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A Preparative Synthesis of Human Chitinase Fluorogenic Substrate (4′-Deoxychitobiosyl)-4-methylumbelliferone

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To meet the increasing clinical demand for the diagnostic agent (4′-deoxychitobiosyl)-4-methylumbelliferone, a flexible and scalable route of synthesis is needed. In this paper such a route is presented. The key to the route is the use of a partially protected thiophenyl glucosamine as starting material for the preparation of both the reducing and nonreducing end building blocks of the 4′-deoxychitobiose disaccharide.

Introduction

The existence of endogenous chitinases in mammals was only discovered a decade ago.[1] Firstly identified was the enzyme chitotriosidase (CHIT1), a chitinase that is strongly expressed and secreted by lipid-laden tissue macrophages that are found in patients suffering from the glycolipid storage disorder Gaucher disease.[2–4] Relatively more modest elevations in plasma chitotriosidase have subsequently been detected in other disease conditions involving macrophages, including several lysosomal storage disorders,[5–8] fungal and parasite infections like malaria and visceral Leishmaniasis,[2,9,10] thalassemia,[11] sarcoidosis,[12] and atherosclerosis.[13] The existence of a second mammalian chitinase, named AMCase (acidic mammalian chitinase), has been recognized recently,[14,15] and its role in the etiology of asthma has recently been proposed.[16]

Measurement of plasma chitinase activity in man is now widely applied for clinical purposes. The most important application is in the monitoring of severity of disease in Gaucher patients.[2,17] Chitotriosidase activity levels in plasma of Gaucher patients correlate to the progression of the disease and the effect of therapeutic intervention. The enzyme is thus an ideal marker through which Gaucher patients are identified and their reaction towards therapeutic agents is monitored.[18] Currently, two therapies for the treatment of Gaucher patients are applied, namely, enzyme replacement therapy and substrate reduction therapy.[19–24] Both therapies are expensive and therefore monitoring their effect (or optimal dosage and treatment regimen) through measuring serum chitotriosidase activity levels has considerable clinical value.

In the years immediately following our discovery of chitotriosidase levels as Gaucher marker we made use of the umbelliferyl chitobioside fluorogenic substrate **1** (Figure 1).[2] However, we soon found out that human chitotriosidase possesses intrinsic transglycosylase activity,[25] in that chitobiose or higher oligomers are formed through hydrolysis of **1** and connected to the nonreducing 4′-hydroxy group of another substrate to form higher oligomers. This side reaction complicates interpretation of the kinetics of the enzyme-mediated generation of the fluorescent umbelliferonate anion and in fact also renders the efficiency of the fluorogenic substrate suboptimal. To circumvent this problem we reported the development of fluorogenic substrate **2** (Figure 1), in which the 4′-OH group is removed, preventing chitotriosidase-mediated transglycosylation.[25] Deoxychitobiosyl umbelliferone **2** is a superior chitotriosidase

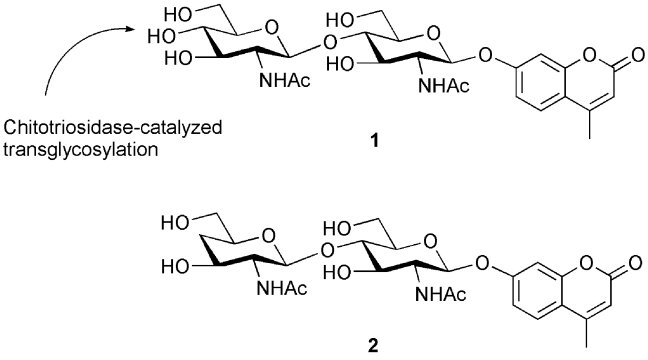

Figure 1. Umbelliferyl chitobioside fluorogenic substrate **1** and **2**.

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substrate compared to **1**. The same holds for the enzyme AMCase.[17] Given the rapidly growing interest to monitor plasma chitotriosidase also in other disease conditions, and given the present interest in AMCase in relation to asthma, deoxychitobiosyl umbelliferone **2** has become a very desired fluorogenic substrate and we foresee that larger quantities will be needed on an annual basis in the near future. We were thus in need of a route for the synthesis of compound **2** that is more efficient and reliable than the original route we reported in our transglycosylase activity studies.

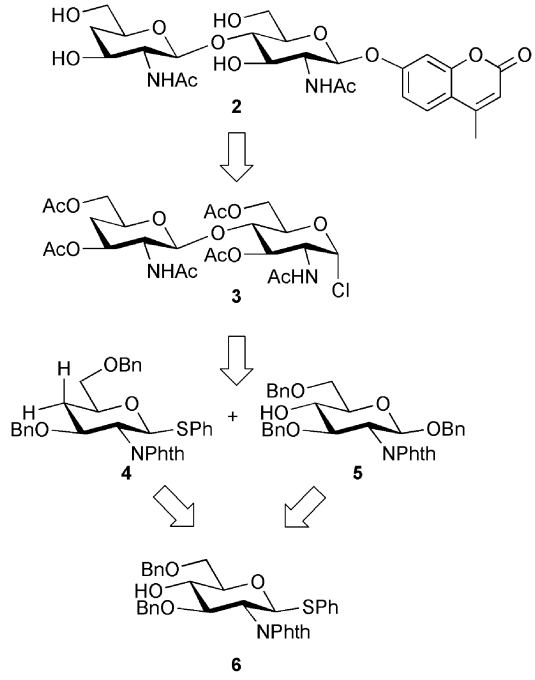
In our original work[25] we started from the disaccharide chitobiose, which we converted in nine steps into the target compound. Although sufficiently effective for the preparation of several milligrams, the route falls short when aiming for larger quantities. The nine-step sequence is quite inefficient (3% overall yield), and furthermore, the starting disaccharide, chitobiose, is rather expensive. We thus devised a new route of synthesis, the details of which we disclose here.

Results and Discussion

Our synthetic plan is outlined in Scheme 1. We envisaged that the formation of the glycosidic linkage between the chitobiose and the umbelliferyl chromophore could best be achieved by S_N2 displacement of an anomeric chloride by the umbelliferyl phenolate anion, as the poor nucleophilicity of the protonated phenol precludes the use of Lewis acidic glycosylation methods. For the construction of the chitobiose core we selected thiophenyl glycoside building blocks, because the anomeric thiophenyl group can be easily introduced early in the synthesis; it is also stable to the reaction conditions employed throughout the synthesis of the synthons and can be selectively activated with a variety of soft nucleophiles to provide a glycosylating species.

Furthermore, thiophenyl glycosides are shelf stable and often crystalline, which for the large-scale preparation of the building blocks is a valuable asset. To maximize the efficiency in the construction of deoxychitobiosyl umbelliferone **2** a route was designed, in which a single thioglycoside (i.e., **6**) serves as an advanced precursor for both the nonreducing and reducing end glucosamine building blocks. We selected the phthaloyl group to protect the glucosamine amino function because it is cheap, robust under both basic and acidic conditions, and can be readily introduced onto the glucosamine substrate on a large scale by using well-established chemistry. 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 hydroxy groups during the assembly of the chitobiose disaccharide.

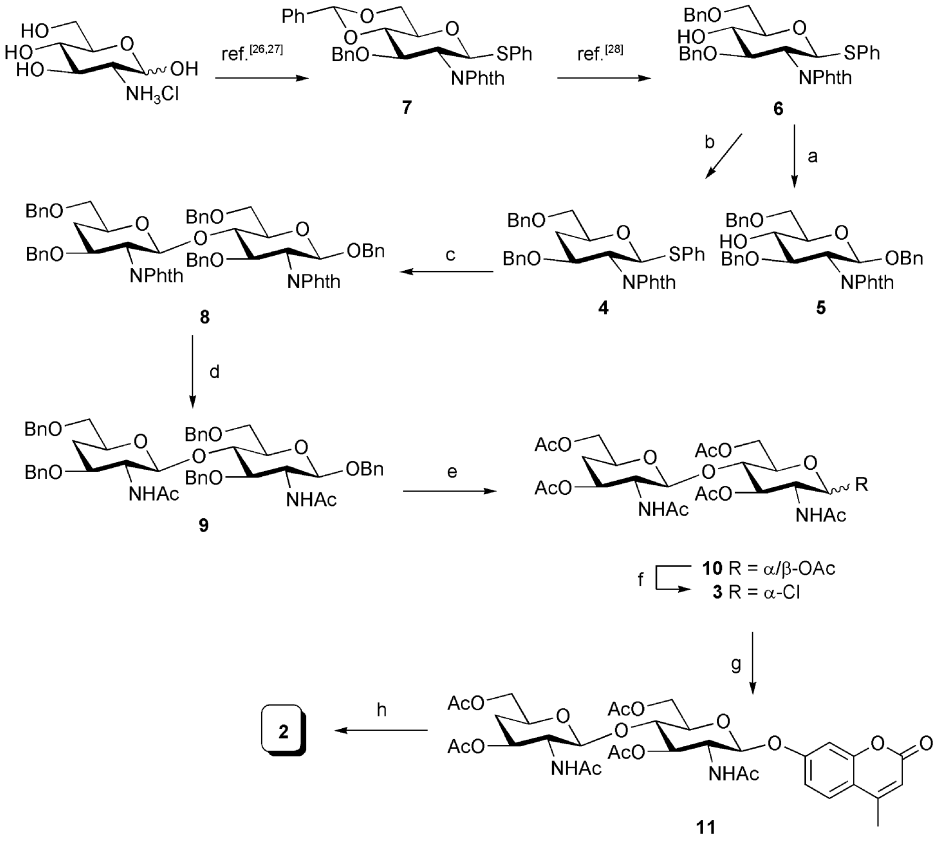
Scheme 2 depicts the synthesis of deoxychitobiosyl umbelliferone **2**, which started with the synthesis of thioglycoside **6**. 4,6-Benzylidene-*N*-phthaloyl thioglucosamine (**7**) was obtained from D-glucosamine in 40% yield over eight steps on a 147-g scale.[26,27] Only a single chromatographic



Scheme 1. Synthetic outline for the large-scale synthesis of umbelliferyl chitobioside fluorogenic substrate **2**.

purification was required in this sequence of reactions. Reductive opening of the benzylidene acetal in the next step was affected by treatment of **7** with trifluoroacetic acid (TFA) and triethylsilane (TES) to selectively provide key thioglycoside **6** in 85% yield.[28] The formation of the regioisomeric C-4 benzyl ether was not observed. To provide the nonreducing end glucosamine building block, alcohol **6** was treated with NaH and CS_2 followed by MeI to provide the methyl dithiocarbonate. Radical fragmentation with the use of Bu_3SnH and AIBN as initiator in refluxing toluene then led to deoxygenated glucosamine **4** in 87% yield.[29] For the construction of the reducing end glucosamine building block **5**, partly protected thioglycoside **6** was condensed with benzyl alcohol employing *N*-iodosuccinimide (NIS) and a catalytic amount of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as activator.[30] The use of a large excess of BnOH (5 equiv.), and the high nucleophilicity of this alcohol as compared to the glucosamine C-4 hydroxy, completely prevented self-condensation of **6**, and glucosamine **5** was obtained in 75% yield. In the ensuing NIS-mediated glycosylation, deoxyglucosamine **4** and benzyl glucosamine **5** were reacted in a 1:1 ratio to provide chitobioside derivative **8** in excellent yield.

To introduce the umbelliferyl chromophore, disaccharide **8** was transformed into disaccharide chloride **3**. To this end, both *N*-phthaloyl groups in **8** were removed by transamidation with ethylenediamine in refluxing *n*-butanol. Subsequent acetylation of the resulting free amines then provided crystalline dimer **9**. Removal of all benzyl groups from this disaccharide proved to be more troublesome than expected because of the low solubility of the partly benzylated-*N*-acetyled chitobiosides. The best results were obtained when



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

disaccharide **9** was treated under 5 bar hydrogen pressure with Pearlman's catalyst (5 mol-%) in a THF/MeOH (1:1) solvent mixture in the presence of AcOH (5 equiv.). The fully deprotected 4′-deoxychitobioside was then acetylated to give penta-*O*-acetate **10** in 65% yield as an amorphous white solid over the two steps. The final stages of the synthesis followed procedures slightly adapted from literature.[31] Chlorination of the reducing end glucosamine derivative required careful tuning of the reaction conditions. Anomeric acetate **10** was treated with dry HCl in a mixture of AcOH and Ac_2O at 5 °C for 42 h to afford 4′-deoxychitobiosyl chloride **3** in 74%. We observed that shorter reaction times led to incomplete chlorination and that at higher reaction temperatures the GlcNAc–GlcNAc interglycosidic bond was cleaved. Previously, it has been reported that the anomeric chlorination of chitobiosyl acetate can be readily accomplished at room temperature. Presumably the 4-deoxy nature of the nonreducing end GlcNAc residue in **10** makes the glycosidic linkage more labile towards acidic cleavage. Introduction of the umbelliferyl chromophore was accomplished by S_N2 displacement of the anomeric α-chloride by the tetrabutylammonium salt of 4-methylumbelliferone, generated under phase-transfer conditions.[32] The protected umbelliferyl derivative was obtained in 62% yield as a white amorphous solid. Saponification of the acetyl esters with NaOMe and HPLC purification completed the synthesis of target compound **2**.

Conclusions

In conclusion we have developed an efficient, reliable, and scalable route for the synthesis of 4′-deoxychitobiosyl umbelliferone **2**. The synthesis is based on the use of a partially protected thiophenyl glucosamide, which is readily transformed into both the reducing and nonreducing end building blocks for the construction of 4′-deoxychitobiose.

Experimental Section

General: Dichloromethane was heated at reflux with P_2O_5 and distilled before use. All other chemicals (Acros, Fluka, Merck, Schleicher & Schuell) were used as received. Column chromatography was performed on Merck silica gel 60 (0.040–0.063 mm). TLC analysis was conducted on HPTLC aluminum sheets (Merck, silica gel 60, F245). Compounds were visualized by UV absorption (245 nm), by spraying with 20% H_2SO_4 in ethanol or with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (25 g/L), $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ (10 g/L), 10% H_2SO_4 in H_2O followed by charring at ≈140 °C. ^1H

and ^{13}C NMR spectra were recorded with a Bruker AV 400. NMR spectra were recorded in $CDCl_3$ with chemical shifts (δ) relative to tetramethylsilane unless stated otherwise. Optical rotations were measured with a Propol automatic polarimeter. High-resolution mass spectra were recorded with a LTQ-orbitrap (thermoelectron). IR spectra were recorded with a Shimadzu FTIR-8300.

Phenyl 3,6-Di-*O*-benzyl-2,4-dideoxy-2-phthalimido-1-thio-β-D-glucopyranoside (4): Glycoside **6** (25.4 g, 43.8 mmol) was coevaporated three times 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 (60% dispersion in oil, 2.63 g, 65.7 mmol) was added, and the reaction mixture was kept at 0 °C for 1 h and then warmed to room temperature. At room temperature, MeI (4.82 mL, 77.5 mmol) was added. After 30 min the mixture was quenched by the addition of AcOH and subsequently diluted with EtOAc (250 mL). The mixture was then washed with $NaHCO_3$ (aq.). The layers were separated, and the organic layer was dried with $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (toluene/ethyl acetate, 100:0 → 95:5) yielded the thiocarbamate intermediate as a 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 1 h when TLC analysis showed complete consumption of the starting material, the reaction was cooled to room temperature and concentrated in vacuo. The residue was taken up in acetonitrile and washed two times with hexane; the acetonitrile layer was concentrated in vacuo. Column chromatography (petroleum ether/ethyl acetate, 100:0 → 70:30) afforded **4** (20.1 g, 87%) as an oil. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.59 (q, J = 11.6 Hz, 1 H, 4-H), 2.31 (dd, J = 3.6, 12.8 Hz, 1 H), 3.54–3.58 (m, 1 H, C-6), 3.65–3.69 (m, 1 H, 6-H), 3.84 (m, 1 H, 5-H), 4.19–4.34 (m, 3 H, 2-H, 3-H, CH_2 Bn), 4.55–4.57 (m, 3 H, CH_2 Bn), 5.57 (d, J = 10.4 Hz, 1 H, 1-H), 6.98–7.02 (m, 5 H, H arom.), 7.16–7.17 (m, 3 H, H arom.), 7.28–7.39 (m, 7 H, H arom.), 7.66–7.83 (m, 4 H, H arom.) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 34.0 (C-4), 55.5 (C-2), 70.6 (CH_2 Bn), 72.3 (C-6), 73.3 (CH_2 Bn), 73.5 (C-3), 75.4 (C-5), 83.6 (C-1), 123.1, 123.3, 127.3–128.6 (CH arom.), 131.5 (C_q arom.), 132.0 (CH arom.), 132.5 (C_q arom.), 133.7 (CH arom.), 137.7, 138.0 (C_q arom.), 167.7, 168.0 (C=O Phth) ppm. HRMS: calcd. for $C_{34}H_{31}NO_5S + Na^+$ 588.18151; found 588.18115.

Benzyl 3,6-Di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (5): Glycoside **6** (23.6 g, 40.5 mmol) was coevaporated three times 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 3 Å molecular sieves for 30 min. After cooling to 0 °C, a catalytic amount of TMSOTf (0.81 mL, 4.5 mmol) was added. After 1 h, the mixture was warmed to room temperature when TLC analysis showed complete consumption of the starting material, and the reaction was quenched by the addition of Et_3N (5.6 mL, 40.5 mmol). The reaction mixture was diluted with DCM and washed with $Na_2S_2O_3$ (aq.). The water layer was extracted twice with DCM, and the collected organic layer was dried with $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (petroleum ether/ethyl acetate, 100:0 → 60:20) yielded **5** (17.6 g, 75%) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$): δ = 3.20 (d, J = 2.4 Hz, 1 H, OH), 3.62–3.65 (m, 1 H, 5-H), 3.82 (m, 3 H, 4-H, 6-H, 6-H), 4.23–4.25 (m, 2 H, 2-H, 3-H), 4.47 (d, J = 12.4 Hz, 1 H, CH_2 Bn), 4.51 (d, J = 12.4 Hz, 1 H, CH_2 Bn), 4.58 (d, J = 12.0 Hz, 1 H, CH_2 Bn), 4.64 (d, J = 12.0 Hz, 1 H, CH_2 Bn), 4.74 (d, J = 12.4 Hz, 1 H, CH_2 Bn), 4.78 (d, J = 12.4 Hz, 1 H, CH_2 Bn), 5.15–5.17 (m, 1 H, 1-H), 6.89–6.93 (m, 3 H, H arom.), 7.02–7.07 (m, 7 H, H arom.), 7.28–7.36 (m, 5 H, H arom.), 7.53 (br. s, 1 H, H arom.), 7.62–7.63 (m, 2 H, H arom.), 7.76 (br. s, 1 H, H arom.) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 55.3 (C-2), 70.3 (C-6), 70.6 (CH_2 Bn), 73.5 (CH_2 Bn), 73.7 (C-5), 73.8 (C-4), 74.1 (CH_2 Bn), 78.4 (C-3), 97.2 (C-1), 123 (CH arom.), 127.2–128.3 (CH arom.), 131.4 (C_q arom.), 133.5 (CH arom.), 136.9, 137.6, 138.0 (C_q arom.), 168.0 (C=O Phth), 168.1 (C=O Phth) ppm. HRMS: calcd. for $C_{35}H_{33}NO_7 + Na^+$ 602.21492; found 602.21471.

Phenyl 3,6-Di-*O*-benzyl-2-deoxy-2-phthalimido-4-*O*-(3,6-di-*O*-benzyl-2,4-dideoxy-2-phthalimido-β-D-glucopyranosyl)-β-D-glucopyranoside (8): A mixture of donor **4** (20.1 g, 35.4 mmol) and acceptor **5** (20.6 g, 35.4 mmol) were coevaporated three times with toluene. DCM (350 mL) and NIS (9.56 g, 42.5 mmol) were added, and the mixture was stirred over activated 3 Å molecular sieves 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 the 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 $Na_2S_2O_3$ (aq.). The water layer was extracted twice with DCM, and the collected organic layer was dried with $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (petroleum ether/ethyl acetate, 100:0 → 70:30) yielded **8** (31.7 g, 86%) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.52 (q, J = 12.0 Hz, 1 H, 4′-H), 2.28 (dd, J = 4.8, 12.8 Hz, 1 H, 4′-H), 3.34–3.58 (m, 6 H), 4.11–4.39 (m, 7 H), 4.44–4.58 (m, 6 H, CH_2 Bn), 4.68 (d, J = 12.4 Hz, 1 H, CH_2 Bn), 4.84 (d, J = 12.4 Hz, 1 H, CH_2 Bn), 4.98 (d, J = 6.4 Hz, 1 H, 1-H), 5.29 (d, J = 8.0 Hz, 1 H, 1′-H), 6.82 (br. s, 3 H, H arom.), 6.96–7.02 (m, 12 H, H arom.), 7.20–7.37 (m, 10 H, H arom.), 7.58–7.59 (m, 2 H, H arom.), 7.67–7.71 (m, 4 H, H arom.), 7.88–7.89 (m, 2 H, H arom.) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 34.2 (C-4′), 55.6 (C-2), 57.7 (C-2′), 68.1, 70.3, 70.6, 71.1, 71.9, 72.4, 72.5, 73.2, 74.0, 74.5, 75.5, 76.5, 97.0 (C-1), 97.2 (C-1′), 122.9–123.5 (CH arom.), 126.7–128.3 (CH arom.), 131.5 (C_q arom.), 133.4–133.7 (CH arom.), 136.9–138.6 (C_q arom.), 167.5–168.1 (C=O Phth) ppm. HRMS: calcd. for $C_{63}H_{58}N_2O_{12} + Na^+$ 1057.38820; found 1057.38876.

Phenyl 3,6-Di-*O*-benzyl-2-deoxy-2-acetamido-4-*O*-(3,6-di-*O*-benzyl-2,4-dideoxy-2-acetamido-β-D-glucopyranosyl)-β-D-glucopyranoside (9): Disaccharide **8** (31.7 g, 30.6 mmol) was dissolved in *n*BuOH (275 mL) and ethylene diamine (30 mL). This mixture was heated at reflux overnight and subsequently concentrated in vacuo. The reaction was then coevaporated three times 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 warmed to room temperature. 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 through hyflo-gel concentrated in vacuo. Crystallization (petroleum ether/ethyl acetate) yielded **9** (26.6 g, 82%) as slightly yellow crystals. ^1H NMR (400 MHz, $CDCl_3/CD_3OD$, 1:1): δ = 1.45 (q, J = 12.0 Hz, 1 H, 4′-H), 1.94 (s, 6 H, CH_3 NHAc), 2.20 (dd, J = 4.8, 12.8 Hz, 1 H, 4′-H), 3.37–3.38 (m, 1 H), 3.44–3.51 (m, 3 H), 3.63–3.79 (m, 5 H), 3.97 (t, J = 6.4 Hz, 1 H), 4.12 (t, J = 6.8 Hz, 1 H) 4.38–4.49 (m, 5 H, CH_2 Bn, H1), 4.54–4.69 (m, 5 H, CH_2 Bn, H1′), 4.75 (d, J = 11.6 Hz, 1 H, CH_2 Bn), 4.86 (d, J = 12.4 Hz, 1 H, CH_2 Bn), 7.21–7.35 (m, 25 H, H arom.) ppm. ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$, 1:1): δ = 22.2, 22.5 (CH_3 NHAc), 33.1 (C-4′), 51.8 (C-2), 55.5 (C-2′), 68.9, 69.8, 70.2, 70.5, 71.8, 72.3, 73.0, 73.1, 74.1, 74.4, 75.4, 78.4, 99.6, 100.2 (C-1, C-1′), 126.8–128.0 (CH arom.), 137.1–138.3 (C_q arom.), 170.8, 171.4 (C_q NHAc) ppm. HRMS: calcd. for $C_{51}H_{58}N_2O_{10} + Na^+$ 881.39837; found 881.39865.

1,3,6-Tri-*O*-acetyl-2-deoxy-2-acetamido-4-*O*-(3,6-di-*O*-acetyl-2,4-dideoxy-2-acetamido-β-D-glucopyranosyl)-D-glucopyranoside (10): Disaccharide **9** (22.9 g, 26.6 mmol) was dissolved in THF (250 mL) and then MeOH (250 mL), AcOH (9 mL, 106 mmol), and $Pd(OH)_2$ (20% on activated carbon, 1 g, 1.33 mmol) were added. The mixture was shaken overnight on a par 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, and after 1 h the mixture was warmed to room temperature and stirred overnight. The reaction was quenched by the addition of MeOH at 0 °C and then concentrated in vacuo. The residue was taken up in $CHCl_3$ and washed with 1 M HCl (aq.)/$NaHCO_3$ (aq.) and brine. The organic layer was dried with $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (DCM/MeOH, 100:0 → 97:3) yielded **10** (10.6 g, 65%) as a white amorphous solid. ^{1}H NMR of alpha acetate (400 MHz, CD_3OD): δ = 1.51 (q, J = 11.6 Hz, 1 H, 4′-H), 1.86–2.14 (22 H, CH_3 Ac, 4′-H), 3.61 (t, J = 9.2 Hz, 1 H), 3.79 (m, 1 H), 3.88 (t, J = 9.6 Hz, 1 H), 3.98 (m, 1 H), 4.04–4.12 (m, 2 H), 4.23 (dd, J = 5.6, 11.6 Hz, 1 H), 4.30 (dd, J = 3.6, 10.8 Hz, 1 H), 4.44 (d, J = 12.0 Hz, 1 H), 4.56 (d, J = 8.0 Hz, 1 H, 1′-H), 5.04 (dt, J = 5.2, 10.8 Hz, 1 H), 5.24 (t, J = 10.0 Hz, 1 H), 5.99 (d, J = 3.6 Hz, 1 H, 1-H) ppm. ^{13}C NMR of alpha acetate (100 MHz, CD_3OD): δ = 20.8–23.0 (CH_3 Ac), 33.8 (C-4′), 52.2, 56.4 (C-2, C-2′), 63.5, 66.7 (C-6, C-6′), 70.7, 71.6, 72.0, 72.4, 76.9, 91.5 (C-1), 102.6 (C-1′), 171.9–172.4 (C=O Ac) ppm. HRMS: calcd. for $C_{26}H_{38}N_2O_{15}$ + Na^+ 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-dideoxy-2-acetamido-β-D-glucopyranosyl)-β-D-glucopyranoside (11): Disaccharide **10** (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 3 h. The reaction mixture was then placed at 5 °C for 42 h, after which TLC analyses (DCM/acetone, 60:40) showed complete consumption of the starting material. The reaction mixture was diluted with $CHCl_3$ (50 mL, 0 °C) and washed twice with H_2O (25 mL, 0 °C) and twice with $NaHCO_3$ (aq.) (25 mL, 0 °C). The organic layer was dried with $MgSO_4$ and concentrated in vacuo to yield amorphous solid **3** (1.14 g). Its purity was evaluated by ^{1}H NMR spectroscopy [^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.55 (q, J = 11.6 Hz, 1 H, 4′-H), 1.86–2.14 (22 H, CH_3 Ac, 4′-H), 3.73–3.83 (m, 4 H), 4.03 (dd, J = 4.0, 11.6 Hz, 1 H), 4.20–4.25 (m, 2 H), 4.36–4.54 (m, 3 H), 4.48 (d, J = 8.0 Hz, 1 H, 1′-H), 5.02 (dt, J = 5.2, 11.2 Hz, 1 H), 5.30 (t, J = 10.0 Hz, 1 H), 5.94 (d, J = 8.0 Hz, 2 H, NHAc), 5.96 (d, J = 8.0 Hz, 2 H, NHAc), 6.12 (d, J = 3.6 Hz, 1 H, 1-H) ppm]. 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-methylumbelliferylsodium salt[33] (1.9 g, 9.59 mmol), and tetrabutylammonium hydrogen sulfate (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 two times with $NaHCO_3$ (0.2 M) and two times with H_2O. The organic layer was dried with $MgSO_4$ and concentrated in vacuo. Purification by column chromatography ($CHCl_3$/MeOH, 100:0 → 97:3) yielded **11** (0.88 g, 46%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD, 1:1): δ = 1.71 (q, J = 12.4 Hz, 1 H, 4′-H), 1.86–2.14 (19 H, CH_3 Ac, 4′-H), 2.38 (s, 3 H, CH_3 4-methylumbelliferyl), 4.04–4.29 (m, 7 H), 5.14 (t, J = 8.1 Hz, 1 H), 5.23–5.26 (m, 3 H), 5.46–5.49 (m, 2 H), 6.09 (s, 1 H) 6.54 (d, J = 9.2 Hz, 1 H, NHAc), 6.72 (d, 1 H, J = 9.2 Hz, NHAc). 6.86 (m, 2 H), 7.44 (d, 1 H, J = 10.4 Hz) ppm. ^{13}C NMR of (100 MHz, $CDCl_3$/CD_3OD, 1:1):δ = 18.5 (CH_3 4-methylumbelliferyl), 20.5–23.2 (CH_3 Ac), 32.5 (C-4′), 54.2, 54.4 (C-2, C-2′), 62.0, 65.2 (C-6, C-6′), 68.5, 69.9, 70.0, 72.0, 72.1, 98.7 (C-1), 102.9 (C-1′), 103.5 (CH arom.), 112.4, 133.3 (CH arom.), 114.7 (C_q arom.), 125.5 (CH arom.), 153.3, 154.4, 159.9, 160.1 (C_q arom.), 171.9–172.4 (C_q Ac) ppm. HRMS: calcd. for $C_{34}H_{42}N_2O_{16}$ + Na^+ 757.24265; found 757.24278.

4-Methylumbelliferyl 2-Deoxy-2-acetamido-4-*O*-(2,4-dideoxy-2-acetamido-β-D-glucopyranosyl)-β-D-glucopyranoside (2): To a suspension of **11** (0.878 g, 1.195 mmol) in MeOH (60 mL) was added NaOMe (30wt.-% in MeOH, 44 μL, 0.24 mmol). The reaction was stirred under exclusion of light. When LC–MS (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 **2** (227 mg, 28%) as white fluffy solid. ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 1.21 (q, J = 11.6 Hz, 1 H, 4′-H), 1.80 (s, 3 H, CH_3 NHAc), 1.84 (s, 4 H, CH_3 NHAc, 4′-H), 2.39 (s, 3 H, CH_3 4-methylumbelliferyl), 3.36–3.68 (m, 10 H), 3.78 (q, J = 9.2 Hz, 1 H, C-2 or C-2′), 4.30 (d, J = 8.4 Hz, 1 H, 1′-H), 4.69 (br. s, 1 H, OH), 4.84–4.90 (m, 3 H, OH), 5.17 (d, J = 8.4 Hz, 1 H, 1-H), 6.25 (s, 1 H, 4-methylumbelliferyl), 6.94 (d, J = 8.8 Hz, 1 H, 4-methylumbelliferyl), 7.02 (d, J = 1.6 Hz, 1 H), 7.67–7.71 (m, 2 H, 4-methylumbelliferyl, NH), 7.90 (d, J = 8.8 Hz, 1 H, NH) ppm. ^{13}C NMR (100 MHz, [D_6]DMSO): δ = 18.1 (CH_3 4-methylumbelliferyl), 23.0 (CH_3 NHAc), 23.1 (CH_3 NHAc), 35.8 (C-4′), 54.4, 57.0 (C-2 and C-2′), 59.7, 63.5 (C-6 and C-6′), 68.2, 72.3, 72.9, 75.1, 80.9 (C-3, C-4, C-5, C-3′, C-5′), 98.3 (C-1), 102.5 (C-1′), 103.2 (CH arom.) 111.9 (CH arom.), 113.5 (CH arom.), 114.3 (C_q arom.), 126.5 (CH arom.), 153.3, 154.4, 159.9, 160.1 (C_q arom.), 169.2, 169.4 (C=O Ac) ppm. HRMS: calcd. for $C_{26}H_{34}N_2O_{12}$ + Na^+ 589.20040; found 589.20031.

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