Synthesis of chondroitin/dermatan sulfate-like oligosaccharides and evaluation of their protein affinity by fluorescence polarization

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Abstract

Here, we present a novel approach for the chemical synthesis of chondroitin and dermatan sulfate oligosaccharides. A key point of this strategy is the preparation and use of an *N*-trifluoroacetyl galactosamine building block containing a 4,6-*O*-di-*tert*-butylsilylene group. Glycosylation reactions proceeded in good yields (74-91%) with our protecting group distribution. Using this approach, we have synthesized, for the first time, a chondroitin/dermatan sulfate-like tetrasaccharide that contains both types of uronic acids, D-glucuronic and L-iduronic acid. Moreover, we have employed a fluorescence polarization competition assay to evaluate the interactions between the synthesized oligosaccharides and FGF-2 (basic Fibroblast Growth Factor). Our results show that this method, using standard instrumentation and minimal sample consumption, is a powerful tool for the rapid analysis of the glycosaminoglycan affinity for proteins in solution.

Introduction

Chondroitin sulfate (CS) and dermatan sulfate (DS) are highly heterogeneous and sulfated, linear polysaccharides that belong to the glycosaminoglycan (GAG) family.[1, 2] CS is formed by the repetition of disaccharide units of D-glucuronic acid (GlcA) and *N*-acetyl-D-galactosamine (GalNAc), following the sequence GlcA-β(1→3)-GalNAc-β(1→4). The disaccharide repeating unit of the structurally related DS mainly contains L-iduronic acid (IdoA) in place of GlcA. Both polysaccharides may contain sulfate groups at different positions of the chain (Figure 1). These sulfate groups are introduced during the biosynthesis of these polymers, through the action of specific sulfotransferases, giving rise to GAG chains with a high level of structural diversity. The microheterogeneity of CS and DS can be considered as a capacity to encode information and control a wide variety of biological processes by specific interactions with certain proteins.[3] As in the case of others members of the GAG family, such as heparin and heparan sulfate,[4-8] it is proposed that defined CS and DS oligosaccharide sequences are responsible for specific protein recognition and subsequent activity.[3] However, little is known about the exact structural requirements for these interactions. In this context, synthetic CS and DS oligosaccharides[9-13] are useful tools for the establishment of structure-activity relationships and the preparation of mimetics that potentially modulate the biological functions of the natural products.[14-16] Interestingly, CS and DS are often found as co-polymeric structures.[17] These hybrid CS/DS chains, containing both types of uronic acids, GlcA and IdoA, are involved in growth factor signalling and neuronal growth and development.[17] The interaction between CS/DS and several chemokines and growth factors, including FGF-2 (basic Fibroblast Growth Factor), is mediated by oversulfated oligosaccharide sequences containing GlcA/IdoA-GalNAc (4,6-OSO$_3$).[18-21] In order to study these interactions at the molecular level, it would be very useful the access to well-defined synthetic CS/DS

oligosaccharides. However, to the best of our knowledge, the synthesis of a CS/DS oligomer, containing both GlcA and IdoA, has not yet been reported.

Despite significant advances in the field,[22-33] the synthesis of GAG oligosaccharides, including DS and CS, is still challenging, mainly due to the low reactivity of the building blocks required.[34-37] There is still a great demand for efficient synthetic strategies, involving a robust and reliable set of carbohydrate building blocks. This is an important point for the successful automation of oligosaccharide synthesis.[33, 38-40] Here, we present a novel approach for the synthesis of CS and DS oligomers that is based on the use of an *N*-trifluroacetyl-protected galactosamine building block. Glycosylation reactions proceeded in high yields using our design of protecting groups. The efficiency of this strategy is illustrated with the total synthesis of the oversulfated tetrasaccharide **1** (Scheme 1) that contains both IdoA and GlcA and bears sulfate groups at positions 4 and 6 of the GalNAc units, position 2 of the uronic acid moieties, and position 4 of the non-reducing terminus. All these positions may be sulfated in the natural products (Figure 1), except position 4 of the non-reducing end. Interestingly, the introduction of a "non-natural" sulfate group at the reducing end did not significantly affect the FGF-2 affinity of a synthetic heparin hexasaccharide[41] and an "artificial" IdoA monosaccharide, sulfated at positions 2 and 4, showed considerable binding to several proteins.[42, 43] Moreover, we have developed a fluorescence polarization assay to analyse the binding of tetrasaccharide **1** and its di-*O*-benzylated precursor to a model heparin-binding protein, FGF-2. Fluorescence polarization measurements allowed the study of GAG oligosaccharide-protein interactions in solution, with minimal time and sample consumption, and using a standard fluorescence reader. Therefore, our results indicate that this method can be considered as a powerful tool to evaluate the binding

affinities of synthetic oligosaccharides for receptors of biological relevance, helping to establish structure-activity relationships.

Results and discussion

Synthesis of chondroitin/dermatan sulfate-like oligosaccharides

For the synthesis of the CS/DS-related tetrasaccharide **1**, we first prepared the required monosaccharide building blocks **2**-**4** (Scheme 1). Glucuronic acid trichloroacetimidate **2** was prepared from known diol **5**,[44] as shown in Scheme 2. Selective oxidation at position 6 was performed by treatment of the diol **5** with calcium hypochlorite and catalytic TEMPO, under phase-transfer conditions.[44] Strict control of the reaction time and temperature, and quenching with Na_2SO_3 were required to avoid the chlorination of the electron-rich 4-methoxyphenyl ring.[45] The carboxylate intermediate was then esterified with BnBr and Bu_4NI in DMF at 60ºC to give the benzyl uronate **6**. Levulinoylation at position 4, followed by oxidative removal of the 4-methoxyphenyl group with CAN, and trichloroacetimidate formation, afforded glycosyl donor **2**. Starting from diol **9**,[46, 47] iduronic acid trichloroacetimidate **3** was obtained by levulinoylation, selective desilylation and anomeric activation with Cl_3CCN and K_2CO_3. Regarding the galactosamine unit, two types of building blocks, possessing different protections on the amino group, have been employed, up to date, for the synthesis of CS and DS oligosaccharides.[10] 2-Azido-2-deoxy-galactose derivatives[11, 12, 48-51] present some limitations for their general use due to the non-participating character of the azido moiety. On the contrary, 2-deoxy-2-trichloroacetamido-galactose building blocks lead to the stereoselective formation of the required 1,2-*trans* glycosidic bond. Impressive synthesis of CS oligomers have been reported using *N*-trichloroacetyl(TCA)-protected units.[3, 32, 52-55] However, this amino protecting group is associated with some problems. For example, we[56] and others[57] have detected the formation of stable trichlorooxazoline

side products during glycosidation of 2-deoxy-2-trichloroacetamido donors. Moreover, several difficulties are occasionally encountered in the final transformation to the desired 2-acetamido group. Thus, the deprotection of multiple *N*-TCA groups by basic hydrolysis, followed by selective *N*-acetylation, requires very long reaction times.[57] Alternatively, radical reduction using tributylstannane afford, in some cases, significant amounts of mono- and dichloroacetamide intermediates.[58, 59] For these reasons, we considered the use of an alternative amine-protecting group for the synthesis of CS/DS oligosaccharides. We chose an *N*-trifluoroacetyl (TFA) group because it can be easily removed under mild conditions while ensures high β selectivities in glycosylation reactions.[60]

Thus, we first planned the preparation of a *N*-TFA-protected galactosamine unit that should act as an efficient glycosyl acceptor in coupling reactions with uronic acid donors. The synthesis of such a compound was challenging (Scheme 3). Known tetraacetate **12**[61, 62] was synthesized from galactosamine hydrochloride in 66% yield by treatment with NaOMe and then with trifluoroacetic anhydride and Et$_3$N in MeOH, followed by extensive acetylation (Ac$_2$O, Py, DMAP). Compound **12** was transformed into 4-methoxyphenyl glycoside **15** by glycosylation with 4-methoxyphenol, followed by de-*O*-acetylation and benzylidenation. This compound was an ideal candidate for our synthetic approach because the 4,6-*O*-benzylidene acetal would allow the selective sulfation of these positions at the end of the synthesis to generate, among others, biologically relevant type E sulfation sequences.[3] However, **15** suffered from poor solubility in organic solvents such as dichloromethane, toluene and acetonitrile, and glycosylation attempts with uronic acid donor **2**[63] in THF or THF/CH$_2$Cl$_2$ mixtures did not give any desired disaccharide. We then decided to prepare compound **17** with a silyl ether group at the anomeric position. Selective cleavage of the anomeric acetyl group of

12 was followed by treatment with TDSCl to afford derivative **16**. Acetate hydrolysis and benzylidenation gave compound **17**. Surprisingly, this derivative proved to be unstable during silica gel chromatography. Therefore, **17** was not considered anymore as building block for our synthetic scheme. We then directed our attention to the 6-*O*-chloroacetylated building block **18**, efficiently synthetized from **16** in two steps. It was anticipated that diol **18** would be selectively glycosylated at position 3. Unfortunately, glycosylation reactions between diol **18** and uronic acid donor **2**[63] proceeded in low yield (<15%) and regioselectivity. Finally, we decided to prepare monosaccharide **4** containing a di-*tert*-butylsilylene group. It has been reported that this protecting group, compared to benzylidene acetals, confers desirable properties to glycosyl acceptors, such as better solubility in most organic solvents and higher stability under acidic glycosylation conditions.[58, 64] Thus, we treated monosaccharide **14** with di-*tert*-butylsilyl bistriflate in pyridine to obtain **4** in high yield. Gratifyingly, coupling of **4** with glucuronic acid trichloroacetimidate **2** gave the desired β (1→3) disaccharide **19** in excellent yield (Scheme 4). These results highlight the profound effect that protecting group distribution of the building blocks has on glycosylation reactions.[65] We also perfomed the glycosylation reaction between **4** and iduronic acid donor **3**. The target α (1→3) disaccharide **20** was also obtained in high yield. The small-to-zero coupling constants for IdoA protons indicated that this residue mainly exists in $^1C_4$ conformation, and the value of the $^1J_{C, H}$ (172 Hz) confirmed the α configuration of the new glycosidic linkage.[66]

Next, we studied the 2+2 assembly of the disaccharide units **19** and **20** to generate a tetrasaccharide sequence containing both GlcA and IdoA. The 4,6-di-*tert*-butylsilylene group[67] of galactose and galactosamine donors leads to the selective formation of α glycosides despite the presence of participating groups at position 2.[68] In some cases,

even 4,6-O-benzylidene derivatives of galactosamine donors may lead to loss of stereocontrol in coupling reactions with glucuronic acid-derived acceptors.[69] For these reasons, we decided to transform disaccharide **20** in a suitable protected donor containing less sterically hindered acetyl groups at positions 4 and 6 to obtain the desired 1,2-*trans* glycoside with excellent stereoselectivity. Cleavage of the silylene group gave diol **21**. The isolation of this compound was tricky because **21** formed gels in several solvents, such as toluene, CH_2Cl_2 and EtOAc. Therefore, diol **21** was directly acetylated, without further purification, to yield compound **22**. Removal of the 4-methoxyphenyl group followed by treatment with Cl_3CCN and catalytic DBU gave donor **24**. On the other hand, **19** was transformed into acceptor **25** by treatment with hydrazine monohydrate in a pyridine/acetic acid solution. Coupling of disaccharides **24** and **25** gave the target β tetrasaccharide **26** in high yield. This result demonstrates the utility of our synthetic route for the assembly of CS and DS oligosaccharides, paving the way for other sequences with different sulfation patterns.

Tetrasaccharide **26** was submitted to the deprotection/sulfation steps to obtain final compound **1**. Cleavage of the silylene group was performed by treatment with $(HF)_n \cdot Py$ complex. Hydrolysis of the acyl groups, benzyl and methyl esters, and trifluoroacetamides was carried out by treatment with lithium hydroperoxide and then NaOH to give compound **28**. The amine groups were selectively acetylated with Ac_2O in MeOH to provide intermediate **29**, which was purified by gel permeation chromatography. Then, extensive O-sulfation using $SO_3 \cdot Me_3N$ in DMF at 100 °C under microwave heating[41, 70] gave cleanly the corresponding hepta-O-sulfated tetrasaccharide **30**, which proved to be soluble in water. This compound was converted into the corresponding calcium salt for NMR characterization because the ^{1}H-NMR spectrum of the sodium salt showed considerable signal overlap. NMR spectra showed the

characteristic downfield shifts of the proton and carbon signals at positions bearing a sulfate group (see Table 1 and 2). COSY, HSQC and TOCSY NMR experiments were employed for the structural assignment. Additionally, mass spectroscopic analysis confirmed the structure of **30**. Finally, hydrogenolysis of **30** gave the fully deprotected tetrasaccharide **1** in good yield. The structure of **1** was confirmed by NMR and mass spectroscopic analysis. The values of ^{1}H and ^{13}C NMR chemical shifts for sulfated positions are in good agreement with those reported in the literature for similar GAG sulfated sequences.[41,53]

Fluorescence polarization measurements

Fluorescent polarization is a powerful tool for the study of biomolecular interactions in solution.[71] It is based on the observation that when a fluorescent molecule is excited with plane-polarized light, the remaining polarization of the emitted light depends on the rotational rate of the fluorescent molecule in solution that is inversely related to its molecular weight. Thus, the light emitted by a small fluorescent compound, which rotates quickly in solution, is highly depolarized and, therefore, the polarization value is low. If the fluorescent probe binds to a high molecular weight molecule, for example, a protein, the large complex rotates slower in solution, the emitted light remains polarized, and the polarization value is higher. Importantly, fluorescence polarization measurements do not require immobilization of protein or ligand to a surface for the analysis of the interaction. Thus, ligand's bound/free ratio can be directly measured in solution, avoiding potential misleading effects derived from the attachment of the biomolecules to solid supports. Moreover, this technique is adequate for high throughput screening, requires very little amount of samples, and is well suited for the binding analysis of small ligands, such as oligosaccharides, to a protein receptor. Despite these advantages, fluorescence polarization has had a limited use in the study of

carbohydrate-protein interactions,[72, 73] in part due to the lack of sensitive and appropiate instrumentation until the late 90's. Interestingly, competition assays can be easily designed to study the binding affinities of nonfluorescent ligands.[72] In fact, we employed a competition experiment to evaluate the affinity of synthetic tetrasaccharides **1** and **30** to FGF-2, as described below.

First, we prepared five different fluorescein-conjugated glycosaminoglycan oligosaccharides (**36-40**) to select an optimal probe for binding studies with FGF-2 (Scheme 7 and Supplementary information). Commercially available oligosaccharides **31-35**, derived from natural heparin by enzymatic depolimerization, were functionalized by reaction of the aldehyde group of the reducing end of the chain with a hydrazide-containing fluorescein molecule.[74] The corresponding glycosyl hydrazides were obtained in good yield, after purification by reverse phase C-18 chromatography. Then, fluorescent labelled sugars **36-40** were mixed with a fixed concentration of FGF-2, and polarization was measured in 384-well microplates using a standard fluorescence microplate reader (Figure 2). Control wells containing only the fluorescent probe, without any protein, were included in the experiment. As expected,[75, 76] heparin hexasaccharide **38** and tetrasaccharide **37** bound to FGF-2 since a significant increased polarization value was observed in FGF-2 containing wells. No interaction was detected for heparin disaccharide **36** and hyaluronic acid oligosaccharides **39** and **40**, ruling out any binding of FGF-2 to the fluorescein tag. Hexa **38**, which gave best binding, was chosen as optimal probe for inhibition experiments (see below). Importantly, the use of 384-well plates allowed the minimization of the sample quantities required for these experiments: the standard assay was performed with 10 nM fluorescent probe and approximately 100 nM protein in a final volume of 40 µL per well.

Next, the binding of **38** to FGF-2 was measured with increasing concentrations of protein (see Supplementary information, Figure S1), giving the corresponding binding curve that was analyzed as a Langmuir isotherm to determine the dissociation constant (K_D). The obtained value (117 ± 10 nM) was consistent with a previous measurement, in solution, of the binding affinity between a similar heparin hexasaccharide and FGF-2.[75]

A competition binding assay was then optimized to analyze the binding affinites of non-fluorescent ligands, such as tetrasaccharides **1** and **30**, to FGF-2. Thus, the polarization of samples containing fixed concentrations of protein and fluorescent probe were recorded in the presence of a certain concentration of potential competitors (Figure 3). In these experiments, we chose an FGF-2 concentration close to the K_D of the interaction with **38** in order to get a high enough polarization value while still using the minimal amount of inhibitor. Besides **1** and **30**, synthetic oligosaccharides **41**-**46**[41, 77] were included in the screening (Figure 4). The displacement of fluorescent **38** by an active competitor resulted in a decrease of the polarization value (Figures 3 and 4). In this way, the inhibitory capacity of non labelled compounds could be easily and quickly screened. As shown in Figure 4, at 25 µM concentration, monosaccharide **41** and disaccharides **42** and **46** did not significantly affect the interaction between fluorescent **38** and FGF-2, while hexasaccharides **44** and **45** strongly inhibited the binding. Interestingly, the presence of 25 µM of **1** and **30** gave 63-67 % inhibition. A similar effect was observed with tetrasaccharide **43**. These results indicated that CS/DS-related tetrasaccharides **1** and **30** are able to interact with FGF-2 and that their relative inhibitory potencies are similar to the one displayed by a heparin tetrasaccharide. After demonstrating that this fluorescence polarization assay can be used for the rapid screening of a library of compounds, we studied the inhibitory potency of oversulfated **1**

in more detail. We measured the polarization of samples containing FGF-2, fluorescent **38** and increasing concentrations of tetrasaccharide **1** (Figure 5). The obtained curve was fitted to the equation for a simple one-site competitive interaction. An IC_{50} value of 15 µM was estimated for compound **1**. In terms of screening for inhibitors, our fluorescence polarization competition assay is advantageous over methods that require the immobilization of the ligand or the protein on a solid support and are based on the inhibition of the interaction that occurs at the surface, because this surface interaction can be affected by multivalency, involves unknown amounts of one of the biomolecules and requires additional washing and incubation steps.

The binding of FGF-2 to cell surface heparan sulfate GAGs is essential for tumor angiogenesis and growth. Inhibition of angiogenesis is a well established and important anti-cancer strategy and, therefore, there is a great interest on compounds that potentially inhibit the FGF-2/GAG interaction.[78] Our data indicate that CS/DS tetrasaccharides **1** and **30** display considerable inhibitory activity and can be considered as starting points for the design and synthesis of more active compounds. Moreover, recent studies[78, 79] indicate that the introduction of lipophilic groups on the structure of synthetic antiangiogenic molecules improved the properties of these anticancer agents, and, in this context, the activity showed by the di-*O*-benzylated **30** is particularly remarkable.

Conclusions

We have prepared a *N*-TFA-protected galactosamine unit as key building block for the synthesis of CS and DS oligosaccharides. While the participating *N*-TFA group ensures the desired 1,2-*trans* stereochemistry of the glycosidic bond, the temporary introduction of a di-*tert*-butylsilylene group at positions 4 and 6 transforms this moiety in an excellent glycosyl acceptor for coupling reactions with uronic acid donors. Our results

provide an additional illustration of the profound impact that protecting groups have on the success of a glycosylation reaction. Moreover, our protecting group design is compatible with the deprotection/sulfation steps required for the preparation of final CS and DS sequences. Following this strategy, oversulfated CS/DS-like tetrasaccharide **1** was successfully synthesized. Importantly, our approach can be easily applied to the synthesis of other CS and DS oligosaccharides, bearing different sulfate patterns.

On the other hand, we have developed a fluorescence polarization assay for the rapid screening of the interactions between the synthesized oligosaccharides and proteins. The binding is analysed in solution, avoiding the potential artefacts and the additional washing steps that are typically associated to assays where the receptor or the ligand is immobilized on a solid surface. The only requirement to perform these experiments is the preparation of an adequate fluorescent probe. With this probe at hand, we could evaluate the relative binding affinities of a small library of non-fluorescent synthetic GAG oligosaccharides, including synthesized CS/DS tetrasaccharides **1** and **30**, to a model heparin-binding protein (FGF-2), by using a competition experiment. Our results show that this method is an excellent platform for the fast screening of GAG-protein interactions. It requires very little sample (nmol/pmol per well) and can be useful for the determination of structure-activity relationships of synthetic GAG sequences, contributing to the understanding of the role of these polysaccharides in various biological processes.

Experimental

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 the Mass Spectrometry Service, CITIUS, Universidad de Sevilla. HR MS (electrospray) of compounds **30** and **1** were obtained with a Thermo LTQ Orbitrap Velos spectrometer at CCiT, Universitat de Barcelona. Microwave-based sulfation reactions were performed using a Biotage Initiator Eight synthesizer in sealed reaction vessels.

Benzyl (4-methoxyphenyl 2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranoside) uronate (6): TEMPO (2 mL of a 0.016 M solution in CH_2Cl_2), Bu$_4$NBr (2 mL of a 0.08 M solution in CH_2Cl_2) and KBr (0.65 mL of a 0.5 M solution in H_2O) were added dropwise at 0ºC to a solution of diol **5** (1.6 g, 3.33 mmol) in CH_2Cl_2 (33 mL). A solution of Ca(ClO)$_2$ (1.2 g, 8.3 mmol) and NaHCO$_3$ (1.2 g, 14.3 mmol) in H_2O (31 mL) was then added dropwise at 0ºC. After stirring for 1 h at 0ºC, the reaction was quenched by adding Na$_2$SO$_3$ (25 mL of a 0.8 M solution in H_2O). After stirring for 15 min at 0ºC, the reaction mixture was diluted with additional CH_2Cl_2 and H_2O, and the organic layer was then separated, washed with a solution of Na$_2$SO$_3$ (0.8 M) and brine, dried (MgSO$_4$), filtered, and concentrated. The residue was dissolved in DMF (45 mL) and benzyl bromide (0.8 mL, 6.7 mmol) and Bu$_4$NI (0.6 g, 1.7 mmol) were added. The

mixture was stirred for 3 h at 60 ºC, diluted with EtOAc, washed with H_2O, dried

($MgSO_4$), filtered, and concentrated. Flash chromatography (toluene/EtOAc, 13:1) gave

6 (1.1 g, 56%). TLC (6:1 toluene/EtOAc) R_f 0.41; $[\alpha]_D^{20}$ −3.4º (*c* 1.0, CH_2Cl_2); ^{1}H-NMR

(300 MHz, $CDCl_3$): δ 7.94 (m, 2H, Ar), 7.50 (m, 1H, Ar), 7.36 (m, 2H, Ar), 7.28 (m,

5H, Ar), 7.09 (m, 5H, Ar), 6.83 (m, 2H, Ar), 6.62 (m, 2H, Ar), 5.42 (dd, 1H, H-2), 5.17

(2d, 2H, CH_2(Bn)), 4.93 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1), 4.70 (2d, 2H, CH_2(Bn)), 4.12 (dd,

1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 3.96 (d, 1H, H-5), 3.71 (dd, 1H, $J_{2,3}$ = 9.2 Hz, H-3), 3.63

(s, 3H, Me(OMP)); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 168.9, 165.5 (CO(COOBn, Bz)),

155.8, 151.3, 138.1, 135.3 (Ar-C), 133.5 (Ar-CH), 130.0, 129.8, 128.8, 128.7, 128.6,

128.4, 128.2, 127.8, (Ar-C, Ar-CH), 119.3, 114.6 (Ar-CH), 101.5 (C-1), 81.0 (C-3),

74.8, 74.7 (CH_2(Bn), C-5), 73.1 (C-2), 72.0 (C-4), 67.5 (CH_2(Bn)), 55.6 (Me(OMP));

HR MS: m/z: calcd for $C_{34}H_{32}O_9Na$: 607.1944; found: 607.1946 $[M+Na]^+$.

Benzyl (4-methoxyphenyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-β-D-glucopyranoside) uronate (7): Compound **6** (2.1 g, 3.6 mmol), 1,3-

dicyclohexylcarbodiimide (1.11 g, 5.39 mmol), DMAP (44 mg, 0.36 mmol) and

levulinic acid (1.83 mL, 18.0 mmol) were dissolved in CH_2Cl_2 (25 mL). After stirring

for 3 h, the mixture was diluted with CH_2Cl_2 and washed with saturated aqueous

$NaHCO_3$. The organic phase was dried with $MgSO_4$, filtered, and concentrated in

vacuo. The residue was purified by flash cromatography on silica gel (2:1

hexane/EtOAc) to give **7** as a white solid (1.98 g, 81%). TLC (2:1 hexane/EtOAc) R_f

0.28; $[\alpha]_D^{20}$ +8.4º (*c* 1.0, CH_2Cl_2); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.94 (m, 2H, Ar),

7.52 (m, 1H, Ar), 7.38 (m, 2H, Ar), 7.28 (m, 5H, Ar), 7.09 (m, 5H, Ar), 6.83 (m, 2H,

Ar), 6.65 (m, 2H, Ar), 5.49 (dd, 1H, $J_{1,2}$ = 7.2 Hz, $J_{2,3}$ = 8.9 Hz, H-2), 5.40 (dd, 1H, $J_{3,4}$

= 9.0 Hz, $J_{4,5}$ = 9.6 Hz, H-4), 5.08 (s, 2H, CH_2(Bn)), 4.99 (d, 1H, H-1), 4.58 (2d, 2H,

CH_2(Bn)), 4.08 (d, 1H, H-5), 3.88 (dd, 1H, $J_{2,3}$ = $J_{3,4}$ = 8.9 Hz, H-3), 3.66 (s, 3H,

Me(OMP)), 2.60-2.17 (m, 4H, CH$_2$(Lev)), 2.05 (s, 3H, CH$_3$(Lev)); ^{13}C-NMR (75 MHz, CDCl$_3$): δ 205.9 (CO(Lev)), 171.2, 166.9, 165.0 (CO (COOBn, Bz, Lev)), 155.9, 151.1, 137.4, 135.1 (Ar-C), 133.4 (Ar-CH), 129.8, 129.5, 128.6, 128.5, 128.3, 128.0, 127.7, (Ar-C, Ar-CH), 119.3, 114.5 (Ar-CH), 101.0 (C-1), 78.9 (C-3), 73.9 (CH$_2$(Bn)), 72.9 (C-5), 72.8 (C-2), 71.0 (C-4), 67.7 (CH$_2$(Bn)), 55.6 (Me(OMP)), 37.6 (CH$_2$(Lev)), 29.7 (CH$_3$(Lev)), 27.7 (CH$_2$(Lev)); HR MS: m/z: calcd for C$_{39}$H$_{38}$O$_{11}$Na: 705.2312; found: 705.2316 [M+Na]$^+$.

Benzyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-α,β-D-glucopyranosuronate (8):
CAN (8.1 g, 14 mmol) was added to a solution of the glycoside **7** (1.9 g, 2.8 mmol) in toluene–acetonitrile–water (1:6:1, 75 mL) and the mixture was stirred for 1.5 h at r.t. The mixture was then diluted with EtOAc, washed with H$_2$O and NaHCO$_3$, dried (MgSO$_4$), filtered and concentrated under reduced pressure. Flash chromatography on silica gel (toluene/EtOAc 1:0 → 3:1) afforded the corresponding hemiacetal **8** (960 mg, 60%) as a mixture of α/β anomers (9:1). TLC (2:1 toluene/EtOAc) R$_f$ 0.47; ^{1}H-NMR (300 MHz, CDCl$_3$) (for α anomer): δ 8.03 (m, 2H, Ar), 7.58 (m, 1H, Ar), 7.43 (m, 2H, Ar), 7.34 (m, 5H, Ar), 7.21 (m, 5H, Ar), 5.68 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1), 5.29 (dd, 1H, $J_{3,4}$ = $J_{4,5}$ = 8.9 Hz, H-4), 5.16 (dd, 1H, H-2), 5.06 (2d, 2H, CH$_2$(Bn)), 4.70 (2d, 2H, CH$_2$(Bn)), 4.61 (d, 1H, $J_{4,5}$ = 8.9 Hz, H-5), 4.23 (dd, 1H, $J_{2,3}$ = $J_{3,4}$ = 8.9 Hz, H-3), 2.58-2.21 (m, 4H, CH$_2$(Lev)), 2.09 (s, 3H, CH$_3$(Lev)); ^{13}C-NMR (75 MHz, CDCl$_3$) (for α anomer): δ 206.4 (CO(Lev)), 171.4, 168.5, 165.7 (CO (COOBn, Bz, Lev)), 137.9-127.7 (Ar), 90.3 (C-1), 76.1(C-3), 74.8 (CH$_2$(Bn)), 72.9 (C-2), 71.0 (C-4), 68.9 (C-5), 68.0 (CH$_2$(Bn)), 37.6 (CH$_2$(Lev)), 29.7 (CH$_3$(Lev)), 27.7 (CH$_2$(Lev)); ^{1}H-NMR (300 MHz, CDCl$_3$) (selected data for β anomer): δ 7.99 (m, 2H, Ar), 7.58 (m, 1H, Ar), 7.43 (m, 2H, Ar), 7.26 (m, 5H, Ar), 7.18 (m, 5H, Ar), 5.37 (dd, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.0 Hz, H-4), 5.20 (dd, 1H, H-2), 4.86 (d, 1H, $J_{1,2}$ = 6.9 Hz, H-1), 4.15 (d, 1H, $J_{4,5}$ = 9.2 Hz, H-5),

3.95 (dd, 1H, $J_{2,3} = J_{3,4}$ = 8.3 Hz, H-3), 2.58-2.21 (m, 4H, CH_2(Lev)), 2.09 (s, 3H, CH_3(Lev)); ^{13}C-NMR (75 MHz, CDCl$_3$) (selected data for β anomer from HMQC experiment): δ 96.3 (C-1), 77.6 (C-3), 72.7 (C-5), 70.8 (C-4); HR MS: *m/z*: calcd for $C_{32}H_{32}O_{10}$Na: 599.1893; found: 599.1911 [*M*+Na]$^+$.

***O*-(Benzyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-α,β-D-glucopyranosyluronate) trichloroacetimidate (2):** Trichloroacetonitrile (0.83 mL, 8.3 mmol) and K$_2$CO$_3$ (126 mg, 0.91 mmol) were added to **8** (480 mg, 0.83 mmol) in dry CH$_2$Cl$_2$ (4.8 mL). After stirring at room temperature for 4 h, the mixture was filtered off and concentrated in vacuo to obtain **2** (574 mg, 96%). TLC (2:1 toluene/EtOAc) R$_f$ 0.55; ^{1}H-NMR (300 MHz, CDCl$_3$): (for α anomer): δ 8.59 (s, 1H, NH), 7.95 (m, 2H, Ar), 7.58 (m, 1H, Ar), 7.43 (m, 2H, Ar), 7.37 (m, 5H, Ar), 7.20 (m, 5H, Ar), 6.74 (d, 1H, $J_{1,2}$ = 3.3 Hz, H-1), 5.44 (dd, 1H, H-2), 5.38 (dd, 1H, H-4), 5.15 (2d, 2H, CH_2(Bn)), 4.72 (2d, 2H, CH_2(Bn)), 4.52 (d, 1H, $J_{4,5}$ = 10.1 Hz, H-5), 4.29 (dd, 1H, $J_{2,3} = J_{3,4}$ = 9.7 Hz, H-3), 2.66-2.20 (m, 4H, CH_2(Lev)), 2.12 (s, 3H, CH_3(Lev)); ^{13}C-NMR (75 MHz, CDCl$_3$) (for α anomer): δ 206.4 (CO(Lev)), 171.4, 168.5, 165.7 (CO(COOBn, Bz, Lev)), 160.0 (C=NH), 137.4-127.7 (Ar), 93.1 (C-1), 90.8 (CCl$_3$), 75.7 (C-3), 74.9 (CH_2(Bn)), 71.3, 71.2, 70.9 (C-2, C-5, C-4), 67.9 (CH_2(Bn)), 37.6 (CH_2(Lev)), 29.7 (CH_3(Lev)), 27.7 (CH_2(Lev)); HR MS: *m/z*: calcd for $C_{34}H_{32}Cl_3NO_{10}$Na: 742.0989; found: 742.0984 [*M*+Na]$^+$.

Methyl (dimethylthexylsilyl 3-*O*-benzyl-2,4-di-*O*-levulinoyl-β-L-idopyranoside) uronate (10): Lev$_2$O preparation: LevOH (4.7 mL, 45.4 mmol) was added at 0ºC to a solution of 1,3-dicyclohexylcarbodiimide (4.68 g, 22.7 mmol) in CH$_2$Cl$_2$ (38 mL). After stirring for 5 min at room temperature, the mixture was cooled and filtered to give a solution of Lev$_2$O in CH$_2$Cl$_2$.

Lev$_2$O (15.0 mL of a 0.76 M solution in CH$_2$Cl$_2$) was added at room temperature to a mixture of **9** (1.0 g, 2.3 mmol) and DMAP (41 mg, 0.34 mmol) in dry Py (40 mL). The mixture was stirred for 22 h, diluted with CH$_2$Cl$_2$, and washed with 1 M HCl, saturated aqueous NaHCO$_3$, and H$_2$O. The organic phase was dried (MgSO$_4$), filtered and concentrated to dryness. The residue was purified by column chromatography (hexane/EtOAc 2:1) to afford **10** (1.55 g, 98%). TLC (hexane/EtOAc 1:1) R$_f$ 0.26; $[\alpha]_D^{20}$ +19° (*c* 1.0, CHCl$_3$); ^{1}H-NMR (300 MHz, CDCl$_3$): δ 7.34 (m, 5H, Ar), 5.16 (m, 1H, H-4), 5.08 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1), 4.93 (m, 1H, H-2), 4.76, 4.69 (2d, 2H, CH$_2$(Bn)), 4.59 (d, 1H, $J_{4,5}$ = 2.1 Hz, H-5), 3.86 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 2.7 Hz, H-3), 3.78 (s, 3H, COOMe), 2.92-2.50 (m, 8H, CH$_2$(Lev)), 2.18 (s, 6H, CH$_3$(Lev)), 1.61 (hp, 1H, C*H*(CH$_3$)$_2$), 0.87-0.83 (12H, CH(C*H$_3$*)$_2$, C(CH$_3$)$_2$), 0.23, 0.14 (2s, 6H, Si(CH$_3$)$_2$); ^{13}C-NMR (75 MHz, CDCl$_3$): δ 206.5, 206.3 (CO(Lev)), 172.3, 172.1 (CO(Lev)), 167.9 (COOMe), 137.3 (Ar-C), 128.6, 128.2, 127.8 (Ar-CH), 93.1 (C-1), 74.4 (C-3), 73.1 (CH$_2$(Bn)), 72.7 (C-5), 68.0 (C-2), 67.2 (C-4), 52.4 (COOMe), 38.0, 37.8 (CH$_2$(Lev)), 34.1 (*C*H(CH$_3$)$_2$), 30.0, 29.9 (CH$_3$(Lev)), 28.1 (CH$_2$(Lev)), 25.0 (*C*(CH$_3$)$_2$), 20.3, 20.0, 18.7, 18.5 (CH(*C*H$_3$)$_2$, C(*C*H$_3$)$_2$), -1.8, -3.5 (Si(CH$_3$)$_2$); HR MS: *m/z*: calcd for C$_{32}$H$_{48}$O$_{11}$SiNa: 659.2864; found: 659.2847 [*M*+Na]$^+$.

Methyl 3-*O*-benzyl-2,4-di-*O*-levulinoyl-α,β-L-idopyranosuronate (11): An excess of (HF)$_n$·Py (7.2 mL) was added at −10°C under an argon atmosphere to a solution of **10** (1.63 g, 2.56 mmol) in dry THF (37 mL). After 19 h at 0°C the mixture was diluted with CH$_2$Cl$_2$ and washed with H$_2$O and saturated NaHCO$_3$ solution until neutral pH. The organic layers were dried (MgSO$_4$), filtered and concentrated in vacuo. The residue was purified by column chromatography (toluene/EtOAc 1:3) to afford **11** as a mixture of α/β anomers (1:1) (970 mg, 77%). TLC (hexane/EtOAc 1:2) R$_f$ 0.13; ^{1}H-NMR (300 MHz, CDCl$_3$): δ 7.34 (m, 10H, Ar, Ar'), 5.30 (m, 1H, H-1), 5.26 (m, 1H, H-4), 5.16 (m,

1H, H-4'), 5.14 (bs, 1H, H-1'), 4.98 (d, 1H, $J_{4,5}$ = 2.3 Hz, H-5), 4.95 (m, 1H, H-2'), 4.85 (m, 1H, H-2), 4.78, 4.74 (2m, 4H, CH₂(Bn), CH₂(Bn)'), 4.69 (d, 1H, $J_{4,5}$ = 2.0 Hz, H-5'), 4.18 (bd, 1H, OH), 3.96 (bt, 2H, H-3, H-3'), 3.81, 3.80 (2s, 6H, COOMe, COOMe'), 2.91-2.45 (m, 16H, CH₂(Lev), CH₂(Lev)'), 2.19-2-17 (3s, 12H, CH₃(Lev), CH₃(Lev)'); ¹³C-NMR (75 MHz, CDCl₃): δ 207.3, 206.5, 206.3 (CO(Lev), CO(Lev)'), 172.4, 171.9, 171.8 (CO(Lev), CO(Lev)'), 168.8, 168.1 (COOMe, COOMe'), 137.0, 136.6 (Ar-C), 129.1, 128.7, 128.6, 128.4, 128.3, 128.2, 128.0, 127.8 (Ar-C, Ar-CH), 93.0 (C-1), 92.1 (C-1'), 73.5, 73.4 (CH₂(Bn), CH₂(Bn)'), 73.1, 72.7, 72.3 (C-3, C-3', C-5'), 68.0 (C-2'), 67.1 (C-4, C-4'), 66.7 (C-2), 65.6 (C-5), 52.6 (COOMe, COOMe'), 38.1, 37.8, 37.7 (CH₂(Lev), CH₂(Lev)'), 29.8 (CH₃(Lev), CH₃(Lev)'), 28.0, 27.9, 27.8 (CH₂(Lev), CH₂(Lev)'); HR MS: *m/z*: calcd for $C_{24}H_{30}O_{11}Na$: 517.1686; found: 517.1666 [*M*+Na]⁺.

O-(Methyl 3-O-benzyl-2,4-di-O-levulinoyl-α,β-L-idopyranosyluronate) trichloroacetimidate (3): K₂CO₃ (207 mg, 1.67 mmol) and trichloroacetonitrile (3.0 mL, 30 mmol) were added at room temperature to a solution of **11** (750 mg, 1.52 mmol) in dry CH₂Cl₂ (15 mL). After stirring for 11 h at room temperature, the reaction mixture was filtered through a pad of Celite and concentrated to dryness to obtain **3** as a mixture of α/β anomers (920 mg, 95%). TLC (toluene/EtOAc 1:4) Rf 0.44, 0.58 (α and β anomers); ¹H-NMR (300 MHz, CDCl₃) (data for major anomer): δ 8.68 (s, 1H, NH), 7.34 (m, 5H, Ar), 6.23 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 5.27 (m, 1H, H-2), 5.22 (m, 1H, H-4), 4.80 (d, 1H, $J_{4,5}$ = 2.0 Hz, H-5), 4.80-4.68 (m, 2H, CH₂(Bn)), 3.99 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 3.0 Hz, H-3), 3.78 (s, 3H, COOMe), 2.90-2.49 (m, 8H, CH₂(Lev)), 2.16 (s, 6H, CH₃(Lev)); ¹³C-NMR (75 MHz, CDCl₃) (data for major anomer): δ 206.4, 206.3 (CO(Lev)), 172.1, 172.0 (CO(Lev)), 167.2 (COOMe), 160.4 (C=NH), 136.9 (Ar-C), 128.6, 128.3, 127.9 (Ar-CH), 94.4 (C-1), 90.8 (CCl₃), 73.6 (C-3), 73.4 (CH₂(Bn)), 73.2 (C-5), 67.3 (C-4),

65.8 (C-2), 52.7 (COOMe), 37.8, 37.7 (CH$_2$(Lev)), 29.9, 29.8 (CH$_3$(Lev)), 28.0, 27.9 (CH$_2$(Lev)); ^{1}H-NMR (300 MHz, CDCl$_3$) (data for minor anomer): δ 8.69 (s, 1H, NH), 7.34 (m, 5H, Ar), 6.40 (bs, 1H, H-1), 5.27 (m, 1H, H-4), 5.11 (m, 1H, H-2), 5.04 (d, 1H, $J_{4,5}$ = 1.8 Hz, H-5), 4.80-4.68 (m, 2H, CH$_2$(Bn)), 3.87 (m, 1H, H-3), 3.78 (s, 3H, COOMe), 2.90-2.49 (m, 8H, CH$_2$(Lev)), 2.17 (s, 6H, CH$_3$(Lev)); ^{13}C-NMR (75 MHz, CDCl$_3$) (data for minor anomer): δ 206.4, 206.3 (CO(Lev)), 171.8, 171.7 (CO(Lev)), 168.1 (COOMe), 160.1 (C=NH), 137.2 (Ar-C), 128.4, 127.9, 127.7 (Ar-CH), 95.0 (C-1), 90.5 (CCl$_3$), 72.6 (CH$_2$(Bn)), 71.5 (C-3), 67.8 (C-5), 67.3 (C-4), 65.2 (C-2), 52.7 (COOMe), 37.9, 37.8 (CH$_2$(Lev)), 29.9, 29.8 (CH$_3$(Lev)), 28.0, 27.9 (CH$_2$(Lev)); HR MS: m/z: calcd for C$_{26}$H$_{30}$Cl$_3$NO$_{11}$Na: 660.0782; found: 660.0782 [M+Na]$^+$.

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-trifluoroacetamido-α,β-D-galactopyranose (12)[61, 62]: Galactosamine hydrochloride (21 g, 93.4 mmol) was suspended in MeOH (250 mL). NaOMe (105 mL, 1.3 M solution in MeOH) was added at room temperature. After stirring for 30 min, TFA anhydride (14.2 mL, 98.1 mmol) was added at 0ºC. After stirring for 10 min, Et$_3$N (13.6 mL, 94.0 mmol) was added. After stirring for 28 h at room temperature, the reaction mixture was concentrated in vacuo. The residue was dissolved in Py (420 ml) and DMAP (6.4 g, 46.7 mmol) and acetic anhydride (105.8 mL, 1.1 mol) were added at 0 ºC. The reaction was stirred for 72 h at room temperature, diluted with EtOAc, washed with H$_2$O, 1 M HCl and NaHCO$_3$, dried (MgSO$_4$), filtered, and concentrated. Flash chromatography (hexane/EtOAc, 3:1) gave **12** (28.4 g, 66%) as a mixture of α/β anomers (1:0.8). TLC (2:1 hexane/EtOAc) R$_f$ 0.27; ^{1}H-NMR (300 MHz, CDCl$_3$) (data for α anomer): δ 6.55 (bs, 1H, NH), 6.28 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 5.46 (dd, 1H, H-4), 5.29 (dd, 1H, $J_{2,3}$ = 11.4 Hz, $J_{3,4}$ = 3.5 Hz, H-3), 4.68 (m, 1H, H-2), 4.28-4.03 (m, 3H, H-5, H-6a, H-6b), 2.18-2.00 (m, 12H, CH$_3$); ^{1}H-NMR (300 MHz, CDCl$_3$) (data for β anomer): δ 7.02 (bs, 1H, NH), 5.77 (d, 1H, $J_{1,2}$ = 8.7 Hz, H-

1), 5.40 (dd, 1H, H-4), 5.18 (dd, 1H, $J_{2,3}$ = 11.3 Hz, $J_{3,4}$ = 3.3 Hz, H-3), 4.49 (m, 1H, H-2), 4.28-4.03 (m, 3H, H-5, H-6a, H-6b), 2.18-2.00 (m, 12H, CH$_3$); ESI MS: *m/z*: calcd for $C_{16}H_{20}F_3NO_{10}Na$: 466.1; found: 466.1 [*M*+Na]$^+$.

4-Methoxyphenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoroacetamido-β-D-galactopyranoside (13): TMSOTf (320 µL, 1.7 mmol) was added to a cooled (0ºC) solution of **12** (5.1 g, 11.5 mmol) and 4-methoxyphenol (2.6 g, 20.7 mmol) in dry CH$_2$Cl$_2$ (51 mL). The mixture was stirred for 1 h at 0ºC and TEA (1 mL) was then added. The mixture was diluted with EtOAc and washed with H$_2$O, saturated aqueous NaHCO$_3$ and H$_2$O. The organic phase was dried (MgSO$_4$), filtered, and concentrated. The residue was purified by silica gel chromatography (toluene/EtOAc 4:1 → 1:1) to give **13** (2.4 g, 42%) and starting material (α anomer, 2.4 g, 40%). TLC (4:1 toluene/EtOAc) R$_f$ 0.17; [α]$_D^{20}$ − 3.5 ° (*c* 1.0, CHCl$_3$); ^{1}H-NMR (300 MHz, CDCl$_3$): δ 6.98 (d, 1H, $J_{2,NH}$ = 9.1 Hz, NH), 6.92 (m, 2H, Ar), 6.78 (m, 2H, Ar), 5.41 (d, 1H, $J_{3,4}$ = 2.9 Hz, H-4), 5.31 (dd, 1H, $J_{2,3}$ = 11.3 Hz, H-3), 5.04 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1), 4.40 (m, 1H, H-2), 4.26-4.12 (2dd, 2H, $J_{6a,6b}$ = 11.5 Hz, H-6a, H-6b), 4.03 (br dd, 1H, $J_{5,6a}$ = $J_{5,6b}$ = 6.5 Hz, H-5), 3.76 (s, 3H, Me(OMP)), 2.18-2.00 (3s, 9H, CH$_3$(Ac)); ^{13}C-NMR (75 MHz, CDCl$_3$): δ 170.7, 170.6, 170.3 (COCH$_3$), 158.0 (q, $^2J_{C,F}$ = 34.1 Hz, *C*OCF$_3$), 155.9, 151.0 (Ar-C), 118.8, 114.7 (Ar-CH), 115.6 (q, $^1J_{C,F}$ = 287.0 Hz, COCF$_3$), 100.4 (C-1), 71.1 (C-5), 69.5 (C-3), 66.4 (C-4), 61.6 (C-6), 55.7 (Me(OMP)), 51.8 (C-2), 20.5 (CH$_3$(Ac)) ; HR MS: *m/z*: calcd for $C_{21}H_{24}NO_{10}F_3Na$: 530.1250 ; found: 530.1268 [*M*+Na]$^+$.

4-Methoxyphenyl 3,4,6-trihydroxy-2-deoxy-2-(trifluoroacetamido)-β-D-galactopyranoside (14): Compound **13** (2.8 g, 5.5 mmol) was dissolved in MeOH (39 mL) and NaOMe (365 µL, 2.17 M solution in MeOH) was added. After 50 min, Amberlite acidic resin was added until pH 7. The Amberlite resin was filtered off, and

the solvent was removed in vacuo to give **14** (2.1 g, quantitative). TLC (16:1 CH_2Cl_2 /MeOH) R_f 0.21; ^{1}H NMR (300 MHz, MeOD): δ 6.97 (m, 2H, Ar), 6.82 (m, 2H, Ar), 4.91 (d, 1H, $J_{1,2} = 8.5$ Hz, H-1), 4.25 (dd, 1H, $J_{2,3} = 10.7$ Hz, H-2), 3.91 (d, 1H, $J_{3,4} = 3.1$ Hz, H-4), 3.87-3.71 (m, 6H, H-6a, H-6b, H-3, CH_3(OMP)), 3.64 (dd, 1H, H-5); HR MS: *m/z*: calcd for $C_{15}H_{18}NO_7F_3Na$: 404.0933; found: 404.0923 $[M+Na]^+$.

4-Methoxyphenyl 4,6-*O*-benzylidene-2-deoxy-2-trifluoroacetamido-β-D-galactopyranoside (15): Benzaldehyde dimethyl acetal (1.0 mL, 6.7 mmol) and *p*-toluenesulfonic acid (0.08 g, 0.45 mmol) were added to a solution of **14** (1.7 g, 4.5 mmol) in MeCN (31 mL). After stirring at room temperature for 4 h, EtOAc was added and the mixture was washed with saturated aqueous $NaHCO_3$. The organic phase was dried with $MgSO_4$, filtered and concentrated in vacuo. Flash chromatography (toluene/acetone 5:1 → 1:1) gave **15** (1.8 g, 87%). TLC (2:1 toluene/acetone) R_f 0.43; $[\alpha]_D^{20}$ − 8.4° (*c* 1.0, acetone); ^{1}H-NMR (400 MHz, $(CD_3)_2CO$): δ 8.56 (d, 1H, $J_{2,NH} = 9.4$ Hz, NH), 7.59 (m, 2H, Ar), 7.40 (m, 3H, Ar), 7.02 (m, 2H, Ar), 6.86 (m, 2H, Ar), 5.72 (s, 1H, PhCHO), 5.19 (d, 1H, $J_{1,2} = 8.3$ Hz, H-1), 4.48-4.35 (m, 3H, H-2, H-4, OH), 4.32-4.18 (m, 2H, H-6a, H-6b), 4.16-4.06 (m, 1H, H-3), 3.85 (s, 1H, H-5), 3.76 (s, 3H, Me (OMP)), ^{13}C-NMR (100 MHz, $(CD_3)_2CO$): δ 157.3 (q, $^2J_{C,F} = 36.0$ Hz, $COCF_3$), 155.5-115.5 (Ar), 116.3 (q, $^1J_{C,F} = 289.0$ Hz, COCF_3), 100.7 (PhCHO), 100.3 (C-1), 75.4 (C-4), 69.8 (C-3), 68.8 (C-6), 66.9 (C-5), 55.0 (Me(OMP)), 53.8 (C-2); HR MS: *m/z*: calcd for $C_{22}H_{22}NO_7F_3Na$: 492.1246; found: 492.1234 $[M+Na]^+$.

Dimethylthexylsilyl   3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoroacetamido-β-D-galactopyranoside (16): Benzylamine (3.0 mL, 28 mmol) was added to a solution of **12** (4.0 g, 9.0 mmol) in THF (40 mL). After stirring for 3 h at room temperature, the mixture was diluted with CH_2Cl_2, and washed with 1M HCl and H_2O. The organic phase was dried over Mg_2SO_4, filtered and the solvent was removed under reduced

pressure. The residue [TLC (3:2 hexane/EtOAc) R_f 0.36] (3.9 g) was dissolved in CH_2Cl_2 (19.5 mL). Imidazole (1.78 g, 26.2 mmol) and thexyldimethylsilyl chloride (2.30 mL, 11.7 mmol) were added. After 24 h, the mixture was diluted with CH_2Cl_2 and washed with H_2O. The organic layer was dried over $MgSO_4$, filtered, and the solvent was removed in vacuo. Flash chromatography on silica gel (3:1 hexane/EtOAc) afforded **16** (2.43 g, 51%). TLC (3:1 hexane/EtOAc) R_f 0.33; $[\alpha]_D^{20}$ -8.7º (*c* 1.0, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.71 (d, 1H, $J_{2,NH}$ = 9.3 Hz, NH), 5.38 (dd, 1H, $J_{3,4}$ = 3.8 Hz, H-4), 5.22 (dd, 1H, $J_{3,4}$ = 3.5 Hz, $J_{2,3}$ = 11.3 Hz, H-3), 4.84 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.29- 4.08 (m, 3H, H-2, H-6a, H-6b), 3.95 (m, 1H, H-5), 2.28-1.94 (3s, 9H, COC*H₃*), 1.62 (m, 1H, C*H*(CH₃)₂), 0.97-0.76 (m, 12H, C(C*H₃*)₂ and CH(C*H₃*)₂), 0.29-0.07 (2s, 6H, Si(C*H₃*)₂); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 170.9, 170.6, 170.4 (COCH₃), 157.3 (q, $^2J_{C,F}$ = 36.9 Hz, *C*OCF₃), 115.8 (q, $^1J_{C,F}$ = 290.0 Hz, CO*C*F₃), 96.0 (C-1), 71.0 (C-5), 69.8 (C-3), 66.8 (C-4), 61.9 (C-6), 53.6 (C-2), 33.9, 24.8, 20.7, 20.5, 19.8, 18.4 (TDS, OAc), -2.0, -3.0 (TDS); HR MS: *m/z*: calcd for $C_{22}H_{36}NO_9F_3NaSi$: 566.2009; found: 566.1991 [*M*+Na]⁺.

Dimethylthexylsilyl 6-*O*-chloroacetyl-2-deoxy-2-trifluoroacetamido-β-D-galactopyranoside (18): Compound **16** (2.2 g, 4.1 mmol) was dissolved in MeOH (31 mL) and NaOMe (273 µL, 2.17 M solution in MeOH) was added. After 50 min, Amberlite acidic resin was added until pH 7. The Amberlite resin was filtered off, and the solvent was removed in vacuo to give the desired triol (1.7 g, quantitative). An aliquot of this triol (0.5 g, 1.2 mmol) was dissolved in anhydrous CH_2Cl_2 (45 mL) and collidine (4.5 mL), and chloroacetic anhydride (112 µL, 1.4 mmol) was then added dropwise at -60 ºC. After stirring for 1 h at -60 ºC, the mixture was diluted with EtOAc and washed with 1M HCl, $NaHCO_3$ and H_2O. The organic phase was dried ($MgSO_4$), filtered, and concentrated. Flash silica chromatography (3:2 hexane/EtOAc) afforded **18**

(454 mg, 77%). TLC (3:2 hexane/EtOAc) R_f 0.14; $[\alpha]_D^{20}$ + 17 ° (c 1.0, MeOH); ^{1}H-NMR (300 MHz, CDCl$_3$): δ 7.31 (d, 1H, $J_{2,NH}$ = 7.9 Hz, NH), 4.70 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1), 4.56-4.30 (m, 2H, H-6a, H-6b), 4.10 (s, 2H, COCH$_2$Cl), 4.02-3.80 (m, 3H, H-3, H-4, H-2), 3.75 (m, 1H, H-5), 1.58 (m, 1H, CH(CH$_3$)$_2$), 0.98-0.70 (m, 12H, C(CH_3)$_2$ and CH(CH_3)$_2$), 0.23-0.03 (2s, 6H, Si(CH_3)$_2$); ^{13}C-NMR (75 MHz, CDCl$_3$): δ 167.7 (COCH$_2$Cl), 159.0 (q, $^2J_{C,F}$ = 36.8 Hz, COCF$_3$), 115.8 (q, $^1J_{C,F}$ = 285.0 Hz, COCF$_3$), 95.8 (C-1), 72.5 (C-5), 70.4, 68.3 (C-3, C-4), 65.1 (C-6), 55.9 (C-2), 40.5 (CH$_2$Cl), 33.9, 24.8, 19.8, 18.5 (Ac, TDS), -2.0, -4.0 (TDS); ESI MS: m/z: calcd for C$_{18}$H$_{31}$ClF$_3$NO$_7$SiNa: 516.2; found: 516.2 [M+Na]$^+$.

4-Methoxyphenyl 4,6-*O*-di-*tert*-butylsilylene-2-deoxy-2-trifluoroacetamido-β-D-galactopyranoside (4): Compound **14** (0.66 g, 1.73 mmol) was dissolved in dry Py (30 mL) and cooled (0ºC). Di-*tert*-butylsilyl bis(trifluoromethanesulfonate) (0.63 mL, 1.9 mmol) was added and the mixture was stirred at room temperature for 12 min. The reaction was quenched with MeOH (3 mL), diluted with EtOAc (120 mL), and washed with 1 M HCl, saturated aqueous NaHCO$_3$, and H$_2$O. The organic phase was dried (MgSO$_4$), filtered and concentrated to dryness. The residue was purified by column chromatography (toluene/EtOAc 5:1) to afford **4** (0.8 g, 89%). TLC (toluene/EtOAc 3:1) R_f 0.30; $[\alpha]_D^{20}$ –17º (c 1.0, CHCl$_3$); ^{1}H-NMR (300 MHz, CDCl$_3$): δ 6.92 (m, 2H, Ar), 6.87 (d, 1H, $J_{2,NH}$ = 9.1 Hz, NH), 6.78 (m, 2H, Ar), 4.97 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 4.39 (d, 1H, $J_{3,4}$ = 2.7 Hz, H-4), 4.24 (m, 2H, H-6a, H-6b), 4.09 (bq, 1H, H-2), 3.89 (dd, 1H, $J_{2,3}$ = 10.5 Hz, H-3), 3.75 (s, 3H, Me(OMP)), 3.46 (bs, 1H, H-5), 2.68 (bs, 1H, OH), 1.09, 1.06 (2s, 18H, C(CH$_3$)$_3$); ^{13}C-NMR (75 MHz, CDCl$_3$): δ 158.2 (q, $^2J_{C,F}$ = 37.2 Hz, COCF$_3$), 155.9, 151.2 (Ar-C), 119.7 (Ar-CH), 115.9 (q, $^1J_{C,F}$ = 288.0 Hz, COCF$_3$), 114.6 (Ar-CH), 100.2 (C-1), 72.0 (C-4), 71.5 (C-5), 70.7 (C-3), 66.9 (C-6), 55.7

(Me(OMP)), 55.1 (C-2), 27.5 (C(CH_3)$_3$), 23.4, 20.9 (C(CH$_3$)$_3$); HR MS: m/z: calcd for $C_{23}H_{34}F_3NO_7SiNa$: 544.1954; found: 544.1968 [M+Na]$^+$.

4-Methoxyphenyl 3-*O*-(benzyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-β-D-glucopyranosyluronate)-4,6-*O*-di-*tert*-butylsilylene-2-deoxy-2-trifluoroacetamido-β-D-galactopyranoside (19): Acceptor **4** (0.18 g, 0.3 mmol) and glucuronic acid trichloroacetimidate **2** (0.38 g, 0.5 mmol) were combined in a flask, coevaporated with toluene and dried under vacuum. The starting materials were disolved in dry CH$_2$Cl$_2$ (5 mL) and further dried by stirring over freshly activated 4Å molecular sieves for 15 min. TMSOTf (10 µL, 0.05 mmol) was added at 0ºC. After 10 min, the reaction was quenched with Et$_3$N (2.5 mL) and filtered, and the solvent was removed under reduced pressure. Purification by flash cromathography (8:1 toluene/EtOAc) yielded **19** (345 mg, 91%). TLC (5:1 toluene/EtOAc) R$_f$ 0.57; [α]$_D^{20}$ +16.5º (*c* 1.0, CH$_2$Cl$_2$); ^{1}H-NMR (500 MHz, CDCl$_3$) δ 8.01 (m, 2H, Ar), 7.62 (m, 1H, Ar), 7.47 (m, 2H, Ar), 7.42-7.31 (m, 5H, Ar), 7.18-7.07 (m, 5H, Ar), 6.94 (m, 2H, Ar), 6.81(m, 2H, Ar), 5.41-5.30 (m, 4H, H-2B, H-1B, H-4B, H-1A), 5.18 (2d, 2H, CH$_2$(Bn)),4.68-4.53 (m, 3H, H-4A, CH$_2$(Bn)), 4.43 (dd, 1H, $J_{2,3}$ = 11.3 Hz, $J_{3,4}$ = 2.4 Hz, H-3A), 4.21-4.03 (m, 4H, H-6aA, H-6bA, H-5B, H-2A), 3.86 (dd, 1H, $J_{2,3}$ = $J_{3,4}$ = 8.7 Hz, H-3B), 3.78 (s, 3H, Me(OMP)), 3.42 (s, 1H, H-5A), 2.62-2.22 (m, 4H, CH$_2$(Lev)), 2.13 (s, 3H, CH$_3$(Lev)), 1.09, 0.98 (2s, 18H, C(CH$_3$)$_3$); ^{13}C-NMR (100 MHz, CDCl$_3$): δ 206.0 (CO(Lev)), 171.3, 167.0, 164.9 (CO(COOBn, Bz, Lev)), 157.6 (q, $^2J_{C,F}$ = 38.6 Hz, COCF$_3$), 155.8, 151.1, 137.2, 134.6 (Ar-C), 133.5 (Ar-CH), 129.8-127.8 (Ar), 120.0 (Ar-CH), 115.5 (q, $^1J_{C,F}$ = 288.5 Hz, COCF$_3$), 114.5 (Ar-CH), 99.5, 99.4 (C-1B, C-1A), 79.6 (C-3B), 74.5 (C-3A), 74.2 (CH$_2$(Bn)), 73.2 (C-4A), 72.5 (C-4B or C-2B), 72.2 (C-5B), 71.3 (C-5A), 70.7 (C-2B or C-4B), 68.1 (CH$_2$(Bn)), 67.0 (C-6A), 55.6 (Me(OMP)), 53.7 (C-2A), 37.6 (CH$_2$(Lev)), 29.8 (CH$_3$(Lev)), 27.7, 27.6, 27.4 (CH$_2$(Lev), C(CH$_3$)$_3$), 23.2, 20.8

(C(CH$_3$)$_3$); HR MS: *m/z*: calcd for C$_{55}$H$_{64}$NO$_{16}$F$_3$NaSi: 1102.3844; found: 1102.3820 [*M*+Na]$^+$.

4-Methoxyphenyl 3-*O*-(methyl 3-*O*-benzyl-2,4-di-*O*-levulinoyl-α-L-idopyranosyluronate)-4,6-*O*-di-*tert*-butylsilylene-2-deoxy-2-trifluoroacetamido-β-D-galactopyranoside (20): Donor **3** (0.92 g, 1.44 mmol) and acceptor **4** (0.5 g, 0.96 mmol) were dissolved in dry CH$_2$Cl$_2$ (12 mL) in the presence of freshly activated 4Å molecular sieves. After stirring for 30 min at 0ºC, TMSOTf (348 µL of a 0.41 M solution in dry CH$_2$Cl$_2$) was added under an argon atmosphere. After stirring for 15 min at 0ºC, the reaction mixture was neutralized with Et$_3$N and concentrated to dryness. The residue was purified by column chromatography (toluene/EtOAc 1:1) to afford **20** (756 mg, 79%). TLC (toluene/EtOAc 1:1) R$_f$ 0.19; [α]$_D^{20}$ −18º (*c* 1.0, CHCl$_3$); ^{1}H-NMR (300 MHz, CDCl$_3$): δ 7.34-7.27 (m, 5H, Ar), 7.00 (d, 1H, $J_{2,NH}$ = 7.2 Hz, NH), 6.94 (m, 2H, Ar), 6.79 (m, 2H, Ar), 5.34 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1A), 5.28 (bt, 1H, H-4B), 5.15 (bs, 1H, H-1B), 5.07 (d, 1H, $J_{4,5}$ = 2.6 Hz, H-5B), 4.88 (bt, 1H, H-2B), 4.72 (m, 2H, CH$_2$(Bn)), 4.54 (bd, 1H, H-4A), 4.35 (dd, 1H, $J_{2,3}$ = 11.0 Hz, $J_{3,4}$ = 2.3 Hz, H-3A), 4.20 (m, 2H, H-6aA, H-6bA), 3.99 (m, 1H, H-2A), 3.80-3.75 (m, 7H, H-3B, Me(OMP), COOMe), 3.48 (bs, 1H, H-5A), 2.83-2.44 (m, 8H, CH$_2$(Lev)), 2.16 (s, 6H, CH$_3$(Lev)), 1.06, 0.97 (2s, 18H, C(CH$_3$)$_3$); ^{13}C-NMR (75 MHz, CDCl$_3$): δ 206.8, 206.3 (CO(Lev)), 171.7, 171.6 (CO(Lev)), 168.9 (COOMe), 157.8 (q, $^2J_{C,F}$ = 36.8 Hz, *C*OCF$_3$), 155.9, 151.2, 138.0 (Ar-C), 128.4, 127.7, 127.5, 120.0 (Ar-CH), 115.6 (q, $^1J_{C,F}$ = 287.8 Hz, CO*C*F$_3$), 114.6 (Ar-CH), 100.3 (C-1B), 99.1 (C-1A), 78.1 (C-3A), 73.3 (C-3B), 72.7, 72.6 (C-4A, CH$_2$(Bn)), 71.3 (C-5A), 68.6 (C-4B), 67.8 (C-2B), 67.4 (C-5B), 67.0 (C-6A), 55.7 (COOMe or Me(OMP)), 54.0 (C-2A), 52.6 (COOMe or Me(OMP)), 37.9, 37.8 (CH$_2$(Lev)), 29.9 (CH$_3$(Lev)), 28.0, 27.7, 27.4 (CH$_2$(Lev), C(*C*H$_3$)$_3$), 23.3, 20.9

($C(CH_3)_3$); HR MS: m/z: calcd for $C_{47}H_{62}F_3NO_{17}SiNa$: 1020.3637; found: 1020.3667 $[M+Na]^+$.

4-Methoxyphenyl 3-O-(methyl 3-O-benzyl-2,4-di-O-levulinoyl-α-L-idopyranosyluronate)- 4,6-di-O-acetyl-2-deoxy-2-trifluoroacetamido-β-D-galactopyranoside (22): An excess of $(HF)_n \cdot Py$ (1.26 mL, 48.3 mmol) was added at 0°C under an argon atmosphere to a solution of **20** (250 mg, 0.25 mmol) in dry THF (5 mL). After 23 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 **21** (209 mg, 97%). TLC (toluene/acetone 3:2) R_f 0.21; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.87 (d, 1H, $J_{2,NH}$ = 7.1 Hz, NH), 7.36-7.24 (m, 5H, Ar), 6.96 (m, 2H, Ar), 6.80 (m, 2H, Ar), 5.47 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1A), 5.23 (m, 2H, H-4B, H-1B), 4.98 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 5.5 Hz, H-2B), 4.88 (d, 1H, $J_{4,5}$ = 4.7 Hz, H-5B), 4.68 (s, 2H, CH_2(Bn)), 4.48 (dd, 1H, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 3.1 Hz, H-3A), 4.20 (d, 1H, H-4A), 3.99 (dd, 1H, $J_{5,6a}$ = 6.6 Hz, $J_{6a,6b}$ = 11.8 Hz, H-6aA), 3.95-3.82 (m, 3H, H-2A, H-6bA, H-3B), 3.80, 3.76 (2s, 6H, Me(OMP), COOMe), 3.71 (bt, 1H, H-5A), 2.94-2.28 (m, 8H, CH_2(Lev)), 2.17 (s, 6H, CH_3(Lev)); ^{13}C-NMR (75 MHz, $CDCl_3$) (selected data from HSQC experiment): δ 129-127 (Ar-CH), 118.8, 114.2 (Ar-CH), 99.0 (C-1B), 98.3 (C-1A), 75.9 (C-3A), 74.9 (C-3B), 74.0 (C-5A), 73.0 (CH_2(Bn)), 70.5 (C-2B), 69.7 (C-5B), 69.3 (C-4B), 68.4 (C-4A), 62.3 (C-6A), 55.3 (COOMe or Me(OMP)), 53.9 (C-2A), 52.3 (COOMe or Me(OMP)), 37.4 (CH_2(Lev)), 29.4 (CH_3(Lev)), 27.3 (CH_2(Lev); HR MS: m/z: calcd for $C_{39}H_{46}F_3NO_{17}Na$: 880.2616; found: 880.2617 $[M+Na]^+$.

21 (152 mg, 0.177 mmol) was dissolved in dry Py (7 mL), cooled (0ºC) and Ac_2O (0.5 mL) was added. After stirring for 48 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/acetone 3:1) to afford **22** (149 mg, 89%). TLC (toluene/acetone 3:2) R$_f$ 0.49; $[\alpha]_D^{20}$ -17° (*c* 1.0, CHCl$_3$); ^{1}H-NMR (300 MHz, CDCl$_3$): δ 7.77 (d, 1H, $J_{2,NH}$ = 7.4 Hz, NH), 7.36-7.27 (m, 5H, Ar), 6.95 (m, 2H, Ar), 6.79 (m, 2H, Ar), 5.44 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1A), 5.41(d, 1H, $J_{3,4}$ = 3.1 Hz, H-4A), 5.28 (bt, 1H, H-4B), 5.01 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1B), 4.92 (bt, 1H, $J_{2,3}$ = 5.5 Hz, H-2B), 4.88 (d, 1H, $J_{4,5}$ = 3.3 Hz, H-5B), 4.63 (q, 2H, CH$_2$(Bn)), 4.58 (dd, 1H, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.9 Hz, H-3A), 4.18-4.06 (m, 2H, H-6aA, H-6bA), 3.99 (bt, 1H, H-5A), 3.86 (m, 1H, H-2A), 3.80-3.75 (m, 7H, H-3B, Me(OMP), COOMe), 2.92-2.33 (m, 8H, CH$_2$(Lev)), 2.19, 2.17 (2s, 6H, CH$_3$(Lev)), 2.03, 1.92 (2s, 6H, CH$_3$(Ac)); ^{13}C-NMR (75 MHz, CDCl$_3$): δ 207.8, 206.3 (CO(Lev)), 171.5, 171.4, 170.5, 170.4 (CO(Lev, Ac)), 168.7 (COOMe), 157.9 (q, $^2J_{C,F}$ = 37.5 Hz, *C*OCF$_3$), 155.9, 151.1, 137.6 (Ar-C), 128.5, 127.9, 119.1 (Ar-CH), 115.6 (q, $^1J_{C,F}$ = 288.1 Hz, CO*C*F$_3$), 114.6 (Ar-CH), 100.3 (C-1B), 99.0 (C-1A), 75.0 (C-3B), 73.7 (C-3A), 73.2 (CH$_2$(Bn)), 71.3 (C-5A), 69.9 (C-4B), 69.4 (C-2B), 68.8 (C-4A), 68.6 (C-5B), 61.7 (C-6A), 55.7 (COOMe or Me(OMP)), 55.2 (C-2A), 52.5 (COOMe or Me(OMP)), 37.9, 37.7 (CH$_2$(Lev)), 29.8 (CH$_3$(Lev)), 27.9, 27.6 (CH$_2$(Lev), 20.7, 20.4 (CH$_3$(Ac)); HR MS: *m/z*: calcd for C$_{43}$H$_{50}$F$_3$NO$_{19}$Na: 964.2827; found: 964.2841 [*M*+Na]$^+$.

3-*O*-(Methyl 3-*O*-benzyl-2,4-di-*O*-levulinoyl-α-L-idopyranosyluronate)-4,6-di-*O*-acetyl-2-deoxy-2-trifluoroacetamido-α,β-D-galactopyranose (23): CAN (0.75 mL of a 0.63 M solution in H$_2$O) was added to a solution of **22** (149 mg, 0.158 mmol) in toluene/MeCN (1:6; 5.25 mL), and the mixture was vigorously stirred for 1 h 20 min at 0ºC. It was then diluted with EtOAc, washed with H$_2$O, saturated aqueous NaHCO$_3$, and H$_2$O. The organic phase was dried (MgSO$_4$), filtered and concentrated to dryness. The residue was purified by column chromatography (toluene/acetone 5:2) to afford **23**

(100 mg, 76%) as a mixture of α/β anomers. TLC (toluene/acetone 3:2) R_f 0.34, 0.31; ^{1}H-NMR (300 MHz, CDCl$_3$) (data for α anomer): δ 7.39-7.26 (m, 5H, Ar), 6.73 (d, 1H, $J_{2,NH}$ = 9.3 Hz, NH), 5.40 (d, 1H, $J_{3,4}$ = 3.0 Hz, H-4A), 5.37 (d, 1H, $J_{1,2}$ = 3.1 Hz, H-1A), 5.20 (bt, 1H, H-4B), 5.06 (bs, 1H, H-1B), 4.92 (d, 1H, $J_{4,5}$ = 2.3 Hz, H-5B), 4.80 (m, 1H, H-2B), 4.63 (q, 2H, CH$_2$(Bn)), 4.58 (m, 1H, H-2A), 4.36 (bt, 1H, H-5A), 4.13 (dd, 1H, $J_{2,3}$ = 10.8 Hz, H-3A), 4.07 (dd, 1H, $J_{5,6a}$ = 5.3 Hz, $J_{6a,6b}$ = 11.5 Hz, H-6aA), 3.97 (dd, 1H, $J_{5,6b}$ = 7.0 Hz, H-6bA), 3.81 (s, 3H, COOMe), 3.75 (bt, 1H, H-3B), 3.27 (bs, 1H, OH), 2.84-2.48 (m, 8H, CH$_2$(Lev)), 2.18 (s, 6H, CH$_3$(Lev)), 2.03, 1.68 (2s, 6H, CH$_3$(Ac)); ^{13}C-NMR (75 MHz, CDCl$_3$) (data for α anomer): δ 207.5, 206.4 (CO(Lev)), 171.7, 171.5, 170.7 (CO(Lev, Ac)), 169.4 (COOMe), 157.8 (q, $^2J_{C,F}$ = 37.3 Hz, COCF$_3$), 137.4 (Ar-C), 128.5, 128.2, 128.0 (Ar-CH), 115.9 (q, $^1J_{C,F}$ = 288.6 Hz, COCF$_3$), 100.4 (C-1B), 91.8 (C-1A), 75.0 (C-3A), 72.8 (CH$_2$(Bn)), 72.5 (C-3B), 69.0 (C-4A), 68.4 (C-4B), 67.7 (C-5A), 67.2 (C-2B), 67.1 (C-5B), 62.3 (C-6A), 52.7 (COOMe), 49.9 (C-2A), 37.9 (CH$_2$(Lev)), 30.0 (CH$_3$(Lev)), 27.9 (CH$_2$(Lev), 20.9, 20.3 (CH$_3$(Ac)); HR MS: m/z: calcd for C$_{36}$H$_{44}$F$_3$NO$_{18}$Na: 858.2408; found: 858.2403 [M+Na]$^+$.

O-[3-O-(Methyl 3-O-benzyl-2,4-di-O-levulinoyl-α-L-idopyranosyluronate)-4,6-di-O-acetyl-2-deoxy-2-trifluoroacetamido-α,β-D-galactopyranosyl] trichloroacetimidate (24): Trichloroacetonitrile (180 μL, 1.8 mmol) and catalytic DBU (107 μL of a 0.084 M solution in dry CH$_2$Cl$_2$) were added to a solution of **23** (100 mg, 0.12 mmol) in dry CH$_2$Cl$_2$ (2 mL). After stirring for 13 h at room temperature, the reaction mixture was concentrated to dryness. The residue was purified by flash chromatography (toluene/acetone 5:2 + 1% Et$_3$N) to afford **24** (99 mg, 84%) as a mixture of α/β anomers. TLC (toluene/acetone 5:2) R_f 0.42 (α); ^{1}H-NMR (400 MHz, CDCl$_3$) (data for α anomer): δ 8.82 (s, 1H, NH(TCA)), 7.36-7.26 (m, 5H, Ar), 6.88 (d, 1H, $J_{2,NH}$ = 9.2 Hz, NH(TFA)), 6.39 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1A), 5.47 (bd, 1H, $J_{3,4}$ = 2.9 Hz, H-4A),

5.18 (bt, 1H, H-4B), 5.13 (bd, 1H, H-1B), 4.87 (d, 1H, $J_{4,5}$ = 2.7 Hz, H-5B), 4.78 (m, 1H, H-2B), 4.74 (m, 1H, H-2A), 4.61 (q, 2H, CH$_2$(Bn)), 4.28 (bt, 1H, H-5A), 4.20 (dd, 1H, $J_{2,3}$ = 10.9 Hz, H-3A), 4.08 (dd, 1H, $J_{5,6a}$ = 5.9 Hz, $J_{6a,6b}$ = 11.6 Hz, H-6aA), 3.94 (dd, 1H, $J_{5,6b}$ = 7.1 Hz, H-6bA), 3.78 (s, 3H, COOMe), 3.74 (bt, 1H, H-3B), 2.80-2.46 (m, 8H, CH$_2$(Lev)), 2.15, 2.13 (2s, 6H, CH$_3$(Lev)), 1.96, 1.72 (2s, 6H, CH$_3$(Ac)); ^{13}C-NMR (100 MHz, CDCl$_3$) (data for α anomer): δ 206.8, 206.2 (CO(Lev)), 171.6, 170.4, 170.3 (CO(Lev, Ac)), 168.9 (COOMe), 160.3 (C=NH), 157.7 (q, $^2J_{C,F}$ = 38.0 Hz, COCF$_3$), 137.3 (Ar-C), 128.5, 128.1, 128.0 (Ar-CH), 115.7 (q, $^1J_{C,F}$ = 289.0 Hz, COCF$_3$), 100.6 (C-1B), 94.9 (C-1A), 90.7 (CCl$_3$), 75.1 (C-3A), 72.7 (CH$_2$(Bn)), 72.5 (C-3B), 69.9 (C-5A), 68.2 (C-4A), 68.1 (C-4B), 67.6 (C-2B), 67.2 (C-5B), 61.7 (C-6A), 52.7 (COOMe), 49.5 (C-2A), 37.8, 37.6 (CH$_2$(Lev)), 29.8 (CH$_3$(Lev)), 27.9, 27.7 (CH$_2$(Lev), 20.6, 20.1 (CH$_3$(Ac)); ESI MS: m/z: calcd for C$_{38}$H$_{44}$Cl$_3$F$_3$N$_2$O$_{18}$Na: 1001.2; found: 1001.1 [M+Na]$^+$.

4-Methoxyphenyl 3-*O*-(benzyl 2-*O*-benzoyl-3-*O*-benzyl–β-D-glucopyranosyluronate)-4,6-*O*-di-*tert*-butylsilylene-2-deoxy-2-trifluoroacetamido-β-D-galactopyranoside (25): Compound **19** (0.25 g, 0.23 mmol) was dissolved in CH$_2$Cl$_2$ (4 mL), and hydrazine monohydrate (2 mL of a 0.25 M solution in Py/AcOH, 3:2) was added. After stirring at room temperature for 2 h, the reaction mixture was quenched with acetone (1 mL), diluted with CH$_2$Cl$_2$, washed with HCl (1M), saturated aqueous NaHCO$_3$ and H$_2$O, dried with MgSO$_4$, filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (9:1 toluene/EtOAc) to give **25** (0.19 g, 84%). TLC (5:1 toluene/ EtOAc) R$_f$ 0.43; [α]$_D^{20}$ +6.1° (*c* 1.0, CH$_2$Cl$_2$); ^{1}H-NMR (500 MHz, CDCl$_3$) δ 8.00 (m, 2H, Ar), 7.61 (m, 1H, Ar), 7.46 (m, 2H, Ar), 7.43- 7.31 (m, 5H, Ar), 7.23-7.12 (m, 5H, Ar), 6.98 (d, 1H, $J_{2,NH}$ = 6.5 Hz, NH), 6.92 (m, 2H, Ar), 6.81(m, 2H, Ar), 5.44 (d, 1H, $J_{1,2}$ = 8.2 Hz, H-1A), 5.39 (d, 1H, CH$_2$(Bn)),

5.34 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1B), 5.29 (dd, 1H, H-2B), 5.20 (d, 1H, CH$_2$(Bn)), 4.77 (2d, 2H, CH$_2$(Bn)), 4.62 (d, 1H, H-4A), 4.44 (dd, 1H, $J_{2,3}$ = 11.3 Hz, $J_{3,4}$ = 2.4 Hz, H-3A), 4.19-4.05 (m, 2H, H-6aA, H-4B), 4.03-4.91 (m, 3H, H-6bA, H-5B, H-2A), 3.78 (s, 3H, Me(OMP)), 3.69 (dd, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, H-3B), 3.35 (s, 1H, H-5A), 1.08, 1.00 (2s, 18H, C(CH$_3$)$_3$); ^{13}C-NMR (100 MHz, CDCl$_3$): δ 169.0, 165.0 (CO(COOBn, Bz)), 157.7 (q, $^2J_{C,F}$ = 37.1 Hz, COCF$_3$), 155.9, 151.1, 137.6, 134.4 (Ar-C), 133.5 (Ar-CH), 129.9-127.8 (Ar), 120.2 (Ar-CH), 115.4 (q, $^1J_{C,F}$ = 286.4 Hz, COCF$_3$), 114.5 (Ar-CH), 100.3 (C-1B), 99.0 (C-1A), 81.0 (C-3B), 74.9 (C-3A), 74.7 (CH$_2$(Bn)), 73.8 (C-5B), 73.4 (C-4A), 72.5 (C-2B), 72.2 (C-4B), 71.3 (C-5A), 68.0 (CH$_2$(Bn)), 66.9 (C-6A), 55.6 (Me(OMP)), 54.2 (C-2A), 27.6, 27.4 (C(CH$_3$)$_3$), 23.2, 20.8 (C(CH$_3$)$_3$); HR MS: m/z: calcd for C$_{50}$H$_{58}$NO$_{14}$F$_3$NaSi: 1004.3476; found: 1004.3511 [M+Na]$^+$.

4-Methoxyphenyl *O*-**(methyl** **3-*O*-benzyl-2,4-di-*O*-levulinoyl-α-L-idopyranosyluronate)-(1→3)-*O*-(4,6-di-*O*-acetyl-2-deoxy-2-trifluoroacetamido-β-D-galactopyranosyl)-(1→4)-*O*-(benzyl** **2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranosyluronate)-(1→3)-4,6-*O*-di-*tert*-butylsilylene-2-deoxy-2-trifluoroacetamido-β-D-galactopyranoside (26):** Donor **24** (52 mg, 53 µmol) and acceptor **25** (35 mg, 35 µmol) were dissolved in dry CH$_2$Cl$_2$ (1.0 mL) in the presence of freshly activated 4Å molecular sieves. After stirring for 30 min, TMSOTf (115 µL of a 0.092 M solution in dry CH$_2$Cl$_2$) was added under an argon atmosphere at 0°C. After stirring for 15 min at 0°C, the reaction mixture was neutralized with Et$_3$N and concentrated to dryness. The residue was purified by column chromatography (toluene/acetone 3:1) to afford **26** (47 mg, 74%). TLC (toluene/acetone 3:1) R$_f$ 0.32; [α]$_D^{20}$ –5° (*c* 1.0, CHCl$_3$); ^{1}H-NMR (400 MHz, CDCl$_3$): δ 7.93 (d, 2H, Ar), 7.57 (t, 1H, Ar), 7.47-7.22 (m, 12H, Ar), 7.06 (m, 5H, Ar), 6.96 (d, 1H, $J_{2,NH}$ = 8.7 Hz, NH), 6.90 (m, 2H, Ar), 6.83 (d, 1H, $J_{2,NH}$ = 7.0 Hz, NH), 6.78 (m, 2H, Ar), 5.34 (d, 1H, CH$_2$(Bn)),

5.33 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1A), 5.25 (m, 3H, H-1B, H-2B, H-4D), 5.19 (d, 1H, CH$_2$(Bn)), 5.13 (bd, 1H, H-4C), 4.98 (bs, 1H, H-1D), 4.96 (d, 1H, $J_{4,5}$ = 2.4 Hz, H-5D), 4.80 (m, 2H, H-2D, CH$_2$(Bn)), 4.64 (m, 2H, CH$_2$(Bn)), 4.57 (d, 1H, $J_{3,4}$ = 2.5 Hz, H-4A), 4.51 (d, 1H, CH$_2$(Bn)), 4.37 (dd, 1H, $J_{2,3}$ = 11.3 Hz, H-3A), 4.18 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1C), 4.12 (bd, 1H, $J_{6a,6b}$ = 12.3 Hz, H-6aA), 4.04 (m, 3H, H-6bA, H-4B, H-2C), 3.96 (d, 1H, $J_{4,5}$ = 9.3 Hz, H-5B), 3.93 (m, 1H, H-2A), 3.81 (m, 4H, H-6aC, Me(OMP) or COOMe), 3.75 (s, 3H, Me(OMP) or COOMe), 3.74-3.66 (m, 3H, H-3D, H-6bC, H-3B), 3.61 (dd, 1H, $J_{2,3}$ = 10.6 Hz, $J_{3,4}$ = 3.4 Hz, H-3C), 3.33 (m, 2H, H-5A, H-5C), 2.83-2.50 (m, 8H, CH$_2$(Lev)), 2.19, 2.18 (2s, 6H, CH$_3$(Lev)), 1.97, 1.61 (2s, 6H, CH$_3$(Ac)), 1.06, 1.02 (2s, 18H, C(CH$_3$)$_3$); ^{13}C-NMR (100 MHz, CDCl$_3$): δ 206.9, 206.3 (CO(Lev)), 171.7, 171.5, 170.4, 170.3, 168.9, 168.8, 165.0 (CO(Lev), CO(Ac), CO(Bz), COOBn, COOMe), 158.1 (q, $^2J_{C,F}$ = 36.4 Hz, COCF$_3$), 157.7 (q, $^2J_{C,F}$ = 36.4 Hz, COCF$_3$), 156.0, 151.1, 137.8, 137.6, 134.7 (Ar-C), 133.4, 129.9, 129.7, 129.6, 129.4, 129.1, 128.7, 128.5, 128.1, 127.9, 127.6, 120.3 (Ar-C, Ar-CH), 116.0 (q, $^1J_{C,F}$ = 286.3 Hz, COCF$_3$), 115.5 (q, $^1J_{C,F}$ = 287.9 Hz, COCF$_3$), 114.6 (Ar-CH), 100.4 (C-1B), 100.3 (C-1D), 99.7 (C-1C), 99.3 (C-1A), 80.1 (C-3B), 78.0 (C-4B), 76.4 (C-3C), 75.5 (C-3A), 75.1 (CH$_2$(Bn)), 74.3 (C-5B), 73.3 (C-4A), 73.0 (C-3D), 72.6 (CH$_2$(Bn)), 72.4 (C-2B), 71.4, 71.1 (C-5A, C-5C), 68.5, 68.4 (CH$_2$(Bn), C-4D), 67.8 (C-4C), 67.5 (C-2D), 67.0 (C-5D, C-6A), 61.0 (C-6C), 55.7 (COOMe or Me(OMP)), 54.2 (C-2A), 53.5 (C-2C), 52.6 (COOMe or Me(OMP)), 37.9, 37.7 (CH$_2$(Lev)), 29.9 (CH$_3$(Lev)), 28.0, 27.7, 27.6 (CH$_2$(Lev), C(CH$_3$)$_3$), 23.4 (C(CH$_3$)$_3$), 20.8, 20.7, 20.1 (C(CH$_3$)$_3$, CH$_3$(Ac)); HR MS: m/z: calcd for C$_{86}$H$_{100}$F$_6$N$_2$O$_{31}$SiNa: 1821.5881; found: 1821.5903 [M+Na]$^+$.

4-Methoxyphenyl O-(3-O-benzyl-2,4-di-O-sulfo-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-2-O-sulfo-β-D-glucopyranosyluronic acid)-(1→3)-2-acetamido-2-deoxy-

4,6-di-*O*-sulfo-β-D-galactopyranoside (30): An excess of (HF)$_n$·Py (56 μL, 2.1 mmol) was added at 0°C under an argon atmosphere to a solution of **26** (20 mg, 0.011 mmol) in dry THF (1.0 mL). After 24 h at 0ºC, the mixture was diluted with CH$_2$Cl$_2$ and washed with H$_2$O and saturated NaHCO$_3$ solution until neutral pH. The organic layer was dried (MgSO$_4$), filtered and concentrated in vacuo to give **27**. TLC (toluene/EtOAc 1:2) R$_f$ 0.14; $[α]_D^{20}$ –4º (*c* 0.9, CHCl$_3$); ^{1}H-NMR (400 MHz, CDCl$_3$): δ 7.85 (d, 2H, Ar), 7.56 (t, 1H, Ar), 7.48-7.23 (m, 12H, Ar), 7.13 (d, 1H, $J_{2,NH}$ = 8.2 Hz, NH), 7.06 (m, 5H, Ar), 7.00 (d, 1H, $J_{2,NH}$ = 6.4 Hz, NH), 6.86 (m, 2H, Ar), 6.74 (m, 2H, Ar), 5.36 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1A), 5.25 (q, 2H, CH$_2$(Bn)), 5.23 (m, 2H, H-2B, H-4D), 5.16 (bs, 1H, H-4C), 4.98 (bs, 1H, H-1D), 4.93 (bs, 1H, H-5D), 4.80 (m, 3H, H-1B, H-2D, CH$_2$(Bn)), 4.63 (q, 2H, CH$_2$(Bn)), 4.54 (m, 2H, H-1C, CH$_2$(Bn)), 4.46 (bd, 1H, $J_{2,3}$ = 10.6 Hz, H-3A), 4.20 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 8.2 Hz, H-4B), 4.13 (bs, 1H, H-4A), 4.08 (bd, 1H, $J_{4,5}$ = 8.8 Hz, H-5B), 4.02 (m, 1H, H-2C), 3.89 (m, 1H, H-6aC), 3.83-3.71 (m, 12H, H-2A, H-6aA, H-6bA, H-3B, H-3C, H-3D, Me(OMP), COOMe), 3.63 (m, 2H, H-6bC, H-5C), 3.54 (bt, 1H, H-5A), 2.82-2.49 (m, 8H, CH$_2$(Lev)), 2.18 (s, 6H, CH$_3$(Lev)), 1.90, 1.64 (2s, 6H, CH$_3$(Ac)); ^{13}C-NMR (100 MHz, CDCl$_3$): δ 207.1, 206.4 (CO(Lev)), 171.7, 171.5, 170.5, 170.3, 168.9, 168.8, 165.2 (CO(Lev), CO(Ac), CO(Bz), COOBn, COOMe), 158.0 (bq, $^2J_{C,F}$ = 37.4 Hz, 2x*C*OCF$_3$), 155.8, 151.1, 137.8, 137.6, 134.8 (Ar-C), 133.6, 129.9, 129.5, 129.3, 129.2, 128.9, 128.5, 128.2, 128.1, 127.9, 127.8, 127.7, 119.0 (Ar-C, Ar-CH), 116.0 (q, $^1J_{C,F}$ = 287.6 Hz, CO*C*F$_3$), 115.2 (q, $^1J_{C,F}$ = 287.6 Hz, CO*C*F$_3$), 114.7 (Ar-CH), 101.6 (C-1B), 100.5 (C-1D), 100.1 (C-1C), 98.7 (C-1A), 80.0 (C-3B), 78.5 (C-3A), 77.8 (C-4B), 76.5 (C-3C), 75.1 (CH$_2$(Bn)), 74.7 (C-5C), 74.3 (C-5B), 73.3 (C-3D), 72.9 (CH$_2$(Bn)), 72.6 (C-2B), 71.3 (C-5A), 69.4 (C-4D), 68.7 (CH$_2$(Bn)), 68.6 (C-4A), 68.1 (C-4C), 68.0 (C-2D), 67.3 (C-5D), 62.8 (C-6C), 61.4 (C-6A), 55.8 (COOMe or Me(OMP)), 54.7 (C-2A), 53.9 (C-2C), 52.8 (COOMe or Me(OMP)), 37.9,

37.8 (CH$_2$(Lev)), 29.9, 29.8 (CH$_3$(Lev)), 28.0, 27.7 (CH$_2$(Lev)), 20.6, 20.2 (CH$_3$(Ac));

HR MS: m/z: calcd for C$_{78}$H$_{84}$F$_6$N$_2$O$_{31}$Na: 1681.4860; found: 1681.4868 [M+Na]$^+$.

H$_2$O$_2$ (30%, 0.44 mL) and an aqueous solution of LiOH (0.7 M, 0.27 mL) were added at -5°C to a solution of **27** (11.1 µmol) in THF (1.5 mL). After stirring for 20 h at room temperature, MeOH (1.5 mL) and an aqueous solution of NaOH (4 M, 0.28 mL) were added. After stirring for 6 d at room temperature, the reaction mixture was neutralized with Amberlite IR-120 (H$^+$) resin, filtered, and concentrated to give **28**. ESI MS: m/z: calcd for C$_{45}$H$_{57}$N$_2$O$_{22}$: 977.3; found: 977.2 [M+H]$^-$

Triethylamine (0.4 mL of a 0.36 M solution in dry MeOH) and acetic anhydride (21 µL, 0.22 mmol) were added to a cooled (0°C) solution of **28** (11.1 µmol) in dry MeOH (2.5 mL). After stirring for 2 h at room temperature, triethylamine (0.3 mL) was added and the mixture was concentrated to dryness. The residue was purified by Sephadex LH-20 chromatography (CH$_2$Cl$_2$-MeOH 1:1) to give **29** as triethylammonium salt. The sodium salt of **29** was obtained by treatment with Amberlite IR-120 H$^+$ resin in MeOH (pH ~ 3), followed by filtration, treatment with 0.1 M NaOH (pH ~ 7) and concentration.

^{1}H-NMR (500 MHz, MeOD, data for sodium salt): δ 7.58 (d, 2H, Ar), 7.45 (d, 2H, Ar), 7.34-7.21 (m, 6H, Ar), 6.98 (m, 2H, Ar), 6.82 (m, 2H, Ar), 5.06 (d, 1H, CH$_2$(Bn)), 4.93 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1A), 4.89 (d, 1H, $J_{1,2}$ = 4.4 Hz, H-1D), 4.77 (q, 2H, CH$_2$(Bn)), 4.73 (d, 1H, CH$_2$(Bn)), 4.62 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1C), 4.43 (m, 1H, H-1B), 4.41 (d, 1H, $J_{4,5}$ = 3.7 Hz, H-5D), 4.27 (dd, 1H, $J_{2,3}$ = 10.6 Hz, H-2A), 4.23 (dd, 1H, $J_{2,3}$ = 10.4 Hz, H-2C), 4.13 (d, 1H, $J_{3,4}$ = 2.7 Hz, H-4A), 4.05 (d, 1H, $J_{3,4}$ = 2.7 Hz, H-4C), 3.99-3.95 (m, 2H, H-4B, H-4D), 3.84-3.69 (m, 9H, H-6aA, H-6aC, H-6bA or H-6bC, H-3A (3.80), Me(OMP) (3.74), H-5B (3.72), H-3C (3.71)), 3.65-3.58 (m, 3H, H-6bA or H-6bC, H-2D, H-5A or H-5C), 3.55 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 5.9 Hz, H-3D), 3.52 (m, 1H, H-5A or H-5C), 3.47-3.44 (m, 2H, H-2B, H-3B), 2.05, 1.99 (2s, 6H, NHAc); ^{13}C-NMR

(125 MHz, MeOD) (selected data from HSQC experiment): δ 129.9-128.4, 118.9, 115.1 (Ar-CH), 105.6 (C-1B), 103.9 (C-1D), 102.1 (C-1A), 101.3 (C-1C), 83.7 (C-3B), 81.8 (C-3C), 81.7 (C-3A), 80.3 (C-3D), 78.9 (C-4B), 77.7 (C-5B), 77.2, 76.5 (C-5A, C-5C), 76.4 (CH$_2$(Bn)), 74.0 (C-2B), 73.6 (CH$_2$(Bn)), 71.7 (C-5D), 71.2 (C-4D), 70.6 (C-2D), 69.4 (C-4C), 68.8 (C-4A), 62.7, 62.3 (C-6A, C-6C), 55.7 (Me(OMP)), 52.7 (C-2C), 52.6 (C-2A), 23.1, 22.8 (NAc); ESI MS: m/z: calcd for C$_{49}$H$_{61}$N$_2$O$_{24}$: 1061.4; found: 1061.2 [M+H]$^-$.

Compound **29** (11.1 µmol) and sulfur trioxide–trimethylamine complex (54 mg, 0.39 mmol) were dissolved in dry DMF (1.5 mL) and heated at 100ºC for 2 h using microwave radiation (20 W average power). The reaction vessel was cooled and Et$_3$N (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 **30** as triethylammonium salt. The residue was converted into the sodium salt by elution from a column of Dowex 50WX4-Na$^+$ with MeOH-H$_2$O 9:1 (6.9 mg, 34% from **26**; 4 steps, 76% average yield per step). Due to extensive overlap of ^{1}H-NMR signals of the sodium salt at 25ºC, **30** was characterized as calcium salt at 40ºC. The calcium salt of **30** was obtained by adding a 0.9 M solution of CaCl$_2$ in D$_2$O. ^{1}H-NMR (500 MHz, D$_2$O, 40ºC, data for calcium salt): δ 7.73 (d, 2H, Ar), 7.63-7.46 (m, 8H, Ar), 7.23 (m, 2H, Ar), 7.11 (m, 2H, Ar), 5.44 (bs, 1H, H-1D), 5.37 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1A), 5.30 (bs, 1H, H-5D), 5.11 (bd, 1H, H-4A), 5.02 (bs, 1H, H-4C), 4.98 (q, 2H, CH$_2$(Bn)), 4.91-4.81 (m, 5H, H-1B (4.90), H-4D (4.89), CH$_2$(Bn), H-1C (4.84)), 4.52 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 7.5 Hz, H-2B), 4.47 (dd, 1H, $J_{5,6a}$ = 2.6 Hz, $J_{6a,6b}$ = 11.3 Hz, H-6aA or H-6aC), 4.43-4.19 (m, 10H, H-2D (4.43), H-6aA or H-6aC, H-3A (4.39), H-3D (4.36), H-6bA, H-6bC, H-5A or H-5C (4.33), H-4B (4.29), H-2C (4.24), H-2A (4.22)), 4.14 (m, 1H, H-3C), 4.10 (bt, 1H, H-5A or H-5C), 3.99-3.94 (m, 5H, H-3B (3.98), H-5B (3.97), Me(OMP) (3.94)),

2.21 (s, 6H, NHAc); ^{13}C-NMR (125 MHz, D_2O, 40ºC) (selected data from HSQC experiment): δ 129.6-128.5, 118.6, 115.2 (Ar-CH), 102.2 (C-1B), 101.5 (C-1D), 100.6 (C-1A), 100.4 (C-1C), 80.2 (C-3B), 79.6 (C-3C), 79.1 (C-2B), 77.5 (C-4B), 77.4 (C-5B), 76.7 (C-3A), 75.7 (C-4A), 75.3 (C-4C), 74.2 (CH_2(Bn)), 72.8 (C-5A or C-5C), 72.6 (C-4D), 72.4 (C-5A or C-5C), 71.9 (C-3D), 71.4 (CH_2(Bn)), 71.2 (C-2D), 68.2 (C-5D), 67.8, 67.4 (C-6A, C-6C), 56.0 (Me(OMP)), 52.4 (C-2A), 52.2 (C-2C), 22.8 (NAc); ESI MS: m/z: calcd for $C_{49}H_{55}N_2O_{45}S_7Na_5$: 865.0; found: 865.0 $[M+5Na+2H]^{2-}$; HR MS: m/z: calcd for $C_{49}H_{60}N_2O_{45}S_7$: 810.0262; found: 810.0253 $[M+7H]^{2-}$.

4-Methoxyphenyl ***O*-(2,4-di-*O*-sulfo-α-L-idopyranosyluronic 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 (1):** A solution of **30** (4.9 mg, 2.7 µmol, sodium salt) in H_2O/MeOH (4.5 mL/0.5 mL) was hydrogenated (1.5 atm) in the presence of Pd(OH)$_2$. After 22 h, the suspension was filtered over Celite and concentrated. The residue was purified by Sephadex G-25 chromatography (H_2O/MeOH 9:1) to give **1** as sodium salt after lyophilisation (3.6 mg, 82%; 28% from **26**, 5 steps, 78% average yield per step). ^{1}H-NMR (500 MHz, D_2O): δ 7.10 (d, 2H, Ar), 6.98 (d, 2H, Ar), 5.29 (bs, 1H, H-1D), 5.22 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1A), 4.98 (bs, 1H, H-5D), 4.95 (bs, 1H, H-4A), 4.76 (m, 1H, H-4C), 4.72 (m, 1H, H-1C), 4.70 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1B), 4.66 (bs, 1H, H-4D), 4.43 (m, 1H, H-3D), 4.35 (bd, 1H, H-6aA or H-6aC), 4.31-4.20 (m, 7H, H-6aA or H-6aC, H-3A (4.27), H-6bA, H-6bC, H-5A or H-5C (4.23), H-2B (4.22), H-2D (4.20)), 4.14-4.07 (m, 4H, H-2A, H-2C, H-5A or H-5C, H-3C), 3.93 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.1 Hz, H-4B), 3.82-3.81 (m, 4H, Me(OMP), H-3B), 3.73 (d, 1H, $J_{4,5}$ = 9.4 Hz, H-5B), 2.11, 2.05 (2s, 6H, NHAc); ^{13}C-NMR (125 MHz, D_2O) (selected data from HSQC experiment): δ 118.8, 115.5 (Ar-CH), 103.1 (C-1B), 101.3 (C-1C), 101.0 (C-1A), 100.7

(C-1D), 81.0 (C-4B), 80.1 (C-2B), 77.0 (C-5B, C-3A), 76.6 (C-3C), 76.4 (C-4A), 76.1 (C-4C), 74.8 (C-4D), 73.9 (C-3B), 73.2, 72.7 (C-5A, C-5C), 72.6 (C-2D), 68.5, 67.9 (C-6A, C-6C), 67.0 (C-5D), 66.5 (C-3D), 56.2 (Me(OMP)), 52.8, 52.4 (C-2A, C-2C), 23.2, 22.8 (NAc); ESI MS: m/z: calcd for $C_{35}H_{43}N_2O_{45}S_7Na_5$: 774.9; found: 774.8 $[M+5Na+2H]^{2-}$; HR MS: m/z: calcd for $C_{35}H_{48}N_2O_{45}S_7$: 719.9792; found: 719.9786 $[M+7H]^{2-}$.

Fluorescence polarization assays

Fluorescence polarization measurements were performed in 384-well microplates (black polystyrene, non-treated, Corning) using a TRIAD multimode reader (Dynex). Fluorescent probes **36**-**40**, recombinant human FGF-2 (Peprotech), and inhibitors were dissolved in PBS buffer (10 mM, pH 7.4). For direct binding, 20 µL of a fluorescent probe solution (20 nM) were transferred to each well. Then 20 µL of FGF-2 solution (concentration ranging from 1.45 µM to 23 nM) were added and the microplate was shaked in the dark for 5 min, before reading. The total sample volume in each well was 40 µL. Control wells contained 20 µL of the fluorescent probe solution and 20 µL of PBS buffer. 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. For inhibition assay, 10 µL of probe and 20 µL of protein at fixed concentration (40 nM and 205 nM, respectively) were mixed with 10 µL of inhibitor solution (100 µM). The total sample volume in each well was again 40 µL. After stirring for 5 min in the dark, fluorescence polarization was recorded. Two control wells containing no inhibitor and probe only (no FGF-2) were included in the study. For the determination of IC_{50} value, wells containing probe and FGF-2 at fixed concentration, as described above, were incubated with 6 different concentrations of inhibitor, ranging from 0.025 µM to 100 µM. Average polarization values of six replicate wells were

plotted against the logarithm of inhibitor concentration, and the curve was fitted to the simplified formula corresponding to a one-site competitive interaction. All the experiments were repeated at least twice.

Occasionally, we found loss of FGF-2 activity when working with low concentrated aliquots in PBS buffer. For this reason, all measurements were alternatively done in PBS + 0.5% BSA (data not shown), getting similar results than those obtained with PBS.
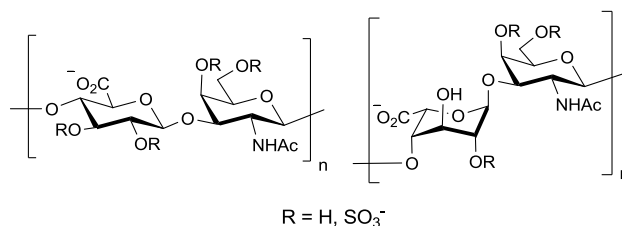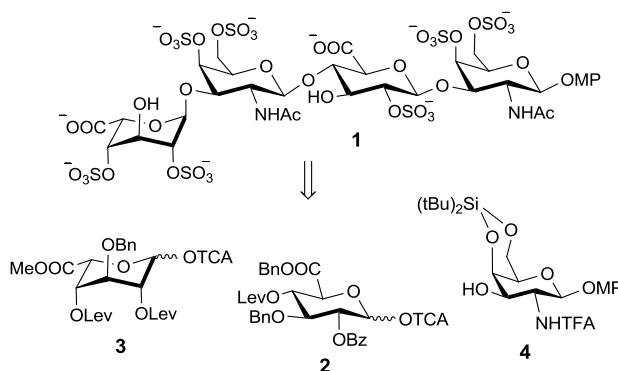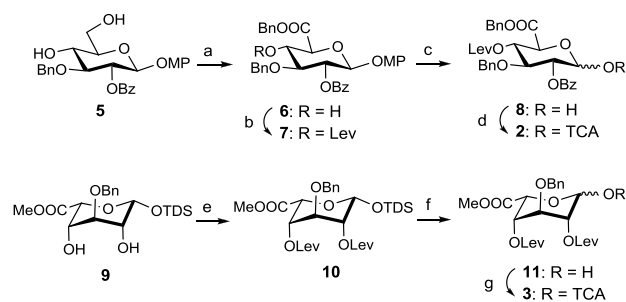

Figure 1. Disaccharide repeating units of chondroitin (left) and dermatan (right) sulfate with potential sites of sulfation indicated.

Scheme 1. Building blocks required for the synthesis of tetrasaccharide **1**.

Scheme 2. a) TEMPO, Bu$_4$NBr, KBr, Ca(ClO)$_2$, NaHCO$_3$, CH$_2$Cl$_2$/H$_2$O, 0°C; BnBr, DMF, Bu$_4$NI, 60°C, 56%; b) LevOH, DCC, DMAP, CH$_2$Cl$_2$, 81%; c) CAN, toluene/CH$_3$CN/H$_2$O, 60%; d) Cl$_3$CCN, K$_2$CO$_3$, CH$_2$Cl$_2$, 96%; e) Lev$_2$O, Py/CH$_2$Cl$_2$, DMAP, 98%; f) (HF)$_n$·Py, THF, 0°C, 77%; g) Cl$_3$CCN, K$_2$CO$_3$, CH$_2$Cl$_2$, 95%.
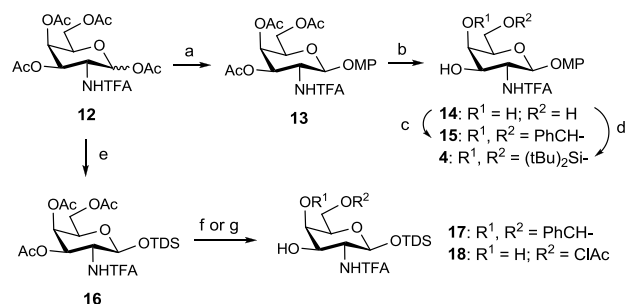
Scheme 3. a) 4-methoxyphenol, TMSOTf, CH$_2$Cl$_2$, 0°C, 42% + 40% starting material; b) NaOMe, MeOH, quantitative; c) PhCH(OMe)$_2$, *p*-TsOH, CH$_3$CN, 87%; d) tBu$_2$Si(OTf)$_2$, Py, 89%; e) BnNH$_2$, THF; TDSCl, imidazole, CH$_2$Cl$_2$, 51%; f) NaOMe, MeOH; PhCH(OMe)$_2$, *p*-TsOH, CH$_3$CN/DMF; g) NaOMe, MeOH; (ClAc)$_2$O, collidine, CH$_2$Cl$_2$, -60°C, 77%.

2 + 4 $\xrightarrow{a}$

b $\left\{\begin{array}{l} \textbf{19}: R = Lev \\ \textbf{25}: R = H \end{array}\right.$

3 + 4 $\xrightarrow{c}$ **20**
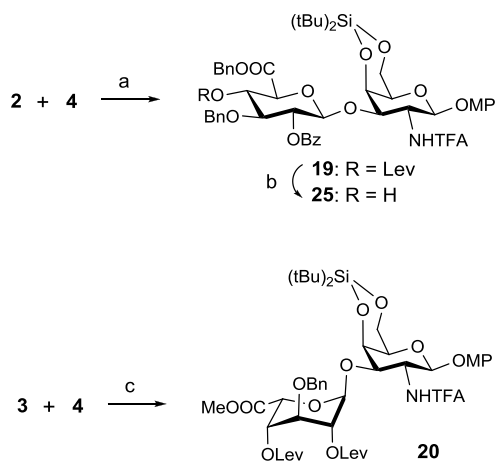
Scheme 4. a) TMSOTf, CH$_2$Cl$_2$, 0ºC, 91%; b) NH$_2$NH$_2$·H$_2$O, Py/AcOH, CH$_2$Cl$_2$, 84%;

c) TMSOTf, CH$_2$Cl$_2$, 0ºC, 79%.

20 $\xrightarrow{a}$ $\xrightarrow{c}$

b $\left\{\begin{array}{l} \textbf{21}: R = H \\ \textbf{22}: R = Ac \end{array}\right.$

d $\left\{\begin{array}{l} \textbf{23}: R = H \\ \textbf{24}: R = TCA \end{array}\right.$

$\xrightarrow{e}$ **26**
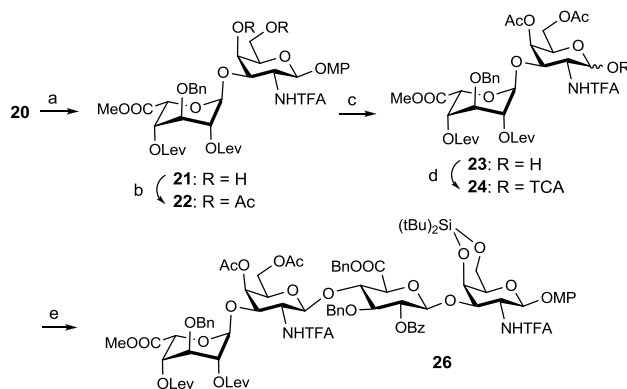
Scheme 5. a) (HF)$_n$·Py, THF, 0ºC, 97%; b) Ac$_2$O, Py, DMAP, 89%; c) CAN,

toluene/CH$_3$CN/H$_2$O, 0ºC, 76%; d) Cl$_3$CCN, DBU, CH$_2$Cl$_2$, 84%; e) **25**, TMSOTf,

CH$_2$Cl$_2$, 0ºC, 74%.
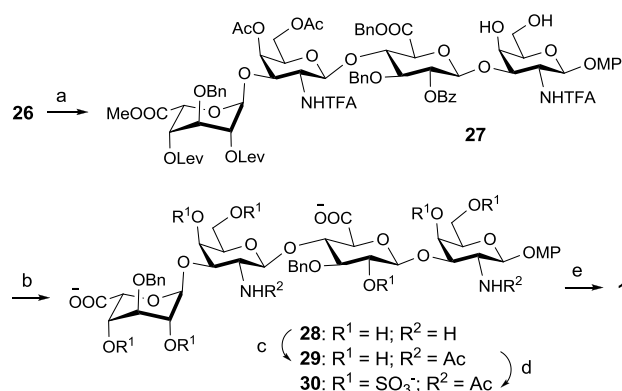
Scheme 6. a) (HF)$_n$·Py, THF, 0ºC; b) LiOH, H$_2$O$_2$, THF; NaOH, MeOH; c) Ac$_2$O, MeOH, Et$_3$N; d) SO$_3$·Me$_3$N, DMF, 100ºC, MW, 34% from **26**, 4 steps, 76% average yield per step; e) H$_2$, Pd(OH)$_2$, H$_2$O/MeOH, 82%.

Table 1. ^{1}H-NMR chemical shifts for sulfated positions of compounds **30** and **1** and the corresponding non-sulfated positions of **29**.

Compound	H-4A	H-6A	H-2B	H-4C	H-6C	H-2D	H-4D
29[a]	4.13	3.84-3.58	3.47-3.44	4.05	3.84-3.58	3.65-3.58	3.99-3.95
30[b]	5.11	4.47-4.19	4.52	5.02	4.47-4.19	4.43	4.89
1[c]	4.95	4.35-4.20	4.22	4.76	4.35-4.20	4.20	4.66

[a] sodium salt, in MeOD; [b] calcium salt, in D$_2$O; [c] sodium salt, in D$_2$O

Table 2. ^{13}C-NMR chemical shifts for sulfated positions of compounds **30** and **1** and the corresponding non-sulfated positions of **29**.

Compound	C-4A	C-6A	C-2B	C-4C	C-6C	C-2D	C-4D
29[a]	68.8	62.7 or 62.3	74.0	69.4	62.7 or 62.3	70.6	71.2
30[b]	75.7	67.8 or 67.4	79.1	75.3	67.8 or 67.4	71.2	72.6
1[c]	76.4	68.5 or 67.9	80.1	76.1	68.5 or 67.9	72.6	74.8

[a] sodium salt, in MeOD; [b] calcium salt, in D$_2$O; [c] sodium salt, in D$_2$O
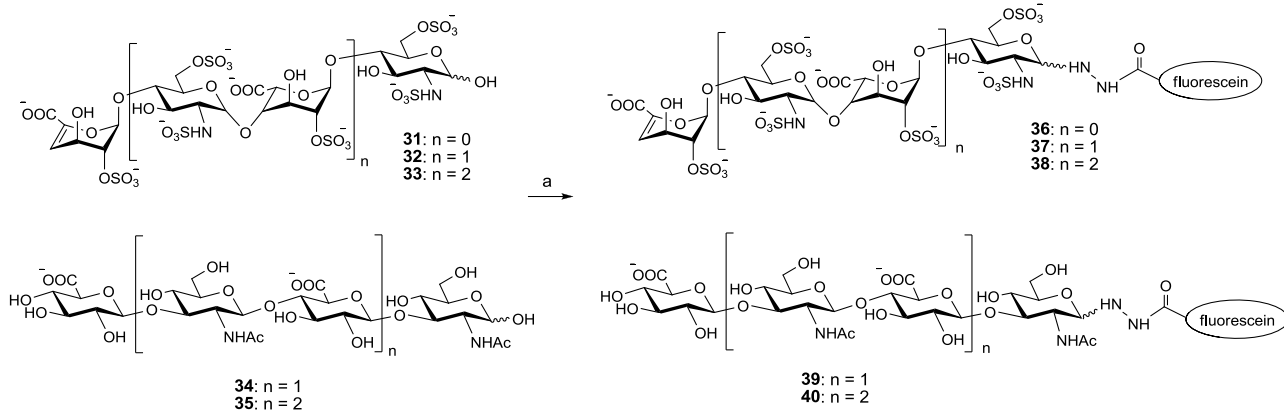
Scheme 7. a) Fluorescein hydrazide, DMSO/phosphate buffer pH 5.5 (1:1), 30°C, 91% (**36**), 91% (**37**), 94% (**38**), 92% (**39**), 95% (**40**).
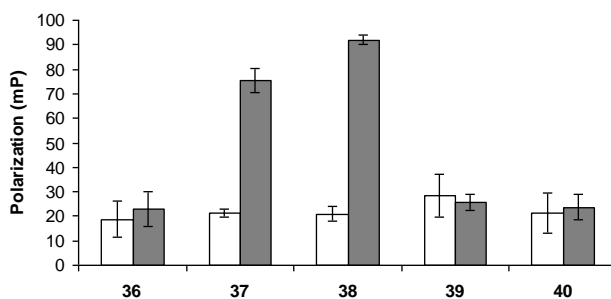

Figure 2. Fluorescence polarization values (right, in grey) from wells containing fluorescent GAG oligosaccharides **36**-**40** (10 nM) and FGF-2 (97 nM) are compared with the values obtained in the absence of the protein (left, in white). For each oligosaccharide, polarization values are the average of three replicate wells and the error bars show the standard deviations for these measurements.
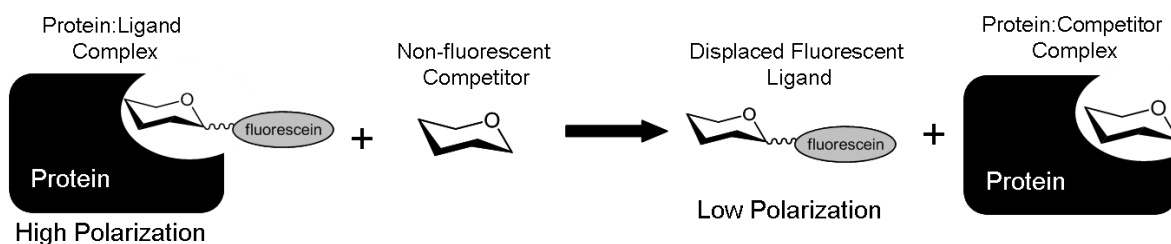
Figure 3. Schematic representation of the competition assay. The displacement of a fluorescent sugar from a protein receptor by an active competitor results in a decrease of the polarization value. Thus, the binding affinities of non-fluorescent compounds can be estimated.
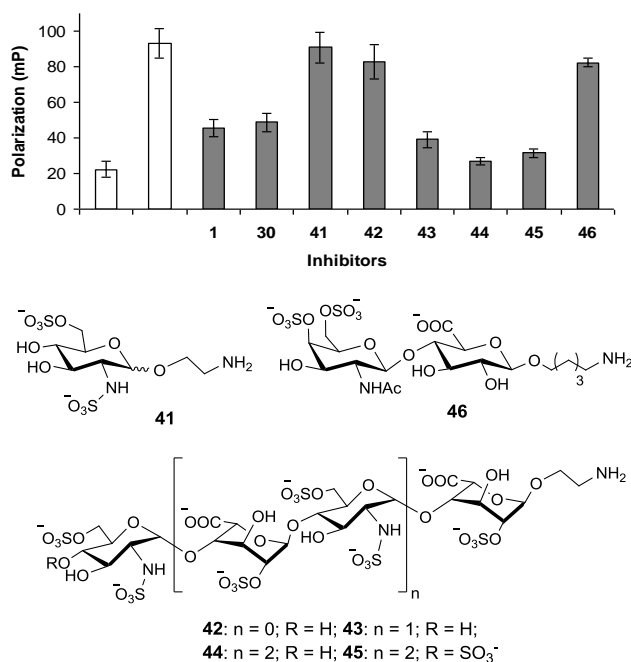

Figure 4. Competition assay to analyse the inhibitory potency of a collection of synthetic oligosaccharides (**1**, **30**, **41**-**46**). The graphic presents the polarization values obtained from wells containing 25 μM inhibitor, 103 nM FGF-2, and 10 nM fluorescent **38**. Control wells (in white) correspond to samples with probe only (left) and no inhibitor (right) and indicate the expected values for 100% and 0% inhibition, respectively. All the measurements are the average of three replicate wells and the error bars show the standard deviations for these measurements.
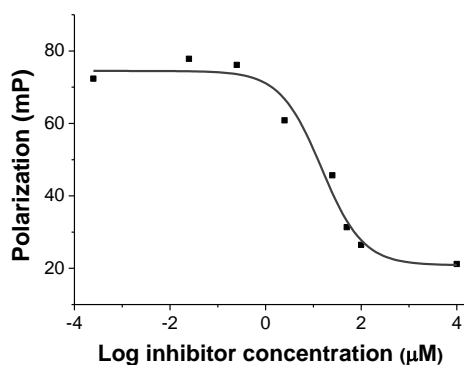
Figure 5. Inhibition curve showing the ability of tetrasacharide **1**, at different concentrations (from 0.025 µM to 100 µM), to inhibit the interaction between FGF-2 (103 nM) and probe **38** (10 nM). The concentration required to inhibit 50% binding (IC$_{50}$ value) was calculated from data analysis (see main text). Control wells, with no inhibitor and no protein, were included in the fitting. All the polarization values are the average of six replicate wells.

Acknowledgements

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