Synthesis & biological applications of glycosylated iminosugars

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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

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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]

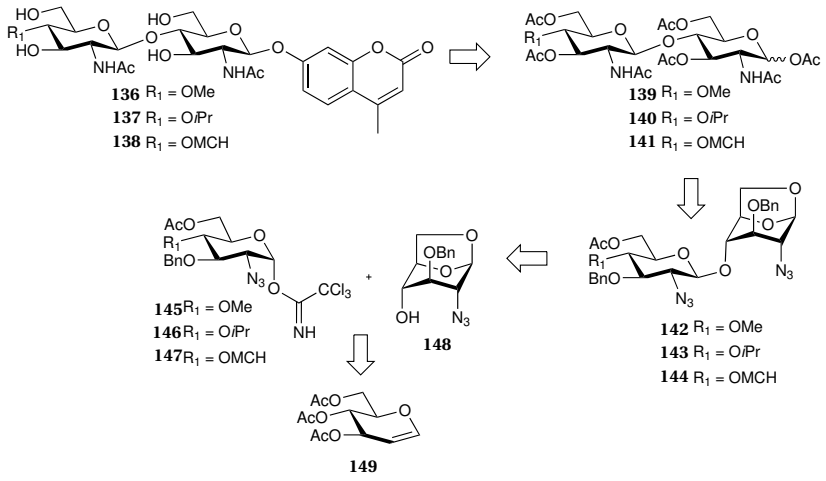
To gain a bigger pool of effective CHIT1 fluorescent substrates, this chapter describes the synthesis of three novel human chitinase substrates **136**,**137** and **138** bearing an anomeric 4-MU for fluorometric read-out. The three substrates have a different modification on the 4'-OH of the non-reducing 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.

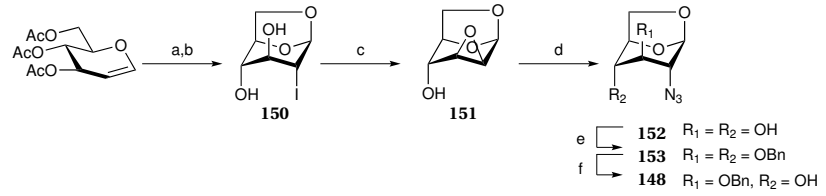
First acceptor **148** was synthesized *via* a modified literature procedure.[20–22] Deacetylation of tri-*O*-acetyl-D-glucal and treatment with bis(tributyltin)oxide in

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**.

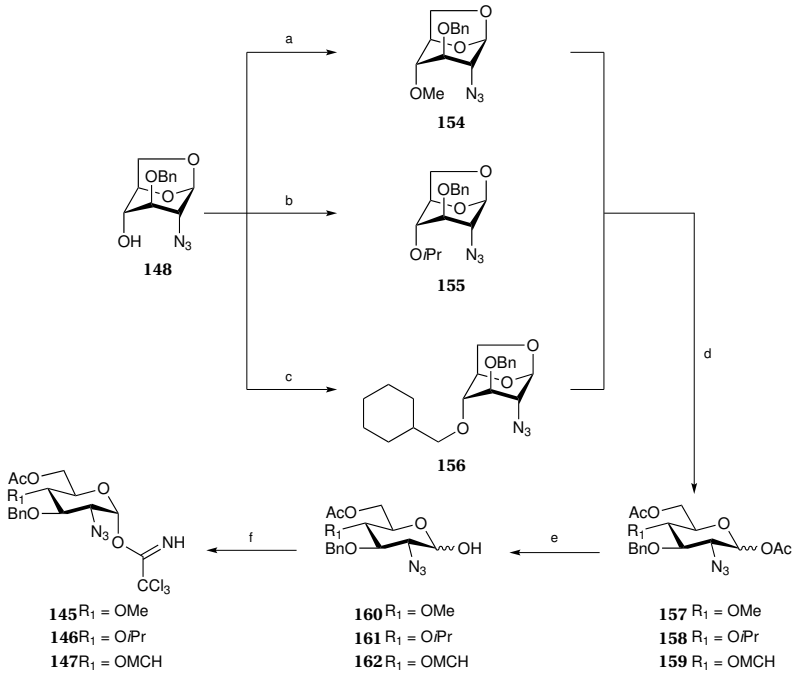


Reagents and conditions: a) $MeOH/H_2O/Et_3N$; b) (1) $(Bu_3Sn)_2O$, Δ, ACN; (2) I_2, DCM, 4 °C; c) $NaHCO_3$, DMF/H_2O, 98% over three steps; d) NaN_3, DMF/H_2O, 120 °C, 52%; e) BnBr, NaH, DMF, 0 °C, 80%; f) $TiCl_4$, DCM, 75%.

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**.



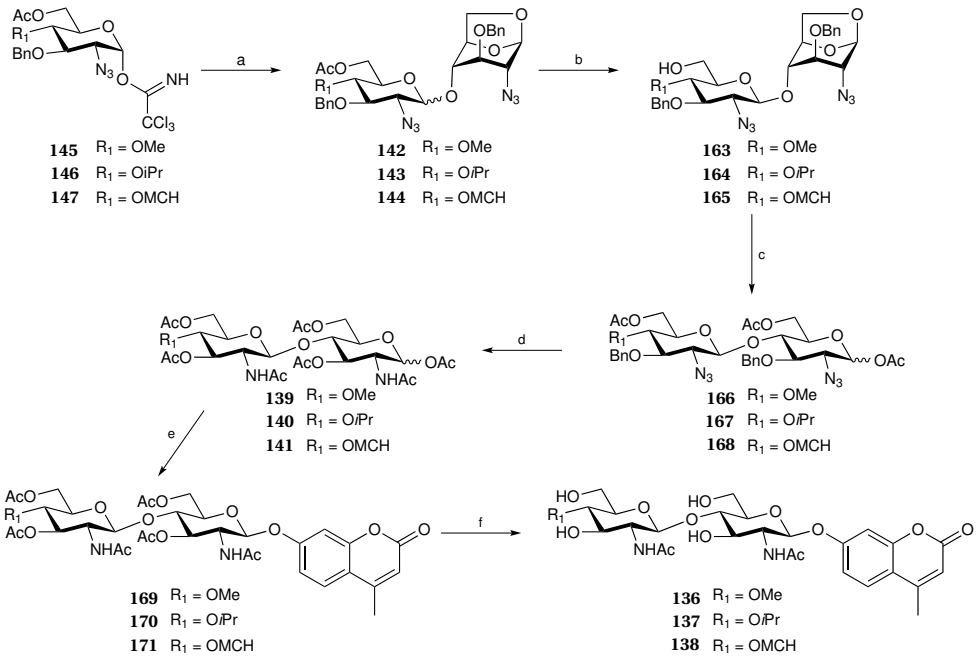
Reagents and conditions: a) MeI, NaH, DMF, 91%; b) I*i*Pr, NaH, DMF, 60%; c) BrMCH, NaH, DMF, 75%; d) Ac_2O, TFA (10% v/v), **157**: 90%, **158**: 86%, **159**: 72%; e)THF, piperidine (6%v/v), **160**: 71%, **161**: quant., **162**: 98%; f) CCl_3CN, DBU, DCM, **145**: 83%, **146**: 70%, **147**: 71%.

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).

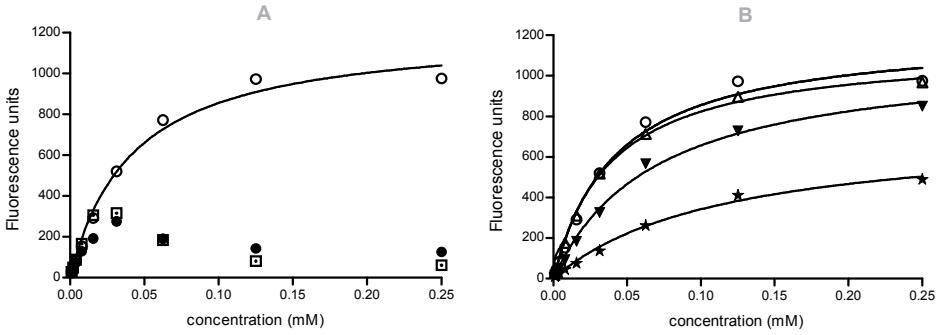


Figure 3.1: (A) Michaelis-Menten kinetics of 4-MU-deoxychitobiose; ○:**125**, ●:4-MU-chitobiose **126**, ⊡:4-MU-chitotriose; (B) Michaelis-Menten kinetics chitinase substrates bearing a modification at the 4-position of the non-reducing end; ○:**125**, △:**136**, ▼:**137**, ⋆:**138**.

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

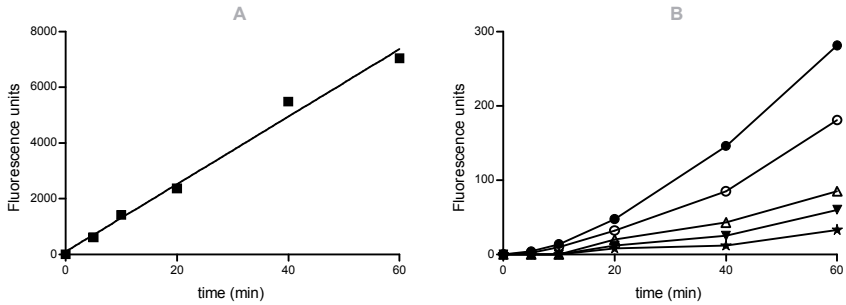


Figure 3.2: (A) Hydrolysis of 4-MU-GlcNAc (0.135 mM) over 60 minutes by jack bean β-hexosaminidase; ■:4-MU-GlcNAc; (B) Hydrolysis of chitobiose and derivatives bearing a modification at the 4-position of the non-reducing end; •:4-MU-chitobiose **126**, ○:**125**, △:**136**, ▼:**137**, ⋆:**138**.

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.

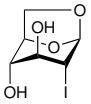
The newly designed compounds **136**, **137** and **138** do not act as acceptors in transglycosylation and offer substrates for CHIT1 that are hydrolysed according to Michaelis-Menten kinetics. An additional advantage is that the novel compounds are lesser substrates for β-hexosaminidases. In situations 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*-

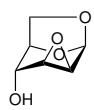
dimethylformamide (DMF), methanol (MeOH), pyridine (pyr) and tetrahydrofuran (THF) were obtained from Biosolve. THF was distilled over $LiAlH_4$ before use. Dichloromethane was boiled under reflux over P_2O_5 for 2 h and distilled prior to use. Molecular sieves 3Å were flame dried under vacuum before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

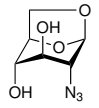
1,6:2,3-Bis-anhydro-β-D-mannopyranose (151):



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, $J =$

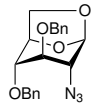
8.8 Hz, 1H, CH, C'-4), 3.44 (t, $J = 2.9$, 1H, CH, C'-3), 3.69-3.95 (m, 2H, CH_2, C'-6), 5.70 (d, $J = 2.9$ Hz, CH, C'-1), ^{13}C NMR (50 MHz, $CDCl_3$) δ 48.49 (CH, C'-2), 53.23 (CH, C'-3), 64.69 (CH_2, C'-6), 65.84 (CH, C'-4), 73.21 (CH, C'-5), 96.56 (CH, C'-1).

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



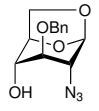
The bis-anhydro sugar **151** (1.15 g, 8 mmol) was heated to reflux temperature in a 10:1 MeOH:H_2O (40 mL) solution containing 5.20 g NaN_3 (80 mmol, 10 equiv), and 4.24 g NH_4Cl (80 mmol, 10 equiv). After ^{1}H NMR showed complete conversion to compound **152** (4.5 days), the solution was cooled, 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):



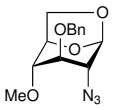
The anhydro sugar **152** (9.35 g, 50 mmol) was dissolved in DMF and cooled to 0 °C. Benzyl bromide (15 mL, 125 mmol, 2.5 equiv) was added followed by portion wise addition of NaH (6 g, 150 mmol, 3 equiv). The reaction was stirred for 3 h, allowing the mixture to warm to rT, after which it was cooled (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):



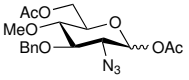
Compound **153** (10.28 g, 28 mmol) was coevaporated thrice with Tol, after which it was dissolved in dry DCM (450 mL). Next $TiCl_4$ (3.2 mL, 29.4 mmol, 1.05 equiv) was carefully added, the mixture was stirred for 1.5 h at rT. The reaction mixture was than poured into cooled (0 °C) H_2O after which 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):



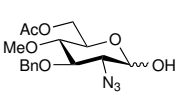
Compound **148** (8.15 mmol, 2.26 g) was dissolved in DMF (25 mL) and cooled using an ice-bath, after stirring for 15 minutes NaH (60% dispersion in mineral oil) (0.49 g, 12 mmol, 1.5 equiv) was added portion wise. After 30 minutes the gas development stopped and MeI (0.61 mL, 9.8 mmol 1.2 equiv) was 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.

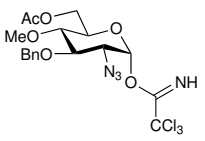
6-*O*-Acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-methyl-α/β-D-glucopyranose (160):



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

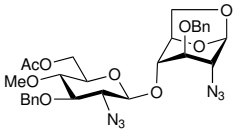
NMR (100 MHz, $CDCl_3$) δ 171.2 (C_q, Ac), 171.1 (C_q, Ac), 137.6 (C_q), 137.6 (C_q Bn), 128.4-127.9 (CH_{arom} Bn), 95.9 (C-1$_\beta$), 91.7 (C-1$_\alpha$), 82.6 (C-3,4$_\beta$), 80.4 (C-3,4$_\alpha$), 79.6 (C-3,4$_{\alpha/\beta}$), 75.4 (CH_2 Bn$_{\alpha/\beta}$), 75.4 (CH_2, Bn$_{\alpha/\beta}$), 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.

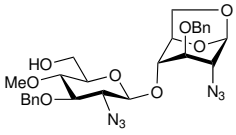
1,6-Anhydro-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-methyl-β-D-glucopyranosyl)-β-D-glucopyranose (142):



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,

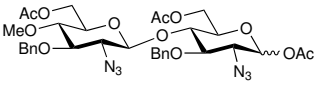
898.8, 738.7, 696.3; HRMS: $C_{29}H_{34}N_6O_9 + Na^+$ requires 633.2279, found 633.2279; $[\alpha]_D^{23}$ + 11.4 °(c = 1, $CHCl_3$).

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):



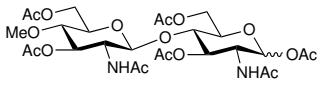
Disaccharide **142** (1.84 g, 3.02 mmol) was dissolved in MeOH (15 mL) and a catalytic amount of NaOMe (30% in MeOH) was added. The reaction mixture was stirred for 1 h at ambient temperature, after which it was neutralized (pH∼7) using Amberlite® IR-120 H^+ resin. Filtering off the resin, concentration 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):



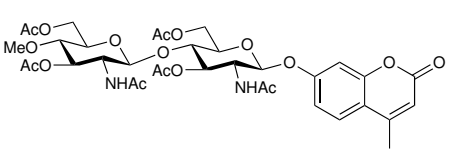
To a solution of compound **163** (1.0 g, 1.7 mmol) in Ac_2O (8.5 mL) TFA (1.28 mL, 15% v/v) was added. The reaction was stirred for 18 h after which it was diluted with toluene and concentrated. After purification using silica gel chromatograph (EtOAc/PE 30%) disaccharide **166** was obtained in 90% 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.

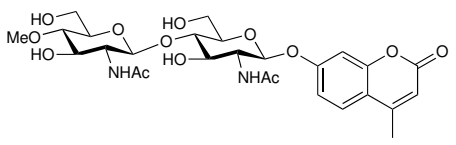
2-Acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-4-*O*-(2-acetamido-2-deoxy-3,6-*O*-di-acetyl-4--*O*-methyl-β-D-glucopyranosyl)-1-*O*-4-methylumbelliferyl-β-D-glucopyranoside (169).



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

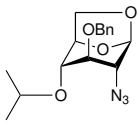
H_2O. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (MeOH/$CHCl_3$ 3%) yielded **169** (27 mg, 25%) as a white amorphous solid. TLC: MeOH/DCM 5%; ^{1}H NMR (600 MHz, (d_4), MeOD) δ 8.06 (d, $J = 9.2$ Hz, 1H, NH), 7.62 (d, $J = 8.7$ Hz, 1H, 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).



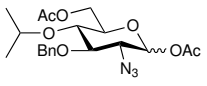
To a suspension of **169** (12mg, 15.70 μmol) in MeOH (1 mL) was added NaOMe (30 wt% in MeOH) (173 μL, 0.40 μmol). The reaction was stirred with the exclusion of light. When LCMS (gradient 0 to 50% MeOH) showed complete conversion to product the mixture was quenched with AcOH (5 μL, 80 μmol). The reaction 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):



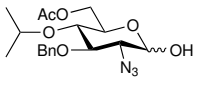
Compound **148** (1.5 g, 5.3 mmol) was dissolved in 25 mL DMF and cooled using an ice-bath and after stirring for 5 minutes NaH (60% dispersion in mineral oil) (234 mg, 5.86 mmol, 1.1 equiv) was added. After 30 minutes the gas formation stopped and 2-iodopropane (586 μL, 5.86 mmol, 1.1 equiv) was added 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.

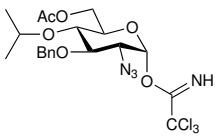
6-*O*-Acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-isopropyl-α/β-D-glucopyranose (161):



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

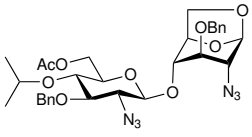
30%) yielded compound **161** in quantitative yield (1.02 g, 2.69 mmol) as a white foam. TLC: EtOAc/PE 40%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43-7.26 (m, 10H, CH_{arom} Bn), 5.26 (d, $J = 2.9$ Hz, 1H, H-1$_\alpha$), 4.90-4.75 (m, 4H, CH_2 Bn), 4.58-4.53 (m, 1H, H-1$_\beta$), 4.42-4.33 (m, 3H, CH_2 H-6), 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.

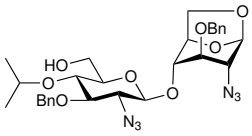
1,6-Anhydro-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-4- *O*-isopropyl-β-D-glucopyranosyl)-β-D-glucopyranose (143):



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),

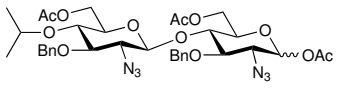
5.52 (s, 1H, H-1$_\alpha$), 5.49 (s, 1H, H-1$_\beta$), 4.87-4.75 (m, 2H, CH$_2$ Bn), 4.69 (t, J = 5.7 Hz, 1H, H-5$_\beta$), 4.63 (dd, J = 14.8, 7.5 Hz, 2H, CH$_2$ Bn), 4.40-4.32 (m, 2H, CH$_2$, H-6'$_\beta$, H-1$_\beta$), 4.12 (d, J = 7.1 Hz, 2H, CH$_2$, H-6$_\beta$), 4.09-4.02 (m, 1H, CH$_2$, H-6'$_\beta$), 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$_3$).

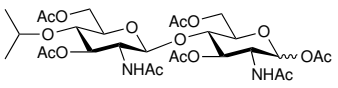
2-Azido-3-*O*-benzyl-2-deoxy-1,6-di-*O*-acetyl-4-*O*-(2-azido-3-*O*-benzyl-2-deoxy-4-*O*-isopropyl- β-D-glucopyranosyl)-α/β-D-glucopyranose (167):



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

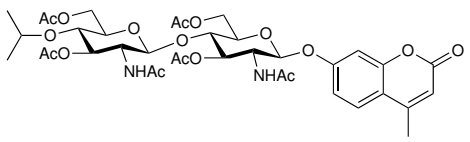
(m, 3H, CH_3 Ac), 2.11 (s, 3H, CH_3 Ac), 1.88 (s, 3H, CH_3 Ac), 1.17 (d, $J = 6.1$ Hz, 3H, CH_3 *i*Prop), 1.09 (d, $J = 6.1$ Hz, 3H, CH_3 *i*Prop); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6 (C_q, Ac), 170.5 (C_q, Ac), 168.8 (C_q, Ac), 138.2 (C_q), 137.6 (C_q), 128.6-127.5 (CH_{arom} Bn), 101.5 (C-1'), 90.2 (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):



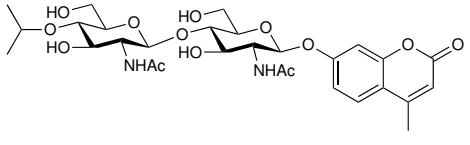
Compound **167** (90 mg, 0.15 mmol) was taken up in a mixture of dioxane/toluene/H_2O (5/2/1, v/v/v, 3 mL) and cooled with an ice-bath. After 10 minutes 1M PMe_3 (0.7 mL) intoluene was added and the reaction was stirred for 18 h at +4 °C. After TLC analysis showed conversion towards lower running, ninhydrin 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.

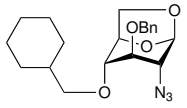
2-Acetamido-2-deoxy-4-*O*-(2-acetamido-2-deoxy-4-*O*-isopropyl-β-D-glucopyranosyl)-1-*O*- 4-methylumbelliferyl-β-D-glucopyranoside (137).



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.

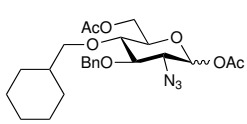
Purification by HPLC (gradient H_2O-MeOH + 0.1% TFA 80-20 → 60-40) evaporation of MeOH and lyophilizing H_2O yielded **137** (2.6 mg, 60%) as white fluffy solid. ^{1}H NMR (600 MHz, (d_6) DMSO) δ 7.9 (d, J = 9.1 Hz, 1H, NH), 7.8 (d, J = 9.2 Hz, 1H, NH), 7.7 (d, J = 8.8 Hz, 1H, 4-methylumbelliferyl), 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, $CHCl_3$).

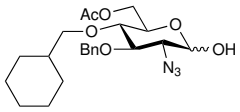
1,6-Di-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-methyl-cyclohexane-α/β-D-glucopyranose (159):



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,

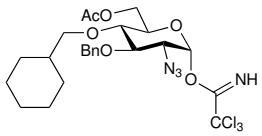
MCH), 2.17 (s, 1H, CH_3 OAc_β), 2.16 (s, 3H, CH_3 Ac_α), 2.08 (s, 4H, $Ac_{\alpha/\beta}$), 1.82-0.83 (m, 16H, CH_2, MCH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7 (C_q, Ac), 169.0 (C_q, Ac), 168.9 (C_q, Ac), 137.7(C_q), 137.7 (C_q), 128.6-128.2 (CH_{arom} Bn), 92.8 (C-1_β), 90.5(C-1_α), 83.1 (C-4_β), 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.

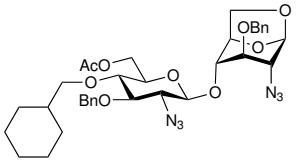
6-*O*-Acetyl-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-methyl-cyclohexane-1-*O*-(*N*- trichloroacetimidoyl)-α-D-glucopyranoside (147):



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

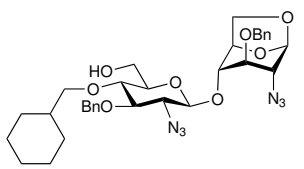
(m, 1H, CH, MCH), 1.28-1.11 (m, 4H, CH_2, MCH), 1.01-0.86 (m, 2H, CH_2, MCH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2 (C_q, C=N), 160.4 (C_q, Ac), 137.5 (C_q), 128.3-127.8 (C-1), 94.5 (C_q, CCl_3), 79.8 (C-3/4/5), 79.2 (CH_2, MCH), 77.9 (C-3/4/5), 75.3 (CH_2 Bn), 71.8 (C-3/4/5), 62.7 (C-2), 62.2 (C-6), 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, $CHCl_3$).

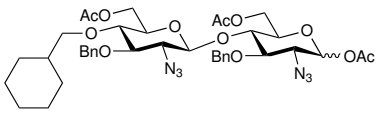
1,6-Anhydro-2-azido-3-*O*-benzyl-2-deoxy-4-*O*-(2-azido-3-*O*-benzyl-2-deoxy-4-*O*-methyl- cyclohexane-β-D-glucopyranosyl)-β-D-glucopyranose (165):



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,

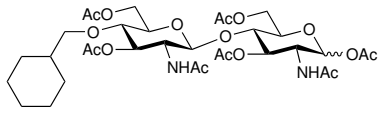
H-2), 1.75 (dd, $J = 30.5$, 13.3 Hz, 4H, CH_2, MCH), 1.53 (s, $J = 2.9$ Hz, 1H, CH, MCH), 1.33-1.11 (m, 4H, CH_2, MCH), 1.03-0.87 (m, 2H, CH_2, MCH); ^{13}C NMR (100 MHz, d_4, MeOD) δ 139.7 (C_q), 139.4 (C_q), 129.5-128.8 (CH_{arom} Bn), 102.7 (C-1'), 101.9 (C-1), 84.4 (C-3'/4'/4), 79.9 (CH_2, MCH), 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.

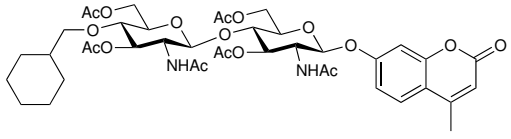
2-Acetamido-2-deoxy-1,3,6-tri-*O*-acetyl-4-*O*-(2-acetamido-2-deoxy-3,6-*O*-di-acetyl-4--*O*- methyl-cyclohexane-β-D-glucopyranosyl)-α/β-D-glucopyranose (141):



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

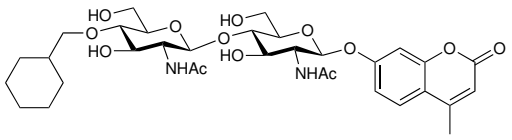
2-Acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-4-*O*-(2-acetamido-2-deoxy-3,6-*O*-di-acetyl-4-*O*-methyl-cyclohexane-β-D-glucopyranosyl)-1-*O*-4-methylumbelliferyl-β-D-glucopyranoside (171):



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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