Synthesis & biological applications of glycosylated iminosugars

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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

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[⋆] 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.

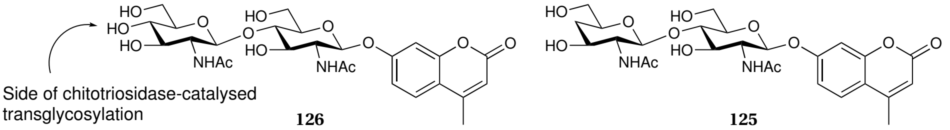


Figure 2.1: Umbelliferyl chitobioside fluorogenic substrates **126** and **125**.

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 phenols 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.

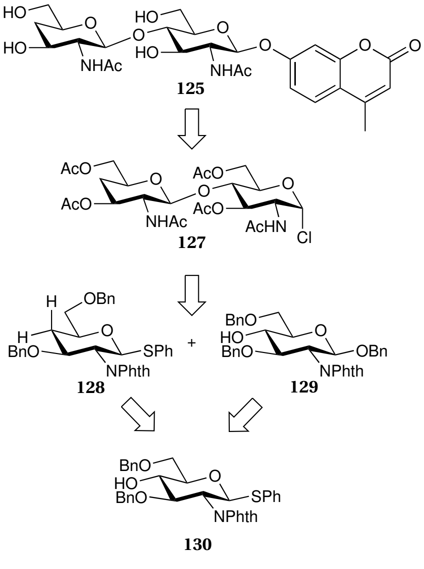


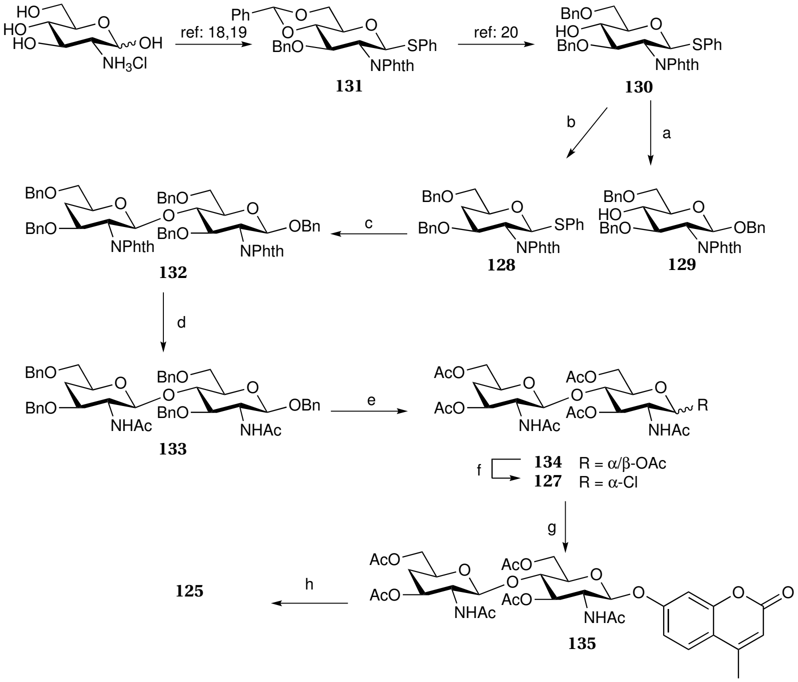
Figure 2.2: Outline for the large-scale synthesis of umbelliferyl chitobioside fluorogenic substrate **125**.

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 treatment 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).

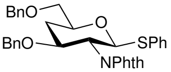
2.3 Conclusion

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

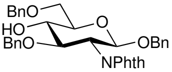
All reagent were of commercial grade and used as received (Acros, Fluka, Merck, Schleicher & Schuell) unless stated otherwise. Diethyl ether (Et_2O), light petroleum ether (PE 40-60), en toluene (Tol) were purchased from Riedel-de Haën. Dichloromethane (DCM), *N*,*N*-dimethylformamide (DMF), methanol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



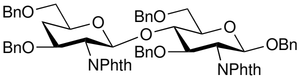
Glycoside **130** (25.4 g, 43.8 mmol) was coevaperated thrice with dioxane, then taken up in THF (220 mL). Imidazole (0.298 g, 4.38 mmol) and CS_2 (7.9 mL, 131 mmol) were added after which the mixture was cooled to 0°C. NaH (2.63 g, 60% dispersion in oil, 65.7 mmol) was added and the reaction was kept 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).



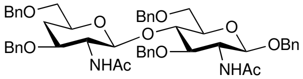
Glycoside **130** (23.6 g, 40.5 mmol) was coevaperated thrice with toluene. DCM (810 mL), BnOH (21 mL, 202 mmol) and NIS (10.9 g, 48.6 mmol) were added. The mixture was stirred over activated 3Å molsieves for 30 min. After cooling to 0°C a catalytic amount of TMSOTf (0.81 mL, 4.5 mmol) was added. After 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%; ^1H 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).



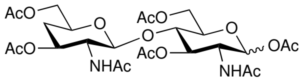
A mixture of donor **128** (20.1 g, 35.4 mmol) and acceptor **129** (20.6 g, 35.4 mmol) were coevaperated three times with toluene. DCM (350 mL) and NIS (9.56 g, 42.5 mmol) were added and the mixture was stirred over activated 3Å molsieves for 30 min. The mixture was cooled to 0°C before a catalytic 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%; ^1H 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).



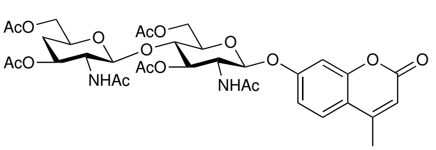
Disaccharide **132** (31.7 g, 30.6 mmol) was dissolved in *n*-BuOH (275 mL) and ethylene diamine (30 mL). This mixture was refluxed overnight and subsequently concentrated *in vacuo*. The reaction was then coevaporated thrice with toluene and taken up in MeOH (300 mL). At 0°C Ac_2O (30 mL, 300 mmol) 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).



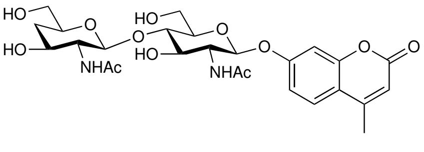
Disaccharide **133** (22.9 g, 26.6 mmol) was disolved in THF (250 mL) then MeOH (250 mL), AcOH (9 mL, 106 mmol) and $Pd(OH)_2$ (1 g, 20% on activated carbon, 1.33 mmol) were added. The mixture was shaken overnight on a Parr apparatus® under 5 bar hydrogen pressure. The resulting mixture was filtered 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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