*, 2565-2570.

therapies for the treatment of Gaucher patients are applied, namely enzyme replacement therapy (ERT) and substrate reduction therapy (SRT) (see also chapter 1).[10–15] Both therapies are expensive and monitoring their effect (i.e. optimal dosage and treatment regimen) through measuring of serum CHIT1 activity has a considerable clinical value.

In the years immediately following the discovery of CHIT1 activity as marker for Gaucher, umbelliferyl chitobioside **126** (Figure 2.1) was used as a fluorogenic substrate in biological assays to give a fluorescent read-out.[1] However, it was found that human CHIT1 possesses intrinsic transglycosylase activity,[16] in that higher oligomers are formed through hydrolysis of **126** followed by coupling to the 4-position of the non-reducing end carbohydrate of another substrate. This side reaction complicates interpretation of the kinetics of the enzyme-mediated generation of the fluorescent 4-methylumbelliferyl anion (4-MU). To circumvent the possibility of CHIT1 mediated transglycosylation a modified fluorogenic substrate **125** (Figure 2.1) was synthesized, in which the 4'-OH is removed.[16] This modification gave a superior CHIT1 and AMCase substrate as compared to **126**.[8] Given the growing interest to monitor CHIT1 activity and given the present interest of AMCase in relation to asthma, umbelliferone 4'-deoxychitobioside **125** has become a very desired fluorogenic substrate.

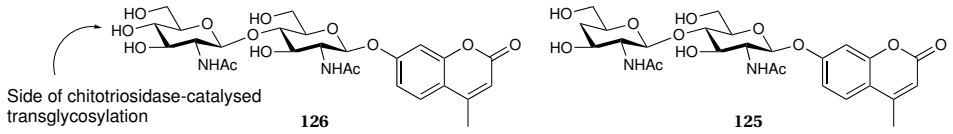


Figure 2.1: Umbelliferyl chitobioside fluorogenic substrates **126** and **125**.

In the original paper[16] the synthesis of **125** started from chitobiose, a disaccharide, forming the target compound **125** in nine consecutive synthetic steps. Although sufficiently effective for the preparation of several milligrams, the route falls short when aiming for larger quantities. The nine-step sequence is quite inefficient (3% overall yield) and furthermore the starting disaccharide (chitobiose) is rather expensive. This chapter describes a more efficient and reliable route towards the superior substrate **125**.

2.2 Results and Discussion

4-Methylumbelliferyl deoxychitobioside **125** was synthesized as outlined in Figure 2.2. The linkage between the chitobiose and the 4-methylumbelliferyl fluorophore could best be achieved via a S_N2-displacement of the anomeric α-chloride in **127** by the umbelliferyl phenolate anion. This procedure was selected as aromatic hydroxyl functions are substantially less nucleophilic than the corresponding aliphatic hydroxyls. In addition, Lewis acid mediated glycosylation of phe-

nols can give rise to an unwanted Fries rearrangement.[17] For the construction of the chitobiose-core a thiophenyl glycoside building block was used because the anomeric thiophenyl group can be introduced early in the synthesis, is stable to the reaction conditions employed and can be selectively activated with a variety of soft electrophiles to provide glycosylating species. Furthermore thiophenyl glycosides are shelf stable and often crystalline, which for the large-scale preparation of building blocks is a valuable asset. To maximize the efficiency in the construction of 4'-deoxychitobiosyl umbelliferone **125** a route was designed, in which a single thioglycoside (**130**) serves as an advanced precursor for both the non-reducing and reducing end glucosamine building blocks. To protect the glucosamine nitrogen function the phthaloyl group was selected because it is cheap, robust under both basic and acidic conditions, and can be readily introduced on glucosamine on a large scale using well-established chemistry.[18,19] The phthalimide group reliably provides anchimeric assistance in the coupling of the two glucosamines to give the 1,2-trans glycosidic bond and does not give rise to oxazoline side products. Benzyl ethers will mask all hydroxyls during the assembly of the chitobiose disaccharide.

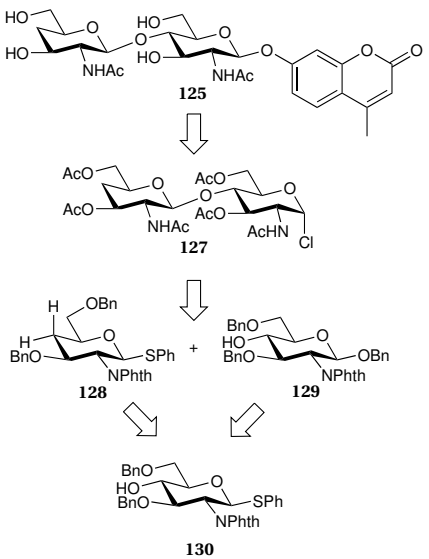


Figure 2.2: Outline for the large-scale synthesis of umbelliferyl chitobioside fluorogenic substrate **125**.

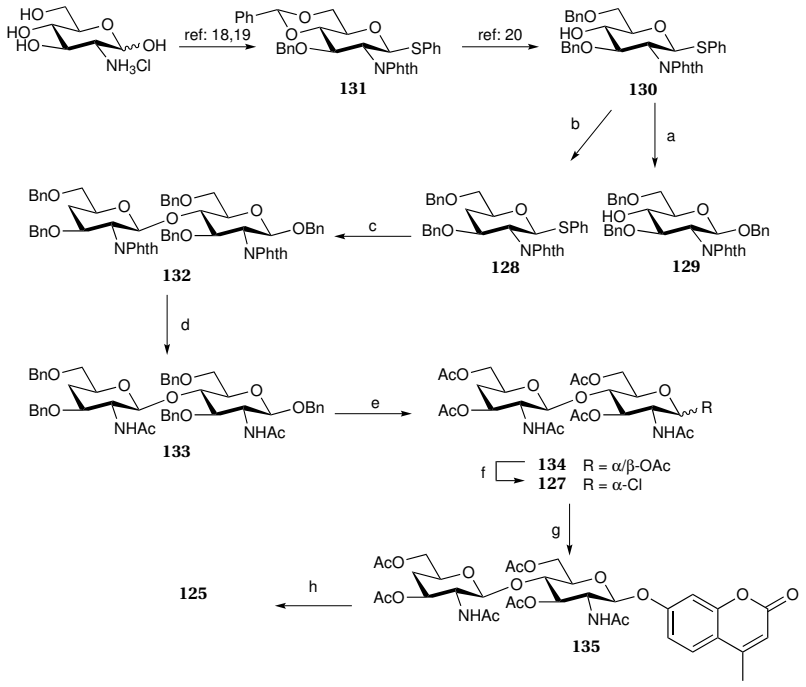
Scheme 2.1 depicts the synthesis of 4'-deoxychitobiosyl umbelliferone which started with the synthesis of thioglycoside **130**. Thioglucosamine **131** was obtained from D-glucosamine in 40% yield over 8 steps on a 147 g scale.[18,19] Only a single chromatographic purification was required in this sequence of reactions. Reductive opening of the benzylidene acetal in the next step was affected by treat-

ment of **131** with TFA and TES to selectively provide key thioglycoside **130** in 85% yield.[20] The formation of the regioisomeric C-4 benzyl ether was not observed. To provide the non-reducing end glucosamine building block, alcohol **130** was subjected to a Barton-McCombie deoxygenation by treatment with NaH and CS_2 followed by MeI to provide the methyl dithiocarbonate. Radical fragmentation using Bu_3SnH and AIBN as initiator in refluxing toluene then led to the deoxygenated glucosamine **128** in 87% yield.[21] For the construction of the reducing end glucosamine building block **129**, partly protected thioglycoside **130** was condensed with benzyl alcohol employing *N*-iodosuccinimide (NIS) and a catalytic amount of TMSOTf as activator cocktail.[22] The use of a large excess of BnOH (5 equivalents), and the high nucleophilicity of this alcohol as compared to the glucosamine 4-OH, completely prevented self-condensation of **130**. Glucosamine **129** was obtained in 75% yield. In the ensuing NIS/TMSOTf mediated glycosylation deoxy glucosamine **128** and benzyl glucosamine **129** were reacted in a 1 : 1 ratio to provide chitiobioside derivative **132** in excellent yield.

To introduce the umbelliferyl chromophore, disaccharide **132** was transformed into the disaccharide α-chloride **127**. To this end, both *N*-phthaloyl groups in **132** were removed by transamidation with ethylenediamine in refluxing *n*-butanol. Subsequent acetylation of the resulting free amines then provided the crystalline dimer **133**. Removal of all benzyl groups from this disaccharide proved to be more troublesome than expected due to the low solubility of the partly debenzylated-*N*-acetyl chitobioside intermediates. The best results were obtained when disaccharide **133** was treated under 5 bar hydrogen pressure using 5 mol% of Pearlman's catalyst in a 1:1 THF/MeOH solvent mixture in the presence of 5 equivalents of AcOH. The fully deprotected 4'-deoxychitobioside was then acetylated to give the penta-*O*-acetate **134** in 65% yield as an amorphous white solid over two steps.

The final stages of the synthesis followed procedures slightly adapted from literature.[23] Chlorination of the reducing end glucosamine derivative required careful tuning of the reaction conditions. The anomeric acetate **134** was treated with dry HCl in a mixture of AcOH and Ac_2O at 5°C for 42 hours to afford 4'-deoxychitobiosyl chloride **127** in 74%. Shorter reaction times led to incomplete chlorination and higher reaction temperatures gave interglycosidic bond cleavage. Previously it has been reported that the anomeric chlorination of chitobiosyl acetate can be readily accomplished at room temperature.[23] Presumably the absence of the hydroxyl function on the 4-position of the non-reducing end GlcNAc in **134** makes the glycosidic linkage more labile towards acidic cleavage. Introduction of the 4-methylumbelliferyl chromophore was accomplished by S_N2 displacement of the anomeric α-chloride by the tetrabutyl ammonium salt of 4-methylumbelliferone, generated under phase transfer conditions (PTC).[24] The protected umbelliferyl derivative was obtained in 62% as a white amorphous solid. Saponification of the acetyl esters with NaOMe and HPLC purification completed the synthesis of target compound **125**, yielding 227 mg (28%) of product.

Scheme 2.1: Large-scale synthesis of umbelliferyl chitobioside fluorogenic substrate **125**.



Reagents and conditions: a) DCM, BnOH, NIS, 0°C , TMSOTf (75%) b) (1) THF, imidazole, CS_2, 0°C, NaH, 1h, then rT, MeI (93%); (2) Tol, Bn_3SnH, AIBN, Δ (87%); c) DCM, NIS, 0°C , TMSOTf (86%); d) (1) *n*BuOH, ethylenediamine, Δ ; (2) MeOH, Ac_2O, Et_3N (82% over two steps); e) (1) THF, MeOH, AcOH, $Pd(OH)_2$, H_2; (2) pyr., Ac_2O (65% over two steps); f) AcOH, Ac_2O, HCl, 0°C to 5°C (74%); g) $CHCl_3$, H_2O, $NaHCO_3$, umbelliferone sodium salt, TBAHS (62%); h) MeOH, NaOMe (28% after HPLC purification).

2.3 Conclusion

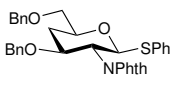
This chapter describes an efficient, reliable and scalable route for the synthesis of 4'-deoxychitobiosyl umbelliferone **125**. The synthesis is based on the use of a partially protected thiophenyl glucosamine derivative **130** as main building block, which is readily transformed into both the reducing and non-reducing end building blocks for the construction of 4'-deoxychitobiose core. This carbohydrate core is then converted to an α-chloride donor, which was then coupled, under PTC, with 4-methylumbelliferone salt to yield, after deprotection, target compound **125**.

2.4 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. ^{1}H 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}.

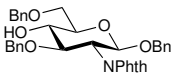
Phenyl 3,6-di-*O*-benzyl-2,4-di-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (128).



Glycoside **130** (25.4 g, 43.8 mmol) was coevaperated thrice with dioxane, then taken up in THF (220 mL). Imidazole (0.298 g, 4.38 mmol) and CS_2 (7.9 mL, 131 mmol) were added after which the mixture was cooled to 0°C. NaH (2.63 g, 60% dispersion in oil, 65.7 mmol) was added and the reaction was kept at 0°C for one hour then allowed to warm to rT. At rT MeI (4.82 mL, 77.5 mmol) was added. After 30 min. the mixture was quenched by addition of AcOH and subsequently diluted with EtOAc (250 mL). The mixture was then washed with $NaHCO_3$. The layers were separated and the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (Tol-EA 100-0→95-5) yielded the thiocarbamate intermediate as the yellow oil (27.4 g, 93%). The thiocarbonate (27.4 g, 40.8 mmol) was coevaporated three times with toluene, dissolved in toluene (800 mL) and degassed with sonication under argon flow for 5 min. Bu_3SnH (16.4 mL, 61.2 mmol) and AIBN (0.33 g, 2.04 mmol) were added and the mixture was warmed to 120°C . After 1h when TLC analysis showed complete consumption of starting material the reaction was cooled to rT and concentrated *in vacuo*. The residue was taken up in ACN and washed twice with hexane, the ACN layer was concentrated *in vacuo*. Column chromatography (EtOAc/PE 30%) afforded **128** as an oil (20.1 g, 87%). TLC: EtOAc/PE 45%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66-7.83 (m, 4H, H_{arom}), 7.28-7.39 (m, 7H, H_{arom}), 7.16-7.17 (m, 3H, H_{arom}), 6.98-7.02 (m, 5H, H_{arom}), 5.57 (d, 1H, $J = 10.4$ Hz, H-1), 4.55-4.57 (m, 3H, CH_2 Bn), 4.19-4.34 (m, 3H, H-2, H-3, CH_2 Bn), 3.84 (m, 1H, H-5), 3.65-3.69 (m, 1H, H-6), 3.54-3.58 (m, 1H, C-6), 2.31 (dd, 1H, $J = 3.6$ Hz, 12.8 Hz), 1.59 (q, 1H, $J = 11.6$ Hz, H-4); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.7, 168.0 (C=O Phth), 137.7, 138.0 (C_q), 133.7 (CH_{arom}), 132.5 (C_q), 132.0 (CH_{arom}), 131.5 (C_q), 127.3-128.6 (CH_{arom}), 123.3, 123.1, 83.6 (C-1), 75.4 (C-5), 73.5 (C-3),

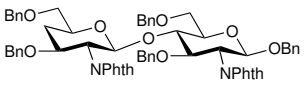
73.3 (CH_2 Bn), 72.3 (C-6), 70.6 (CH_2 Bn), 55.5 (C-2)34.0 (C-4); HRMS: $C_{34}H_{31}NO_5S + Na^+$ requires 588.18151, found 588.18115.

Benzyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (129).



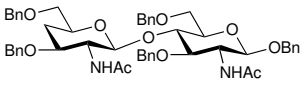
Glycoside **130** (23.6 g, 40.5 mmol) was coevaperated thrice with toluene. DCM (810 mL), BnOH (21 mL, 202 mmol) and NIS (10.9 g, 48.6 mmol) were added. The mixture was stirred over activated 3Å molsieves for 30 min. After cooling to 0°C a catalytic amount of TMSOTf (0.81 mL, 4.5 mmol) was added. After 1h the mixture was allowed to warm to rT when TLC analyses showed complete consumption of starting material the reaction was quenched by addition of Et_3N (5.6 mL, 40.5 mmol). The reaction mixture was diluted with DCM and washed with $Na_2S_2O_3$. The water layer was extracted twice with DCM, the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (EtOAc/PE 20%) yielded **129** as a colorless oil (17.6 g, 75%). TLC: EtOAc/PE 50%; ^{1}H NMR (400 MHz, $CDCl_3$) δ , 7.76 (bs, 1H, H_{arom}), 7.62-7.63 (m, 2H, H_{arom}), 7.53 (bs, 1H, H_{arom}), 7.28-7.36 (m, 5H, H_{arom}), 7.02-7.07 (m, 7H, H_{arom}), 6.89-6.93 (m, 3H, H_{arom}), 5.15-5.17 (m, 1H, H-1), 4.78 (d, 1H, J = 12.4 Hz, CH_2 Bn), 4.74 (d, 1H, J = 12.4 Hz, CH_2 Bn), 4.64 (d, 1H, J = 12.0 Hz, CH_2 Bn), 4.58 (d, 1H, J = 12.0 Hz, CH_2 Bn), 4.51 (d, 1H, J = 12.4 Hz, CH_2 Bn), 4.47 (d, 1H, J = 12.4 Hz, CH_2 Bn), 4.23-4.25 (m, 2H, H-2, H-3), 3.82 (m, 3H, H-4, H-6, H-6), 3.62-3.65 (m, 1H, H-5),3.20 (d, 1H, J = 2.4 Hz, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.0 (C=O Phth), 168.1 (C=O Phth), 136.9, 137.6, 138.0 (C_q), 131.4 (C_q), 133.5 (CH_{arom}), 127.2-128.3 (CH_{arom}), 97.2 (C-1), 123 (CH_{arom}), 78.4 (C-3), 74.1 (CH_2 Bn), 73.8 (C-4), 73.7 (C-5), 73.5 (CH_2 Bn), 70.6 (CH_2 Bn), 70.3 (C-6), 55.3 (C-2); HRMS: $C_{35}H_{33}NO_7 + Na^+$ requires 602.21492, found 602.21471.

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)-β-D-glucopyranoside (132).



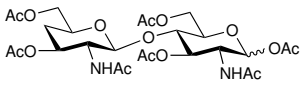
A mixture of donor **128** (20.1 g, 35.4 mmol) and acceptor **129** (20.6 g, 35.4 mmol) were coevaperated three times with toluene. DCM (350 mL) and NIS (9.56 g, 42.5 mmol) were added and the mixture was stirred over activated 3Å molsieves for 30 min. The mixture was cooled to 0°C before a catalytic amount of TMSOTf (0.32 mL, 1.77 mmol) was added. After TLC analysis showed complete consumption of starting material (3 h) at 0°C , the reaction was quenched with Et_3N (5.0 mL, 35 mmol). The reaction mixture was diluted with DCM and washed with $Na_2S_2O_3$. The water layer was extracted twice with DCM, the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (EtOAc/PE 30%) yielded **132** as a colorless oil (31.7 g, 86%). TLC : EtOAc/PE 30%; ^{1}H NMR (400 MHz, $CDCl_3$)δ = 7.88-7.89 (m, 2H, H_{arom}), 7.67-7.71 (m, 4H, H_{arom}), 7.58-7.59 (m, 2H, H_{arom}), 7.20-7.37 (m, 10 H, H_{arom}), 6.96-7.02 (m, 12H, H_{arom}), 6.82 (bs, 3H, H_{arom}), 5.29 (d, 1H, J = 8.0 Hz, H-1'), 4.98 (d, 1H, J = 6.4 Hz, H-1), 4.84 (d, 1H, J = 12.4 Hz, CH_2 Bn), 4.68 (d, 1H, J = 12.4 Hz, CH_2 Bn), 4.44-4.58 (m, 6H, CH_2 Bn), 4.11-4.39 (m, 7H), 3.34-3.58 (m, 6H), 2.28 (dd, 1H, J = 4.8 Hz, 12.8 Hz, H-4'), 1.52 (q, 1H, J = 12.0 Hz, H-4'); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.5-168.1 (C=O Phth), 136.9-138.6 (C_q), 133.4-133.7 (CH_{arom}), 131.5 (C_q), 126.7-128.3 (CH_{arom}), 122.9-123.5 (CH_{arom}), 97.2 (C-1'), 97.0 (C-1), 76.5, 75.5, 74.5, 74.0, 73.2, 72.5, 72.4, 71.9, 71.1, 70.6, 70.3, 68.1, 57.7 (C-2'), 55.6 (C-2), 34.2 (C-4'); HRMS: $C_{63}H_{58}N_2O_{12} + Na^+$ requires 1057.38820, found 1057.38876.

Phenyl 3,6-di-*O*-benzyl-2-deoxy-2-acetamido-4-*O*-(3,6-di-*O*-benzyl-2,4-di-deoxy-2-acetamido-β-D-glucopyranosyl)-β-D-glucopyranoside (133).



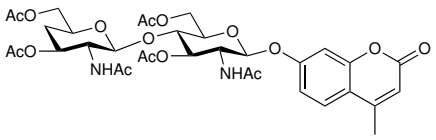
Disaccharide **132** (31.7 g, 30.6 mmol) was dissolved in *n*-BuOH (275 mL) and ethylene diamine (30 mL). This mixture was refluxed overnight and subsequently concentrated *in vacuo*. The reaction was then coevaporated thrice with toluene and taken up in MeOH (300 mL). At 0°C Ac_2O (30 mL, 300 mmol) and Et_3N (8.5 mL, 61.2 mmol) were added and the mixture was allowed to warm to rT. The resulting mixture was concentrated *in vacuo* and taken up in $CHCl_3$ and washed with H_2O. The collected organic layer was stirred over activated carbon and filtered over hyflo-gel concentrated *in vacuo*. Crystallization PE-EA yielded **133** (26.6 g, 82%) as slightly yellow crystals. TLC: EtOAc/PE 25%; ^{1}H NMR (400 MHz, $CDCl_3$ / CD_3OD, 1/1): δ 7.21-7.35 (m, 25H, H_{arom}), 4.86 (d, 1H, J = 12.4 Hz, CH_2 Bn), 4.75 (d, 1H, J = 11.6 Hz, CH_2 Bn), 4.54-4.69 (m, 5H, CH_2 Bn, H1'), 4.38-4.49 (m, 5H, CH_2 Bn, H1), 4.12 (t, 1H, J = 6.8 Hz), 3.97 (t, 1H, J = 6.4 Hz), 3.63-3.79 (m, 5H), 3.44-3.51 (m, 3H), 3.37-3.38 (m, 1H), 2.20 (dd, 1H, J = 4.8 Hz, 12.8 Hz, H-4'), 1.94 (s, 6H, CH_3 NHAc), 1.45 (q, 1H, J = 12.0 Hz, H-4'); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD, 1/1): δ 171.4 (C_q NHAc), 170.8, 137.1-138.3 (C_q), 126.8-128.0 (CH_{arom}), 100.2 (C-1, C-1'), 99.6, 78.4, 75.4, 74.4, 74.1, 73.1, 73.0, 72.3, 71.8, 70.5, 70.2, 69.8, 68.9, 55.5 (C-2'), 51.8 (C-2), 33.1 (C-4'), 22.5 (CH_3 NHAc),22.2; HRMS: $C_{51}H_{58}N_2O_{10} + Na^+$ requires 881.39837, found 881.39865.

1,3,6-Tri-*O*-acetyl-2-deoxy-2-acetamido-4-*O*-(3,6-di-*O*-acetyl-2,4-di-deoxy-2-acetamido-β-D-glucopyranosyl)-D-glucopyranoside (134).



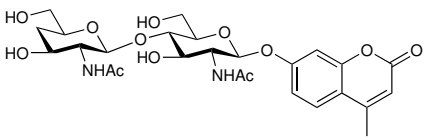
Disaccharide **133** (22.9 g, 26.6 mmol) was disolved in THF (250 mL) then MeOH (250 mL), AcOH (9 mL, 106 mmol) and $Pd(OH)_2$ (1 g, 20% on activated carbon, 1.33 mmol) were added. The mixture was shaken overnight on a Parr apparatus® under 5 bar hydrogen pressure. The resulting mixture was filtered over Whatmann® filter paper, concentrated *in vacuo* and taken up in pyridine (180 mL). At 0°C, Ac_2O (55 mL) was added, after 1h the mixture was allowed to warm to rT and stirred o.n.. The reaction was quenched by addition of MeOH at 0°C then concentrated *in vacuo*. The residue was taken up in $CHCl_3$ and washed with 1M HCl:$NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (MeOH/DCM 3%) yielded **134** as a white amorphous solid (10.6 g, 65%). TLC: MeOH/DCM 5% ; ^{1}H NMR of α acetate (400 MHz, CD_3OD): δ 5.99 (d, 1H, J = 3.6 Hz, H-1), 5.24 (t, 1H, J = 10.0 Hz), 5.04 (dt, 1H, J = 5.2 Hz, 10.8 Hz), 4.56 (d, 1H, J = 8.0 Hz, H-1'), 4.44 (d, 1H, J = 12.0 Hz), 4.30 (dd, 1H, J = 3.6 Hz, 10.8 Hz), 4.23 (dd, 1H, J = 5.6 Hz, 11.6 Hz), 4.04-4.12 (m, 2H), 3.88 (t, 1H, J = 9.6 Hz), 3.98 (m, 1H), 3.79 (m, 1H), 3.61 (t, 1H, J = 9.2 Hz), 1.86-2.14 (22H, CH_3 Ac, H-4'), 1.51 (q, 1H, J = 11.6 Hz, H-4'); ^{13}C NMR of α-acetate (100 MHz, CD_3OD): δ 171.9-172.4 (C=O Ac), 102.6 (C-1'), 91.5 (C-1), 76.9, 72.4, 72.0, 71.6, 70.7, 66.7 (C-6, C-6'), 63.5, 56.4 (C-2, C-2'), 52.2, 33.8 (C-4'),20.8-23.0 (CH_3 Ac);HRMS: $C_{26}H_{38}N_2O_{15} + Na^+$ requires 641.21644, found 641.21643.

4-Methylumbelliferyl 1,3,6-tri-*O*-acetyl-2-deoxy-2-acetamido-4-*O*-(3,6-di-*O*-acetyl-2,4-di-deoxy-2-acetamido-β-D-glucopyranosyl)-β-D-glucopyranoside (135).



Disaccharide **134** (1.61 g, 2.59 mmol) was dissolved in AcOH (13 mL) and Ac_2O (3.2 mL). At 0°C dry HCl_g was bubbled through (liberated under Kipp conditions) for 3h. The reaction mixture was then placed at 5°C for 42 h at which TLC analysis (DCM-acetone 60-40) showed complete consumption of starting material. The reaction diluted with $CHCl_3$ (50 mL, 0°C) and washed twice with H_2O (25 mL, 0°C) and twice with $NaHCO_3$ (25 mL, 0°C). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* yielding an amorphous solid **127** (1.14 g) and purity was evaluated by ^{1}H NMR (400 MHz, CDCl3): δ 1.55 (q, 1H, J = 11.6 Hz, H-4'), 1.86-2.14 (22H, CH_3 Ac, H-4'), 3.73-3.83 (m, 4H), 4.03 (dd, 1H, J = 4.0 Hz, 11.6 Hz), 4.20-4.25 (m, 2H), 4.36-4.54 (m, 3H), 4.48 (d, 1H, $J = 8.0$ Hz, H-1'), 5.02 (dt, 1H, $J = 5.2$ Hz, 11.2 Hz), 5.30 (t, 1H, $J = 10.0$ Hz), 5.94 (d, 2H, $J = 8.0$ Hz, NHAc), 5.96 (d, 2H, $J = 8.0$ Hz, NHAc), 6.12 (d, 1H, $J = 3.6$ Hz, H-1). The resulting solid was dissolved in $CHCl_3$ (76 mL) and added to a solution of H_2O (76 mL), $NaHCO_3$ (1.29 g, 15 mmol), 4-methylumbelliferyl sodium salt[25] (1.9 g, 9.59 mmol) and TBAHS (1.3 g, 3.84 mmol). The biphasic mixture was stirred overnight under exclusion of light. The phases were separated and the organic layer was washed twice with $NaHCO_3$ (0.2 M) and twice with H_2O. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (MeOH/$CHCl_3$ 3%) yielded **135** (0.88 g, 62%) as a white amorphous solid. TLC: MeOH/DCM 5%; ^{1}H NMR (400 MHz, $CDCl_3$ / CD_3OD, 1/1): δ 7.44 (d, 1H, $J =$ 10.4 Hz), 6.86 (m, 2H), 6.72 (d, 1H, $J = 9.2$ Hz, NHAc), 6.54 (d, 1H, $J = 9.2$ Hz, NHAc), 6.09 (s, 1H), 5.46-5.49 (m, 2H), 5.23-5.26 (m, 3H), 5.14 (t, 1H, $J = 8.1$ Hz), 4.04-4.29 (m, 7H), 2.38 (s, 3H, CH_3 4-methylumbelliferyl), 1.86-2.14 (19H, CH_3 Ac, H-4'), 1.71 (q, 1H, $J = 12.4$ Hz, H-4'); ^{13}C NMR of (100 MHz, $CDCl_3$/CD_3OD, 1/1): δ 171.9-172.4 (C_q Ac), 160.1 (C_q), 159.9, 154.4, 153.3, 125.5 (CH_{arom}), 114.7 (C_q), 133.3 (CH_{arom}), 112.4, 103.5 (CH_{arom}), 102.9 (C-1'), 98.7 (C-1), 72.1, 72.0, 70.0, 69.9, 68.5, 65.2 (C-6, C-6'), 62.0, 54.4 (C-2, C-2'), 54.2, 32.5 (C-4'), 20.5-23.2 (CH_3 Ac), 18.5 (CH_3 4-methylumbelliferyl); HRMS: $C_{34}H_{42}N_2O_{16} + Na^+$ requires 757.24265, found 757.24278.

4-Methylumbelliferyl 2-deoxy-2-acetamido-4-*O*-(2,4-di-deoxy-2-acetamido-β-D-glucopyranosyl)-β-D-glucopyranoside (125).



To a suspension of **135** (0.878 g, 1.195 mmol) in MeOH (60 mL) was added NaOMe (44 μL, 30 wt% in MeOH, 0.24 mmol). The reaction was stirred under exclusion of light. When LCMS (gradient 0 to 50% MeOH) showed complete conversion to the product, the mixture was quenched with AcOH (70 μL, 1.2 mmol). The reaction was diluted with H_2O (60 mL), the MeOH was evaporated *in vacuo* and the remaining H_2O was lyophilized. Purification by HPLC (gradient H_2O-MeOH + 0.1% TFA 80-20→60-40) evaporation of MeOH and lyophilizing H_2O yielded **125** (227 mg, 28%) as white fluffy solid. ^{1}H NMR (400 MHz, (D_6) DMSO): δ 7.90 (d, 1H, $J = 8.8$ Hz, NH), 7.67-7.71 (m, 2H, 4-methylumbelliferyl, NH), 7.02 (d, 1H, $J = 1.6$ Hz), 6.94 (d, 1H, $J = 8.8$ Hz, 4-methylumbelliferyl), 6.25 (s, 1H, 4-methylumbelliferyl), 5.17 (d, 1H, $J = 8.4$ Hz, H-1), 4.84-4.90 (m, 3H, OH), 4.69 (bs, 1H, OH),

4.30 (d, 1H, $J = 8.4$ Hz, H-1'), 3.78 (q, 1H, $J = 9.2$ Hz, C-2 or C-2'), 3.36-3.68 (m, 10 H), 2.39 (s, 3H, CH_3 4-methylumbelliferyl), 1.84 (s, 4H, CH_3 NHAc, H-4'), 1.80 (s, 3H, CH_3 NHAc), 1.21 (q, 1H, $J = 11.6$ Hz, H-4'); ^{13}C NMR (100 MHz, (D_6) DMSO): δ 169.2, 169.4 (C=O Ac), 160.1 (C_q), 159.9, 154.4, 153.3, 126.5 (CH_{arom}), 114.3 (C_q), 113.5 (CH_{arom}), 111.9 (CH_{arom}), 103.2 (CH_{arom}), 102.5 (C-1'), 98.3 (C-1), 80.9, 75.1, 72.9, 72.3, 68.2 (C-3, C-4, C-5, C-3', C-5'), 59.7, 63.5 (C-6 and C-6'), 54.4, 57.0 (C-2 and C-2'), 35.8 (C-4'), 23.1 (CH_3 NHAc), 23.0 (CH_3 NHAc), 18.1 (CH_3 4-methylumbelliferyl); HRMS: $C_{26}H_{34}N_2O_{12} + Na^+$ requires 589.20040, found 589.20031.

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3

Design and Synthesis of Three Novel Human Chitinase Fluorogenic Substrates*

3.1 Introduction

Until the end of the last century it was believed that man lacked the ability to process chitin, a linear polymer of β-1,4-linked *N*-acetyl-D-glucosamines which is found on cell walls and coating of many organisms. The first mammalian chitinase was serendipitously discovered in the search for elevated serum glycosidase activity in patients suffering from Gaucher disease, a rare lysosomal storage disease in which glucosylceramide (GC) is inefficiently processed by mutant β-glucocerebrosidase (GBA1).[1–4]

This enzyme, identified as human chitotriosidase (CHIT1), is nowadays used as marker to reflect the total body burden on Gaucher cells.[5] A drawback in the marker assays is the disproportional fluorophore to enzyme ratio (Figure 3.1 A), which is found when using high concentrations of 4-methylumbelliferyl-chitobiose **126** (Figure 2.1) as substrate.[6] This is caused by the transglycosylase activity of CHIT1 forming larger chito-oligomers as substrates, resulting in inefficient release

* Duivenvoorden, B. A.; Ghauharali, K.; Bussink, A. P.; Codée, J. D. C.; van der Marel, G. A.; Scheij, S.; Verhoek, M.; Overkleeft, H. S.; Groener, J. E.; Aerts, J. M. F. G.; Boot, R. G. *Manuscript in preparation.*

of 4-methylumbelliferyl (4-MU), the fluorophore. To circumvent the possibility of CHIT1 mediated transglycosylation a modified fluorogenic substrate **125** was synthesized (**Chapter 2**), in which the 4'-OH is removed.[6] This derivative follows Michaelis-Menten kinetics giving rise to a proportional 4-MU to enzyme ratio (Figure 3.1 A).[6,7] More recent studies[8] showed that **125** is also the substrate of choice when dealing with Gaucher patients having the common polymorphism (G102S) in CHIT1, which shows a slightly impaired catalytic activity toward 4-MU-chitobioside substrate **126** as compared to the wild type CHIT1. However, G102S-CHIT1 activity is normal when using 4-methylumbelliferyl 4'-deoxychitobioside **125** as substrate. It should further be mentioned that increased plasma chitotriosidase activity is not unique for Gaucher patients. Plasma chitotriosidase activity is increased, albeit much more modestly, in several lysosomal and nonlysosomal diseases, such as sarcoidosis, visceral leishmaniasis, leprosy, arthritis, multiple sclerosis, thalassemia, chronic obstructive pulmonary disease (COPD), malaria, and atherosclerosis.[2,9–17]

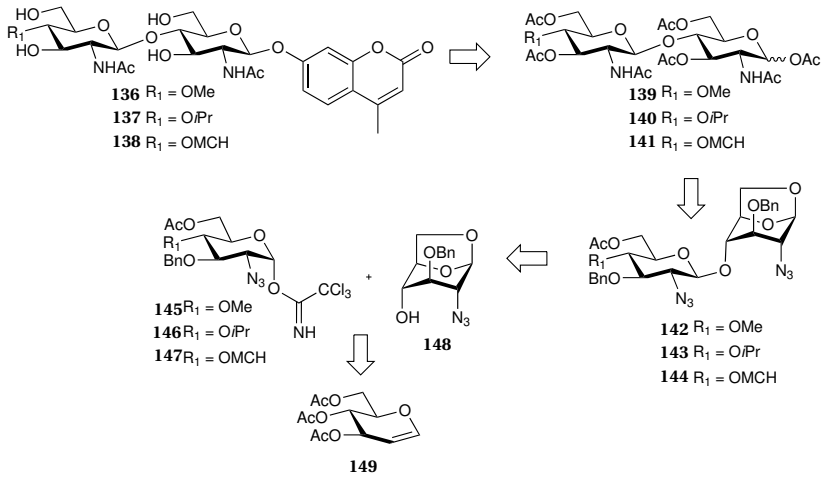
To gain a bigger pool of effective CHIT1 fluorescent substrates, this chapter describes the synthesis of three novel human chitinase substrates **136**,**137** and **138** bearing an anomeric 4-MU for fluorometric read-out. The three substrates have a different modification on the 4'-OH of the non-reducing sugar, going from the relative small *O*-methyl group (OMe) to the more sterically demanding *O*-isopropyl (*i*OPr) and *O*-methyl cyclohexane group (OMCH). A 1,6-anhydro building block is used as a common precursor for the synthesis of the donors and acceptors which after condensation and further modifications, will be evaluated as substrates for CHIT1.

3.2 Results and discusion

The use of an 1,6-anhydro building block for the synthesis of the donors and acceptors helps to overcome the low reactivity of the 4-OH function of *N*-acetylglucosamine, which is well recognized.[18] These constrained 1,6-anhydro sugars are know to exhibit enhanced reactivity of the 4-OH function over there unconstrained counterparts.[19] Further enhancement of the reactivity of the 4-OH can be gained by the use of an azide group on the 2-position of glucosamine, resulting in a 10 fold increase of reactivity as compared to *N*-acetyl or *N*-phthalimido-protected acceptors, as shown by the group of Crich.[18] By using 1,6-anhydro glucosamine **148** as the key building block a route was developed for the synthesis of three novel fluorogenic 4-methylumbelliferyl chiotobiose substrates, bearing a modification on the 4'-OH of the non-reducing end the retrosyntheis of which is outlined in Scheme 3.1.

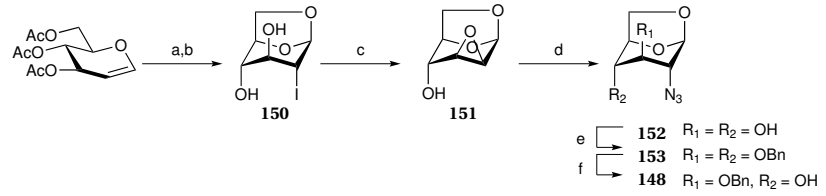
First acceptor **148** was synthesized *via* a modified literature procedure.[20–22] Deacetylation of tri-*O*-acetyl-D-glucal and treatment with bis(tributyltin)oxide in

Scheme 3.1: Route of synthesis of three novel human chitinase fluorogenic substrates.



refluxing acetonitrile, followed by 1,6-iodocyclization[21,23,24] gave compound **150** (Scheme 3.2). Compound **150** was then heated in a DMF:H_2O mixture and treated with a mild base ($NaHCO_3$) to yield 1,6:2,3-bis-anhydro-β-D-glucopyranose **151**. Cerny epoxide **151** was carefully purified to prevent the formation of the unwanted regio-isomer in the next step. This side reaction was attributed to the presence of trace amounts of tin. Opening of the epoxide of **151** with sodium azide, followed by benzylation of the diol and subsequently regioselective debenzylation using $TiCl_4$ gave acceptor **148**.[25]

Scheme 3.2: Synthesis of key building block **148**.

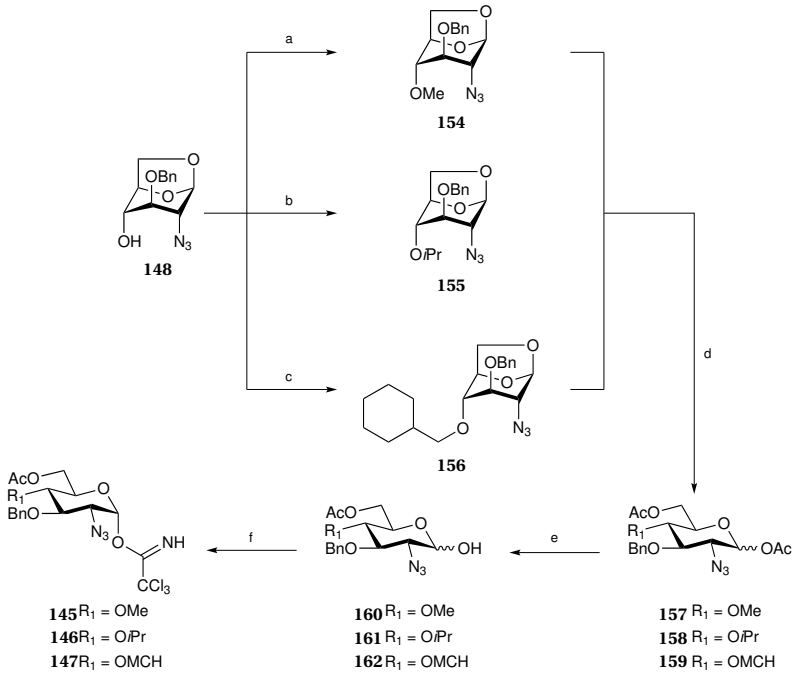


Reagents and conditions: a) $MeOH/H_2O/Et_3N$; b) (1) $(Bu_3Sn)_2O$, Δ, ACN; (2) I_2, DCM, 4 °C; c) $NaHCO_3$, DMF/H_2O, 98% over three steps; d) NaN_3, DMF/H_2O, 120 °C, 52%; e) BnBr, NaH, DMF, 0 °C, 80%; f) $TiCl_4$, DCM, 75%.

Alkylation of the 4-position of anhydro sugar **148** using sodium hydride and MeI in DMF yielded compound **154** (Scheme 3.3) in a good yield. Under similar conditions the application of I*i*Pr and BrMCH (bromomethyl cyclohexane) gave rise to compounds **155** and **156** in slightly reduced yields. Subsequently all three 1,6-anhydro sugars were opened under acidic conditions followed by *in situ* acetylation, using Ac_2O and TFA. Selective deprotection of anomeric position and treatment with trichloroacenitrile and DBU led to the isolation of anomeric mixture of

imidates **145**, **146** and **147** with α as the major isomer.

Scheme 3.3: Synthesis of donors **145**, **146** and **147**.



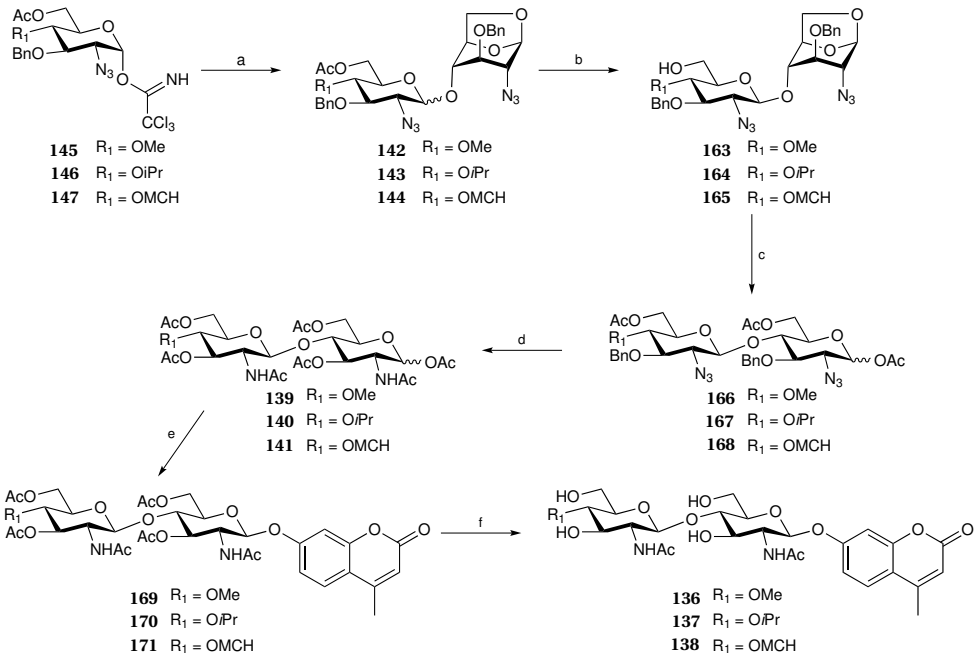
Reagents and conditions: a) MeI, NaH, DMF, 91%; b) I*i*Pr, NaH, DMF, 60%; c) BrMCH, NaH, DMF, 75%; d) Ac_2O, TFA (10% v/v), **157**: 90%, **158**: 86%, **159**: 72%; e)THF, piperidine (6%v/v), **160**: 71%, **161**: quant., **162**: 98%; f) CCl_3CN, DBU, DCM, **145**: 83%, **146**: 70%, **147**: 71%.

Couplings of the imidates (**145**, **146**, **147**) with acceptor **148** (Scheme 3.4) were performed at -80 °C, in dry toluene under influence of $BF_3 \cdot OEt_2$ yielding dimers **142**, **143** and **144** in high yield and high β-selectivity.[26] After deacetylation, pure β-anomers (**163**, **164**, **165**) were obtained by silica gel chromatography. Trifluoroacetic acid and Ac_2O mediated opening of the 1,6-anhydro sugar in **163**, **164**, **165** resulted in the formation of dimers **166**, **167** and **168**. The final steps towards the target substrates involved several protective group manipulations. Starting off with a Staudinger reduction of the azides, the released amines were acetylated using Ac_2O and pyridine. The benzyl groups were removed by hydrogenolysis using Pearlman's catalyst in MeOH:2,2,2-trifluoroethanol (TFE). Preceding the introduction of the fluorophore at the anomeric center the free hydroxyls were protected with acetyl groups yielding intermediate **139**, **140** and **141**.

Fluorophore 4-MU was selected by virtue of its easy quantification in fluorometric assays. Introduction of 4-MU can be attained by phase transfer conditions (PTC) which involves a halide donor in combination with a phenolate also known as the Michael procedure.[27,28] Conversion of the anomeric acetate in **139**, **140**

and **141** into the corresponding α-chloride was found to be troublesome. Several methods were explored including $ZnCl_2$ and α,α-2,2-dichloromethyl methyl ether (DCMME)[29], AcCl and HCl[30] however, these did not give satisfactory results. A combination of AcOH and Ac_2O at 0 °C and dry HCl gas, produced using Kipp conditions (HCl and H_2SO_4), gave the highest yields and most reproducible results.[31,32] The obtained chlorides were coupled with fluorophore 4-MU *via* a optimized Michael procedure using $NaHCO_3$ as a base, an excess of 4-MU sodium salt and TBAHS gave the best results to yield compounds **169**, **170** and **171**. Deacetylation under Zemplén conditions and HPLC purification yielded the final products **136**, **137** and **138** as white powders.

Scheme 3.4: Synthesis of three humane fluorogenic chitinase substrates **136**, **137** and **138**.



Reagents and conditions: a) $BF_3 \cdot Et_2O$, Tol, -80 °C, **142**: 83%, **143**: 81 %, **144**: 65%; b) NaOMe, MeOH, **163**: 65%, **164**: 50%, **165**: 52%; c) Ac_2O, TFA (15% v/v), **166**: 90%, **167**: 80%, **168**: 87%; d) (1) PMe_3, Tol:H_2O:dioxane; (2) Ac_2O, Pyr. (3) $Pd(OH)_2$, H_2, MeOH, TFE; (4) Ac_2O, Pyr., **139**: 48%, **140**: 40%, **141**: 38%; e) (1) AcOH, Ac_2O, HCl_g, 0 °C; (2) Na 4-MU, TBHS, $NaHCO_3$ 0.2M, $CHCl_3$, **169**: 25%, **170**: 17%, **171**: 50%; f) NaOMe, MeOH, **136**: 37%, **137**: 60%, **138**: 22%.

The three human chitinase substrates (**136**, **137** and **138**) were tested for their ability to be processed by human chitinase CHIT1. CHIT1 is able to degrade 4-MU-chitotriose and 4-MU-chitobiose by removal of the oligosaccharide moiety and concomitant release of fluorescent 4-MU. However, the ongoing transglycosylation of the substrates, results in a reduced 4-MU release at higher substrate concentrations (Figure 3.1 A). As described in **Chapter 2** 4-MU-deoxychitobiose **125**

is an improved fluorogenic substrate, which indeed allows a superior fluorometric assay of chitinase activity since the interfering transglycosylation of substrate does not occur (Figure 3.1 A). Compounds **136**, **137** and **138** all showed Michaelis-Menten kinetics like the parent 4'-deoxy substrate **125** (Figure 3.1 B).

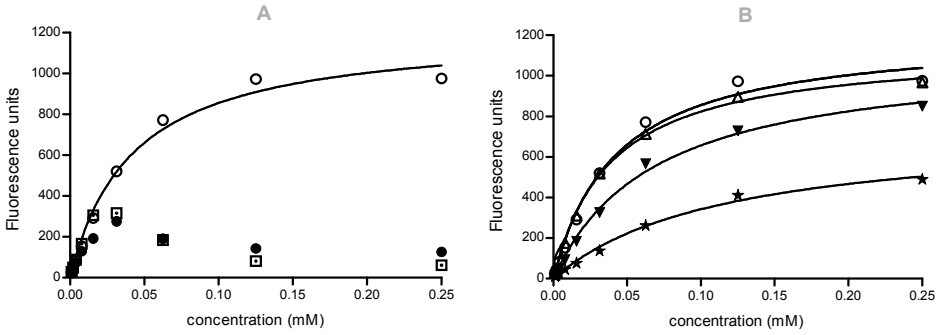


Figure 3.1: (A) Michaelis-Menten kinetics of 4-MU-deoxychitobiose; ○:**125**, ●:4-MU-chitobiose **126**, ⊡:4-MU-chitotriose; (B) Michaelis-Menten kinetics chitinase substrates bearing a modification at the 4-position of the non-reducing end; ○:**125**, △:**136**, ▼:**137**, ⋆:**138**.

Km values of the new 4-MU-substrates become higher with a more bulky substituent: 37, 50, 88, and 200 μM for **125**, **136**, **137** and **138**, respectively. V_{max} values are quite similar for **125**, **136** and **137**: 5.0, 4.7 and 4.4 mmol/mg CHIT1/h, respectively. The V_{max} value for **138**, (1.7 mmol/mg chitotriosidase/h) is clearly lower. Nevertheless none of the three novel compounds was superior to the 4-deoxy derivative **125**.

In plasma or tissue extracts a stepwise degradation of chitinase substrates can occur. This reaction is catalyzed by β-hexosaminidase which slowly and stepwise removes a GluNAc moiety from the non-reducing end, resulting in undesired background release of 4-MU.[33] Therefore, the ability of jack bean (*Canavalia*) β-hexosaminidase to sequentially hydrolyze the modified 4-MU-chitobioses was examined, its enzymatic activity towards 4-MU-GlcNAc (0.135 mM) was first determined at the optimal pH of 4.0. The assay showed to be linear in time over 60 minutes (Figure 3.2 A).

An identical amount of enzyme was incubated with 0.135 mM of 4-MU-chitobioses with different modifications at 4-position of the non-reducing end (**136**, **137** and **138**) as well as the 4'-deoxy derivative **125** and the unmodified substrate. Both the unmodified 4-MU-chitobiose substrate and **125** are relatively good substrates, resulting in gradual release of the fluorescent leaving group (Figure 3.2 B). After 1 hour incubation with 4-MU-chitbiose the amount of 4-MU released by jack bean β-hexosaminidase was about 4% of that released from 4-MU-GlcNac under similar conditions. In contrast, in the case of **125** this was about 2%. Figure 3.2 B shows that substrates bearing a modification at the 4-position of the

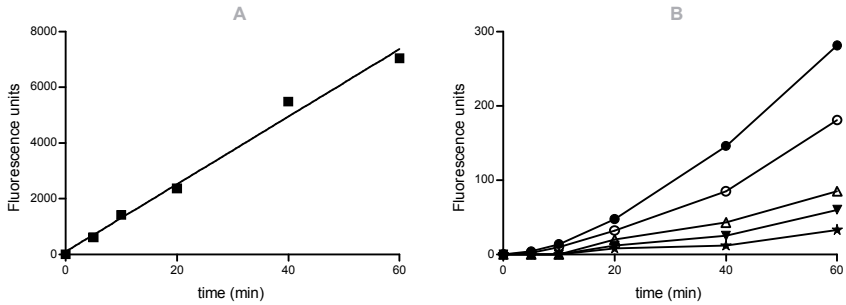


Figure 3.2: (A) Hydrolysis of 4-MU-GlcNAc (0.135 mM) over 60 minutes by jack bean β-hexosaminidase; ■:4-MU-GlcNAc; (B) Hydrolysis of chitobiose and derivatives bearing a modification at the 4-position of the non-reducing end; •:4-MU-chitobiose **126**, ○:**125**, △:**136**, ▼:**137**, ⋆:**138**.

non-reducing end, particularly 4'-isopropyloxychitobiosyl umbelliferone **137** and 4'-cyclohexylmethoxychitobiosyl umbelliferone **138**, are much more resistant towards jack bean β-hexosaminidase mediated hydrolysis, most likely due to the steric bulk which precludes binding of a other sugar to the active site of this β-hexosaminidase.

3.3 Conclusion

This chapter describes the synthesis of three novel human chitinase fluorogenic substrates, using 1,6-anhydrosugar **148** as a common building block. Anhydro sugar **148** was not only transformed into a glycosyl acceptor but was also used as precursor in the synthesis of the three different imidate donors (**145**, **146**, **147**). Key step entailed the coupling under phase transfer conditions of the fluorophore and the dimeric α-chlorides.

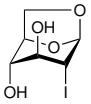
The newly designed compounds **136**, **137** and **138** do not act as acceptors in transglycosylation and offer substrates for CHIT1 that are hydrolysed according to Michaelis-Menten kinetics. An additional advantage is that the novel compounds are lesser substrates for β-hexosaminidases. In situations where significant β-hexosaminidase activity is suspected in a sample, next to chitinase activity, and where one aims to monitor specifically chitinase activity, the latter two substrates (4'-isopropyloxychitobiosyl umbelliferone **137** and 4'-cyclohexylmethoxychitobiosyl umbelliferone **138**) may be the reagents of choice.

3.4 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*-

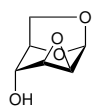
dimethylformamide (DMF), methanol (MeOH), pyridine (pyr) and tetrahydrofuran (THF) were obtained from Biosolve. THF was distilled over $LiAlH_4$ before use. Dichloromethane was boiled under reflux over P_2O_5 for 2 h and distilled prior to use. Molecular sieves 3Å were flame dried under vacuum before 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. ^{1}H 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}.

1,6-Anhydro-2-deoxy-2-iodo-β-D-glucopyranose (150):



A solution of the commercial available tri-*O*-acetyl-D-glucal (2.72 g, 10 mmol) was dissolved in MeOH:H_2O:Et_3N (10:10:1, 125 mL) was stirred for 1 h at ambient temperature, then concentrated. The residue was dried by coevaporation with dioxane (3x 50 mL). The clear oil was use further without any purifications. Crude deprotected D-glucal (1.46 g, 10 mmol) was dissolved in 100 mL MeCN. The solution was boiled under reflux with 4.08 mL bis(tributyl stannyl) oxide (4.77g, 8 mmol) and molsieves 4Å for 2.5 h. Subsequently the reaction was cooled to 0 °C, followed by portion wise addition of 3.8 g I_2 (15 mmol, 1.5 equiv). The dark brown mixture was stirred overnight at 4 °C. TLC showed complete conversion of D-glucal into **150**. The mixture was filtered through Celite and concentrated. To the residue were added $Na_2S_2O_3$ (50 mL) and PE (50 mL), and the biphasic mixture was vigorously stirred for several h until the mixture became colorless. The aqueous phase was than washed several times with EtOAc(4x 40 mL). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was used without further purification in the next step.

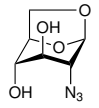
1,6:2,3-Bis-anhydro-β-D-mannopyranose (151):



A heterogeneous solution of compound **150** (2.71 g, 10 mmol) and $NaHCO_3$ (2.5 g, 25 mmol, 2.5 equiv) in DMF:H_2O (10:1) 25 mL was heated to 120 °C. After 4 h, the reaction mixture was cooled, concentrated (*in vacuo*) and silica gel purification (MeOH/EtOAc 10%) yielded 1.42 g (9.85 mmol, 98% over 3 steps) of the title compound **151** as light yellow oil. TLC: EtOAc/PE 50%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.15 (d, $J = 3.7$ Hz, 1H, CH, C'-2), 3.25 (d, $J =$

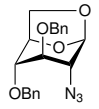
8.8 Hz, 1H, CH, C'-4), 3.44 (t, $J = 2.9$, 1H, CH, C'-3), 3.69-3.95 (m, 2H, CH_2, C'-6), 5.70 (d, $J = 2.9$ Hz, CH, C'-1), ^{13}C NMR (50 MHz, $CDCl_3$) δ 48.49 (CH, C'-2), 53.23 (CH, C'-3), 64.69 (CH_2, C'-6), 65.84 (CH, C'-4), 73.21 (CH, C'-5), 96.56 (CH, C'-1).

1,6-Anhydro-2-azido-2-deoxy-β-D-glucopyranose (152):



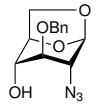
The bis-anhydro sugar **151** (1.15 g, 8 mmol) was heated to reflux temperature in a 10:1 MeOH:H_2O (40 mL) solution containing 5.20 g NaN_3 (80 mmol, 10 equiv), and 4.24 g NH_4Cl (80 mmol, 10 equiv). After ^{1}H NMR showed complete conversion to compound **152** (4.5 days), the solution was cooled, filtered through Celite and concentrated under reduced pressure. Silica gel purification (EtOAc/PE 80%) yielded 0.99 g (5.32 mmol, 66.5%) of the title compound **152** as off-white solid. TLC: EtOAc/PE 50%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.17 (s, 1H, CH, C'-2), 3.53 (s, 1H, CH, C'-4), 3.32-3.69 (m, 2H, CH, CH2, C'-3, C'-6), 4.03 (d, $J =$ 7.3 Hz, 1H, CH_2, C'-6), 4.46 (d, $J = 4.3$ Hz, 1H, CH, C'-5), 5.36 (s, 1H, CH, C'-1); ^{13}C NMR (50 MHz, $CDCl_3$) δ 64.07 (CH, C'-2), 66.55 (CH_2, C'-6), 72.92 (CH, C'-3, C'-4), 77.99 (CH, C'-5), 101.79 (CH, C'-1); ESI-MS: 209.9 (M^+Na^+)

1,6-Anhydro-3,4-di-*O*-benzyl-2-azido-2-deoxy-β-D-glucopyranose (153):



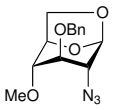
The anhydro sugar **152** (9.35 g, 50 mmol) was dissolved in DMF and cooled to 0 °C. Benzyl bromide (15 mL, 125 mmol, 2.5 equiv) was added followed by portion wise addition of NaH (6 g, 150 mmol, 3 equiv). The reaction was stirred for 3 h, allowing the mixture to warm to rT, after which it was cooled (0 °C) and quenched by addition of MeOH. The mixture was concentrated *in vacuo* and the oily residue was taken up in Et_2O and washed with 1M HCl. The organic layer was dried, filtered and concentrated under reduced pressure. Purification by silica gel chromatography (EtOAc/PE 20%) yielded compound **153** in 65% (11.93 g, 32.5 mmol). TLC: EtOAc/PE 30%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 - 7.22 (m, 12H), 5.51 - 5.43 (s, 1H), 4.65 - 4.44 (m, 5H), 4.03 - 3.96 (d, $J = 7.3$ Hz, 1H), 3.73 - 3.68 (m, 1H), 3.67 - 3.62 (s, 1H), 3.38 - 3.34 (s, 1H), 3.29 - 3.25 (s, 1H).; ^{13}C NMR (100 MHz, d_4), MeOD) δ 137.50, 137.36, 128.66, 128.65, 128.16, 128.11, 128.00, 127.89, 100.65, 76.05, 74.49, 72.47, 71.44, 65.45, 60.03.

1,6-Anhydro-3-*O*-benzyl-2-azido-2-deoxy-β-D-glucopyranose (148):



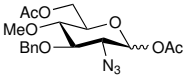
Compound **153** (10.28 g, 28 mmol) was coevaporated thrice with Tol, after which it was dissolved in dry DCM (450 mL). Next $TiCl_4$ (3.2 mL, 29.4 mmol, 1.05 equiv) was carefully added, the mixture was stirred for 1.5 h at rT. The reaction mixture was than poured into cooled (0 °C) H_2O after which the layers were separated. The organic layer was washed with $NaHCO_3$ and H_2O and dried using $MgSO_4$. Concentration and purification by silicagel chromatography (EtOAc/PE 25%) gave **148** in 75% yield (5.9 g, 21.3 mmol). TLC: EtOAc/PE 40%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 - 7.26 (m, 5H), 5.47 - 5.43 (s, 1H), 4.71 - 4.57 (m, 2H), 4.56 - 4.50 (d, $J = 5.6$ Hz, 1H), 4.32 - 4.15 (d, $J = 7.3$ Hz, 1H), 3.83 - 3.72 (m, 1H), 3.70 - 3.63 (d, $J = 7.3$ Hz, 1H), 3.62 - 3.58 (m, 1H), 3.55 - 3.50 (s, 1H), 2.70 - 2.57 (d, $J = 9.8$ Hz, 1H).; ^{13}C NMR (100 MHz, MeOD) δ 137.27, 128.76, 128.27, 127.84, 100.21, 78.17, 76.43, 72.66, 69.01, 65.21, 59.78.

1,6-Anhydro-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-methyl-β-D-glucopyranose (154):



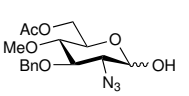
Compound **148** (8.15 mmol, 2.26 g) was dissolved in DMF (25 mL) and cooled using an ice-bath, after stirring for 15 minutes NaH (60% dispersion in mineral oil) (0.49 g, 12 mmol, 1.5 equiv) was added portion wise. After 30 minutes the gas development stopped and MeI (0.61 mL, 9.8 mmol 1.2 equiv) was added dropwise. After 1.5 h the reaction was quenched with MeOH. The reaction mixture was concentrated *in vacuo* and purified using a short silica column (EtOAc/PE 20%) which gave product **154** as clear oil (91%, 2.16 g, 7.42 mmol). TLC: EtOAc/PE 40%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41-7.27 (m, 5H, CH_{arom} Bn), 5.48 (s, 1H, H-1, 4.64 (m, 3H, CH_2 Bn, H-5), 4.08 (d, $J = 7.2$ Hz, 1H, H-6), 3.76 (d, $J =$ 6.4 Hz, 1H, H-6), 3.59 (s, 1H, H-4), 3.39 (s, 3H, CH_3), 3.29 (s, 1H, H-3), 3.20 (s, 1H, H-2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.4 (C_q), 128.7-127.9 (CH_{arom} Bn), 100.6 (C-1), 78.8 (C-2), 75.9 (C-3), 73.8 (C-4), 72.5 (C-6), 65.3 (CH_2 Bn), 59.9 (C-5), 57.2 (CH_3 Me); IR (neat) ν 2096.5, 1718.5, 1244.0, 1099.3, 1004.8, 929.6, 867.9; HRMS: $C_{14}H_{17}N_3O_4 + Na^+$ requires 314.1111, found 314.1113; $[\alpha]_D^{23} + 21.7$ °(c = 2, $CHCl_3$).

1,6-Di-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-methyl-α/β-D-glucopyranose (157):



Anhydro compound **154** (2.16 g, 7.42 mmol) was taken up in Ac_2O (37 mL) and cooled with an ice bath. To this cooled solution TFA (3.7 mL, 10% v/v) was added and the reaction was stirred overnight at room temperature. After complete conversion of the starting material the reaction was diluted with toluene and coevaporated. The resulting oil was purified using a short silica column (EtOAc/PE 30%) yielding **157** (2.62 g, 6.66 mmol, 90%) as a white solid. TLC: EtOAc/PE 50%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43-7.23 (m, 5H, CH_{arom} $Bn_{\alpha/\beta}$), 6.22 (d, $J = 3.6$ Hz, 1H, H-1_α), 5.45 (d, $J = 8.1$ Hz, 1H, H-1_β), 4.88-4.81 (m, 2H, CH_2 $Bn_{\alpha/\beta}$), 4.30-4.21 (m, 2H, CH_2, H-$6_{\alpha/\beta}$), 3.87-3.78 (m, 2H, H-$5_{\alpha/\beta}$ H-$4_{\alpha/\beta}$), 3.55-3.45 (m, 4H, CH_3 Me, H-$3_{\alpha/\beta}$), 3.30 (m, 1H, H-$2_{\alpha/\beta}$), 2.09 (s, 3H, CH_3, OAc), 2.04 (s, 3H CH_3, OAc); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1 (C_q, Ac), 168.3 (C_q, Ac), 137.4 (C_q), 128.2-127.7 (CH_{arom} Bn), 92.2 (C-1_β), 90.0 (C-1_α), 82.4 (C-4_β), 79.9 (C-4_α), 79.5 (C-3_α), 79.1 (C-3_β), 75.1 (CH_2 Bn), 73.5 (C-5_β), 71.0 (C-5_α), 64.5 (CH_3 Me_β), 62.1 (C-$6_{\alpha/\beta}$), 62.1 (CH_3 Me_α), 60.6 (C-2_α), 60.4 (C-2_β), 20.4 (CH_3 OAc), 20.3 (CH_3 OAc); IR (neat) ν 2106.1, 1753.2, 1733.9, 1373.2, 1136.0, 1109.0, 1004.8, 935.4, 906.5, 740.6; HRMS: $C_{18}H_{23}N_3O_7 + Na^+$ requires 416.1428, found 416.1427.

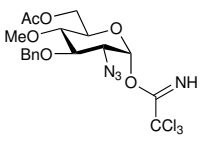
6-*O*-Acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-methyl-α/β-D-glucopyranose (160):



Compound **157** (2.62 g, 6.66 mmol) was dissolved in THF (30 mL) and piperidine (1.8 mL, 6% v/v) was added. The clear solution was stirred overnight at room temperature. After complete conversion to a lower running spot on TLC the reaction was diluted with EtOAc (100 mL) and poured in 1M HCl (100 mL). The layers were separated and the organic layer was washed twice with H_2O and once with brine. Subsequently the EtOAc layer was dried and concentrated *in vacuo*. Flash silica column purification (EtOAc/PE 20%) yielded compound **160** in 71% as a white foam (1.65 g, 4.70 mmol). TLC: EtOAc/PE 50%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46-7.28 (m, 8H, CH_{arom} Bn α/β), 5.26 (d, $J = 3.4$ Hz, 1H, H-1_α), 4.90-4.77 (m, 3H, CH_2 Bn α/β), 4.56 (d, $J = 7.5$ Hz, 1H, H-1_β), 4.37 (m, 2H, CH_2, H-$6_{\alpha/\beta}$), 4.25-4.16 (m, 2H, CH_2, H-$6_{\alpha/\beta}$), 4.07-4.00 (m, 1H, H-5_α), 3.93 (dd, $J = 10.1$, 8.9 Hz, 1H, H-4_α), 3.69 (s, 1H, OH), 3.55 (s, 3H, CH_3, Me_α), 3.53 (s, 1H, CH_3 Me_β), 3.47-3.41 (m, 1H, H-4_β), 3.40-3.30 (m, 3H, H-$2_{\alpha/\beta}$, H-5_β), 3.30-3.19 (m, 2H, H-$3_{\alpha/\beta}$), 2.09 (s, $J = 5.5$ Hz, 6H CH_3, Acα/β); ^{13}C

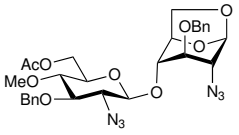
NMR (100 MHz, $CDCl_3$) δ 171.2 (C_q, Ac), 171.1 (C_q, Ac), 137.6 (C_q), 137.6 (C_q Bn), 128.4-127.9 (CH_{arom} Bn), 95.9 (C-1$_\beta$), 91.7 (C-1$_\alpha$), 82.6 (C-3,4$_\beta$), 80.4 (C-3,4$_\alpha$), 79.6 (C-3,4$_{\alpha/\beta}$), 75.4 (CH_2 Bn$_{\alpha/\beta}$), 75.4 (CH_2, Bn$_{\alpha/\beta}$), 73.0 (C-5$_\beta$), 68.9 (C-5$_\alpha$), 67.1 (C-2$_\beta$), 63.6 (C-2$_\alpha$), 62.97 (C-6$_{\alpha/\beta}$), 62.95 (C-6$_{\alpha/\beta}$), 60.8 (OMe$_{\alpha/\beta}$), 60.7 (OMe$_{\alpha/\beta}$) 20.7 (CH_3 Ac$_{\alpha/\beta}$), 20.7 (CH_3 Ac$_{\alpha/\beta}$); IR (neat) ν 2937.4, 2106.1, 1739.7, 1456.2, 1319.2, 1238.2, 1120.6, 1085.8, 1035.7, 995.2, 746.4, 698.2; HRMS: $C_{16}H_{21}N_3O_6 + Na^+$ requires 374.1323, found 374.1321.

6-*O*-Acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-methyl-1-*O*-(*N*-trichloroacetimidoyl) -α-D-glucopyranoside (145):



A solution of compound **160** (1.6 g, 4.6 mmol) and trichloroacetonitrile (1.37 mL, 13.7 mmol, 3 equiv) in dry DCM (25 mL) was treated with 0.2 equivalents of DBU (0.12 mL, 0.91 mmol) for 18 h at ambient temperature. The dark brown solution was concentrated under reduce pressure and directly purified using a silica gel column (20% EtOAc/PE and 2.5% TEA) to obtain the title compound **145** in a good yield (1.88 g, 3.80 mmol, 83%). TLC: EtOAc/PE 30% + 2.5% TEA; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (s, 1H, NH), 7.45-7.29 (m, 5H, CH_{arom} Bn $_{\alpha/\beta}$), 6.37 (d, $J = 3.5$ Hz, 1H, H-1$_\alpha$), 4.89 (d, $J = 10.4$ Hz, 2H, CH_2 Bn), 4.37-4.21 (m, 2H, H-6), 4.01-3.90 (m, 2H, H-4,5), 3.67-3.59 (m, 1H, H-3), 3.56 (s, $J = 11.7$ Hz, 3H, CH_3 Me), 3.36 (dd, $J = 18.4$, 8.5 Hz, 1H, H-2), 2.06 (s, $J = 5.0$ Hz, 3H, CH_3 OAc); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4 (C_q, C=N)160.6 (C_q, Ac), 137.6 (C_q), 128.6-128.1 (CH_{arom} Bn) 94.6 (C-1) 90.7 (C_q, CCl_3) 79.9 (C-3,4,5), 75.6 (CH_2 Bn), 71.8 (C-3,4,5), 62.8 (C-3,4,5), 62.4 (C-6), 61.1 (C-2), 20.8 (CH_3 Ac); IR (neat) ν 2110.0, 1733.9, 1678.0, 146.2, 1373,2 1228.6, 1016.4, 986.2, 906.5, 789.5, 734.8, 696.3.

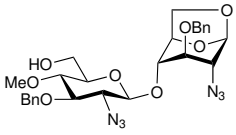
1,6-Anhydro-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-methyl-β-D-glucopyranosyl)-β-D-glucopyranose (142):



A mixture of imidate **145** (1.8 g, 3.6 mmol) and alcohol **148** (2.0 g, 7.3 mmol, 2 equiv) were coevaporated thrice with toluene and dissolved in dry toluene (18 mL). To this mixture activated molecular sieves (4Å) were added and the solution was cooled to -78 °C. After 10 minutes a solution of $BF_3 \cdot Et_2O$ (90 μL, 0.72 mmol, 0.2 equiv) in dry toluene (3 mL) was added and the temperature was allowed to rise to -20 °C in 90 minutes. TLC analysis showed complete conversion of imidate **145** into a lower running spot. The reaction was quenched using TEA (0.5 mL), filtered and concentrated *in vacuo*. The excess of acceptor was acetylated, using an Ac_2O-pyridine cocktail (1 mL/3 mL), after which the reaction was quenched using MeOH and concentrated. The oily residue was directly purified using a silica gel column (EtOAc/PE 40%). Compound **142** was obtained in 83% in 1:3 α:β ratio (1.84 g, 3.02 mmol). TLC: EtOAc/Tol 60%; ^{1}H NMR (400 MHz $CDCl_3$) δ 7.44-7.14 (m, 10H, CH_{arom} Bn$_{\alpha/\beta}$), 5.55 (s, 1H, H-1$_\alpha$), 5.47 (s, 1H, H-1$_\beta$), 4.83 (dt, $J = 18.1$, 10.8 Hz, 2H, CH_2, Bn), 4.73-4.52 (m, 3H, CH_2, H-6, H-5'), 4.41-4.24 (m, 2H, CH_2 Bn, H-1'), 4.16 - 4.01 (m, 2H, CH_2 Bn, H-6'), 3.95 (dd, $J = 6.4$, 5.0 Hz, 1H, H-3'), 3.85-3.69 (m, 2H, CH_2 H-6', H-5), 3.58-3.52 (m, 3H, CH_3 Me), 3.46 (dt, $J = 18.9$, 9.5 Hz, 1H, H-2'), 3.34-3.12 (m, 4H, H-2/3/4/4'), 2.01 (s, 3H, CH_3 Ac$_\beta$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7 (C_q, Ac), 137.8 (C_q), 128.7-127.8 (CH_{arom} Bn), 102.3 (C-1), 100.7 (C-1'), 82.7 (C-4), 79.3 (C-3'), 77.5 (C-5'), 76.6 (C-4'), 75.6 (CH_2 Bn), 75.5 (C-3), 74.8 (C-5), 73.4 (C-6'), 72.9 (C-2), 72.5 (C-6), 65.8 (CH_2 Bn), 61.1 (CH_3 Me), 59.3 (C-2), 20.9 (CH_3 Ac); IR (neat) ν 2100.3, 1739.7, 1456.2, 1363.6, 1232.4, 1066.6, 1001.0, 1026.0, 931.6,

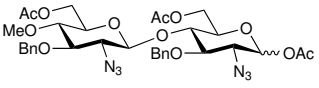
898.8, 738.7, 696.3; HRMS: $C_{29}H_{34}N_6O_9 + Na^+$ requires 633.2279, found 633.2279; $[\alpha]_D^{23}$ + 11.4 °(c = 1, $CHCl_3$).

1,6-Anhydro-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-(2-azido-3-*O*-benzyl-2-deoxy-4-*O*-methyl-β-D-glucopyranosyl)-β-D-glucopyranose (163):



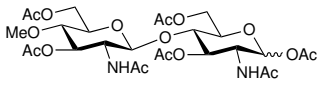
Disaccharide **142** (1.84 g, 3.02 mmol) was dissolved in MeOH (15 mL) and a catalytic amount of NaOMe (30% in MeOH) was added. The reaction mixture was stirred for 1 h at ambient temperature, after which it was neutralized (pH∼7) using Amberlite® IR-120 H^+ resin. Filtering off the resin, concentration and purification using a short silica column (EtOAc/PE) yielded title compound **163** as a clear oil (1.0 g, 1.8 mmol, 59%). TLC: EtOAc/PE 35%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48-7.30 (m, 10H, CH_{arom} 2xBn), 5.52 (s, 1H, H-1), 4.87 (q, J = 10.9 Hz, 2H, CH_2 Bn), 4.73 (m, 2H, CH2, H-6', H-5), 4.61 (d, J = 12.3 Hz, 1H, CH_2 H-6'), 4.26 (d, J = 8.0 Hz, 1H, H-1'), 4.20-4.10 (m, 1H, CH_2 H-6), 3.94 (d, J = 1.3 Hz, 1H, H-3), 3.81 (m, 1H, CH_2 H-6), 3.75 (s, 1H, H-5), 3.69 (s, 2H, CH_2 Bn), 3.58 (s, 3H, CH_3, OMe), 3.49-3.44 (m, 1H, H-2'), 3.36 (s, 1H, H-2), 3.34-3.28 (m, 2H, H-3', H-4), 3.13-3.03 (m, 1H, H-4); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.9 (C_q), 137.3 (C_q), 128.7-127.7 (CH_{arom} 2xBn), 102.1 (C-1'), 100.3 (C-1), 82.5 (C-4), 78.9 (C-3'), 77.9 (C-5'), 75.6 (C-4', C-3), 75.5 (CH_2 Bn), 75.3 (C-5), 74.7 (C-6'), 72.2 (C-2'), 65.8 (C-6), 65.0 (CH_2 Bn), 61.4 (CH_3, OMe), 59.0 (C-2); IR (neat) ν 2108.1, 1454.2, 1261.4, 1141.8, 1074.3, 1006.8, 740.6, 698.2; HRMS: $C_{27}H_{32}N_6O_8 + Na^+$ requires 591.2174, found 591.2172; $[\alpha]_D^{23}$ -16.67 °(c = 0.6, $CHCl_3$).

2-Azido-1,6-di-*O*-acetyl-3-*O*-benzyl-2-deoxy-4-*O*-(2-azido-3-*O*-benzyl-2-deoxy-4-*O*-methyl-β-D-glucopyranosyl)-α/β-D-glucopyranose (166):



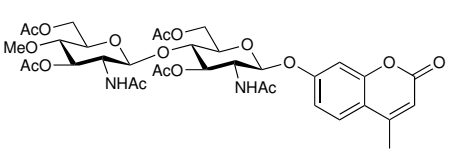
To a solution of compound **163** (1.0 g, 1.7 mmol) in Ac_2O (8.5 mL) TFA (1.28 mL, 15% v/v) was added. The reaction was stirred for 18 h after which it was diluted with toluene and concentrated. After purification using silica gel chromatograph (EtOAc/PE 30%) disaccharide **166** was obtained in 90% yield (1.09 g, 1.53 mmol) as transparent foam. TLC: EtOAc/PE 45%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42-7.27 (m, 15H, CH_{arom} $Bn_{\alpha/\beta}$), 6.20 (d,J = 3.7 Hz, 1H, H-1$_\alpha$), 5.49-5.43 (m, 1H, H-1$_\beta$), 5.05 (dd, J = 33.7, 11.2 Hz, 1H, CH_2 $H_{6'}$), 4.89-4.59 (m, 4H, CH_2 Bn, CH_2 H-6'), 4.52-4.44 (m, 2H, CH_2 Bn), 4.39-4.28 (m, 1H, H-1'), 4.19 (m, 1H, CH_2 H-6), 4.15-4.07 (m, 1H, CH_2 H-6), 4.00-3.86 (m, 3H, H-3, H-4, H-5), 3.78 (dd, J = 7.2, 6.0 Hz, 1H),3.74-3.68 (m, 1H), 3.58-3.50 (m, 1H, H-2), 3.49 (s, 3H, CH_3, Me), 3.42-3.31 (m, 2H, H-2', H-4'), 3.31-3.19 (m, 2H, H-3', H-5'), 2.19-1.90 (m, 13H, CH_3,$Ac_{\alpha/\beta}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7-168.9 (3x C_q, Ac), 138.3-127.5 (Bn), 101.5 (C-1'), 90.23(C-1), 83.3 (C-3'), 79.9 (C-5'), 78.6 (C-4, C-5), 77.5 (C-4, C-5), 75.9 (C-6'), 75.1 (CH_2 Bn), 73.5 (C-4), 71.1 (C-3), 66.8 (C-2'), 62.9 (C-6), 62.4 (CH_2 Bn), 62.2 (C-2), 61.0 (CH_3, OMe), 21.1-20.8 (3x CH_3 Ac); IR (neat) ν 2111.9, 1743.5, 1234.4, 1029.9, 741.6, 689.5; HRMS: $C_{33}H_{40}N_6O_{12} + Na^+$ requires 735.2596, found 735.2595.

2-Acetamido-1,3,6-tri-*O*-acetyl-2-deoxy4-*O*-(2-acetamido-2-deoxy-3,6-*O*-di-acetyl-4–*O*-methyl-β-D-glucopyranosyl)-α/β-D-glucopyranose (139):



Compound **166** (71 mg, 0.1 mmol) was taken up in a mixture of dioxane:toluene:H_2O (5/2/1, v/v/v, 2 mL) and cooled with an ice-bath. After 10 minutes 1M PMe_3 (0.5 mL) in toluene was added and the reaction was stirred for 18 h at 4 °C. After TLC analysis showed conversion towards lower running, ninhydrin positive spot, the reaction was coevaporated thrice with toluene and subsequently acetylated using an Ac_2O-pyridine cocktail (0.5 mL/1.5 mL). The mixture was stirred for 20 h after which it was quenched with MeOH and concentrated under reduced pressure. The resulting white solid was dissolved in a 1:1 mixture of MeOH and TFE (4 mL) and purged with argon. A catalytic amount of $Pd(OH)_2$ spiked with Pd-black was added and the mixture was purged with H_2. Reduction of the benzyl-groups was continued for 5 h followed by filtration and concentration. The residue was again taken up in an Ac_2O-pyridine cocktail (0.5 mL/1.5 mL) with a catalytic amount of DMAP and stirred at ambient temperature for 18 h. After complete acetylation of the disaccharide the mixture was quenched with MeOH and concentrated under reduced pressure. Silica gel purification (MeOH/DCM 3%) yielded 31 mg (47 μmol, 48%) of the title compound **139** as an off-white solid. TLC: MeOH/DCM 5%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.31 (d, $J = 9.8$ Hz, 1H, NH), 6.11 (m, 2H, NH, H-1$_\alpha$), 5.74 (s, 1H), 5.61 (d, $J = 7.5$ Hz, 1H, H-1$_\beta$), 5.26-5.17 (m, 1H, H-4'), 5.09-5.01 (m, 1H), 5.01-4.93 (m, 1H H-4), 4.45-4.37 (m, 1H, H-6), 4.30 (m, 5H, H-1'$_\alpha$, H-1'$_\beta$, H-2', H-5), 4.25-4.18 (m, 1H, H-6), 4.17-4.07 (m, 1H, H-2), 4.06-3.94 (m, 1H, H-2'), 3.93-3.75 (m, 2H), 3.45 (m, 1H, H-3'), 3.41 (d, $J = 3.0$ Hz, 4H, CH_3, OMe), 3.35 (d, $J = 9.2$ Hz, 1H, H-3), 2.21-1.90 (m, 33H, CH_3 Ac and NHAc); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 171.2, 170.9, 170.88, 170.81, 170.6, 170.5, 170.47, 170.4, 170.3, 170.2, 169.4, 168.9, 102.0, 100.8, 92.5, 90.5, 77.4, 77.1, 77.0, 76.7, 75.9, 75.2, 74.9, 73.9, 73.7, 73.0, 71.9, 71.0, 70.9, 70.7, 62.9, 62.8, 62.4, 61.6, 60.4, 60.4, 54.2, 54.1, 51.4, 51.2, 29.7, 23.2, 23.1, 23.0, 21.0, 20.97, 20.9, 20.88, 20.77, 20.70, 20.6; IR (neat) ν 3282.6, 1741.6, 1662.5, 1544.9, 1434.9, 1373.2, 1226.6, 1112.9, 1033.8, 943.1, 732.9; HRMS: $C_{27}H_{40}N_2O_{16} + H^+$ requires 649.2451, found 649.2452.

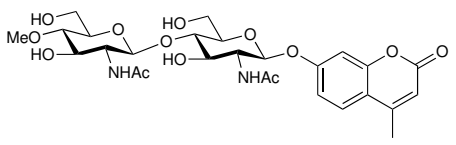
2-Acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-4-*O*-(2-acetamido-2-deoxy-3,6-*O*-di-acetyl-4--*O*-methyl-β-D-glucopyranosyl)-1-*O*-4-methylumbelliferyl-β-D-glucopyranoside (169).



Dimer **139** (100 mg, 154 μmol) was dissolved in AcOH (2 mL) and Ac_2O (1 mL). At 0 °C dry $HCl_{(g)}$ was bubbled through (liberated under Kipp conditions) for 3h. The reaction mixture was then placed at 5 °C for 42 h at which TLC analyses (DCM-acetone 60-40) showed complete consumption of starting material. The reaction was diluted with $CHCl_3$ (10 mL, 0 °C) and washed twice with H_2O (15 mL, 0 °C) and twice with $NaHCO_3$ (15 ml, 0 °C). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* yielding the anomeric α-chloride as an amorphous solid of which purity was evaluated by ^{1}H-NMR. The resulting solid was dissolved in $CHCl_3$ (5 mL) and added to a solution of $NaHCO_3$ 0.2M (5 mL), 4-methylumbelliferyl sodium salt[34,35] (152mg, 770 μmol) and TBAHS (105 mg, 310 μmol). The biphasic mixture was stirred overnight with the exclusion of light. The phases were separated and the organic layer was washed twice with $NaHCO_3$ (0.2 M) and twice with

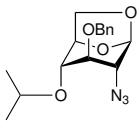
H_2O. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (MeOH/$CHCl_3$ 3%) yielded **169** (27 mg, 25%) as a white amorphous solid. TLC: MeOH/DCM 5%; ^{1}H NMR (600 MHz, (d_4), MeOD) δ 8.06 (d, $J = 9.2$ Hz, 1H, NH), 7.62 (d, $J = 8.7$ Hz, 1H, CH, 4-methylumbelliferyl), 6.99-6.94 (m, 2H, CH_{arom} 4-methylumbelliferyl), 6.19 (s, $J = 9.6$ Hz, 1H, CH_{arom} 4-methylumbelliferyl), 5.27 (d, $J = 8.7$ Hz, 1H, H-1β,H-3), 5.15 (t, $J = 9.8$ Hz, 1H, H-3'), 4.66 (d, $J = 8.4$ Hz, 2H, H-1'$_\beta$), 4.53 (d, $J = 10.2$ Hz, 1H, H-6'), 4.33 (dt, $J = 20.2, 7.2$ Hz, 1H, CH_2 H-6'), 4.23-4.17 (m, 1H, H-2), 4.14 (dd, $J = 11.8, 7.3$ Hz, 2H, CH_2 H-6), 3.95 (t, J = 7.7 Hz, 1H, H-5), 3.86 (t, $J = 9.2$ Hz, 1H, H-4), 3.79-3.72 (m, 1H, H-2'), 3.59 (dd, $J = 8.6, 2.9$ Hz, 1H, H-5'), 3.45-3.40 (m, 3H, CH_3 Me), 3.37 (dd, $J = 12.4, 6.4$ Hz, 1H, H-4'), 2.44 (s, 3H, CH_3, 4-methylumbelliferyl), 2.17-2.05 (m, 12H, CH_3 4xAc), 1.97-1.92 (m, 6H, CH_3 2xAc); ^{13}C NMR (151 MHz, (d_4), MeOD)δ 173.3-171.6 (C_q, Ac), 162.9-154.9 (C_q, 4-methylumbelliferyl), 127.01 (C_{arom}, 4-methylumbelliferyl) 116.2 (C_q, 4-methylumbelliferyl),115.1-104.6 (C_{arom}, 4-methylumbelliferyl), 101.9 (C-1'), 98.9 (C-1), 77.4 (C-4'), 75.9 (C-4), 74.1 (C-3), 74.1 (C-5), 73.7 (C-5'), 64.0 (C-6'), 63.7 (C-6), 60.8 (CH_3 Me), 55.9 (C-2'), 54.9 (C-2), 23.1-19.0 (CH_3 Ac); IR (neat) ν 1739.7, 1660.6, 1612.4, 1612.4, 1371.3, 1222.8, 1066.6, 1033.8, 623.0; HRMS: $C_{35}H_{44}N_2O_{17} + H^+$ requires 765.2713, found 765.2717; $[\alpha]_D^{23}$ -32.4 °(c = 0.5, $CHCl_3$/MeOH).

2-Acetamido-2-deoxy-4-*O*-(2-acetamido-2-deoxy-4-*O*-methyl-β-D-glucopyranosyl)-1--*O*-4-methylumbelliferyl-β-D-glucopyranoside (136).



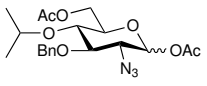
To a suspension of **169** (12mg, 15.70 μmol) in MeOH (1 mL) was added NaOMe (30 wt% in MeOH) (173 μL, 0.40 μmol). The reaction was stirred with the exclusion of light. When LCMS (gradient 0 to 50% MeOH) showed complete conversion to product the mixture was quenched with AcOH (5 μL, 80 μmol). The reaction was diluted with H_2O (2 ml) the MeOH was evaporated *in vacuo* and the remaining H_2O was lyophilized. Purification by HPLC (gradient H_2O-MeOH + 0.1% TFA 80-20 → 60-40) evaporation of MeOH and lyophilizing H_2O yielded **136** (3.5 mg, 5.9 μmol, 37%) as white fluffy solid. ^{1}H NMR (600 MHz, (d_6), DMSO) δ 7.90 (d, $J = 9.0$ Hz, 1H, NH), 7.75 (d, $J = 9.1$ Hz, 1H, NH), 7.69 (d, $J = 8.8$ Hz, 1H, CH, 4-methylumbelliferyl), 7.03 (d, $J = 2.2$ Hz, 1H, CH, 4-methylumbelliferyl), 6.95 (dd, $J = 8.8, 2.3$ Hz, 1H, CH, 4-methylumbelliferyl), 6.26 (s, 1H, CH, 4-methylumbelliferyl), 5.16 (dd, $J = 10.8, 7.4$ Hz, 2H, H-1, OH), 4.83 (t, $J = 5.3$ Hz, 1H, OH), 4.74 (d, $J = 2.1$ Hz, 1H, OH), 4.68 (t, $J = 5.9$ Hz, 1H, OH), 4.36 (d, $J = 8.5$ Hz, 1H, H-1'$_\beta$), 3.77 (d, $J = 9.6$ Hz, 1H), 3.70-3.13 (m, 14H), 2.94 (t, $J = 9.2$ Hz, 1H), 2.40 (s, 3H, CH_3 4-methylumbelliferyl), 1.84 (s, 3H, CH_3 Ac), 1.80 (s, 3H, CH_3 Ac); ^{13}C NMR (151 MHz, (d_6), DMSO) δ 169.2, 160.1, 159.9, 154.4, 153.3, 126.5, 114.3, 113.4, 103.1, 101.9, 98.2, 80.7, 80.0, 75.6, 75.1, 73.8, 72.2, 59.8, 55.7, 54.4, 23.0, 18.1; HRMS: $C_{27}H_{36}N_2O_{13} + H^+$ requires 597.2290, found 597.2283.

1,6-Anhydro-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-isopropyl-β-D-glucopyranose (155):



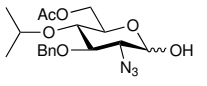
Compound **148** (1.5 g, 5.3 mmol) was dissolved in 25 mL DMF and cooled using an ice-bath and after stirring for 5 minutes NaH (60% dispersion in mineral oil) (234 mg, 5.86 mmol, 1.1 equiv) was added. After 30 minutes the gas formation stopped and 2-iodopropane (586 μL, 5.86 mmol, 1.1 equiv) was added dropwise. After 3 h the reaction was quenched with MeOH. The mixture was concentrated and the residue was taken up in Et_2O and washed twice with 1M $HCl_{(aq)}$. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. The oily residue was re-dissolved in 25 mL DMF and cooled using an ice-bath. The alkylation step was repeated three times. After four cycles the oil compound was purified using a short silica column (EtOAc/PE 20%) which gave product **155** as yellow oil (1.0 g, 3.1 mmol, 60%). TLC: EtOAc/PE 40%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (s, 5H, CH_{arom} Bn), 5.43 (s, 1H, H-1), 4.64-4.44 (m, 3H, CH_2 Bn,H-5), 4.02 (d, J = 6.7 Hz, 1H, H-6), 3.67 (s, 1H, H-6), 3.57 (dd, J = 12.9, 9.5 Hz, 2H, CH, *i*Prop, H-3), 3.33 (s, 1H, H-2), 3.16 (s, 1H, H-4), 1.14 (d, J = 5.3 Hz, 6H, 2xCH_3 *i*Prop); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.2 (C_q), 128.2-127.5 (CH_{arom} Bn), 100.3 (C-1), 76.8 (C-3), 75.1 (C-5), 74.2 (C-4), 72.1 (CH_2 Bn), 70.1 (*i*Prop), 65.1 (C-6), 59.4 (C-2), 22.1 (CH_3 *i*Prop), 21.9 (CH_3 *i*Prop); IR (neat) ν 2970.2, 2898.8, 2098.4, 1454.2, 1369.4, 1070.4, 1004.8, 964.3, 867.9, 736.8; HRMS: $C_{16}H_{21}N_3O_4 + Na^+$ requires 342.1424, found 342.1425; $[\alpha]_D^{23}$ 28.33 °(c = 0.6, $CHCl_3$).

1,6-Di-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-isopropyl-α/β-D-glucopyranose (158):



The yellow oil **155** (1.0 g, 3.1 mmol) was taken up in 20 mL Ac_2O and cooled with an ice bath. To this cooled solution 2 mL TFA (10% v/v) was added and the reaction was stirred overnight at room temperature. After complete conversion of the starting material the reaction was diluted with toluene and coevaporated to yellow oil. Purification using a short silica column (EtOAc/PE 30%) yielded compound **158** in a good yield 86% (1.13 g, 2.69 mmol). TLC: EtOAc/PE 50%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43-7.28 (m, 6H, CH_{arom} Bn), 6.22 (d, J = 3.7 Hz, 1H, H-1$_\alpha$), 5.47 (d, J = 8.4 Hz, 1H, H-1$_\beta$), 4.90-4.80 (m, 2H, CH_2 Bn), 4.34-4.16 (m, 2H, CH-2, H-6), 3.98-3.90 (m, 1H, CH, *i*Prop), 3.87-3.81 (m, 2H, H-3, H-5), 3.56 (m, 2H, H-4, H-2), 2.19-2.13 (m, 4H, CH_3 Ac), 2.07 (s, 4H, CH_3 Ac), 1.21 (t, J = 5.4 Hz, 3H, CH_3 *i*Prop), 1.12 (t, J = 6.3 Hz, 3H, CH_3 *i*Prop); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7 (C_q, Ac), 168.9 (C_q, Ac), 137.6 (C_q), 128.6-128.1 (CH_{arom} Bn), 92.9 (C-1$_\beta$), 90.5 (C-1$_\alpha$), 83.3 (C-3/5$_\beta$), 80.8 (C-3/5$_\alpha$), 75.9 (CH_2 Bn), 74.4 (C-4$_\beta$), 74.2 (C-4$_\alpha$), 73.3 (C-3/5$_\beta$), 73.3 (C-3/5$_\alpha$), 71.7 (*i*Prop), 65.3 (C-2$_\beta$), 63.0 (C-2$_\alpha$), 62.5 (C-6), 23.2 (Ac), 22.0 (Ac), 21.1 (*i*Prop), 20.9 (*i*Prop); IR (neat) ν 3423.4, 2358.8, 2343.4, 2102.3, 1745., 1456.2, 1371.3, 1234.4, 1143.7, 1124.4, 1026.1, 968.2, 997.1, 948.9, 740.6, 694.3; HRMS: $C_{20}H_{27}N_3O_7 + Na^+$ requires 444.1741, found 444.1740.

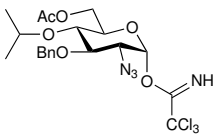
6-*O*-Acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-isopropyl-α/β-D-glucopyranose (161):



Compound **158** (1.13 g, 2.69 mmol) was dissolved in 20 mL THF and 1.5 mL piperidine (6% v/v) was added. The clear solution was stirred overnight at room temperature. After complete conversion to a lower running spot on TLC the reaction was diluted with 100 mL EtOAc and poured in 100 mL 1M HCl. The layers were separated and the organic layer was washed twice with H_2O and once with brine. Subsequently the EtOAc layer was dried and concentrated *in vacuo*. Flash silica column purification (EtOAc/PE

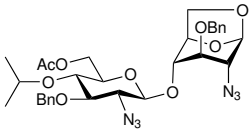
30%) yielded compound **161** in quantitative yield (1.02 g, 2.69 mmol) as a white foam. TLC: EtOAc/PE 40%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43-7.26 (m, 10H, CH_{arom} Bn), 5.26 (d, $J = 2.9$ Hz, 1H, H-1$_\alpha$), 4.90-4.75 (m, 4H, CH_2 Bn), 4.58-4.53 (m, 1H, H-1$_\beta$), 4.42-4.33 (m, 3H, CH_2 H-6), 4.14 (m, 1H, CH_2 H-6), 4.03 (m, 2H, H-5), 3.98-3.87 (m, 2H, H-3), 3.51-3.39 (m, 4H, H-4, CH, *i*Prop.), 3.36 (m, 2H, H-2), 2.08 (m, 6H, CH_3 Ac), 1.23-1.16 (m, 6H, CH_3 *i*Prop), 1.13 (m, 6H, CH_3 *i*Prop); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3 (C_q, Ac), 137.7 (C_q), 137.64 (C_q), 128.5-127.9 (CH_{arom} Bn), 96.2 (C-1$_\beta$), 91.8 (C-1$_\alpha$), 83.2 (CH_β *i*Prop), 80.3 (CH_α *i*Prop), 75.8 (CH_2 Bn), 75.7 (CH_2 Bn), 75.1 (C-4$_\alpha$, 74.5 (C-4$_\beta$), 73.4 (C-3$_\beta$), 73.2 (C-3$_\alpha$), 73.2 (C-5$_\alpha$), 69.2 (C-5$_\beta$), 67.6 (C-2$_\beta$), 64.1 (C-2$_\alpha$), 63.0 (C-6$_{\alpha/\beta}$), 62.9 (C-6$_{\alpha/\beta}$), 23.1 (CH_3 Ac$_{\alpha/\beta}$), 23.1 (CH_3 Ac$_{\alpha/\beta}$), 22.0 (CH_3 *i*Prop), 20.9 (CH_3 *i*Prop); IR (neat) ν 2922.0, 2108.1, 1739.7, 1363.6, 1224.7, 1026.1, 1010.6, 966.3, 914.2, 740.6, 696.3; HRMS: $C_{18}H_{25}N_3O_6 + Na^+$ requires 402.1636, found 402.1634.

6-*O*-Acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-isopropyl-1-*O*-(*N*-trichloroacetimidoyl) -α-D-glucopyranoside (146):



A solution of compound **161** (1.02 g, 2.69 mmol) and trichloroacetonitrile (0.809 mL, 8.07 mmol, 3 equiv) in dry 15 mL DCM was treated with 1,8-diazabicyclo[5.4.0]undec-7-ene 83 μL (0.54 mmol, 0.2 equiv) for 18 h at ambient temperature. The dark brown solution was concentrated under reduce pressure and directly purified using a silica gel column (EtOAc/PE 12.5% + 2.5% TEA) to obtain the title compound **146** as a yellow oil (0.986 g, 1.88 mmol, 70%). TLC: EtOAc/PE 30%; ^{1}H NMR (400 MHz, (d_4), MeOD) δ 8.77 (s, 1H, NH), 7.42-7.25 (m, 5H, CH_{arom} Bn), 6.40 (d, $J =$ 3.5 Hz, 1H, H-1$_\alpha$), 4.89 (q, $J = 10.6$ Hz, 2H, CH_2 Bn), 4.34 (dd, $J = 12.1$, 2.1 Hz, 1H, H-5, 4.18 (dd, $J = 12.1$, 4.4 Hz, 1H, H-6), 3.97 (m, 1H, CH, *i*Prop), 3.92 (m, 2H, H-3, H-4), 3.63 (dd, $J =$ 10.2, 3.6 Hz, 1H, H-6), 3.60-3.53 (m, 1H, H-2), 2.03 (s, 3H, CH_3 Ac), 1.21 (d, $J = 6.1$ Hz, 3H, CH_3 *i*Prop), 1.13 (d, $J = 6.1$ Hz, 3H, CH_3 *i*Prop); ^{13}C NMR (100 MHz, (d_4), MeOD) δ 170.3 (C_q, C=N), 160.4 (C_q, Ac), 137.4 (C_q), 128.3-127.8 (CH_{arom} Bn), 94.4 (C-1), 90.7 (CCl_3), 80.2 (*i*Prop), 75.6 (C_q, CH_2 Bn), 74.5 (C-4), 73.2 (C-3), 72.0 (C-5), 63.0 (C-2), 62.2 (C-6), 22.9 (CH_3 *i*Prop), 21.9 (CH_3 *i*Prop), 20.6 (CH_3 Ac); IR (neat) ν 2110.0, 1741.6, 1674.1, 1234.4, 1139.9, 1018.3, 966.3, 908.4, 794.6, 729.0, 644.2.

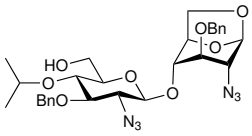
1,6-Anhydro-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-4- *O*-isopropyl-β-D-glucopyranosyl)-β-D-glucopyranose (143):



A mixture of imidate **146** (761 mg, 1.45 mmol) and alcohol **148** (803 mg, 2.9 mmol, 2 equiv) was coevaporated thrice with toluene and dissolved in 7.25 mL of dry toluene. To this mixture activated molecular sieves (4Å) were added and the solution was cooled to -78 °C. After 10 minutes a solution of $BF_3 \cdot Et_2O$ (37 μL, 0.3 mmol, 0.2 equiv) in dry toluene (0.7 mL) was added and the temperature was allowed to rise to -20 °C in 90 minutes. TLC analysis showed complete conversion of imidate **146** into a lower running spot. The reaction was quenched using TEA (0.5 mL), filtered and concentrated *in vacuo*. The excess of acceptor was acetylated, using an Ac_2O-pyridine cocktail (1 mL/3 mL), after which the reaction was quenched using MeOH and concentrated. The oily residue was directly purified using a silica gel column (EtOAc/PE 40%). Compound **143** was obtained in 81% in 1:5 α:β ratio (755 mg, 1.18 mmol). TLC: EtOAc/Tol 40%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39-7.12 (m, 12H, CH_{arom} Bn),

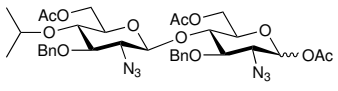
5.52 (s, 1H, H-1$_\alpha$), 5.49 (s, 1H, H-1$_\beta$), 4.87-4.75 (m, 2H, CH$_2$ Bn), 4.69 (t, J = 5.7 Hz, 1H, H-5$_\beta$), 4.63 (dd, J = 14.8, 7.5 Hz, 2H, CH$_2$ Bn), 4.40-4.32 (m, 2H, CH$_2$, H-6'$_\beta$, H-1$_\beta$), 4.12 (d, J = 7.1 Hz, 2H, CH$_2$, H-6$_\beta$), 4.09-4.02 (m, 1H, CH$_2$, H-6'$_\beta$), 4.00-3.86 (m, 2H, H-4'$_\beta$, H-3$_\beta$), 3.77 (m, 2H, CH$_2$, H-6$_\beta$, H-4$_\beta$), 3.50-3.37 (m, 2H, H-2'$_\beta$, H-5'$_\beta$), 3.33-3.15 (m, 3H, H-3'$_\beta$, H$_\beta$, CH, *i*Prop), 2.05 (m, 3H, CH$_3$ Ac), 1.99 (s, 3H, CH$_3$ Ac), 1.18 (m, 3H, CH$_3$ *i*Prop), 1.14-1.07 (m, 3H, CH$_3$ *i*Prop); ^{13}C NMR (100 MHz, CDCl$_3$) δ 170.7 (Ac), 137.8 (C$_q$), 128.7-127.7 (CH$_{arom}$ Bn), 102.4 (C-1'), 100.7 (C-1), 83.2 (*i*Prop), 77.3 (C-4) 76.9 (C-4'), 75.9 (CH$_2$ Bn), 74.8 (C-5), 74.2 (C-5'), 73.7 (C-3'), 73.3 (C-3), 72.5 (CH$_2$ Bn), 66.1 (C-2'), 65.1 (C-6), 62.6 (C-6'), 59.3 (C-2), 23.2 (CH$_3$ *i*Prop), 22.1 (CH$_3$ *i*Prop), 20.8 (CH$_3$ Ac); IR (neat) ν 2102.3, 1735.8, 1238.2, 1068.5, 1028.0, 906.5, 727.1, 646.1, 624.9; HRMS: $C_{31}H_{38}N_6O_9$+Na$^+$ requires 661.2592, found 661.2592; $[\alpha]_D^{23}$ 19.4 °(c = 1, CHCl$_3$).

1,6-Anhydro-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-(2-azido-3-*O*-benzyl-2-deoxy-4-*O*-isopropyl-β-D-glucopyranosyl)-β-D-glucopyranose (164):



Disaccharide **143** (755 mg, 1.18 mmol) was dissolved in MeOH (7 mL) and a catalytic amount of NaOMe (30% in MeOH) was added. The reaction mixture was stirred for 1 h at ambient temperature, after which it was neutralized (pH∼7) using Amberlite® IR-120 H$^+$ resin. Filtering off the resin, concentration and purification using a short silica column (EtOAc/PE 25%) yielded title compound **164** as a clear oil (415 mg, 0.70 mmol, 59%). TLC: EtOAc/Tol 40%; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.40-7.26 (m, 10H, CH$_{arom}$ Bn), 5.48 (s, 1H, H-1'$_\beta$), 4.86-4.76 (m, 2H, CH$_2$ Bn), 4.73-4.66 (m, 2H, CH$_2$ Bn), 4.57 (d, J = 12.3 Hz, 1H, H-5), 4.26 (d, J = 8.0 Hz, 1H, H-1), 4.13 (d, J = 7.3 Hz, 1H, H-6), 3.96-3.90 (m, 1H, H-3), 3.89 (s, 1H, H-4'), 3.81-3.75 (m, 1H, H-4), 3.72 (s, 1H, H-6), 3.64 (d, J = 2.9 Hz, 2H, CH$_2$, H6'), 3.47-3.38 (m, 2H, H$_2$, H$_{5'}$), 3.31 (s, 1H, CH, *i*Prop), 3.28-3.21 (m, 1H, H-3'), 3.06 (dt, J = 9.6, 3.2 Hz, 1H, H-2'), 1.28-1.23 (m, 6H, 2x CH$_3$ *i*Prop); ^{13}C NMR (100 MHz, CDCl$_3$) δ 137.9 (C$_q$), 137.3 (C$_q$), 128.8-127.8 (CH$_{arom}$ Bn), 102.3 (C-1'), 100.4 (C-1), 83.0 (*i*Prop), 78.0 (C-4), 75.9 (C-4', C-5), 75.8 (CH$_2$ Bn), 74.8 (C-5), 74.2 (C-3), 73.3 (C-3'), 72.4 (CH$_2$ Bn), 66.3 (C-2'), 65.2 (C-6), 61.4 (C-6), 59.1 (C-2), 23.0 (CH$_3$ *i*Prop), 22.4 (CH$_3$ *i*Prop); IR (neat) ν 2972.1, 212.3, 1454.2, 1259.4, 1070.4, 1026.1, 1004.8, 964.3, 896.8, 867.9, 696.3; HRMS: $C_{29}H_{36}N_6O_8$ + Na$^+$ requires 619.2487, found 619.2484; $[\alpha]_D^{23}$ -10.8 °(c = 0.5, CHCl$_3$).

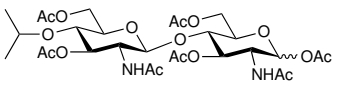
2-Azido-3-*O*-benzyl-2-deoxy-1,6-di-*O*-acetyl-4-*O*-(2-azido-3-*O*-benzyl-2-deoxy-4-*O*-isopropyl- β-D-glucopyranosyl)-α/β-D-glucopyranose (167):



To a solution of compound **164** (0.95 mg, 160 μmol) in Ac$_2$O (1.5 mL) was treated TFA (0.30 mL, 15% v/v). The reaction was stirred for 18 h after which it was diluted with toluene and concentrated. After purification using silica gel chromatograph (EtOAc/PE 30%) disaccharide **167** was obtained in 96% yield (92 mg, 154 μmol) as transparent foam. TLC: EtOAc/PE 60%; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.40-7.24 (m, 15H, CH$_{arom}$ Bn$_{\alpha/\beta}$), 6.19 (d,J = 3.7 Hz, 1H, H-1$_\alpha$), 5.45 (d, J = 8.1, Hz, 1H, H-1$_\beta$), 5.14-4.98 (m, 1H, CH$_2$ H-6'), 4.90-4.70 (m, 3H, CH$_2$ Bn, H-6'), 4.54-4.35 (m, 2H, CH$_2$ Bn), 4.35-4.29 (m, 1H, H-1'), 4.26-4.19 (m, 1H, CH$_2$ H-6), 4.10-4.01 (m, 1H, CH$_2$ H-6), 3.95 (dd, J = 9.8, 5.0 Hz, 1H, H-5), 3.94-3.84 (m, 2H, H-3, H-4), 3.58-3.51 (m, 1H, H-2), 3.47-3.26 (m, 5H, CH, *i*Prop, H-2'/3'/4'/5'), 2.19-2.14

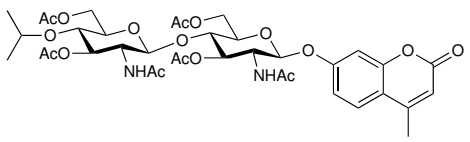
(m, 3H, CH_3 Ac), 2.11 (s, 3H, CH_3 Ac), 1.88 (s, 3H, CH_3 Ac), 1.17 (d, $J = 6.1$ Hz, 3H, CH_3 *i*Prop), 1.09 (d, $J = 6.1$ Hz, 3H, CH_3 *i*Prop); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6 (C_q, Ac), 170.5 (C_q, Ac), 168.8 (C_q, Ac), 138.2 (C_q), 137.6 (C_q), 128.6-127.5 (CH_{arom} Bn), 101.5 (C-1'), 90.2 (C-1), 83.6 (C-3'), 78.4 (C-4/5), 77.1 (C-4/5), 76.0 (C-6'), 75.1 (CH_2 Bn), 74.3 (C-5'), 73.7 (C-4'), 73.1 (*i*Prop), 71.1 (C-3), 67.1 (C-2'), 62.8 (C-6), 62.3 (C-2), 62.2 (CH_2 Bn), 23.1 (CH_3 *i*Prop), 22.1 (CH_3 *i*Prop), 21.0 (CH_3 Ac), 20.9 (CH_3 Ac); IR (neat) ν 2110.0, 1737.7, 1363.6, 1222.8, 1026.1, 1008.7, 931.6, 914.2, 82.5, 696.3; HRMS: $C_{35}H_{44}N_6O_{12}+Na^+$ requires 763.2909, found 763.2913.

2-Acetamido-2-deoxy-1,3,6-tri-*O*-acetyl-4-*O*-(2-acetamido-2-deoxy-3,6-*O*-di-acetyl-4--*O*- isopropyl-β-D-glucopyranosyl)-α/β-D-glucopyranose (140):



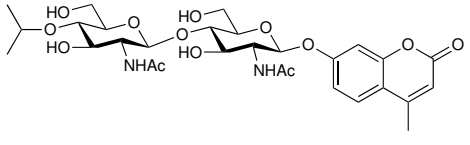
Compound **167** (90 mg, 0.15 mmol) was taken up in a mixture of dioxane/toluene/H_2O (5/2/1, v/v/v, 3 mL) and cooled with an ice-bath. After 10 minutes 1M PMe_3 (0.7 mL) intoluene was added and the reaction was stirred for 18 h at +4 °C. After TLC analysis showed conversion towards lower running, ninhydrin positive spot, the reaction was coevaporated thrice with toluene and subsequently acetylated using an Ac_2O-pyridine cocktail (1 mL/3 mL). The mixture was stirred for 20 h after which it was quench with MeOH and concentrated under reduced pressure. The resulting white solid was dissolved in a 1:1 mixture of MeOH and TFE (6 mL) and purged with argon. A catalytic amount of $Pd(OH)_2$ spiked with Pd-black was added and the mixture was purged with H_2. Reduction of the benzyl-groups was continued for 5 h followed by filtration and concentration. The residue was again taken up in an Ac_2O-pyridine cocktail (0.5 mL/1.5 mL) with a catalytic amount of DMAP and stirred at ambient temperature for 18 h. After complete acetylation of the disaccharide the mixture was quenched with MeOH and concentrated under reduced pressure. Silica gel purification (MeOh/DCM 3%) yielded 37 mg (55 μmol, 36%, 71% per step) of the title compound **140** as an off-white solid. TLC: MeOH/DCM 5%; ^{1}H NMR (400 MHz,(d_4), MeOD) δ 7.83-7.61 (m, 2H, NH), 6.04 (d, $J = 3.6$ Hz, 1H, $H_{1'\alpha}$), 5.65 (d, $J = 8.6$ Hz, 1H, $H_{1'\beta}$), 5.24 (dd, $J = 10.9$, 8.9 Hz, 1H), 5.13 (dd, $J = 9.9$, 8.4 Hz, 1H), 5.04 (dd, $J = 10.5$, 8.2 Hz, 1H), 4.61-4.54 (m, 2H), 4.47-4.27 (m, 4H), 4.28-4.07 (m, 4H), 3.97 (m, 1H), 3.83 (m, 4H), 3.73-3.65 (m, 2H), 3.57-3.48 (m, 3H), 3.37-3.32 (m, 2H), 2.20 (s, 3H, CH_3), 2.17-2.01 (m, 3H, CH_3), 1.95-1.90 (m, 9H, CH_3), 1.10 (dd, $J = 6.1$, 2.1 Hz, 8H, CH_3 iProp); ^{13}C NMR (100 MHz, (d_4), MeOD) δ 171.75, 171.61, 171.04, 170.82, 170.53, 170.47, 170.15, 169.32, 169.26, 100.67, 100.29, 91.70, 90.05, 75.26, 74.98, 74.78, 74.65, 73.27, 73.20, 73.14, 72.79, 72.56, 70.71, 70.15, 62.60, 62.12, 61.83, 54.16, 52.20, 50.46, 48.73, 48.52, 48.30, 48.09, 47.88, 47.66, 47.45, 22.17, 21.93, 21.61, 21.51, 20.04, 19.98; IR (neat) ν 1733.9, 1652.9, 1558.4, 1396.4, 1224.7, 1031.8, 1031.8, 908.4, 727.1, 646.1; HRMS: $C_{296}H_{44}N_2O_{16}+Na^+$ requires 699.2583, found 699.2582.

2-Acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-4-*O*-(2-acetamido-2-deoxy-3,6-*O*-di-acetyl -4- *O*-isopropyl-β-D-glucopyranosyl)-1-*O*-4-methylumbelliferyl-β-D-glucopyranoside (170).



Dimer **140** (30 mg, 44 μmol) was dissolved in AcOH (2 ml) and Ac_2O (1 ml). At 0 °C dry $HCl_{(g)}$ was bubbled through (liberated under Kipp conditions) for 3h. The reaction mixture was then placed at 5 °C for 42 h at which TLC analyses (DCM-acetone 60-40) showed complete consumption of starting material. The reaction diluted with $CHCl_3$ (10 ml, 0 °C) and washed twice with H_2O (15 ml, 0 °C) and twice with $NaHCO_3$ (15 ml, 0 °C). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* yielding an amorphous solid of which purity was evaluated by ^{1}H-NMR. The resulting solid α-chloride was dissolved in $CHCl_3$ (2 ml) and added to a solution of $NaHCO_3$ 0.2M (1 ml), 4-methylumbelliferyl sodium salt[34,35] (43mg, 220 μmol, 5 equiv) and TBAHS (29 mg, 88 μmol, 2 equiv). The biphasic mixture was stirred overnight with the exclusion of light. The phases were separated and the organic layer was washed twice with $NaHCO_3$ (0.2 M) and twice with H_2O. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (MeOH/$CHCl_3$ 3%)yielded **170** (6 mg, 17 %) as a white amorphous solid. TLC: MeOH/DCM 5%; ^{1}H NMR (400 MHz, (d_4), MeOD) δ 7.62 (s, 1H, CH, 4-methylumbelliferyl), 7.01-6.95 (m, 2H, CH_{arom} 4-methylumbelliferyl), 6.21 (s, 1H, CH_{arom} 4-methylumbelliferyl), 5.25 (t, $J = 9.3$ Hz, 2H, H-1$_\beta$, H-3), 5.14-5.00 (m, 1H, H-3'), 4.61 (d, $J = 8.4$ Hz, 1H, H-1'$_\beta$), 4.49 (dd, $J = 11.2$, 9.5 Hz, 1H, CH_2 H-6'), 4.34 (dd, $J = 19.6$, 7.1 Hz, 1H, CH_2 H-6'), 4.27-4.11 (m, 3H, CH_2 H-6, H-2), 3.96-3.90 (m, 1H, H-5), 3.83 (dd, $J = 18.6$, 9.5 Hz, 2H, H-4, H-2'), 3.71 (dt, $J = 12.1$, 6.0 Hz, 1H, CH, *i*Prop), 3.54 (t, $J = 7.2$ Hz, 2H, H-4', H-5'), 2.46 (s, 3H, CH_3 4-methylumbelliferyl), 2.17-2.03 (m, 12H, CH_3 4xAc), 1.94 (d, $J = 6.4$ Hz, 6H, CH_3 2xAc), 1.10 (dt, $J = 13.1$, 6.5 Hz, 6H, CH_3 *i*Prop); ^{13}C NMR (100 MHz, (d_4), MeOD) δ 171.8-170.1 (C_q, 6xAc), 161.5-153.3 (C_q, 4-methylumbelliferyl), 125.5 (CH_{arom} 4-methylumbelliferyl), 114.7 (C_q, 4-methylumbelliferyl), 113.7 (CH_{arom} 4-methylumbelliferyl), 111.8 (C_{arom}, 4-methylumbelliferyl), 103.2 (C-1'), 100.4 (C-1), 97.4 (C-4'), 75.7 (C-4), 74.6- 72.6 (C-3/3'/5/5'/CH), 62.5 (C-6'), 62.3 (C-6), 54.2 (C-2'), 53.3 (C-2), 22.0-21.4 (CH_3 4xAc), 19.8 (CH_3 *i*Prop, Ac), 17.7 (CH_3 Ac); IR (neat) ν 2933.5, 1743.5, 1643.2, 1618.2, 1369.4, 1232.4, 1070.4, 1041.5; HRMS: $C_{37}H_{48}N_2O_{17}+H^+$ requires 793.3026, found 793.3031.

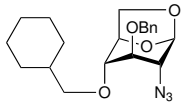
2-Acetamido-2-deoxy-4-*O*-(2-acetamido-2-deoxy-4-*O*-isopropyl-β-D-glucopyranosyl)-1-*O*- 4-methylumbelliferyl-β-D-glucopyranoside (137).



To a suspension of **170** (6mg, 7 μmol) in MeOH (1 mL) was added NaOMe (30 wt% in MeOH) (86 μL, 0.4 μmol). The reaction was stirred with the exclusion of light. When LCMS (gradient 0 to 50 % MeOH) showed complete conversion to product the mixture was quenched with AcOH (20 μL, 350 μmol). The reaction was diluted with H_2O (2 mL) the MeOH was evaporated *in vacuo* and the remaining H_2O was lyophilized.

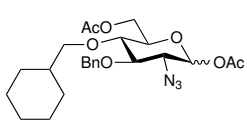
Purification by HPLC (gradient H_2O-MeOH + 0.1% TFA 80-20 → 60-40) evaporation of MeOH and lyophilizing H_2O yielded **137** (2.6 mg, 60%) as white fluffy solid. ^{1}H NMR (600 MHz, (d_6) DMSO) δ 7.9 (d, J = 9.1 Hz, 1H, NH), 7.8 (d, J = 9.2 Hz, 1H, NH), 7.7 (d, J = 8.8 Hz, 1H, 4-methylumbelliferyl), 7.0 (d, J = 2.4 Hz, 1H, 4-methylumbelliferyl), 6.9 (dd, J = 8.8, 2.4 Hz, 1H, 4-methylumbelliferyl), 6.3 (d, J = 1.1 Hz, 1H, 4-methylumbelliferyl), 5.2 (d, J = 8.5 Hz, 1H, $H_{1\beta}$), 5.1 (d, J = 6.5 Hz, 1H, OH), 4.8 (s, 1H, OH), 4.8 (d, J = 2.3 Hz, 1H, OH), 4.7 (s, 1H, OH), 4.4 (s, 1H, OH), 3.4 (m, 13H), 2.4 (d, J = 0.9 Hz, 3H, CH_3 4-methylumbelliferyl), 1.9 (s, 3H, CH_3 Ac), 1.8 (s, 3H, Ac), 1.1 (d, J = 6.1 Hz, 3H, CH_3 *i*Prop), 1.1 (d, J = 6.1 Hz, 3H, CH_3 *i*Prop); ^{13}C NMR (151 MHz, (d_6, DMSO) δ 169.3, 169.3, 160.2, 159.9, 154.5, 153.4, 126.6, 114.4, 113.5, 111.9, 103.2, 102.1, 98.3, 80.9, 76.0, 75.9, 75.1, 74.2, 72.3, 71.9, 55.8, 54.4, 23.2, 23.1, 22.3, 18.2; HRMS: $C_{29}H_{40}N_2O_{13} + Na^+$ requires 625.2603, found 625.2599.

1,6-Anhydro-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-methyl-cyclohexane-β-D-glucopyranose (156):



Compound **148** (1.7 g, 6.0 mmol) was dissolved in 30 mL DMF and cooled using an ice-bath and after stirring for 5 minutes NaH (60% dispersion in mineral oil) (0.6 g, 15 mmol, 2.5 equiv) was added. After 30 minutes the gas evolvement stopped and (bromomethyl)cyclohexane (4.18 mL, 30 mmol, 5 equiv) was added dropwise. The reaction was quenched with MeOH after 3 h. The reaction mixture was concentrated. The residue was taken up in Et_2O and washed twice with 1M $HCl_{(aq)}$. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. The oily residue was re-dissolved in 25 mL DMF and cooled using an ice-bath. The alkylation step was repeated three times. After four cycles the oil compound was purified using a short silica column (EtOAc/PE 10%) which gave product **156** as yellow oil (1.7 g, 4.5 mmol, 75%). TLC: EtOAc/PE 30%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (s, J = 29.7 Hz, 5H, CH_{arom} Bn), 5.47 (s, 1H, H-1), 4.64 (q, J = 12.1 Hz, 3H, CH_2 Bn, H-5), 4.04 (d, J = 5.7 Hz, 1H, CH_2, H-6), 3.76 (s, 1H, CH_2 H-6), 3.60 (s, 1H, H-3), 3.30-3.15 (m, 4H, CH_2, MCH, H-2, H-4), 1.66 (t, J = 26.2 Hz, 3H, CH_2, CH, MCH), 1.32-1.08 (m, 2H, CH_2, MCH), 0.97-0.87 (m, 2H, CH_2, MCH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.5 (C_q, Bn), 128.7-127.9 (CH_{arom} Bn), 100.7 (C-1), 78.0 (C-4), 76.4 (C-3), 75.8 (CH_2, MCH), 74.5 (C-5), 72.6 (C-6), 65.6 (C-2), 60.2 (MCH), 38.0 (CH_2, MCH), 26.7(CH_2, MCH), 25.9(CH_2, MCH); IR (neat) ν 2927.1, 2357.3, 2101.4, 1731.9, 1275.7, 1091.6, 710.4, 316.1; HRMS: $C_{20}H_{27}N_3O_4 + Na^+$ requires 396.1894, found 396.1892; $[\alpha]_D^{23}$ + 3.5 °(c = 0.4, $CHCl_3$).

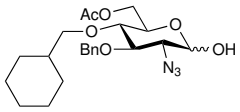
1,6-Di-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-methyl-cyclohexane-α/β-D-glucopyranose (159):



Oil **156** (1.50g, 4 mmol) was taken up in Ac_2O (20 mL) and cooled with an ice bath. To this cooled solution TFA (2mL, 10% v/v) was added and the reaction was stirred overnight at room temperature. After complete conversion of the starting material the reaction was diluted with toluene and coevaporated. Purification using a short silica column (EtOAc/PE 7%) gave compound **159** in 72% yield (1.37 g, 2.88 mmol). TLC: EtOAc/PE 25%; ^{1}H NMR (400 MHz,$CDCl_3$) δ 7.46 - 7.28 (m, 6H, CH_{arom} $Bn_{\alpha/\beta}$), 6.20 (d, J = 3.6 Hz, 1H, H-1_α), 5.45 (d, J = 8.2 Hz, 1H, H-1_β), 4.89-4.79 (m, 3H, CH_2 Bn), 4.31-4.19 (m, 3H, H-$6_{\alpha/\beta}$), 3.90-3.80 (m, 2H, H-$5_{\alpha/\beta}$, H-$3_{\alpha/\beta}$, H-$4_{\alpha/\beta}$), 3.72-3.64 (m, 1H, CH_2, MCH), 3.58-3.30 (m, 3H, H-$2_{\alpha/\beta}$, H-$4_{\alpha/\beta}$, H-$3_{\alpha/\beta}$),3.24 (dt, J = 14.7, 7.4 Hz, 1H, CH_2,

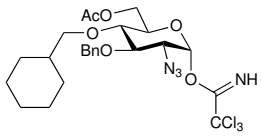
MCH), 2.17 (s, 1H, CH_3 OAc_β), 2.16 (s, 3H, CH_3 Ac_α), 2.08 (s, 4H, $Ac_{\alpha/\beta}$), 1.82-0.83 (m, 16H, CH_2, MCH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7 (C_q, Ac), 169.0 (C_q, Ac), 168.9 (C_q, Ac), 137.7(C_q), 137.7 (C_q), 128.6-128.2 (CH_{arom} Bn), 92.8 (C-1_β), 90.5(C-1_α), 83.1 (C-4_β), 80.4 (C-4_α), 79.5 (CH_2, MCH), 79.3 (CH_2, OMCH), 78.0 (C-3_α), 77.7 (C-3_β), 75.8 (CH_2 Bn), 75.7 (CH_2 Bn), 74.3 (C-5_β), 71.6 (C-5_α), 65.1 (C-2_β), 62.7 (C-2_α), 62.7 (C-6_β), 62.6 (C-6_α), 38.9 (CH), 38.8 (CH), 30.3-25.9 (CH_2, MCH), 21.1 (CH_3), 21.1 (CH_3 Ac), 21.0 (CH_3 Ac), 20.9 (CH_3 Ac); IR (neat) ν 2922.0, 2108.1, 1743.5, 1369.4, 1217.0, 1136.0, 1026.1, 1008.7, 931.6, 734.8, 698.2; HRMS: $C_{24}H_{33}N_3O_7 + Na^+$ requires 498.2214, found 498.2207.

6-*O*-Acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-methyl-cyclohexane-α/β-D- glucopyranose (162):



Compound **159** (1.31 g, 2.77 mmol) was dissolved in THF (14 mL) and piperidine (840 μL, 6% v/v) was added. The clear solution was stirred overnight at room temperature. After complete conversion to a lower running spot on TLC the reaction was diluted with EtOAc (100 mL) and poured in 1M HCl (100 mL). The layers were separated and the organic layer was washed twice with H_2O and once with brine. Subsequently the EtOAc layer was dried and concentrated *in vacuo*. Flash silica column purification (EtOAc/PE 8%) yielded compound **162** in 98% yield (1.23 g, 2.83 mmol) as a white foam. TLC: EtOAc/PE 25%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44-7.28 (m, 10H, CH_{arom} $Bn_{\alpha/\beta}$), 5.27 (t, $J = 3.1$ Hz, 1H, H-1_α), 4.89-4.77 (m, 4H, $CH_2Bn_{\alpha/\beta}$), 4.57 (d, $J = 4.3$ Hz, 1H, H-1_β), 4.38-4.31 (m, 2H, H-$6_{\alpha/\beta}$), 4.25-4.13 (m, 3H, H-$6_{\alpha/\beta}$, H-5_β), 4.07 (dtd, $J = 6.4$, 4.5, 2.3 Hz, 1H, H-5_α), 3.98-3.90 (m, 2H, H-$4_{\alpha/\beta}$), 3.68 (dt, $J = 14.4$, 6.5 Hz, 2H, CH_2), 3.56-3.51 (m, 1H, CH_2), 3.47 (m, 1H, CH), 3.41-3.21 (m, 4H, CH_2, H-$2_{\alpha/\beta}$), 2.09 (s, 2H, H-$3_{\alpha/\beta}$), 2.08 (s, 3H, CH_3 OAc), 1.90-0.77 (m, 3H, CH_3 OAc), 1.80-0.83 (m, 22H, CH_2, CH, MCH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0 (C_q, OAc), 137.9 (C_q, OBn), 137.9 (C_q), 128.6- 128.1 (CH_{arom} Bn), 96.2 (C-1_β), 92.1 (C-1_α), 83.1 (C-4_β), 80.0 (C-4_α), 79.24 (CH_2, MCH), 78.71 (C-3_α), 78.05 (C-3_β), 75.67 (CH_2 Bn), 75.60 (C-5_β), 73.5 (C-5_α), 69.4 (C-2_β), 67.5 (C-2_β), 64.1 (C-$6_{\alpha/\beta}$), 63.2 (CH_2), 63.1 (CH_2), 47.6 (CH), 42.8- 24.5 (CH_2, MCH), 21.4 (CH_3 Ac), 21.0 (CH_3 Ac): Peak assignment based on chemical shift because couplings are inconclusive; IR (neat) ν 2922.0, 2852.5, 2106.1, 1741.6, 1616.2, 1450.4, 1363.6, 1234.4, 1116.7, 1082.0, 1033.8, 910.3, 736.8, 698.2; HRMS: $C_{22}H_{31}N_3O_6 + Na^+$ requires 456.2105, found 456.2102.

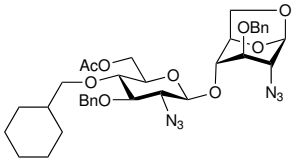
6-*O*-Acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-methyl-cyclohexane-1-*O*-(*N*- trichloroacetimidoyl)-α-D-glucopyranoside (147):



A solution of compound **162** (1.18 g, 2.72 mmol) and trichloroacetonitrile (1.03 mL, 8.16 mmol, 3 equiv) in dry DCM (14 mL) was treated with 1,8-diazabicyclo[5.4.0]undec-7-ene 81 μL (0.54 mmol, 0.2 equiv) for 18 h at ambient temperature. The dark brown solution was concentrated under reduced pressure and the brown oil was directly purified using a silica gel column (EtOAc/PE 7.5% + 2.5% TEA) to obtain the title compound **147** as a yellow oil (1.11 g, 1.93 mmol, 71%). TLC: EtOAc/PE 30%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (s, 1H, NH), 7.43-7.25 (m, 5H, CH_{arom} Bn), 6.38 (d, $J = 3.5$ Hz, 1H, H-$1_{1\alpha}$), 5.62-5.58 (m, 1H, H-1_β), 4.91-4.82 (m, 2H, CH_2 Bn), 4.35-4.19 (m, 2H, CH_2 H-6), 4.01-3.90 (m, 2H, CH_2, MCH), 3.70 (dt, $J = 10.9$, 5.5 Hz, 1H, H-4), 3.64-3.57 (m, 1H, H-5), 3.49-3.38 (m, 1H, H-2), 3.25 (dt, $J =$ 12.7, 6.3 Hz, 1H, H-3), 2.08-2.02 (m, 3H, CH_3 Ac), 1.82-1.61 (m, 4H, CH_2, MCH), 1.60-1.46

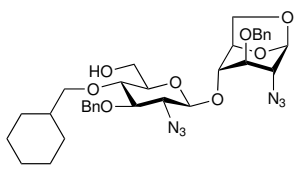
(m, 1H, CH, MCH), 1.28-1.11 (m, 4H, CH_2, MCH), 1.01-0.86 (m, 2H, CH_2, MCH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2 (C_q, C=N), 160.4 (C_q, Ac), 137.5 (C_q), 128.3-127.8 (C-1), 94.5 (C_q, CCl_3), 79.8 (C-3/4/5), 79.2 (CH_2, MCH), 77.9 (C-3/4/5), 75.3 (CH_2 Bn), 71.8 (C-3/4/5), 62.7 (C-2), 62.2 (C-6), 38.5 (MCH), 30.0-25.6 (CH_2, MCH), 20.6 (CH_3 Ac); IR (neat) ν 2106.1, 1739.7, 1652.9, 1506.3, 1232.4, 110.9, 1004.8, 904.6, 835.1, 698.2.

1,6-Anhydro-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-(6-*O*-acetyl-2-azido-3-*O* -benzyl-2-deoxy-4-*O*-methyl-cyclohexane-β-D-glucopyranosyl)-β-D-glucopyranose (144):



A mixture of imidate **147** (1.09 g, 1.87 mmol) and alcohol **148** (1.04 g, 3.76 mmol, 2 equiv) was coevaporated thrice with toluene and dissolved in dry toluene (9.5 mL). To this mixture activated molecular sieves (4Å) were added and the solution was cooled to -78 °C. After 10 minutes a solution of $BF_3 \cdot Et_2O$ (47 μL, 0.374 mmol, 0.2 equiv) in 0.25 mL dry toluene was added and the temperature was allowed to rise to -20 °C in 90 minutes. TLC analysis showed complete conversion of imidate **147** into a lower running spot. The reaction was quenched using TEA (0.5 mL), filtered and concentrated *in vacuo*. The excess of acceptor was acetylated, using an Ac_2O-pyridine cocktail (1 mL/3 mL), after which the reaction was quenched using MeOH and concentrated. The oily residue was directly purified using a silica gel column (EtOAc/PE 10%). Compound **144** was obtained in 65% in 1:8 α:β ratio (0.845 g, 1.22 mmol). TLC: EtOAc/Tol 25%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42-7.23 (m, 10H, CH_{arom} Bn), 5.55 (s, 1H, H-1$_\alpha$), 5.49 (s, 1H, H-1$_\beta$), 4.87-4.74 (m, 2H, CH_2 Bn), 4.72-4.56 (m, 3H, CH_2 H-6', H-5'), 4.37-4.30 (m, 3H, CH_2 Bn, H-1), 4.20-3.99 (m, 2H, CH_2 Bn, H-6), 3.96 (s, 1H, H-3), 3.80-3.44 (m, 3H, CH_2 H-6, MCH, H-5'), 3.34-3.20 (m, 5H, CH_2, MCH, H-2, H-3', H-4, H-4'), 2.00 (s, 3H, CH_3 Ac), 1.78-1.45 (m, 5H, CH_2, CH, MCH), 1.29-1.09 (m, 4H, CH_2, MCH), 0.99-0.84 (m, 2H, CH_2, MCH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7 (C_q, Ac), 137.9 (C_q), 137.6 (C_q), 128.8-127.8 (CH_{arom} Bn), 102.3 (C-1'), 100.7 (C-1), 82.9 (C-3'/4'), 80.1 (C-4'/3'), 79.4 (CH_2. MCH), 77.6 (C-5'), 77.4 (C-3), 75.7 (CH_2 Bn), 75.0, 74.8 (C-5), 73.6 (C-4), 72.6 (C-6'), 65.9 (C-2'), 65.2 (C-6), 62.7 (CH_2 Bn), 59.3 (C-2), 38.8 (MCH), 30.3-25.9 (CH_2, MCH), 20.9 (CH_3 Ac). IR (neat) ν 2922.0, 2100.3, 1739.7, 1454.2, 1363.6, 1236.3, 1070.4, 1026.1, 1006.8, 964.3, 902.6, 736.8, 696.3; HRMS: $C_{35}H_{44}N_6O_9 + Na^+$ requires 715.3062, found 715.3064;$[\alpha]_D^{23}$ 5 °(c = 0.2, $CHCl_3$).

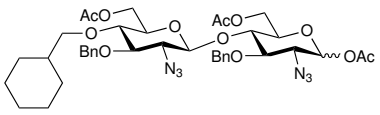
1,6-Anhydro-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-(2-azido-3-*O*-benzyl-2-deoxy-4-*O*-methyl- cyclohexane-β-D-glucopyranosyl)-β-D-glucopyranose (165):



Disaccharide **144** (785 mg, 1.13 mmol) was dissolved in MeOH (5 mL) and a catalytic amount of NaOMe (30% in MeOH) was added. The reaction mixture was stirred for 1 h at ambient temperature, after which it was neutralized (pH∼7) using Amberlite® IR-120 H^+ resin. Filtering off the resin, concentration and purification using a short silica column (EtOAc/PE 10%) yielded title compound **165** as a clear oil (377 mg, 580 μmol, 52%). TLC: EtOAc/PE 30%; ^{1}H NMR (400 MHz, d_4, MeOD) δ .43-7.25 (m, 10H, CH_{arom} Bn), 5.46 (s, 1H, H-1), 4.89-4.78 (m, 2H, CH_2 Bn), 4.75-4.62 (m, 3H, CH_2 Bn, H-5), 4.56 (d, J = 7.4 Hz, 1H, H-1$_\beta$), 4.11 (d, J = 7.3 Hz, 1H, CH_2 H-6), 3.93 (d, J = 8.3 Hz, 2H, H-5', H-3), 3.86-3.67 (m, 3H, CH_2, H-6', H-6), 3.62 (dt, J = 14.0, 7.0 Hz, 1H, CH_2, MCH), 3.44-3.28 (m, 5H, CH_2, MCH, H-2', H-3', H-4', H-4), 3.23 (s, 1H,

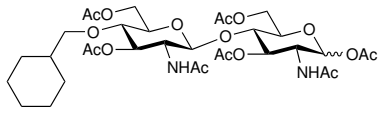
H-2), 1.75 (dd, $J = 30.5$, 13.3 Hz, 4H, CH_2, MCH), 1.53 (s, $J = 2.9$ Hz, 1H, CH, MCH), 1.33-1.11 (m, 4H, CH_2, MCH), 1.03-0.87 (m, 2H, CH_2, MCH); ^{13}C NMR (100 MHz, d_4, MeOD) δ 139.7 (C_q), 139.4 (C_q), 129.5-128.8 (CH_{arom} Bn), 102.7 (C-1'), 101.9 (C-1), 84.4 (C-3'/4'/4), 79.9 (CH_2, MCH), 78.9 (C-3'/4'/4), 78.7 (C-3'/4'/4), 77.4 (C-5'/3), 77.3 (C-5'/3), 76.4 (CH_2 Bn), 76.0 (C-5), 73.4 (CH_2 Bn), 67.6 (C-2'), 66.2 (C-2), 62.0 (C-6), 61.0 (C-2), 40.1 (MCH), 31.3-27.0 (CH_2, MCH); IR (neat) ν 2927.8, 2356.1, 2106.1, 1733.4, 1278.3, 1113.0, 1069.4, 708.8, 504.4;HRMS: $C_{33}H_{42}N_6O_8 + Na^+$ requires 673.2956, found 673.2957; $[\alpha]_D^{23}$ -4 °(c = 0.2, $CHCl_3$).

2-Azido-3-*O*-benzyl-2-deoxy-1,6-di-*O*-acetyl-4-*O*-(2-azido-3-*O*-benzyl-2-deoxy-4-*O*-methyl-cyclohexane-β-D-glucopyranosyl)-α/β-D-glucopyranose (168):



To a solution of compound **165** (377 mg, 580 μmol) in 3 mL Ac_2O 0.45 mL (15% v/v) TFA was added. The reaction was stirred for 18 h after which it was diluted with toluene and concentrated. After purification using silica gel chromatograph (EtOAc/PE 10%) disaccharide **168** was obtained in 87% yield (400 mg, 505 μmol) as transparent foam. TLC: EtOAc/PE 25%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44-7.19 (m, 10H, CH_{arom} Bn), 6.19 (d, $J = 3.7$ Hz, 1H, $H_{1\alpha}$), 5.50-5.41 (m, 1H, H-1_β), 5.17-4.98 (m, 1H, CH_2 Bn), 4.87-4.71 (m, 3H, CH_2 Bn), 4.52-4.43 (m, 1H, CH_2 H-6), 4.35 (m, 2H, CH_2 H-6, H-1), 4.23-4.17 (m, 1H, CH_2 H-6'), 4.14-4.07 (m, 2H, CH_2 H-6', H-3'), 4.00-3.85 (m, 2H, H-3, H-4), 3.64 (dd, $J = 8.5$, 5.8 Hz, 1H, CH_2, MCH), 3.57-3.50 (m, 1H, H-2), 3.44-3.27 (m, 4H, H-2', H-4, H-5, H-5'), 3.20 (dd, $J = 8.4$, 7.0 Hz, 1H, CH_2, MCH), 2.16 (d, $J = 2.4$ Hz, 3H, CH_3 Ac), 2.10 (s, 3H, CH_3 Ac), 1.90 (s, 3H, CH_3 Ac), 1.75-1.60 (m, 4H, CH_2, MCH), 1.54-1.43 (m, 1H, CH, MCH), 1.27-1.09 (m, 4H, CH_2, MCH), 0.96-0.83 (m, 2H, MCH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5 (C_q, Ac), 168.8 (C_q, Ac), 138.2 (C_q), 137.6 (C_q), 128.5-127.4 (CH_{arom} Bn), 101.4 (C-1'), 90.1 (C-1), 83.3 (C-4/5/'4), 79.1 (CH_2, MCH), 78.4 (C-3), 77.8 (C-4/5/'4), 77.1 (C-4), 7.7 (CH_2 Bn), 75.0 (CH_2 Bn), 73.5 (C-4/5/'4), 71.0 (C-3'), 66.8 (C-2'), 62.8 (C-6), 62.2 (C-2), 62.1 (C-6'), 38.7 (MCH), 30.1-25.8 (CH_2, MCH), 21.0 (CH_3 Ac), 20.8 (CH_3 Ac), 20.7 (CH_3 Ac); IR (neat) ν 2922.0, 2356.9, 2341.4, 2108.1, 1737.7, 1452.3, 1363.6, 1222.8, 1105.1, 1026.1, 1008.7, 931.6, 823.5, 734.8, 696.3; HRMS: $C_{39}H_{50}N_6O_{12} + Na^+$ requires 817.3379, found 817.3382.

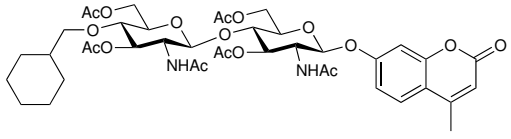
2-Acetamido-2-deoxy-1,3,6-tri-*O*-acetyl-4-*O*-(2-acetamido-2-deoxy-3,6-*O*-di-acetyl-4--*O*- methyl-cyclohexane-β-D-glucopyranosyl)-α/β-D-glucopyranose (141):



Compound **168** (365 mg, 0.46 mmol) was taken up in a mixture of dioxane:toluene;H_2O (5:2:1, v/v/v, 8 mL) and cooled with an ice-bath. After 10 minutes PMe_3 (2.30 mL, 5 equiv) 1M intoluene was added and the reaction was stirred for 18 h at +4 °C. After TLC analysis showed conversion towards lower running, ninhydrin positive spot, the reaction was coevaporated thrice with toluene and subsequently acetylated using an Ac_2O-pyridine cocktail (2 mL/6 mL). The mixture was stirred for 20 h after which it was quench with MeOH and concentrated under reduced pressure. The resulting white solid was dissolved in a 1:1 mixture of MeOH and TFE (8 mL) and purged with argon. A catalytic amount of $Pd(OH)_2$ spiked with Pd-black was added and the mixture was purged with H_2. Reduction of the benzyl-groups was continued for 5 h followed by filtration and concentration. The residue was again taken up in an Ac_2O-pyridine cocktail (2 mL:6 mL) with a catalytic

amount of DMAP and stirred at ambient temperature for 18 h. After complete acetylation of the disaccharide the mixture was quenched with MeOH and concentrated under reduced pressure. Silica gel purification (MeOH/DCM 3%) yielded 122 mg (170 μmol, 39%, 73% per step) of the title compound **141** as an off-white foam. TLC: MeOH/DCM 5%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44-7.19 (m, 14H), 6.19 (d, $J = 3.7$ Hz, 1H), 5.50-5.41 (m, 1H), 5.17-4.98 (m, 1H), 4.87 - 4.71 (m, 4H), 4.52-4.43 (m, 1H), 4.35 (m, 3H), 4.23-4.17 (m, 1H), 4.14-4.07 (m, 2H), 4.00-3.85 (m, 3H), 3.64 (dd, $J = 8.5$, 5.8 Hz, 1H), 3.57-3.50 (m, 2H), 3.44-3.27 (m, 5H), 3.20 (dd, $J = 8.4$, 7.0 Hz, 1H), 2.16 (d, $J = 2.4$ Hz, 3H), 2.10 (s, 4H), 1.90 (s, 3H), 1.75-1.60 (m, 7H), 1.54-1.43 (m, 2H), 1.27-1.09 (m, 6H), 0.96-0.83 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 171.0-169.09, 102.0, 101.9, 100.9, 92.4, 90.4, 90.3, 82.5, 79.0, 78.8, 76.6, 75.8, 75.6, 75.1, 75.0, 74.8, 73.6, 73.4, 73.1, 72.1, 70.9, 70.7, 69.8, 69.7, 63.0, 62.9, 62.2, 61.7, 54.1, 53.6, 51.9, 51.2, 38.4, 29.9, 29.8, 29.7, 29.7, 26.3, 25.7, 25.7, 23.1, 23.1, 22.9, 21.0, 20.9, 20.8, 20.83, 20.79, 20.77, 20.7, 20.6, 20.6; IR (neat) ν 2925.8, 1739.7, 1652.9, 1558.4, 1521.7, 1506.3, 1369.4, 1220.9, 1112.9, 1012.6, 939.3, 906.5, 715.5; HRMS: $C_{33}H_{50}N_2O_{16} + Na^+$ requires 753.3053, found 753.3055

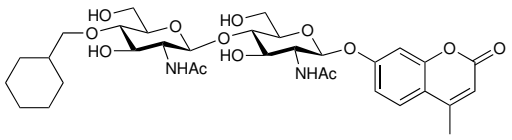
2-Acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-4-*O*-(2-acetamido-2-deoxy-3,6-*O*-di-acetyl-4-*O*-methyl-cyclohexane-β-D-glucopyranosyl)-1-*O*-4-methylumbelliferyl-β-D-glucopyranoside (171):



Dimer **141** (70 mg, 95 μmol) was dissolved in AcOH (2 mL) and Ac_2O (1 mL). At 0 °C dry $HCl_{(g)}$ was bubbled through (liberated under Kipp conditions) for 3h. The reaction mixture was then placed at 5 °C for 42 h at which TLC analyses (DCM-acetone 60-40) showed complete consumption of starting material. The reaction diluted with $CHCl_3$ (10 ml, 0 °C) and washed twice with H_2O (15 ml, 0 °C) and twice with $NaHCO_3$ (15 mL, 0 °C). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* yielding an amorphous solid of which the purity was evaluated by ^{1}H-NMR. The resulting solid was dissolved in $CHCl_3$ (5 ml) and added to a solution of $NaHCO_3$ 0.2M (5 ml), 4-methylumbelliferyl sodium salt[34,35] (94 mg, 480 μmol) and TBAHS (64 mg, 191 μmol). The biphasic mixture was stirred overnight with the exclusion of light. The phases were separated and the organic layer was washed twice with $NaHCO_3$ (0.2 M) and twice with H_2O. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (MeOH/$CHCl_3$ 3%) yielded **171** (41 mg, 48 μmol, 50%) as a white amorphous solid. TLC: MeOH/DCM 5%; ^{1}H NMR (400 MHz, d_4, MeOD) δ 7.61 (d, $J = 8.4$ Hz, 1H, CH, 4-methylumbelliferyl), 7.01-6.95 (m, 2H, CH_{arom} 4-methylumbelliferyl), 6.20 (s, 1H, CH_{arom} 4-methylumbelliferyl), 5.25 (t, $J = 8.3$ Hz, 2H, H-1$_\beta$, H-3), 5.17-5.10 (m, 1H, H-3'), 4.63 (d, $J = 8.3$ Hz, 1H, H-1'$_\beta$), 4.51 (d, $J = 10.2$ Hz, 1H, CH_2 H-6), 4.31 (d, $J = 2.7$ Hz, 2H, CH_2 H-6'), 4.24-4.11 (m, 2H, CH_2 H-6, H-2), 3.93 (t, $J = 8.3$ Hz, 1H, H-5), 3.87-3.74 (m, 2H, H-4, H-2), 3.58 (dd, $J = 8.0$, 4.9 Hz, 1H, H-5'), 3.48-3.25 (m, 3H, CH_2, MCH, H-4'), 2.45 (s, 3H, CH_3 4-methylumbelliferyl), 2.14 (s, 6H, CH_3 Ac), 2.10-2.04 (m, 6H, CH_3 Ac), 1.96 (d, $J = 5.8$ Hz, 6H, CH_3 Ac), 1.68 (t, $J = 11.4$ Hz, 4H, CH_2, MCH), 1.44 (d, $J = 11.2$ Hz, 1H, CH, MCH), 1.30-1.09 (m, 4H, CH_2, MCH), 0.91 (dd, $J = 20.8$, 11.9 Hz, 2H, CH_2, MCH); ^{13}C NMR (100 MHz, d_4, MeOD) δ 171.7-170.1 (C_q, Ac), 161.4 (C_q, 4-methylumbelliferyl), 159.4 (C_q, 4-methylumbelliferyl), 154.2 (C_q, 4-methylumbelliferyl), 153.3 (C_q, 4-methylumbelliferyl), 125.5 (CH_{arom} 4-methylumbellifer-

yl), 114.7 (C_q, 4-methylumbelliferyl), 113.6 (CH_{arom} 4-methylumbelliferyl), 111.8 (CH_{arom} 4-methylumbelliferyl), 103.3 (CH_{arom} 4-methylumbelliferyl), 100.4 (C-1'), 97.5 (C-1), 78.2 (CH_2, MCH), 75.7-75.7 (C-4/4'), 74.4 (C-3'), 72.6-72.4 (C-3/5/5'), 62.6 (C-6'), 62.3 (C-6), 54.3 (C-2'), 53.3 (C-2), 38.0 (MCH), 29.4-25.2 (CH_2, MCH), 21.7-17.6 (CH_3 Ac); IR (neat) ν 1739.7, 1652.9, 1539.1, 1521.7, 1488.9, 1473.5, 1369.4, 1222.8, 1012.6, 939.3, 902.6, 628.8; HRMS:$C_{41}H_{54}N_2O_{17} + Na^+$ requires 869.3315, found 869.3317.

2-Acetamido-2-deoxy-4-*O*-(2-acetamido-2-deoxy-4-*O*-methyl-cyclohexane-β-D-glucopyranosyl)-1- *O*-4-methylumbelliferyl-β-D-glucopyranoside (138):



To a suspension of **171** (20 mg, 23 μmol) in MeOH (1 mL) was added NaOMe (30 wt% in MeOH) (414 μL, 2.30 μmol). The reaction was stirred with the exclusion of light. When LCMS (gradient 0 to 50 % MeOH) showed complete conversion to product the mixture was quenched with AcOH (5 μL, 80 μmol). The reaction was diluted with H_2O (2 mL) the MeOH was evaporated *in vacuo* and the remaining H_2O was lyophilized. Purification by HPLC (gradient H_2O-MeOH + 0.1% TFA 80-20 → 60-40) evaporation of MeOH and lyophilizing H_2O yielded **138** (3.5 mg, 5.16 μmol, 22%) as white fluffy solid. ^{1}H NMR (600 MHz, (d_6), DMSO) δ 7.90 (d, $J = 9.2$ Hz, 1H, NH), 7.74 (d, $J = 9.0$ Hz, 1H, NH), 7.70 (d, $J = 8.8$ Hz, 1H, CH, 4-methylumbelliferyl), 7.04 (d, $J = 2.3$ Hz, 1H, CH, 4-methylumbelliferyl), 6.96 (dd, $J = 8.8$, 2.3 Hz, 1H CH, 4-methylumbelliferyl), 6.27 (s, 1H, CH, 4-methylumbelliferyl), 5.18 (d, $J = 8.4$ Hz, 1H, H-1), 5.06 (d, $J = 6.3$ Hz, 1H, OH), 4.81 (dd, $J = 5.7$, 5.0 Hz, 1H, OH), 4.77 (d, $J = 2.1$ Hz, 1H, OH), 4.69 (t, $J = 6.0$ Hz, 1H, OH), 4.38 (d, $J = 8.4$ Hz, 1H, $H_{1'}$), 3.33 (s, 14H), 3.00 (t, $J = 9.2$ Hz, 1H), 2.41 (s, $J = 9.4$ Hz, 3H, CH_3 4-methylumbelliferyl), 1.85 (s, 3H, CH_3 Ac), 1.81 (s, 3H, CH_3 Ac), 1.70-1.59 (m, 4H, CH_2, MCH), 1.51-1.44 (m, 1H, CH, MCH), 1.24-1.07 (m, 4H, CH_2, MCH), 0.89 (d, $J = 12.2$ Hz, 2H, CH_2, MCH); ^{13}C NMR (151 MHz, (d_6), DMSO) δ 169.2, 169.1, 160.0, 159.9, 154.4, 153.3, 126.5, 114.3, 113.4, 111.9, 103.1, 101.9, 98.2, 80.8, 78.6, 77.6, 75.7, 75.0, 74.0, 72.3, 55.8, 54.4, 38.0, 29.7, 29.4, 26.2, 25.4, 25.3, 23.0, 18.1; HRMS: $C_{33}H_{46}N_2O_{13} + Na^+$ requires 701.2892, found 701.2890

3.5 Biological Evaluation

Enzymes: Human chitotriosidase was expressed in BHK cells and purified from culture medium as described earlier.[36] Jack bean β-hexosaminidase was purchased from Sigma. The tissue was homogenized in 4 volumes of 0.1 M potassium phosphate buffer pH 6.5. The soluble fraction was isolated following ultracentrifugation at 50.000 g for 20 minutes and next β-hexosamindidase in the supernatant was enriched using Concanavalin. A chromotagraphy and elution of bound enzyme with 0.1 mM methylmannose 0.1 M potassium phosphate buffer pH 6.5.

Enzyme activity measurements using artificial 4-MU-substrates: For activity measurements of β-hexosaminidases, 4-MU-GlcNac (Sigma) was used as substrate. Briefly, samples were incubated for 20 minutes at 37 °C with 2 mM 4-MU-GlcNac in McIlvaine buffer (100 mM citric acid, 200 mM sodium phosphate, pH 4.0). The reactions were stopped by the addi-

tion of excess 0.3 M glycine-NaOH, pH 10.3. Formed 4-methylumbelliferone was detected fluorimetrically (excitation at 366 nm; emission at 445 nm). Activity of recombinant chitotriosidase towards 4-MU-oligosaccharide substrates was determined by incubation at 37 °C in McIlvaine buffer (100 mM citric acid, 200 mM sodium phosphate, pH 5.2). Substrate concentrations in various experiments were different as indicated.

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4

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.

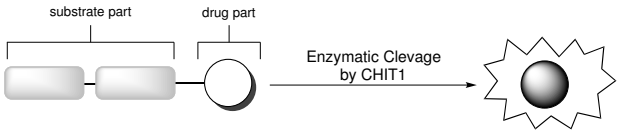


Figure 4.1: Schematic representation of an enzymatic prodrug cleavage. ∘: inactive inhibitor ; •: active inhibitor.

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]

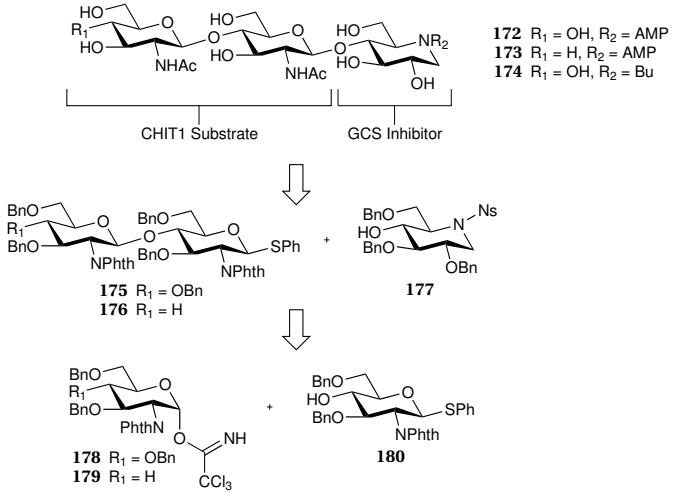
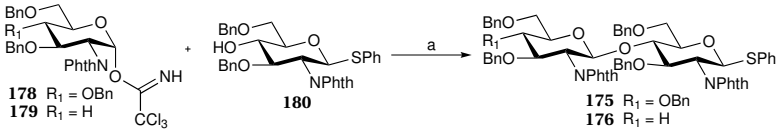


Figure 4.2: Retrosynthetic analysis of potential Gaucher prodrugs **172**, **173** and **174**.

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

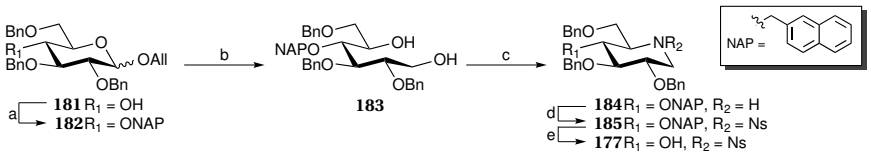


Reagents and conditions: a) TMSOTf, DCM, 0 °C, (**175**, 73%; **176**, 41%.)

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

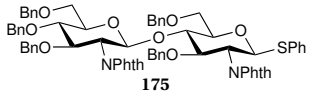
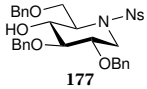
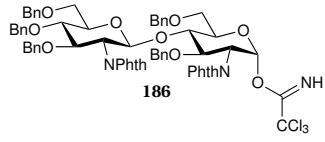
Scheme 4.2: Synthesis of DNJ acceptor **177**.



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%.

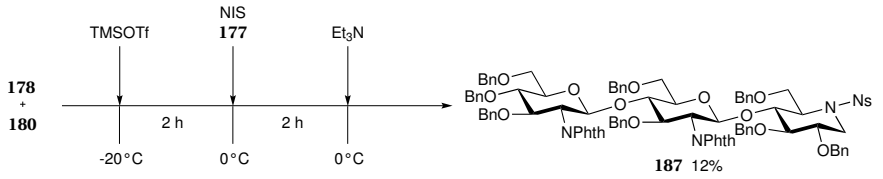


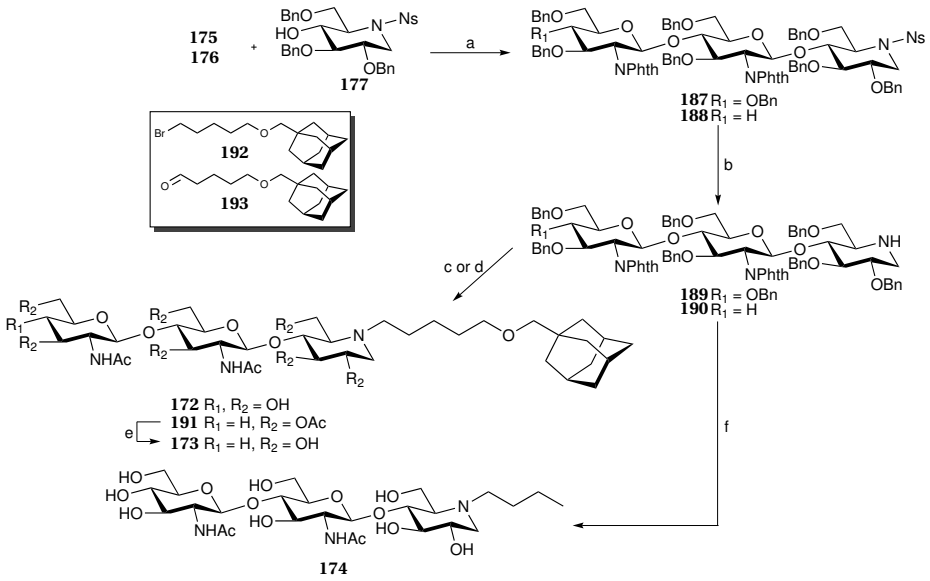
Figure 4.3: One-pot procedure for the synthesis of trimer **187**.

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

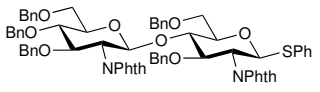
This chapter describes the synthesis of three Gaucher prodrugs **172**, **173** and **174** in which the chitobiose core is used as CHIT1 substrate and NB-DNJ or AMP-DNJ is used as GCS inhibitor or drug part. The chitobiose core was synthesized *via* an imidate coupling with acceptor **180** bearing 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}.

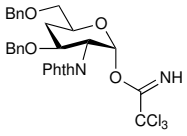
Phenyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-4-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-1-thio-D-glucopyranoside(175):



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,

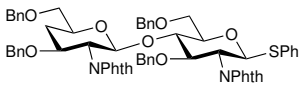
8H), 4.27 - 4.15 (m, 4H), 3.91 - 3.79 (dd, $J = 9.9$, 8.6 Hz, 1H), 3.79 - 3.60 (m, 2H), 3.57 - 3.30 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.5 - 167.3, 138.6- 133.9, 133.7, 132.2, 131.7, 131.5, 128.6 - 127.4, 97.1, 83.4, 79.7, 79.1, 78.9, 77.9, 75.6, 75.2, 74.9, 74.8, 74.7, 73.3, 72.7, 68.4, 68.0, 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):



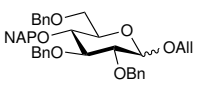
3,6-Di-*O*-benzyl-2,4-di-deoxy-2-phthalimido-D-xylo-hexapyranose[30] (3.37 g, 7,12 mmol) was coevaporated thrice with toluene after which it was dissolved in dry DCM (50 mL). The solution was cooled to 0 °C and stirred for 10 minutes followed by addition of CCl_3CN (7.14 mL, 71.2 mmol, 10 equiv) and 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, $CHCl_3$).

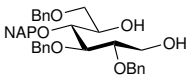
Ally 2,3,4-tri-*O*-benzyl-4-*O*-(2-naphthylmethyl)-α/β-D-glucopyranoside)(182):



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

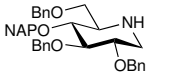
mmol, 2 equiv) was added and the reaction was stirred overnight allowing the reaction mixture to warm to rT. Subsequently, the reaction mixture was cooled to 0 °C, quenched using little MeOH, diluted with Et_2O and washed twice with 1M HCl and H_2O. The organic layer was dried using $MgSO_4$ and concentrated 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, $CHCl_3$).

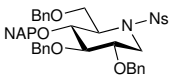
2,3,4-Tri-*O*-benzyl-4-*O*-(2-naphthylmethyl)-1-deoxynojirimycin(184):



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

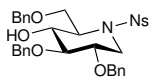
(18.84 g, 238.4 mmol, 20 equiv) and Na_2SO_4 (6.77 g, 47.68 mmol, 4 equiv) in 300 mL MeOH. The reaction was stirred overnight allowing the mixture to warm to room temperature. After TLC-analysis showed full conversion into a lower running product, the reaction mixture was filtered and concentrated under 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, $CHCl_3$).

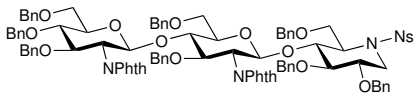
2,3,4-Tri-*O*-benzyl-4-*O*-(2-naphthylmethyl)-*N*-(2-nitrobenzenesulfonyl)-1-deoxynojirimycin(177):



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,

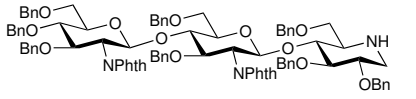
72.27, 71.16, 66.99, 66.00, 60.02, 39.43; IR (neat) ν 3522, 3500, 3487, 3086, 2922, 2866, 1541, 1496, 1371, 1357, 1174, 1089, 1076, 972, 852, 744, 698; HRMS: $C_{33}H_{34}N_2O_8S+Na^+$ requires 641.19281, found 641.19281; $[\alpha]_D^{23}$ -50 °(c = 1, $CHCl_3$).

_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, $CHCl_3$).

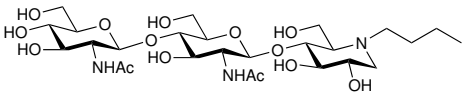
2,3,6-Tri-_O_-benzyl-4-_O_-[3,6-di-_O_-benzyl-2-deoxy-2-phthalimido-4-_O_-(3,4,6-tri-_O_-benzyl-2-phthalimido-β-D-glucopyranosyl)-β-D-glucopyranosyl]-1-deoxynojirimycin (189):



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;

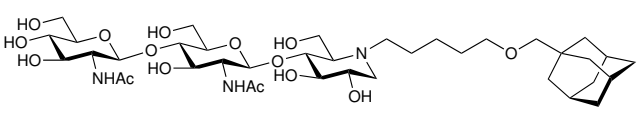
HRMS: $C_{96}H_{90}N_4O_{20}S + Na^+$ requires 1466.61591, found 1466.61775;

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).

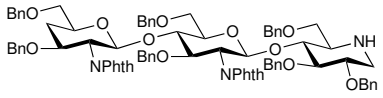
4-*O*-[2-deoxy-2-*N*-acetyl-4-*O*-(2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranosyl]-*N*-[5-(adamantan-1-yl-methoxy)-pentyl]-1-deoxynojirimycin(172):



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

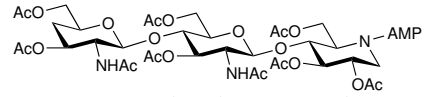
(1H), 2.33 - 2.13 (m, 1H), 2.10 - 2.01 (m, 2H), 1.99 - 1.89 (3H), 1.86 - 1.71 (s, 7H), 1.64 - 1.56 (3H), 1.54 - 1.33 (m, 11H), 1.24 - 1.11 (m, 6H).

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, $CHCl_3$).

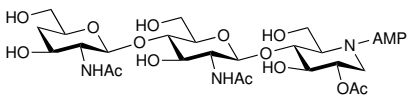
2,3,6-Tri-*O*-acetyl-4-*O*-[3,6-di-*O*-acetyl-2-deoxy-2-*N*-acetyl-4-*O*-(3,4,6-tri-*O*-acetyl-2,4-di-deoxy-2-*N*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranosyl]-*N*-[5-(adamantan-1-yl-methoxy)-pentyl]-1-deoxynojirimycin(191):



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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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.

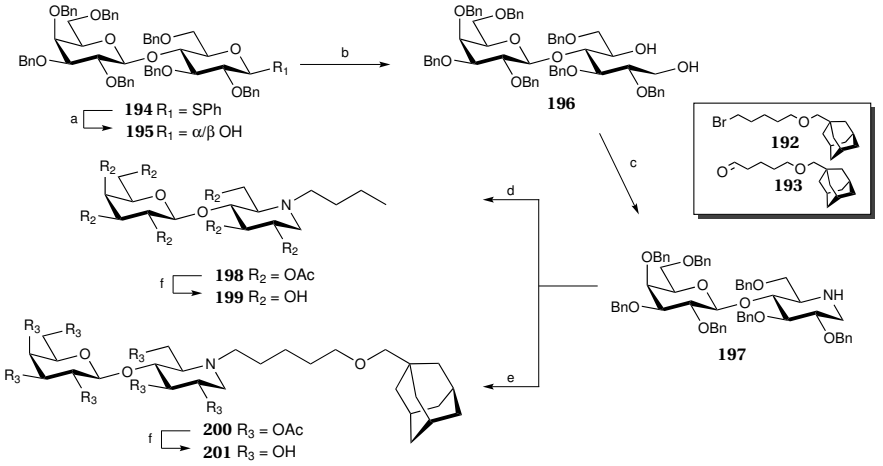
5.2 Results and Discussion

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**.

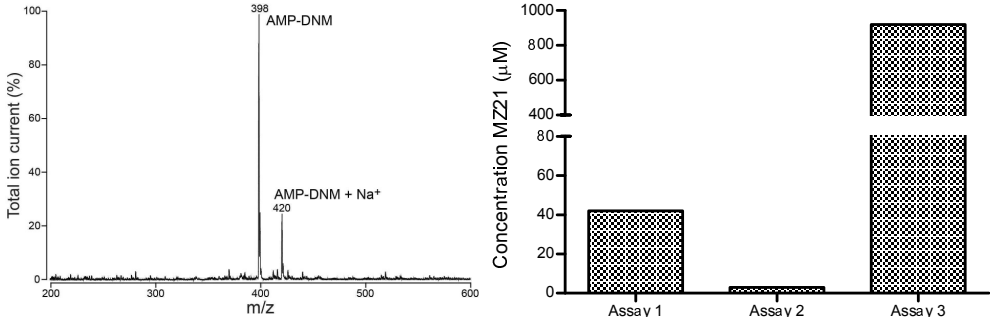


Figure 5.1: Detection of **17** by LC-MS/MS, after cleavage of prodrug **201** by LPH (Left); Recovery of AMP-DNJ (**17**) after incubation of LPH from in intestinal rats mucosa (Right); Assay 1: LPH + 1mM **201**, Assay 2: Inactive LPH + 1mM **201**; Assay 3: LPH + 1mM **17**.

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**).

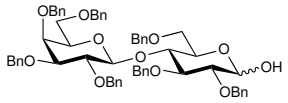
5.3 Conclusion

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

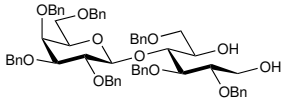
All reagent were of commercial grade and used as received (Acros, Fluka, Merck, Schleicher & Schuell) unless stated otherwise. Diethyl ether (Et_2O), light petroleum ether (PE 40-60), en toluene (Tol) were purchased from Riedel-de Haën. Dichloromethane (DCM), *N,N*-dimethylformamide (DMF), methanol (MeOH), 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):



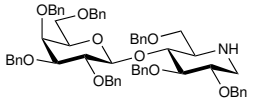
A solution of **194** (24 g, 22.55 mmol) in acetone:H_2O (350:25 mL) was cooled to -20 °C followed by portion wise addition of NBS (20 g, 112.73 mmol, 5 equiv). The reaction mixture turned into a yellow suspension which became clear in 10 minutes. After 15 minutes the reaction turned bright orange and 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

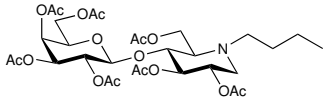
2,3,6-Tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-1-deoxynojirimycin (197):



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

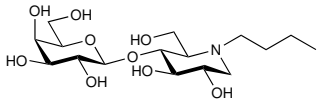
2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-*N*-butyl-1-deoxynojirimycin (198):



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;

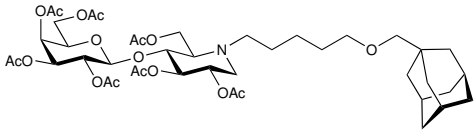
HRMS: $C_{30}H_{45}NO_{16} + H^+$ requires 676.28111, found 676.28099

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

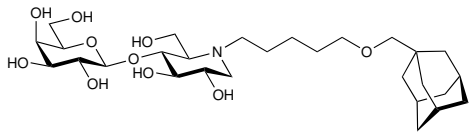
2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-*N*-[5-(adamantan-1-yl-methoxy)-pentyl]-1-deoxynojirimycin (200):



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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6

Synthesis of α- and β-Cholesteryl Glucosides

6.1 Introduction

Steryl glycosides are abundant in nature.[1] For example the gram-negative bacterium *Helicobacter pylori*, a common human pathogens which is known to cause ulcers contains several steryl glycosides, such as cholesteryl-6-*O*-acyl-α-D-glucopyranoside, cholesteryl-α-D-glucopyranoside and cholesteryl-6-*O*-phosphatidyl-α-D-glucopyranoside.[2] Additionally various steryl-β-glucosides are synthesized by plants, including sitosteryl-β-glucoside which can serve as primer in the biosynthesis of cellulose.[3] Studies reporting on the occurrence of endogenous cholesterol-β-glucoside in mammals are surprisingly scarce. Only Murakami-Murofushi and co-workers have reported on the formation of cholesterol-β-glucoside in cultured fibroblasts as a rapid response to heat stress.[4] Very recently they presented evidence that glucosylceramide (GC) and not UDP-glucose acts as a sugar donor in the biosynthesis of cholesteryl glucoside.[5] The enzyme responsible for the synthesis of cholesterol-β-glucoside in man has not yet been identified.

The limited knowledge on endogenous cholesterol-β-glucosides in mammals is surprising since there are numerous speculations that steryl glucosides may act as (neuro)toxins. An example thereof is the neurological disorder amyotrophic lateral sclerosis-parkinsomism dementia complex (ALS-PDC) in which features of parkinsomism are presented and which is linked to the consumption of flour made from cycad fruits (*Cycas micronesica*) that is known to contain a high concentration of steryl glycosides.[6–9]

Parkinsonism and glucosylceramide metabolism also appear to be linked given, the high incidence of neurodegenerative conditions in Gaucher disease patients.[10] Gaucher disease is a rare lysosomal storage disorder, which is caused by the inefficient catabolism of GC by mutant glucocerebrosidase (GBA1).[11] This causes accumulation of GC-laden macrophages which leads to the enlargement of organs (spleen and liver) and inflammation. It may be speculated that GC acts as a donor in the biosynthesis of the potentially neurotoxic steryl-β-glucosides, implying that cholesteryl-β-glucoside is a missing link between parkinsonism and Gaucher.

To further investigate this hypothesis pure samples of α- and β-glucosylated cholesterol are needed. This chapter describes the synthesis of α-cholesteryl glucoside **204** and β-cholesteryl glucoside **207** (Scheme 6.1).

6.2 Results and Discussion

In carbohydrate chemistry the glycosylation of naturally occurring terpenes and steroids present a special challenge. Besides controlling the stereoselectivity, the reactivity of the functional groups in the steroids is an important issue. The secondary 3-OH function in cholesterol is moderately nucleophilic and the alkene function is sensitive to hydrogenation.[12]

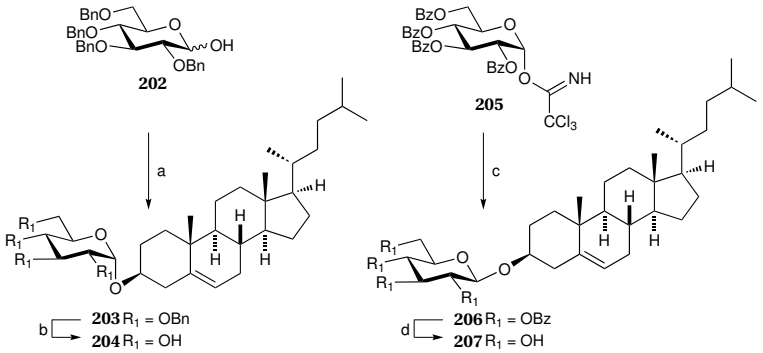
The synthesis of α-cholesteryl glucoside **204** and β-cholesteryl glucoside **207** is shown in Scheme 6.1. For the synthesis of α-cholesteryl glucoside **204**,[13] donor 2,3,4,6-tetra-*O*-benzyl-α/β-D-glucopyranose **202**[14] was used bearing benzyl groups which were cleaved by transfer hydrogenation leaving the alkene in cholesterol intact. *In situ* tosylation of the anomeric alcohol in **202** and coupling with an excess of cholesterol, to prevent self condensation gave steryl glycoside **203**. In almost complete anomeric selectivity (β<5%) and 90% overall yield.[15] Careful deprotection of the benzyl groups using Pearlman's catalyst in EtOH:cyclohexene to prevent the reduction of the endocyclic unsaturated bond in cholesterol, gave **204** which was purified by HPLC.[16]

β-Cholesteryl glycoside **207** was synthesized according to literature.[17] Activation of imidate **205** using TMSOTf followed by addition of cholesterol gave **206** (Scheme 6.1). Saponification of **206** under Zemplén conditions and purification by HPLC gave target compound **207** as a white solid.

6.3 Conclusion

The syntheses of α-cholesteryl glycoside **204** and β-cholesteryl glycoside **207** were successfully executed. The use of Pearlman's catalyst and cyclohexene, as hydrogen source, for the hydrogenation of the benzyl ethers in **203** prevented saturation of the double bond in cholestrol. Both **204** and **207** are currently used as internal

Scheme 6.1: Synthesis of α- and β-cholesteryl glucoside **204** and **207**.



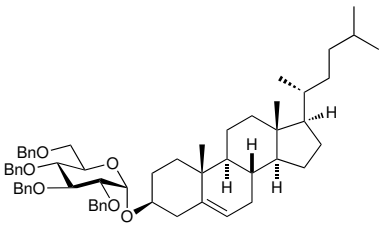
Reagents and conditions: a) TosCl, TEBA, DCM, $NaOH_{aq}$ 3M, 90%; b) $Pd(OH)_2$, H_2, EtOH:cyclohexene, 30%; c) TMSOTf, DCM, -40°C, 83%; d) NaOMe, MeOH ,89%.

standards for the investigation of cholesterol glucosides as common denominator for parkinsonism and Gaucher disease.

6.4 Experimental section

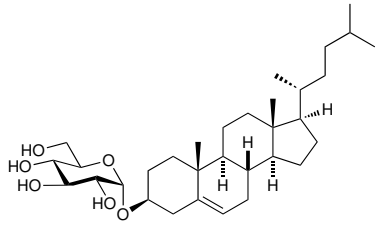
All reagent were of commercial grade and used as received (Acros, Fluka, Merck, Schleicher & Schuell) unless stated otherwise. Diethyl ether (Et_2O), light petroleum ether (PE 40-60), en toluene (Tol) were purchased from Riedel-de Haën. Dichloromethane (DCM), *N*,*N*-dimethylformamide (DMF), methanol (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}.

Cholesteryl 2,3,4,6-*tetra*-*O*-benzyl-α-D-glucopyranoside(203):



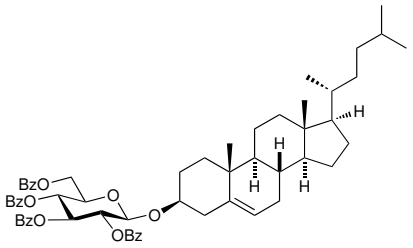
A solutions of known **202**[14] (0.54 g, 1 mmol), TosCl (0.21 g, 1.1 mmol, 1.1 quiv.), benzyltriethyl ammonium chloride (TEBA) (70 mg, 0.3 mmol, 0.3 equiv) and cholesterol (1.54 g, 4 mmol, 4 equiv) in dry DCM (10 mL) was stirred with 40% aqueous NaOH (5 mL) at room temperature. After 10 minutes an addition 5 mL of DCM was added to dilute the mixture. The reaction mixture was stirred overnight after which TLC analysis showed full conversion of the starting material. The mixture was diluted with DCM and H_2O, followed by seperation of the layers. The organic layer was washed thrice with H_2O and dried using $MgSO_4$. After filtration the mixture was concentrated *in vacuo* and purified using a short silica column (EtOAc/Tol 2.5%) gave **203** in 90% yield as a colourless oil (0.28 g, 0.31 mmol).The recorded data agree with those of Vankayalapati *et al.*[18] However, ^{1}H and ^{13}C NMR are given. TLC: 10% EtOAc/Tol; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.33 - 7.11 (m, 20H), 5.38 - 5.35 (m, 1H), 5.03 - 4.41 (m, 9H), 4.00 - 3.44 (m, 7H), 2.37 - 2.10 (m, 2H), 1.99 - 1.68 (m, 5H), 1.55 - 0.85 (m, 33H), 0.68 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 140.8, 138.9, 138.2, 128.3 - 121.7, 102.2, 94.6, 93.4, 84.7, 82.0, 79.9, 79.7, 77.9, 76.6, 75.6, 75.1, 73.4, 72.9, 70.0, 69.1, 68.6, 56.7, 56.1, 42.3, 39.7, 39.4, 36.7, 36.2, 35.7, 31.8, 28.2, 27.9, 24.3, 23.8, 22.8, 22.5, 21.0, 19.3, 18.7, 11.8.

Cholesteryl α-D-glucopyranoside(204):



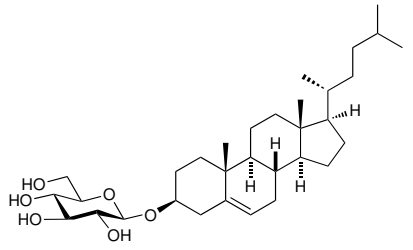
Compound **203** was dissolved in a mixture of ethanol (8 mL) and cyclohexene (4 mL). The reaction mixture was purged thrice with argon followed by addition of a catalytic amount of $Pd(OH)_2$ (20% on carbon). The suspension was stirred under reflux on till TLC analysis showed complete deprotection of the benzyl groups. The mixture was filtered over Whatman® filter paper and concentrated *in vacuo*. Purification by HPLC (gradient H_2O-MeOH + 0.1% TFA) followed by evaporation of MeOH and lyophilizing H_2O yielded **207** (0.131 mg, 0.24 mmol, 30%) as white fluffy solid. The recorded data agree with those of Nagarajan *et al.*[13] However, ^{1}H and ^{13}C NMR are given. ^{1}H NMR (400 MHz, (D_6) DMSO): δ = 5.36 - 5.26 (d, J = 4.5 Hz, 1H), 4.90 - 4.82 (d, J = 5.3 Hz, 1H), 4.82 - 4.76 (d, J = 3.7 Hz, 1H), 4.75 - 4.66 (d, J = 4.7 Hz, 1H), 4.52 - 4.40 (m, 2H), 3.66 - 3.55 (d, J = 9.4 Hz, 1H), 3.50 - 3.41 (m, 3H), 3.19 - 3.11 (m, 1H), 3.09 - 3.00 (m, 1H), 2.41 - 2.18 (m, 2H), 2.02 - 1.77 (m, 5H), 1.61 - 0.80 (m, 34H), 0.69 - 0.64 (s, 3H); ^{13}C NMR (100 MHz, (D_6) DMSO): δ = 140.7, 121.1, 96.9, 76.4, 73.2, 72.8, 71.8, 70.4, 61.0, 56.2, 55.6, 49.5, 41.8, 40.2, 39.9, 39.8, 36.6, 36.2, 35.6, 35.2, 31.4, 31.3, 27.7, 27.4, 27.4, 23.9, 23.2, 22.6, 22.4, 20.6, 19.1, 18.5, 11.7.

Cholesteryl 2,3,4,6-*tetra*-*O*-benzoyl-β-D-glucopyranoside(206):



Known imidate **205**[17] (0.74 g, 1 mmol) and cholesterol (0.32 g, 0.83 mmol, 0.83 equiv) were coevapporated thrice with toluene and dissolved in dry DCM (5 mL). To this mixture 3Å molsieves were added and the mixture was cooled to -40°C. After 10 minutes the mixture was activated by addition of TMSOTf (9 μL, 0.05 mmol) and stirring was continued for 1 hour at -40°C. After complete consumption of the donor the mixture was quenched using 2 mL Et_3N, diluted with DCM and washed twice with H_2O and once with brine. The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by column chromatography (EtOAc/Tol 2%) gave **206** in 83% yield (0.67 g, 0.69 mmol). The recorded data agree with those of Deng *et al.*[17] However, ^{1}H and ^{13}C NMR are given. TLC: 10% EtOAc/Tol;^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.08 - 7.80 (m, 9H), 7.58 - 7.17 (m, 11H), 6.01 - 5.87 (t, J = 9.6 Hz, 1H), 5.72 - 5.61 (t, J = 9.7 Hz, 1H), 5.61 - 5.47 (dd, J = 9.8, 7.9 Hz, 1H), 5.28 - 5.18 (m, 1H), 5.02 - 4.93 (d, J = 7.9 Hz, 1H), 4.68 - 4.46 (m, 2H), 4.22 - 4.13 (m, 1H), 3.60 - 3.52 (m, 1H), 2.26 - 2.12 (m, 2H), 2.07 - 0.80 (m, 38H), 0.73 - 0.60 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.1 - 165.1, 140.3, 133.5 - 122.0, 100.2, 80.5, 73.2, 72.2, 72.1, 70.2, 63.4, 56.8, 56.2, 50.2, 42.4, 39.8, 39.6, 38.9, 37.2, 36.7, 36.3, 35.8, 31.9, 31.8, 29.7, 28.3, 28.1, 24.4, 23.9, 22.9, 22.6, 21.1, 19.3, 18.8

Cholesteryl β-D-glucopyranoside(207):



Compound **206** (0.31 g, 0.33 mmol) was dissolved in a mixture of MeOH:dioxane (10:1 mL). To this mixture a catalytic amount of NaOMe (30% in MeOH) was added and the mixture was stirred for 1.5 hours. After TLC analysis showed complete deprotection of all acetyl groups, the mixture was neutralized using Amberlite® H^+ till $\sim$ pH 7, filtered and concentrated *in vacuo*. Purification by HPLC (gradient H_2O-MeOH + 0.1% TFA) followed by evaporation of MeOH and lyophilizing H_2O yielded **207** (145 mg, 0.26 mmol, 81%) as white fluffy solid. The recorded data agree with those of Nagarajan *et al.*[13] However, ^{1}H and ^{13}C NMR are given. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.44 - 5.25 (d, J = 4.2 Hz, 1H), 4.99 - 4.77 (m, 3H), 4.47 - 4.38 (t, J = 5.7 Hz, 1H), 4.30 - 4.20 (d, J = 7.3 Hz, 1H), 3.73 - 3.60 (m, 1H), 3.53 - 3.40 (m, 2H), 3.19 - 2.98 (m, 3H), 3.00 - 2.86 (m, 1H), 2.46 - 2.31 (m, 1H), 2.24 - 2.05 (t, J = 12.1 Hz, 1H), 2.06 - 1.89 (m, 2H), 1.87 - 1.73 (m, 3H), 1.56 - 0.83 (m, 34H), 0.80 - 0.58 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 140.4, 129.2, 128.5, 121.2, 100.8, 76.9, 76.8, 73.5, 70.1, 61.1, 56.2, 55.6, 49.6, 41.8, 38.3, 36.8, 36.2, 35.6, 35.2, 31.4, 31.4, 29.2, 27.5, 27.4, 23.9, 23.2, 22.6, 22.4, 20.6, 19.1, 18.5, 11.7

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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

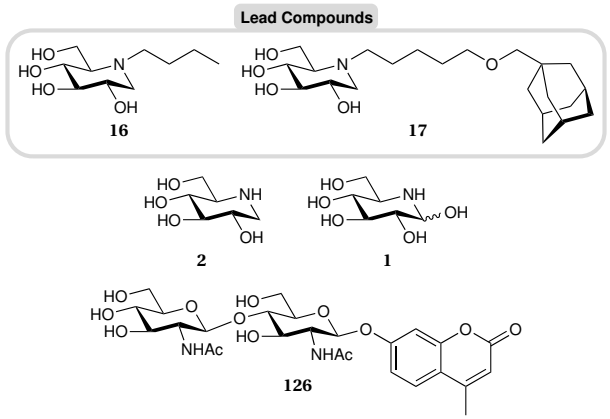


Figure 7.1: Compounds described in section 7.1.

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.

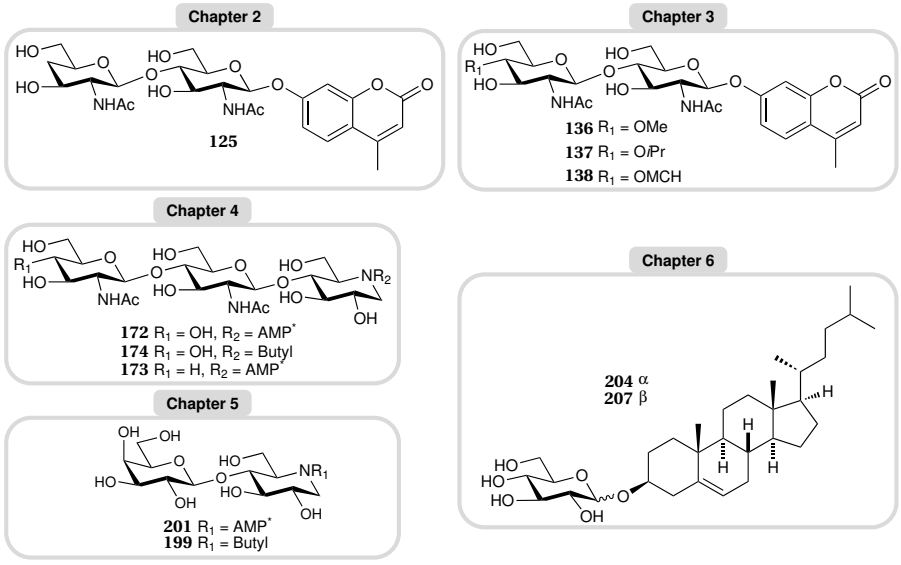


Figure 7.2: Overview of the chapters described in this thesis.
* AMP = *N*-5-(adamantan-1-yl-methoxy)-pentyl

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

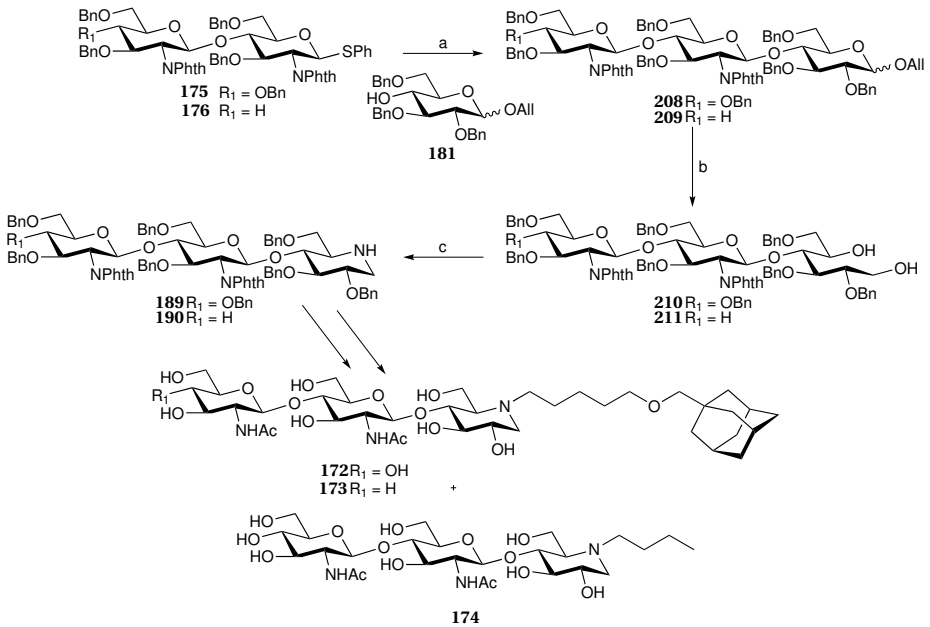
As shown in **Chapter 1** there are several synthetic strategies that can be used for the synthesis of *O*-glycosylated iminosugars. One can tackle this synthetic challenge of synthesizing *O*-glycosylated iminosugars by two different routes. The first strategy employs a carbohydrate that acts as a donor 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**.

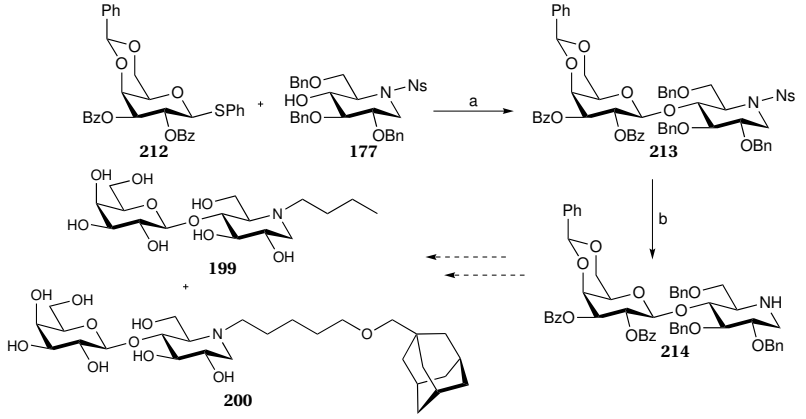


Reagents and conditions: a) NIS, TMSOTf, DCM, 0°C, b) (1) KO*t*Bu, DMSO, 100°C, (2) I_2, THF/H_2O, (3) $LiAlH_4$, THF; c) (1) DMSO, $(COCl)_2$, DCM, -75°C, (2) Et_3N, -75°C to rT, (3) $NaCNBH_3$, $HCOONH_4$, Na_2SO_4, MeOH, 0°C.

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]

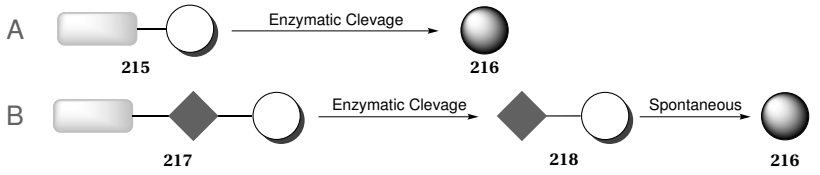


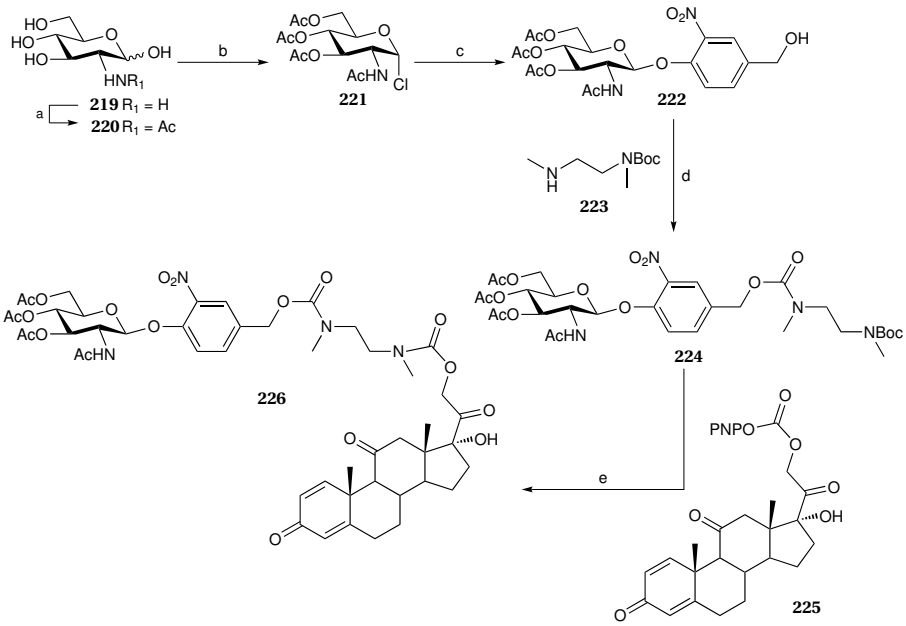
Figure 7.3: Schematic representation of enzymatic prodrug cleavage of bipartite and tripartite prodrugs. ○: inactive drug ; ●: active drug.

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.

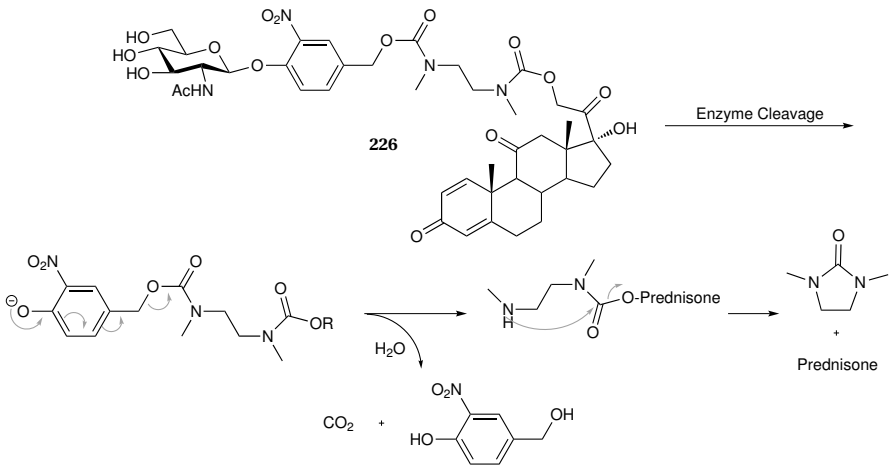


Figure 7.4: Release of prednisone upon cleavage of the substrate by chitinase.

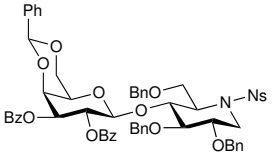
7.3 Experimental section

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}.

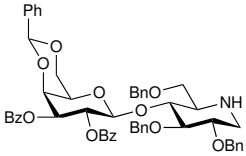
*Synthesis of Prodrug **199** and **200**. (Scheme 7.2)*

_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.

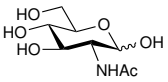
2,3,6-Tri-_O_-benzyl-4-_O_-(-di-_O_-benzoyl-4,6-_O_-benzylidene-β-D-galactopyranosyl)-1-deoxynorjirimycin (214):



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.

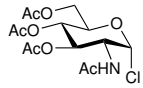
*Synthesis of Triparte Prodrug **226**. (Scheme 7.3)*

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



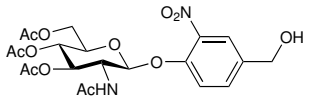
A mixture of 172 mL MeOH and 28 mL NaOMe (30% in MeOH) was added to 43.6 g (200 mmol) D-glucosamine hydrochloride **219**. The mixture was stirred at ambient temperature for 10 minutes, after which it was gently heated and filtrated. The filtrate was cooled to 0°C. Subsequently 250mL Ac_2O was added 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):



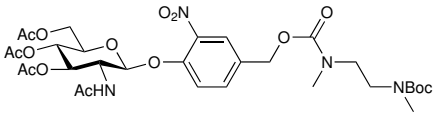
Crude compound **220** (7.5 g, 33.9 mmol) was dissolved in 25 mL distilled AcCl. The reaction mixture starts to boil spontaneously after 1 h. The reaction is left overnight yielding an amber colored clear liquid. The solution is diluted with 20 mL DCM and rapidly washed with 2x 20 mL of cold water, 2x 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):



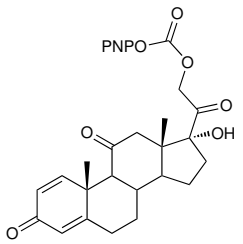
In a two phase-system of 50 mL DCM and 25 mL 1M $NaHCO_3$ were 4-hydroxy-3-nitrobenzyl alcohol (2.53 g, 15 mmol, 1.5 equiv) and tetra-butylammonium bromide (3.32 g, 10 mmol) dissolved. The mixture was vigorously stirred for 15 minutes. After which 3.7 g (10 mmol) of **221** in 5 mL DCM was added dropwise. 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):



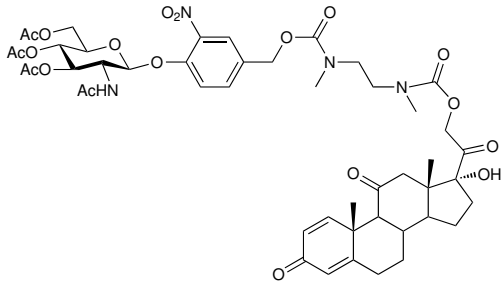
Compound **222** (0.848 g, 1.70 mmol) was dissolved in 15 mL DCM. The solution was cooled to ice bath temperature followed by dropwise addition of 0.71 mL Et_3N (5.1 mmol, 3 equiv). A solution of 0.50 g (2.55 mmol, 1.5 equiv) 4-nitrophenyl chloroformate in 2 mL DCM was added, over 15 minutes. The reaction 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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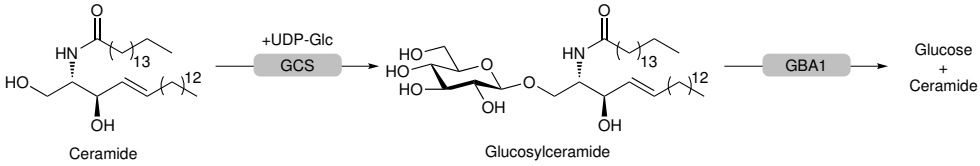
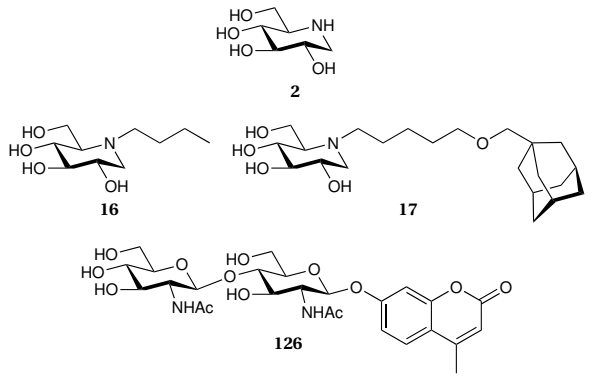
Samenvatting

Iminosuikers zijn suiker (koolhydraat) derivaten waarin de ring-zuurstof is vervangen door een stikstof. Deze groep van gehydroxyleerde alkaloïden heeft interessante biologische eigenschappen. Ze blijken uitstekende remmers te zijn voor verscheidene glycosidases en glycotransferases. Iminosuikers zijn in grote verscheidenheid te vinden in bladeren, vruchten en de stam van de moerbeiplant (*Morus* spp.). Eén van de iminosuikers die uit deze plant kan worden geïsoleerd is 1-deoxynojirimycin (DNJ) **2**, waarvan door de jaren heen verschillende derivaten zijn gesynthetiseerd. Twee van deze derivaten, NB-DNJ **16** en AMP-DNM **17** (Figuur **2**), zijn uitstekende remmers voor het enzym glycosylceramide synthase (GCS). NB-DNJ wordt tegenwoordig gegeven aan patiënten met de ziekte van Gaucher, een lysosomale stapelingsziekte die wordt veroorzaakt door een verstoorde activiteit van het enzym glucocerebrosidase (GBA1) (Figuur **1**). Hierdoor ontstaat een overschot aan glucosylceramide (GC) in de lysosomen, met als gevolg dat organen opzwellen (lever en nieren) en ontstekingen ontstaan. Door het remmen van GCS wordt er minder GC aangemaakt en zal de balans tussen anabolisme en katabolisme van GC worden hersteld.
Het in dit proefschrift beschreven onderzoek behelst de synthese van NB-DNJ en AMP-DNJ prodrugs. Voorts zijn er verschillende nieuwe substraten voor het menselijke chitinase enzym chitotriosidase gesynthetiseerd waarbij ook rekening is gehouden met de ongewenste afbraak door andere enzymen.



Figuur 1: Anabolisme en katabolisme van glucosylceramide.

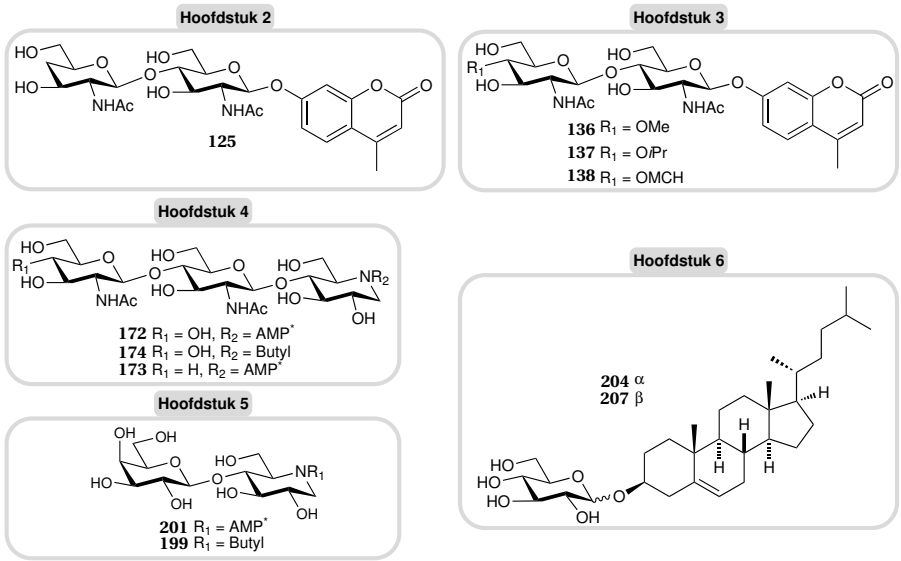
De inleiding van dit proefschrift (**Hoodstuk 1**) geeft een overzicht van alle in de literatuur beschreven *O*-geglycosyleerde iminosuikers. Er worden verschillende voorbeelden gegeven van chemische en enzymatische synthese strategieën waarbij iminosuikers van één of meerdere "gewone" suikers worden voorzien. Ook wordt een kort overzicht gegeven van geglycosyleerde iminosuikers die op een andere manier met elkaar zijn verbonden.



Figuur 2: Moleculen beschreven in de samenvatting.

Chitine is de op een na meest voorkomende bio-polymeer en kan worden gevonden in het exoskelet van geleedpotigen, zoals insecten, kreeftachtigen en spinnen, waar het zorgt voor stevigheid. Tot het einde van de vorige eeuw werd er gedacht dat het menselijk lichaam niet in staat was om chitine of derivaten daarvan af te breken. De eerste menselijke chitinase werd bij toeval gevonden tijdens onderzoek naar een verhoogde glycosidase-activiteit in het serum van Gaucher-patiënten. Dit enzym werd geïdentificeerd als chitotriosidase (CHIT1) en wordt tegenwoordig gebruikt als indicator om de ernst van de ziekte en de werking van de medicatie van Gaucher patiënten te visualiseren. Lange tijd werd umbelliferyl chitobiose **126** (Figuur **2**) gebruikt als fluorescent substraat, maar dit substraat bleek bij verhoogde concentraties een onjuist beeld te geven. De oorzaak hiervan bleek de transglycosylase activiteit van CHIT1 te zijn, waarbij chitotriose of chitobiose aan het substraat wordt gekoppeld. Door de alcoholfunctie op de 4-plaats van het niet reducerende suiker te verwijderen, kon transglycosylering worden voorkomen. Het verbeterde 4'-deoxychitobyosyl umbelliferone **125** bleek een beter substraat voor CHIT1. Eerder beschreven syntheseroutes leidden echter slechts tot milligrammen van dit substraat (**125**, Figuur **3**). **Hoofdstuk 2** behelst een verbeterde en op te schalen synthese route naar het fluorescente substraat **125**. De route is gebaseerd op het gebruik van een centrale bouwsteen die kan worden gebruikt voor de synthese van zowel de donor als de acceptor. De cruciale koppeling tussen het chitobiose deel en de fluorofoor vind plaats onder geoptimaliseerde "phase transfer conditions".

Daarna wordt in **Hoofdstuk 3** het ontwerp, de synthese en de biologische evaluatie van drie nieuwe CHIT1 substraten beschreven (**136**, **137** en **138**, Figuur **3**). Deze chitobiosederivaten zijn voorzien van een fluorofoor op het anomere centrum en hebben een verschillende ether functies op de 4-positie van de niet-reducerende suiker. Deze modificaties omvatten de relatief kleine methylgroep (OMe), de grotere isopropyl (O*i*Pr) en de meeste ruimte innemende methylcyclohexaangroep (OMCH).



Figuur 3: Een overzicht van de stoffen beschreven in dit proefschrift.
* AMP = *N*-5-(adamantan-1-yl-methoxy)-pentyl.

Voor de synthese van substraten **136**, **137** en **138** werd gebruik gemaakt van een 1,6-anhydro glucosamine derivaat, dat dienst deed als uitgangsstof voor de synthese van zowel de donoren als de acceptoren. Uit biologische testen bleek dat alle nieuwe substraten de Michaelis-Menten kinetiek volgen, maar ook dat de nieuwe substraten stabieler zijn voor de ongewenste stapsgewijze degradatie door β-hexosaminidase.

De lokaal verhoogde activiteit van CHIT1 en de directe correlatie met de ernst van de ziekte van Gaucher, biedt de mogelijkheid voor het lokaal activeren van medicatie door gebruik te maken van een zo genoemde "prodrug". **Hoofdstuk 4** behandelt het ontwerp en de synthese van mogelijke prodrugs voor de ziekte van Gaucher, waarbij chitobiose of 4'-deoxychitobiose wordt gebruikt als enzym substraat en NB-DNJ (**16**) of AMP-DNJ (**17**) als medicatie. Door deze moleculen *via* een glycosidische binding aan elkaar te koppelen ontstaan er inactieve prodrugs (**172**, **174** en **173**, Figuur **3**). De actieve verbinding **16** of **17** komt vrij wanneer het enzym, CHIT1, het chitobiose deel er weer afknipt. Hierdoor is de medicatie alleen lokaal actief waardoor de kans op mogelijke bijwerkingen wordt verminderd.

Het is bekend dat sommige iminosuikers een bittere smaak hebben. De afkeer van de mens tegen stoffen met een bittere smaak is een oerinstinct dat ons waarschuwt voor potentieel gevaarlijke stoffen. Met als doel de bittere smaak van NB-DNJ en AMP-DNJ te maskeren wordt in **Hoofdstuk 5** de synthese van twee verschillende prodrugs (**201** en **199**) uiteengezet, waarbij galactose is gekoppeld aan een iminosuiker (Figuur **3**). De aldus verkregen prodrugs zijn lactose derivaten die bekend staan om hun mild zoete smaak. Door lactose te gebruiken als uit-

gangsstof kon een glycosyleringsstap worden vermeden. Uit de biologische resultaten bleek dat de AMP-DNJ prodrug (**201**) kan worden geknipt door het enzym lactase-phlorizin hydrolase (LPH), dat wordt gevonden in het darmslijm van ratten.

Uit onderzoek om een eenduidige oorzaak voor de ziekte van Parkinson en het metabolisme van glycosylceramide te vinden, is gebleken dat patiënten met de ziekte van Parkinson een hoge dosis geglucosyleerd cholesterol hebben. Recent onderzoek toont aan dat tijdens de biosynthese van deze geglucosyleerde cholesterol niet de verwachte UDP-glucose als glucosedonor wordt gebruikt, maar glycosylceramide. Met de synthese van α (**204**) en β geglucosyleerde cholesterol (**207**) in **Hoofdstuk 6** is een interne standaard verkregen, die een bijdrage te levert aan verder onderzoek.

List of Publications

1. **Study of the Glycosidation Properties of 1-Thiomannosazidopyranosides and 1-Thiomannosaziduronic Acid Esters**
   van den Bos, L. J.; Duivenvoorden, B. A.; de Koning, M. C.; Filippov, D. V.; Overkleeft, H. S.; van der Marel, G. A.
   Eur. J. Org. Chem. **2007**, *1*, 116-124

2. **A Preparative Synthesis of Human Chitinase Fluorogenic Substrate (4'-Deoxychitobiosyl)-4-methylumbelliferone**
   Duivenvoorden, B. A.; Dinkelaar, J.; Wennekes, T.; Overkleeft, H. S.; Boot, R. G.; Aerts, J. M. F. G.; Codée, J. D. C.; van der Marel, G. A.
   Eur. J. Org. Chem. **2010**, *13*, 2565-2570

3. **A Panel of Subunit-selective Activity-based Proteasome Probes**
   Verdoes, M.; Willems, L. I.; van der Linden, W. A.; Duivenvoorden, B. A.; van der Marel, G. A.; Florea, B. I.; Kisselev, A. F.; Overkleeft, H. S.
   Org. Bio. Chem. **2010**, *8*, 2719-2727

4. **Transglycosylase Features of Human, Murine and Bovine Chitinases: Design of Improved Fluorogenic Substrates**
   Duivenvoorden, B. A.; Ghauharali, K.; Bussink, A. P.; Codée, J. D. C.; van der Marel, G. A.; Scheij, S.; Verhoek, M.; Overkleeft, H. S.; Groener, J. E.; Aerts, J. M. F. G.; Boot, R. G.
   Manuscript in preparation

Curriculum Vitæ

Boudewijn Adriaan Duivenvoorden werd geboren op 23 augustus 1983 in Haarlem. Na het behalen van zijn HAVO diploma met twee verschillende profielen (N&T en N&G) aan het Coornhert lyceum te Haarlem in 2001, begon hij aan de opleiding HLO (laboratorium onderwijs) aan de Hogeschool van Leiden. Na 2,5 jaar ging hij een zgn. "Sandwich-Programma" volgen, waarbij hij gelijktijdig alle scheikunde colleges aan de Universiteit van Leiden volgde. Tijdens zijn laatste jaar aan de HLO nam hij deel aan een afstudeerstage in de vakgroep Bio-Organische Synthese van prof. dr. H.S. Overkleeft en prof. dr. G.A. van der Marel. De afstudeerstage werd geleid door dr. L.J. van den Bos. Zijn betrokkenheid bij deze stage betrof de synthese van de repeterende eenheid van het zwitterionische polysaccharide A2.

Direct na zijn HLO afstudeerstage nam hij deel aan een universitaire hoofdvakstage onder leiding van dr. U. Hillaert. Het onderzoek betrof de synthese van op prednison gebaseerde pro-drugs. In het kader van een bijvakstage onder begeleiding van dr. M. Verdoes, verrichtte hij onderzoek naar specifieke proteasoom subunit remmers. Zijn titel master of science behaalde hij in februari 2007.

Direct daarop aansluitend tot en met augustus 2011 voerde hij, als assistent in opleiding (aio), het in dit proefschrift beschreven onderzoek in de vakgroep Bioorganische Synthese (BioSyn), uit. Het onderzoek stond onder leiding van prof. dr. H.S. Overkleeft, prof. dr. G.A. van der Marel en dr. J.D.C. Codée.

In het kader van dit onderzoek nam hij in september 2007 deel aan de 14^{th} European Carbohydrate Symposium in Lübeck, Duitsland. Delen van dit onderzoek zijn gepresenteerd op de jaarlijkse HRSMC meeting "Design and Synthesis" in Lunteren.

Dankwoord

Het is eindelijk zover, de laatste maar waarschijnlijk voor de meeste lezers, de eerst gekozen pagina van mijn proefschrift. Mijn dankwoord wil ik gebruiken om een flink aantal mensen de revue te laten passeren. Velen zijn op mijn pad gekomen tijdens mijn promotieperiode, andere heb ik de afgelopen periode nóg beter leren kennen.

Allereerst bedank ik mijn ouders voor hun onvoorwaardelijke steun. Mam, vanwege jouw altijd luisterend oor en de talloze keren dat je samen met mij, mijn schoolwerk nakeek. Pa, omdat het bijbrengen van jouw passie voor alles wat vliegt, kruipt, loopt en zwemt, mij uiteindelijk in de scheikunde heeft doen belanden. En daarnaast natuurlijk ook voor de vele biertjes, als "het portemonneetje" weer eens leeg was. Broer Jurriaan voor het samen lachen en gewoon broertjes zijn.

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