Carbohydrate Research 346 (2011) 1467–1478

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

A practical synthesis of capped 4-methylumbelliferyl hyaluronan disaccharides and tetrasaccharides as potential hyaluronidase substrates

Henrik Gold [a], Stefan Munneke [a], Jasper Dinkelaar [a], Herman S. Overkleeft [a], Johannes M. F. G. Aerts [b], Jeroen D. C. Codée [a,*], Gijs A. van der Marel [a,*]

[a] Leiden Institute of Chemistry, Leiden University, Gorlaeus Laboratories, Einsteinweg 55, 2300 RA Leiden, The Netherlands
[b] Department of Medical Biochemistry, Academic Medical Center, University of Amsterdam, Germany

ARTICLE INFO

Article history:
Received 11 February 2011
Received in revised form 24 March 2011
Accepted 29 March 2011
Available online 6 April 2011

Keywords:
Hyaluronan
Hyaluronidase
Fluorogenic substrate
Glycosylation
Barton deoxygenation

ABSTRACT

The synthesis of hyaluronan dimers and tetramers equipped with a 4-methylumbelliferyl group at the reducing end to potentially allow monitoring of hyaluronidase activities is described. The 4-OH at the non-reducing glucuronate in the presented series is either removed or methylated to prohibit transglycosylase reactions, leading to a total of four probes.

1. Introduction

4-Methylumbelliferyl glycosides are often used to monitor the activity of glycosidases from different sources.[1] They are stable under physiological conditions and undergo enzyme-catalyzed hydrolysis, after which the generated 4-methylumbelliferonate has excellent fluorescence properties. As such, 4-methylumbelliferyl glycosides are ideal molecular probes for glycobiology studies in which a glycosidase activity needs to be assessed in in vitro or in situ research settings. Glycosidase substrate recognition is often governed to a large extent by the nature of the non-reducing fragment of a glycoconjugate and less so by the nature of the aglycone, enabling the design of glycosidase fluorogenic probes by grafting the 4-methylumbelliferonate onto the non-reducing fragment in the appropriate stereochemical form (alpha or beta). This is true in particular for exoglycosidases, enzymes that recognize and remove a single monosaccharide residue from a substrate glycoconjugate.[2] Indeed, effective 4-methylumbelliferyl glycosides for many exoglycosidase activities are now commercially available. The situation is less optimal with respect to probes to monitor the activity of endoglycosidases, enzymes that recognize and remove oligosaccharides composed of a number of monosaccharides in a single step. Obviously, fluorogenic substrates targeting endoglycosidases are more challenging to synthesize and as a consequence literature on these is relatively scarce. Endoglycosidases may further have a considerable affinity for the natural aglycone, a feature well exemplified by most cellulase binding grooves that can accommodate a number of glucoside residues at both sides of the scissile glycosidic linkages. Finally, and a feature we have encountered in the past in our work on chitinase probes, endoglycosidases may possess considerable transglycosylase activity, by which a released oligosaccharide fragment is condensed with the next substrate, thus obscuring the interpretation of a fluorescence assay.[3] Our rationale and the subject of this work is that in order to establish whether fluorogenic endoglycosidase substrates for a given enzyme family are suitable probes, these first need be designed and synthesized. In designing a probe and its synthesis one should take into consideration both the number of monosaccharide units comprising the non-reducing part, which should be modifiable with relative ease, and the possible occurrence of transglycosidation, which should be negated by blocking potential transglycosylation sites. In this work we report on our synthetic efforts towards the generation of potential fluorogenic hyaluronidase probes.

Hyaluronan, a member of the glycosaminoglycan polysaccharide superfamily, is an important structural component of several mammalian tissues.[4] Hyaluronan is a linear polysaccharide composed of (1→4)-linked β-D-glucuronic acid-(1→3)-*N*-acetyl-β-D-glucosamine repeating disaccharides and occurs in macromolecular

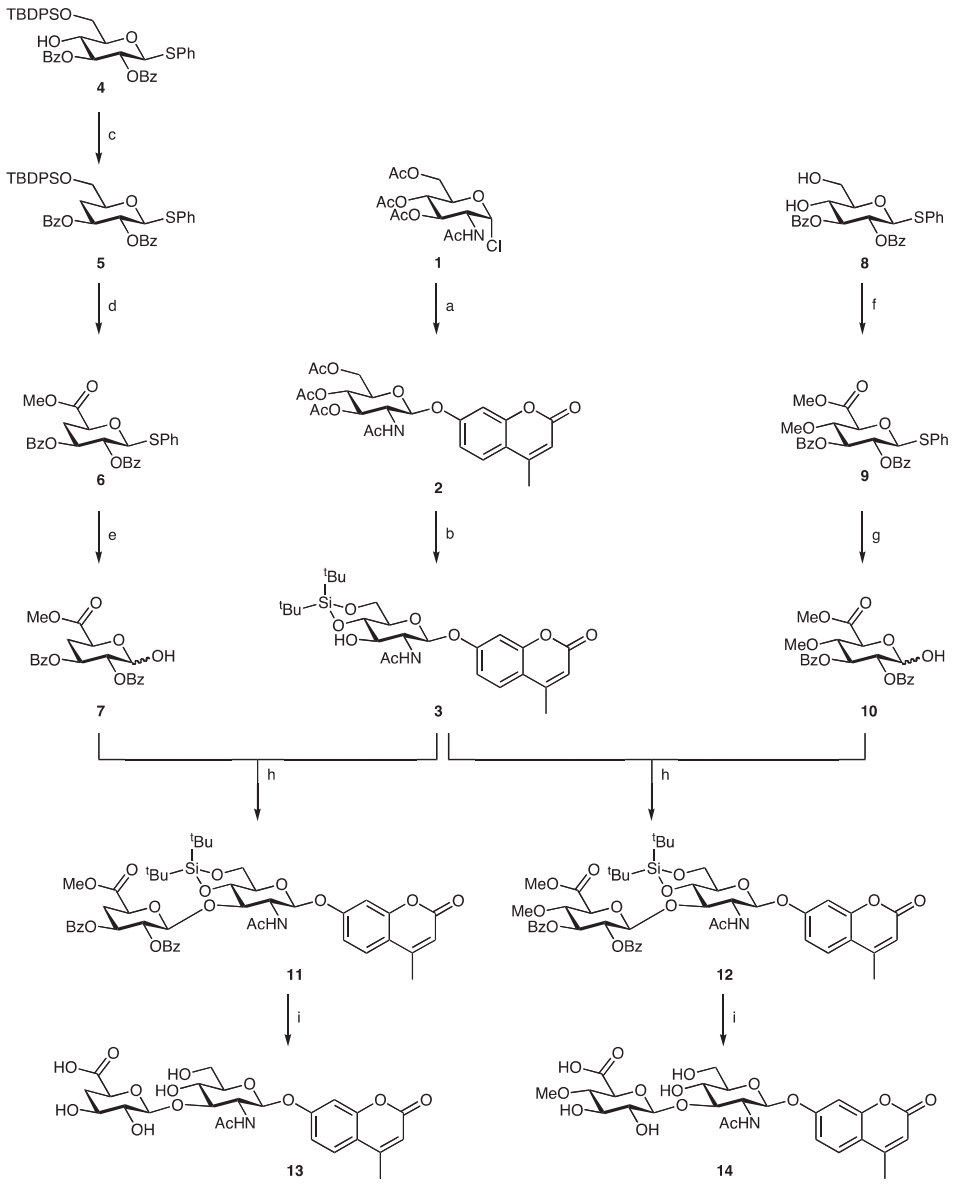
* Corresponding authors.
E-mail addresses: jcodee@chem.leidenuniv.nl (J.D.C. Codée), marel_g@chem.leidenuniv.nl (G.A. van der Marel).

0008-6215/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2011.03.042

sizes up to 20 MDa.[5] Several mammalian hyaluronidase activities have been identified to date and the list is considerably longer when considering hyaluronidases originating from other organisms.[6] The substrate preference of these enzymes appears to vary to an extent that hyaluronan can be processed to macromolecules of a size smaller than the aforementioned 20 MDa but also to oligosaccharides numbering from four to about 40 monosaccharides, with the latter oligomers commonly referred to as low molecular weight hyaluronan. One intriguing recent finding is that low molecular weight hyaluronan, but not the macromolecules, has immunostimulating properties in mammalian systems and by extension, hyaluronidases may partake in immune regulation processes.[7] In all, we reasoned that hyaluronidases are interesting targets for fluorogenic probe development, the more so since such entities are unprecedented in the literature.

2. Results and discussion

Hyaluronidases process hyaluronan by cleavage of glycosidic bonds between *N*-acetyl-β-D-glucosamine and β-D-glucuronic acid residues, to reveal the glucosamine at the reducing end of one fragment and the glucuronic acid on the non-reducing end of the other fragment.[6] We therefore based the design of our potential hyaluronidase fluorogenic substrates on a 4-methylumbelliferonate β-linked to *N*-acetylglucosamine at the reducing end. We further considered the likelihood that hyaluronidases of different nature would require hyaluronan fragments of different size for recognition, and endeavoured to establish a flexible synthesis strategy to accommodate this. Finally, and as said in our previous work on endoglycosidase fluorogenic substrate development we encountered considerable transglycosylation activity, which complicated



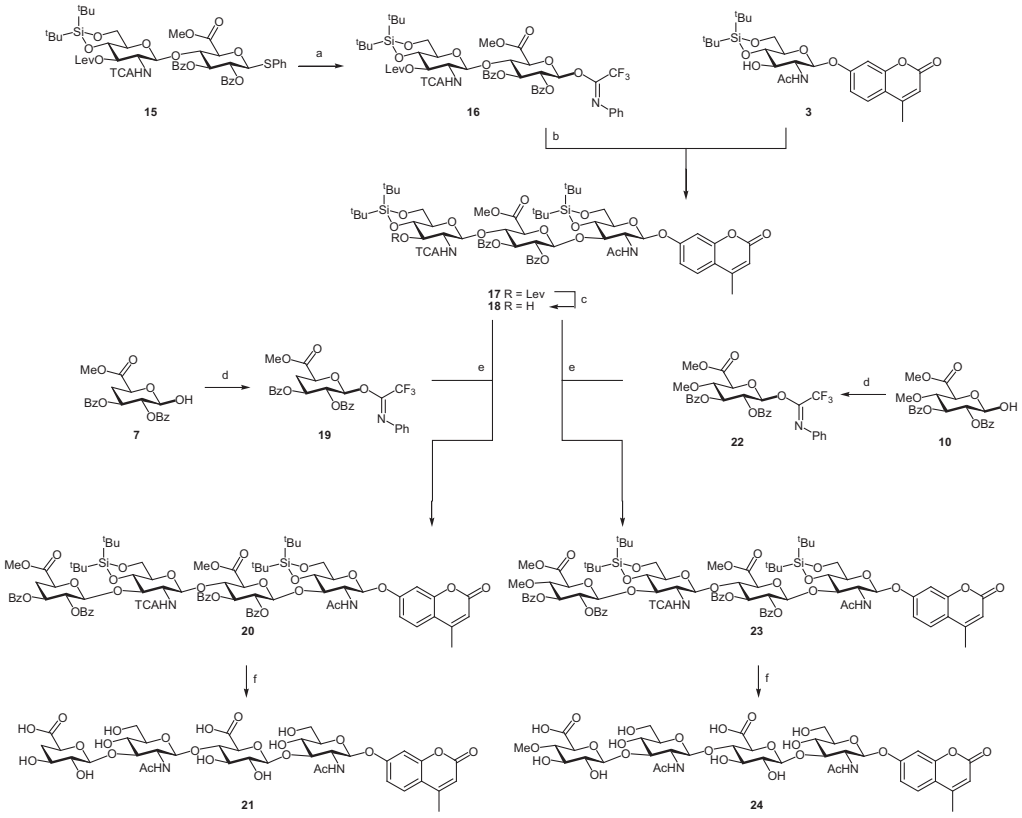
Scheme 1. Reagents and conditions: (a) TBABr, CsOH, 4-methylumbelliferone, CH_2Cl_2/H_2O, rt, 1 h, 72%; (b) (1) NaOMe, MeOH, rt, 4 h; (2) $(^tBu)_2Si(OTf)_2$, pyridine, DMF, −40 °C, 45 min, 94%; (c) (1) Im_2CS, toluene, 90 °C, 20 h, 89%; (2) AIBN, *n*-Bu_3SnH, toluene, 90 °C, 6 h, 83%; (d) (1) TBAF, THF, rt, 2 h, 94%; (2) TEMPO, BAIB, CH_2Cl_2/H_2O, 0 °C, 1 h; (3) $TMSCHN_2$, CH_2Cl_2/MeOH, rt, 20 min, 90%; (e) NIS, TFA, CH_2Cl_2, 0 °C, 2.5 h, 96%; (f) (1) TEMPO, BAIB, CH_2Cl_2/H_2O, rt, 2.5 h; (2) $TMSCHN_2$, CH_2Cl_2/MeOH, rt, 20 min, 73% (over two steps); (3) $TMSCHN_2$, BF_3OEt_2, CH_2Cl_2, −40 °C to rt, 2 h, 49% (92% based on recovered starting material); (g) NIS, TFA, CH_2Cl_2, 0 °C, 2.5 h, 75%; (h) Ph_2SO, Tf_2O, CH_2Cl_2, −20 °C, **11**: 82%, **12**: 53%; (i) (1) NaOMe (cat), MeOH, CH_2Cl_2; (2) HF-pyridine, 18 h; (3) Na_2CO_3 (aq); (4) RP-HPLC, **13**: 77%, **14**: 63%.

assessment of glycosidase activity making use of fluorogenic substrate assays. In these studies we had an interest in the study of human chitinases (chitotriosidase, acid mammalian chitinase) and we found that both enzymes are capable of transglycosylation of the commercial chitinase substrate, chitobiosyl-4-methylumbelliferone, at the 4′ position. Blocking this position by either deoxygenation or methylation obliterated this transglycosylation, leading to efficient fluorogenic substrates that are clean in the sense that they undergo a single enzymatic transformation, namely the desired hydrolysis.[3a,8] We therefore decided to target both the corresponding 4-deoxy and 4-methoxy derivatives of our projected HA probes, taking into account an even number of monosaccharide residues and a GlcNAc moiety at the reducing end in our design. Altogether this leads to the design of a set of potential hyaluronidase fluorogenic substrates with the general structure R-[(→4)-β-D-glucuronic acid-(1→3)-*N*-acetyl-β-D-glucosamine-(1→)]$_n$-4-methylumbelliferone, with R = H or OMe. In this work we demonstrate the synthetic feasibility of these substrates through the synthesis of a set of dimeric and tetrameric potential hyaluronan substrates of the above design and with n = 1 or 2.

The synthesis of the hyaluronan umbelliferonates composed of a single β-D-glucuronic acid-(1→3)-*N*-acetyl-β-D-glucosamine moiety is depicted in Scheme 1.[9] In our experience,[8] the formation of a glycosidic linkage with the 4-methylumbelliferyl aglycone can be troublesome and we decided to introduce this linkage at the onset of our synthetic schemes.[10] This leads to partially protected *N*-acetylglucosamine derivative **3** as the common starting point and this building block was prepared in three steps from the anomeric chloride **1**.[11] S_N2 substitution of the anomeric chloride with the sodium salt of 4-methylumbelliferone provided fully protected β-glycoside **2** in 72% yield. Global deprotection followed by installation of the di-*tert*-butylsilylene protective group gave the desired acceptor glycoside **3** in 94% yield over the two steps. We elected to employ the di-*tert*-butylsilylene group[12] instead of the benzylidene group because we,[13] and others,[11] have previously shown the former to confer desirable properties to the acceptor. In particular, GlcNAc-derived donor/acceptor glycosides often suffer from poor solubility in organic solvents, and introduction of the di-*tert*-butylsilylene protective group, which withstands most acidic glycosylation conditions, makes these glycosides better soluble in organic solvents.

Donor uronic acid derivatives **7** and **10** were readily prepared as follows. Barton deoxygenation[14] of the 4-hydroxyl in the partially and orthogonally protected thioglucoside **4** gave 4-deoxyglucose derivative **5**. Fluoride-mediated desilylation followed by TEMPO-BAIB oxidation[15] and transformation of the carboxylate to the methyl ester provided phenyl thiouronate **6**, which was anomerically deprotected using conditions we previously described (*N*-iodosuccinimide, trifluoroacetic acid)[16] to give donor hemi-acetal **7**. In a related sequence of events, the primary alcohol in phenyl thioglucoside **8**[17] was selectively oxidized using the TEMPO/BAIB reagent combination, after which the carboxylate was methylated with TMS-diazomethane. In a subsequent step the 4-OH group was capped with the same reagent under the agency of BF_3OEt_2.[18,19] Liberation of the anomeric hydroxyl provided donor hemiacetal **10**.

Condensation of **7** and **10** with acceptor **3** under dehydrative glycosylation conditions (Ph_2SO, Tf_2O)[20] provided the fully protected disaccharides **11** and **12**, respectively, which were globally deprotected and purified to homogeneity to deliver the potential hyaluronidase fluorogenic substrates **13** and **14** in good quantities and good overall yield. It should be noted here that phenylthioglu-



Scheme 2. Reagents and conditions: (a) (1) NIS, TFA, CH_2Cl_2, 0 °C, 2 h, 89%; (2) Cs_2CO_3, ClC(NPh)CF_3, acetone, 0 °C, 3 h, 85%; (b) TfOH, CH_2Cl_2, 0 °C, 2 h, 60%; (c) hydrazine acetate, pyridine/acetic acid, rt, 10 min, 99%; (d) Cs_2CO_3, ClC(NPh)CF_3, acetone, 0 °C, 3 h, **19**: 73%, **22**: 65%; (e) TfOH, CH_2Cl_2, 0 °C, 4 h, **20**: 78%, **23**: 85%; (f) (1) zinc dust, AcOH, rt, 40 h; (2) KI, acetone, rt, 20 h; (3) zinc dust, AcOH, rt, 20 h; (4) NaOMe (cat), MeOH, CH_2Cl_2, 6 h; (5) $(HF)_3{\cdot}Et_3N$, pyridine, 18 h; (6) Na_2CO_3 (aq), 4 h; (7) RP-HPLC, **21**: 60%, **24**: 43%.

curonates **6** and **9** could, in principle, also serve as donors to generate disaccharides **11** and **12**. We however found that in our hands, and by making use of conditions advocated by us (Ph_2SO, Tf_2O),[21] and others (BSP, Tf_2O),[22] these thioglycosides failed to give productive couplings.

The construction of tetramers **21** and **24** is depicted in Scheme 2 and is based on orthogonally protected hyaluronan disaccharide **15** we previously employed in the modular assembly of hyaluronan oligomers of varying size.[13] Hydrolysis of the thioacetal and ensuing transformation into *N*-phenyl-trifluoroimidate[23] donor **16** set the stage for condensation with acceptor GlcNAc derivative **3**. Condensation to give the fully protected **17** under the agency of triflic acid in methylene chloride proceeded in 60% yield. Attempts to effect the same transformation but starting from phenylthiodisaccharide **15** or the intermediate hemiacetal obtained after NIS/TFA treatment under the appropriate glycosylation conditions proved low yielding. Liberation of the 3″-OH by hydrazine-mediated removal of the Lev protective group gave acceptor trisaccharide **18**, which was effectively condensed with either donor imidate **19** or donor imidate **22** to give the fully protected tetrasaccharides **20** and **23** in 78% and 85% yield, respectively. Hemiacetals **7** and **10**, the precursors of imidates **19** and **20**, could also be condensed with trisaccharide **18** under the agency of Ph_2SO and Tf_2O, however in considerable reduced yield (around 40%). Global deprotection of the tetrasaccharides proved to proceed with some difficulties, in that reductive transformation of the trichloroacetamide moieties in **20**/**23** into the corresponding N-acetyl groups did not go to completion even after prolonged exposure to zinc dust in acetic acid.[24] Rather, the monochlorides were obtained as the major product. We therefore had to resort to a Finkelstein transformation of the monochloroacetamide intermediates to give the corresponding mono-iodo acetamides and resubject these to the zinc dust mediated reductive dehalogenation. This three-step dehalogenation proceeded with good efficiency and removal of the remaining protective groups proceeded under standard conditions to give the target tetrasaccharides **21** and **24** in 43% and 60% yield, respectively, after HPLC purification.

3. Conclusion

In summary, we have demonstrated the synthesis of a set of four potential hyaluronidase fluorogenic substrates, equipped with the 4-methylumbelliferonate fluorogenic leaving group. Our strategy is flexible in that both dimeric and tetrameric glycoconjugates can be assessed through the use of common intermediates, and that the nature of the non-reducing cap (deoxy or methoxy) can be determined in the final glycosylation step by selection of the appropriate donor glucuronate. A protecting group strategy has been developed, which allows the deprotection of the HA-saccharides, leaving the coumarine moiety untouched. Our future work will entail the assessment of the fluorogenic substrates against a panel of hyaluronidases. Finally, if one considers the modular assembly of the tetrameric derivatives making use of disaccharides **15**/**16** it is evident that our strategy can be readily adapted to generate hyaluronan oligomers composed of a larger number of monosaccharides. In this scheme, we envisage elongation of **18** with one or more copies of imidate **16** prior to introducing the non-reducing glucuronate moiety.

4. Experimental

4.1. General methods and materials

Commercially available reagents and solvents (Acros, Fluka or Merck) were used as received unless stated otherwise. Dichloromethane and THF were freshly distilled, before use, over P_2O_5 and Na/benzophenone, respectively. Et_3N was destilled over calcium hydride and stored over potassium hydroxide. Trifluoromethanesulfonic anhydride was distilled from P_2O_5. All moisture sensitive reactions were performed under an argon atmosphere. Traces of water were removed from starting compounds by co-evaporation with dichloroethane, dioxane and/or toluene. Molecular sieves 3 Å were flamedried prior to use. Liquid column chromatography was performed using forced flow of the indicated solvent systems on Screening Devices Silica Gel 60 (40–63 μm mesh). Size exclusion chromatography was performed on Sephadex LH20 (eluent $MeOH/CH_2Cl_2$, 1:1). Analytical TLC was performed on aluminium sheets, pre-coated with silica gel (Merck, Silica Gel 60, F_{254}). Compounds were visualized with UV absorption (245 nm), by spraying with either 20% H_2SO_4 in ethanol, or ammonium molybdate/cerium sulfate solution [$(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (25 g/L), $(NH_4)_4Ce(SO_4)_6{\cdot}2H_2O$ (10 g/L), 10% sulphuric acid in ethanol], or phosphormolybdic acid in EtOH (150 g/L) followed by charring (~150 °C) or by spraying with potassium permanganate (1.6% in concd sulfuric acid). IR spectra were recorded on a Shimadzu FTIR-8300 and are reported in cm^{-1}. Optical rotations were measured on a Propol automatic polarimeter (Sodium D-line, $\lambda = 589$ nm). ^1H and ^{13}C NMR spectra were recorded on a Bruker AV 400 MHz spectrometer at 400.2 (^1H) and 100.6 (^{13}C) MHz or on a Bruker AV 500 MHz spectrometer at 500.0 (^1H) and 125.1 (^{13}C) MHz respectively. Chemical shifts are reported as δ values (ppm) and directly referenced to TMS (0.00 ppm) in $CDCl_3$ or via the solvent residual peak (D_2O). Coupling constants (*J*) are given in Hertz and all ^{13}C spectra are proton decoupled. NMR assignments were made using COSY and HSQC and in some cases TOCSY experiments. LC–MS analyses were performed on a LCQ Advantage Max (Thermo Finnigan) equipped with a Gemini C_{18} column (Phenomenex, 50 × 4.6 mm, 3μ), utilizing the following buffers: A: H_2O, B: acetonitrile and C: 1.0% $TFA_{(aq)}$. HPLC purifications were performed on a Gilson GX-281 automated HPLC system, equipped with a preparative Gemini C_{18} column (Phenomenex, 150 × 21.20, 5μ). Products were eluted using the following buffers: A: ammonium acetate (20 $mM_{(aq)}$) or triethylammonium acetate (50 $mM_{(aq)}$), B: acetonitrile (HPLC-grade), 20 mL/min. Purified products were lyophilized on a CHRIST ALPHA 2–4 LD_{PLUS} to remove water and traces of buffer salts. High resolution mass spectra were recorded by direct injection (2 μL of a 2 μM solution in water/acetonitrile; 50/50; v/v and 0.1% formic acid) on a mass spectrometer equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 °C) with resolution $R = 60000$ at m/z 400 (mass range $m/z = 150–2000$) and dioctylplthalate ($m/z = 391.28428$) as a 'lock mass'. The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture.

4.1.1. 4-Methylumbelliferyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranoside (2)

4-Methylumbelliferone (2.24 g, 12.7 mmol, 1.5 equiv) and tetrabutylammonium bromide (4.10 g, 12.7 mmol, 1.5 equiv) were dissolved in CH_2Cl_2 (30 mL). To this solution cesium hydroxide (3.56 g, 11.9 mmol, 1.4 equiv), dissolved in water (30 mL) was added and the two-phase system was stirred vigorously for 10 min at room temperature. 2-Acetamido-2-deoxy-3,4,6-tri-O-acetyl-β-D-glucopyranosyl chloride[11] (3.1 g, 8.48 mmol, 1.0 equiv), dissolved in CH_2Cl_2 (10 mL) was then added dropwise over 10 min. The mixture was stirred for an additional 45 min and then transferred to an extraction funnel with CH_2Cl_2 (20 mL) and washed with water (2× 100 mL) and brine (80 mL). The aqueous layers were extracted with CH_2Cl_2 (100 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated in vacuo. Purifica-

tion by column chromatography (5–20% acetone in CH_2Cl_2) produced the title compound as a white solid (3.08 g, 6.09 mmol, 72%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.56 (m, 1H, H-$5_{4\text{-MU}}$), 7.01–6.96 (m, 2H, H-$6_{4\text{-MU}}$ and H-$8_{4\text{-MU}}$), 6.18 (d, 1H, J = 1.1 Hz, H-$3_{4\text{-MU}}$), 5.44–5.38 (m, 2H, H-1 and H-3), 5.13 (dd, 1H, J = 10.2, 9.4 Hz, H-2), 4.30 (dd, 1H, J = 12.3, 5.7 Hz, H-6_a), 4.22 (dd, 1H, J = 10.2, 8.3 Hz, H-4), 4.19 (dd, 1H, J = 12.3, 2.3 Hz, H-6_b), 4.02 (ddd, 1H, J = 10.2, 5.7, 2.3 Hz, H-5), 3.95 (s, 1H, NH), 2.43 (s, 3H, $CH_{3\text{-}4\text{-MU}}$), 2.13 (s, 3H, $CH_{3\text{-OAc}}$), 2.09 (s, 6H, $CH_{3\text{-OAc}}$ ×2), 1.93 (s, 3H, $CH_{3\text{-NAc}}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.5 (C=O_{NAc}), 170.8, 170.6, 169.6 (C=O_{OAc} ×3), 161.4, 159.42, 154.35, 153.0 (C-$2_{4\text{-MU}}$, C-$7_{4\text{-MU}}$, C-$9_{4\text{-MU}}$ and C-$10_{4\text{-MU}}$), 125.5 (C-$5_{4\text{-MU}}$), 114.9 (C-$4_{4\text{-MU}}$), 114.0 (C-$6_{4\text{-MU}}$), 112.2 (C-$3_{4\text{-MU}}$), 103.5 (C-$8_{4\text{-MU}}$), 97.7 (C-1), 72.0 (C-3), 71.8 (C-5), 68.4 (C-2), 61.9 (C-6), 53.8 (C-4), 22.3 ($CH_{3\text{-NAc}}$), 20.3, 20.17, 20.16 ($CH_{3\text{-OAc}}$ ×3), 18.3 ($CH_{3\text{-}4\text{-MU}}$); IR (neat): 2959, 2932, 2860, 1718, 1616, 1267, 1072, 1034, 827, 766 cm^{-1}; HRMS calcd for [$C_{24}H_{27}NO_{11}$+Na]$^+$: 528.1476, found 528.1474.

4.1.2. 4-Methylumbelliferyl 2-acetamido-2-deoxy-4,6-*O*- di-*tert*-butylsilanediyl-β-D-glucopyranoside (3)

Peracetylated *N*-acetyl glucosamine **2** (4.0 g, 7.91 mmol, 1.0 equiv) was dissolved in MeOH/CH_2Cl_2 1:1 (250 mL) and sodium methoxide (30% in MeOH) (0.33 mL, 2.37 mmol, 0.3 equiv) was added and the reaction was left stirring at room temperature for 4 h. The reaction was then quenched with Amberlite-H$^+$, filtered and concentrated in vacuo. The product was obtained without further purification in >95% purity according to ^{1}H NMR (2.88 g, 7.59 mmol, 96%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.65 (d, 1H, J = 8.7 Hz, H-$5_{4\text{-MU}}$), 7.05–6.98 (m, 2H, H-$6_{4\text{-MU}}$ and H-$8_{4\text{-MU}}$), 6.20 (d, 1H, J = 1.0 Hz, H-$3_{4\text{-MU}}$), 5.18 (d, 1H, J = 8.4 Hz, H-1), 4.00–3.91 (m, 2H, H-2 and H-3), 3.76 (dd, 1H, J = 12.0, 5.0 Hz, H-6_a), 3.61 (dd, 1H, J = 10.4, 8.3 Hz, H-4), 3.53–3.45 (m, 2H, H-5 and H-6_b), 2.46 (d, 3H, J = 1.0 Hz, $CH_{3\text{-}4\text{-MU}}$), 2.00 (s, 3H, $CH_{3\text{-NAc}}$); IR (neat): 3298, 2924, 2890, 1717, 1616, 1539, 1290, 1081, 1042, 853, 628 cm^{-1}; HRMS calcd for [$C_{18}H_{21}NO_8$+H]$^+$: 380.1340, found 380.1341. 4-Methylumbelliferyl glycoside (2.8 g, 7.38 mmol, 1.05 equiv) was coevaporated once in anhydrous DMF and then dissolved in anhydrous DMF (140 mL). The mixture was cooled to −40 °C, before drop-wise addition of di-*tert*-butylsilanediyl bistriflate (2.28 mL, 7.03 mmol, 1.0 equiv). The reaction was stirred for 30 min at −40 °C and pyridine was added (1.7 mL, 21.1 mmol, 3.0 equiv). The reaction was stirred an additional 15 min and then transferred to an extraction funnel with diethyether (400 mL). The organics were washed with water (2× 400 mL) and brine (350 mL) The aqueous layers were extracted with ether (400 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by column chromatography (80–100% EtOAc in petroleum ether), giving the title compound as a white solid (3.58 g, 6.89 mmol, 98%). R_f = 0.41 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.41 (d, 1H, J = 8.8 Hz, H-$5_{4\text{-MU}}$), 7.23 (d, 1H, J = 7.6 Hz, NH), 6.91 (dd, 1H, J = 8.8, 2.0 Hz, H-$6_{4\text{-MU}}$), 6.87 (d, 1H, J = 2.0 Hz, H-$8_{4\text{-MU}}$), 6.04 (s, 1H, H-$3_{4\text{-MU}}$), 5.48 (d, 1H, J = 7.9 Hz, H-1), 4.20 (dd, 1H, J = 10.1, 4.9 Hz, H-6_a), 4.06 (s, 1H, 3-OH), 4.04–3.98 (m, 3H, H-2, H-3, H-6_b), 3.85 (dd, 1H, J = 9.5, 8.1 Hz, H-4), 3.60 (ddd, 1H, J = 9.7, 9.5, 5.3 Hz, H-5), 2.33 (s, 3H, $CH_{3\text{-}4\text{-MU}}$), 2.09 (s, 3H, $CH_{3\text{-NAc}}$), 1.06 (s, 9H, $CH_{3\text{-tBu-Si}}$), 0.99 (s, 9H, $CH_{3\text{-tBu-Si}}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ = ^{13}C NMR (100 MHz, Aceton) δ 171.8 (C=O_{NAc}), 161.1, 159.7, 154.4, 152.6 (C-$2_{4\text{-MU}}$, C-$7_{4\text{-MU}}$, C-$9_{4\text{-MU}}$ and C-$10_{4\text{-MU}}$), 125.4 (C-$5_{4\text{-MU}}$), 114.6 (C-$4_{4\text{-MU}}$), 114.0 (C-$6_{4\text{-MU}}$), 112.2 (C-$3_{4\text{-MU}}$), 103.5 (C-$8_{4\text{-MU}}$), 98.1 (C-1), 77.2 (C-4), 74.3 (C-3), 70.5 (C-5), 66.0 (C-6), 56.3(C-2), 27.3, 26.8 ($CH_{3\text{-tBu-Si}}$ ×2), 23.3 ($C_{q\text{-tBu-Si}}$), 22.5 ($CH_{3\text{-NAc}}$), 19.8 ($CH_{3\text{-}4\text{-MU}}$), 18.4 ($C_{q\text{-tBu-Si}}$); IR (neat): 3308, 2936, 2861, 1718, 1616, 1389, 1268, 1071, 827, 653 cm^{-1}; HRMS calcd for [$C_{26}H_{37}NO_8Si$+H]$^+$: 520.2361, found 520.2363.

4.1.3. Phenyl 2,3-di-*O*-benzoyl-6-(*tert*-butyldiphenylsilyl)-1-thio-β-D-glucopyranoside (4)

To a solution of phenyl 1-thio-2,3-di-*O*-benzoyl-β-D-glucoside (5.21 g, 10.8 mmol, 1.0 equiv) in DMF (20 mL), imidazole (1.47 g, 21.6 mmol, 2.0 equiv) was added followed by TBDPS-Cl (3.60 mL, 14.0 mmol, 1.3 equiv). The reaction was quenched after 20 h with MeOH (5 mL) and then diluted with water (500 mL) and extracted with ether (2× 400 mL). The organics were dried with $MgSO_4$, filtered and concentrated in vacuo. Purification by column chromatography (5–25% EtOAc in petroleum ether) yielded the title compound as a colourless oil (7.55 g, 10.5 mmol, 97%). R_f = 0.63 (30% acetone in petroleum ether); $[\alpha]_D^{22}$ +56 (c 1.0 $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.03–7.99 (m, 4H, H_{arom}), 7.80–7.75 (m, 4H, H_{arom}), 7.54–7.38 (m, 15H, H_{arom}), 7.31–7.25 (m, 4H, H_{arom}), 5.52 (t, 1H, J = 9.2 Hz, H-3), 5.45 (t, 1H, J = 9.2 Hz, H-2), 4.97 (d, 1H, J = 9.6 Hz, H-1), 4.04–4.10 (m, 3H, H-4, H-6_a, H-6_b), 3.71 (m, 1H, H-5), 3.17 (br s, 1H, OH), 1.13 (s, 9H, $CH_{3\text{-tBu}}$), ^{13}C NMR (101 MHz, CDCl3) δ = 167.4, 165.4 (C=O_{Bz} ×2), 135.8 (×2), 133.6, 133.4 (CH_{arom} ×4), 132.9 ($C_{q\text{-arom}}$), 132.7(CH_{arom}), 132.6 ($C_{q\text{-arom}}$), 130.1, 130.00, 129.95 (CH_{arom} ×3), 129.5, 129.1 ($C_{q\text{-arom}}$ ×2), 129.0, 128.5, 128.1, 128.0 (CH_{arom} ×4), 86.2 (C-1), 80.1 (C-5), 78.2 (C-3), 70.5, 70.2 (C-2, C-4), 64.2 (C-6), 27.0 ($CH_{3\text{-tBu}}$), 19.4 ($C_{q\text{-tBu}}$); IR (neat): 3494, 2930, 2858, 1728, 1067, 1275 734, 701, 502 cm^{-1}; HRMS calcd for [$C_{42}H_{42}O_7SSi$+Na]$^+$: 741.2313, found 741.2313.

4.1.4. Phenyl 2,3-di-*O*-benzoyl-6-(*tert*-butyldiphenylsilyl)-3-deoxy-1-thio-β-D-glucopyranoside (5)

Phenyl thioglycoside **4** (6.7 g, 9.3 mmol, 1.0 equiv) and thiocarbonyldiimidazole (2.49 g, 14.0 mmol, 1.5 equiv) were dissolved in dry toluene (120 mL). The mixture was heated for 5 h at 90 °C and then cooled down to room temperature. The reaction was washed with satd aq $NaHCO_3$ (200 mL) and brine (200 mL). The organics were dried with $MgSO_4$, filtered and concentrated in vacuo. Purification by column chromatography (30–50% EtOAc in petroleum ether) yielded the 4-thiocarbonylimidazole compound as an off-white solid (6.88 g, 8.3 mmol, 89%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.16 (s, 1H, H-$2_{imidazole}$), 7.95 (d, 2H, J = 8.1 Hz, H_{arom}), 7.80 (d, 2H, J = 8.1 Hz, H_{arom}), 7.70 (dd, 2H, J = 8.0, 1.3 Hz, H_{arom}), 7.62 (dd, 1H, J = 8.0, 1.3 Hz, H_{arom}), 7.55–7.48 (m, 3H, J = 6.8 Hz, H-$5_{imidazole}$ and H_{arom} ×2), 7.46–7.22 (m, 17H, H_{arom}), 6.97 (dd, 1H, J = 1.6, 0.7 Hz, H-$4_{imidazole}$), 6.21 (t, 1H, J = 9.6 Hz, H-4), 5.96 (t, 1H, J = 9.5 Hz, H-3), 5.54 (t, 1H, J = 9.8 Hz, H-2), 5.09 (d, 1H, J = 10.0 Hz, H-1), 3.95 (m, 1H, H-5), 3.91 (dd, 1H, J = 11.8, 2.4 Hz, H-6_a) 3.85 (dd, 1H J = 11.8, 4.7 Hz, H-6_b), 1.07 (s, 9H, $CH_{3\text{-tBu}}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 182.4 (C=S), 165.7, 164.9 (C=O_{Bz} ×2), 137.0, 135.6, 135.4, 133.42, 133.35, 132.7 (CH_{arom} ×6), 132.5, 132.2 ($C_{q\text{-arom}}$ ×2), 130.9 ($CH_{imidazole}$), 129.82, 129.78, 129.0, 128.4, 128.3, 128.2, 127.7 (CH_{arom} ×7), 118.0 ($CH_{imidazole}$), 86.5 (C-1), 78.9 (C-5), 76.5 (C-4), 74.2 (C-3), 70.2 (C-2), 62.7 (C-6), 26.7 ($CH_{3\text{-tBu}}$), 19.1 ($C_{q\text{-tBu}}$); IR (neat): 3070, 2934, 2860, 1734, 1393, 1270, 1221, 1068, 1026, 985, 703 cm^{-1}; HRMS calcd for [$C_{46}H_{44}N_2O_7S_2Si$+H]$^+$: 829.2432, found 829.2439. The 4-thiocarbonylimidazole derivative (6.88 g, 8.3 mmol, 1.0 equiv) was coevaporated with dry toluene two times to remove traces of water and then dissolved in anhydrous toluene (100 mL). Bu_3SnH (5.54 mL, 20.8 mmol, 2.5 equiv) and AIBN (0.20 g, 1.2 mmol, 0.15 equiv) were added at 90 °C. The reaction was stirred at this temperature for 2 h and then cooled down, before being washed with satd aq $NaHCO_3$ (100 mL) and brine (100 mL). The organics were dried with $MgSO_4$, filtered and concentrated in vacuo. Purification by column chromatography (30% EtOAc in petroleum ether) gave 4-deoxy glucose derivate **5** as a colourless oil (4.85 g, 6.90 mmol, 83%). $[\alpha]_D^{22}$ +60 (c 1.0 $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.99

(d, 2H, J = 7.8 Hz, H_{arom}), 7.94 (d, 2H, J = 7.8 Hz, H_{arom}), 7.74–7.67 (m, 4H, H_{arom}), 7.51–7.45 (m, 4H, H_{arom}), 7.43–7.32 (m, 10H, H_{arom}), 7.25–7.20 (m, 3H, H_{arom}), 5.43–5.35 (m, 2H, H-2, H-3), 4.94 (d, 1H, J = 9.4 Hz, H-1), 3.89–3.83 (m, 2H, H-5, H-6_a), 3.76 (m, 1H, H-6_b), 2.44 (dd, 1H, J = 12.4, 3.4 Hz, H-4_{eq}), 1.86 (dd, 1H, J = 12.4, 11.5 Hz, H-4_{ax}), 1.14 (s, 9H, $CH_{3\text{-}tBu}$), ^{13}C NMR (126 MHz, $CDCl_3$) δ = 165.81, 165.37 (C=O_{Bz} ×2), 135.60, 135.58, 133.12, 133.10 (CH_{arom} ×4), 133.01 ($C_{q\text{-}arom}$), 132.30, 129.74, 129.68 (CH_{arom} ×3), 129.57, 129.43 ($C_{q\text{-}arom}$ ×2), 128.80, 128.30, 127.72, 127.52 (CH_{arom} ×4), 86.4 (C-1), 76.6 (C-5), 73.2 (C-3), 71.2 (C-2), 66.0 (C-6), 32.9 (C-4), 26.8 ($CH_{3\text{-}tBu}$), 19.2 ($C_{q\text{-}tBu}$); IR (neat): 2931, 2857, 1718, 1451, 1428, 1274, 1070, 1027, 908, 733, 702, cm^{-1}; HRMS calcd for $[C_{42}H_{42}O_6SSi+Na]^+$: 725.2364, found 725.2364.

4.1.5. Methyl (phenyl 2,3-di-*O*-benzoyl-3-deoxy-1-thio-β-D-glucopyranoside) uronate (6)

To a solution of 4-deoxy phenyl thioglycoside **5** (3.66 g, 5.2 mmol, 1.0 equiv) in THF (40 mL) TBAF (1 M in THF) (10.4 mL, 10.4 mmol, 2.0 equiv) was added at room temperature. The reaction was stirred for 2 h and then dissolved in EtOAc (200 mL) and washed with satd aq $NaHCO_3$ (250 mL) and brine (200 mL). The aqueous layers were extracted with EtOAc (200 mL) and the combined organics were dried (Na_2SO_4) and concentrated in vacuo. Purification by column chromatography (20–50% EtOAc in petroleum ether) produced the title compound as a white solid (2.28 g, 4.91 mmol, 94%). $[\alpha]_D^{22}$ +101 (c 1.0 $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.99 (d, 2H, J = 7.4 Hz, H_{arom}), 7.92 (d, 2H, J = 7.4 Hz, H_{arom}), 7.55–7.45 (m, 4H, H_{arom}), 7.41–7.33 (m, 4H, H_{arom}), 7.32–7.28 (m, 3H, H_{arom}), 5.44–5.36 (m, 2H, H-2, H-3), 4.96 (d, 1H, J = 10.0 Hz, H-1), 3.85 (m, 1H, H-5), 3.76 (ddd, 1H, J = 11.4, 7.8, 3.2 Hz, H-6_a), 3.68 (dt, 1H, J = 11.4, 5.9 Hz, H-6_a), 2.33 (m, 1H, H-4_{eq}), 2.16 (dd, 1H, J = 7.8, 5.9 Hz, OH), 1.82 (m, 1H, H-4_{ax}); ^{13}C NMR (126 MHz, $CDCl_3$) δ = 165.8, 165.4 (C=O_{Bz} ×2), 132.6, 133.4 (CH_{arom} ×2), 132.3 ($C_{q\text{-}aorm}$), 129.8, 129.7 (CH_{arom} ×2), 129.5, 129.4 ($C_{q\text{-}aorm}$ ×2), 128.2, 128.5, 129.1 (CH_{arom}×3), 86.3 (C-1), 76.6 (C-5), 73.0 (C-3), 71.2 (C-2), 65.0 (C-6), 32.3 (C-4); IR (neat): 3513, 2931, 2872, 1718, 1451, 1274, 1068, 1026, 908, 748, 705 cm^{-1}; HRMS calcd for $[C_{26}H_{24}O_6S+Na]^+$: 487.1186, found 487.1183. A solution of the 6-hydroxyl phenyl thioglucoside (2.02 g, 4.35 mmol, 1.0 equiv), TEMPO (136 mg, 0.87 mmol, 0.2 equiv) and BAIB (3.5 g, 10.9 mmol, 2.5 equiv), in CH_2Cl_2/H_2O 2:1 (45 mL) was vigorously stirred for 1 h. The reaction was then quenched with $Na_2S_2O_3$ (10% aq) (150 mL) and the mixture was extracted with EtOAc (2× 150 mL). The organics were washed with water (150 mL) and brine (150 mL), before being dried ($MgSO_4$), filtered and concentrated in vacuo. The crude mixture was co-evaporated with toluene two times to remove traces of water and acetic acid and dissolved in CH_2Cl_2/MeOH 2:1 (45 mL). Trimethylsilyl diazomethane (2 M in diethyl ether) (6.5 mL, 13.0 mmol, 3.0 equiv) was added until the solution remained orange. The reaction was then quenched with AcOH (5 mL), diluted with EtOAc (100 mL), washed with satd aq $NaHCO_3$ (2× 100 mL) and brine (100 mL). The aqueous layers were extracted with EtOAc (100 mL) and the combined organics were dried with $MgSO_4$, filtered and concentrated in vacuo. Purification by column chromatography (20–40% EtOAc in petroleum ether) yielded the title methyl uronate as a white solid (1.92 g, 4.06 mmol, 90%). $[\alpha]_D^{22}$ +78 (c 1.0 $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.99 (d, 2H, J = 7,9 Hz, H_{arom}), 7.93 (m, 2H, J = 7,9 Hz, H_{arom}), 7.55–7.49 (m, 4H, H_{arom}), 7.40 (t, 2H, J = 7.8 Hz, H_{arom}), 7.36 (t, 2H, J = 7.8 Hz, H_{arom}), 7.32–7.29 (m, 3H, H_{arom}), 5.42–5.37 (m, 2H, H-2, H-3), 4.93 (d, 1H, J = 9.4 Hz, H-1), 4.34 (dd, 1H, J = 12.1, 2.2 Hz, H-5), 3.82 (s, 3H, $CH_{3\text{-}COOMe}$), 2.73 (m, 1H, H-4_{eq}), 2.03 (m, 1H, H-4_{ax}); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.8 (C=O_{COOMe}), 165.7, 165.2 (C=O_{Bz} ×2), 133.30, 133.25 (CH_{arom} ×2), 131.94 ($C_{q\text{-}arom}$), 129.77, 129.72 (CH_{arom} ×2), 129.4, 129.1 ($C_{q\text{-}arom}$ ×2), 128.9, 128.37, 128.32 (CH_{arom} ×3), 86.8 (C-1), 74.0 (C-5), 72.3 (C-2), 70.5 (C-3), 52.6 ($CH_{3\text{-}COOMe}$), 33.35 (C-4); IR (neat): 2954, 1718, 1451, 1272, 1026, 906, 728, 706 cm^{-1}; HRMS calcd for $[C_{27}H_{24}O_7S+Na]^+$: 515.1135, found 515.1130.

4.1.6. Methyl (2,3-di-*O*-benzoyl-4-deoxy-α/β-D-glucopyranose) uronate (7)

To a solution of methyl (phenyl thioglucopyranoside)uronate **6** (0.50 g, 1.02 mmol, 1.0 equiv) in CH_2Cl_2 (8 mL), *N*-iodosuccinimide (0.25 g, 1.12 mmol, 1.1 equiv) and TFA (86 μL, 1.12 mmol, 1.1 equiv) were added at 0 °C. The reaction was left stirring at 0 °C (2.5 h) and then quenched with sodium thiosulphate (20% aq) (10 mL). The reaction mixture was transferred to an extraction funnel with EtOAc (40 mL) and washed with water (40 mL), satd aq $NaHCO_3$ (40 mL) and brine (40 mL). The aqueous layers were extracted with EtOAc (40 mL) and the combined organics were dried with $MgSO_4$, filtered and concentrated in vacuo. Purification by column chromatography (20–40% EtOAc in petroleum ether) yielded the title compound as a white solid (0.39 g, 0.98 mmol, 96%, α/β = 4:1) ^{1}H NMR (400 MHz, $CDCl_3$) α-isomer: δ = 8.07–7.95 (m, 4H, H_{arom}), 7.53–7.46 (m, 2H, H_{arom}), 7.40–7.35 (m, 4H, H_{arom}), 5.83 (ddd, 1H, J = 11.2, 9.9, 5.2 Hz, H-3), 5.80 (br t, 1H, J = 3.5 Hz, H-1), 5.31 (dd, 1H, J = 9.9, 3.2 Hz, H-2), 4.89 (dd, 1H, J = 12.0, 2.7 Hz, H-5), 4.42 (d, 1H, J = 3.5 Hz, OH), 3.76 (s, 3H, $CH_{3\text{-}COOMe}$), 2.75 (ddd, 1H, J = 12.8, 5.0, 2.7 Hz, H-4_{eq}), 2.00 (ddd, 1H, J = 12.8, 12.0, 11.2 Hz, H-4_{ax}); ^{13}C NMR (101 MHz, $CDCl_3$) α-isomer: δ = 170.8 (C=O_{COOMe}), 165.9, 165.7 (C=O_{Oac} ×2), 133.3, 133.2, 129.8, 129.6 (CH_{arom} ×4), 129.4, 129.2 ($C_{q\text{-}arom}$ ×2), 128.3 (CH_{arom}), 91.2 (C-1), 72.1 (C-2), 67.8 (C-3), 66.2 (C-5), 52.5 ($CH_{3\text{-}COOMe}$), 33.1 (C-4). The β-isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.13–7.95 (m, 4H, H_{arom}), 7.53–7.46 (m, 2H, H_{arom}), 7.40 - 7.35 (m, 4H, H_{arom}), 5.48 (m, 1H, H-3), 5.30 (m, 1H, H-2), 4.97 (br t, 1H, J = 7.2 Hz, H-1), 4.54 (br d, 1H, J = 7.8 Hz, OH), 4.37 (dd, 1H, J = 11.9, 2.5 Hz, H-5), 3.76 (s, 3H, $CH_{3\text{-}COOMe}$), 2.72 (ddd, 1H, J = 12.9, 5.3, 2.5 Hz, H-4_{eq}), 2.04 (m, 1H, H-4_{ax}); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 169.6 (C=O_{COOMe}), 166.4, 165.7 (C=O_{Oac} ×2), 133.4, 133.3, 129.8, 129.7 (CH_{arom} ×4), 129.4, 129.0 ($C_{q\text{-}arom}$ ×2), 128.3 (CH_{arom}), 95.9 (C-1), 74.2 (C-2), 70.4 (C-3), 70.3 (C-5), 52.7 ($CH_{3\text{-}COOMe}$), 33.0 (C-4); IR (neat): 3440, 2956, 1718, 1451, 1261, 1069, 909, 707 cm^{-1}; HRMS calcd for $[C_{21}H_{20}O_8+Na]^+$: 423.1050, found 423.1048.

4.1.7. Methyl (phenyl 2,3-di-*O*-benzoyl-4-*O*-methyl-1-thio-β-D-glucopyranoside) uronate (9)

A solution of diol **8**[17] (1.64 g, 3.41 mmol, 1.0 equiv), TEMPO (0.10 g, 0.66 mmol, 0.2 equiv) and BAIB (2.64 g, 8.2 mmol, 2.5 equiv) in CH_2Cl_2 (30 mL) and H_2O (15 mL) was stirred vigorously. The reaction was quenched after 2.5 h with $Na_2S_2O_3$ (10% aq) (100 mL). The mixture was diluted with EtOAc (50 mL) and washed with brine (50 mL). The organics were dried with $MgSO_4$, filtered and concentrated in vacuo. The crude mixture was coevaporated with toluene two times to remove traces of water and acetic acid, dissolved in CH_2Cl_2/MeOH 2:1 (50 mL). Trimethylsilyldiazomethane (2 M in diethyl ether) (4.9 mL, 9.84 mmol, 3.0 equiv) was added until the solution remained orange. The reaction was quenched with AcOH (2 mL), diluted with EtOAc (50 mL), washed with aq satd $NaHCO_3$ (2× 100 mL) and brine (100 mL). The organics were dried with $MgSO_4$, filtered and concentrated in vacuo. Purification by column chromatography (20–40% EtOAc in petroleum ether) produced the title compound as a white solid (1.27 g, 2.50 mmol, 73% over two steps). R_f = 0.13 (20% EtOAc in petroleum ether); NMR data are in accordance with literature precedence.[15] IR (neat): 2463, 3058, 2959, 1718, 1451, 1258, 1216, 1064, 708 cm^{-1}; HRMS calcd for $[C_{27}H_{24}O_8S+Na]^+$: 531.1084, found 531.1081. To a solution of the methyl (glucopyranoside)uronate (0.51 g, 1.0 mmol, 1.0 equiv) in dry CH_2Cl_2 (5 mL), $BF_3{\cdot}OEt_2$ (0.25 mL, 2.0 mmol, 2.0 equiv) and trimethylsilyldiazomethane (2 M in diethyl ether) (1.0 mL, 2.0 mmol, 2.0 equiv) were added

at −40 °C. The reaction was allowed to warm to ambient temperature in 2 h. The reaction was then quenched with acetic acid (0.5 mL), diluted with CH_2Cl_2 (20 mL) and washed with aq satd $NaHCO_3$ (2× 50 mL) and brine (50 mL). The organics were dried ($MgSO_4$), filtered and concentrated in vacuo. Purification by column chromatography (20% EtOAc in petroleum ether) afforded the title compound **9** as a white solid (254 mg, 0.49 mmol, 49%, 92% based on recovered starting material). R_f = 0.45 (30% EtOAc in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.99–7.91 (m, 4H, H_{arom}), 7.54–7.49 (m, 5H, H_{arom}), 7.42–7.31 (m, 6H, H_{arom}), 5.68 (t, 1H, J = 9.2 Hz, H-3), 5.38 (t, 1H, J = 9.6 Hz, H-2), 4.98 (d, 1H, J = 9.6 Hz, H-1), 4.10 (d, 1H, J = 9.6 Hz, H-5), 3.92 (t, 1H, J = 9.2 Hz, H-4), 3.89 (s, 3H, $CH_{3\text{-}COOMe}$), 3.42 (s, 3H, $CH_{3\text{-}OMe}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.0 ($C{=}O_{COOMe}$), 165.5 ($C{=}O_{Bz}$), 165.1 ($C{=}O_{Bz}$), 133.3, 133.2 (CH_{arom} ×2), 131.8 (C $C_{q\text{-}arom}$), 130.0, 129.8, 129.7 (CH_{arom} ×3), 129.1 ($C_{q\text{-}arom}$), 128.9, 128.3 (CH_{arom} ×2), 86.8 (C-1), 78.9 (C-4), 77.8 (C-5), 75.5 (C-3), 70.2 (C-2), 52.7 ($CH_{3\text{-}COOMe}$), 60.4 ($CH_{3\text{-}OMe}$); IR (neat): 3070, 2951, 1747, 1733, 1718, 1452, 1264, 1065, 1022, 709, 686 cm^{-1}; HRMS calcd for $[C_{28}H_{26}O_8S+Na]^+$: 545.1241, found 545.1236.

4.1.8. Methyl (2,3-di-*O*-benzoyl-4-*O*-methyl-α/β-D-glucopyranose) uronate (10)

To a solution of methyl (phenyl thioglucoside)uronate **9** (0.23 g, 0.43 mmol, 1.0 equiv) in CH_2Cl_2 (8 mL), *N*-iodosuccinimide (0.10 g, 0.45 mmol, 1.05 equiv) and TFA (35 μL, 0.45 mmol, 1.05 equiv) were added at 0 °C. The reaction was left stirring at room temperature (2.5 h) and then quenched with sodium thiosulphate (20% aq) (2.0 mL). The reaction mixture was transferred to an extraction funnel with EtOAc (40 mL) and washed with satd aq $NaHCO_3$ (40 mL) and brine (30 mL). The aqueous layers were extracted with EtOAc (40 mL) and the combined organics were dried with $MgSO_4$, filtered and concentrated in vacuo. Purification by column chromatography (10–30% EtOAc in petroleum ether) yielded the title compound in a 10:1 α/β-ratio as a white solid (0.14 g, 0.32 mmol, 75%). R_f = 0.45 (30% EtOAc in petroleum ether); NMR assignment for major isomer (α) ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.04–8.00 (m, 2H, H_{arom}), 7.99–7.95 (m, 2H, H_{arom}), 7.56–7.47 (m, 2H, H_{arom}), 7.43–7.33 (m, 4H, H_{arom}), 6.00 (t, 1H, J = 9.5 Hz, H-3), 5.70 (dd, 1H, J = 3.7, 3.4 Hz, H-1), 5.18 (dd, 1H, J = 9.5, 3.4 Hz, H-2), 4.61 (d, 1H, J = 9.7 Hz, H-5), 3.88 (dd, 1H, J = 9.7, 9.5 Hz, H-4), 3.83 (s, 3H, $CH_{3\text{-}COOMe}$), 3.43 (s, 3H, $CH_{3\text{-}OMe}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.5 ($C{=}O_{COOMe}$), 165.9, 165.5 ($C{=}O_{Bz}$ ×2), 133.4, 133.3, 129.9, 129.7 (CH_{arom} ×4), 129.5 ($C_{q\text{-}arom}$), 128.4 (CH_{arom}), 90.7 (C-1), 79.1 (C-4), 71.8 (C-2), 71.4 (C-3), 67.1 (C-5), 60.3 ($CH_{3\text{-}OMe}$), 52.8 ($CH_{3\text{-}COOMe}$); IR (neat): 3440, 2955, 2849, 1725, 1452, 1265, 1108, 1069, 709 cm^{-1}; HRMS calcd for $[C_{22}H_{22}O_9+Na]^+$: 453.1156, found 453.1152.

4.1.9. 4-Methylumbelliferyl 2-acetamido-2-deoxy-3-*O*-(methyl 2,3-di-*O*-benzoyl-4-deoxy-β-D-glucopyranosyl uronate)-4,6-*O*-di-*tert*-butylsilanediyl-β-D-glucopyranoside (11)

Hemiacetal **7** (0.20 g, 0.50 mmol, 1.2 equiv) and diphenyl sulfoxide (0.23 g, 1.15 mmol, 2.8 equiv) were coevaporated two times with anhydrous toluene, dissolved in anhydrous CH_2Cl_2 (10 mL) and stirred with flame dried molecular sieves (3 Å) for 30 min. The mixture was then cooled to −60 °C and trifluoromethanesulfonic anhydride (0.10 mL, 0.62 mmol, 1.44 equiv) was added. The reaction mixture was allowed to warm to −20 °C and left stirring at this temp for 1 h. *N*-Acetylglucosamine acceptor **3** (0.21 g, 0.41 mmol, 1.0 equiv) was coevaporated two times with anhydrous toluene (with a drop of anhydrous CH_2Cl_2) and then dissolved in anhydrous CH_2Cl_2 (5 mL) before addition to the activated donor. The reaction was warmed to ~0 °C and left stirring at this temperature over night. The reaction was then quenched with Et_3N (0.29 mL, 2.1 mmol, 5.0 equiv), transferred to an extraction funnel with EtOAc (40 mL) and washed with satd aq $NaHCO_3$ (40 mL) and brine (40 mL). The aqeous layers were extracted with EtOAc (40 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by column chromatography (10–50% EtOAc in petroleum ether) produced the title compound as a white solid (0.30 g, 0.34 mmol, 82%). R_f = 0.50 (30% EtOAc in CH_2Cl_2); $[\alpha]_D^{22}$ +61 (*c* 0.5 $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.99–7.96 (m, 2H, H_{arom}), 7.94–7.89 (m, 2H, H_{arom}), 7.53–7.48 (m, 2H, H_{arom}), 7.43 (d, 1H, J = 8.5 Hz, H-$5_{4\text{-}MU}$), 7.41–7.34 (m, 4H, H_{arom}), 6.85 (dd, 1H, J = 8.5, 2.4 Hz, H-$6_{4\text{-}MU}$), 6.84 (d, 1H, J = 2.4 Hz, H-$8_{4\text{-}MU}$), 6.12 (d, 1H, J = 1.1 Hz, H-$3_{4\text{-}MU}$), 5.98 (d, 1H, J = 7.2 Hz, NH), 5.82 (d, 1H, J = 8.2 Hz, H-1), 5.44 (dd, 1H, J = 9.4, 7.5 Hz, H-2′), 5.34 (ddd, 1H, J = 11.4, 9.4, 5.2 Hz, H-3′), 5.20 (d, 1H, J = 7.5 Hz, H-1′), 4.45 (dd, 1H, J = 9.8, 8.8 Hz, H-3), 4.31 (dd, 1H, J = 12.1, 2.2 Hz, C-5′), 4.19 (dd, 1H, J = 10.3, 4.9 Hz, C-6_{eq}), 4.10 (t, 1H, J = 9.0 Hz, H-4), 3.93 (dd, 1H, J = 10.3, 9.8 Hz, H-6_{ax}), 3.79 (s, 3H, $CH_{3\text{-}COOMe}$), 3.65 (ddd, 1H, J = 9.8, 9.0, 4.9 Hz, H-5), 3.47 (ddd, 1H, J = 9.8, 8.2, 7.2 Hz, H-2), 2.70 (ddd, 1H, J = 12.6, 5.2, 2.2 Hz, H-$4'_{eq}$), 2.35 (d, 3H, J = 1.1 Hz, $CH_{3\text{-}4MU}$), 2.05 (ddd, 1H, J = 12.6, 12.1, 11.4 Hz, H-$4'_{ax}$), 1.56 (s, 3H, $CH_{3\text{-}NAc}$), 1.05 (s, 9H, $CH_{3\text{-}tBu\text{-}Si}$), 1.03 (s, 9H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (126 MHz, $CDCl_3$) δ = 170.8 ($C{=}O_{NAc}$), 169.2 ($C{=}O_{COOMe}$), 165.7, 165.4 ($C{=}O_{OBz}$ ×2), 160.9, 159.6, 154.6, 152.5 (C-$2_{4\text{-}MU}$, C-$7_{4\text{-}MU}$, C-$9_{4\text{-}MU}$ and C-$10_{4\text{-}MU}$), 133.4, 133.3, 129.7, 129.6 (CH_{arom} ×4), 129.2, 129.0 ($C_{q\text{-}arom}$ ×2), 128.5, 128.3 (CH_{arom} ×2), 125.5 (C-$5_{4\text{-}MU}$), 115.1 (C-$4_{4\text{-}MU}$), 113.5 (C-$6_{4\text{-}MU}$), 112.4 (C-$3_{4\text{-}MU}$), 104.2 (C-$8_{4\text{-}MU}$), 99.4 (C-1′), 97.0 (C-1), 79.7 (C-3), 76.4 (C-4), 73.3 (C-2′), 71.4 (C-3′), 70.6 (C-5), 70.3v (C-5′), 66.0 (C-6), 57.4 (C-2), 52.5 ($CH_{3\text{-}COOMe}$), 33.2 (C-4′), 27.3, 26.9 ($CH_{3\text{-}tBu\text{-}Si}$ ×2), 23.0 ($CH_{3\text{-}NAc}$), 22.5, 19.9 ($C_{q\text{-}tBu\text{-}Si}$ ×2), 18.6 ($CH_{3\text{-}4MU}$); HRMS calcd for $[C_{47}H_{55}NO_{15}Si+Na]^+$: 924.3233, found 924.3237.

4.1.10. 4-Methylumbelliferyl 2-acetamido-2-deoxy-3-*O*-(methyl 2,3-di-*O*-benzoyl-4-*O*-methyl-β-D-glucopyranosyl uronate)-4,6-*O*-di-*tert*-butylsilanediyl-β-D-glucopyranoside (12)

Hemiacetal **10** (0.14 g, 0.32 mmol, 1.2 equiv) and diphenyl sulfoxide (0.15 g, 0.75 mmol, 2.8 equiv) were coevaporated two times with anhydrous toluene, dissolved in anhydrous CH_2Cl_2 (6 mL) and stirred with flame dried molecular sieves (3 Å) for 30 min. The mixture was then cooled to −60 °C and trifluoromethanesulfonic anhydride (65 μL, 0.39 mmol, 1.44 equiv) was added. The reaction mixture was allowed to warm to −20 °C and left stirring at this temp for 1 h. *N*-Acetylglucosamine acceptor **3** (0.14 g, 0.27 mmol, 1.0 equiv) was coevaporated two times with anhydrous toluene (with a few drop of anhydrous CH_2Cl_2) and then dissolved in anhydrous CH_2Cl_2 (3 mL) before addition to the activated donor. The reaction was warmed to ~0 °C and left stirring at this temperature over night. The reaction was then quenched with Et_3N (0.19 mL, 1.35 mmol, 5.0 equiv), transferred to an extraction funnel with EtOAc (40 mL) and washed with satd aq $NaHCO_3$ (40 mL) and brine (40 mL). The aqeous layers were extracted with EtOAc (40 mL) and the combined organics were dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by column chromatography (10–50% EtOAc in petroleum ether) produced the title compound as a white solid (132 mg, 0.14 mmol, 53%). R_f = 0.54 (30% EtOAc in CH_2Cl_2); $[\alpha]_D^{22}$ +45 (*c* 1.0 $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.98–7.93 (m, 4H, H_{arom}), 7.56 - 7.50 (m, 2H, H_{arom}), 7.44 (d, 1H, J = 8.3 Hz, H-$5_{4\text{-}MU}$), 7.42 - 7.36 (m, 4H, H_{arom}), 6.84 (dd, 1H, J = 8.3, 2.3 Hz, H-$6_{4\text{-}MU}$), 6.82 (d, 1H, J = 2.3 Hz, H-$8_{4\text{-}MU}$), 6.13 (d, 1H, J = 1.1 Hz, H-$3_{4\text{-}MU}$), 5.74 (d, 1H, J = 8.2 Hz, H-1), 5.68 (d, 1H, J = 7.1 Hz, NH), 5.60 (t, 1H, J = 9.1 Hz, H-3′), 5.40 (dd, 1H, J = 9.1, 7.5 Hz, H-2′), 5.11 (d, 1H, J = 7.4 Hz, H-1′), 4.41 (dd, 1H, J = 9.9, 8.6 Hz, H-3), 4.18 (dd, 1H, J = 10.3, 5.0 Hz, H-6_{eq}), 4.10 (d, 1H, J = 9.1 Hz, C-5′), 4.00–3.86 (m, 3H, C-4, C-6_{ax} and C-4′), 3.80 (s, 3H, $CH_{3\text{-}COOMe}$), 3.61 (ddd, 1H, J = 9.9, 9.7, 5.0 Hz, H-5), 3.39 (s, 3H, $CH_{3\text{-}OMe}$), 3.30 (ddd, 1H, J = 9.9, 8.2, 7.1 Hz, H-2), 2.36 (d, 3H, J = 1.1 Hz,

$CH_{3\text{-}4MU}$), 1.64 (s, 3H, $CH_{3\text{-}NAc}$), 1.06 (s, 9H, $CH_{3\text{-}tBu\text{-}Si}$), 1.01 (s, 9H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.9 ($C{=}O_{NAc}$), 168.5 ($C{=}O_{COOMe}$), 165.4, 165.2 ($C{=}O_{OBz}$ ×2), 160.9 , 159.5, 154.6, 152.3 ($C\text{-}2_{4\text{-}MU}$, $C\text{-}7_{4\text{-}MU}$, $C\text{-}9_{4\text{-}MU}$ and $C\text{-}10_{4\text{-}MU}$), 133.6, 133.3, 129.7, 129.7 (CH_{arom} ×4), 129.1, 129.0 ($C_{q\text{-}arom}$ ×2), 128.6, 128.4 (CH_{arom} ×2), 125.6 ($C\text{-}5_{4\text{-}MU}$), 115.2 ($C\text{-}4_{4\text{-}MU}$), 113.4 ($C\text{-}6_{4\text{-}MU}$), 112.8 ($C\text{-}3_{4\text{-}MU}$), 104.3 ($C\text{-}8_{4\text{-}MU}$), 100.6 (C-1′), 96.7 (C-1), 80.1 (C-3), 79.1 (C-4′), 76.1 (C-4), 74.4 (C-5′), 74.2 (C-3′), 73.0 (C-2′), 70.4 (C-5), 66.1 (C-6), 60.3 ($CH_{3\text{-}OMe}$), 57.7 (C-2), 52.6 ($CH_{3\text{-}COOMe}$), 27.3, 26.9 ($CH_{3\text{-}tBu\text{-}Si}$ ×2), 23.2 ($CH_{3\text{-}NAc}$), 22.5, 19.9 ($C_{q\text{-}tBu\text{-}Si}$ ×2), 18.6 ($CH_{3\text{-}4MU}$); IR (neat): 2936, 2860, 1734, 1616, 1390, 1272, 1090, 832, 709 cm^{-1}; HRMS calcd for $[C_{48}H_{57}NO_{16}Si{+}Na]^+$: 954.3339, found 954.3345.

4.1.11. 4-Methylumbelliferyl 2-acetamido-2-deoxy-3-*O*-(4-deoxy-β-D-glucopyranosyl uronicacid)-β-D-glucopyranoside sodium salt (13)

Protected dimer **11** (130 mg, 0.14 mmol, 1.0 equiv) was dissolved in anhydrous MeOH (4 mL) and sodium methoxide (30% in MeOH) (40 μL, 0.14 mmol, 1.0 equiv) was added under an atmosphere of argon. The reaction was allowed to run over night at ambient temperature and monitored by HPLC-MS. The reaction was quenched with acetic acid (0.3 mL) and then coevaporated with toluene. The residue was dissolved in pyridine (2 mL) and Et_3N tris-hydrogenfluoride (47 μL, 0.29 mmol, 2.0 equiv) was added. The mixture was stirred for 6 h at ambient temperature, while the reaction progress was monitored by HPLC-MS. Upon completion, water (2 mL) and satd aq Na_2CO_3 (1 mL) were added and the mixture stirred over night at ambient temperature. The reaction progress was monitored by HPLC-MS and then concentrated in vacuo. The residue was purified by HPLC according to the general procedure. Repeated lyofilization followed by filtration over wet Amberlite-Na^+ (3 mL) gave the title compound as a white solid (62 mg, 0.11 mmol, 77%). ^{1}H NMR (500 MHz, D_2O) δ = 7.42 (d, 1H, J = 8.9 Hz, $H\text{-}5_{4\text{-}MU}$), 6.88 (dd, 1H, J = 8.8, 2.1 Hz, $H\text{-}6_{4\text{-}MU}$), 6.79 (d, 1H, J = 2.3 Hz, $H\text{-}8_{4\text{-}MU}$), 6.00 (s, 1H, $H\text{-}3_{4\text{-}MU}$), 5.20 (d, 1H, J = 8.5 Hz, H-1), 4.41 (d, 1H, J = 7.8 Hz, H-1′), 4.11 (dd, 1H, J = 10.4, 8.5 Hz, H-2), 3.98 (dd, 1H, J = 12.2, 2.2 Hz, H-5′), 3.92 (dd, 1H, J = 12.6, 1.7 Hz, $H\text{-}6_a$), 3.84 (dd, 1H, J = 10.5, 7.8 Hz, H-3), 3.79 (m, 1H, $H\text{-}6_b$), 3.71 (m, 1H, H-5), 3.63 (m, 1H, H-4), 3.20 (dd, 1H, J = 9.2, 7.9 Hz, H-2′), 2.29–2.21 (m, 4H, $H\text{-}4'_{eq}$ and $CH_{3\text{-}4MU}$), 1.99 (s, 3H, $CH_{3\text{-}NAc}$), 1.52 (q, 1H, J = 12.2 Hz, $H\text{-}4'_{ax}$). ^{13}C NMR (126 MHz, D_2O) δ = 178.3 ($C{=}O_{COO\text{-}Na+}$), 176.2 ($C{=}O_{NAc}$), 165.4, 160.6, 157.2, 154.9 ($C\text{-}2_{4\text{-}MU}$, $C\text{-}7_{4\text{-}MU}$, $C\text{-}9_{4\text{-}MU}$ and $C\text{-}10_{4\text{-}MU}$), 127.9 ($C\text{-}5_{4\text{-}MU}$), 116.4 ($C\text{-}4_{4\text{-}MU}$), 115.1 ($C\text{-}6_{4\text{-}MU}$), 112.6 ($C\text{-}3_{4\text{-}MU}$), 104.9 ($C\text{-}8_{4\text{-}MU}$), 104.4 (C-1′), 99.8 (C-1), 83.9 (C-3), 77.1 (C-5), 75.7 (C-2′), 73.7 (C-5′), 71.7 (C-3′), 69.8 (C-4), 61.8 (C-6), 55.6 (C-2), 37.5 (C-4′), 23.6 ($CH_{3\text{-}4MU}$), 19.2 ($CH_{3\text{-}NAc}$).

4.1.12. 4-Methylumbelliferyl 2-acetamido-2-deoxy-3-*O*-(4-*O*-methyl-β-D-glucopyranosyl uronicacid)-β-D-glucopyranoside sodium salt (14)

Protected dimer **12** (48 mg, 51 μmol, 1.0 equiv) was dissolved in anhydrous MeOH (2 mL) and sodium methoxide (30% in MeOH) (7 μL, 51 μmol, 1.0 equiv) was added under an atmosphere of argon. The reaction mixture was stirred over night at ambient temperature and the reaction progress was monitored by HPLC-MS. The reaction was quenched with acetic acid (0.2 mL) and then coevaporated with toluene. The residue was dissolved in pyridine (2 mL) and Et_3N tris-hydrogenfluoride (17 μL, 0.10 mmol, 2.0 equiv) was added. The reaction mixture was stirred for 6 h at ambient temperature, while the reaction progress was monitored by HPLC-MS. Upon completion water (2 mL) and satd aq Na_2CO_3 (1 mL) were added and the mixture was stirred over night at ambient temperature. The reaction was monitored by HPLC-MS and then concentrated in vacuo. The residue was purified by HPLC according to the general procedure. Repeated lyofilization followed by filtration over wet Amberlite-Na^+ (2 mL) gave the title compound as a white solid (19 mg, 32 μmol, 63%). ^{1}H NMR (500 MHz, MeOD-d_4) δ = 7.72 (d, 1H, J = 8.7 Hz, $H\text{-}5_{4\text{-}MU}$), 7.07–7.02 (m, 2H, $H\text{-}6_{4\text{-}MU}$ and $H\text{-}8_{4\text{-}MU}$), 6.00 (d, 1H, J = 1.2 Hz, $H\text{-}3_{4\text{-}MU}$), 5.24 (d, 1H, J = 8.5 Hz, H-1), 4.38 (d, 1H, J = 7.6 Hz, H-1′), 4.10 (dd, 1H, J = 10.3, 8.5 Hz, H-2), 3.94 (dd, 1H, J = 12.2, 1.8 Hz, $H\text{-}6_a$), 3.81 (dd, 1H, J = 10.3, 7.9 Hz, H-3), 3.76 (dd, 1H, J = 12.2, 4.8 Hz, $H\text{-}6_b$), 3.66 (d, 1H, J = 9.6 Hz, H-5′), 3.58–3.51 (m, 5H, H-4, H-5 and $CH_{3\text{-}OMe}$), 3.45 (t, 1H, J = 9.0 Hz, H-3′), 3.36 (dd, 1H, J = 9.2, 7.6 Hz, H-2′), 3.28 (t, 1H, J = 9.0 Hz, H-4′), 2.46 (d, 3H, J = 1.2 Hz, $CH_{3\text{-}4MU}$), 2.00 (s, 3H, $CH_{3\text{-}NAc}$). ^{13}C NMR (126 MHz, MeOD-d_4) δ = 176.1 ($C{=}O_{COO\text{-}Na+}$), 174.5 ($C{=}O_{NAc}$), 163.2, 161.8, 156.0, 155.4 ($C\text{-}2_{4\text{-}MU}$, $C\text{-}7_{4\text{-}MU}$, $C\text{-}9_{4\text{-}MU}$ and $C\text{-}10_{4\text{-}MU}$), 127.4 ($C\text{-}5_{4\text{-}MU}$), 116.2 ($C\text{-}4_{4\text{-}MU}$), 114.9 ($C\text{-}6_{4\text{-}MU}$), 113.0 ($C\text{-}3_{4\text{-}MU}$), 104.9 ($C\text{-}8_{4\text{-}MU}$), 104.8 (C-1′), 100.1 (C-1), 83.93 (C-3), 83.85 (C-4′), 78.2 (C-5′), 78.1 (C-5), 77.2 (C-3′), 74.4 (C-2′), 70.3 (C-4), 62.4 (C-6), 60.7 ($CH_{3\text{-}OMe}$), 55.9 (C-2), 23.2 ($CH_{3\text{-}4MU}$), 18.6 ($CH_{3\text{-}NAc}$); HRMS calcd for $[C_{25}H_{31}NO_{14}{+}Na]^+$: 592.1637, found 592.1634.

4.1.13. Methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-di-*tert*-butylsilanediyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-β-D-glucopyranosyl)-α/β-D-glucopyranoside *N*-Phenyl-2,2,2-trifluoroacetimidate) uronate (16)

To a solution of disaccharide **15**[13] (4.50 g, 4.28 mmol, 1.0 equiv) in CH_2Cl_2 (150 mL) *N*-iodosuccinimide (1.93 g, 8.56 mmol, 2.0 equiv) and trifluoroacetic acid (0.32 mL, 4.28 mmol, 1.0 equiv) were added at 0 °C. After 2 h the mixture was quenched by addition of Et_3N (1 mL) and transferred to an extraction funnel with CH_2Cl_2 (100 mL). The organics were washed with satd aq $NaHCO_3$ (300 mL) and brine (300 mL). The aqueous layers were then extracted with EtOAc (300 mL) and the combined organics were dried ($MgSO_4$), filtered and concentrated in vacuo. Purification by column chromatography (10–40% EtOAc in petroleum ether) yielded the disaccharide hemiacetal as a white solid in a 4:1 α/β-ratio (3.68 g, 3.83 mmol, 89%). R_f = 0.19 (50% EtOAc in petroleum ether); NMR assignment for the major isomer (α): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.97–7.93 (m, 4H, H_{arom}), 7.56–7.45 (m, 2H, H_{arom}), 7.43–7.37 (m, 2H, H_{arom}), 7.35–7.30 (m, 2H, H_{arom}), 6.85 (d, 1H, J = 8.9 Hz, NH), 5.97 (dd, 1H, J = 10.0, 8.9 Hz, H-3), 5.68 (d, 1H, J = 3.4 Hz, H-1), 5.21 (dd, 1H, J = 10.0, 3.4 Hz, H-2), 5.02 (dd, 1H, J = 10.7, 9.1 Hz, H-3′), 4.97 (d, 1H, J = 8.3 Hz, H-1′), 4.61 (d, 1H, J = 9.5 Hz, H-5), 4.21 (t, 1H, J = 9.2 Hz, H-4), 3.86–3.81 (m, 4H, H-2′ and $CH_{3\text{-}COOMe}$), 3.61 (br s, 1H, OH), 3.56 (t, 1H, J = 9.2 Hz, H-4′), 3.49 (dd, 1H, J = 10.4, 5.0 Hz, $H\text{-}6'_a$), 3.25 (ddd, 1H, J = 9.9, 9.8, 5.0 Hz, H-5′), 2.71–2.66 (m, 3H, $H\text{-}6'_b$ and $CH_{2\text{-}Lev}$), 2.57–2.54 (m, 2H, $CH_{2\text{-}Lev}$), 2.04 (s, 3H, $CH_{3\text{-}Lev}$), 0.88 (s, 9H, $CH_{3\text{-}tBu\text{-}Si}$), 0.87 (s, 9H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 205.9 ($C{=}O_{Lev\text{-}ketone}$), 172.4 ($C{=}O_{Lev\text{-}ester}$), 170.1 ($C{=}O_{COOMe}$), 165.8, 165.1 ($C{=}O_{Bz}$ ×2), 161.7 ($C{=}O_{TCA}$), 133.4, 133.1 (CH_{arom} ×2), 130.1 ($C_{q\text{-}arom}$), 129.9, 129.6 (CH_{arom} ×2), 128.9 ($C_{q\text{-}arom}$), 128.5, 128.4 (CH_{arom} ×2), 100.5 (C-1′), 92.3 ($C_{q\text{-}CCl3}$), 90.5 (C-1), 76.7 (C-4), 74.4 (C-3′), 74.2 (C-4′), 71.0 (C-5′), 70.6 (C-2), 69.7 (C-3), 69.5 (C-5), 65.0 (C-6′), 55.8 (C-2′), 53.1 ($CH_{3\text{-}COOMe}$), 38.8 ($CH_{2\text{-}Lev}$), 29.7 ($CH_{3\text{-}Lev}$), 27.8 ($CH_{2\text{-}Lev}$), 27.2, 26.7 ($CH_{3\text{-}tBu\text{-}Si}$ ×2), 22.4, 19.7 ($C_{q\text{-}tBu\text{-}Si}$ ×2); IR (neat): 3362, 2935, 2860, 1722, 1526, 1276, 1070, 827, 710 cm^{-1}; HRMS calcd for $[C_{42}H_{52}Cl_3NO_{16}Si{+}Na]^+$: 982.2013, found 928.2018. To a solution of the disaccharide hemiacetal (3.68 g, 3.84 mmol, 1.0 equiv) in acetone (10 mL) cesium carbonate (1.870 g, 5.76 mmol, 1.5 equiv) and *N*-phenyl-2,2,2-trifluoroacetimidoyl chloride (0.88 mL, 5.76 mmol, 1.5 equiv) were added at 0 °C. After 1.5 h the mixture filtered was over Celite and concentrated in vacuo. Purification by column chromatography (0–15% acetone in petroleum ether) produced the title disaccharide imidate as a colourless oil in a 1:3 α/β-ratio (3.712 g, 3.28 mmol, 85%). R_f = 0.22 (50% EtOAc in petroleum ether); ^{1}H NMR

(400 MHz, $CDCl_3$): δ = 8.01–7.93 (m, 4H, H_{arom}), 7.56–7.50 (m, 2H, H_{arom}), 7.44–7.35 (m, 4H, H_{arom}), 7.27 (t, 0.3H, J = 7.8 Hz, $H_{arom\text{-}NPh\text{-}\alpha}$), 7.16–7.07 (m, 2H, H-1, $H_{arom\text{-}NPh\text{-}\alpha/\beta}$), 7.01 (t, 0.7H, J = 7.4 Hz, $H_{arom\text{-}NPh\text{-}\beta}$), 6.93 (d, 0.3H, J = 8.9 Hz, NH_α), 6.92 (d, 0.7H, J = 8.7 Hz, NH_β), 6.79 (br d, 0.7H, J = 8.0 Hz, H-1_β), 6.77 (br s, 0.6H, $H_{arom\text{-}NPh\text{-}\alpha}$), 6.43 (br d, 1.4H, J = 6.2 Hz, $H_{arom\text{-}NPh\text{-}\beta}$), 6.23 (br s, 0.3H, H-$1_\alpha$), 5.95 (t, 0.7H, J = 9.6 Hz, H-3_β), 5.74 (t, 0.3H, J = 7.6 Hz, H-3_α), 5.56 (m, 1H, H-2_β and H-2_α), 5.11 (dd, 0.7H, J = 10.7, 9.0 Hz, H-$3'_\beta$), 5.06 (dd, 0.7H, J = 10.5, 8.8 Hz, H-$3'_\alpha$), 5.03 (d, 0.7H, J = 8.2 Hz, H-$1'_\beta$), 4.98 (d, 0.3H, J = 8.2 Hz, H-$1'_\alpha$), 4.54–4.48 (m, 1H, H-5_α and H-5_β), 4.44 (t, 0.3H, J = 7.9 Hz, H-4_α), 4.38 (t, 0.7H, J = 9.4 Hz, H-4_β), 3.87 (s, 2.1H, $CH_{3COOMe\text{-}\beta}$), 3.86–3.79 (m, 1H, H-$2'_\alpha$ and H-$2'_\beta$), 3.78 (s, 0.9H, $CH_{3COOMe\text{-}\alpha}$), 3.64–3.55 (m, 1.7H, H-$4'_\beta$, H-$6'_{a\text{-}\alpha}$ and H-$6'_{a\text{-}\beta}$), 3.51 (t, 0.3H, J = 9.3 Hz, H-$4'_\alpha$), 3.31 (td, 0.3H, J = 9.7, 4.8 Hz, H-$5'_\alpha$), 3.27 (td, 0.7H, J = 9.9, 4.9 Hz, H-$5'_\beta$), 2.83–2.66 (m, 3H, H-$6'_{b\text{-}\alpha}$, H-$6'_{b\text{-}\beta}$ and $CH_{2\text{-}Lev\text{-}\alpha/\beta}$), 2.60–2.54 (m, 2H, $CH_{2\text{-}Lev\text{-}\alpha/\beta}$), 2.13 (s, 2.1H, $CH_{3\text{-}Lev\text{-}\beta}$), 2.12 (s, 0.9H, $CH_{3\text{-}Lev\text{-}\alpha}$), 0.91–0.89 (m, 9H, $CH_{3\text{-}tBu\text{-}Si\text{-}\alpha/\beta}$), 0.88 (s, 2.7H, $CH_{3\text{-}tBu\text{-}Si\text{-}\alpha}$), 0.87 (s, 6.3H, $CH_{3\text{-}tBu\text{-}Si\text{-}\beta}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 205.7, 205.6 ($C{=}O_{Lev\text{-}ketone\text{-}\alpha/\beta}$), 172.2 ($C{=}O_{Lev\text{-}ester\text{-}\alpha}$), 172.0 ($C{=}O_{Lev\text{-}ester\text{-}\beta}$), 168.7 ($C{=}O_{COOMe\text{-}\alpha}$), 168.5 ($C{=}O_{COOMe\text{-}\beta}$), 165.1 ($C{=}O_{Bz\text{-}\beta}$), 164.9 ($C{=}O_{Bz\text{-}\alpha}$), 164.8 ($C{=}O_{Bz\text{-}\beta}$), 164.7 ($C{=}O_{Bz\text{-}\alpha}$), 161.6 ($C{=}O_{TCA\text{-}\beta}$), 161.5 ($C{=}O_{TCA\text{-}\alpha}$), 142.7 ($C_{q\text{-}arom\text{-}NPh\text{-}\alpha}$), 142.5 ($C_{q\text{-}arom\text{-}NPh\text{-}\beta}$), 133.6, 133.4, 133.2, 133.1, 129.7 ($CH_{arom\text{-}\alpha/\beta}$ ×5), 129.61 ($C_{q\text{-}arom\text{-}\alpha/\beta}$), 129.59 , 129.5 ($CH_{arom\text{-}\alpha/\beta}$ ×2), 129.4 ($C_{q\text{-}arom\text{-}\alpha/\beta}$), 128.6, 128.5, 128.4, 128.3, 128.2 ($CH_{arom\text{-}\alpha/\beta}$ ×5), 128.0 ($C_{q\text{-}arom\text{-}\alpha/\beta}$), 124.5, 124.3, 119.1, 118.8 ($CH_{arom\text{-}NPh\text{-}\alpha/\beta}$ ×4), 100.8 (C-$1'_\alpha$), 100.0 (C-$1'_\beta$), 94.4 (C-1_α), 92.6 ($C_{q\text{-}CCl3\text{-}\alpha}$), 92.3 ($C_{q\text{-}CCl3\text{-}\beta}$), 91.7 (C-$1_\beta$), 75.8 (C-$4_\beta$), 75.6 (C-$4_\alpha$), 74.4 (C-$4'_\beta$), 74.3 (C-$4'_\alpha$), 74.2 (C-$3'_\alpha$), 73.8 (C-$3'_\beta$), 71.6 (C-$5_{\alpha/\beta}$), 70.5 (C-$5_{\alpha/\beta}'$), 70.4 (C-$3_\alpha$), 70.2 (C-$2_\alpha$), 69.6 (C-$3_\beta$), 69.3 (C-$2_\beta$), 64.9 (C-$6'_{\alpha/\beta}$), 55.9 (C-$2'_\beta$), 55.7 (C-$2'_\alpha$), 53.3 ($CH_{3\text{-}COOMe\text{-}\beta}$), 53.0($CH_{3\text{-}COOMe\text{-}\beta}$), 37.9 ($CH_{2\text{-}Lev\text{-}\alpha/\beta}$), 29.5 ($CH_{3\text{-}Lev\text{-}\alpha/\beta}$), 29.2, 27.9 ($CH_{2\text{-}Lev\text{-}\alpha/\beta}$), 27.1, 26.6 ($CH_{3\text{-}tBu\text{-}Si\text{-}\alpha/\beta}$ ×2), 22.3, 19.6 ($C_{q\text{-}tBu\text{-}Si\text{-}\alpha/\beta}$ ×2); IR (neat): 2935, 2861, 2360, 1718, 1526, 1268, 1070, 907, 827, 729 cm^{-1}.

4.1.14. 4-Methylumbelliferyl 2-acetamido-4,6-*O*-di-*tert*-butylsilanediyl-2-deoxy-3-*O*-(methyl 2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-di-*tert*-butylsilanediyl-2-deoxy-3-*O*-levulinoyl-2-trichloro-acetamido-β-D-glucopyranosyl)-β-D-glucopyranosyl uronate)-β-D-glucopyranoside (17)

A mixture of imidate donor **16** (3.031 g, 2.67 mmol, 1.2 equiv) and *N*-acetyl glucosamine acceptor **3** (1.161 g, 2.23 mmol, 1.0 equiv) was co-evaporated two times with anhydrous toluene, dissolved in anhydrous CH_2Cl_2 (10 mL) and stirred over activated molsieves (3 Å) for 1 h. Trifluoromethanesulfonic acid (20 μL, 0.23 mmol, 0.1 equiv) was added at 0 °C and the reaction mixture was stirred overnight at 4 °C. Anhydrous Et_3N (0.2 mL) was added and the reaction was transferred to an extraction funnel with EtOAc (40 mL) and washed with satd aq $NaHCO_3$ (50 mL) and brine (50 mL). The aqueous layers were extracted with EtOAc (50 mL) and the combined organics were dried ($MgSO_4$), filtered and concentrated in vacuo. Purification by size exclusion chromatography followed by column chromatography (20–40% EtOAc in petroleum ether) yielded trisaccharide **17** as a white solid (1.961 g, 1.34 mmol, 60%). $[\alpha]_D^{22}$ −5 (*c* 0.43, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.97–7.92 (m, 4H, H_{arom}), 7.57–7.52 (m, 2H, H_{arom}), 7.45 (d, 1H, J = 9.4 Hz, H-$5_{4\text{-}MU}$), 7.43–7.38 (m, 4H, H_{arom}), 6.89 (d, 1H, J = 8.8 Hz, NH_{TCA}), 6.86–6.82 (m, 2H, H-$6_{4\text{-}MU}$ and H-$8_{4\text{-}MU}$), 6.14 (d, 1H, J = 1.1 Hz, H-$3_{4\text{-}MU}$), 5.75 (d, 1H, J = 7.5 Hz, NH_{NAc}), 5.65 (d, 1H, J = 8.2 Hz, H-1), 5.59 (t, 1H, J = 9.1 Hz, H-3′), 5.36 (dd, 1H, J = 9.1, 6.5 Hz, H-2′), 5.19 (d, 1H, J = 6.5 Hz, H-1′), 5.02 (dd, 1H, J = 10.6, 9.2 Hz, H-3″), 4.89 (d, 1H, J = 8.3 Hz, H-1″), 4.38 (dd, 1H, J = 10.1, 8.4 Hz, H-3), 4.33 (t, 1H, J = 9.2 Hz, H-4′), 4.19 (dd, 1H, J = 10.3, 5.0 Hz, H-6_a), 4.11 (d, 1H, J = 9.2 Hz, H-5′), 3.96 (dd, 1H, J = 9.5, 8.4 Hz, H-4), 3.88 (dd, 1H, J = 10.3, 10.0, H-6_b), 3.84–3.79 (m, 4H, $CH_{3\text{-}COOMe}$ and H-2″), 3.67–3.50 (m, 3H, H-5, H-6a″ and H-4″), 3.44 (ddd, 1H, J = 10.1, 8.2, 7.5 Hz, H-2), 3.26 (td, 1H, J = 9.9, 4.9 Hz, H-5″), 2.72–2.65 (m, 3H, $CH_{2\text{-}Lev}$ and H-6b″), 2.56 (t, 2H, J = 6.8 Hz, $CH_{2\text{-}Lev}$), 2.37 (d, 3H, J = 1.1 Hz, $CH_{3\text{-}4\text{-}MU}$), 2.13 (s, 3H, $CH_{3\text{-}Lev}$), 1.79 (s, 3H, $CH_{3\text{-}NAc}$), 1.05 (s, 9H, $CH_{3\text{-}tBu\text{-}Si}$), 0.98 (s, 9H, $CH_{3\text{-}tBu\text{-}Si}$), 0.89 (s, 9H, $CH_{3\text{-}tBu\text{-}Si}$), 0.87 (s, 9H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 205.8 ($C{=}O_{Lev\text{-}ketone}$), 172.3 ($C{=}O_{Lev\text{-}ester}$), 170.7 ($C{=}O_{NAc}$), 169.3 ($C{=}O_{COOMe}$), 165.3, 165.1 ($C{=}O_{Bz}$ ×2), 161.7 (C-$2_{4\text{-}MU}$), 160.9 ($C{=}O_{TCA}$), 159.5, 154.7, 152.3 (C-$7_{4\text{-}MU}$, C-$9_{4\text{-}MU}$ and C-$10_{4\text{-}MU}$), 133.7, 133.2 (CH_{arom} ×2), 129.9 ($C_{q\text{-}arom}$), 129.7, 129.6 (CH_{arom} ×2), 129.0 ($C_{q\text{-}arom}$), 128.6, 128.4 (CH_{arom} ×2), 125.6 (C-$5_{4\text{-}MU}$), 115.2 (C-$4_{4\text{-}MU}$), 113.5 (C-$6_{4\text{-}MU}$), 112.9 (C-$3_{4\text{-}MU}$), 104.1 (C-$8_{4\text{-}MU}$), 100.5 (C-1′), 100.4 (C-1″), 97.2 (C-1), 92.4 ($C_{q\text{-}CCl3}$), 79.2 (C-3), 76.4 (C-4), 76.2 (C-4′), 74.4 (C-4″), 74.3 (C-3″), 74.0 (C-5′), 72.8 (C-2′), 72.6 (C-3′), 70.6 (C-5″), 70.4 (C-5), 66.1 (C-6), 65.0 (C-6″), 57.1 (C-2), 55.8 (C-2″), 53.1 ($CH_{3\text{-}COOMe}$), 38.0 ($CH_{2\text{-}Lev}$), 29.8 ($CH_{3\text{-}Lev}$), 28.0 ($CH_{2\text{-}Lev}$), 27.3, 27.2, 26.9, 26.7 ($CH_{3\text{-}tBu\text{-}Si}$ ×4), 23.3 ($CH_{3\text{-}NAc}$), 22.6, 22.4, 19.9, 19.7 ($C_{q\text{-}tBu\text{-}Si}$ ×4), 18.6 ($CH_{3\text{-}4\text{-}MU}$); IR (neat): 2934, 2860, 1718, 1270, 1069, 827, 709 cm^{-1}; HRMS calcd for $[C_{68}H_{87}Cl_3N_2O_{32}Si_2{+}H]^+$: 1461.4377, found 1461.4393.

4.1.15. 4-Methylumbelliferyl 2-acetamido-4,6-*O*-di-*tert*-butylsilanediyl-2-deoxy-3-*O*-(methyl 2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-di-*tert*-butylsilanediyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-β-D-glucopyranosyl uronate)-β-D-glucopyranoside (18)

To a solution of trisaccharide **17** (1.902 g, 1.30 mmol, 1.0 equiv) in pyridine/acetic acid 4:1 (60 mL), hydrazine monohydrate (0.32 g, 6.50 mmol, 5.0 equiv) was added at room temperature. Upon full conversion of the starting material (TLC) the mixture was diluted with EtOAc (100 mL) and washed with satd aq $NaHCO_3$ (200 mL) and brine (100 mL). The aqueous layers were extracted with EtOAc (100 mL) and the combined organics were dried ($MgSO_4$), filtered and concentrated in vacuo. Purification by column chromatography (20–50% EtOAc in petroleum ether) yielded the title compound as a white solid (1.76 g, 1.29 mmol, 99%). $[\alpha]_D^{22}$ +7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.97–7.91 (m, 4H, H_{arom}), 7.56–7.50 (m, 2H, H_{arom}), 7.45 (d, 1H, J = 9.6 Hz, H-$5_{4\text{-}MU}$), 7.42–7.37 (m, 4H, H_{arom}), 7.06 (d, 1H, J = 7.5 Hz, NH_{TCA}), 6.85 (dd, 1H, J = 9.6, 2.5 Hz, H-$6_{4\text{-}MU}$), 6.84 (d, 1H, J = 2.5 Hz, H-$8_{4\text{-}MU}$), 6.13 (d, 1H, J = 1.1 Hz, H-$3_{4\text{-}MU}$), 5.82 (d, 1H, J = 7.5 Hz, NH_{NAc}), 5.64 (d, 1H, J = 8.2 Hz, H-1), 5.58 (t, 1H, J = 9.1 Hz, H-3′), 5.38 (dd, 1H, J = 9.2, 6.6 Hz, H-2′), 5.21 (d, 1H, J = 6.5 Hz, H-1′), 4.94 (d, 1H, J = 8.3 Hz, H-1″), 4.41–4.32 (m, 2H, H-3 and H-4′), 4.19 (dd, 1H, J = 10.3, 5.1 Hz, H-6_a), 4.15 (d, 1H, J = 9.3 Hz, H-5′), 3.97 (dd, 1H, J = 9.5, 8.5 Hz, H-4), 3.90 (t, 1H, J = 10.2, H-6_b), 3.83 (s, 3H, $CH_{3\text{-}COOMe}$), 3.78 (dd, 1H, J = 10.4, 8.5 Hz, H-3″), 3.61 (td, 1H, J = 9.9, 5.1 Hz, H-5), 3.56 (dd, 1H, J = 10.4, 5.1 Hz, H-6a″), 3.51–3.43 (m, 2H, H-2 and H-2″), 3.36 (dd, 1H, J = 9.4, 8.5 Hz, H-4″), 3.26 (td, 1H, J = 9.9, 5.1 Hz, H-5″), 2.72 - 2.65 (t, 1H, J = 10.3 Hz, H-6b″), 2.36 (d, 3H, J = 1.1 Hz, $CH_{3\text{-}4\text{-}MU}$), 1.77 (s, 3H, $CH_{3\text{-}NAc}$), 1.06 (s, 9H, $CH_{3\text{-}tBu\text{-}Si}$), 0.98 (s, 9H, $CH_{3\text{-}tBu\text{-}Si}$), 0.90 (s, 9H, $CH_{3\text{-}tBu\text{-}Si}$), 0.89 (s, 9H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.7 ($C{=}O_{NAc}$), 169.3 ($C{=}O_{COOMe}$), 165.2, 165.1 ($C{=}O_{Bz}$ ×2), 162.3 (C-$2_{4\text{-}MU}$), 160.9 ($C{=}O_{TCA}$), 159.5, 154.7, 152.3 (C-$7_{4\text{-}MU}$, C-$9_{4\text{-}MU}$ and C-$10_{4\text{-}MU}$), 133.6, 133.1 (CH_{arom} ×2), 129.8 ($C_{q\text{-}arom}$), 129.7, 129.6 (CH_{arom} ×2), 129.0 ($C_{q\text{-}arom}$), 128.6, 128.4 (CH_{arom} ×2), 125.6 (C-$5_{4\text{-}MU}$), 115.2 (C-$4_{4\text{-}MU}$), 113.5 (C-$6_{4\text{-}MU}$), 112.8 (C-$3_{4\text{-}MU}$), 104.1 (C-$8_{4\text{-}MU}$), 100.4 (C-1′), 99.3 (C-1″), 97.2 (C-1), 92.6 ($C_{q\text{-}CCl3}$), 79.3 (C-3), 77.4 (C-4″), 76.4 (C-4), 75.7 (C-4′), 74.1 (C-5′), 73.7 (C-3″), 72.8 (C-2′), 72.7 (C-3′), 70.4 (C-5″), 70.1 (C-5), 66.1 (C-6), 65.0 (C-6″), 58.2 (C-2), 57.0 (C-2″), 53.1 ($CH_{3\text{-}COOMe}$), 27.34, 27.29, 26.9, 26.8

($CH_{3\text{-}tBu\text{-}Si}$ ×4), 23.3 ($CH_{3\text{-}NAc}$), 22.6, 22.4, 19.9, 19.7 ($C_{q\text{-}tBu\text{-}Si}$ ×4), 18.6 ($CH_{3\text{-}4\text{-}MU}$); IR (neat): 3351, 2934, 2859, 1718, 1270, 1069, 827, 731, 709 cm^{-1}; HRMS calcd for $[C_{63}H_{81}Cl_3N_2O_{21}Si_2+Na]^+$: 1385.3828, found 1385.3839.

4.1.16. Methyl (2,3-di-*O*-benzoyl-4-deoxy-α/β-D-glucopyranoside *N*-Phenyl-2,2,2-trifluoroacetimidate) uronate (19)

To a solution of 4-deoxy hemiacetal **7** (361 mg, 0.73 mmol, 1.0 equiv) in acetone (5 mL) Cs_2CO_3 (474 mg, 1.46 mmol, 2.0 equiv) and *N*-phenyl-2,2,2-trifluoroacetimidoyl chloride (0.26 mL, 1.46 mmol, 2.0 equiv) were added at 0 °C. After 2 h at 0 °C was the mixture filtered over Celite and concentrated in vacuo. Purification by column chromatography (0–5% acetone in petroleum ether) produced the title imidate as a colourless oil in a 2:3 α/β-ratio (306 mg, 0.54 mmol, 73%). NMR assignment for the major isomer (β): ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.06–7.95 (m, 4H, H_{arom}), 7.59–7.49 (m, 2H, H_{arom}), 7.46–7.36 (m, 4H, H_{arom}), 7.27 (m, 1H, $H_{arom\text{-}NPh}$), 7.14–7.06 (m, 2H, $H_{arom\text{-}NPh}$), 6.81 (d, 1H, *J* = 7.7 Hz, H-1), 6.39 (br d, 2H, *J* = 5.0 Hz, H_{arom}), 5.83 (ddd, 1H, *J* = 11.0, 10.8, 5.0 Hz, H-3), 5.60 (m, 1H, H-2), 4.82 (br d, 1H, *J* = 11.8 Hz, H-5), 3.81 (s, 3H, $CH_{3\text{-}COOMe}$), 2.89 (m, 1H, H-4_{eq}), 2.13 (m, 1H, H-4_{ax}); ^{13}C NMR (101 MHz, CDCl3) δ = 169.0 ($C{=}O_{COOMe}$), 165.6, 165.3 ($C{=}O_{Bz}$ ×2), 142.8 ($C_{q\text{-}arom\text{-}NPh}$), 133.6, 133.4, 129.8, 129.74, 129.65 (CH_{arom} ×5), 129.1, 128.8 ($C_{q\text{-}arom}$ ×2), 128.6, 128.53, 128.48, 128.45, 128.41, 128.38 (CH_{arom} ×6), 124.3, 124.2, 119.0 ($CH_{arom\text{-}NPh}$ ×3), 92.8 (C-1), 70.4 (C-2), 68.9 (C-5), 67.6 (C-3), 52.7 ($CH_{3\text{-}COOMe}$), 32.9 (C-4); IR (neat): 2959, 2925, 1718, 1452, 1272, 1208, 1070, 1027, 777, 695 cm^{-1}; HRMS calcd for $[C_{29}H_{24}F_3NO_8+Na]^+$: 594.1346, found 594.1348.

4.1.17. Methyl (2,3-di-*O*-benzoyl-4-*O*-methyl-α/β-D-glucopyranoside *N*-phenyl-2,2,2-trifluoroacetimidate) uronate (22)

To a solution of 4-methoxy hemiacetal **10** (0.754 g, 1.75 mmol, 1.0 equiv) in acetone (8 mL) Cs_2CO_3 (1.140 g, 3.50 mmol, 2.0 equiv) and *N*-phenyl-2,2,2-trifluoroacetimidoyl chloride (0.54 mL, 3.50 mmol, 2.0 equiv) were added at 0 °C. After 1.5 h at 0 °C the mixture was filtered over Celite and concentrated in vacuo. Purification by column chromatography (0–10% EtOAc in petroleum ether) yielded the title imidate as a colourless oil in a 1:5 α/β-ratio (0.679 g, 1.13 mmol, 65%). NMR assignment for the major isomer (β): R_f = 0.45 (20% EtOAc in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.04–8.01 (m, 2H, H_{arom}), 7.98–7.94 (m, 2H, H_{arom}), 7.56–7.50 (m, 2H, H_{arom}), 7.14–7.08 (m, 2H, $H_{arom\text{-}NPh}$), 6.99 (t, 1H, *J* = 7.4 Hz, $H_{arom\text{-}NPh}$), 6.78 (d, 1H, *J* = 7.5 Hz, H-1), 6.40 (br d, 2H, *J* = 6.1 Hz, $H_{arom\text{-}NPh}$), 6.03 (t, 1H, *J* = 9.8 Hz, H-3), 5.47 (dd, 1H, *J* = 10.1, 3.3 Hz, H-2), 4.52 (d, 1H, *J* = 10.0 Hz, H-5), 3.97 (t, 1H, *J* = 9.7 Hz, H-4), 3.87 (s, 3H, $CH_{3\text{-}COOMe}$), 3.46 (s, 3H, $CH_{3\text{-}OMe}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.4 ($C{=}O_{COOMe}$), 165.31, 165.25 ($C{=}O_{Bz}$ ×2), 142.7 ($C_{q\text{-}arom\text{-}NPh}$), 133.6, 133.4, 129.9, 129.7 (CH_{arom} ×4), 129.1 ($C_{q\text{-}arom}$), 128.54, 128.51, 128.48, 128.43 (CH_{arom} ×4), 124.5, 124.3, 119.0 ($CH_{arom\text{-}NPh}$ ×3), 92.1 (C-1), 78.7 (C-4), 72.2 (C-5), 71.3 (C-3), 70.2 (C-2), 60.6 ($CH_{3\text{-}OMe}$), 52.9 ($CH_{3\text{-}COOMe}$); IR (neat): 2959, 2925, 1718, 1451, 1261, 909, 707 cm^{-1}; HRMS calcd for $[C_{30}H_{26}F_3NO_9+Na]^+$: 624.1452, found 624.1451.

4.1.18. 4-Methylumbelliferyl 2-acetamido-4,6-*O*-di-*tert*-butylsilanediyl-2-deoxy-3-*O*-[methyl 2,3-di-*O*-benzoyl-4-*O*-{4,6-*O*-di-*tert*-butylsilanediyl-2-deoxy-3-*O*-(methyl 2,3-di-*O*-benzoyl-4-deoxy-β-D-glucopyranosyl uronate)-2-trichloroacetamido-β-D-glucopyranosyl}-β-D-glucopyranosyl uronate]-β-D-glucopyranoside (20)

Trisaccharide acceptor **18** (201 mg, 0.147 mmol, 1.0 equiv) and imidate donor **19** (168 mg, 0.29 mmol, 2.0 equiv) were co-evaporated two times with anhydrous toluene and then dissolved in anhydrous CH_2Cl_2 (4 mL). Activated molsieves (3 Å, 1.0 g) were added and the reaction mixture was stirred at room temperature for 1 h. The reaction was cooled to 0 °C and trifluoromethanesulfonic acid (2.0 μL, 22 μmol, 0.15 equiv) was added. The reaction was stirred for 4 h and then quenched with Et_3N (0.2 mL, 1.4 mmol, 10 equiv). The reaction mixture was transferred to an extraction funnel with CH_2Cl_2 (40 mL) and washed with satd aq $NaHCO_3$ (40 mL) and brine (30 mL). The aqueous layers were extracted with CH_2Cl_2 (40 mL) and the combined organics were dried $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by size exclusion followed by column chromatography (30–50% EtOAc in petroleum ether) to give the title compound (200 mg, 0.114 mmol, 78% yield). R_f = 0.24 (50% EtOAc in petroleum ether); $[\alpha]_D^{22}$: +21 (C = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.98–7.89 (m, 8H, H_{arom}), 7.57–7.48 (m, 4H, H_{arom}), 7.45–7.33 (m, 9H, H-$5_{4\text{-}MU}$ and H_{arom}), 7.04 (d, 1H, *J* = 7.5 Hz, NH_{TCA}), 6.84 (dd, 1H, *J* = 7.5, 2.1 Hz, H-$6_{4\text{-}MU}$), 6.83 (d, 1H, *J* = 2.1 Hz, H-$8_{4\text{-}MU}$), 6.14 (d, 1H, *J* = 1.1 Hz, H-$3_{4\text{-}MU}$), 5.74 (d, 1H, *J* = 7.3 Hz, NH_{NAc}), 5.70 (d, 1H, *J* = 8.2 Hz, H-1), 5.54 (t, 1H, *J* = 8.9 Hz, H-3′), 5.38 (dd, 1H, *J* = 8.9, 6.6 Hz, H-2′), 5.31 (dd, 1H, *J* = 9.2, 6.6 Hz, H-2‴), 5.26 (m, 1H, H-3‴), 5.17 (d, 1H, *J* = 6.7 Hz, H-1′), 5.08 (d, 1H, *J* = 6.8 Hz, H-1‴), 5.07 (d, 1H, *J* = 8.1 Hz, H-1″), 4.42 (dd, 1H, *J* = 10.1, 8.5 Hz, H-4′), 4.40 (t, 1H, *J* = 9.1 Hz, H-3), 4.25 (dd, 1H, *J* = 11.9, 2.8 Hz, H-5‴), 4.24–4.15 (m, 2H, H-3″ and H-6_a), 4.10 (d, 1H, *J* = 9.3 Hz, H-5′), 3.94 (t, 1H, *J* = 9.0 Hz, H-4), 3.88 (t, 1H, *J* = 10.3 Hz, H-6_b), 3.80 (s, 3H, $CH_{3\text{-}COOMe}$), 3.79 (s, 3H, $CH_{3\text{-}COOMe}$), 3.66–3.57 (m, 3H, H-5, H-4″ and H-$6''_a$), 3.41–3.31 (m, 2H, H-2 and H-2″), 3.16 (ddd, 1H, *J* = 9.8, 9.7, 4.8 Hz, H-5″), 2.77 (t, 1H, *J* = 10.3 Hz, H-$6''_b$), 2.66 (ddd, 1H, *J* = 12.9, 5.2, 2.8 Hz, H-$4''_{eq}$), 2.36 (d, 3 H, *J* = 1.1 Hz, $CH_{3\text{-}4\text{-}MU}$), 2.02 (m, 1H, H-$4''_{ax}$), 1.78 (s, 3H, $CH_{3\text{-}NAc}$), 1.04 (s, 9H, $CH_{3\text{-}tBu\text{-}Si}$), 0.97 (s, 9H, $CH_{3\text{-}tBu\text{-}Si}$), 0.88 (s, 9H, $CH_{3\text{-}tBu\text{-}Si}$), 0.81 (s, 9H, $CH_{3\text{-}tBu\text{-}Si}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.8 ($C{=}O_{NAc}$), 169.1, 168.6 ($C{=}O_{COOMe}$ ×2), 165.7, 165.3, 165.2, 165.1 ($C{=}O_{Bz}$ ×4), 161.6 (C-$2_{4\text{-}MU}$), 160.9 ($C{=}O_{TCA}$), 159.5, 154.7, 152.2 (C-$7_{4\text{-}MU}$, C-$9_{4\text{-}MU}$ and C-$10_{4\text{-}MU}$), 133.6, 133.3, 133.14, 133.12 (CH_{arom} ×4), 129.77 ($C_{q\text{-}arom}$), 129.75, 129.68 129.56 (CH_{arom} ×3), 129.3, 129.05, 129.04 ($C_{q\text{-}arom}$ ×3), 128.6, 128.42, 128.35, 128.27 (CH_{arom} ×4), 125.6 (C-$5_{4\text{-}MU}$), 115.2 (C-$4_{4\text{-}MU}$), 113.4 (C-$6_{4\text{-}MU}$), 112.8 (C-$3_{4\text{-}MU}$), 104.2 (C-$8_{4\text{-}MU}$), 100.3 (C-1′), 98.5 (C-1″), 98.3 (C-1‴), 96.9 (C-1), 92.5 ($C_{q\text{-}CCl3}$), 79.1 (C-3), 76.7 (C-3″), 76.0 (C-4), 75.1 (C-4′), 74.5 (C-4″), 74.1 (C-5′), 73.0 (C-3′), 72.9 (C-2‴), 72.6 (C-2′), 71.2 (C-3″), 70.4 (C-5), 70.28 (C-5‴), 70.25 (C-5″), 66.1 (C-6), 65.2 (C-6″), 58.8 (C-2), 57.5 (C-2″), 53.1, 52.5 ($CH_{3\text{-}COOMe}$ ×2), 32.4 (C-4‴), 27.3, 27.3, 26.9, 26.7 ($CH_{3\text{-}tBu\text{-}Si}$ ×4), 23.3 ($CH_{3\text{-}NAc}$), 22.6, 22.4, 19.9, 19.6 ($C_{q\text{-}tBu\text{-}Si}$ ×4), 18.6 ($CH_{3\text{-}4\text{-}MU}$); HRMS calcd for $[C_{84}H_{99}Cl_3N_2O_{28}Si_2+H]^+$: 1745.5061, found 1745.5077.

4.1.19. 4-Methylumbelliferyl 2-acetamido-4,6-*O*-di-*tert*-butylsilanediyl-2-deoxy-3-*O*-[methyl 2,3-di-*O*-benzoyl-4-*O*-{4,6-*O*-di-*tert*-butylsilanediyl-2-deoxy-3-*O*-(methyl 2,3-di-*O*-benzoyl-4-*O*-methyl-β-D-glucopyranosyl uronate)-2-trichloroacetamido-β-D-glucopyranosyl}-β-D-glucopyranosyl uronate]-β-D-glucopyranoside (23)

Trisaccharide acceptor **18** (100 mg, 0.073 mmol, 1.0 equiv) and imidate donor **22** (88 mg, 0.15 mmol, 2.0 equiv) were coevaporated two times with anhydrous toluene and then dissolved in anhydrous CH_2Cl_2 (2 mL). Activated molsieves (3å, 0.5 g) were added and the reaction mixture was stirred at room temperature for 1 h, before cooling to 0 °C and addition of trifluoromethanesulfonic acid (~1.0 μL, 11 μmol, 0.15 equiv). The reaction was stirred for 4 h and then quenched with Et_3N (0.1 mL, 0.73 mmol, 10 equiv). The reaction mixture was transferred to an extraction funnel with CH_2Cl_2 (20 mL) and washed with satd aq $NaHCO_3$ (20 mL) and brine (20 mL). The aqueous layers were extracted with CH_2Cl_2 (20 mL) and the combined organics were dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by size exclusion

chromatography, followed by column chromatography (30–50% EtOAc in petroleum ether) to give the title tetrasaccharide **23** (111 mg, 0.062 mmol, 85% yield). R_f = 0.22 (50% EtOAc in petroleum ether); $[\alpha]_D^{22}$: + 8.3 (C = 0.157, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ = 7.96–7.90 (m, 8H, H_{arom}), 7.53–7.49 (m, 4H, H_{arom}), 7.45 (d, 1H, J = 9.5 Hz, H-$5_{4\text{-MU}}$), 7.41–7.35 (m, 8H, H_{arom}), 6.86 (d, 1H, J = 9.5 Hz, H-$6_{4\text{-MU}}$), 6.84 (d, 1H, J = 7.5 Hz, NH_{TCA}), 6.82 (s, 1H, H-$8_{4\text{-MU}}$), 6.13 (d, 1H, J = 1.0 Hz, H-$3_{4\text{-MU}}$), 5.71 (d, 1H, J = 7.4 Hz, NH_{NAc}), 5.67 (d, 1H, J = 8.2 Hz, H-1), 5.52 (t, 1H, J = 9.1 Hz, H-3‴), 5.50 (t, 1H, J = 9.1 Hz, H-3′), 5.37 (dd, 1H, J = 9.1, 6.7 Hz, H-2′), 5.25 (dd, 1H, J = 8.9, 6.5 Hz, H-2‴), 5.13 (d, 1H, J = 6.7 Hz, H-1′), 5.07 (d, 1H, J = 6.5 Hz, H-1‴), 4.99 (d, 1H, J = 8.2 Hz, H-1″), 4.41 (dd, 1H, J = 10.1, 8.5 Hz, H-3), 4.35 (t, 1H, J = 9.1 Hz, H-4′), 4.20–4.14 (m, 2H, H-6_a and H-3″), 4.06 (d, 1H, J = 9.2 Hz, H-5′), 4.02 (d, 1H, J = 9.0 Hz, H-5‴), 3.95 (t, 1H, J = 9.0 Hz, H-4‴), 3.94 (dd, 1H, J = 9.5, 8.6 Hz, H-4), 3.88 (t, 1H, J = 10.3 Hz, H-6_b), 3.81 (s, 3H, $CH_{3\text{-COOMe}}$), 3.79 (s, 3H, $CH_{3\text{-COOMe}}$), 3.64–3.56 (m, 2H, H-5 and H-$6''_a$), 3.51 (t, 1H, J = 9.2 Hz, H-4″), 3.39 (s, 3H, $CH_{3\text{-OMe}}$), 3.35 (m, 1H, H-2), 3.27–3.13 (m, 2H, H-2″ and H-5″), 2.69 (t, 1H, J = 10.4 Hz, H-$6''_b$), 2.36 (s, 3H, $CH_{3\text{-4MU}}$), 1.76 (s, 3H, $CH_{3\text{-NAc}}$), 1.04 (s, 9H, $CH_{3\text{-tBu-Si}}$), 0.97 (s, 9H, $CH_{3\text{-tBu-Si}}$), 0.90 (s, 9H, $CH_{3\text{-tBu-Si}}$), 0.82 (s, 9H, $CH_{3\text{-tBu-Si}}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.7 (C=O_{NAc}), 168.6, 168.5 (C=O_{COOMe} ×2), 165.4, 165.34, 165.29, 165.1 (C=O_{Bz} ×4), 161.5 (C-$2_{4\text{-MU}}$), 160.9 (C=O_{TCA}), 159.5, 154.7, 152.2 (C-$7_{4\text{-MU}}$, C-$9_{4\text{-MU}}$ and C-$10_{4\text{-MU}}$), 133.6, 133.3, 133.1, 129.9 (CH_{arom} ×4), 129.8 ($C_{q\text{-arom}}$), 129.70, 129.67, 129.58 (CH_{arom} ×3), 129.2, 129.08, 129.03 ($C_{q\text{-arom}}$ ×3), 128.6, 128.42, 128.37, 128.33 (CH_{arom} ×4), 125.6 (C-$5_{4\text{-MU}}$), 115.2 (C-$4_{4\text{-MU}}$), 113.4 (C-$6_{4\text{-MU}}$), 112.9 (C-$3_{4\text{-MU}}$), 104.3 (C-$8_{4\text{-MU}}$), 100.4 (C-1′), 99.6 (C-1‴), 98.4 (C-1″), 97.0 (C-1), 92.5 ($C_{q\text{-CCl3}}$), 79.2 (C-3), 78.4 (C-4‴), 77.6 (C-3″), 76.2 (C-4), 75.11 (C-4″), 75.08 (C-4′), 74.5 (C-5′), 74.2 (C-5‴), 74.1 (C-3‴), 73.1 (C-2‴), 72.8 (C-3′), 72.6 (C-2′), 70.4 (C-5), 70.2 (C-5″), 66.1 (C-6), 65.2 (C-6″), 60.4 ($CH_{3\text{-OMe}}$), 58.7 (C-2″), 57.4 (C-2), 53.1, 52.6 ($CH_{3\text{-COOMe}}$ ×2), 27.3 (×2), 26.9, 26.7 ($CH_{3\text{-tBu-Si}}$ ×4), 23.3 ($CH_{3\text{-NAc}}$), 22.6, 22.4, 19.9, 19.6 ($C_{q\text{-tBu-Si}}$ ×4), 18.6 ($CH_{3\text{-4-MU}}$); IR (neat): 3352, 2935, 2860, 1730, 1616, 1269, 1070, 828, 710 cm^{-1}; HRMS calcd for $[C_{85}H_{101}Cl_3N_2O_{29}Si_2+H]^+$: 1775.5167, found 1775.5180.

4.1.20. 4-Methylumbelliferyl 2-acetamido-2-deoxy-3-*O*-[4-*O*-{2-acetamido-2-deoxy-3-*O*-(4-deoxy-β-D-glucopyranosyl uronate)-β-D-glucopyranosyl}-β-D-glucopyranosyl uronate]-β-D-glucopyranoside bis ammonium salt (21)

Protected tetrasaccharide **20** (75 mg, 42 μmol, 1.0 equiv) was dissolved in acetic acid (5 mL) and activated zinc dust (138 mg, 2.1 mmol, 50 equiv) was added three times with 2 h interval. The reaction was then stirred at ambient temperature over night. HPLC-MS indicated a ~5:2 ratio of mono-chloro residue and the fully dehalogenated product. The residue was diluted with toluene and filtered through a plug of Celite. The solids were washed with some additional toluene and the residue was then concentrated in vacuo and dissolved in anhydrous acetone (5 mL). Potassium iodide (210 mg, 1.30 mmol, 30 equiv) was added and the reaction mixure was stirred over night at ambient temperature giving full conversion to the monoiodo derivate as measured by HPLC-MS. The mixture was filtered, concentrated in vacuo and the remaining residue was dissolved in acetic acid (5 mL) and activated zinc (138 mg, 2.1 mmol, 50 equiv) was added. The reaction was stirred over night giving full conversion to the fully dehalogenated product. The solids were filtered, washed and the eluent was concentrated in vacuo. The residue was dissolved in MeOH (5 mL) and sodium methoxide (29 μL, 44 μmol, 1.0 equiv) was added and the reaction was stirred under argon at room temperature over night. Upon completion of the reaction, as monitored by HPLC-MS, the reaction was quenched with acetic acid (0.2 mL). The solvents were removed in vacuo by coevaporation with toluene (5 mL) and the residue was dissolved in pyridine (3 mL), before addition of hydrogen fluoride/Et_3N (29 μL, 17 mmol, 4 equiv). The reaction was stirred at ambient temperature for 3 h (the completion of the reaction was confirmed by HPLC-MS) and water (3 mL) was added followed by satd aq sodium carbonate (1.5 mL). The reaction was stirred at ambient temperature over night and then concentrated in vacuo. The product was purified by HPLC according to the general procedure. The title compound was obtained as the bis ammonium salt (24 mg, 26 μmol, 60%). ^1H NMR (600 MHz, D_2O) δ = 7.60 (d, 1H, J = 8.8 Hz, H-$5_{4\text{-MU}}$), 6.99 (dd, 1H, J = 8.8, 2.5 Hz, H-$6_{4\text{-MU}}$), 6.93 (d, 1H, J = 2.5 Hz, H-$8_{4\text{-MU}}$), 6.16 (s, 1H, H-$3_{4\text{-MU}}$), 5.25 (d, 1H, J = 8.5 Hz, H-1), 4.62 (d, 1H, J = 7.9 Hz, H-1′), 4.58 (d, 1H, J = 8.4 Hz, H-1″), 4.42 (d, 1H, J = 7.8 Hz, H-1‴), 4.30 (dd, 1H, J = 12.3, 2.4 Hz, H-5‴), 4.16 (t, 1H, J = 9.4 Hz, H-2), 4.02 (d, 1H, J = 9.7 Hz, H-5′), 3.95 (d, 1H, J = 12.1 Hz, H-6_a), 3.93–3.87 (m, 2H, H-6_a″ and H-3), 3.86–3.80 (m, 3H, H-2″, H-4′ and H-6_b), 3.79–3.72 (m, 3H, H-3‴, H-6_b″ and H-3″), 3.71–3.67 (m, 2H, H-4 and H-5), 3.65 (m, 1H, H-3′), 3.56 (dd, 1H, J = 11.9, 6.4 Hz, H-4″), 3.49 (m, 1H, H-5″), 3.40 (t, 1H, J = 8.7 Hz, H-2′), 3.21 (t, 1H, J = 8.5 Hz, H-2‴), 2.39–2.34 (m, 4H, H-$4'''_{eq}$ and $CH_{3\text{-4MU}}$), 2.01 (s, 3H, $CH_{3\text{-NAc}}$), 1.99 (s, 3H, $CH_{3\text{-NAc}}$), 1.64 (q, 1H, H-$4'''_{ax}$); ^{13}C NMR (151 MHz, $CDCl_3$) δ = 175.82, 175.76 (C=O_{NAc} ×2), 174.4, 172.3 (C=$O_{COO\text{-}NH4+}$ ×2), 165.4, 160.3, 157.0, 154.7 (C-$2_{4\text{-MU}}$, C-$7_{4\text{-MU}}$, C-$9_{4\text{-MU}}$ and C-$10_{4\text{-MU}}$), 127.6 (C-$5_{4\text{-MU}}$), 116.3 (C-$4_{4\text{-MU}}$), 114.9 (C-$6_{4\text{-MU}}$), 112.3 (C-$3_{4\text{-MU}}$), 104.6 (C-$8_{4\text{-MU}}$), 104.0 (C-1‴), 103.8 (C-1′), 102.1 (C-1″), 99.5 (C-1), 84.1 (C-3″), 83.0 (C-3), 81.1 (C-4′), 76.7 (C-5), 76.3 (C-5″), 75.0 (C-2‴), 74.6 (C-3′), 74.5 (C-5′), 73.1 (C-2′), 71.2 (C-5‴), 70.7 (C-3‴), 69.4 (C-4″), 69.0 (C-4), 61.5 (C-6″), 61.3 (C-6), 55.20 (C-2), 55.16 (C-2″), 36.2 (C-4‴), 23.3, 23.2 ($CH_{3\text{-NAc}}$ ×2), 18.9 ($CH_{3\text{-4-MU}}$).

4.1.21. 4-Methylumbelliferyl 2-acetamido-2-deoxy-3-*O*-[4-*O*-{2-acetamido-2-deoxy-3-*O*-(4-*O*-methyl-β-D-glucopyranosyl uronate)-β-D-glucopyranosyl}-β-D-glucopyranosyl uronate]-β-D-glucopyranoside bis ammonium salt (24)

Protected tetrasaccharide **23** (18 mg, 10 μmol, 1.0 equiv) was dissolved in acetic acid (2 mL) and activated zinc dust (33 mg, 0.50 mmol, 50 equiv) was added three times with 2 h interval. The reaction was stirred at ambient temperature over night, after which HPLC-MS indicated a ~4:1 ratio of mono-chloro residue and the fully dehalogenated product. The residue was diluted with toluene and filtered through a plug of Celite. The solids were washed with some additional toluene and the residue was then concentrated in vacuo and dissolved in anhydrous acetone (5 mL). Potassium iodide (50 mg, 0.30 mmol, 30 equiv) was added and the reaction mixure was stirred over night at ambient temperature giving full conversion to the monoiodo derivate as measured by HPLC-MS. The mixture was filtered, concentrated in vacuo and the remaining residue was dissolved in acetic acid (2 mL) and activated zinc (33 mg, 0.5 mmol, 50 equiv) was added. The reaction was stirred over night giving full conversion to the fully dehalogenated product. The solids were iltered, washed and the eluent was concentrated in vacuo. The residue was dissolved in MeOH (2 mL) and sodium methoxide (1.5 μL, 10 μmol, 1.0 equiv) was added and the reaction was stirred under argon at room temperature over night. After completion of the reaction, as monitored by HPLC-MS, the reaction was quenched with acetic acid (0.2 mL). The solvents were removed in vacuo by coevaporation with toluene (5 mL) and the residue was dissolved in pyridine (1 mL), before addition of hydrogenfluoride/Et_3N (6.6 μL, 40 μmol, 4 equiv). The reaction was stirred for 3 h at ambient temperature (the completion of the reaction was confirmed by HPLC-MS) and water (1 mL) was added followed by satd aq sodium carbonate (0.5 mL). The reaction was stirred at ambient temperature over night and then concentrated in vacuo. The product was purified by HPLC according to the general procedure. The title compound was obtained as the bis ammonium salt (4.3 mg,

4.4 μmol, 43%). ^{1}H NMR (600 MHz, D_2O) δ = 7.74 (d, 1H, J = 9.4 Hz, H-$5_{4\text{-MU}}$), 7.08–7.05 (m, 2H, H-$6_{4\text{-MU}}$ and H-$8_{4\text{-MU}}$), 6.28 (s, 1H, H-$3_{4\text{-MU}}$), 5.29 (d, 1H, J = 8.5 Hz, H-1), 4.57 (d, 1H, J = 7.9 Hz, H-1′), 4.55 (d, 1H, J = 8.3 Hz, H-1″),4.47 (d, 1H, J = 7.8 Hz, H-1‴), 4.16 (dd, 1H, J = 10.5, 8.5 Hz, H-2), 3.95 (dd, 1H, J = 12.1, 2.3 Hz, H-6_a), 3.93–3.89 (m, 2H, H-$6''_a$ and H-5′), 3.88–3.83 (m, 3H, H-3, H-5‴ and H-2″),3.83–3.78 (m, 2H, H-6_a and H-4′), 3.76 (dd, 1H, J = 12.4, 5.2 Hz, H-$6''_a$), 3.73–3.69 (m, 2H, H-4 and H-5), 3.67 (m, 1H, H-3″), 3.65 (m, 1H, H-3′), 3.55 (t, 2H, J = 9.3 Hz, H-4″ and H-3‴), 3.50–3.46 (m, 4H, H-5″ and $CH_{3\text{-OMe}}$), 3.39 (dd, 1H, J = 9.5, 7.9 Hz, H-2′), 3.33 (dd, J = 9.5, 7.9 Hz, 1H, H-2‴), 3.30 (t, J = 9.5 Hz, 1H, H-4‴), 2.45 (s, 3H, $CH_{3\text{-}4MU}$), 2.00 (s, 3H, $CH_{3\text{-NAc}}$), 1.99 (s, 3H, $CH_{3\text{-NAc}}$); ^{13}C NMR (151 MHz, D_2O) δ 175.9, 175.8 ($C{=}O_{NAc}$ ×2), 174.7, 173.4 ($C{=}O_{COO\text{-}NH4+}$ ×2), 165.7, 160.4, 157.3, 155.0 (C-$2_{4\text{-MU}}$, C-$7_{4\text{-MU}}$, C-$9_{4\text{-MU}}$ and C-$10_{4\text{-MU}}$), 127.7 (C-$5_{4\text{-MU}}$), 116.6 (C-$4_{4\text{-MU}}$), 114.9 (C-$6_{4\text{-MU}}$), 112.4 (C-$3_{4\text{-MU}}$), 104.7 (C-$8_{4\text{-MU}}$), 104.0 (C-1‴), 103.6 (C-1′), 101.9 (C-1″), 99.6 (C-1), 83.5 (C-5), 82.9 (C-3), 82.7 (C-4″), 81.0 (C-4′), 76.7 (C-5), 76.3 (C-5″), 75.69 (C-3‴), 75.65 (C-5′), 75.1 (C-5‴), 74.6 (C-3′), 73.5 (C-2‴), 73.2 (C-2′), 69.2 (C-4″), 69.1 (C-4), 61.43 (C-6″), 61.35 (C-6), 61.1 ($CH_{3\text{-OMe}}$), 55.24 (C-2), 55.19 (C-2″), 23.4, 23.1 ($CH_{3\text{-NAc}}$ ×2), 19.0 ($CH_{3\text{-}4\text{-MU}}$).

Acknowledgements

This research was supported by The Netherlands Ministry of Economic Affairs and the B-Basic partner organizations (www.b-basic.nl) through B-Basic, a public-private NWO-ACTS program (ACTS) Advanced Chemical Technologies for Sustainability) and The Netherlands Organization of Scientific Research (NWO, Vidi grant).

Supplementary data

Supplementary data (^{1}H and ^{13}C NMR spectra) associated with this article can be found, in the online version, at doi:10.1016/j.carres.2011.03.042.

References

1. (a) Wagschal, K.; Heng, C.; Lee, C. C.; Robertson, G. H.; Orts, W. J.; Wong, D. W. S. *Appl. Biochem. Biotechnol.* **2009**, *155*, 304–313; (b) Macauley, M. S.; Whitworth, G. E.; Debowski, A. W.; Chin, D.; Vocadlo, D. J. *J. Biol. Chem.* **2005**, *280*, 25313–25322; (c) Wang, L.-X.; Keyani, N. O.; Roseman, S.; Lee, Y. C. *Glycobiology* **1997**, *7*, 855–860.
2. Sinnot, M. L. *Chem. Rev.* **1990**, *90*, 1171–1202.
3. (a) Aguilera, B.; Ghauharali-van der Vlugt, K.; Helmond, M. T. J.; Out, J. M. M.; Donker-Koopman, W. E.; Groener, J. E. M.; Boot, R. G.; Renkema, G. H.; van der Marel, G. A.; van Boom, J. H.; Overkleeft, H. S.; Aerts, J. M. F. G. *J. Biol. Chem.* **2003**, *278*, 40911–40916; (b) Hollak, C. E. M.; van Weely, S.; van Oers, M. H. J.; Aerts, J. M. F. G. *J. Clin. Invest.* **1994**, *93*, 1288–1292.
4. (a) Meyer, K.; Palmer, J. W. *J. Biol. Chem.* **1934**, *107*, 629–634; (b) Laurent, T. C.; Fraser, J. R. E. *FASEB J.* **1992**, *6*, 2397–2404; (c) Knudson, C. B.; Knudson, W. *FASEB J.* **1993**, *7*, 1233–1241; (d) Zeng, C.; Toole, B. P.; Kinney, S. D.; Kuo, J.; Stamenkovic, I. *Int. J. Cancer* **1998**, *77*, 396–401.
5. (a) Volpi, N.; Schiller, J.; Stern, R.; Soltes, L. *Curr. Med. Chem.* **2009**, *16*, 1718–1745; (b) Stern, R.; Asari, A. A.; Sugahara, K. N. *Eur. J. Cell Biol.* **2006**, *85*, 699–715.
6. El-Safory, N. S.; Fazary, A. E.; Lee, C.-K. *Carbohydr. Polym.* **2010**, *81*, 165–181.
7. (a) Jiang, D.; Liang, J.; Noble, P. W. *Ann. Rev. Cell Dev. Biol.* **2007**, *23*, 435–461; (b) Noble, P. W. *Matrix Biol.* **2002**, *21*, 25–29.
8. Dinkelaar, J.; Duivenvoorden, B. A.; 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**, 2565–2570.
9. For a review on the synthesis of glycosaminoglycans, including HA, see: Yeung, B. K. S.; Chong, P. Y. C.; Petillo, P. A. *Carbohydr. Res.* **2002**, *21*, 779–865.
10. Jacobsson, M.; Malmberg, J.; Ellervik, U. *Carbohydr. Res.* **2006**, *341*, 1266–1281.
11. Horton, D. *Org. Synth. Coll. Vol.* **1973**, *5*, 1.
12. Kumagai, D.; Miyazaki, M.; Nishimura, S.-I. *Tetrahedon Lett.* **2001**, *42*, 1953–1956.
13. Dinkelaar, J.; Gold, H.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *J. Org. Chem.* **2009**, *74*, 4208–4216.
14. Iacono, S.; Rasmussen, J. R.; Card, P. J.; Smart, B. E. *Org. Synth.* **1986**, *64*, 57–63.
15. Van den Bos, L. J.; Codée, J. D. C.; van Boom, J. H.; Overkleeft, H. S.; van der Marel, G. A. *Org. Lett.* **2004**, *6*, 2165–2168.
16. Dinkelaar, J.; Witte, M. D.; van den Bos, L. J.; Overkleeft, H. S.; van der Marel, G. A. *Carbohydr. Res.* **2006**, *341*, 1723–1729.
17. Ziegler, T.; Eckhardt, E.; Herold, G. *Liebigs Ann. Chem.* **1992**, *1992*, 441–451.
18. All basic reaction conditions resulted in partial benzoyl migration form the C3–OH to the C4–OH.
19. Lindley, M. G.; Birch, G. G.; Khan, R. *Carbohydr. Res.* **1975**, *43*, 360–365.
20. Garcia, B. A.; Poole, J. L.; Gin, D. Y. *J. Am. Chem. Soc.* **1997**, *119*, 7597–7598.
21. Codée, J. D. C.; Litjens, R. E. J. N.; Den Heeten, R.; Overkleeft, H. S.; Van Boom, J. H.; Van der Marel, G. A. *Org. Lett.* **2003**, *5*, 1519–1522.
22. Crich, D.; Smith, M. *J. Am. Chem. Soc.* **2001**, *123*, 9015–9020.
23. (a) Yu, B.; Tao, H. *Tetrahedron Lett.* **2001**, *42*, 2405–2407; (b) Yu, B.; Sun, J. *Chem. Commun.* **2010**, *46*, 4668–4679.
24. Vibert, A.; Lopin-Bon, C.; Jacquinet, J. C. *Tetrahedron Lett.* **2010**, *51*, 1867–1869.