Synthesis of chondroitin sulfate oligosaccharides using *N*-tetrachlorophthaloyl and *N*-trifluoroacetyl galactosamine building blocks

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We have explored novel synthetic routes for the preparation of chondroitin sulfate (CS) oligosaccharides that are based on the use of *N*-tetrachlorophthaloyl (*N*-TCP) and *N*-trifluoroacetyl (*N*-TFA) galactosamine building blocks. On the one hand, we have performed the total synthesis of two CS disaccharides using *N*-TCP units, demonstrating the compatibility of TCP protection with the final deprotection/sulfation steps. However, the 2+2 coupling of *N*-TCP containing disaccharides, for the synthesis of CS tetrasaccharides, failed. On the contrary, a synthetic route using *N*-TFA galactosamine units efficiently afforded biologically relevant CS-like oligosaccharides. The TFA groups could be easily removed at the end of the synthesis and microwave irradiation greatly facilitated the sulfation reactions. The utility of this approach was illustrated with the total synthesis of two CS-like tetrasaccharides, with different sulfate distribution. Finally, we employed a fluorescence polarization assay to estimate the relative ability of the synthesized compounds to inhibit the interaction between FGF-2 and heparin.

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Introduction

Chondroitin sulfate (CS) is a linear and sulfated polysaccharide that belongs to the glycosaminoglycan family.[1] CS is constituted by the repetition of disaccharide units of D-glucuronic acid (GlcA)-β(1→3)-*N*-acetyl-D-galactosamine (GalNAc)-β(1→4). This repeating unit can be sulfated at various positions, giving rise to CS polymer chains with different sulfation patterns and a high level of structural diversity. In fact, CS chains are classified into different types based on the predominant sulfate group distribution, although a combination of different patterns is often found in CS samples. For instance, CS-A predominantly contains one sulfate at position 4 of the GalNAc unit, while CS-C has the sulfate group at position 6 and CS-E owns two sulfates at positions 4 and 6 of the GalNAc residue (Figure 1).

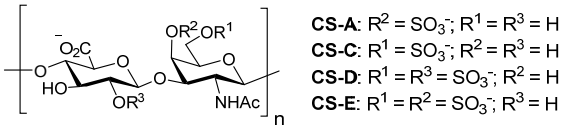


Figure 1. Some of the typical disaccharide units found in CS.

CS is involved in important biological processes such as central nervous system development[2] and malaria infection.[3] The participation of CS in these processes is mediated by certain oligosaccharide sequences, with particular sulfate distribution, that interact with several protein receptors.[4] For example, CS-E binds to several heparin-binding growth factors and chemokines,[5, 6] playing important roles in the central nervous system. Recent studies indicated that a CS-E tetrasaccharide interacts with midkine, a growth factor that participates in the development and repair of neural tissues.[7] Interestingly, this interaction requires a specific arrangement of sulfate groups since other oligosaccharides, with different sulfation motifs, bind significantly weaker (or do not bind at all) to midkine. A different class of CS, CS-A, has an important role in pregnancy-associated malaria.[8] This subtype of CS binds to *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), a critical step for the adhesion of *Plasmodium falciparum*-infected red blood cells to the placenta.

Due to the heterogeneity of the polymer, structurally defined CS oligosaccharides are not easily obtained in sufficient amounts from natural sources. Therefore, well-defined synthetic oligosaccharides are required for the study of CS-protein interactions at the molecular level, the establishment of structure-activity relationships and the evaluation of the potential therapeutic applications and biological activities of this type of compounds. Despite the recent advances in CS oligosaccharides and analogues synthesis,[7, 9-19] new approaches to the preparation of these molecules are still highly demanded. A careful protecting group design is required to obtain the adequate configuration of the glycosidic bonds and introduce the sulfate groups at defined positions. The choice of the protection for the amino group[20] of the GalNAc units is an important point in this design. Most reported synthesis of CS oligomers employed *N*-trichloroacetyl galactosamine building blocks.[21] However, the use of this type of units presents some limitations. The formation of stable trichlorooxazoline side products has been reported in glycosidation

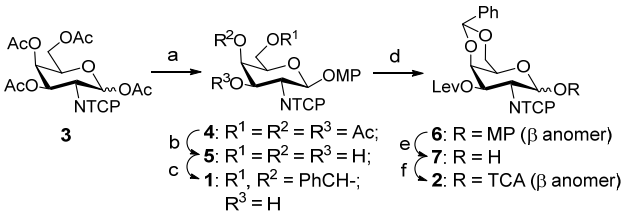
reactions of 2-deoxy-2-trichloroacetamido donors.[22-24] Additionally, the final transformation of the 2-trichloroacetamido to the desired 2-acetamido group can be problematic. The basic hydrolysis of multiple trichloroacetamide groups requires very long reaction times[22] and the alternative reduction (using tributylstannane/AIBN or Zn/acetic acid) affords, in some cases, significant amounts of mono- and dichloroacetamide intermediates.[25-27] For these reasons, we decided to explore the use of new galactosamine monomers that contain a *N*-tetrachlorophtaloyl (*N*-TCP)[28, 29] or a *N*-trifluroacetyl (*N*-TFA)[30-32] protecting group for the synthesis of CS oligosaccharides.

We envisioned that the TCP group would be a good alternative for 2-amino protection because this group avoids the formation of stable oxazolines while it strongly favours the formation of the 1,2-*trans* glycosidic linkage.[20, 33] Moreover, cleavage of the *N*-TCP moiety does not require the harsh reaction conditions needed for the removal of the analogous *N*-phthaloyl group.[34] On the other hand, we envisioned that the TFA group could facilitate the final deprotection steps since it can be removed under milder conditions, compared to the trichloroacetyl. This group also ensures high β selectivities in glycosidation reactions.[35, 36] Next, we will describe the results obtained with both types of building blocks. As a first goal, we considered the synthesis of CS tetrasaccharides since it has been demonstrated that tetramers are long enough to interact with several proteins and exhibit biological activity.[7, 9, 37-38]

Results and Discussion

N-TCP galactosamine building blocks for the synthesis of CS oligosaccharides

We prepared novel GalNAc monomers **1** and **2** (Scheme 1) that can be used as glycosyl acceptor and donor, respectively, in coupling reactions with glucuronic acid derivatives to afford CS-like disaccharides. The 4,6-*O*-benzylidene acetal will allow the selective sulfation of these positions at the end of the synthesis (see below). Tetraacetate **3**[39] was converted into the 4-methoxyphenyl glycoside **4** by treatment with 4-methoxyphenol and $BF_3{\cdot}Et_2O$. De-*O*-acetylation of **4** is not trivial due to the base-sensitive nature of the *N*-TCP group and the reported formation of methyl glycoside byproducts during the acid-catalyzed hydrolysis of a similar glucosamine derivative.[40] Therefore, de-*O*-acetylation was carried out under strictly controlled basic conditions to afford, after benzylidenation, compound **1**. Donor **2** was prepared from **1** by levulination at position 3, followed by oxidative removal of the 4-methoxyphenyl group with CAN and trichloroacetimidate activation.

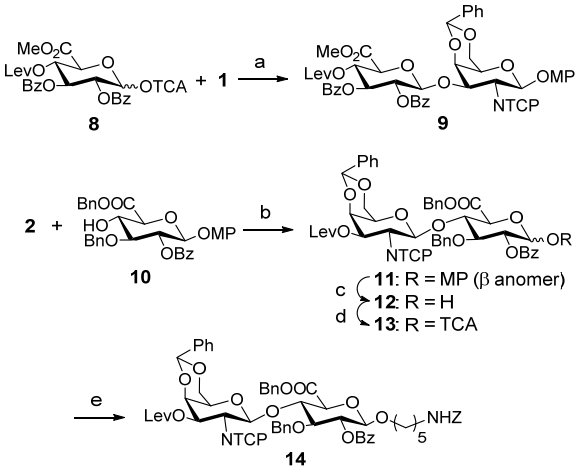


Scheme 1. Reagents and conditions: a) 4-methoxyphenol, $BF_3{\cdot}Et_2O$, CH_2Cl_2; b) NaOMe, MeOH, 0ºC; c) $PhCH(OMe)_2$, *p*-TsOH, CH_3CN, 50% (3 steps, from **3**); d) Lev_2O, DMAP, CH_2Cl_2, 92%; e) CAN, toluene/CH_3CN/H_2O, 0ºC, 84%; f) Cl_3CCN, DBU, CH_2Cl_2, 91%. MP = 4-methoxyphenyl; Lev = levulinoyl; TCA: trichloroacetimidoyl.

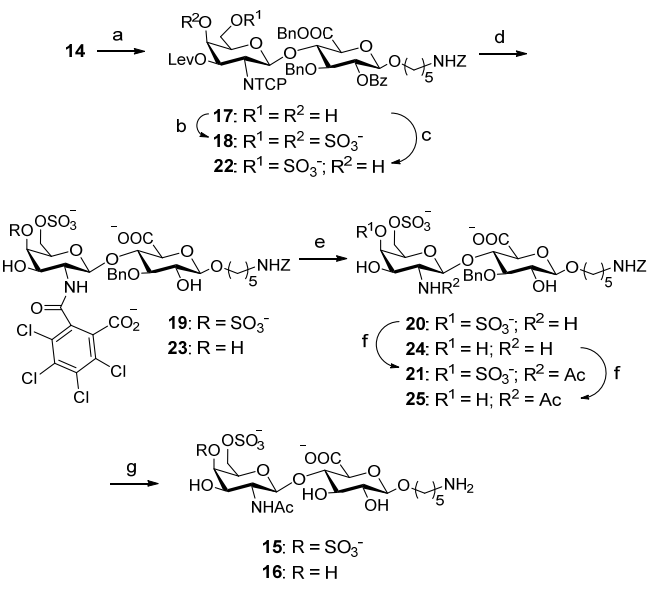
Then, we studied the utility of **1** and **2** in glycosylation reactions with glucuronic acid moieties to afford fully protected CS disaccharide precursors (Scheme 2). Glucuronic acid trichloroacetimidate **8**[41, 42] was coupled with acceptor **1** to give disaccharide **9** in good yield. After testing different reaction conditions, the best results were obtained with $BF_3{\cdot}Et_2O$ as catalyst and toluene as solvent. On the other hand, donor **2** was coupled to acceptor **10**[43] to yield disaccharide **11**, with the alternative monosaccharide sequence GalNAc-GlcA. In order to introduce an amine-terminated linker at the reducing end of the chain, highly convenient for further conjugation of the final molecules, **11** was transformed into trichloroacetimidate **13**. Glycosylation between **13** and 5-(benzyloxycarbonylamino)-1-pentanol afforded the desired disaccharide **14** in good yield.

With these fully protected disaccharides at hand, we then checked the compatibility of the *N*-TCP group with the deprotection/sulfation steps required for the synthesis of final deprotected CS oligomers. Thus, starting from **14** we successfully prepared disaccharides **15** and **16** that correspond to the sulfation patterns of CS-E and CS-C, respectively (Scheme 3). The benzylidene acetal of **14** was removed using TFA and the resulting diol was extensively sulfated to give **18**. Here, the reaction time was significantly decreased (40 min) using microwave heating.[44] The next step was the basic hydrolysis of the ester groups that occurred with concomitant partial hydrolysis of the *N*-TCP moiety to afford **19**. The amide bond in **19** was cleaved by treatment with ethylenediamine in DMF. This reaction only took 90 min using microwave heating.[45, 46] Finally, the amine group of **20** was selectively acetylated and the resulting derivative **21** was hydrogenated to yield CS-E disaccharide **15** in good yield. For the preparation of CS-C dimer **16**, diol **17** was heated at 50ºC, under microwave irradiation, in the presence of 2 equiv. of $SO_3{\cdot}NMe_3$

complex. After stirring for 30 min, the 6-*O*-sulfated compound **22** was obtained in high yield. When we carried out the selective 6-OH sulfation with conventional heating in an oil bath, the reaction time was much longer and the yield was lower due to the recovery of some unsulfated starting material. Saponification, followed by amide bond cleavage, *N*-acetylation and hydrogenolysis gave final disaccharide **16**.



Scheme 2. Reagents and conditions: a) $BF_3{\cdot}Et_2O$, toluene, 54% + 27% recovered **1**; b) TMSOTf, CH_2Cl_2, 58% + 16% recovered **10**; c) CAN, $CH_2Cl_2/CH_3CN/H_2O$, 84%; d) Cl_3CCN, K_2CO_3, CH_2Cl_2, 95%; e) 5-(benzyloxycarbonylamino)-1-pentanol, TMSOTf, CH_2Cl_2, 67%. Z = benzyloxycarbonyl.

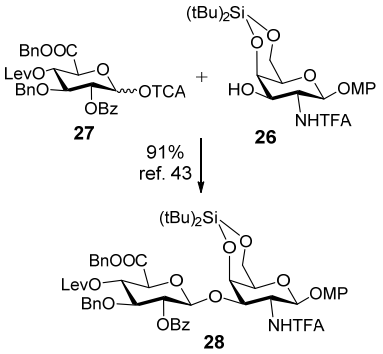


Scheme 3. Reagents and conditions: a) TFA, CH_2Cl_2, 0ºC, 99% b) $SO_3{\cdot}Me_3N$ (10 equiv.), DMF, 100ºC, 40 min, MW, 83%; c) $SO_3{\cdot}Me_3N$ (2 equiv.), DMF, 50ºC, 30 min, MW, 84%; d) H_2O_2, LiOH, THF; NaOH, MeOH, 86% for **19**, 89% for **23**; e) $NH_2(CH_2)_2NH_2$, DMF, 100ºC, 90 min, MW; f) Ac_2O, Et_3N, MeOH, 75% for **21**, 88% for **25** (2 steps, from **19** and **23**, respectively); g) H_2, $Pd(OH)_2$, H_2O/MeOH, quantitative for **15**, quantitative for **16**.

Next, we attempted the synthesis of longer CS sequences by a 2+2 coupling of disaccharide units. For this purpose, disaccharide **9** was transformed into the corresponding glycosyl acceptor (by delevulination) and donor (by cleavage of the anomeric 4-methoxyphenyl group and trichloroacetimidate formation). Unfortunately, the 2+2 glycosylation failed and the desired $(GlcA\text{-}GalNAc)_2$ tetrasaccharide could not be isolated. Alternatively, we tried the preparation of a $(GalNAc\text{-}GlcA)_2$ tetrasaccharide from compound **11**. Similarly, **11** was converted into the corresponding acceptor and donor disaccharides, but the 2+2 condensation of these units failed again. In all these glycosylation trials, most starting glycosyl acceptor was recovered from the reaction mixture.

Synthesis of CS oligomers using *N*-TFA-protected galactosamine units

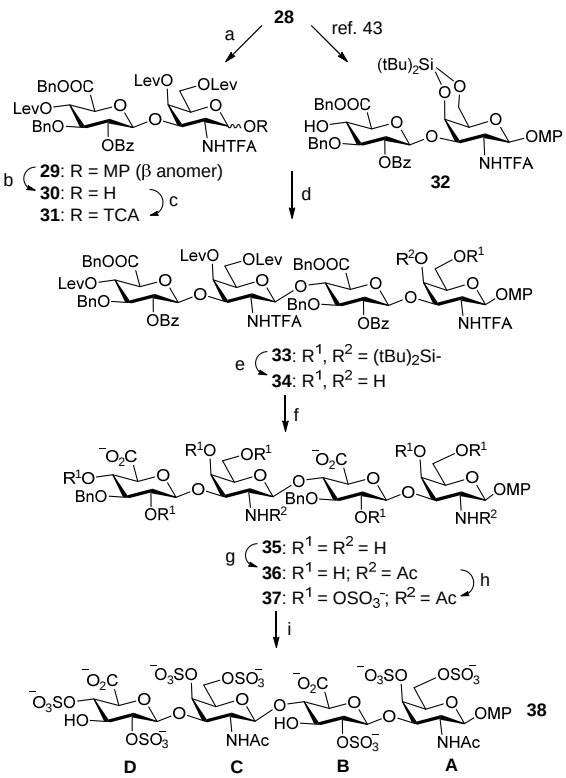
For the synthesis of biologically relevant CS tetrasaccharides, we turned our attention to *N*-TFA-protected GalNAc units. We have previously reported the preparation and use of this type of building blocks for the synthesis of a hybrid chondroitin/dermatan sulfate oligosaccharide,[43] owning both GlcA and L-iduronic acid (IdoA). Monosaccharide **26**, containing a 4,6-*O*-di-*tert*-butylsilylene group, acted as an excellent glycosyl acceptor in coupling reaction with poorly reactive GlcA trichloroacetimidate **27** to generate key intermediate **28** (Scheme 4).[43]



Scheme 4. Synthesis of key disaccharide **28** using *N*-TFA unit **26**.

Here, we expand the utility of this *N*-TFA monomer to the preparation of a CS tetrasaccharide sequence, containing exclusively GlcA (Scheme 5). For this purpose, we planned a 2+2 condensation of appropriate disaccharide derivatives. First, **28** was transformed into a suitable donor containing acyl groups at positions 4 and 6 of the GalNAc moiety in order to obtain selectively the β (1→4) glycosidic bond. Thus, compound **28** was treated with $(HF)_n{\cdot}Py$ and then with levulinic anhydride and DMAP to yield disaccharide **29**. Trichloroacetimidate **31** was obtained by cleavage of the anomeric 4-methoxyphenyl group followed by treatment with trichloroacetonitrile and DBU.

Coupling between **31** and **32**[43] gave tetrasaccharide **33** in good yield. Importantly, **31** was prepared immediately before its use in the glycosidation reaction since we observed partial decomposition of this trichloroacetimidate after extended storage, even at low temperature (-28ºC/4ºC range). Tetrasaccharide **33** has a versatile protecting group distribution that paves the way to the preparation of CS sequences with different sulfation motifs. We decided to prepare, for the first time, non-natural oversulfated compound **38** since we have found that analogous IdoA-containing tetrasaccharides (Figure 3, see below) are able to interact with FGF-2 (basic fibroblast growth factor), a heparin-binding protein involved in angiogenesis. Thus, removal of the silylene group was followed by basic hydrolysis and selective *N*-acetylation to afford **36**. Treatment with $SO_3 \cdot NMe_3$ complex at 100ºC using microwave irradiation[47] gave hepta-*O*-sulfated tetrasaccharide **37**. The introduction of the sulfate groups at the desired positions was confirmed by the ^1H and ^{13}C NMR data that showed the typical downfield shifts for the sulfated positions (Tables 1 and 2). Finally, hydrogenolysis of **37** yielded the fully deprotected CS tetramer **38**. NMR analysis confirmed the structure of **38**. ^1H and ^{13}C NMR data are in full agreement with those reported for similar CS derivatives.



Scheme 5. Reagents and conditions: a) $(HF)_n \cdot Py$, THF, 0ºC; Lev_2O, Py, DMAP, 83%; b) CAN, $CH_2Cl_2/CH_3CN/H_2O$, 81%; c) Cl_3CCN, DBU, CH_2Cl_2, 70%; d) TMSOTf, CH_2Cl_2, 0ºC, 71%; e) $(HF)_n \cdot Py$, THF, 0ºC, 75%; f) LiOH, H_2O_2, THF; NaOH, MeOH; g) Ac_2O, MeOH, Et_3N, 90% (2 steps, from **34**); h) $SO_3 \cdot Me_3N$, DMF, 100ºC, MW, 2 h, 56%; i) H_2, $Pd(OH)_2$, H_2O/MeOH, 97%.

Table 1. ^1H-NMR chemical shifts for sulfated positions of compounds **37** and **38** and the corresponding non-sulfated positions of **36**.

Compound	H-4A	H-6A	H-2B	H-4C	H-6C	H-2D	H-4D
36[a]	4.19-4.12	3.86-3.56	3.46	4.19-4.12	3.86-3.56	3.41	3.72-3.56
37[b]	4.96	4.36-4.10	4.41	4.96	4.36-4.10	4.41	4.93-4.85
38[b]	4.95	4.36-4.21	4.28-4.21	4.93	4.36-4.21	4.28-4.21	4.51

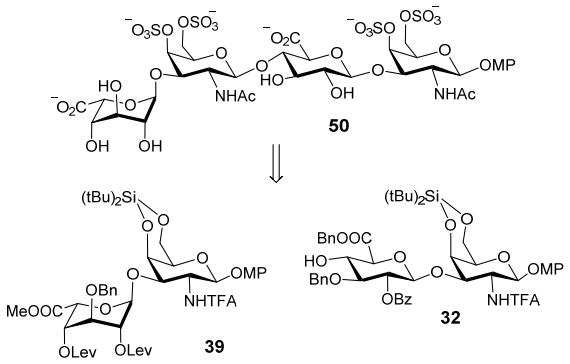
[a] sodium salt, in MeOD; [b]sodium salt, in D_2O

Table 2. ^{13}C-NMR chemical shifts for sulfated positions of compounds **37** and **38** and the corresponding non-sulfated positions of **36**.

Compound	C-4A	C-6A	C-2B	C-4C	C-6C	C-2D	C-4D
36[a]	67.6	61.1	72.7	67.6	61.1	73.0	-[c]
37[b]	76.9	69.3-68.6	80.0	76.9	69.3-68.6	80.0	77.2
38[b]	77.0	69.0-68.7	80.7	77.0	69.0-68.7	80.7	79.0

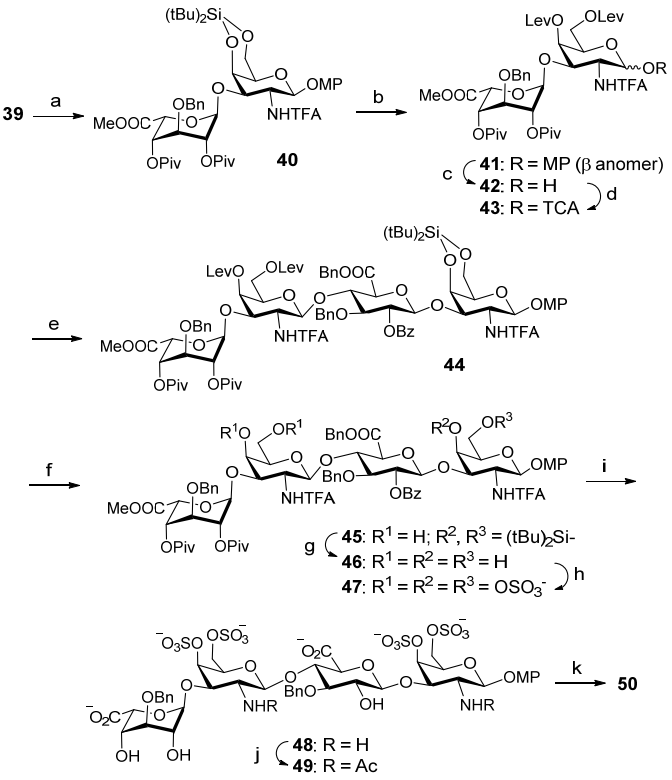
[a] sodium salt, in MeOD; [b] sodium salt, in D_2O; [c] not determined

As mentioned before, **38** is an oversulfated CS sequence containing a sulfation pattern not found in nature. In order to demonstrate that *N*-TFA building blocks can be also employed for the preparation of oligosaccharides with naturally occurring sulfate distributions, we synthesized, for the first time, tetrasaccharide **50** (Scheme 6). This compound contains sulfate groups at positions 4 and 6 of the GalNAc units, the sulfation pattern of biologically relevant CS-E. Moreover, **50** has a IdoA unit at the non-reducing end. It has been reported that the presence of IdoA (instead of GlcA) in CS chains, giving rise to hybrid chondroitin/dermatan sulfate copolymers, has critical roles in the central nervous system development.[2, 48] Oligosaccharide sequences containing GlcA/IdoA-GalNAc (4,6-OSO_3^-) interact with L- and P-selectin and several chemokines and heparin-binding growth factors.[6, 49] The chemical synthesis of these structures, containing both GlcA and IdoA, will contribute to determine the role of hybrid sequences in biological processes including stem cell proliferation, neurogenesis and neural network formation.



Scheme 6. Building blocks required for the synthesis of tetrasaccharide **50**.

Tetrasaccharide **50** was obtained from disaccharides **39**[43] and **32** (Scheme 6). The protecting groups of **39** were rearranged to produce the E sulfation pattern at the end of the synthesis. Delevulination followed by pivaloylation afforded compound **40** (Scheme 7). The silylene group was then changed by two levulinoyl groups (→**41**) that allow the selective deprotection and subsequent sulfation at positions 4 and 6 of the GalNAc moiety and, at the same time, favour the adequate stereochemistry of the glycosidic bond in the 2+2 condensation. Formation of the trichloroacetimidate **43** was then achieved by oxidative removal of the anomeric 4-methoxyphenyl group with CAN followed by treatment with trichloroacetonitrile and DBU. Fully protected tetramer **44** was prepared by glycosylation of **43** and **32**. The protecting group distribution of **44** can lead, among others, to a CS-E sulfation motif. Treatment with hydrazine monohydrate and then with $(HF)_n$·Py complex gave tetraol **46**. The released hydroxyl groups were sulfated in 30 min, using microwave heating, to yield **47**. ^{1}H NMR spectrum of **47** showed the expected downfield shifts for H-4 GalNAc (4.14-3.93 ppm in **46**; 4.85-4.80 ppm in **47**) and H-6a,b GalNAc (3.83-3.48 ppm in **46**; 4.52-4.25 in **47**). ^{13}C NMR spectrum of **47** also showed the expected downfield shifts of the signals for C-4 GalNAc (68.6 ppm in **46**; 75.4-75.1 ppm in **47**) and C-6 GalNAc (62.7-62.5 ppm in **46**; 68.0-67.5 ppm in **47**). Hydrolysis of ester and amide functions followed by *N*-acetylation and hydrogenolysis afforded tetrasaccharide **50** in high yield. The structure of this compound was confirmed by NMR (COSY, TOCSY and HSQC experiments) and mass spectroscopy. ^{1}H and ^{13}C NMR chemical shifts are in good agreement with those published for similar oligosaccharides.



Scheme 7. Reagents and conditions: a) $NH_2NH_2·H_2O$, Py/AcOH, CH_2Cl_2; PivCl, DMAP, Py, 87%; b) $(HF)_n$·Py, THF, 0ºC; Lev_2O, Py, DMAP, 81%; c) CAN, $CH_2Cl_2/CH_3CN/H_2O$, 70%; d) Cl_3CCN, DBU, CH_2Cl_2, 78%; e) **32**, TMSOTf, CH_2Cl_2, 0ºC, 53%; f) $NH_2NH_2·H_2O$, Py/AcOH, CH_2Cl_2, 87%; g) $(HF)_n$·Py, THF, 0ºC, 97%; h) $SO_3·Me_3N$, DMF, 100ºC, MW, 30 min, quantitative; i) LiOH, H_2O_2, THF; NaOH, MeOH; j) Ac_2O, MeOH, Et_3N, 86% (2 steps, from **47**); k) H_2, $Pd(OH)_2$, H_2O/MeOH, quantitative.

Fluorescence polarization competition assay

FGF-2 is a heparin-binding protein[50, 51] that also recognizes CS sequences.[5] We finally screened the interactions between FGF-2 and the synthesized water-soluble CS oligosaccharides (**15**, **16**, **38**, **50** and the dibenzylated tetrasaccharide precursors **37** and **49**). For this purpose, we employed a fluorescence polarization competition assay, previously developed in our group,[43] in which we measured the relative ability of the synthetic CS oligomers to inhibit the interaction between FGF-2 and a fluorescent labelled heparin probe. Briefly, the fluorescence polarization of samples containing fixed concentrations of protein and fluorescent probe were measured in the presence of the different CS oligosaccharides (Figure 2). The binding of a CS oligomer to FGF-2 would displace the fluorescent probe from its complex with the protein, resulting in a decrease of the polarization value. In this experiment we included two control samples (in white, Figure 2). The first one (in the left) only contained fluorescent probe and indicated the expected value for 100 % inhibition. The second one (in the right) contained FGF-2 and fluorescent probe, without inhibitor, and indicated the expected

value for 0% inhibition. As shown in Figure 2, at 25 µM inhibitor concentration, disaccharides **15** and **16** and tetrasaccharides **49** and **50** only showed low inhibitory activity, while compounds **37** and **38** were able to inhibit 47 and 63%, respectively, of the interaction. However, the inhibitory activities of oversulfated **37** and **38** were lower than those obtained with previously synthesized IdoA-containing tetrasaccharides **51** and **52** (Figure 3).[43] In summary, these data indicate that oversulfated, non-natural tetrasaccharides (**37-38**, **51-52**) display stronger inhibitory abilities that those containing natural sulfation patterns (**49**, **50**). These results also suggest that the presence of an IdoA unit, instead of a GlcA, at the nonreducing end of oversulfated structures, as in compounds **51** and **52**, can increase the relative binding affinities of the synthetic non-natural CS oligosaccharides to FGF-2. The interaction between FGF-2 and heparin is crucial for tumor growth and angiogenesis. Therefore, the discovery of compounds that inhibit the FGF-2/heparin interaction is an area of great interest.[52, 53] Although the tested compounds displayed modest activities, this experiment gives some interesting data on the structural features for inhibition of heparin binding to FGF-2. Moreover, this assay illustrates the potential of our fluorescence polarization method for the fast comparison of relative inhibitory activities.

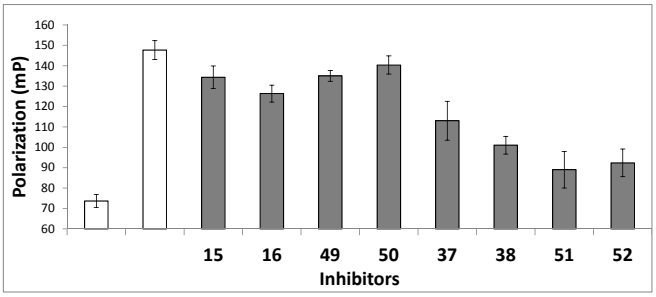


Figure 2. Competition assay to compare the relative inhibitory potencies of the synthetic CS-like oligosaccharides. The graphic presents (in grey) the polarization values obtained from wells containing 25 µM inhibitor, 73 nM FGF-2, and 10 nM fluorescent heparin-like probe. The composition of control wells (in white) is described in the main text. All the measurements are the average of three replicate wells and the error bars show the standard deviations for these measurements.

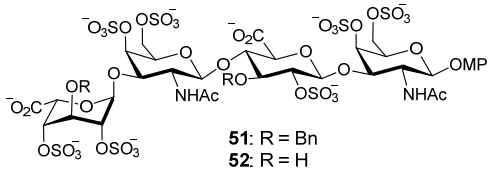


Figure 3. Structure of IdoA containing tetrasaccharides **51** and **52**.

Conclusions

We have explored and compared the utility of two differently protected galactosamine units for the preparation of CS oligosaccharides. Although *N*-TCP protection was useful at the disaccharide level, we were not able to synthesize longer sequences with this type of units. On the contrary, we have showed that a strategy based on *N*-TFA building blocks can afford biologically relevant CS oligosaccharides in good yield. The efficiency of this approach was exemplified by the synthesis of two novel CS-like tetrasaccharides, with different sulfation pattern and uronic acid distribution. TFA groups led to the stereoselective formation of 1,2-*trans* glycosidic bonds and could be easily removed at the end of the synthesis. Microwave irradiation facilitated sulfation reactions. Finally, we compared the abilities of the synthetic CS oligosaccharides to inhibit heparin binding to FGF-2, obtaining some interesting information on the structural features for inhibitory activity.

Experimental Section

General procedures: Thin layer chromatography (TLC) analyses were performed on silica gel 60 F_{254} precoated on aluminium plates (Merck) and the compounds were detected by staining with sulfuric acid/ethanol (1:9), with cerium (IV) sulfate (10 g)/phosphomolybdic acid (13 g)/sulfuric acid (60 mL) solution in water (1 L), or with anisaldehyde solution [anisaldehyde (25 mL) with sulfuric acid (25 mL), ethanol (450 mL) and acetic acid (1 mL)], followed by heating at over 200ºC. Column chromatography was carried out on silica gel 60 (0.2-0.5 mm, 0.2-0.063 mm or 0.040-0.015 mm; Merck). Optical rotations were determined with a Perkin-Elmer 341 polarimeter. ^{1}H- and ^{13}C-NMR spectra were acquired on Bruker DPX-300, Avance III-400 and DRX-500 spectrometers. Unit A refers to the reducing end monosaccharide in the NMR data. Electrospray mass spectra (ESI MS) were carried out with an Esquire 6000 ESI-Ion Trap from Bruker Daltonics. High resolution mass spectra (HR MS) were carried out by CITIUS (Universidad de Sevilla), CCiT (Universitat de Barcelona) and SIdI (Universidad Autónoma de Madrid). Microwave-based sulfation reactions were performed using a Biotage Initiator Eight synthesizer in sealed reaction vessels.

4-Methoxyphenyl 4,6-*O*-benzylidene-2-deoxy-2-tetrachlorophthalimido-β-D-galactopyranoside (1): $BF_3{\cdot}Et_2O$ (731 µL, 5.82 mmol) was added under an argon atmosphere at 0ºC to a mixture of **3** (1.79 g, 2.91 mmol) and 4-methoxyphenol (723 mg, 5.82 mmol) in dry CH_2Cl_2 (8.0 mL). The temperature was gradually raised to room temperature during 2 h, and the mixture was stirred for 24 h, diluted with CH_2Cl_2 (40 mL), and washed with H_2O, saturated aqueous $NaHCO_3$, and H_2O. The organic phase was dried ($MgSO_4$) and concentrated to dryness.

The residue was purified by column chromatography (hexane-EtOAc 3:1) to afford **4** (1.63 g). TLC (toluene-acetone 3:2) R_f 0.44; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.86 (m, 2H, Ar), 6.75 (m, 2H, Ar), 5.79 (d, 1H, $J_{1,2}$=8.5 Hz, H-1), 5.78 (dd, 1H, $J_{2,3}$=11.3 Hz, $J_{3,4}$=3.1 Hz, H-3), 5.53 (d, 1H, H-4), 4.79 (dd, 1H, H-2), 4.28-4.10 (m, 3H, H-5, H-6a, H-6b), 3.74 (s, 3H, Me(OMP)), 2.23, 2.06, 1.91 (3s, 9H, OAc); ESI MS: *m/z*: calcd for $C_{27}H_{23}Cl_4NO_{11}Na$: 700.0; found: 700.1 $[M+Na]^+$.

Compound **4** (1.63 g, 2.4 mmol) was dissolved in MeOH (186 mL), and MeONa/MeOH (1M, 3.2 mL) was added. The mixture was stirred at 0ºC for 10 min, and AcOH (15.5 mL) was added. The mixture was stirred at room temperature for 10 min and co-concentrated twice with toluene. The residue was filtered through a silica column (toluene-acetone 2:1) to give **5** (855 mg) and a mixture of partially deacetylated compounds (348 mg). The partially deprotected products were dissolved in MeOH (40 mL) and treated with additional MeONa (0.68 mL of a 1M solution in MeOH) at 0ºC. After stirring for 7 min at 0ºC, AcOH (3.3 mL) was added and the mixture was concentrated. The residue was purified by column chromatography to give additional **5** (197 mg). TLC (toluene-acetone 2:1) R_f 0.29; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.87 (m, 2H, Ar), 6.74 (m, 2H, Ar), 5.62 (d, 1H, $J_{1,2}$=8.4 Hz, H-1), 4.61 (bt, 1H, H-2), 4.46 (dd, 1H, $J_{2,3}$=11.0 Hz, $J_{3,4}$=3.0 Hz, H-3), 4.03 (d, 1H, H-4), 3.84-3.75 (m, 3H, H-5, H-6a, H-6b), 3.69 (s, 3H, Me(OMP)); ESI MS: *m/z*: calcd for $C_{21}H_{17}Cl_4NO_8Na$: 574.0; found: 574.1 $[M+Na]^+$.

Compound **5** (1.052 g, 1.90 mmol) was dissolved in dry MeCN (19 mL). Benzaldehyde dimethyl acetal (0.43 mL, 2.85 mmol) and *p*-TsOH (188 µL of a 0.5 M solution in dry MeCN) were added, and the mixture was stirred for 45 min, diluted with EtOAc (200 mL), and washed with saturated aqueous $NaHCO_3$, and H_2O. The organic phase was dried ($MgSO_4$) and concentrated to dryness. The residue was purified by column chromatography (toluene-EtOAc 8:1) to afford **1** (930 mg, 50% from **3**, 3 steps). TLC (toluene-EtOAc 8:1) R_f 0.27; $[\alpha]^{20}_D$ +11º (*c* 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.55 (m, 2H, Ar), 7.40 (m, 3H, Ar), 6.91 (m, 2H, Ar), 6.75 (m, 2H, Ar), 5.75 (d, 1H, $J_{1,2}$=8.3 Hz, H-1), 5.61 (s, 1H, Ph-CH), 4.69 (bt, 1H, H-2), 4.51 (dd, 1H, $J_{2,3}$=10.9 Hz, $J_{3,4}$=3.1 Hz, H-3), 4.38 (d, 1H, $J_{6a,6b}$=12.4 Hz, H-6a), 4.33 (bd, 1H, H-4), 4.13 (d, 1H, H-6b), 3.73 (s, 3H, Me(OMP)), 3.70 (s, 1H, H-5); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 164.2, 163.6 (2CO), 155.8, 150.6, 140.5, 140.4, 137.3, 130.1, 129.8 (Ar-C), 129.6, 128.5 (Ar-CH), 127.6, 127.3 (Ar-C), 126.6, 119.3, 114.5 (Ar-CH), 101.7 (Ph-CH), 97.6 (C-1), 74.9 (C-4), 69.2 (C-6), 67.8 (C-3), 67.0 (C-5), 55.7 (Me(OMP)), 55.4 (C-2); HR MS: *m/z*: calcd for $C_{28}H_{21}Cl_4NO_8Na$: 661.9919; found: 661.9899 $[M+Na]^+$.

4-Methoxyphenyl 4,6-*O*-benzylidene-2-deoxy-3-*O*-levulinoyl-2-tetrachlorophthalimido-β-D-galactopyranoside (6): Lev_2O preparation: LevOH (2.0 mL, 19.1 mmol) was added at 0ºC to a solution of 1,3-dicyclohexylcarbodiimide (1.97 g, 9.54 mmol) in CH_2Cl_2 (16 mL). After stirring 5 min at room temperature, the mixture was cooled and filtered, and the urea precipitate was washed with additional CH_2Cl_2 (2.7 mL), to give 18.7 mL of a 0.51 M Lev_2O solution.

Lev_2O (16.4 mL of a 0.51 M solution in CH_2Cl_2) was added at room temperature to a mixture of **1** (2.16 g, 3.37 mmol) and DMAP (61.1 mg, 0.51 mmol). The mixture was stirred for 1 h 30 min, diluted with CH_2Cl_2, and washed with saturated aqueous $NaHCO_3$, and H_2O. The organic phase was dried ($MgSO_4$), filtered and concentrated to dryness. The residue was purified by column chromatography (toluene-EtOAc 9:1) to afford **6** (2.29 g, 92%). TLC (toluene-EtOAc 6:1) R_f 0.31; $[\alpha]^{20}_D$ +14º (*c* 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.60-7.55 (m, 2H, Ar), 7.42-7.38 (m, 3H, Ar), 6.90 (m, 2H, Ar), 6.74 (m, 2H, Ar), 5.82 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1), 5.80 (dd, 1H, $J_{2,3}$ = 11.4 Hz, $J_{3,4}$ = 3.6 Hz, H-3), 5.58 (s, 1H, Ph-CH), 5.00 (dd, 1H, H-2), 4.45 (bd, 1H, H-4), 4.37 (dd, 1H, $J_{5,6a}$ = 1.4 Hz, $J_{6a,6b}$ = 12.5 Hz, H-6a), 4.12 (dd, 1H, $J_{5,6b}$ = 1.4 Hz, H-6b), 3.73 (s, 3H, Me(OMP)), 3.72 (m, 1H, H-5), 2.76-2.39 (m, 4H, CH_2(Lev)), 1.93 (s, 3H, CH_3(Lev)); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 206.4 (CO(Lev)), 172.0 (CO(Lev)), 164.0, 163.0 (2CO), 155.8, 150.5, 140.3, 140.2, 137.6, 130.1, 129.9 (Ar-C), 129.3, 128.4 (Ar-CH), 127.7, 127.4 (Ar-C), 126.5, 119.3, 114.5 (Ar-CH), 101.1 (Ph-CH), 97.6 (C-1), 73.0 (C-4), 69.1 (C-6), 68.7 (C-3), 66.9 (C-5), 55.7 (Me(OMP)), 51.7 (C-2), 37.9 (CH_2(Lev)), 29.6 (CH_3(Lev)), 28.2 (CH_2(Lev)); HR MS: *m/z*: calcd for $C_{33}H_{27}Cl_4NO_{10}Na$: 760.0287; found: 760.0278 $[M+Na]^+$.

4,6-*O*-Benzylidene-2-deoxy-3-*O*-levulinoyl-2-tetrachlorophthalimido-α,β-D-galactopyranose (7): CAN (6.65 g, 11.9 mmol) was added at 0ºC to a solution of **6** (2.2 g, 3.0 mmol) in toluene/MeCN/H_2O (1:6:1; 104 mL). After stirring for 1 h at 0ºC, the mixture was diluted with EtOAc, washed with H_2O, saturated aqueous $NaHCO_3$, and H_2O. The organic phase was dried ($MgSO_4$), filtered and concentrated to dryness. The residue was purified by column chromatography (toluene-EtOAc 4:1) to afford **7** (1.60 g, 84%) as a mixture of α/β anomers (2:10). TLC (toluene-EtOAc 4:1) R_f 0.23 (for a mixture of α/β anomers); ^{1}H-NMR (300 MHz, $CDCl_3$) (data for β anomer): δ 7.55-7.48 (m, 2H, Ar), 7.39-7.33 (m, 3H, Ar), 5.77 (dd, 1H, $J_{2,3}$ = 11.3 Hz, $J_{3,4}$ = 3.5 Hz, H-3), 5.54 (s, 1H, Ph-CH), 5.47 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1), 4.67 (dd, 1H, H-2), 4.40 (bd, 1H, H-4), 4.34 (dd, 1H, $J_{5,6a}$ = 1.4 Hz, $J_{6a,6b}$ = 12.6 Hz, H-6a), 4.08 (dd, 1H, $J_{5,6b}$ = 1.4 Hz, H-6b), 3.84 (bs, 1H, OH), 3.67 (m, 1H, H-5), 2.69-2.35 (m, 4H, CH_2(Lev)), 1.91 (s, 3H, CH_3(Lev)); ^{13}C-NMR (75 MHz, $CDCl_3$) (data for β anomer): δ 206.5 (CO(Lev)), 171.9 (CO(Lev)), 163.8, 163.5 (2CO), 140.1, 137.5, 129.9, 129.8 (Ar-C), 129.3, 128.3 (Ar-CH), 127.6, 127.5 (Ar-C), 126.3 (Ar-CH), 100.9 (Ph-CH), 92.8 (C-1), 72.9 (C-4), 69.2 (C-6), 68.5 (C-3), 66.9 (C-5), 53.5 (C-2), 37.9 (CH_2(Lev)), 29.6 (CH_3(Lev)), 28.2 (CH_2(Lev)); ^{1}H-NMR (300 MHz, $CDCl_3$) (data for α anomer): δ 7.55-7.48 (m, 2H, Ar), 7.39-7.33 (m, 3H, Ar), 6.33 (dd, 1H, $J_{2,3}$ = 12.1 Hz, $J_{3,4}$ = 3.4 Hz, H-3), 5.57 (s, 1H, Ph-CH), 5.50 (m, 1H, H-1), 5.04 (dd, 1H, $J_{1,2}$ = 3.1 Hz, H-2), 4.57 (bd, 1H, H-4), 4.26 (bd, 1H, $J_{6a,6b}$ = 12.2 Hz, H-6a), 4.14-4.07 (m, 2H, H-5, H-6b), 4.00 (bs, 1H, OH), 2.69-2.35 (m, 4H, CH_2(Lev)), 1.97 (s, 3H, CH_3(Lev)); ^{13}C-NMR (75 MHz, $CDCl_3$) (significant data for α anomer): δ 93.0 (C-1), 73.3 (C-4), 69.5 (C-6), 65.5 (C-3), 62.6 (C-5), 51.3 (C-2); HR MS: *m/z*: calcd for $C_{26}H_{21}Cl_4NO_9Na$: 653.9868; found: 653.9876 $[M+Na]^+$.

***O*-(4,6-*O*-Benzylidene-2-deoxy-3-*O*-levulinoyl-2-tetrachlorophthalimido-β-D-galactopyranosyl) trichloroacetimidate (2):** Trichloroacetonitrile (5.3 mL, 54 mmol) and catalytic DBU (96 µL of a 0.11 M solution in dry CH_2Cl_2) were added to a solution of **7** (680 mg, 1.07 mmol) in dry CH_2Cl_2 (15 mL). After stirring for 7 h at room temperature, the reaction mixture was concentrated to dryness. The residue was purified by flash chromatography (toluene-EtOAc 5:1 + 1% Et_3N) to afford **2** (763 mg, 91%). TLC (toluene-EtOAc 3:1) R_f 0.40; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.64 (s, 1H, NH), 7.57 (m, 2H, Ar), 7.41 (m, 3H, Ar), 6.54 (d, 1H, $J_{1,2}$ = 8.9 Hz, H-1), 5.90 (dd, 1H, $J_{2,3}$ = 11.4 Hz, $J_{3,4}$ = 3.6 Hz, H-3), 5.59 (s, 1H,

Ph-CH), 5.05 (dd, 1H, H-2), 4.49 (d, 1H, H-4), 4.43 (d, 1H, $J_{6a,6b}$ = 12.4 Hz, H-6a), 4.14 (d, 1H, H-6b), 3.87 (bs, 1H, H-5), 2.71-2.41 (m, 4H, CH_2(Lev)), 1.93 (s, 3H, CH_3(Lev)); ESI MS: *m/z*: calcd for $C_{28}H_{21}Cl_7N_2O_9Na$: 796.9; found: 797.0 $[M+Na]^+$.

4-Methoxyphenyl 3-*O*-(methyl 2,3-di-*O*-benzoyl-4-*O*-levulinoyl-β-D-glucopyranosyluronate)-4,6-*O*-benzylidene-2-deoxy-2-tetrachlorophthalimido-β-D-galactopyranoside (9): $BF_3{\cdot}Et_2O$ (326 µL of a 0.26 M solution in dry toluene) was added under an argon atmosphere at room temperature to a mixture of **1** (154 mg, 0.24 mmol) and **8** (475 mg, 0.72 mmol) containing freshly activated 4Å molecular sieves in dry toluene (2.5 mL). After stirring for 45 min at room temperature, the reaction mixture was neutralized with Et_3N and concentrated to dryness. The residue was purified by column chromatography (toluene-acetone 8:1) to afford **9** (148 mg, 54%) and unreacted acceptor (41 mg, 27%). TLC (toluene-acetone 3:1) Rf 0.48; $[\alpha]^{20}_D$ +18º (*c* 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.76-7.09 (m, 15H, Ar), 6.79 (m, 2H, Ar), 6.68 (m, 2H, Ar), 5.64 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3B), 5.59 (s, 1H, PhCH), 5.49 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1A), 5.41 (t, 1H, $J_{4,5}$ = 9.6 Hz, H-4B), 5.34 (dd, 1H, $J_{1,2}$ = 7.6 Hz, H-2B), 5.07 (d, 1H, H-1B), 4.99 (dd, 1H, $J_{2,3}$ = 11.3 Hz, H-2A), 4.82 (dd, 1H, $J_{3,4}$ = 3.2 Hz, H-3A), 4.63 (bd, 1H, H-4A), 4.36 (bd, 1H, $J_{6a,6b}$ = 12.2 Hz, H-6aA), 4.24 (d, 1H, H-5B), 4.13 (bd, 1H, H-6bA), 3.72, 3.69 (2s, 6H, COOMe, Me(OMP)), 2.66-2.30 (m, 4H, CH_2(Lev)), 2.03 (m, 3H, CH_3(Lev)); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 171.3, 167.0, 165.6, 164.4, 164.1, 162.0 (CO(Lev, NTCP, COOMe, Bz), 155.6, 150.5, 140.3, 140.0, 137.7 (Ar-C), 133.5, 133.1, 129.9-125.4, 119.0, 114.4 (Ar-CH, Ar-C), 101.7 (C-1B), 100.9 (PhCH), 97.7 (C-1A), 76.2 (C-3A), 75.1 (C-4A), 72.3 (C-5B), 72.2 (C-3B), 71.9 (C-2B), 69.4 (C-4B), 69.0 (C-6A), 66.9 (C-5A), 55.5, 52.9 (COOMe, Me(OMP)), 52.0 (C-2A), 37.4 (CH_2(Lev)), 29.5 (CH_3(Lev)), 27.6 (CH_2(Lev)); HR MS: *m/z*: calcd for $C_{54}H_{45}Cl_4NO_{18}Na$: 1158.1288; found: 1158.1327 $[M+Na]^+$.

Benzyl |4-Methoxyphenyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-(4,6-*O*-benzylidene-2-deoxy-3-*O*-levulinoyl-2-tetrachlorophthalimido-β-D-galactopyranosyl)-β-D-glucopyranoside|uronate (11): Donor **2** (655 mg, 0.842 mmol) and acceptor **10** (274 mg, 0.468 mmol) were dissolved in dry CH_2Cl_2 (5 mL) in the presence of freshly activated 4Å molecular sieves. After stirring for 10 min at room temperature, TMSOTf (229 µL of a 0.18 M solution in dry CH_2Cl_2) was added under an argon atmosphere. After stirring for 9 min, the reaction mixture was neutralized with Et_3N and concentrated to dryness. The residue was purified by column chromatography (toluene-EtOAc 9:1) to afford **11** (325 mg, 58%) and unreacted **10** (45 mg, 16%). TLC (toluene-EtOAc 4:1) Rf 0.43; $[\alpha]^{20}_D$ –9º (*c* 1.0, CH_2Cl_2); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.94 (m, 2H, Ar), 7.56 (m, 1H, Ar), 7.48-7.15 (m, 17H, Ar), 6.77 (m, 2H, Ar), 6.64 (m, 2H, Ar), 5.75 (dd, 1H, $J_{2,3}$ = 11.4 Hz, $J_{3,4}$ = 3.6 Hz, H-3B), 5.48 (s, 1H, PhCH), 5.46 (dd, 1H, $J_{1,2}$ = 7.4 Hz, $J_{2,3}$ = 8.6 Hz, H-2A), 5.38 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1B), 5.20 (d, 1H, CH_2(Bn)), 5.04 (d, 1H, CH_2(Bn)), 4.99 (d, 1H, CH_2(Bn)), 4.91 (d, 1H, H-1A), 4.81 (d, 1H, CH_2(Bn)), 4.70 (dd, 1H, H-2B), 4.36 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.0 Hz, H-4A), 4.31 (d, 1H, H-4B), 4.17 (d, 1H, $J_{6a,6b}$ = 12.4 Hz, H-6aB), 3.95-3.89 (m, 2H, H-3A, H-5A), 3.86 (d, 1H, H-6bB), 3.69 (s, 3H, Me(OMP)), 3.19 (bs, 1H, H-5B), 2.69-2.32 (m, 4H, CH_2(Lev)), 1.92 (s, 3H, CH_3(Lev)); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 206.3 (CO(Lev)), 171.9, 167.3, 165.1, 164.2, 163.4 (CO(Lev, NTCP, COOBn, Bz), 155.8, 151.0, 140.0, 139.8, 138.3, 137.7, 135.1 (Ar-C), 133.3 (Ar-CH), 130.0, 129.8, 129.7, 129.5, 129.1, 128.7, 128.5, 128.2, 128.1, 127.9, 127.6, 127.3, 126.4 (Ar-C, Ar-CH), 119.1, 114.5 (Ar-CH), 101.1, 101.0 (PhCH, C-1A), 97.9 (C-1B), 80.2 (C-3A or C-5A), 77.8 (C-4A), 74.7 (C-3A or C-5A), 74.5 (CH_2(Bn)), 73.0 (C-2A), 72.7 (C-4B), 68.9 (C-6B), 68.3 (C-3B), 67.5 (CH_2(Bn)), 66.4 (C-5B), 55.7 (Me(OMP)), 52.0 (C-2B), 37.9 (CH_2(Lev)), 29.6 (CH_3(Lev)), 28.2 (CH_2(Lev)); HR MS: *m/z*: calcd for $C_{60}H_{51}Cl_4NO_{17}Na$: 1220.1809; found: 1220.1812 $[M+Na]^+$.

Benzyl |2-*O*-benzoyl-3-*O*-benzyl-4-*O*-(4,6-*O*-benzylidene-2-deoxy-3-*O*-levulinoyl-2-tetrachlorophthalimido-β-D-galactopyranosyl)-α,β-D-glucopyranose|uronate (12): A solution of CAN (79 mg, 0.14 mmol) in H_2O (0.3 mL) was added to a solution of **11** (42 mg, 0.035mmol) in CH_2Cl_2/MeCN (1:2; 2.7 mL). The mixture was vigorously stirred for 1 h at room temperature, then diluted with EtOAc, washed with H_2O, saturated aqueous $NaHCO_3$, and H_2O. The organic phase was dried ($MgSO_4$), filtered and concentrated to dryness. The residue was purified by column chromatography (CH_2Cl_2-MeOH 80:1) to afford **12** (40 mg, 84%) as a mixture of α/β anomers (2:1). TLC (CH_2Cl_2-MeOH 80:1) Rf 0.23, 0.27; ^{1}H-NMR (300 MHz, $CDCl_3$) (for major anomer (α)): δ 7.94 (m, 2H, Ar), 7.58-7.07 (m, 18H, Ar), 5.69 (dd, 1H, $J_{2,3}$ = 11.3 Hz, $J_{3,4}$ = 3.6 Hz, H-3B), 5.49-5.45 (m, 3H, PhCH, H-1B, H-1A), 5.19 (d, 1H, CH_2(Bn)), 5.11-5.00 (m, 3H, CH_2(Bn), H-2A), 4.85 (d, 1H, CH_2(Bn)), 4.69 (m, 1H, H-2B), 4.39 (d, 1H, $J_{4,5}$ = 9.0 Hz, H-5A), 4.29-4.08 (m, 4H, H-4B, H-3A, H-4A, H-6aB), 3.80 (d, 1H, $J_{6a,6b}$ = 12.0 Hz, H-6bB), 3.10 (s, 1H, H-5B), 2.68-2.37 (m, 4H, CH_2(Lev)), 1.91 (s, 3H, CH_3(Lev)); ^{13}C-NMR (75 MHz, $CDCl_3$) (for major anomer (α)): δ 206.3 (CO(Lev)), 171.9, 168.5, 165.9, 164.2, 163.5 (CO(Lev, NTCP, COOBn, Bz), 140.0, 139.8, 138.3, 137.7, 135.2 (Ar-C), 133.4 (Ar-CH), 130.0-126.5 (Ar-C, Ar-CH), 101.0 (PhCH), 98.0 (C-1B), 90.7 (C-1A), 77.8, 77.4 (C-3A, C-4A), 74.8 (CH_2(Bn)), 73.2 (C-2A), 72.8 (C-4B), 70.5 (C-5A), 68.9 (C-6B), 68.5 (C-3B), 67.5 (CH_2(Bn)), 66.4 (C-5B), 52.1 (C-2B), 37.9 (CH_2(Lev)), 29.6 (CH_3(Lev)), 28.2 (CH_2(Lev)); ^{1}H-NMR (300 MHz, $CDCl_3$) (for minor anomer (β)): δ 7.94 (m, 2H, Ar), 7.58-7.07 (m, 18H, Ar), 5.71 (dd, 1H, $J_{2,3}$ = 11.4 Hz, $J_{3,4}$ = 3.6 Hz, H-3B), 5.49-5.45 (m, 1H, PhCH), 5.39 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1B), 5.21 (d, 1H, CH_2(Bn)), 5.11-5.00 (m, 3H, CH_2(Bn), H-2A), 4.83 (d, 1H, CH_2(Bn)), 4.72-4.66 (m, 2H, H-1A, H-2B), 4.29-4.08 (m, 3H, H-4B, H-4A, H-6aB), 3.97-3.78 (m, 3H, H-3A, H-5A, H-6bB), 3.12 (s, 1H, H-5B), 2.68-2.37 (m, 4H, CH_2(Lev)), 1.91 (s, 3H, CH_3(Lev)); ^{13}C-NMR (75 MHz, $CDCl_3$) (selected data for minor anomer (β)): δ 101.0 (PhCH), 97.8 (C-1B), 96.3 (C-1A), 79.9 (C-3A), 77.8 (C-4A), 75.4 (C-2A), 74.8 (CH_2(Bn)), 74.7 (C-5A), 73.2(C-4B), 68.9 (C-6B), 68.3 (C-3B), 67.7 (CH_2(Bn)), 66.4 (C-5B), 52.0 (C-2B), 37.9 (CH_2(Lev)), 29.6 (CH_3(Lev)), 28.2 (CH_2(Lev)); HR MS: *m/z*: calcd for $C_{53}H_{45}Cl_4NO_{16}Na$: 1114.1390; found: 1114.1425 $[M+Na]^+$.

***O*-[Benzyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-(4,6-*O*-benzylidene-2-deoxy-3-*O*-levulinoyl-2-tetrachlorophthalimido-β-D-galactopyranosyl)-α,β-D-glucopyranosyluronate] trichloroacetimidate (13):** Trichloroacetonitrile (1.57 mL, 15.8 mmol) and K_2CO_3 (47 mg, 0.35 mmol) were added to **12** (345 mg, 315 µmol) in dry CH_2Cl_2 (8 mL) under an argon atmosphere. After stirring at room temperature for 15 h, the mixture was filtered and concentrated in vacuo to give **13** (370 mg, 95%) as an α/β mixture (75:100). TLC (toluene-acetone 8:1) Rf 0.40; ^{1}H-NMR (300 MHz, $CDCl_3$) (for major anomer (β)): δ 8.53 (s, 1H, NH), 7.91 (m, 2H, Ar), 7.58-7.09 (m, 18H, Ar),

6.00 (d, 1H, $J_{1,2}$ = 6.1 Hz, H-1A), 5.69 (dd, 1H, $J_{2,3}$ = 11.3 Hz, $J_{3,4}$ = 3.6 Hz, H-3B), 5.52-5.43 (m, 3H, PhCH, H-1B, H-2A), 5.19-4.87 (m, 4H, CH_2(Bn)), 4.70 (m, 1H, H-2B), 4.48 (dd, 1H, $J_{3,4}$ = 8.0 Hz, $J_{4,5}$ = 9.8 Hz, H-4A), 4.34-4.20 (m, 1H, H-4B), 4.16-4.08 (m, 2H, H-5A, H-6aB), 4.00 (dd, 1H, $J_{2,3}$ = 6.5 Hz, H-3A), 3.84 (d, 1H, $J_{6a,6b}$ = 11.5 Hz, H-6bB), 3.13 (s, 1H, H-5B), 2.69-2.32 (m, 4H, CH_2(Lev)), 1.92 (s, 3H, CH_3(Lev)); ^{13}C-NMR (75 MHz, $CDCl_3$) (for major anomer (β)): δ 206.2 (CO(Lev)), 171.9, 167.4, 164.9, 164.2, 163.4 (CO(Lev, NTCP, COOBn, Bz), 160.8 (C=NH), 140.0, 139.8, 138.2, 137.7, 135.0 (Ar-C), 133.5 (Ar-CH), 129.9-126.4 (Ar-C, Ar-CH), 100.9 (PhCH), 98.2 (C-1B), 95.5 (C-1A), 90.5 (CCl_3), 80.5(C-3A), 77.4 (C-4A), 74.4 (C-5A), 73.7 (CH_2(Bn)), 72.7 (C-4B), 71.4 (C-2A), 68.9 (C-6B), 68.5 (C-3B), 67.5 (CH_2(Bn)), 66.5 (C-5B), 52.0 (C-2B), 37.9 (CH_2(Lev)), 29.6 (CH_3(Lev)), 28.2 (CH_2(Lev)); ^{1}H-NMR (300 MHz, $CDCl_3$) (for minor anomer (α)): δ 8.50 (s, 1H, NH), 7.91 (m, 2H, Ar), 7.58-7.09 (m, 18H, Ar), 6.56 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1A), 5.70 (dd, 1H, $J_{2,3}$ = 11.4 Hz, $J_{3,4}$= 3.8 Hz, H-3B), 5.52-5.43 (m, 2H, PhCH, H-1B), 5.34 (dd, 1H, $J_{2,3}$ = 9.3 Hz, H-2A), 5.21-4.82 (m, 4H, CH_2(Bn)), 4.70 (m, 1H, H-2B), 4.34-4.08 (m, 5H, H-3A, H-4A, H-5A, H-4B, H-6aB), 3.80 (d, 1H, $J_{6a,6b}$ = 11.6 Hz, H-6bB), 3.11 (s, 1H, H-5B), 2.69-2.32 (m, 4H, CH_2(Lev)), 1.92 (s, 3H, CH_3(Lev)); ^{13}C-NMR (75 MHz, $CDCl_3$) (selected data for minor anomer (α)): δ 160.4 (C=NH), 129.9-126.4 (Ar-C, Ar-CH), 100.9 (PhCH), 98.4 (C-1B), 93.4 (C-1A), 79.9, 77.9, 75.0 (C-3A, C-4A, C-5A), 73.5 (CH_2(Bn)), 72.7 (C-4B), 71.9 (C-2A), 68.9 (C-6B), 68.5 (C-3B), 67.6 (CH_2(Bn)), 66.4 (C-5B), 52.1 (C-2B); HR MS: *m/z*: calcd for $C_{55}H_{45}Cl_7N_2O_{16}Na$: 1257.0486; found: 1257.0470 [*M*+Na]$^+$.

Benzyl [*N*-Benzyloxycarbonyl-5-aminopentyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-(4,6-*O*-benzylidene-2-deoxy-3-*O*-levulinoyl-2-tetrachlorophthalimido-β-D-galactopyranosyl)-β-D-glucopyranoside] uronate (14): Donor **13** (184 mg, 0.15 mmol) and benzyl *N*-(5-hydroxypentyl) carbamate (107 mg, 0.45 mmol) were dissolved in dry CH_2Cl_2 (1 mL) in the presence of freshly activated 4Å molecular sieves. After stirring for 45 min at room temperature, TMSOTf (162 µL of a 0.092 M solution in dry CH_2Cl_2) was added under an argon atmosphere. After stirring for 15 min, the reaction mixture was neutralized with Et_3N and concentrated to dryness. The residue was purified by column chromatography (toluene-acetone 10:1) to afford **14** (130 mg, 67%). TLC (toluene-EtOAc 3:1) Rf 0.44; $[\alpha]^{20}_D$ –5º (*c* 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.92 (m, 2H, Ar), 7.53 (m, 1H, Ar), 7.48-7.02 (m, 22H, Ar), 5.71 (dd, 1H, $J_{2,3}$ = 11.5 Hz, $J_{3,4}$ = 3.5 Hz, H-3B), 5.46 (s, 1H, PhCH), 5.36 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1B), 5.21 (m, 2H, H-2A, CH_2(Bn)), 5.05 (m, 3H, CH_2(Z), CH_2(Bn)), 4.94 (d, 1H, CH_2(Bn)), 4.79 (d, 1H, CH_2(Bn)), 4.68 (dd, 1H, H-2B), 4.55 (bt, 1H, NH), 4.44 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1A), 4.26 (bd, 1H, H-4B), 4.24 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4A), 4.13 (d, 1H, $J_{6a,6b}$ = 12.1 Hz, H-6aB), 3.88-3.80 (m, 3H, H-3A, H-5A, H-6bB), 3.70, 3.30 (2m, 2H, CH_2-O), 3.10 (bs, 1H, H-5B), 2.89 (m, 2H, CH_2-N), 2.69-2.32 (m, 4H, CH_2(Lev)), 1.92 (s, 3H, CH_3(Lev)), 1.49-1.06 (m, 6H, $(CH_2)_3$); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 206.3 (CO(Lev)), 171.9, 167.7, 165.1, 164.2, 163.5 (CO(Lev, NTCP, COOBn, Bz)), 156.4 (CO(Z)), 140.0, 139.8, 138.4, 137.7, 136.8 (Ar-C), 133.3 (Ar-CH), 129.9, 129.8, 129.4, 129.1, 128.7, 128.6, 128.5, 128.4, 128.2, 127.9, 127.4, 127.3, 126.4 (Ar-C, Ar-CH), 101.4 (C-1A), 100.9 (PhCH), 97.9 (C-1B), 80.4 (C-3A), 78.0 (C-4A), 74.7 (C-5A), 74.3 (CH_2(Bn)), 73.2 (C-2A), 72.7 (C-4B), 69.8 (CH_2-O), 68.9 (C-6B), 68.3 (C-3B), 67.5 (CH_2(Bn)), 66.6 (CH_2(Z)), 66.3 (C-5B), 52.1 (C-2B), 40.9 (CH_2-N), 37.9 (CH_2(Lev)), 29.6 (CH_2), 29.4 (CH_3(Lev)), 28.9 (CH_2), 28.1 (CH_2(Lev)), 23.1 (CH_2); HR MS: *m/z*: calcd for $C_{66}H_{62}Cl_4N_2O_{18}Na$: 1333.2649; found: 1333.2604 [*M*+Na]$^+$.

Benzyl [*N*-Benzyloxycarbonyl-5-aminopentyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-(2-deoxy-3-*O*-levulinoyl-2-tetrachlorophthalimido-β-D-galactopyranosyl)-β-D-glucopyranoside] uronate (17): TFA (100 µL) was added at 0ºC to a solution of **14** (25 mg, 19 µmol) in CH_2Cl_2 (1 mL). After stirring for 2 h at 0ºC, the solution was diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$ aqueous solution and brine. The organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by column chromatography (toluene-acetone 7:2) to afford **17** (23 mg, 99%). TLC (toluene-acetone 7:2) Rf 0.14; $[\alpha]^{20}_D$ –12º (*c* 1.0, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.97 (m, 2H, Ar), 7.55 (m, 1H, Ar), 7.43-7.13 (m, 17H, Ar), 5.59 (dd, 1H, $J_{2,3}$ = 11.3 Hz, $J_{3,4}$ = 3.1 Hz, H-3B), 5.28-5.13 (m, 4H, H-1B, H-2A, CH_2(Bn)), 5.06 (m, 2H, CH_2(Z)), 4.85 (d, 1H, CH_2(Bn)), 4.66-4.55 (m, 3H, CH_2(Bn), H-2B, NH), 4.48 (d, 1H, $J_{1,2}$ = 7.4 Hz, H-1A), 4.25 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz, H-4A), 4.13 (bd, 1H, H-4B), 3.87 (d, 1H, H-5A), 3.82 (t, 1H, $J_{2,3}$ = 8.9 Hz, H-3A), 3.73 (m, 1H, CH_2-O), 3.62 (m, 2H, H-6aB, H-6bB), 3.34 (m, 2H, CH_2-O, H-5B), 2.91 (m, 2H, CH_2-N), 2.66, 2.40 (2m, 4H, CH_2(Lev)), 2.03 (s, 3H, CH_3(Lev)), 1.49-1.11 (m, 6H, $(CH_2)_3$); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 207.4 (CO(Lev)), 171.8, 167.6, 165.1, 164.1, 163.3 (CO(Lev, NTCP, COOBn, Bz)), 156.4 (CO(Z)), 140.0, 139.8, 137.9, 136.8, 135.1 (Ar-C), 133.4 (Ar-CH), 129.8, 129.7, 129.6, 128.7, 128.6, 128.5, 128.3, 128.2, 128.0, 127.6, 127.3 (Ar-C, Ar-CH), 101.4 (C-1A), 97.0 (C-1B), 79.3 (C-3A), 76.9 (C-4A), 74.9 (C-5A), 73.7 (C-5B), 73.5 (CH_2(Bn)), 72.5 (C-2A), 70.1 (C-3B), 69.7 (CH_2-O), 67.4 (CH_2(Bn)), 67.2 (C-4B), 66.5 (CH_2(Z)), 62.7 (C-6B), 51.7 (C-2B), 40.8 (CH_2-N), 38.0 (CH_2(Lev)), 29.5 (CH_2, CH_3(Lev)), 28.8 (CH_2), 28.1 (CH_2(Lev)), 22.9 (CH_2); HR MS: *m/z*: calcd for $C_{59}H_{58}Cl_4N_2O_{18}Na$: 1245.2336; found: 1245.2332 [*M*+Na]$^+$.

Benzyl [*N*-Benzyloxycarbonyl-5-aminopentyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-(2-deoxy-3-*O*-levulinoyl-4,6-di-*O*-sulfo-2-tetrachlorophthalimido-β-D-galactopyranosyl)-β-D-glucopyranoside] uronate (18): Compound **17** (13 mg, 11 µmol) and sulfur trioxide–trimethylamine complex (15 mg, 0.11 mmol) were dissolved in dry DMF (1.0 mL) and heated at 100ºC for 40 min using microwave radiation (28 W average power). The reaction vessel was cooled and Et_3N (150 µL), MeOH (1 mL) and CH_2Cl_2 (1 mL) were added. The solution was layered on the top of a Sephadex LH 20 chromatography column which was eluted with CH_2Cl_2-MeOH (1:1) to obtain **18** as triethylammonium salt (14 mg, 83%). TLC (CH_2Cl_2- MeOH 9:2) Rf 0.40; ^{1}H-NMR (300 MHz, CD_3OD): δ 7.98 (m, 2H, Ar), 7.60 (m, 1H, Ar), 7.50-7.05 (m, 17H, Ar), 5.77 (dd, 1H, $J_{2,3}$ = 11.6 Hz, $J_{3,4}$ = 3.3 Hz, H-3B), 5.35 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1B), 5.29 (d, 1H, CH_2(Bn)), 5.17 (d, 1H, CH_2(Bn)), 5.06 (t, 1H, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 8.7 Hz, H-2A), 5.02 (m, 2H, CH_2(Z)), 4.96 (d, 1H, CH_2(Bn)), 4.86 (bd, 1H, H-4B), 4.68 (d, 1H, H-1A), 4.56 (m, 2H, CH_2(Bn), H-2B), 4.45 (dd, 1H, $J_{5,6a}$ = 3.4 Hz, $J_{6a,6b}$ = 11.9 Hz, H-6aB), 4.22 (m, 2H, H-6bB, H-4A), 4.11 (m, 2H, H-5A, H-5B), 4.00 (t, 1H, $J_{3,4}$ = 9.2 Hz, H-3A), 3.69 (m, 1H, CH_2-O), 3.42 (m, 1H, CH_2-O), 3.19 (q, 12H, Et_3NH^+), 2.82 (m, 3H, CH_2-N, CH_2(Lev)), 2.60 (dt, 1H, CH_2(Lev)), 2.39 (m, 2H, CH_2(Lev)), 1.92 (s, 3H, CH_3(Lev)), 1.44-1.08 (m, 24H, $(CH_2)_3$, Et_3NH^+); ^{13}C-NMR (75 MHz, CD_3OD) (Significant data from HSQC

experiment): δ 102.2 (C-1A), 97.8 (C-1B), 79.8 (C-3A), 77.8 (C-4A), 74.9 (C-5A), 74.5 (C-5B), 74.2 (CH_2(Bn)), 73.7 (C-2A), 72.7 (C-4B), 70.6 (CH_2-O), 68.5 (C-3B), 68.4 (C-6B), 68.1 (CH_2(Bn)), 66.9 (CH_2(Z)), 52.9 (C-2B), 47.4 (Et_3NH^+), 41.3 (CH_2-N), 38.1 (CH_2(Lev)), 29.8 (CH_2), 29.6 (CH_2), 29.1 (CH_3(Lev)), 28.8 (CH_2(Lev)), 23.5 (CH_2), 8.7 (Et_3NH^+); ESI MS: *m/z*: calcd for $C_{59}H_{56}Cl_4N_2O_{24}S_2Na$: 1403.1; found: 1402.7 [*M*+Na]$^-$; HR MS: *m/z*: calcd for $C_{59}H_{56}Cl_4N_2O_{24}S_2$: 690.0715; found: 690.0709 [*M*]$^{-2}$.

***N*-Benzyloxycarbonyl-5-aminopentyl 4-*O*-(2-acetamido-2-deoxy-4,6-di-*O*-sulfo-β-D-galactopyranosyl)-3-*O*-benzyl-β-D-glucopyranosiduronic acid (21):** H_2O_2 (30%, 0.52 mL) and an aqueous solution of LiOH (0.7 M, 0.32 mL) were added at -5ºC to a solution of **18** (21 mg, 13 µmol) in THF (1.4 mL). After stirring for 20 h at room temperature, MeOH (1.4 mL) and an aqueous solution of NaOH (4 M, 0.33 mL) were added. After stirring for 24 h at room temperature, the reaction mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered, and concentrated. The residue was dissolved in CH_2Cl_2/MeOH/Et_3N (1.0 mL/1.0 mL/0.1 mL) and purified by Sephadex LH-20 chromatography (CH_2Cl_2-MeOH 1:1) to give compound **19** as triethylammonium salt (16 mg, 86%). TLC (EtOAc:Py:H_2O:AcOH 9:5:3:1) Rf 0.27; ^{1}H-NMR (300 MHz, CD_3OD): δ 7.59-7.19 (m, 10H, Ar), 5.06 (m, 3H, CH_2(Z), CH_2(Bn)), 4.79 (bd, 1H, H-4B), 4.72 (d, 1H, CH_2(Bn)), 4.62 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1B), 4.45 (dd, 1H, $J_{5,6a}$ = 3.9 Hz, $J_{6a,6b}$ = 11.8 Hz, H-6aB), 4.41 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1A), 4.22 (m, 2H, H-2B, H-6bB), 4.06 (bt, 1H, H-4A), 3.96 (m, 1H, H-5B), 3.86 (m, 1H, CH_2-O), 3.77 (dd, 1H, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 2.9 Hz, H-3B), 3.58 (m, 3H, CH_2-O, H-3A, H-5A), 3.37 (t, 1H, $J_{2,3}$ = 8.0 Hz, H-2A), 3.19 (q, 24H, Et_3NH^+), 3.11 (m, 2H, CH_2-N), 1.66-1.38 (3m, 6H, $(CH_2)_3$), 1.29 (t, 36H, Et_3NH^+); ^{13}C-NMR (75 MHz, CD_3OD) (Significant data from HSQC experiment): δ 129.5, 128.9, 128.7, 128.3 (Ar-CH), 104.0 (C-1A), 100.8 (C-1B), 83.7 (C-3A), 78.9 (C-4A), 76.8 (C-4B), 75.4 (CH_2(Bn)), 74.5 (C-5B), 74.0 (C-2A), 72.8 (C-3B), 70.5 (CH_2-O), 68.6 (C-6B), 67.0 (CH_2(Z)), 55.3 (C-2B), 47.6 (Et_3NH^+), 41.3 (CH_2-N), 30.1, 30.0, 23.8 ($(CH_2)_3$), 8.9 (Et_3NH^+); ESI MS: *m/z*: calcd for $C_{40}H_{43}Cl_4N_2O_{22}S_2$: 1107.1; found: 1106.7 [*M*+3H]$^-$.

Ethylendiamine (76 µL, 1.1 mmol) was added under an argon atmosphere to a solution of **19** (16 mg, 11 µmol) in dry DMF (1.3 mL), and the reaction mixture was subjected to microwave radiation for 90 min at 100ºC. The reaction vessel was cooled under a stream of nitrogen and concentrated to dryness. The residue was purified by Sephadex LH-20 chromatography (CH_2Cl_2-MeOH 1:1) to give **20**. TLC (EtOAc:Py:H_2O:AcOH 9:5:3:1) Rf 0.38; ESI MS: *m/z*: calcd for $C_{32}H_{42}N_2O_{19}S_2$: 411.1; found: 410.8 [*M*+H]$^{2-}$. Triethylamine (207 µL of a 0.18 M solution in dry MeOH) and acetic anhydride (5 µL, 0.05 mmol) were added to a cooled solution of **20** (11 µmol) in dry MeOH (2.3 mL). After stirring for 2 h at 0ºC, additional triethylamine (207 µL of a 0.18 M solution in dry MeOH) and acetic anhydride (5 µL, 0.05 mmol) were added and the reaction was stirred for 1 h at room temperature. The reaction mixture was purified by Sephadex LH-20 chromatography (CH_2Cl_2-MeOH 1:1). The residue was converted into the sodium salt by elution from a column of Dowex 50WX4-Na^+ with MeOH-H_2O 9:1 to give **21** (7.9 mg, 75% from **19**). TLC (EtOAc:Py:H_2O:AcOH 9:5:3:1) Rf 0.28; ^{1}H-NMR (500 MHz, CD_3OD): δ 7.54-7.19 (m, 10H, Ar), 5.04 (m, 3H, CH_2(Z), CH_2(Bn)), 4.78 (bs, 1H, H-4B), 4.68 (m, 2H, CH_2(Bn), H-1B), 4.38 (dd, 1H, $J_{5,6a}$ = 5.0 Hz, $J_{6a,6b}$ = 11.3 Hz, H-6aB), 4.28 (d, 1H, $J_{1,2}$ = 7.3 Hz, H-1A), 4.16 (dd, 1H, $J_{5,6b}$ = 6.3 Hz, H-6bB), 4.05 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 9.5 Hz, H-2B), 3.99 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.1 Hz, H-4A), 3.96 (m, 1H, H-5B), 3.86 (m, 1H, CH_2-O), 3.66 (m, 2H, H-5A, H-3B), 3.52 (m, 1H, CH_2-O), 3.44 (bt, 1H, H-3A), 3.38 (bt, 1H, H-2A), 3.11 (t, 2H, CH_2-N), 2.04 (s, 3H, NAc), 1.62, 1.51, 1.40 (3m, 6H, $(CH_2)_3$); ^{13}C-NMR (125.5 MHz, CD_3OD) (Significant data from HSQC experiment): δ 104.1 (C-1A), 101.5 (C-1B), 83.8 (C-3A), 79.8 (C-4A), 78.0 (C-5A), 76.8 (C-4B), 75.6 (CH_2(Bn)), 74.0 (C-5B, C-2A), 73.5 (C-3B), 70.4 (CH_2-O), 67.8 (C-6B), 67.0 (CH_2(Z)), 54.4 (C-2B), 41.5 (CH_2-N), 30.4, 30.0, 23.8 ($(CH_2)_3$), 23.0 (NAc); ESI MS: *m/z*: calcd for $C_{34}H_{44}N_2O_{20}S_2$: 432.1; found: 431.8 [*M*+H]$^{2-}$; HR MS: *m/z*: calcd for $C_{34}H_{44}N_2O_{20}S_2$: 432.0969; found: 432.0956 [*M*+H]$^{2-}$.

5-aminopentyl 4-*O*-(2-acetamido-2-deoxy-4,6-di-*O*-sulfo-β-D-galactopyranosyl)-β-D-glucopyranosiduronic acid (15): A solution of **21** (7.9 mg, 8.5 µmol, as sodium salt) in H_2O/MeOH (2.7 mL/0.3 mL) was hydrogenated in the presence of $Pd(OH)_2$. After 24 h, the suspension was filtered over Celite and concentrated. The residue was purified by Sephadex G-10 chromatography (H_2O/MeOH 9:1) to give **15** as sodium salt after lyophilisation (6.0 mg, quantitative; 53% from **14**, 6 steps, 90% average yield per step). ^{1}H-NMR (500 MHz, D_2O): δ 4.72 (bd, 1H, $J_{3,4}$ = 2.1 Hz, H-4B), 4.58 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1B), 4.47 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1A), 4.32 (dd, 1H, $J_{5,6a}$ = 3.3 Hz, $J_{6a,6b}$ = 11.3 Hz, H-6aB), 4.25 (dd, 1H, $J_{5,6b}$ = 8.7 Hz, H-6bB), 4.12 (dd, 1H, H-5B), 3.94-3.87 (m, 3H, H-2B, H-3B, CH_2-O), 3.77 (m, 3H, H-4A, H-5A, CH_2-O), 3.62 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, H-3A), 3.35 (t, 1H, H-2A), 3.00 (t, 2H, CH_2-N), 2.05 (s, 3H, NAc), 1.67, 1.46 (2m, 6H, $(CH_2)_3$); ^{13}C-NMR (125.5 MHz, D_2O) (Significant data from HSQC experiment): δ 102.8 (C-1A), 102.2 (C-1B), 82.3 (C-4A), 77.2 (C-5A), 76.0 (C-4B), 74.9 (C-3A), 73.1 (C-2A), 72.9 (C-5B), 70.6 (CH_2-O), 70.5 (C-3B), 68.3 (C-6B), 53.0 (C-2B), 39.9 (CH_2-N), 28.6, 26.8 (CH_2, CH_2), 23.1 (NAc), 22.5 (CH_2); ESI MS: *m/z*: calcd for $C_{19}H_{33}N_2O_{18}S_2$: 641.1; found: 640.8 [*M*+2H]$^-$; HR MS: *m/z*: calcd for $C_{19}H_{33}N_2O_{18}S_2$: 641.1188; found: 641.1170 [*M*+2H]$^-$.

Benzyl [*N*-Benzyloxycarbonyl-5-aminopentyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-(2-deoxy-3-*O*-levulinoyl-6-*O*-sulfo-2-tetrachlorophthalimido-β-D-galactopyranosyl)-β-D-glucopyranoside] uronate (22): Compound **17** (27 mg, 22 µmol) and sulfur trioxide–trimethylamine complex (6.2 mg, 44 µmol) were dissolved in dry DMF (3.0 mL) and heated at 50ºC for 30 min using microwave radiation (15 W average power). The reaction vessel was cooled and Et_3N (150 µL), MeOH (1 mL) and CH_2Cl_2 (1 mL) were added. The solution was purified by Sephadex LH 20 chromatography (CH_2Cl_2-MeOH 1:1) and silica gel column chromatography (CH_2Cl_2-MeOH 12:1 + 1% Et_3N) to afford **22** as triethylammonium salt (26 mg, 84%). TLC (CH_2Cl_2-MeOH 10:1) Rf 0.41; ^{1}H-NMR (300 MHz, CD_3OD): δ 7.97 (m, 2H, Ar), 7.60 (m, 1H, Ar), 7.47 (m, 2H, Ar), 7.39-7.26 (m, 10H, Ar), 7.13-7.03 (m, 5H, Ar), 5.66 (dd, 1H, $J_{2,3}$ = 11.4 Hz, $J_{3,4}$ = 3.2 Hz, H-3B), 5.42 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1B), 5.30 (d, 1H, CH_2(Bn)), 5.14 (d, 1H, CH_2(Bn)), 5.08 (t, 1H, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 8.6 Hz, H-2A), 5.02 (m, 2H, CH_2(Z)), 4.89 (d, 1H, CH_2(Bn)), 4.68 (d, 1H, H-1A), 4.59 (dd, 1H, H-2B), 4.55 (d, 1H, CH_2(Bn)), 4.23-4.08 (m, 5H, H-4A, H-4B, H-5A, H-6aB, H-6bB), 3.99 (t, 1H, H-3A), 3.81 (bt, 1H, H-5B), 3.69 (m, 1H, CH_2-O), 3.40 (m, 1H, CH_2-O), 3.18 (q, 6H, Et_3NH^+), 2.82 (m, 2H, CH_2-N), 2.65 (m, 2H, CH_2(Lev)), 2.38 (m, 2H, CH_2(Lev)), 1.89 (s, 3H, CH_3(Lev)), 1.44-1.10 (m, 15H, $(CH_2)_3$,

Et_3NH^+); ^{13}C-NMR (75 MHz, CD_3OD) (Significant data from HSQC experiment): δ 134.4, 130.6, 129.4, 129.2, 129.0, 128.6, 128.5 (Ar-CH), 102.2 (C-1A), 97.9 (C-1B), 79.8 (C-3A), 77.7 (C-4A, C-5A), 75.3 (C-4B), 73.8 (C-5B, CH_2(Bn)), 73.7 (C-2A), 70.8 (C-3B), 70.6 (CH_2-O), 68.1 (CH_2(Bn)), 67.1 (CH_2(Z)), 66.2 (C-6B), 53.1 (C-2B), 47.7 (Et_3NH^+), 41.2 (CH_2-N), 38.2 (CH_2(Lev)), 30.3 (CH_2), 29.9 (CH_2), 29.1 (CH_3(Lev)), 28.7 (CH_2(Lev)), 23.8 (CH_2), 9.0 (Et_3NH^+); ESI MS: *m/z*: calcd for $C_{59}H_{57}Cl_4N_2O_{21}S$: 1301.2; found: 1301.0 [*M*]$^-$; HR MS: *m/z*: calcd for $C_{59}H_{57}Cl_4N_2O_{21}S$: 1301.1934; found: 1301.2029 [*M*]$^-$.

***N*-Benzyloxycarbonyl-5-aminopentyl 4-*O*-(2-acetamido-2-deoxy-6-*O*-sulfo-β-D-galactopyranosyl)-3-*O*-benzyl-β-D-glucopyranosiduronic acid (25):** H_2O_2 (30%, 0.73 mL) and an aqueous solution of LiOH (0.7 M, 0.45 mL) were added at -5ºC to a solution of **22** (26 mg, 18 μmol) in THF (2.0 mL). After stirring for 20 h at room temperature, MeOH (2.0 mL) and an aqueous solution of NaOH (4 M, 0.46 mL) were added. After stirring for 24 h at room temperature, the reaction mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered, and concentrated. The residue was dissolved in CH_2Cl_2/MeOH/Et_3N (1.0 mL/1.0 mL/0.1 mL) and purified by Sephadex LH-20 chromatography (CH_2Cl_2-MeOH 1:1) to give compound **23** as triethylammonium salt (22 mg, 89%). TLC (EtOAc:Py:H_2O:AcOH 9:5:3:1) Rf 0.34; ^{1}H-NMR (300 MHz, CD_3OD): δ 7.54 (m, 2H, Ar), 7.35-7.20 (m, 8H, Ar), 5.06 (m, 2H, CH_2(Z)), 5.04 (d, 1H, CH_2(Bn)), 4.72 (d, 1H, CH_2(Bn)), 4.68 (d, 1H, $J_{1,2}$ = 8.8 Hz, H-1B), 4.36 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1A), 4.29 (dd, 1H, $J_{5,6a}$ = 7.4 Hz, $J_{6a,6b}$ = 10.5 Hz, H-6aB), 4.23 (bt, 1H, H-2B), 4.15 (dd, 1H, $J_{5,6b}$ = 5.8 Hz, H-6bB), 4.07 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 8.4 Hz, H-4A), 3.99-3.94 (m, 2H, H-4B, H-5A), 3.85 (m, 1H, CH_2-O), 3.76 (m, 1H, H-5B), 3.67 (m, 1H, H-3B), 3.54 (m, 2H, CH_2-O, H-3A), 3.38 (t, 1H, $J_{2,3}$ = 8.0 Hz, H-2A), 3.18 (q, 18H, Et_3NH^+), 3.11 (m, 2H, CH_2-N), 1.66-1.35 (3m, 6H, $(CH_2)_3$), 1.29 (t, 27H, Et_3NH^+); ^{13}C-NMR (75 MHz, CD_3OD) (Significant data from HSQC experiment): δ 129.4, 128.9, 128.4 (Ar-CH), 103.9 (C-1A), 100.7 (C-1B), 83.8 (C-3A), 78.7 (C-4A), 77.0 (C-5A), 75.4 (CH_2(Bn)), 74.6 (C-5B), 74.2 (C-2A), 74.0 (C-3B), 70.6 (CH_2-O), 68.5 (C-4B), 67.1 (CH_2(Z)), 66.7 (C-6B), 55.0 (C-2B), 47.4 (Et_3NH^+), 41.5 (CH_2-N), 30.3, 30.1, 23.8 ($(CH_2)_3$), 8.9 (Et_3NH^+); ESI MS: *m/z*: calcd for $C_{40}H_{42}Cl_4KN_2O_{19}S$: 1065.0; found: 1065.0 [*M*+H+K]$^-$.

Ethylendiamine (111 μL, 1.65 mmol) was added under an argon atmosphere to a solution of **23** (22 mg, 16 μmol) in dry DMF (1.0 mL), and the reaction mixture was subjected to microwave radiation for 90 min at 100ºC. The reaction vessel was cooled and concentrated to dryness. The residue was purified by Sephadex LH-20 chromatography (CH_2Cl_2-MeOH 1:1) to give **24**. TLC (EtOAc:Py:H_2O:AcOH 12:5:3:1) Rf 0.23; ESI MS: *m/z*: calcd for $C_{32}H_{43}N_2O_{16}S$: 743.2; found: 743.2 [*M*+H]$^-$.

Triethylamine (150 μL of a 0.36 M solution in dry MeOH) and acetic anhydride (7.8 μL, 83 μmol) were added to a cooled solution of **24** (16 μmol) in dry MeOH (1.5 mL). After stirring for 3 h at room temperature, additional Et_3N (0.2 mL) was added and the reaction mixture was purified by Sephadex LH-20 chromatography (CH_2Cl_2-MeOH 1:1). The residue was converted into the sodium salt by elution from a column of Dowex 50WX4-Na^+ with MeOH-H_2O 9:1 to give **25** (12 mg, 88% from **23**; 2 steps). TLC (EtOAc:Py:H_2O:AcOH 12:5:3:1, two elutions) Rf 0.41; ^{1}H-NMR (400 MHz, CD_3OD): δ 7.50-7.20 (m, 10H, Ar), 5.06 (s, 2H, CH_2(Z)), 5.02 (d, 1H, CH_2(Bn)), 4.69 (d, 1H, CH_2(Bn)), 4.62 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1B), 4.28 (d, 1H, $J_{1,2}$ = 6.9 Hz, H-1A), 4.25 (dd, 1H, $J_{5,6a}$ = 8.2 Hz, $J_{6a,6b}$ = 10.2 Hz, H-6aB), 4.10 (dd, 1H, $J_{5,6b}$ = 5.4 Hz, H-6bB), 4.03-3.93 (m, 2H, H-2B, H-4A), 3.91 (bs, 1H, H-4B), 3.86 (m, 1H, CH_2-O), 3.74-3.65 (m, 2H, H-5B, H-5A), 3.52 (m, 2H, H-3B, CH_2-O), 3.44 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 8.9 Hz, H-3A), 3.39 (t, 1H, H-2A), 3.11 (t, 2H, CH_2-N), 2.06 (s, 3H, NAc), 1.62, 1.51, 1.41 (3m, 6H, $(CH_2)_3$); ^{13}C-NMR (100 MHz, CD_3OD) (Significant data from HSQC experiment): δ 128.9-128.1 (Ar-CH), 103.9 (C-1A), 101.2 (C-1B), 83.8 (C-3A), 79.7 (C-4A), 77.7 (C-5A), 75.2 (CH_2(Bn)), 74.5 (C-3B), 73.9 (C-2A), 73.8 (C-5B), 70.2 (CH_2-O), 68.2 (C-4B), 66.9 (CH_2(Z)), 66.2 (C-6B), 54.4 (C-2B), 41.3 (CH_2-N), 30.1, 29.8, 23.6 ($(CH_2)_3$), 22.6 (NAc); ESI MS: *m/z*: calcd for $C_{34}H_{45}N_2O_{17}S$: 785.2; found: 785.1 [*M*+H]$^-$; HR MS: *m/z*: calcd for $C_{34}H_{44}N_2O_{17}S$: 392.1185; found: 392.1180 [*M*]$^{-2}$.

5-aminopentyl 4-*O*-(2-acetamido-2-deoxy-6-*O*-sulfo-β-D-galactopyranosyl)-β-D-glucopyranosiduronic acid (16): A solution of **25** (10 mg, 12 μmol, as sodium salt) in H_2O/MeOH (3.6 mL/0.4 mL) was hydrogenated in the presence of $Pd(OH)_2$. After 24 h, the suspension was filtered over Celite and concentrated. The residue was purified by Sephadex G-10 chromatography (H_2O/MeOH 9:1) to give **16** as sodium salt after lyophilisation (7.4 mg, quantitative; 66 % from **17**, 5 steps, 92% average yield per step). ^{1}H-NMR (500 MHz, D_2O): δ 4.53 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1B), 4.48 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1A), 4.25 (m, 2H, H-6aB, H-6bB), 4.00 (m, 2H, H-4B, H-5B), 3.92 (m, 2H, H-2B, CH_2-O), 3.74 (m, 4H, H-3B, H-4A, H-5A, CH_2-O), 3.63 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 8.7 Hz, H-3A), 3.36 (t, 1H, H-2A), 3.00 (t, 2H, CH_2-N), 2.06 (s, 3H, NAc), 1.68, 1.47 (2m, 6H, $(CH_2)_3$); ^{13}C-NMR (125.5 MHz, D_2O) (Significant data from HSQC experiment): δ 102.7 (C-1A), 102.0 (C-1B), 81.6 (C-4A), 77.1 (C-5A), 74.6 (C-3A), 73.2 (C-5B), 73.1 (C-2A), 71.2 (C-3B), 70.6 (CH_2-O), 67.9 (C-4B), 67.6 (C-6B), 52.5 (C-2B), 39.8 (CH_2-N), 28.5, 26.9 (CH_2, CH_2), 23.0 (NAc), 22.4 (CH_2); ESI MS: *m/z*: calcd for $C_{19}H_{33}N_2O_{15}S$: 561.1613; found: 561.0 [*M*+H]$^-$; HR MS: *m/z*: calcd for $C_{19}H_{33}N_2O_{15}S$: 561.1619; found: 561.1599 [*M*+H]$^-$.

4-Methoxyphenyl 3-*O*-(benzyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-β-D-glucopyranosyluronate)-2-deoxy-4,6-di-*O*-levulinoyl-2-trifluoroacetamido-β-D-galactopyranoside (29): An excess of $(HF)_n$·Py (5.5 mL) was added at 0°C under an argon atmosphere to a solution of **28** (1.23 g, 1.14 mmol) in dry THF (25 mL). After 24 h at 0ºC, the mixture was diluted with CH_2Cl_2 and washed with H_2O and saturated $NaHCO_3$ solution until neutral pH. The organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo to give the corresponding diol that was used for the next step without further purification [TLC (toluene-EtOAc 1:4) Rf 0.46]. LevOH (2.32 mL, 22.8 mmol) was added at 0ºC to a solution of 1,3-dicyclohexylcarbodiimide (2.35 g, 11.4 mmol) in CH_2Cl_2 (20 mL). After stirring for 5 min at room temperature, the mixture was cooled, filtered and concentrated to give quantitatively levulinic anhydride (Lev_2O, 11.4 mmol). A solution of the diol (1.14 mmol) in dry Py (30 mL) was added under an argon atmosphere to a flask containing Lev_2O (11.4 mmol) and DMAP (140 mg, 1.14 mmol). After stirring for 5 h at room temperature, the reaction mixture was diluted with CH_2Cl_2 and washed with 1M HCl aqueous solution, saturated $NaHCO_3$ aqueous solution and H_2O. The organic layers were dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by column chromatography (toluene-EtOAc 2:1) to afford **29** (950 mg, 83%). TLC (toluene-EtOAc 2:1) Rf 0.37; $[\alpha]^{20}_D$ +20º (*c*

1.0, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.94 (m, 2H, Ar), 7.59 (m, 1H, Ar), 7.44 (m, 2H, Ar), 7.39-7.30 (m, 5H, Ar), 7.13-7.07 (m, 5H, Ar), 6.89 (m, 2H, Ar), 6.77 (m, 2H, Ar), 6.69 (bs, 1H, N*H*), 5.47 (d, 1H, $J_{3,4}$ = 2.6 Hz, H-4A), 5.32 (pt (pseudotriplet), 1H, H-4B), 5.28 (pt, 1H, H-2B), 5.19-5.11 (m, 3H, H-1A and CH_2(Bn)), 4.79 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1B), 4.61-4.52 (m, 3H, H-3A and CH_2(Bn)), 4.15-4.09 (m, 2H, 2 x H-6A), 4.07 (d, 1H, $J_{4,5}$ = 9.6 Hz, H-5B), 3.89-3.80 (m, 3H, H-5A, H-3B and H-2A), 3.75 (s, 3H, OCH_3), 2.80-2.20 (m, 12H, 3 x $OCO(CH_2)_2$), 2.15 (s, 3H, $COCH_3$), 2.14 (s, 3H, $COCH_3$), 2.10 (s, 3H, $COCH_3$); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 206.84 (CO(Lev)), 206.81 (CO(Lev)), 206.1 (CO(Lev)), 172.3 (CO), 171.5 (CO), 171.2 (CO), 166.9 (CO), 165.0 (CO), 157.6 (q, $J_{C,F}$ = 38.7 Hz, *C*OCF_3), 155.9 (Ar), 151.0 (Ar), 137.4 – 114.3 (Ar-C and Ar-CH), 115.5 (q, $J_{C,F}$ = 289.0 Hz, CO*C*F_3), 100.1 (C-1B), 98.9 (C-1A), 79.2 (C-3B), 74.2 (CH_2(Bn)), 73.2 (C-3A), 72.8 (C-5B), 72.6 (C-2B), 71.6 (C-5A), 70.8 (C-4B), 68.7 (C-4A), 67.9 (CH_2(Bn)), 62.3 (C-6A), 55.7 (OCH_3), 54.6 (C-2A), 38.2 ($OCO(CH_2)_2$), 38.0 ($OCO(CH_2)_2$), 37.7 ($OCO(CH_2)_2$), 29.9 ($COCH_3$), 29.8 (2 x $COCH_3$), 28.0 ($OCO(CH_2)_2$), 27.9 ($OCO(CH_2)_2$), 27.8 ($OCO(CH_2)_2$); HR MS: *m/z*: calcd for $C_{57}H_{60}F_3NNaO_{20}$: 1158.3558; found: 1158.3596 [*M*+Na]$^+$.

3-*O*-(Benzyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-β-D-glucopyranosyluronate)-2-deoxy-4,6-di-*O*-levulinoyl-2-trifluoroacetamido-α,β-D-galactopyranose (30): CAN (7.6 mL of a 0.44 M solution in H_2O) was added to a solution of **29** (950 mg, 0.84 mmol) in CH_2Cl_2/MeCN (1:2; 22.8 mL), and the mixture was vigorously stirred for 1 h at room temperature. It was then diluted with EtOAc, washed with H_2O, saturated aqueous $NaHCO_3$ and H_2O. The organic phase was dried ($MgSO_4$), filtered and concentrated to dryness. The residue was purified by column chromatography (toluene-acetone 3:1) to afford **30** (695 mg, 81%) as a mixture of α/β anomers. TLC (toluene-acetone 3:2) Rf 0.44 and 0.31; ^{1}H-NMR (500 MHz, $CDCl_3$) (data for α anomer): δ 7.94 (m, 2H, Ar), 7.57 (m, 1H, Ar), 7.44-7.32 (m, 7H, Ar), 7.15-7.05 (m, 5H, Ar), 6.69 (d, 1H, $J_{2,NH}$ = 8.7 Hz, N*H*), 5.39 (d, 1H, $J_{3,4}$ = 2.3 Hz, H-4A), 5.31-5.25 (m, 3H, H-1A, H-2B and H-4B), 5.19-5.11 (2d, 2H, J_{gem} = 11.9 Hz, CH_2(Bn)), 4.81 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1B), 4.55 (2d, 2H, J_{gem} = 11.6 Hz, CH_2(Bn)), 4.40-4.32 (m, 2H, H-2A and H-5A), 4.27-4.20 (m, 2H, H-6aA and H-3A), 4.05 (d, 1H, $J_{4,5}$ = 9.7 Hz, H-5B), 3.94 (dd, 1H, $J_{5,6b}$ = 8.8 Hz, $J_{6a,6b}$ = 11.4 Hz, H-6bA), 3.82 (pt, 1H, H-3B), 2.84-2.21 (m, 12H, 3 x $OCO(CH_2)_2$), 2.16 (s, 3H, $COCH_3$), 2.10 (s, 3H, $COCH_3$), 2.09 (s, 3H, $COCH_3$); ^{13}C-NMR (125 MHz, $CDCl_3$) (data for α anomer) δ 208.8 (CO(Lev)), 206.9 (CO(Lev)), 206.0 (CO(Lev)), 172.2 (CO), 171.8 (CO), 171.2 (CO), 166.8 (CO), 165.0 (CO), 157.1 (q, *C*OCF_3), 137.3-127.8 (Ar-C and Ar-CH), 115.7 (q, CO*C*F_3), 99.6 (C-1B), 91.4 (C-1A), 79.3 (C-3B), 74.2 (CH_2(Bn)), 73.0 (C-5B), 72.2 (C-2B), 72.0 (C-3A), 71.0 (C-4B),68.9 (C-4A), 68.0 (CH_2(Bn)), 67.0 (C-5A), 63.0 (C-6A), 50.1 (C-2A), 38.5 ($OCO(CH_2)_2$), 38.1 ($OCO(CH_2)_2$), 37.7 ($OCO(CH_2)_2$), 30.0 ($COCH_3$), 29.9 ($COCH_3$), 29.8 ($COCH_3$), 28.3 ($OCO(CH_2)_2$), 28.0 ($OCO(CH_2)_2$), 27.7 ($OCO(CH_2)_2$); HR MS: *m/z*: calcd for $C_{50}H_{54}NO_{19}NaF_3$: 1052.3140; found: 1052.3175 [*M*+Na]$^+$.

***O*-[3-*O*-(Benzyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-β-D-glucopyranosyluronate)-2-deoxy-4,6-di-*O*-levulinoyl-2-trifluoroacetamido-α,β-D-galactopyranosyl] trichloroacetimidate (31):** Compound **30** (120 mg, 0.11 mmol) was dissolved in dry CH_2Cl_2 (3 mL) and Cl_3CCN (234 µL, 2.3 mmol) and DBU (261 µL of a 0.066 M solution in CH_2Cl_2) were added. After stirring at room temperature for 5 h, the mixture was concentrated in vacuo. Flash chromatography on silica gel (toluene-acetone 5:1 + 1% Et_3N) afforded **31** (95 mg, 70%) as an α/β mixture. TLC (toluene-acetone 3:2) Rf 0.65 and 0.48; ^{1}H-NMR (500 MHz, $CDCl_3$) (data for α anomer): δ 8.71 (s, 1H, N*H*(TCA)), 7.96 (m, 2H, Ar), 7.59 (m, 1H, Ar), 7.44 (m, 2H, Ar), 7.37 (m, 5H, Ar), 7.13-7.05 (m, 5H, Ar), 7.00 (br d, $J_{NH,2}$ = 7.3 Hz, 1H, N*H*(TFA)), 6.54 (d, 1H, $J_{1,2}$ = 3.4 Hz, 1H, H-1A), 5.55 (br d, 1H, H-4A), 5.35 (pt, 1H, H-2B), 5.23 (pt, 1H, H-4B), 5.18-5.06 (2d, 2H, J_{gem} = 12.1 Hz, CH_2(Bn)), 4.93 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1B), 4.62-4.51 (m, 3H, H-2A and CH_2(Bn)), 4.35 (dd, 1H, $J_{3,4}$ = 2.9 Hz, $J_{2,3}$ = 11.0 Hz, H-3A), 4.28 (m, 1H, H-5A), 4.12-4.04 (m, 3H, H-5B and 2 x H-6A), 3.85 (pt, 1H, H-3B), 2.74-2.16 (m, 12H, 3$OCO(CH_2)_2$), 2.14 (s, 3H, $COCH_3$), 2.10 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$).

4-Methoxyphenyl *O*-(benzyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-β-D-glucopyranosyluronate)-(1→3)-*O*-(2-deoxy-4,6-di-*O*-levulinoyl-2-trifluoroacetamido-β-D-galactopyranosyl)-(1→4)-*O*-(benzyl 2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranosyluronate)-(1→3)-2-deoxy-4,6-*O*-di-*tert*-butylsilylene-2-trifluoroacetamido-β-D-galactopyranoside (33): Donor **31** (143 mg, 0.12 mmol) and aceptor **32** (67 mg, 0.07 mmol) were coevaporated with toluene, concentrated in vacuo and dissolved in dry CH_2Cl_2 (3 mL) in the presence of freshly activated 4Å molecular sieves. After stirring for 10 min at 0 ºC, TMSOTf (264 µL of a 0.09 M solution in dry CH_2Cl_2) was added under an argon atmosphere. After stirring for 30 min at 0ºC, the reaction mixture was neutralized with Et_3N, filtered, and concentrated to dryness. The residue was purified by column chromatography (toluene-acetone 5:1) to afford **33** (97 mg, 71%). TLC (toluene-EtOAc 2:1) Rf 0.34; $[\alpha]^{20}_D$ +11º (*c* 1.0, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.96 (m, 2H, Ar), 7.91 (m, 2H, Ar), 7.57 (m, 2H, Ar), 7.46-7.29 (m, 14H, Ar), 7.15-7.08 (m, 10H, Ar), 6.90 (m, 2H, Ar), 6.84 (d, 1H, $J_{2,NH}$ = 6.9 Hz, N*H*), 6.78 (m, 2H, Ar), 6.52 (d, 1H, $J_{2,NH}$ = 8.7 Hz, N*H*), 5.37-5.33 (m, 2H, H-1A and H-4D), 5.27-5.15 (m, 8H, H-1B, H-2B, H-4C, H-2D and 2 x CH_2(Bn)), 4.78 (d, 1H, J_{gem} = 11.1 Hz, CH_2(Bn)), 4.73 (d, 1H, $J_{1,2}$ = 7.4 Hz, H-1D), 4.61-4.52 (m, 3H, H-4A and CH_2(Bn)), 4.46 (d, 1H, J_{gem} = 11.1 Hz, CH_2(Bn)), 4.33 (dd, 1H, $J_{2,3}$ = 11.1 Hz, $J_{3,4}$ = 1.9 Hz, H-3A), 4.15-4.08 (m, 3H, H-1C, H-5D and H-6A), 4.05-4.02 (m, 2H, H-4B and H-6A), 3.96-3.81 (m, 6H, H-2A, H-2C, 2 x H-6C H-5B and H-3D), 3.75 (s, 3H, OCH_3), 3.69 (m, 1H, H-3C), 3.63 (m, 1H, H-3B), 3.34 (m, 2H, H-5A and H-5C), 2.75-2.21 (m, 12H, 3 x $OCO(CH_2)_2$), 2.18 (s, 3H, $COCH_3$), 2.11 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 1.04 (s, 9H, $C(CH_3)_3$), 0.99 (s, 9H, $C(CH_3)_3$); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 207.1 (CO(Lev)), 206.6 (CO(Lev)), 206.0 (CO(Lev)), 172.2 (CO), 171.4 (CO), 171.2 (CO), 168.6-164.9 (4 x CO), 157.6 (2q, 2 x *C*OCF_3), 156.0-114.5 (Ar-C and Ar-CH), 117.9 (2q, 2 x CO*C*F_3), 100.2 (C-1B), 99.8, 99.7 (C-1C and C-1D), 99.3 (C-1A), 80.1 (C-3B), 79.2 (C-3D), 77.8 (C-4B), 75.4 (C-3A), 75.1 (CH_2(Bn)), 74.3, 74.2, 74.1, 73.2, 73.0 (C-3C, C-5B, CH_2(Bn), C-4A, C-5D), 72.5, 72.4 (2C: C-2B or C-4C or C-2D or CH_2(Bn)), 71.4, 71.1 (C-5A and C-5C), 70.9 (C-4D), 68.2, 68.1, 67.9 (3C: CH_2(Bn) or C-2B or C-4C or C-2D), 67.0 (C-6A), 61.5 (C-6C), 55.7 (OCH_3), 54.0, 53.0 (C-2A and C-2C), 38.1 ($OCO(CH_2)_2$), 38.0 ($OCO(CH_2)_2$), 37.7 ($OCO(CH_2)_2$), 29.9 ($COCH_3$), 29.8 ($COCH_3$), 29.7 ($COCH_3$), 28.1 ($OCO(CH_2)_2$), 27.9 ($OCO(CH_2)_2$), 27.7 ($OCO(CH_2)_2$), 27.6, 27.5, 27.5 ($C(CH_3)_3$), 23.3, 20.8

($C(CH_3)_3$); HR MS: *m/z*: calcd for $C_{100}H_{110}N_2O_{32}F_6NaSi$: 2015.6607; found: 2015.6607 $[M+Na]^+$.

4-Methoxyphenyl *O*-(3-*O*-benzyl-2,4-di-*O*-sulfo-β-D-glucopyranosyluronic acid)-(1→3)-*O*-(2-acetamido-2-deoxy-4,6-di-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-*O*-(3-*O*-benzyl-2-*O*-sulfo-β-D-glucopyranosyluronic acid)-(1→3)-2-acetamido-2-deoxy-4,6-di-*O*-sulfo-β-D-galactopyranoside (37): An excess of $(HF)_n{\cdot}Py$ (102 µL, 3.8 mmol) was added at 0 ºC under an argon atmosphere to a solution of **33** (39 mg, 0.02 mmol) in dry THF (1.0 mL). After 24 h at 0 ºC, the mixture was diluted with CH_2Cl_2 and washed with H_2O and saturated $NaHCO_3$ solution until neutral pH. The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by column chromatography (toluene-EtOAc 2:3) to afford **34** (27 mg, 75%). TLC (toluene-EtOAc 1:2) R_f 0.36; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.96 (m, 2H, Ar), 7.86 (m, 2H, Ar), 7.56 (m, 2H, Ar), 7.46-7.33 (m, 14H, Ar), 7.13-7.06 (m, 10H, Ar), 6.86 (m, 2H, Ar), 6.74 (m, 2H, Ar), 5.34-5.11 (m, 9H, H-4D, H-2D, H-1A, H2B, H-4C and CH_2(Bn)), 4.75 (m, 3H, H-1B, H-1D and CH_2(Bn)), 4.60-4.49 (m, 4H, H-1C and CH_2(Bn)), 4.38 (bs, 1H, H-3A), 4.15 (bs, 1H, H-4B), 4.08-3.97 (m, 4H, H-4A, H-5D, H-5B and H-3C), 3.90 (m, 3H, H-2C and 2 x H-6A or C), 3.82 (m, 3H, H-2A, H-3D and H-6A or C), 3.72 (m, 4H, H-3B and OCH_3), 3.56 (m, 3H, H-5A, H-5C and H6A or C), 2.74-2.17 (m, 12H, 3x $OCO(CH_2)_2$), 2.12 (s, 3H, $COCH_3$), 2.10 (s, 3H, $COCH_3$), 2.06 (s, 3H, $COCH_3$); ^{13}C-NMR (100 MHz, $CDCl_3$) (selected data from HSQC experiment): δ 100.1 (C-1B), 99.7 (C-1D), 99.6 (C-1C), 98.7 (C-1A), 79.3 (C-3B), 79.1 (C-3D), 77.5 (C-3A), 77.3 (C-4B), 74.5 (CH_2(Bn)), 74.1 (CH_2(Bn)), 74.1 (C-5B or 5D), 74.0 (C-5A or 5C), 73.9 (C-3C), 72.7 (C-5B or 5D), 72.4 (C-2B, C4C), 71.0 (C-5A or 5C), 70.7 (C-4D), 68.3 (C-4A), 68.0 (C-2D, 2 x CH_2(Bn)), 62.6 (C-6A or C), 61.7 (C-6A or C), 55.7 (COCH_3), 54.1 (C-2A), 53.2 (C-2C); HR MS: *m/z*: calcd for $C_{92}H_{94}F_6N_2Na_2O_{32}$: 949.2739; found: 949.2735 $[M+2Na]^{2+}$.

H_2O_2 (30%, 0.26 mL) and a solution of LiOH (0.7 M, 0.16 mL) were added at -5 ºC to a solution of **34** (12 mg, 6.5 µmol) in THF (1.0 mL). After stirring for 24 h at room temperature, MeOH (1 mL) and a solution of NaOH (4 M, 0.33 mL) were added. After stirring for 3 days at room temperature, the reaction was neutralized with Amberlite IR-120 (H^+) resin, filtered and concentrated to give **35**. ESI MS: *m/z*: calcd for $C_{45}H_{56}N_2NaO_{22}$: 999.3; found: 999.4 $[M+Na]^-$.

Et_3N (12 µL, 85 µmol) and Ac_2O (12 µL, 129 µmol) were added to a cooled (0 ºC) solution of **35** (6.5 µmol) in dry MeOH (2.0 mL). After stirring for 3 h at room temperature, triethylamine (0.3 mL) was added and the mixture was coevaporated with toluene and methanol. The residue was purified by Sephadex LH-20 chromatography (MeOH:CH_2Cl_2 1:1) to give **36** as triethylammonium salt. The sodium salt of **36** (6.2 mg, 90%) was obtained by treatment with Amberlite IR-120 (H^+) resin in MeOH (pH ~ 3), followed by filtration, treatment with 0.04 M NaOH (pH ~ 7) and concentration. ^{1}H-NMR (500 MHz, MeOD, data for sodium salt): δ 7.58 (m, 2H, Ar), 7.44 (m, 2H, Ar), 7.34-7.20 (m, 6H, Ar), 6.98 (m, 2H, Ar), 6.82 (m, 2H, Ar), 5.05 (d, 1H, J_{gem} = 10.3 Hz, CH_2(Bn)), 4.93 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1A), 4.88 (m, 2H, CH_2(Bn)), 4.72 (d, 1H, J_{gem} = 10.3 Hz, CH_2(Bn)), 4.62 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1C), 4.43 (m, 2H, H-1B and H-1D), 4.27 (dd, 1H, $J_{1,2}$ = 8.5 Hz, $J_{2,3}$ = 10.7 Hz, H-2A), 4.19-4.12 (m, 3H, H-2C, H-4C and H-4A), 3.96 (m, 1H, H-4B), 3.86-3.76 (m, 4H, H-3A and 3 x H-6A or C), 3.74 (s, 3H, OCH_3), 3.72-3.56 (m, 6H, H-5B, H-6A or C, H-3C, H-5A or C, H-4D and H-5D), 3.51 (m, 1H, H-5A or C), 3.46 (m, 2H, H-2B and H-3B), 3.41 (m, 2H, H-2D and H-3D), 2.05 (s, 3H, NHCOCH_3), 2.05 (s, 3H, NHCOCH_3); ^{13}C-NMR (100 MHz, MeOD) (selected data from HSQC experiment): δ 128.5-113.7 (Ar), 104.2 (C-1B and C-1D), 100.6 (C-1A), 100.0 (C-1C), 84.1 (C-3D), 82.6 (C-3C), 82.5 (C-3B), 80.6 (C-3A), 77.6 (C-4B), 76.4 (C-5B), 75.8 (C-5A or C), 75.2 (2C: C-4D or C-5D or C-5A or C), 75.0 (CH_2(Bn)), 74.0 (CH_2(Bn)), 73.0 (C-2D), 72.7 (C-2B), 72.0 (C-4D or C-5D or C-5A or C), 67.6 (C-4A and C-4C), 61.1 (C-6A and C-6C), 54.4 (COCH_3), 51.5 (C-2C), 51.2 (C-2A), 22.1 (NHCOCH_3), 21.6 (NHCOCH_3); HR MS: *m/z*: calcd for $C_{49}H_{60}N_2O_{24}$: 530.1773; found: 530.1775 $[M]^{2-}$. Compound **36** (6mg, 5.4 µmol) and the sulfur trioxide–trimethylamine complex (26 mg, 0.19 mmol) were dissolved in dry DMF (1.5 mL) and heated at 100 °C for 2 h using microwave radiation (20 W average power). The reaction vessel was cooled and Et_3N (150 µL) and MeOH (1 mL) were added. The solution was layered on the top of a Sephadex LH-20 chromatography column which was eluted with MeOH to obtain **37** as triethylammonium salt. The residue was converted into the sodium salt by elution from a column of Dowex 50WX4-Na^+ with MeOH–H_2O 9 : 1 (5 mg, 56%). ^{1}H-NMR (500 MHz, D_2O, 40 ºC, data for sodium salt): δ 7.62 (m, 2H, Ar), 7.50-7.36 (m, 8H, Ar), 7.12 (m, 2H, Ar), 7.00 (m, 2H, Ar), 5.31 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1A), 4.96 (m, 2H, H-4A and H-4C), 4.93-4.85 (m, 4H, H-1B or D, H-4D and CH_2(Bn)), 4.80-4.71 (m, 4H, H-1C, H-1D or B, and CH_2(Bn)), 4.41 (m, 2H, H-2B and H-2D), 4.36-4.23 (m, 5H, H-3D or H-5D, H-3A and 3 x H-6A or C), 4.20 (m, 2H, H-3D or H-5D, and H-5A or C), 4.15 (m, 2H, H-4B and H-3C), 4.10 (m, 1H, H-6A or C), 3.99 (m, 2H, H-2C and H-2A), 3.93 (m, 1H, H-5A or C), 3.88 (pt, 1H, H-3B), 3.83 (s, 3H, OCH_3), 3.81 (m, 1H, H-5B), 2.09 (NHCOCH_3), 2.08 (NHCOCH_3); ^{13}C-NMR (125 MHz, D_2O, 40ºC) (selected data from HSQC experiment): δ 130.6-116.4 (Ar), 103.8 (C-1D or B), 102.4 (C-1B or D), 101.6 (C-1A), 101.4 (C-1C), 81.6 (C-3B), 80.0 (C-2B and 2D), 80.0 (C-5A/C or C-3D or C-5D) 79.5 (C-3D or 5D), 78.7 (C-5B), 78.6 (C-3C and C-4B), 77.9 (C-3A), 77.2 (C-4D), 76.9 (C-4A and C-4C), 75.1 (CH_2(Bn)), 74.2 (CH_2(Bn)), 74.1 (C-5A/C or C-3D or C-5D), 73.4 (C-5A or 5C), 69.3 (C-6A or 6C), 68.6 (C-6A or 6C), 57.2 (OCH_3), 53.9 (C-2A and C-2C), 23.9 (2x NHCOCH_3).

4-Methoxyphenyl *O*-(2,4-di-*O*-sulfo-β-D-glucopyranosyluronic acid)-(1→3)-*O*-(2-acetamido-2-deoxy-4,6-di-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-*O*-(2-*O*-sulfo-β-D-glucopyranosyluronic acid)-(1→3)-2-acetamido-2-deoxy-4,6-di-*O*-sulfo-β-D-galactopyranoside (38): A solution of **37** (4.0 mg, 2.5 µmol, sodium salt) in H_2O/MeOH (3.6 mL/0.4 mL) was hydrogenated at 1.5 bar pressure in the presence of $Pd(OH)_2$ (12 mg). After 24h, the suspension was filtered over celite and concentrated to give **38** after lyophilisation (3.4 mg, 97 %). ^{1}H-NMR (500 MHz, D_2O, data for sodium salt): δ 7.10 (m, 2H, Ar), 6.99 (m, 2H, Ar), 5.22 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1A), 4.95 (d, 1H, $J_{3,4}$ = 2.6 Hz, H-4A), 4.93 (d, 1H, $J_{3,4}$ = 2.4 Hz, H-4C), 4.77 (m, 2H, H-1C and H-1D), 4.71 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1B), 4.51 (pt, 1H, H-4D), 4.36-4.31 (m, 2H, 2 x H-6A or C), 4.28-4.21 (m, 6H, 2 x H-6A or C, H-5A or C, H-2D, H-3A and H-2B), 4.16-4.08 (m, 3H, H-3C, H-5A or C, and H-2A), 4.03 (pt, 1H, H-3D), 3.98 (d, 1H, $J_{4,5}$ = 8.5 Hz, H-5D), 3.93 (m, 2H, H-4B and H-2C), 3.85-3.81 (m, 4H, H-3B and OCH_3), 3.72 (d, 1H, $J_{4,5}$ = 9.6 Hz, H-5B), 2.07 (NHCOCH_3), 2.06 (NHCOCH_3); ^{13}C-NMR (125 MHz, D_2O) (selected data from HSQC experiment): δ 119.6

(Ar), 116.2 (Ar), 103.8 (C-1B), 102.9 (C-1D), 102.6 (C-1C), 101.7 (C-1A), 81.5 (C-4B), 80.7 (C-2B and C-2D), 79.0 (C-4D), 77.9 (C-3A), 77.8 (C-5B), 77.5 (C-3C and C-5D), 77.0 (C-4A and C-4C), 74.4 (C-3B), 74.2 (C-3D), 73.9 (C-5A or C), 73.5 (C-5A or C), 69.0 (C-6A or C), 68.7 (C-6A or C), 56.8 (OCH_3), 53.3 (C-2A), 23.7 (2xNHCOCH_3). ESI MS: *m/z*: calcd for $C_{35}H_{41}N_2O_{45}S_7Na_7$: 796.9; found: 796.7 $[M+7Na]^{2-}$.

4-Methoxyphenyl 3-*O*-(methyl 3-*O*-benzyl-2,4-di-*O*-pivaloyl-α-L-idopyranosyluronate)-2-deoxy-4,6-*O*-di-*tert*-butylsilylene-2-trifluoroacetamido-β-D-galactopyranoside (40): Compound **39** (427 mg, 0.428 mmol) was dissolved in CH_2Cl_2 (8.5 mL) and hydrazine monohydrate (3.42 mL of a 0.5 M solution in Py/AcOH 3:2) was added. After stirring at room temperature for 3h, the reaction mixture was quenched with acetone (5.0 mL). The mixture was diluted with CH_2Cl_2 and washed with 1 M HCl aqueous solution, saturated $NaHCO_3$ aqueous solution and H_2O. The organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo to give the corresponding diol that was used for the next step without further purification [TLC (toluene-EtOAc 1:2) Rf 0.20]. This diol was dissolved in Py (9 mL). Pivaloyl chloride (2.5 mL) and DMAP (21 mg, 0.17 mmol) were added and the solution was stirred at room temperature. After 45 h, the mixture was diluted with CH_2Cl_2, washed with 1 M HCl aqueous solution, saturated $NaHCO_3$ aqueous solution and H_2O, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (toluene-EtOAc 6:1) to yield **40** (363 mg, 87%; 2 steps). TLC (toluene-EtOAc 4:1) Rf 0.40; $[\alpha]^{20}_D$ –19º (*c* 1.0, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.39-7.26 (m, 5H, Ar), 6.94 (m, 2H, Ar), 6.79 (m, 2H, Ar), 6.66 (d, 1H, $J_{2,NH}$ = 7.2 Hz, NH), 5.31 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1A), 5.29 (m, 1H, H-4B), 5.15 (d, 1H, $J_{4,5}$ = 2.0 Hz, H-5B), 5.04 (bs, 1H, H-1B), 4.86 (m, 1H, H-2B), 4.79 (2d, 2H, CH_2(Bn)), 4.53 (d, 1H, $J_{3,4}$ = 2.8 Hz, H-4A), 4.39 (dd, 1H, $J_{2,3}$ = 11.0 Hz, H-3A), 4.20 (m, 2H, H-6aA, H-6bA), 3.98 (dt, 1H, H-2A), 3.76 (s, 3H, Me(OMP) or COOMe), 3.74 (m, 4H, H-3B, Me(OMP) or COOMe), 3.47 (bs, 1H, H-5A), 1.19, 1.17, 1.08, 0.97 (4s, 36H, C(CH_3)$_3$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 177.9, 177.6 (CO(Piv)), 169.1 (COOMe), 157.9 (q, $J_{C,F}$ = 37.2 Hz, *C*OCF_3), 156.0, 151.2, 137.9 (Ar C), 128.5, 127.7, 127.5, 120.0 (Ar-CH), 115.6 (q, $J_{C,F}$ = 288.4 Hz, CO*C*F_3), 114.6 (Ar-CH), 101.4 (C-1B), 99.2 (C-1A), 78.8 (C-3A), 74.0 (C-3B), 72.7 (C-4A), 72.5 (CH_2(Bn)), 71.3 (C-5A), 67.7 (C-4B), 67.5 (C-2B), 67.0 (C-6A), 66.9 (C-5B), 55.7 (COOMe or Me(OMP)), 54.1 (C-2A), 52.4 (COOMe or Me(OMP)), 39.1, 38.8 (*C*(CH_3)$_3$, Piv), 27.8, 27.4, 27.3, 27.1 (C(*C*H_3)$_3$), 23.3, 21.0 (*C*(CH_3)$_3$); HR MS: *m/z*: calcd for $C_{47}H_{66}F_3NO_{15}SiNa$: 992.4052; found: 992.4087 $[M+Na]^+$.

4-Methoxyphenyl 3-*O*-(methyl 3-*O*-benzyl-2,4-di-*O*-pivaloyl-α-L-idopyranosyluronate)-2-deoxy-4,6-di-*O*-levulinoyl-2-trifluoroacetamido-β-D-galactopyranoside (41): An excess of $(HF)_n$·Py (1.85 mL, 71.1 mmol) was added at 0°C under an argon atmosphere to a solution of **40** (363 mg, 0.374 mmol) in dry THF (8.0 mL). After 24 h at 0ºC the mixture was diluted with CH_2Cl_2 and washed with H_2O and saturated $NaHCO_3$ solution until neutral pH. The organic layers were dried ($MgSO_4$), filtered and concentrated in vacuo to give the corresponding diol (290 mg) that was used for the next step without further purification [TLC (toluene-EtOAc 1:6) Rf 0.45]. LevOH (0.72 mL, 7.0 mmol) was added at 0ºC to a solution of 1,3-dicyclohexylcarbodiimide (721 mg, 3.5 mmol) in CH_2Cl_2 (6 mL). After stirring for 5 min at room temperature, the mixture was cooled, filtered and concentrated to give quantitatively levulinic anhydride (Lev_2O, 3.5 mmol). A solution of the diol (290 mg) in dry Py (10 mL) was added under an argon atmosphere to a flask containing Lev_2O (3.5 mmol) and DMAP (44 mg, 0.35 mmol). After stirring for 26 h at room temperature, the reaction mixture was diluted with CH_2Cl_2 and washed with 1M HCl aqueous solution, saturated $NaHCO_3$ aqueous solution and brine. The organic layers were dried ($MgSO_4$), filtered and concentrated in vacuo. In order to complete the reaction, this residue was dissolved again in dry Py (10 mL) and was added under an argon atmosphere to a flask containing additional Lev_2O (3.5 mmol) and DMAP (44 mg, 0.35 mmol). After stirring for 26 h at room temperature, the reaction mixture was diluted with CH_2Cl_2 and washed with 1M HCl aqueous solution, saturated $NaHCO_3$ aqueous solution and brine. The organic layers were dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by column chromatography (toluene-EtOAc 2:1) to afford **41** (311 mg, 81%). TLC (toluene-EtOAc 1:1) Rf 0.30; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.36-7.26 (m, 5H, Ar), 6.95 (m, 2H, Ar), 6.79 (m, 3H, NH, Ar), 5.41 (d, 1H, $J_{3,4}$ = 3.2 Hz, H-4A), 5.26 (m, 2H, H-4B, H-1A), 4.98 (bs, 1H, H-1B), 4.86 (d, 1H, $J_{4,5}$ = 2.4 Hz, H-5B), 4.83 (bt, 1H, H-2B), 4.71 (m, 2H, CH_2(Bn)), 4.50 (dd, 1H, $J_{2,3}$ = 10.9 Hz, $J_{3,4}$ = 3.3 Hz, H-3A), 4.16 (dd, 1H, $J_{5,6a}$ = 7.4 Hz, $J_{6a,6b}$ = 11.4 Hz, H-6aA), 4.09 (dd, 1H, $J_{5,6a}$ = 5.8 Hz, H-6bA), 3.98 (m, 1H, H-2A), 3.93 (bt, 1H, H-5A), 3.76 (s, 6H, Me(OMP), COOMe), 3.69 (bt, 1H, H-3B), 2.72-2.23 (m, 8H, CH_2(Lev)), 2.16, 2.08 (2s, 6H, CH_3(Lev)), 1.18, 1.14 (2s, 18H, CH_3(Piv)); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 206.8, 206.3 (CO(Lev)), 177.8, 177.5, 172.2, 172.1 (CO(Lev, Piv)), 168.9 (COOMe), 158.0 (q, $J_{C,F}$ = 37.3 Hz, *C*OCF_3), 155.9, 151.1, 137.6 (Ar-C), 128.0, 127.9, 119.0 (Ar-CH), 115.7 (q, $J_{C,F}$ = 288.2 Hz, CO*C*F_3), 114.7 (Ar-CH), 100.9 (C-1B), 99.2 (C-1A), 75.4 (C-3A), 74.4 (C-3B), 72.6 (CH_2(Bn)), 71.4 (C-5A), 68.6 (C-4A), 68.0 (C-2B), 67.8 (C-4B), 67.1 (C-5B), 61.9 (C-6A), 55.7 (COOMe or Me(OMP)), 54.7 (C-2A), 52.4 (COOMe or Me(OMP)), 39.0, 38.7 (*C*(CH_3)$_3$ (Piv)), 38.0, 37.9 (CH_2(Lev)), 29.9, 29.7 (CH_3(Lev)), 27.9, 27.8 (CH_2(Lev)), 27.2, 27.0 (C(*C*H_3)$_3$ (Piv)); HR MS: *m/z*: calcd for $C_{49}H_{62}F_3NO_{19}Na$: 1048.3766; found: 1048.3790 $[M+Na]^+$.

3-*O*-(Methyl 3-*O*-benzyl-2,4-di-*O*-pivaloyl-α-L-idopyranosyluronate)-2-deoxy-4,6-di-*O*-levulinoyl-2-trifluoroacetamido-α,β-D-galactopyranose (42): CAN (1.6 mL of a 0.63 M solution in H_2O) was added to a solution of **41** (346 mg, 0.337 mmol) in CH_2Cl_2/MeCN (1:2; 15 mL). After stirring for 1 h 30 min at room temperature, the reaction mixture was diluted with EtOAc, washed with H_2O, saturated aqueous $NaHCO_3$, and H_2O. The organic phase was dried ($MgSO_4$), filtered and concentrated to dryness. The residue was purified by column chromatography (toluene-acetone 7:2) to afford **42** (218 mg, 70%) as a mixture of α/β anomers. TLC (toluene-acetone 7:2) Rf 0.20 and 0.11; ^{1}H-NMR (300 MHz, $CDCl_3$) (data for α anomer): δ 7.40-7.26 (m, 5H, Ar), 6.65 (d, 1H, $J_{2,NH}$ = 9.5 Hz, NH), 5.35 (m, 2H, H-4A, H-1A), 5.20 (bt, 1H, H-4B), 5.02 (bs, 1H, H-1B), 4.80 (d, 1H, $J_{4,5}$ = 2.4 Hz, H-5B), 4.76 (m, 1H, H-2B), 4.74-4.62 (2d, 2H, CH_2(Bn)), 4.53 (dt, 1H, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 10.6 Hz, H-2A), 4.34 (dd, 1H, $J_{5,6a}$ = 3.7 Hz, $J_{5,6b}$ = 8.8 Hz, H-5A), 4.22 (dd, 1H, $J_{6a,6b}$ = 11.5 Hz, H-6aA), 4.10 (dd, 1H, $J_{3,4}$ = 3.1 Hz, H-3A), 3.95 (dd, 1H, H-6bA), 3.77 (s, 3H, COOMe), 3.70 (bt, 1H, H-3B), 2.82-2.26 (m, 8H, CH_2(Lev)), 2.19, 2.05 (2s, 6H, CH_3(Lev)), 1.18, 1.15 (2s, 18H, (CH_3)$_3$ (Piv)); ^{13}C-NMR (75 MHz, $CDCl_3$) (data for α anomer): δ 208.5, 206.7 (CO(Lev)), 177.8, 177.6, 172.3

(CO(Lev, Piv)), 169.0 (COOMe), 157.7 (q, $J_{C,F}$ = 37.2 Hz, *C*OCF$_3$), 137.5 (Ar-C), 128.5, 128.1, 128.0 (Ar-CH), 115.9 (q, $J_{C,F}$ = 288.0 Hz, CO*C*F$_3$), 101.1 (C-1B), 91.7 (C-1A), 75.5 (C-3A), 73.7 (C-3B), 72.4 (CH_2(Bn)), 69.4 (C-4A), 67.9 (C-4B), 67.5 (C-2B), 67.0 (C-5B), 66.9 (C-5A), 62.7 (C-6A), 52.4 (COOMe), 50.2 (C-2A), 39.0, 38.7 (*C*(CH$_3$)$_3$ (Piv)), 38.3, 37.9 (CH_2(Lev)), 29.9, 29.7 (CH_3(Lev)), 28.1, 27.7 (CH_2(Lev)), 27.2, 27.1 (C(*C*H$_3$)$_3$ (Piv)); HR MS: *m/z*: calcd for $C_{42}H_{56}F_3NO_{18}Na$: 942.3347; found: 942.3311 [*M*+Na]$^+$.

4-Methoxyphenyl *O*-(methyl 3-*O*-benzyl-2,4-di-*O*-pivaloyl-α-L-idopyranosyluronate)-(1→3)-*O*-(2-deoxy-4,6-di-*O*-levulinoyl-2-trifluoroacetamido-β-D-galactopyranosyl)-(1→4)-*O*-(benzyl 2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranosyluronate)-(1→3)-2-deoxy-4,6-*O*-di-*tert*-butylsilylene-2-trifluoroacetamido-β-D-galactopyranoside (44): Donor **43** (168 mg, 0.158 mmol) and acceptor **32** (103 mg, 0.105 mmol) were dissolved in dry CH_2Cl_2 (3.0 mL) in the presence of freshly activated 4Å molecular sieves. After stirring for 30 min at 0ºC, TMSOTf (343 µL of a 0.092 M solution in dry CH_2Cl_2) was added under an argon atmosphere. After stirring for 30 min at 0ºC, the reaction mixture was neutralized with Et_3N, filtered and concentrated to dryness. The residue was purified by column chromatography (toluene-acetone 7:1) to afford **44** (106 mg, 53%). TLC (toluene-acetone 3:1) Rf 0.55; $[\alpha]^{20}_D$ –2º (*c* 1.0, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.92 (d, 2H, Ar), 7.56 (t, 1H, Ar), 7.49-7.22 (m, 12H, Ar), 7.07 (m, 5H, Ar), 6.95 (d, 1H, $J_{2,NH}$ = 6.5 Hz, NH), 6.90 (m, 2H, Ar), 6.78 (m, 2H, Ar), 6.68 (d, 1H, $J_{2,NH}$ = 8.9 Hz, NH), 5.37 (d, 1H, $J_{1,2}$ = 8.2 Hz, H-1A), 5.30 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1B), 5.28 (m, 2H, CH_2(Bn)), 5.24 (m, 1H, H-4D), 5.21 (t, 1H, H-2B), 5.17 (d, 1H, $J_{3,4}$ = 3.1 Hz, H-4C), 4.95 (bs, 1H, H-1D), 4.91 (d, 1H, $J_{4,5}$ = 2.2 Hz, H-5D), 4.84 (d, 1H, CH_2(Bn)), 4.77 (bs, 1H, H-2D), 4.72-4.66 (2d, 2H, CH_2(Bn)), 4.57 (bs, 1H, H-4A), 4.51 (d, 1H, CH_2(Bn)), 4.36 (bd, 1H, $J_{2,3}$ = 11.3 Hz, H-3A), 4.24 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1C), 4.17-3.98 (m, 4H, H-2C, H-6aA, H-4B, H-6bA), 3.97-3.93 (m, 2H, H-2A, H-5B), 3.87 (m, 2H, H-6aC, H-6bC), 3.78, 3.75 (2s, 6H, Me(OMP), COOMe), 3.68 (m, 2H, H-3B, H-3D), 3.57 (dd, 1H, $J_{2,3}$ = 10.5 Hz, H-3C), 3.45 (t, 1H, $J_{5,6a}$ = $J_{5,6b}$ = 6.5 Hz, H-5C), 3.32 (m, 1H, H-5A), 2.68 (m, 2H, CH_2(Lev)), 2.48-2.16 (m, 8H, CH_2(Lev), CH_3(Lev) (2.17)), 2.10-2.04 (m, 1H, CH_2(Lev)), 1.96 (s, 3H, CH_3(Lev)), 1.20, 1.18, 1.05, 1.01 (4s, 36H, C(CH$_3$)$_3$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 206.9, 206.1 (CO(Lev)), 177.9, 177.3, 172.2, 172.0, 169.1, 168.9, 165.1 (CO(Lev), CO(Piv), CO(Bz), COOBn, COOMe), 158.2 (q, $J_{C,F}$ = 37.1 Hz, *C*OCF$_3$), 157.7 (q, $J_{C,F}$ = 36.8 Hz, *C*OCF$_3$), 156.0, 151.1, 137.8, 137.6, 134.6 (Ar-C), 133.4, 129.9, 129.7, 129.5, 129.4, 129.3, 128.5, 128.2, 128.0, 127.7, 120.3 (Ar-C, Ar-CH), 116.0 (q, $J_{C,F}$ = 288.3 Hz, CO*C*F$_3$), 115.5 (q, $J_{C,F}$ = 288.6 Hz, CO*C*F$_3$), 114.6 (Ar-CH), 100.4 (C-1D), 100.2, 100.1 (C-1B, C-1C), 99.3 (C-1A), 80.2 (C-3B), 78.3 (C-4B), 76.5 (C-3C), 75.3 (CH_2(Bn)), 75.2 (C-3A), 74.2 (C-3D), 74.1 (C-5B), 73.4 (C-4A), 72.5 (CH_2(Bn)), 72.4 (C-2B), 71.4 (C-5A), 71.2 (C-5C), 68.5 (CH_2(Bn)), 68.1 (C-4C), 67.8 (C-4D, C-2D), 67.6 (C-6A), 67.0 (C-5D), 61.4 (C-6C), 55.7 (COOMe or Me(OMP)), 54.1 (C-2A), 53.3 (C-2C), 52.4 (COOMe or Me(OMP)), 39.1, 38.7 (*C*(CH$_3$)$_3$ (Piv)), 38.0, 37.8 (CH_2(Lev)), 29.9, 29.6 (CH_3(Lev)), 28.0, 27.7 (CH_2(Lev)), 27.6, 27.3, 27.1 (C(*C*H$_3$)$_3$), 23.4, 20.8 (*C*(CH$_3$)$_3$ (DBSi)); HRMS: *m/z*: calcd for $C_{92}H_{112}F_6N_2O_{31}SiNa$: 1905.6820; found: 1905.6755 [*M*+Na]$^+$.

4-Methoxyphenyl *O*-(methyl 3-*O*-benzyl-2,4-di-*O*-pivaloyl-α-L-idopyranosyluronate)-(1→3)-*O*-(2-deoxy-2-trifluoroacetamido-β-D-galactopyranosyl)-(1→4)-*O*-(benzyl 2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranosyluronate)-(1→3)-2-deoxy-4,6-*O*-di-*tert*-butylsilylene-2-trifluoroacetamido-β-D-galactopyranoside (45): Compound **44** (105 mg, 0.056 mmol) was dissolved in CH_2Cl_2 (1.5 mL) and hydrazine monohydrate (0.45 mL of a 0.5 M solution in Py/AcOH 3:2) was added. After stirring at room temperature for 3h, the reaction mixture was quenched with acetone (0.7 mL). The mixture was diluted with CH_2Cl_2 and washed with 1 M HCl aqueous solution, saturated $NaHCO_3$ aqueous solution and H_2O. The organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (toluene-EtOAc 6:1) to yield **45** (82 mg, 87%). TLC (toluene-EtOAc 3:2) Rf 0.32; $[\alpha]^{20}_D$ –7º (*c* 1.0, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.97 (d, 2H, Ar), 7.57 (t, 1H, Ar), 7.46-7.33 (m, 12H, Ar), 7.13 (m, 5H, Ar), 6.91 (m, 2H, Ar), 6.80 (m, 3H, Ar, NH), 6.52 (d, 1H, $J_{2,NH}$ = 8.4 Hz, NH), 5.38 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1A), 5.30-5.20 (m, 5H, H-1B, H-2B, CH_2(Bn), H-4D), 5.08 (bd, 1H, H-1D), 4.94 (d, 1H, $J_{4,5}$ = 2.7 Hz, H-5D), 4.86 (d, 1H, CH_2(Bn)), 4.85 (bd, 1H, H-2D), 4.79-4.68 (2d, 2H, CH_2(Bn)), 4.58 (bs, 1H, H-4A), 4.54 (d, 1H, CH_2(Bn)), 4.38 (bd, 1H, $J_{2,3}$ = 11.1 Hz, H-3A), 4.27 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1C), 4.24 (m, 1H, H-2C), 4.11 (d, 1H, $J_{6a,6b}$ = 12.3 Hz, H-6aA), 4.05 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4B), 3.99 (m, 1H, H-6bA), 3.98-3.91 (m, 3H, H-5B, H-2A, H-4C), 3.76-3.75 (2s, 6H, COOMe, Me(OMP)), 3.70 (m, 2H, H-3D, H-3B), 3.56-3.45 (m, 3H, H-6aC, H-6bC, H-3C), 3.32 (m, 1H, H-5A), 3.12 (m, 1H, H-5C), 1.20, 1.18, 1.06, 1.02 (4s, 36H, C(CH$_3$)$_3$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 177.6, 177.4, 169.0, 168.7, 165.1 (CO(Piv), CO(Bz), COOBn, COOMe), 157.9 (q, $J_{C,F}$ = 36.9 Hz, *C*OCF$_3$), 157.7 (q, $J_{C,F}$ = 36.7

Hz, *C*OCF$_3$), 156.0, 151.1, 137.7, 137.2, 134.7 (Ar-C), 133.5, 129.9, 129.5, 129.4, 129.2, 129.1, 128.6, 128.5, 128.3, 128.1, 128.0, 127.8, 120.3 (Ar-C, Ar-CH), 116.0 (q, $J_{C,F}$ = 288.3 Hz, CO*C*F$_3$), 115.5 (q, $J_{C,F}$ = 288.3 Hz, CO*C*F$_3$), 114.6 (Ar-CH), 100.4 (C-1B), 100.2 (C-1C), 99.6 (C-1D), 99.3 (C-1A), 80.6 (C-3B), 79.0 (C-3C), 78.0 (C-4B), 75.8 (CH$_2$(Bn)), 75.5 (C-3A), 74.6 (C-5C), 74.1 (C-5B), 73.7 (C-3D), 73.3 (C-4A), 72.6 (C-2B), 72.4 (CH$_2$(Bn)), 71.4 (C-5A), 68.3 (CH$_2$(Bn), C-4C), 68.2 (C-2D), 67.8 (C-5D), 67.0 (C-4D, C-6A), 62.3 (C-6C), 55.7 (COOMe or Me(OMP)), 54.1 (C-2A), 52.5 (COOMe or Me(OMP)), 52.3(C-2C), 39.1, 38.8 (*C*(CH$_3$)$_3$ (Piv)), 27.6, 27.3, 27.1 (C(*C*H$_3$)$_3$), 23.4, 20.8 (*C*(CH$_3$)$_3$ (DBSi)); HRMS: *m/z*: calcd for $C_{82}H_{100}F_6N_2O_{27}SiNa$: 1709.6085; found: 1709.6031 [*M*+Na]$^+$.

4-Methoxyphenyl *O*-(methyl 3-*O*-benzyl-2,4-di-*O*-pivaloyl-α-L-idopyranosyluronate)-(1→3)-*O*-(2-deoxy-2-trifluoroacetamido-β-D-galactopyranosyl)-(1→4)-*O*-(benzyl 2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranosyluronate)-(1→3)-2-deoxy-2-trifluoroacetamido-β-D-galactopyranoside (46): An excess of (HF)$_n$·Py (223 µL, 8.6 mmol) was added at 0°C under an argon atmosphere to a solution of **45** (75 mg, 0.044 mmol) in dry THF (4.0 mL). After 24 h at 0ºC the mixture was diluted with CH_2Cl_2 and washed with H_2O and saturated $NaHCO_3$ solution until neutral pH. The organic layers were dried ($MgSO_4$), filtered and concentrated in vacuo to give **46** (67 mg, 97%). TLC (toluene-EtOAc 1:2) Rf 0.20; $[\alpha]^{20}_D$ – 3º (*c* 1.0, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.85 (d, 2H, Ar), 7.56 (t, 1H, Ar), 7.44-7.26 (m, 13H, Ar, NH), 7.10-7.03 (m, 5H, Ar), 6.83 (m, 2H, Ar), 6.72 (m, 2H, Ar), 6.64 (m, 1H, NH), 5.37 (m, 1H, H-1A), 5.29 (m, 1H, H-2B), 5.22 (m, 3H, H-4D, CH$_2$(Bn)), 5.06 (bd, 1H, H-1D), 4.93 (d, 1H, $J_{4,5}$ = 2.8 Hz, H-5D), 4.87 (d, 1H, CH$_2$(Bn)), 4.84 (m, 1H, H-2D), 4.81 (d, 1H, $J_{1,2}$ = 7.4 Hz, H-1B), 4.77-4.56 (3d, 3H, CH$_2$(Bn)), 4.48 (m, 2H, H-1C, H-3A), 4.31 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 8.8 Hz, H-4B), 4.16 (m, 1H, H-2C), 4.14 (bs, 1H, H-4A), 4.06 (bd, 1H, H-5B), 3.93 (bd, 1H, $J_{3,4}$ = 2.6 Hz, H-4C), 3.88 (m, 1H, H-2A), 3.83-3.70 (m, 9H, H-6aA, H-3B, H-3D, Me(OMP), COOMe), 3.59 (m, 4H, H-6bA, H-6aC, H-3C, H-5A), 3.48 (dd, 1H, $J_{5,6b}$ = 2.8 Hz, $J_{6a,6b}$ = 12.1 Hz, H-6bC), 3.26 (m, 1H, H-5C), 2.29 (m, 4H, OH), 1.19, 1.18 (2s, 18H, C(CH$_3$)$_3$); ^{13}C-NMR (100 MHz, $CDCl_3$) (selected data from HSQC experiment): δ 133.7, 130.2, 129.7-127.6, 119.1, 114.7 (Ar-CH), 101.7 (C-1B), 100.1 (C-1C), 99.6 (C-1D), 98.7 (C-1A), 80.5 (C-3B), 78.8 (C-3C), 78.6 (C-3A), 77.4 (C-4B), 75.9 (CH$_2$(Bn)), 75.2 (C-5C), 74.5 (C-5A), 74.2 (C-5B), 74.1 (C-3D), 72.8 (C-2B), 72.7 (CH$_2$(Bn)), 68.8 (CH$_2$(Bn)), 68.6 (C-4A, C-4C, C-2D), 68.0 (C-5D), 67.6 (C-4D), 62.7 (C-6C), 62.5 (C-6A), 55.9 (COOMe or Me(OMP)), 54.5 (C-2A), 52.8 (C-2C, COOMe or Me(OMP)), 27.5 (C(*C*H$_3$)$_3$); HRMS: *m/z*: calcd for $C_{74}H_{84}F_6N_2O_{27}Na$: 1569.5063; found: 1569.5101 [*M*+Na]$^+$.

4-Methoxyphenyl *O*-(methyl 3-*O*-benzyl-2,4-di-*O*-pivaloyl-α-L-idopyranosyluronate)-(1→3)-*O*-(2-deoxy-4,6-di-*O*-sulfo-2-trifluoroacetamido-β-D-galactopyranosyl)-(1→4)-*O*-(benzyl 2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranosyluronate)-(1→3)-2-deoxy-4,6-di-*O*-sulfo-2-trifluoroacetamido-β-D-galactopyranoside (47): Compound **46** (16 mg, 10 µmol) and sulfur trioxide–trimethylamine complex (58 mg, 0.41 mmol) were dissolved in dry DMF (1.5 mL) and heated at 100ºC for 30 min using microwave radiation (23 W average power). The reaction vessel was cooled and Et_3N (300 µL), MeOH (1 mL) and CH_2Cl_2 (1 mL) were added. The solution was layered on the top of a Sephadex LH 20 chromatography column which was eluted with CH_2Cl_2-MeOH (1:1) to obtain **47** as triethylammonium salt (23 mg, quantitative). TLC (EtOAc:Py:H_2O:AcOH 12:5:3:1) Rf 0.14; ^{1}H-NMR (500 MHz, $CD_3OD/CDCl_3$ 7:1): δ 7.94 (d, 2H, Ar), 7.62 (t, 1H, Ar), 7.54-7.05 (m, 17H, Ar), 6.97 (d, 2H, Ar), 6.82 (d, 2H, Ar), 5.68 (d, 1H, $J_{4,5}$ = 2.7 Hz, H-5D), 5.43 (d, 1H, *J* = 11.8 Hz, CH$_2$(Bn)), 5.34 (dd, 1H, H-4D), 5.26-5.22 (m, 2H, H-2B, CH$_2$(Bn)), 5.05 (d, 1H, $J_{1,2}$ = 2.4 Hz, H-1D), 5.02-4.97 (m, 2H, H-2D, CH$_2$(Bn)), 4.90 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1B), 4.85-4.80 (m, 3H, H-1A, H-4A, H-4C), 4.76 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1C), 4.70 (m, 2H, CH$_2$(Bn)), 4.52-4.43 (m, 3H, CH$_2$(Bn), 2 x H-6A or C), 4.40-4.35 (m, 2H, H-4B, H-6A or C), 4.33-4.25 (m, 2H, H-6A or C, H-2C), 4.20-4.08 (m, 3H, H-2A, H-3A, H-5B), 4.01 (dd, 1H, $J_{2,3}$ = 11.0 Hz, $J_{3,4}$ = 3.0 Hz, H-3C), 3.97-3.94 (m, 2H, H-5A, H-5C), 3.85 (dd, 1H, H-3B), 3.78-3.74 (2s, 6H, Me(OMP), COOMe), 3.61 (dd, 1H, H-3D), 3.16 (q, 24H, Et_3NH^+), 1.28 (t, 36H, Et_3NH^+), 1.18, 1.14 (2s, 18H, C(CH$_3$)$_3$); ^{13}C-NMR (125 MHz, $CD_3OD/CDCl_3$ 7:1) (selected data from HSQC experiment): δ 128.3-114.1 (Ar-CH), 101.6 (C-1B), 101.1 (C-1D), 100.7 (C-1C), 99.3 (C-1A), 79.9 (C-3B), 76.9 (C-3A), 75.8 (C-4B), 75.4-75.1 (C-4A, C-4C, C-3C), 74.9 (C-3D), 74.6-74.5 (C-5B, CH$_2$(Bn)), 73.4 (C-5A, C-5C), 72.6 (C-2B), 71.4 (CH$_2$(Bn)), 69.2 (C-4D), 68.8 (C-2D), 68.0-67.5 (CH$_2$(Bn), C-6C, C-6A, C-5D), 54.6 (COOMe or Me(OMP)), 53.0 (C-2A), 51.8 (C-2C), 51.5 (COOMe or Me(OMP)), 26.3 (C(*C*H$_3$)$_3$); ESI MS: *m/z*: calcd for $C_{74}H_{81}F_6N_2O_{39}S_4Na$: 943.2; found: 943.3 [*M*+Na+H]^2.

4-Methoxyphenyl *O*-(3-*O*-benzyl-α-L-idopyranosyluronic acid)-(1→3)-*O*-(2-acetamido-2-deoxy-4,6-di-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-*O*-(3-*O*-benzyl-β-D-glucopyranosyluronic acid)-(1→3)-2-acetamido-2-deoxy-4,6-di-*O*-sulfo-β-D-galactopyranoside (49): H_2O_2 (30%, 0.19 mL) and an aqueous solution of LiOH (0.7 M, 0.12 mL) were added at 0ºC to a solution of **47** (11 mg, 4.8 µmol) in THF (0.5 mL). After stirring for 24 h at room temperature, MeOH (1.0 mL), H_2O (0.3 mL) and an aqueous solution of NaOH (4 M, 0.24 mL) were added. After stirring for 72 h at room temperature, the reaction mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered, and concentrated to give **48** [ESI MS: *m/z*: calcd for $C_{45}H_{54}N_2O_{34}S_4Na_2$: 670.0; found: 669.9 [*M*+2Na+2H]$^{2-}$]. Triethylamine (9 µL, 64 µmol) and acetic anhydride (9 µL, 97 µmol) were added to a cooled (0ºC) solution of **48** in dry MeOH (1.5 mL). After stirring for 2 h at r.t., Et_3N (200 µL) was added and the mixture was concentrated to dryness. The residue was purified by Sephadex LH-20 chromatography (MeOH 100%) to give **49**. This compound was then dissolved in H_2O (2 mL) and Amberlite IR-120 H^+ resin was added (pH = 3.3). The mixture was filtered, treated with 0.04 M NaOH (pH = 7.5) and lyophilized. The white solid was finally eluted from a column of Dowex 50WX4-Na^+ (H_2O-MeOH 9:1) to obtain **49** as sodium salt (6 mg, 86%) after lyophilization. TLC (EtOAc:Py:H_2O:AcOH 6:5:3:1) Rf 0.17; ^{1}H-NMR (500 MHz, D_2O): δ 7.57-7.36 (m, 10H, Ar), 7.09 (d, 2H, Ar), 6.97 (d, 2H, Ar), 5.03 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1A), 4.96 (d, 1H, *J* = 10.5 Hz, CH$_2$(Bn)), 4.85 (d, 1H, $J_{3,4}$ = 2.5 Hz, H-4A), 4.84-4.77 (m, 6H, CH$_2$(Bn), H-5D(4.83), H-1D(4.80), H-4C(4.76)), 4.70 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1C), 4.55 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1B), 4.35-4.29 (m, 2H, H-2A, H-6A or C), 4.26-4.19 (m, 3H, H-5A or C, 2 x H-6A or C), 4.16 (dd, 1H, H-3A), 4.14-4.04 (m, 4H, H-6A or C, H-4D, H-2C, H-4B), 3.99 (m, 1H, H-5A or C), 3.95 (m, 1H, H-3C), 3.81 (s, 3H, Me(OMP)), 3.76 (d, 1H, $J_{4,5}$ = 9.5 Hz, H-5B), 3.67-3.53 (m, 4H, H-2D, H-3B, H-2B, H-3D), 2.04, 2.03 (2s, 6H, NHAc); ^{13}C-NMR (125 MHz, D_2O) (selected data from HSQC experiment): δ 130.1-116.1(Ar-CH), 105.3

(C-1D), 104.9 (C-1B), 101.6 (C-1A), 101.3 (C-1C), 82.9 (C-3D), 82.4 (C-3B), 78.4 (C-4B), 77.8 (C-5B), 77.6 (C-4C, C-3C), 76.9 (C-4A), 75.9 (C-3A), 74.7, 74.4 (CH_2(Bn)), 73.8-73.4 (C-5A, C-5C, C-4D), 72.6 (C-2B), 71.8 (C-2D), 68.9, 68.5 (C-6A, C-6C), 56.8 (Me(OMP)), 52.9, 52.6 (C-2A, C-2C), 23.6 (NAc); ESI MS: *m/z*: calcd for $C_{49}H_{58}N_2O_{36}S_4Na_3$: 1447.1; found: 1447.0 [*M*+3Na+2H]$^+$.

4-Methoxyphenyl *O*-(α-L-idopyranosyluronic acid)-(1→3)-*O*-(2-acetamido-2-deoxy-4,6-di-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyluronic acid)-(1→3)-2-acetamido-2-deoxy-4,6-di-*O*-sulfo-β-D-galactopyranoside (50): A solution of **49** (5.8 mg, 3.8 µmol, sodium salt) in H_2O/MeOH (4.5 mL/0.5 mL) was hydrogenated in the presence of $Pd(OH)_2$ (12 mg). After 24 h, the suspension was filtered over celite, concentrated, and lyophilized to give **50** as sodium salt (5.1 mg, quantitative). ^{1}H-NMR (500 MHz, D_2O): δ 7.09 (d, 2H, Ar), 6.98 (d, 2H, Ar), 5.02 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1A), 4.85 (d, 1H, $J_{3,4}$ = 2.6 Hz, H-4A), 4.83 (d, 1H, $J_{1,2}$ = 4.5 Hz, H-1D), 4.76 (d, 1H, H-4C), 4.67 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1C), 4.63 (d, 1H, $J_{4,5}$ = 3.6 Hz, H-5D), 4.53 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1B), 4.34-4.21 (m, 6H, H-2A (4.31), 2 x H-6A, 2 x H-6C, H-5A or C (4.23)), 4.17-4.15 (m, 2H, H-5A or C, H-3A), 4.09-4.02 (m, 2H, H-2C, H-3C), 3.93 (dd, 1H, H-4D), 3.81 (s, 3H, Me(OMP)), 3.78 (dd, 1H, H-4B), 3.69 (d, 1H, $J_{4,5}$ = 9.7 Hz, H-5B), 3.63-3.59 (m, 2H, H-3B, H-3D), 3.49 (dd, 1H, $J_{2,3}$ = 7.5 Hz, H-2D), 3.44 (dd, 1H, H-2B), 2.05, 2.03 (2s, 6H, NHAc); ^{13}C-NMR (125 MHz, D_2O) (selected data from HSQC experiment): δ 119.6-116.2 (Ar), 104.7 (C-1B), 104.1 (C-1D), 102.6 (C-1C), 101.8 (C-1A), 83.4 (C-4B), 77.8 (C-5B), 77.0 (C-4C, C-4A), 76.5 (C-3C), 76.0 (C-3A), 75.0 (C-3B or D), 73.7, 73.5 (C-5A, C-5C), 73.4 (C-3B or D), 73.1 (C-2B), 72.7 (C-4D), 72.3 (C-5D), 71.4 (C-2D), 68.9-68.8 (C-6A, C-6C), 56.9 (Me(OMP)), 52.7 (C-2A, C-2C), 23.5 (NAc); ESI MS: *m/z*: calcd for $C_{35}H_{46}N_2O_{36}S_4Na_3$: 1267.0; found: 1266.9 [*M*+3Na+2H]$^+$.

Fluorescence polarization assay

Fluorescence polarization measurements were performed in 384-well microplates (black polystyrene, non-treated, Corning) using a TRIAD multimode reader (Dynex). Fluorescent probe (a fluorescent heparin-like hexasaccharide) and inhibitors were dissolved in PBS buffer (10 mM, pH 7.4). Recombinant human FGF-2 (Peprotech) was dissolved in PBS buffer (10 mM, pH 7.4) containing 1% BSA. For inhibition assay, 10 µL of probe solution and 20 µL of protein at fixed concentration (40 nM and 145 nM, respectively) were mixed with 10 µL of inhibitor solution (100 µM). The total sample volume in each well was 40 µL. Therefore, all measurements were done in PBS + 0.5% BSA, and the final concentrations of inhibitor, fluorescent probe and FGF-2 in each well were 25 µM, 10 nM and 73 nM, respectively. After stirring for 5 min in the dark, fluorescence polarization was recorded. Two control wells were included in the study. The first one only contained fluorescent probe; the second one contains FGF-2 and probe, without inhibitor. Blank wells contained 20 µL of FGF-2 solution and 20 µL of PBS buffer and their measurements were substracted from all values. All samples were performed in replicates of three.

Supporting Information (see footnote on the first page of this article): Copies of the NMR spectra of new compounds.

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[1] K. Sugahara, T. Mikami, T. Uyama, S. Mizuguchi, K. Nomura, H. Kitagawa, *Curr. Opin. Struct. Biol.* **2003**, *13*, 612-620.

[2] K. Sugahara, T. Mikami, *Curr. Opin. Struct. Biol.* **2007**, *17*, 536-545.

[3] K. Singh, A. G. Gittis, P. Nguyen, D. C. Gowda, L. H. Miller, D. N. Garboczi, *Nat. Struct. Mol. Biol.* **2008**, *15*, 932-938.

[4] C. I. Gama, L. C. Hsieh-Wilson, *Curr. Opin. Chem. Biol.* **2005**, *9*, 609-619.

[5] S. S. Deepa, Y. Umehara, S. Higashiyama, N. Itoh, K. Sugahara, *J. Biol. Chem.* **2002**, *277*, 43707-43716.

[6] H. Kawashima, K. Atarashi, M. Hirose, J. Hirose, S. Yamada, K. Sugahara, M. Miyasaka, *J. Biol. Chem.***2002**, *277*, 12921-12930.

[7] C. I. Gama, S. E. Tully, N. Sotogaku, P. M. Clark, M. Rawat, N. Vaidehi, W. A. Goddard, A. Nishi, L. C. Hsieh-Wilson, *Nat. Chem. Biol.* **2006**, *2*, 467-473.

[8] J. G. Beeson, K. T. Andrews, M. Boyle, M. F. Duffy, E. K. Choong, T. J. Byrne, J. M. Chesson, A. M. Lawson, W. Chai, *J. Biol. Chem.* **2007**, *282*, 22426-22436.

[9] S. E. Tully, R. Mabon, C. I. Gama, S. M. Tsai, X. W. Liu, L. C. Hsieh-Wilson, *J. Am. Chem. Soc.* **2004**, *126*, 7736-7737.

[10] C. Lopin, J. C. Jacquinet, *Angew. Chem., Int. Ed.* **2006**, *45*, 2574-2578.

[11] M. Rawat, C. I. Gama, J. B. Matson, L. C. Hsieh-Wilson, *J. Am. Chem. Soc.* **2008**, *130*, 2959-2961.

[12] J. Tamura, Y. Nakada, K. Taniguchi, M. Yamane, *Carbohydr. Res.* **2008**, *343*, 39-47.

[13] A. Vibert, C. Lopin-Bon, J. C. Jacquinet, *Chem. –Eur. J.* **2009**, *15*, 9561-9578.

[14] J. C. Jacquinet, C. Lopin-Bon, A. Vibert, *Chem. –Eur. J.* **2009**, *15*, 9579-9595.

[15] A. Vibert, C. Lopin-Bon, J.C. Jacquinet, *Eur. J. Org. Chem.* **2011**, 4183-4204.

[16] G. Despras, C. Bernard, A. Perrot, L. Cattiaux, A. Prochiantz, H. Lortat-Jacob, J. M. Mallet, *Chem. –Eur. J.* **2013**, *19*, 530-539.

[17] J. Tamura, H. Tanaka, A. Nakamura, N. Takeda, *Tetrahedron Lett.* **2013**, *54*, 3940-3943.

[18] S. Eller, M. Collot, J. Yin, H. S. Hahm, P. H. Seeberger, *Angew. Chem., Int. Ed.* **2013**, *52*, 5858-5861.

[19] E. Bedini, C. De Castro, M. De Rosa, A. Di Nola, A. Iadonisi, O. F. Restaino, C. Schiraldi, M. Parrilli, *Angew. Chem., Int. Ed.* **2011**, *50*, 6160-6163.

[20] A. F. G. Bongat, A. V. Demchenko, *Carbohydr. Res.* **2007**, *342*, 374-406.

[21] a) E. Bedini, M. Parrilli, *Carbohydr. Res.* **2012**, *356*, 75-85; b) N. A. Karst, R. J. Linhardt, *Curr. Med. Chem.* **2003**, *10*, 1993-2031; c) A.

Vibert, J. C. Jacquinet, C. Lopin-Bon, *J. Carbohydr. Chem.* **2011**, *30*, 393-414.

[22] X. W. Lu, M. N. Kamat, L. J. Huang, X. F. Huang, *J. Org. Chem.* **2009**, *74*, 7608-7617.

[23] G. Macchione, J. L. de Paz, P. M. Nieto, *unpublished results*

[24] P. Chassagne, L. Raibaut, C. Guerreiro, L. A. Mulard, *Tetrahedron* **2013**, *69*, 10337-10350.

[25] A. Vibert, C. Lopin-Bon, J. C. Jacquinet, *Tetrahedron Lett.* **2010**, *51*, 1867-1869.

[26] H. Gold, S. Munneke, J. Dinkelaar, H. S. Overkleeft, J. M. F. G. Aerts, J. D. C. Codee, G. A. van der Marel, *Carbohydr. Res.* **2011**, *346*, 1467-1478.

[27] M. Guillemineau, F.-I. Auzanneau, *J. Org. Chem.* **2012**, *77*, 8864-8878.

[28] J. S. Debenham, R. Madsen, C. Roberts, B. Fraser-Reid, *J. Am. Chem. Soc.* **1995**, *117*, 3302-3303.

[29] J. C. Castro-Palomino, R. R. Schmidt, *Tetrahedron Lett.* **1995**, *36*, 5343-5346.

[30] M. L. Wolfrom, H. B. Bhat, *J. Org. Chem.* **1967**, *32*, 1821-1823.

[31] M. L. Wolfrom, P. J. Conigliaro, *Carbohydr. Res.* **1969**, *11*, 63-76.

[32] D. J. Silva, H. M. Wang, N. M. Allanson, R. K. Jain, M. J. Sofia, *J. Org. Chem.* **1999**, *64*, 5926-5929.

[33] L. Lay, L. Manzoni, R. R. Schmidt, *Carbohydr. Res.* **1998**, *310*, 157-171.

[34] J. S. Debenham, R. Rodebaugh, B. Fraser-Reid, *J. Org. Chem.* **1997**, *62*, 4591-4600.

[35] A. F. G. Bongat, M. N. Kamat, A. V. Demchenko, *J. Org. Chem.* **2007**, *72*, 1480-1483.

[36] H. Weiss, C. Unverzagt, *Angew. Chem., Int. Ed.* **2003**, *42*, 4261-4263.

[37] S. E. Tully, M. Rawat, L. C. Hsieh-Wilson, *J. Am. Chem. Soc.* **2006**, *128*, 7740-7741.

[38] J. Tamura, N. Tsutsumishita-Nakai, Y. Nakao, M. Kawano, S. Kato, N. Takeda, S. Nadanaka, H. Kitagawa, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1371-1374.

[39] T. M. Wrodnigg, I. Lundt, A. E. Stutz, *J. Carbohydr. Chem.* **2006**, *25*, 33-41.

[40] U. Ellervik, G. Magnusson, *J. Org. Chem.* **1998**, *63*, 9314-9322.

[41] M. Mar Kayser, J. L. de Paz, P. M. Nieto, *Eur. J. Org. Chem.* **2010**, 2138-2147.

[42] K. Ait-Mohand, C. Lopin-Bon, J. C. Jacquinet, *Carbohydr. Res.* **2012**, *353*, 33-48.

[43] S. Maza, M. Mar Kayser, G. Macchione, J. Lopez-Prados, J. Angulo, J. L. de Paz, P. M. Nieto, *Org. Biomol. Chem.* **2013**, *11*, 3510-3525.

[44] S. Maza, J. L. de Paz, P. M. Nieto, *Tetrahedron Lett.* **2011**, *52*, 441-443.

[45] T. Yamada, S. Kinjyo, J. Yoshida, S. Yamago, *Chem. Lett.* **2005**, *34*, 1556-1557.

[46] N. Khiar, R. Navas, I. Fernandez, *Tetrahedron Lett.* **2012**, *53*, 395-398.

[47] S. Maza, G. Macchione, R. Ojeda, J. Lopez-Prados, J. Angulo, J. L. de Paz, P. M. Nieto, *Org. Biomol. Chem.* **2012**, *10*, 2146-2163.

[48] X. F. Bao, S. Nishimura, T. Mikami, S. Yamada, N. Itoh, K. Sugahara, *J. Biol. Chem.* **2004**, *279*, 9765-9776.

[49] C. D. Nandini, T. Mikami, M. Ohta, N. Itoh, F. Akiyama-Nambu, K. Sugahara, *J. Biol. Chem.* **2004**, *279*, 50799-50809.

[50] S. Faham, R. E. Hileman, J. R. Fromm, R. J. Linhardt, D. C. Rees, *Science* **1996**, *271*, 1116-1120.

[51] L. Pellegrini, *Curr. Opin. Struct. Biol.* **2001**, *11*, 629-634.

[52] S. Cochran, C. P. Li, J. K. Fairweather, W. C. Kett, D. R. Coombe, V. Ferro, *J. Med. Chem.* **2003**, *46*, 4601-4608.

[53] K. D. Johnstone, T. Karoli, L. Liu, K. Dredge, E. Copeman, C. P. Li, K. Davis, E. Hammond, I. Bytheway, E. Kostewicz, F. C. K. Chiu, D. M. Shackleford, S. A. Charman, W. N. Charman, J. Harenberg, T. J. Gonda, V. Ferro, *J. Med. Chem.* **2010**, *53*, 1686-1699.

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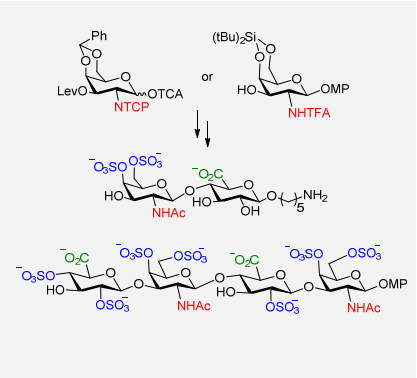
Entry for the Table of Contents

Layout 1:

Oligosaccharide Synthesis

We have explored the scope and limitations of *N*-tetrachlorophthaloyl (*N*-TCP) and *N*-trifluoroacetyl (*N*-TFA) galactosamine building blocks for the preparation of chondroitin sulfate oligosaccharides. These synthetic routes provided two di- and two tetrasaccharides with different sulfate distribution.



Giuseppe Macchione, Susana Maza, M. Mar Kayser, José L. de Paz,* Pedro M. Nieto* Page No. – Page No.

Synthesis of chondroitin sulfate oligosaccharides using *N*-tetrachlorophthaloyl and *N*-trifluoroacetyl galactosamine building blocks

Keywords: Glycosylation / Oligosaccharides / Protecting groups / Chondroitin sulfate / Carbohydrates