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Pierre Buisson, Elodie Treuillet, Marie Schuler, Chrystel Lopin-Bon. Multigram scale preparation of a semi-synthetic N-trifluoroacetyl protected chondroitin disaccharide building block: Towards the stereoselective synthesis of chondroitin sulfates disaccharides. *Carbohydrate Research*, 2022, 512, pp.108514. 10.1016/j.carres.2022.108514 . hal-03890843

HAL Id: hal-03890843

<https://univ-orleans.hal.science/hal-03890843v1>

Submitted on 22 Jul 2024

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Multigram scale preparation of a semi-synthetic *N*-trifluoroacetyl protected chondroitin disaccharide building block: towards the stereoselective synthesis of chondroitin sulfates disaccharides

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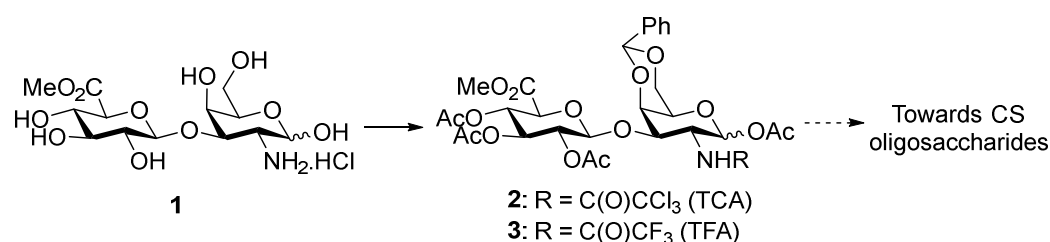
Abstract: the chemoselective *N*-trifluoroacetylation of a chondroitin disaccharide obtained from controlled acid hydrolysis of a commercially available polymeric chondroitin sulfate is reported for the first time. We also described the multi-gram scale synthesis of a donor block having a benzylidene moiety further used for the expeditious and stereocontrolled synthesis of glycosides fitted with various aglycons. Stereocontrolled β -glycosylation, sulfation and efficient *N*-TFA deprotection steps afforded the desired disaccharides in good yields.

Keywords: Chondroitin sulfates • Chemoselective protection • Disaccharides • Glycosylation • Hemisynthesis • Trifluoroacetylation

1. Introduction

Chondroitin sulfates (CS) are heteropolysaccharides that belong to a family of complex, polyanionic polymers called glycosaminoglycans (GAGs). CS are ubiquitously present on the cell surface as well as in the extracellular matrix. They consist of a repeating disaccharide unit (with a highly variable molecular weight) composed of a D-glucuronic acid (D-GlcA) and a 2-acetamido-2-deoxy-D-galactose (D-GalNAc) and contain, on average, one sulfate group per disaccharide unit.¹ The most commonly sulfated positions are positions 4 (CS-A) and/or 6 (CS-E and CS-C, respectively) of the D-GalNAc moiety. Due to their rich structural diversity and to their various sulfation patterns, CS play an important role in numerous biological processes (neuronal development, morphogenesis, inflammation...)² through their interaction with a large variety of binding proteins. Thus, there is a great demand of well-defined oligosaccharides fragments of CS; however, due to their complex structure, their synthesis is not trivial. During the last decade, we developed an original and straightforward method for the preparation of CS oligosaccharides by semi synthesis starting from commercially

available CS polymers.³ This step-economic process allows the synthesis of the key intermediate **1** possessing the basic repeating unit of CS (D-GlcA-D-GalN) (Scheme 1). This disaccharide **1** is a polyhydroxylated compound with an ester moiety and is poorly soluble in organic solvents thus rendering its *N*-chemoselective protection difficult. So far, the trichloroacetyl (TCA) group, known to be a good auxiliary in synthesis of 1,2-*trans*-2-amino-2-deoxy sugars, is the only *N*-protecting group introduced on disaccharide **1**. For example, our group described the multi-gram scale synthesis of a key disaccharide **2**, bearing the *N*-TCA, a benzylidene and acetyl protecting groups as the starting material for the synthesis of a large library of well-defined CS oligosaccharides.⁴ However, the reduction of the TCA group at the end of the synthesis into its *N*-acetyl analog (radical conditions or using Zn-Cu couple) does not always go to completion and mono- or dichloroacetamide species are often isolated. Moreover, those deprotection conditions are not compatible with all kinds of aglycons such as azide or alkene/alkyne moieties for example. Trifluoroacetyl group (TFA) has been described for the synthesis of various 2-acetamido-2-deoxy compounds and has shown to be an efficient auxiliary for stereocontrolled glycosylation easily removed by basic treatment.⁵ Thus, TFA group could be envisaged as a good alternative to TCA group but it has rarely been used in CS oligosaccharides and all the synthetic strategies were based on the use of monomeric units as starting materials (*NH*-TFA galactosamine and glucuronic acid building blocks).⁶ This is why, we report here for the first time a new synthetic pathway which is the gram-scale synthesis of the new key disaccharide building block **3** (Scheme 1) bearing the TFA group, starting from disaccharide **1** using our semi-synthetic strategy. Transformation into one fully deprotected 4-sulfated (CS-A) disaccharide bearing *N*-acetyl group is also reported as a proof of concept.



Scheme 1. Key building blocks **2** and **3**.

2. Results and discussion

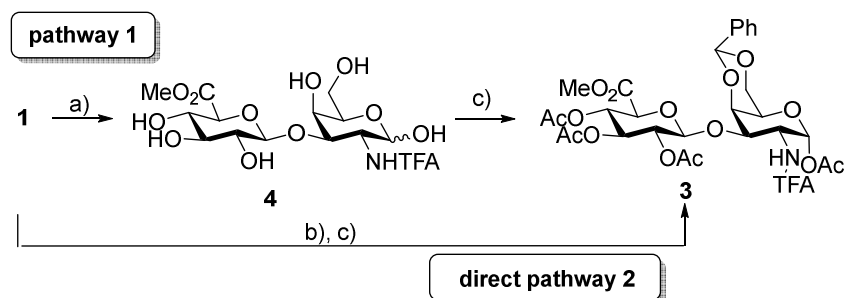
Starting disaccharide methyl ester **1** was prepared by acid hydrolysis of CS polymer, followed by esterification using our usual hemisynthetic process.³ In order to synthesize fully-protected compound **3**, we then investigated the chemoselective *N*-trifluoroacetylation of **1** to obtain intermediate **4** (Scheme 2 (pathway 1, step a), Table 1) that will further be transformed into the target compound. We first envisaged to apply similar conditions to those reported for the chemoselective transformation into *N*-TCA *i.e.* the use of a large excess of acylating reagent in pyridine (Table 1, entry

1).³ Regarding the TCA protection, those conditions allowed the formation of a transient total *O,N*-pertrichloroacetylated compound soluble in organic solvent. Aqueous work up to remove the water-soluble side-products followed by selective solvolysis gave only the *N*-TCA product. However, in the present trifluoroacetylation process, this attempt using excess of (CF₃CO)₂O failed and only traces of the compound **4** were observed. This is probably due to the lability of the O-C(O)CF₃ bond which is hydrolyzed during the work up thus leading to the loss of the polyhydroxylated compound in the aqueous phase. In order to limit salts formation and to avoid the extraction step, the number of equivalents of (CF₃CO)₂O was decreased but once again, only traces of **4** were observed after purification on silica gel (Table 1, entry 2). Changing the base to triethylamine (NEt₃) did not improve this reaction (Table 1, entries 3 and 4). Better results were obtained using ethyl trifluoroacetate as acylating agent with NEt₃ or Na₂CO₃ as the base in MeOH: we were thus able to isolate the desired disaccharide **4** in reasonable quantities (Table 1, entries 5-7). However, it has to be noted that most of the time, the compound could never be obtained in pure form, even after chromatographic purification (normal or reverse phase), and that it was usually accompanied by unseparable by-products (salts). We nevertheless treated this impure compound with benzaldehyde and trifluoroacetic acid (to install the benzylidene group), followed by an *in situ* peracetylation (Scheme 2 (pathway 1, step c)) and the target fully-protected disaccharide **3** was isolated after purification in 19% yield over 3 steps from **1**.

Table 1. Synthesis of **4** by *N*-trifluoroacetylation of **1** (pathway 1).

Entry	Trifluoroacetylating reagent	Equiv.	Base (equiv.)	Solvent	Time	Temp.	Mass of 4
1		11	-	pyridine	2h	0°C	traces
2	(CF ₃ CO) ₂ O	2	-	pyridine	2h	0°C	-
3		2	Et ₃ N 2 equiv.	MeOH	2h	0°C	-
4		1	Et ₃ N 2 equiv.	MeOH	24h	rt	-
5		2	Na ₂ CO ₃ 2 equiv.	MeOH	24h	rt	465 mg ^[a]
6	CF ₃ CO ₂ Et	4	Na ₂ CO ₃ 4 equiv.	MeOH	24h	rt	864 mg ^[a]
7		2	Et ₃ N 2 equiv.	MeOH	24h	rt	1 g ^[a]

^[a] mass of product from 1.2 g of **1**; after purification, product **4** couldn't be isolated totally pure.



Scheme 2. Synthesis of **3**. a) Trifluoroacetylating reagent, base, solvent (see table 1) then purification; b) trifluoroacetylating reagent, base, solvent (see table 2) then evaporation; c) TFA, PhCHO, rt, 1 day then Ac₂O, pyridine, rt, 1 day then purification (see Table 2 for the yield).

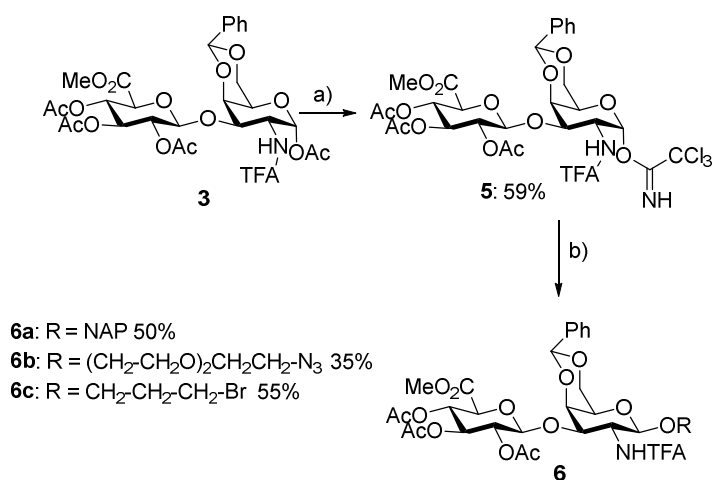
Considering the difficulties to isolate the intermediate **4** pure, we then concentrated our efforts on the direct synthesis of disaccharide **3** (from **1**) using the crude compound **4** (direct pathway 2). To this end, we tried various trifluoroacetylation conditions (Scheme 2 (pathway 2, step b), Table 2) and after a simple evaporation of the reaction, the crude mixture was directly engaged in the benzylidene formation and acetylation steps (Scheme 2 (pathway 2, step c)). By following this direct strategy, we could finally observe the target compound **3** using (CF₃CO)₂O (in pyridine or in MeOH with triethylamine) showing that the *N*-protection occurred (Table 2, entries 1-2). However, we were not able to obtain a pure compound and a significant amount of by-products was observed. As before, changing the acetylating agent for ethyl trifluoroacetate (Table 2, entries 3-6) led to better results and the best conditions (Table 2, entry 6) allowed us to isolate the pure disaccharide **3** with 33% yield from **1**. Interestingly, this direct pathway has been extrapolated with the same yield on large-scale and 13 g of **3** were obtained starting from 22 g of **1**. In comparison, 17 g of NH-TCA protected disaccharide **2** was usually obtained from 22 g of **1** (40% yield).^{4a}

Table 2. *N*-trifluoroacetylation conditions and yield of fully-protected disaccharide **3** (pathway 2).

Entry	Trifluoroacetylating reagent	Equiv.	Base (equiv.)	Solvent	Time	Temperature	Yield of 3 from 1 after 3 steps
1	(CF ₃ CO) ₂ O	3	-	pyridine	4h	rt	8% ^[a]
2		2	Et ₃ N 1.05 equiv.	MeOH	24h	0°C then rt	10% ^[a]
3	CF ₃ CO ₂ Et	2	Et ₃ N 2 equiv.	MeOH	24h	rt	19%
4		2	Et ₃ N 2 equiv.	MeOH	24h	55°C	11%
5		4	Et ₃ N 4 equiv.	MeOH	o/n	rt	8%
6		4	Et ₃ N 2 equiv.	MeOH	o/n	rt	33%

[a] compound **3** couldn't be obtained in pure form

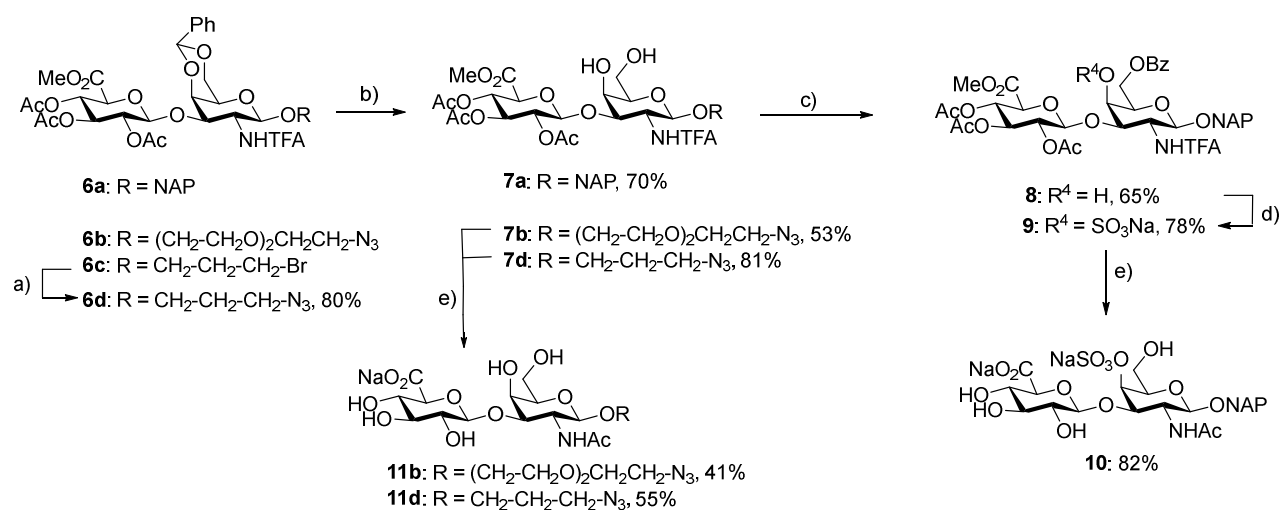
Starting from disaccharide **3** we next envisaged to synthesize various disaccharides. To this end, and in order to introduce various aglycons, we first activated the anomeric position with a trichloroacetimidate moiety and we obtained in two steps the crystalline α -trichloroacetimidate disaccharide **5** in 59% yield using classical conditions⁷(Scheme 3). Despite the presence of a participating group at C-2, donors derivatives in D-galacto series bearing a 4,6-benzylidene are known to lead to mixture during the glycosylation step.⁸ This is explained by the constraint of the 1,3-dioxolane ring that hinders the β -face. In 2009, we showed that it was possible to circumvent this problem using of a soft Lewis acid (boron trifluoride ethyl etherate) at low temperature and we were able to obtain mainly 1,2-*trans*-linked glycosides starting from a disaccharide having NTCA and benzylidene moieties.^{4a} Applying those glycosylation conditions on NTFA disaccharide **5** in the presence of different alcohols (3-bromopropanol, *O*-(2-azidoethyl)triethylene glycol and 2-naphthylmethanol), successfully afforded only the corresponding 1,2-*trans*-glycosides **6a-c** in 35-55% yield (no traces of the 1,2-*cis* analog could be observed) (Scheme 3).



Scheme 3. Synthesis of **5** and glycosylation. a) hydrazine acetate, DMF, rt, 1h; then Cl₃CCN, DBU, CH₂Cl₂, rt, 4h; b) alcohol (ROH), BF₃.OEt₂, CH₂Cl₂, -60°C->-20°C, 2-3h.

Within the frame of a program devoted to the study of cathepsin K (Cat K), a collagenolytic protease involved in osteoporosis disease, we are particularly interested in the synthesis of short 4-sulfated chondroitin (CS-A) oligomers as new tools to understand the enzymatic mechanism of Cat K. We thus envisaged to take advantage of the presence of the benzylidene group to differentiate positions 4 and 6 and to synthesize the 4-sulfated disaccharide **10** (Scheme 4). To this end, an acidic hydrolysis of benzylidene acetal was first performed and diol **7a** was isolated in 70% yield. Regioselective 6-*O*-benzoylation using benzoyl cyanide followed by 4-*O*-sulfonation with sulfur trioxide-trimethylamine complex gave the sodium salt **9**. Final two-step saponification⁹ process with lithium hydroperoxide and sodium hydroxide followed by *N*-acetylation afforded the desired 4-sulfated disaccharide **10** in very good yield, thus showing the ease of deprotection of TFA.

In order to validate the choice of TFA as a protecting group we also considered the full deprotection and conversion of NHTFA into NHAc of disaccharides carrying aglycons that would be affected during NHTCA reduction. To this end, we focused on disaccharides **6b** and **6d** (obtained from the corresponding bromo derivatives **6c** in excellent yield) that both possess an azido terminal group. As previously, hydrolysis of the benzylidene acetal under acidic conditions afforded diols **7b** and **7d**. Two-step saponification followed by selective *N*-acetylation successfully afforded unprotected disaccharides **11b** and **11d** still bearing the azido group in good yields thus confirming again the interest to install the TFA protecting group on our hemi-synthetic disaccharide **1**.



Scheme 4. Synthesis of CS-A disaccharide **10** and azido disaccharides **11b** and **11d**. a) NaN₃, acetone/H₂O, 70°C, 4h; b) TFA/H₂O (3:1), CH₂Cl₂, 0°C, 2 h; c) BzCN, pyridine, RT, 1d; d) Me₃N.SO₃, DMF, 70°C, 3d; e) LiOH/H₂O₂, THF/H₂O, -10°C → rt, 1d; then 4 M NaOH, rt, 2d; then Ac₂O, Et₃N, MeOH/H₂O (4:1), 2h.

3. Conclusion

In conclusion, *N*-TFA protected disaccharide **3** could be prepared on gram-scale from efficient chemoselective protection of a crude chondroitin disaccharide obtained from a hemisynthetic pathway from CS polymer. This enlarges the scope of this process, reported so far solely with a TCA protecting group, thus offering the possibility of working with aglycons labile under TCA deprotection conditions such as azido moieties. Starting from this key building block **3**, we described the synthesis of the elaborate 4-sulfated disaccharide **10**, which is currently under evaluation on Cathepsin K. Finally, stereoselective glycosylations and conversion of TFA into acetyl group could be validated. The synthesis of longer oligosaccharides using this attractive and efficient process is now under study.

4. Experimental

4.1. General methods

Commercial solvents and reagents were purchased from Sigma-Aldrich, Acros, Alfa-Aesar, TCI, Carbosynth and Fluorochem suppliers and were used without further purification unless otherwise stated. Anhydrous solvents were dried by standard methods: DCM was distilled over P₂O₅, THF and acetonitrile were purified with a dry station GT S100 immediately prior use; DMF, methanol and toluene were dried over molecular sieves. Molecular sieves were activated by heating overnight in an Erschem oven at 500°C. Hydrazine acetate was recrystallised in methanol prior to use. For the anhydrous reactions, all glassware was dried in an oven overnight (100°C), then removed and cooled down to rt under argon flow. Argon flow was dried with a solution of conc. H₂SO₄ solution and CaCl₂.

Flash silica column chromatography was performed on silica gel 60N (spherical neutral, 40-63 μm, Merck). The reactions were monitored by thin layer chromatography (TLC) on silica gel 60F254 precoated aluminium plates. Compounds were visualised under UV light (λ= 254 nm or λ=365 nm) and by charring with a 10% H₂SO₄ ethanolic solution or Hanessian's stain.

Infrared spectra (ATR) were recorded on a Perkin-Elmer PARAGON 1000 PC instrument and values are reported in cm⁻¹. Mass Spectra were performed on a Perkin-Elmer Sciex API 300 mass spectrometer (low resolution) and a Bruker MaXis Q-ToF (High resolution) from the "Federation de Recherche" ICOA/CBM FR2708 platform in the electrospray ionisation (ESI) mode. Melting points were determined in open capillary tubes using a Buchi Melting Point B-545 apparatus and values are uncorrected. Optical rotation were measured at 20°C into sodium light using a Jasco P-2000 polarimeter with a quartz tank with a path length of 1 cm, values are given in deg. dm⁻¹.g⁻¹. mL⁻¹ with concentrations reported in g/100 mL.

¹H NMR, ¹³C NMR and ¹⁹F NMR were recorded with Bruker Avance II 250 MHz or an Avance III HD Nanobay 400 MHz spectrometers. CD₃OD, DMSO-*d*₆ and mainly CDCl₃, with tetramethylsilane as internal reference, were used as deuterated solvents. Acetone was added in D₂O NMR samples as an internal reference for carbon NMR. Assignments were based on DEPT 135 sequence, homo- and heteronuclear 2D correlations. Chemical shifts were reported in parts per million (ppm). Coupling constants (*J*) are reported, expressed in Hertz (Hz) and rounded to the nearest 0.5 Hz, splitting patterns are designated as b (broad), s (singlet), d (doublet), dd (doublet of doublet), ddd (doublet of doublet of doublet), t (triplet), dt (doublet of triplet), q (quartet) or m (multiplet). To simplify the NMR attribution, protecting and activated groups have been abbreviated: acetyl group (OAc), benzylidene group (CH-Ph), p-methoxyphenyl group (MP), 2-Naphthylmethyl group (NAP), tosyl group (Ts), trifluoroacetyl group (TFA).

The following solvents have been abbreviated: DCM (dichloromethane), DMF (*N,N*-dimethylformamide), EA (ethyl acetate), EtOH (ethanol), Et₂O (diethyl ether), MeOH (methanol), PE (petroleum ether) and THF (tetrahydrofuran).

4.2. O-(Methyl-β-D-glucopyranosyluronate)-(1→3)-2-amino-2-deoxy-D-galactopyranose hydrochloride salt (1)

Disaccharide **1** (36 g) was obtained by acid hydrolysis of commercially available chondroitin sulfate A sodium salt from bovine trachea (50 g) using reported procedure.³

4.3. O-(Methyl-β-D-glucopyranosyluronate)-(1→3)-2-deoxy-2-trifluoroacetamido-α-D-galactopyranose (4)

Ethyl trifluoroacetate (1.40 mL, 5.9 mmol, 2.0 equiv.) and Et₃N (0.82 mL, 5.92 mmol, 2.0 equiv.) were added to a solution of crude disaccharide **1** (1.2 g, 2.96 mmol, 1.00 equiv.) in MeOH (7.4 mL, 0.4 M). The reaction was stirred for 1 day at rt. The reaction mixture was concentrated under reduced pressure and the product **4** was obtained after purification by silica gel column chromatography (EA/MeOH/H₂O: 15/7/1) as a 2/1 mixture of α/β anomers as a white solid (1.00 g).

*R*_f = 0.50 (EA/MeOH/H₂O: 15/7/1); ¹H NMR (400 MHz, CD₃OD) δ (ppm) = 5.19 (d, ³J₁₋₂ = 3.5 Hz, 1H, H-1 GalN), 4.58 (d, ³J₁₋₂ = 8.0 Hz, 1H, H-1 GlcA), 4.43 (dd, ³J₁₋₂ = 3.5, ³J₂₋₃ = 7.0 Hz, 1H, H-2 GalN), 4.14-4.04 (m, 3H, H-3 GalN, H-4 GalN, H-5 GalN), 3.90 (d, ³J₄₋₅ = 9.5 Hz, 1H, H-5 GlcA), 3.80 (s, 3H, CO₂Me), 3.72-3.70 (m, 2H, H-6 GalN), 3.52 (t, ³J₃₋₄ = 9.5 Hz, 1H, H-4 GlcA), 3.39 (t, ³J₂₋₃ = 9.0 Hz, 1H, H-3 GlcA), 3.31-3.27 (m, 1H, H-2 GlcA); ¹³C NMR (100MHz, CD₃OD) δ (ppm) = 170.1 (C-6 GlcA), 158.1 (q, ²J_{C-F} = 37 Hz, (C=O)CF₃), 116.2 (q, ¹J_{C-F} = 288 Hz, CF₃), 104.3 (C-1 GlcA), 90.9 (C-1 GalN), 76.6 (C-3 or C-4 or C-5 GalN), 75.8 (C-3 GlcA), 75.3 (C-5 GlcA), 73.1 (C-2 GlcA), 71.7 (C-4 GlcA), 70.0 (C-3 or C-4 or C-5 GalN), 68.5 (C-3 or C-4 or C-5 GalN), 61.2 (C-6 GalN), 51.5 (CO₂Me), 50.2 (C-2 GalN); ¹⁹F NMR (376.5 MHz, CD₃OD) δ (ppm) = -76.9 (CF₃); HRMS (ESI⁺) *m/z*: calcd for C₁₇H₂₄F₃NNaO₁₃: 466.1163 [M+Na]⁺; found: 466.1166 IR (neat) $\tilde{\nu}$ (cm⁻¹) = 3313 (N-H), 2958, 2885 (C_{sp3}-H), 1754, 1744, 1707 (C=O), 1550 (C=C), 1368 (C_{sp3}-H), 1224 (C-O), 1174 (C-CF₃), 821 (C_{sp2}-H), 726, 697 (C_{sp2}-H).

4.4. O-(Methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosyluronate)-(1→3)-1-O-acetyl-4,6-O-benzylidene-2-deoxy-2-trifluoroacetamido-α-D-galactopyranose (3)

Ethyl trifluoroacetate (25.7 mL, 215.2 mmol, 4.0 equiv.) and Et₃N (14.8 mL, 107.6 mmol, 2.0 equiv.) were added to a solution of disaccharide **1** (21.8 g, 53.8 mmol, 1.00 equiv.) in MeOH (7.4 mL, 0.4 M). The reaction was stirred for 1 day at rt. The reaction mixture was evaporated under reduced pressure and used without any further purification.

Trifluoroacetic acid (11 mL, 143.6 mmol, 2.7 equiv.) was added to a solution of the previous crude disaccharide in benzaldehyde (220 mL, 0.24 M) under argon atmosphere. The reaction mixture was stirred for 1 day at rt then AcONa (23.9 g, 291.6 mmol, 5.4 equiv.) was added at rt and after 30 min

Ac₂O (65.0 mL, 648.0 mmol, 12.0 equiv.) and DCM/pyridine 1/1 (220 mL, 0.24 M). The reaction mixture was stirred for 1 day then poured into ice. The reaction mixture was extracted 3 times with DCM, then the combined organic layers were washed twice with a saturated aqueous solution of NaHCO₃, dried over MgSO₄, filtered and the solvent evaporated under reduced pressure. The desired product **3** was obtained after purification by silica gel column chromatography (PE/DCM 1/3 up to DCM then DCM/Acetone: 9/1) as a yellow solid (12.75 g, 33%).

R_f = 0.33 (PE/EA 3:7); *m.p.* 137°C (from MeOH); $[\alpha]_{\text{D}}^{20} = + 30.7$ (*c* 0.98, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ (ppm) = 7.50-7.53 (m, 2H, H-Ar), 7.34-7.40 (m, 3H, H-Ar), 6.88 (bs, 1H, NH), 6.47 (d, ³J₁₋₂ = 3.5 Hz, 1H, H-1 GalN), 5.52 (s, 1H, CH-Ph), 5.21-5.30 (m, 2H, H-3, H-4 GlcA), 5.08 (t, ³J₁₋₂ = ³J₂₋₃ = 8.5 Hz, 1H, H-2 GlcA), 4.97 (d, 1H, H-1 GlcA), 4.68 (ddd, ³J_{NH-2} = 8.0, ³J₂₋₃ = 11.0 Hz, 1H, H-2 GalN), 4.48 (d, ³J₃₋₄ = 3.0 Hz, 1H, H-4 GalN), 4.43 (dd, ³J₃₋₄ = 3.0, ³J₂₋₃ = 11.0 Hz, 1H, H-3 GalN), 4.28 (d, ²J_{6a-6b} = 12.5 Hz, 1H, H-6a GalN), 4.15 (d, ³J₄₋₅ = 9.5 Hz, 1H, H-5 GlcA), 4.07 (d, 1H, H-6b GalN), 3.85 (s, 1H, H-5 GalN), 3.74 (s, 3H, CO₂Me), 2.19, 2.06, 2.04, 2.03 (4s, 12H, OAc); ¹³C NMR (100 MHz, CDCl₃) δ (ppm) = 169.79, 169.77, 169.5, 168.9 (4C, OAc), 167.1 (C-6 GlcA), 157.2 (q, ²J_{C-F} = 37 Hz, (C=O)CF₃), 137.3 (C-Ar), 129.1, 128.3, 128.2 (3C, CH-Ar), 115.6 (q, ¹J_{C-F} = 288 Hz, CF₃), 101.0 (CH-Ph), 98.3 (C-1 GlcA), 91.3 (C-1 GalN), 74.5 (C-4 GalN), 72.1 (C-5 GlcA), 71.7 (C-3 or C-4 GlcA), 70.3 (C-2 GlcA), 70.0 (C-3 GalN), 68.9 (C-6 GalN), 68.8 (C-3 or C-4 GlcA), 64.9 (C-5 GalN), 53.1 (CO₂Me), 48.4 (C-2 GalN), 20.8, 20.5, 20.45, 20.42 (4C, CH₃, OAc); ¹⁹F NMR (376.5 MHz, CDCl₃) δ (ppm) = -75.8 (CF₃); IR (neat) $\tilde{\nu}$ (cm⁻¹) = 1748, 1715 (C=O), 1373 (C_{sp3}-H), 1218 (C-O), 1176 (C-CF₃), 701.54 (C_{sp2}-H); HRMS (ESI⁺) *m/z* calcd for C₃₀H₃₄F₃NNaO₁₆: 744.1722 [M+Na]⁺; found: 744.1711.

4.5. *O*-(Methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-trifluoroacetamido-1-*O*-trichloroacetimidoyl-α-D-galactopyranose (**5**)

Hydrazine acetate (1.90 g, 20.69 mmol, 1.5 eq.) was added to a solution of the disaccharide **4** (9.89 g, 13.79 mmol, 1.00 eq.) in dry DMF (100 mL, 0.15M) under argon and the reaction was stirred at rt for 1h. The reaction mixture was poured into ice, extracted 3 times with ethyl acetate then the combined organic layers were washed 5 times with brine. After drying over Na₂SO₄, filtration and evaporation under reduced pressure, the crude product was used without any further purification.

Trichloroacetonitrile (13.8 mL, 137.9 mmol, 10.0 equiv.) and DBU (0.350 mL, 3.44 mmol, 0.25 equiv.) were added to a solution of the previous crude disaccharide in dry DCM (106 mL, 0.13 M) under argon. The reaction mixture was stirred 4h at rt then concentrated under reduced pressure. The desired product **5** was obtained after purification by silica gel column chromatography (PE/EA: 55/45 + 0.3% Et₃N) as a white solid (6.74 g, 59 %).

R_f = 0.44 (PE/EA 55/45); $[\alpha]_{\text{D}}^{20} = + 57.4$ (*c* 1.02, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ (ppm) = 8.79 (s, 1H, NHC(O)CCl₃), 7.49-7.51 (m, 2H, H-Ar), 7.33-7.38 (m, 3H, H-Ar), 6.79 (d, ³J_{NH-2} = 8.0 Hz, 1H, NH-TFA),

6.61 (d, $^3J_{1-2} = 3.5$ Hz, 1H, H-1 GalN), 5.52 (s, 1H, CH-Ph), 5.15-5.26 (m, 2H, H-3, H-4 GlcA), 5.08 (t, $^3J_{1-2} = ^3J_{2-3} = 8.0$ Hz, 1H, H-2 GlcA), 4.97 (d, 1H, H-1 GlcA), 4.74 (ddd, $^3J_{2-3} = 11.0$ Hz, 1H, H-2 GalN), 4.51 (d, $^3J_{3-4} = 3.0$ Hz, 1H, H-4 GalN), 4.37 (dd, 1H, H-3 GalN), 4.29 (dd, $^2J_{6a-6b} = 13.0$, $^3J_{5-6a} = 1.5$ Hz, 1H, H-6a GalN), 4.03-4.14 (m, 2H, H-5 GlcA, H-6b GalN), 3.90 (bs, 1H, H-5 GalN), 3.68 (s, 3H, CO₂Me), 2.01, 2.00, 1.98 (3s, 9H, OAc); **¹³C NMR (100 MHz, CDCl₃)** δ (ppm) = 169.8, 169.6, 169.4 (3C, OAc), 166.7 (C-6 GlcA), 160.3 (NH), 157.1 (q, $^2J_{C-F} = 37$ Hz, (C=O)CF₃), 137.6 (C-Ar), 128.9, 128.3, 128.2 (3C, CH-Ar), 115.6 (q, $^1J_{C-F} = 288$ Hz, CF₃), 100.9 (CH-Ph), 98.8 (C-1 GlcA), 95.3 (C-1 GalN), 90.8 (CCl₃), 74.4 (C-4 GalN), 72.3 (C-5 GlcA), 71.9 (C-3 GlcA or C-4 GlcA), 71.1 (C-3 GalN), 70.6 (C-2 GlcA), 68.84 (C-3 or C-4 GlcA), 68.78 (C-6 GalN), 65.2 (C-5 GalN), 52.9 (CO₂Me), 48.8 (C-2 GalN), 20.8, 20.5, 20.4 (3C, CH₃, OAc); **¹⁹F NMR (376.5 MHz, CDCl₃)** δ (ppm) = -75.8 (CF₃); **IR (neat)** $\tilde{\nu}$ (cm⁻¹) = 3338 (N-H), 1754, 1676 (C=O and C=N), 1370 (C_{sp3}-H), 1212 (C-O), 1157 (C-CF₃), 739.41, 700.12 (C_{sp2}-H); **HRMS (ESI⁺):** *m/z* calcd for C₂₈H₃₂F₃NNaO₁₄: 702.1616 [M-Cl₃CC(NH)+Na]⁺; found: 702.1623.

4.6. 2-Naphthylmethyl *O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-trifluoroacetamido-β-D-galactopyranoside (6a)

2-Naphthylmethanol (305 mg, 1.94 mmol, 1.6 equiv.) was added to a solution of imidate **5** (1.0 g, 1.21 mmol, 1.0 equiv.) and molecular sieves 4Å in dry DCM (6 mL, 0.2M) under argon. The reaction was stirred at rt for 40 min then BF₃.Et₂O 46% (80 μL, 302 μmol, 0.25 equiv.) was added at -60°C and stirred at -60°C to -20°C for 2h. Et₃N (126 μL, 907 μmol, 0.75 equiv.) was added, the reaction was stirred at rt for 10 min, then filtrated and evaporated under reduced pressure and the residue was purified by silica gel column chromatography (PE/EA: 5/5 + 1% Et₃N). The obtained product was precipitated in methanol and, after filtration, the desired product **6a** was obtain as a white solid (502 mg, 50%).

R_f = 0.21 (PE/EA 5/5); [α]_D²⁰ = -10.9 (c 0.98, CH₂Cl₂); **¹H NMR (400 MHz, CDCl₃/(CD₃)₂CO: 2/1)** δ (ppm) = 8.29 (d, $^3J_{NH-2} = 8.0$ Hz, 1H, NH), 7.84-7.74 (m, 4H, H-Ar), 7.57-7.30 (m, 8H, H-Ar), 5.61 (s, 1H, CH-Ph), 5.25-5.10 (m, 2H, H-3, H-4 GlcA), 5.06-4.95 (m, 1H, H-2 GlcA), 4.92 (d, $^3J_{1-2} = 8.0$ Hz, 1H, H-1 GlcA), 4.89 (ABq, 2H, CH₂NAP), 4.82 (d, $^3J_{1-2} = 7.5$ Hz, 1H, H-1 GalN), 4.46 (d, $^3J_{3-4} = 3.0$ Hz, 1H, H-4 GalN), 4.32 (dd, $^2J_{6a-6b} = 11.0$, $^3J_{5-6a} = 3.0$ Hz, 1H, H-6a GalN), 4.21-4.31 (m, 2H, H-2 and H-3 GalN), 4.16 (d, $^3J_{4-5} = 9.5$ Hz, H-5 GlcA), 4.13 (dd, $^3J_{5-6b} = 1.5$ Hz, 1H, H-6b GalN), 3.67 (s, 3H, CO₂Me), 3.57 (m, 1H, H-5 GalN), 1.97, 1.95, 1.94 (3s, 9H, OAc); **¹³C NMR (100 MHz, CDCl₃/(CD₃)₂CO: 2/1)** δ (ppm) = 169.4, 169.0, 168.8 (3C, OAc), 167.0 (C-6 GlcA), 157.0 (q, $^2J_{C-F} = 37$ Hz, (C=O)CF₃), 138.2, 134.8, 133.1, 132.9 (4C, C-Ar), 128.4, 127.9, 127.8, 127.7, 127.5, 126.5, 126.2, 126.0, 125.8, 125.7 (10C, CH-Ar), 116.0 (q, $^1J_{C-F} = 288$ Hz, CF₃), 100.8 (C-1 GlcA), 100.4 (CH-Ph), 98.5 (C-1 GalN), 76.2 (C-3 GalN), 75.1 (C-4 GalN), 72.1 (C-3 GlcA), 71.8 (C-5 GlcA), 70.8 (C-2 GlcA), 69.9 (CH₂ (NAP)), 69.2 (C-4 GlcA), 68.8 (C-6 GalN), 66.5 (C-5 GalN), 53.3 (CO₂Me), 52.2 (C-2 GalN), 20.0, 19.9, 19.7 (3C, CH₃, OAc); **¹⁹F NMR (376.5 MHz, CDCl₃/(CD₃)₂CO: 2/1)** δ (ppm) = -75.8 (CF₃); **IR (neat)** $\tilde{\nu}$ (cm⁻¹) = 3313 (N-H), 2958, 2885 (C_{sp3}-H), 1754, 1744, 1707 (C=O), 1550

(C=C), 1368 cm^{-1} ($\text{C}_{\text{sp}^3\text{-H}}$), 1224 (C-O), 1174 (C-CF₃), 821 cm^{-1} ($\text{C}_{\text{sp}^2\text{-H}}$), 726, 697 ($\text{C}_{\text{sp}^2\text{-H}}$); **HRMS (ESI⁺)**: m/z calcd for C₃₉H₄₀F₃NNaO₁₅ : 842.2242 [M+Na]⁺; found : 842.2242.

4.7. 2-[2-(2-azidoethoxy)ethoxy]ethyl O-(methyl 2,3,4-tri-O-acetyl- β -D-glucopyranosyluronate)-(1 \rightarrow 3)-4,6-O-benzylidene-2-deoxy-2-trifluoroacetamido- β -D-galactopyranoside (6b)

O-(2-Azidoethyl)triethylene glycol (68 mg, 389 μmol , 1.6 equiv.) was added to a solution of disaccharide **5** (200 mg, 243 μmol , 1.0 equiv.) and molecular sieves 4Å in dry DCM (1.2 mL, 0.2 M) under argon atmosphere. The reaction was stirred for 40 min at rt then BF₃.Et₂O 46% (10 μL , 36.5 μmol , 0.15 equiv.) was added at -60°C and stirred at -60°C to -20°C for 3h Et₃N (12 μL , 10.9 μmol , 0.45 equiv.) was added, the reaction was stirred at rt for 10 min, then filtrated and evaporated under reduced pressure and the residue was purified by silica gel column chromatography (PE/EA: 25/75 + 1% Et₃N) to give the desired product **6b** as a white solid (71.1 mg, 35%).

R_f = 0.22 (PE/EA 25/75); $[\alpha]_D^{20}$ = +11.6 (c 1.00, CH₂Cl₂); **¹H NMR (400 MHz, CDCl₃)** δ (ppm) = 7.56-7.54 (m, 2H, H-Ar), 7.41-7.33 (m, 3H, H-Ar), 7.24 (d, ³J_{NH-2} = 7.5 Hz, 1H, NH), 5.56 (s, 1H, CH-Ph), 5.25-5.18 (m, 2H, H-3 GlcA, H-4 GlcA), 5.04-5.00 (m, 1H, H-2 GlcA), 4.99 (d, ³J₁₋₂ = 8.5 Hz, 1H, H-1 GalN), 4.90 (d, ³J₁₋₂ = 7.5 Hz, 1H, H-1 GlcA), 4.48 (dd, ³J₂₋₃ = 11.0Hz, ³J₃₋₄ = 3.5 Hz, 1H, H-3 GalN), 4.40 (d, 1H, H-4 GalN), 4.31 (dd, ³J_{5-6a} = 1.5 Hz, ²J_{6a-6b} = 12.5 Hz, 1H, H-6a GalN), 4.10-4.04 (m, 2H, H-5GlcA, PEG₃(CH₂)_a), 3.92-3.86 (m, 1H, H-2 GalN), 3.74-3.78 (m, 1H, PEG₃(CH₂)_b), 3.76-3.62 (m, 11H, CO₂Me, PEG₃(CH₂)), 3.50 (bs, 1H, H-5 GalN), 3.41-3.38 (m, 2H, PEG₃(CH₂)), 2.02, 2.01, 1.99 (3s, 9H, OAc); **¹³C NMR (100MHz, CDCl₃)** δ (ppm) = 170.0, 169.4, 169.3 (3C, OAc), 167.1 (C-6 GlcA), 157.4 (q, ²J_{C-F} = 37 Hz, (C=O)CF₃), 137.7 (C-Ar), 128.8, 128.13, 128.10 (3C, CH-Ar), 115.7 (q, ¹J_{C-F} = 288 Hz, CF₃), 100.7 (CH-Ph), 100.4 (C-1 GlcA), 99.3 (C-1 GalN), 75.5 (C-4 GalN), 75.4 (C-3 GalN), 72.3 (C-3 or C-4 GlcA), 72.0 (C-5 GlcA), 71.2 (C-2 GlcA), 70.7, 70.5, 70.4, 69.9 (4C, PEG₃(CH₂)), 69.02 (C-6 GalN), 69.01 (C-3 or C-4 GlcA), 68.1 (PEG₃(CH₂)), 66.6 (C-5 GalN), 53.5 (C-2 GalN), 52.9 (CO₂Me), 50.6 (PEG₃(CH₂)), 20.53, 20.46, 20.3 (3C, OAc); **¹⁹F NMR (376.5 MHz, CDCl₃)** δ (ppm) = -75.67 (CF₃); **IR (neat)** $\tilde{\nu}$ (cm^{-1}) = 3289 (N-H), 3055, 2946, 2874 ($\text{C}_{\text{sp}^3\text{-H}}$), 2103 (N₃), 1754 (C=O), 1557, 1440, 1374 ($\text{C}_{\text{sp}^3\text{-H}}$), 1212 (C-O), 1159 (C-CF₃), 725 ($\text{C}_{\text{sp}^2\text{-H}}$); **HRMS (ESI⁺)**: m/z calcd for C₃₄H₄₃F₃N₄NaO₁₇ : 859.2468 [M+Na]⁺; found : 859.2469.

4.8. 3-Bromopropyl O-(methyl 2,3,4-tri-O-acetyl- β -D-glucopyranosyluronate)-(1 \rightarrow 3)-4,6-O-benzylidene-2-deoxy-2-trifluoroacetamido- β -D-galactopyranoside (6c)

3-bromo-1-propanol (661 μL , 6.42 mmol, 1.6 equiv.) was added to a solution of disaccharide **5** (3.3 g, 4.01 mmol, 1.0 equiv.) and molecular sieves 4Å in dry DCM (33 mL, 0.12 M) under argon atmosphere. The reaction was stirred for 40 min at rt then BF₃.Et₂O 46% (267 μL , 1.0 mmol, 0.25 equiv.) was added and stirred at -60°C to -20°C for 2h. Et₃N (418 μL , 3.0 mmol, 0.75 equiv.) was added, the reaction was stirred at rt for 10 min, then filtrated and evaporated under reduced pressure and the residue was

purified by silica gel column chromatography (PE/EA: 50/50 + 0.2% Et₃N) to give the desired product **6c** as a white solid (1.77 g, 55%).

$R_f = 0.22$ (PE/EA 5/5); **m.p.** 180-185°C (from MeOH); $[\alpha]_D^{20} = +19.1$ (c 1.22, CH₂Cl₂); **¹H NMR (400 MHz, CDCl₃)** δ (ppm) = 7.54-7.55 (m, 2H, H-Ar), 7.36-7.42 (m, 3H, H-Ar), 6.97 (d, ³J_{2-NH} = 7.0 Hz, 1H, NH), 5.56 (s, 1H, CH-Ph), 5.19-5.25 (m, 2H, H-3, H-4 GlcA), 5.01-5.06 (m, 1H, H-2 GlcA), 4.99 (d, ³J₁₋₂ = 8.0 Hz, 1H, H-1 GlcA), 4.97 (d, ³J₁₋₂ = 9.0 Hz, 1H, H-1 GalN), 4.53 (dd, ³J₂₋₃ = 11.2 Hz, ³J₃₋₄ = 3.5 Hz, 1H, H-3 GalN), 4.42 (d, 1H, H-4 GalN), 4.33 (dd, ²J_{6a-6b} = 12.5 Hz, ³J_{5-6a} = 1.5 Hz, 1H, H-6a GalN), 3.99-4.09 (m, 3H, H-5 GlcA, H-6b GalN, O-CH₂a), 3.79-3.86 (m, 1H, H-2 GalN), 3.72 (s, 3H, CO₂Me), 3.63-3.69 (m, 1H, O-CH₂b), 3.51 (bs, 1H, H-5 GalN), 3.45-3.48 (m, 2H, CH₂-Br), 2.11-2.19 (m, 1H, -CH₂-a), 1.99-2.06 (m, 10H, 3 OAc and -CH₂-b); **¹³C NMR (100 MHz, CDCl₃)** δ (ppm) = 170.0, 169.44, 169.36 (3C, OAc), 167.1 (C-6 GlcA), 157.5 (q, ²J_{C-F} = 37 Hz, (C=O)CF₃), 137.6 (C-Ar), 128.9, 128.2, 126.1 (3C, CH-Ar), 115.6 (q, ¹J_{C-F} = 288 Hz, CF₃), 100.7 (CH-Ph), 100.0 (C-1 GalN), 99.0 (C-1 GlcA), 75.6 (C-4 GalN), 74.5 (C-3 GalN), 72.3 (C-3 or C-4 GlcA), 71.9 (C-5 GlcA), 71.2 (C-2 GlcA), 69.0 (C-6 GalN), 68.9 (C-3 or C-4 GlcA), 67.1 (O-CH₂), 66.6 (C-5 GalN), 53.9 (C-2 GalN), 52.9 (CO₂Me), 32.4 (-CH₂-), 30.0 (CH₂-Br), 20.53, 20.47, 20.4 (3C, CH₃, OAc); **¹⁹F NMR (376.5 MHz, CDCl₃)** δ (ppm) = -75.8 (CF₃); **IR (neat)** $\tilde{\nu}$ (cm⁻¹) = 3315 (N-H), 2958, 2874 (C_{sp3}-H), 1752 (C=O), 1368 (C_{sp3}-H), 1214 (C-O), 1162 (C-CF₃), 725 (C-Br), 739, 700 (C_{sp2}-H); **HRMS (ESI⁺):** *m/z* calcd for C₃₁H₃₇BrF₃NaNO₁₅ : 822.1190 [M+Na]⁺; found : 822.1197.

4.9. 3-Azidopropyl O-(methyl 2,3,4-tri-O-acetyl- β -D-glucopyranosyluronate)-(1 \rightarrow 3)-4,6-O-benzylidene-2-deoxy-2-trifluoroacetamido- β -D-galactopyranoside (**6d**)

Sodium azide (907 mg, 13.96 mmol, 10.0 equiv.) was added to a solution of bromo derivative **6c** (1.11 g, 1.40 mmol, 1.0 equiv.) in acetone/H₂O: 2/1 (24 mL, 0.06 M). The reaction was heated at 70°C for 4h then the reaction mixture was cooled down to rt and water was added. The resulting precipitate was filtrated to obtain the desired product **6d** as a white solid (840 mg, 80%).

$R_f = 0.22$ (PE/EA 5/5); $[\alpha]_D^{20} = +15.2$ (c 1.06, CH₂Cl₂); **¹H NMR (400 MHz, CDCl₃)** δ (ppm) = 7.54-7.56 (m, 2H, H-Ar), 7.36-7.42 (m, 3H, H-Ar), 7.21 (d, ³J_{2-NH} = 7.0 Hz, 1H, NH), 5.57 (s, 1H, CH-Ph), 5.20-5.25 (m, 2H, H-3, H-4 GlcA), 5.00-5.08 (m, 1H, H-2 GlcA), 5.01 (d, ³J₁₋₂ = 8.0 Hz, 1H, H-1 GalN), 4.94 (d, ³J₁₋₂ = 7.5 Hz, 1H, H-1 GlcA), 4.55 (dd, ³J₂₋₃ = 11.0 Hz, ³J₃₋₄ = 3.5 Hz, 1H, H-3 GalN), 4.43 (d, 1H, H-4 GalN), 4.34 (dd, ²J_{6a-6b} = 12.5 Hz, ³J_{5-6a} = 1.5 Hz, 1H, H-6a GalN), 3.98-4.11 (m, 3H, H-5 GlcA, H-6b GalN, O-CH₂a), 3.78-3.84 (m, 1H, H-2 GalN), 3.73 (s, 3H, CO₂Me), 3.56-3.61 (m, 1H, O-CH₂b), 3.52 (bs, 1H, H-5 GalN), 3.37 (t, ³J₂₋₃ = 6.5 Hz, 2H, CH₂-N₃), 2.04, 2.02, 2.00 (3 s, 9H, OAc), 1.78-1.91 (m, 2H, -CH₂-); **¹³C NMR (100 MHz, CDCl₃)** δ (ppm) = 170.0, 169.48, 169.45 (3C, OAc), 167.1 (C-6 GlcA), 157.5 (q, ²J_{C-F} = 37 Hz, (C=O)CF₃), 137.5 (C_q-Ar), 129.0, 128.2, 126.1 (3C, CH-Ar), 115.6 (q, ¹J_{C-F} = 288 Hz, CF₃), 100.8 (CH-Ph), 100.1 (C-1 GlcA), 98.9 (C-1 GalN), 75.6 (C-4 GalN), 74.5 (C-3 GalN), 72.2 (C-3 or C-4 GlcA), 71.9 (C-5 GlcA), 71.2 (C-2 GlcA), 69.0 (C-6 GalN), 68.9 (C-3 or C-4 GlcA), 66.6 (C-5 GalN), 66.4 (O-CH₂), 53.9 (C-2 GalN),

53.0 (CO₂Me), 48.1 (CH₂-N₃), 28.9 (-CH₂-), 20.52, 20.47, 20.4 (3C, CH₃, OAc) ¹⁹F NMR (376.5 MHz, CDCl₃) δ (ppm) = -75.8 (CF₃); IR (neat) $\tilde{\nu}$ (cm⁻¹) = 3308 (N-H), 2958 (C_{sp3}-H), 2098 (N₃), 1704 (C=O), 1368 (C_{sp3}-H), 1204 (C-O), 1168 (C-CF₃), 699 (C_{sp2}-H); HRMS (ESI⁺): *m/z* calcd for C₃₁H₃₇F₃N₄NaO₁₅ : 785.2099 [M+Na]⁺; found : 785.2097.

4.10. 2-Naphthylmethyl O-(methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosyluronate)-(1→3)-2-deoxy-2-trifluoroacetamido-β-D-galactopyranoside (7a)

A solution of trifluoroacetic acid (1.00 mL, 13.14 mmol, 10.0 equiv.) and water (0.33 mL) (3/1) was added to a solution of disaccharide **6a** (1.07 g, 1.31 mmol, 1.00 equiv.) in DCM (13 mL, 0.1 M). The mixture reaction was stirred for 2h at 0°C. It was then extracted 3 times with ethyl acetate, then the combined organic layers were washed 3 times with water, dried over MgSO₄, filtered and the solvent evaporated under reduced pressure and co-evaporated 2 times with toluene. The desired product **7a** was obtained after purification by silica gel column chromatography (DCM/MeOH: 96/4) as a white solid (671 mg, 70%).

R_f = 0.29 (DCM/Acetone: 90/10); [α]_D²⁰ = -21.9 (*c* 1.07, CH₂Cl₂/MeOH: 1/1); ¹H NMR (400 MHz, CDCl₃/CD₃OD: 3/1) δ (ppm) = 7.81-7.39 (m, 7H, H-Ar), 5.22 (dd, ³J₂₋₃ = ³J₃₋₄ = 9.5 Hz, 1H, H-3 GlcA), (dd, ³J₄₋₅ = 9.5 Hz, 1H, H-4 GlcA), 4.98 (dd, ³J₁₋₂ = ³J₂₋₃ = 9.0 Hz, 1H, H-2 GlcA), 4.91 (ABq, 2H, CH₂NAP), 4.70 (d, ³J₁₋₂ = 7.5 Hz, 1H, H-1 GlcA), 4.62 (d, ³J₁₋₂ = 8.0 Hz, 1H, H-1 GalN), 4.10 (d, 1H, H-5 GlcA), 4.10-4.06 (m, 2H, H-2, H-4 GalN), 3.96 (dd, 1H, H-3 GalN), 4.32 (dd, ²J_{6a-6b} = 11.5 Hz, ³J_{5-6a} = 6.5 Hz, 1H, H-6a GalN), 4.32 (dd, ²J_{6a-6b} = 11.5, ³J_{5-6b} = 5.5 Hz, 1H, H-6b GalN), 3.74 (s, 3H, CO₂Me), 3.52 (dd, 1H, H-5 GalN), 2.03, 2.01 (2s, 9H, OAc) ; ¹³C NMR (100 MHz, CDCl₃/CD₃OD: 3/1) δ (ppm) = 170.6, 170.2 (3C, OAc), 168.1 (C-6 GlcA), 158.3 (q, ²J_{C-F} = 37 Hz, (C=O)CF₃), 134.8, 133.5, 133.4 (3C, C-Ar), 128.4, 128.1, 127.9, 127.0, 126.4, 126.3, 125.9 (7C, CH-Ar), 116.3 (q, ¹J_{C-F} = 288 Hz, CF₃), 101.3 (C-1 GlcA), 99.1 (C-1 GalN), 79.1 (C-4 GalN), 75.0 (C-5 GalN), 72.3 (C-3 GalN or C-5 GlcA), 72.1 (C-3 GlcA), 71.2 (C-2 GlcA), 70.7 (CH₂ (NAP)), 69.7 (C-4 GlcA), 68.2 (C-3 GalN or C-5 GlcA), 61.6 (C-6 GalN), 53.2 (CO₂Me), 52.7 (C-2 GalN), 20.6, 20.5, 20.3 (3C, CH₃, OAc) ; ¹⁹F NMR (376.5 MHz, CDCl₃/CD₃OD: 3/1) δ (ppm) = -72.0 (CF₃); IR (neat) $\tilde{\nu}$ (cm⁻¹) = 3503, 3309 (N-H and O-H), 2939 (C_{sp3}-H), 1746, 1698 (C=O), 1563 (C=C), 1377 (C_{sp3}-H), 1219 (C-O), 1166 (C-CF₃), 816 (C_{sp2}-H), 727, 700 (C_{sp2}-H); HRMS (ESI⁺): *m/z* calcd for C₃₂H₃₆F₃NNaO₁₅ : 754.1929 [M+Na]⁺; found : 754.1927.

4.11. 2-[2-(2-azidoethoxy)ethoxy]ethyl O-(methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosyluronate)-(1→3)-2-deoxy-2-trifluoroacetamido-β-D-galactopyranoside (7b)

A solution of trifluoroacetic acid (150 μL, 2.03 mmol, 10.0 equiv.) and water (50 μL) (3/1) was added to a solution of disaccharide **6b** (170 mg, 203 μmol, 1.00 equiv.) in DCM (2 mL, 0.1 M). The reaction

mixture was stirred at 0°C for 2h then evaporated and co-evaporated with toluene under reduced pressure. The desired product **7b** was obtained after purification by silica gel column chromatography (DCM/MeOH: 95/5) as a white solid (79.0 mg, 53%).

$R_f = 0.15$ (DCM/MeOH: 95/5); $[\alpha]_D^{20} = +10.6$ (c 1.00, CH₂Cl₂); **¹H NMR (400 MHz, CDCl₃)** δ (ppm) = 7.76 (d, ³J_{NH-2} = 8.5 Hz, 1H, NH), 5.27 (dd, ³J₂₋₃ = ³J₃₋₄ = 9.5 Hz, 1H, H-3 GlcA), 5.20 (dd, ³J₄₋₅ = 9.5 Hz, 1H, H-4 GlcA), 5.04 (dd, ³J₁₋₂ = 7.5 Hz, 1H, H-2 GlcA), 4.84 (d, ³J₁₋₂ = 9.0 Hz, 1H, H-1 GalN), 4.81 (d, 1H, H-1 GlcA), 4.19-4.15 (m, 3H, H-3 GalN, H-4 GalN, H-5 GlcA), 4.02-3.92 (m, 3H, H-2 GalN, H-6a GalN, PEG₃), 3.86-3.78 (m, 2H, H-6b GalN, PEG₃(CH₂)), 3.76 (s, 3H, CO₂Me), 3.63-3.72 (m, 9H, H-5 GalN, PEG₃(CH₂)), 3.54 (bs, 1H, OH), 3.50-3.39 (m, 2H, PEG(CH₂)), 3.02 (bs, 1H, OH), 2.04, 2.03, 2.02 (3s, 9H, OAc); **¹³C NMR (100 MHz, CDCl₃)** δ (ppm) = 170.0, 169.8, 169.6 (3C, OAc), 167.5 (C-6 GlcA), 157.4 (q, ²J_{C-F} = 37 Hz, (C=O)CF₃), 115.8 (q, ¹J_{C-F} = 288 Hz, CF₃), 100.9 (C-1 GlcA), 100.0 (C-1 GalN), 79.3 (C-5 GlcA or C-3 GalN or C-4 GalN), 73.9 (C-5 GalN), 72.0 (C-3 GlcA), 71.7 (C-5 GlcA or C-3 GalN or C-4 GalN), 71.0 (C-2 GlcA), 70.7, 70.4, 70.3, 69.8 (4C, PEG₃(CH₂)), 69.1 (C-4 GlcA), 68.4 (2C, C-5 GlcA or C-3 GalN or C-4 GalN and C-6 GalN), 62.2 (PEG₃(CH₂)), 53.1 (CO₂Me), 53.0 (C-2 GalN), 50.5 (PEG₃(CH₂)), 20.5, 20.4, 20.2 (3C, OAc); **¹⁹F NMR (376.5 MHz, CDCl₃)** δ (ppm) = -75.50 (CF₃); **IR (neat)** $\tilde{\nu}$ (cm⁻¹) = 3521, 3310 (N-H and O-H), 2947, 2882 (C_{sp3}-H), 2100 (N₃), 1744, 1712 (C=O), 1557, 1442, 1373 (C_{sp3}-H), 1213 (C-O), 1165 (C-CF₃), 1091, 1037 (C-O) 725 cm⁻¹ (C_{sp2}-H); **HRMS (ESI⁺):** m/z calcd for C₂₇H₃₉F₃N₄NaO₁₇ : 771.2155 [M+Na]⁺; found : 771.2158.

4.12. 3-Azidopropyl *O*-(methyl 2,3,4-tri-*O*-acetyl- β -D-glucopyranosyluronate)-(1 \rightarrow 3)-2-deoxy-2-trifluoroacetamido- β -D-galactopyranoside (**7d**)

A solution of trifluoroacetic acid (685 μ L, 8.95 mmol, 10.0 equiv.) and water (228 μ L) (3/1) was added to a solution of disaccharide **6d** (682 mg, 895 μ mol, 1.00 equiv.) in DCM (15 mL, 0.06 M). The reaction mixture was stirred at 0°C for 2h then evaporated and co-evaporated with toluene under reduced pressure. The desired product **7d** was obtained after purification by silica gel column chromatography (DCM/MeOH: 95/5) as a white solid (489 mg, 81%).

$R_f = 0.42$ (DCM/MeOH 95/5); $[\alpha]_D^{20} = + 5.1$ (c 1.08, CH₂Cl₂); **¹H NMR (400 MHz, CDCl₃/CD₃OD: 1/1)** δ (ppm) = 8.85 (d, ³J_{NH-2} = 7.5Hz, 1H, NH), 5.25 (dd, ³J₂₋₃ = ³J₃₋₄ = 9.5 Hz, 1H, H-3 GlcA), 5.13 (dd, ³J₄₋₅ = 9.5 Hz, 1H, H-4 GlcA), 4.99 (dd, ³J₁₋₂ = 8.5 Hz, 1H, H-2 GlcA), 4.76 (d, 1H, H-1 GlcA), 4.58 (d, ³J₁₋₂ = 8.0 Hz, 1H, H-1 GalN), 4.15 (d, 1H, H-5 GlcA), 4.10-4.07 (m, 1H, H-4 GalN), 3.87-4.02 (m, 4H, H-2 GalN, H-3 GalN, H-6a GalN, O-CH₂a), 3.85-3.73 (m, 4H, CO₂Me, H-6b GalN), 3.51-3.61 (m, 2H, H-5 GalN, O-CH₂b), 3.35 (t, ³J = 6.5 Hz, 2H, CH₂-N₃), 2.04, 2.02, 2.01 (3 s, 9H, OAc), 1.87-1.75 (m, 2H, -CH₂-); **¹³C NMR (100 MHz, CDCl₃/CD₃OD: 1/1)** δ (ppm) = 174.1, 173.7, 171.7 (3C, OAc), 161.8 (q, ²J_{C-F} = 37 Hz, (C=O)CF₃), 122.7 (q, ¹J_{C-F} = 288 Hz, CF₃), 104.9 (C-1 GlcA), 104.0 (C-1 GalN), 82.8 (C-3 GalN), 78.3 (C-5 GalN), 75.8 (C-3 GlcA), 75.7 (C-5 GlcA), 74.8 (C-2 GlcA), 73.2 (C-4 GlcA), 71.7 (C-4 GalN), 70.0 (O-CH₂), 65.3 (C-6 GalN), 56.9

(CO₂Me), 56.4 (C-2 GalN), 51.7 (CH₂-N₃), 32.8 (-CH₂-), 24.2, 24.1, 23.9 (3C, OAc); **¹⁹F NMR (376.5 MHz, CDCl₃/CD₃OD: 1/1)** δ (ppm) = -72.0 (CF₃); **IR (neat)** $\tilde{\nu}$ (cm⁻¹) = 3515, 3309, (N-H and O-H), 2953 (C_{sp3}-H), 2100 (N₃), 1750, 1696 (C=O), 1372 (C_{sp3}-H), 1212 (C-O), 1160 (C-CF₃); **HRMS (ESI⁺):** *m/z* calcd for C₂₄H₃₃F₃N₄NaO₁₅ : 697.1787 [M+Na]⁺; found : 697.1787.

4.13. 2-Naphthylmethyl *O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-(1→3)-6-benzoyl-2-deoxy-2-trifluoroacetamido-β-D-galactopyranoside (**8**)

Benzoyl cyanide (131 mg, 1.52 mmol, 2.0 equiv.) was added to a solution of disaccharide **7a** (555 mg, 758 μmol, 1.00 equiv.) in pyridine (11 mL, 0.07 M). The reaction mixture was stirred for 24h at rt then quenched by addition of methanol (15 mL). After 30 minutes, the reaction mixture was extracted 3 times with ethyl acetate and the combined organic layers were washed 3 times with water. The solvent was dried over MgSO₄, filtrated and evaporated under reduced pressure. The desired product **8** was obtained after purification by silica gel column chromatography (DCM/Acetone: 90/10) as a white solid (410 mg, 65%).

R_f = 0.33 (DCM/Acetone 89/11); **[α]_D²⁰** = -26.7 (*c* 1.07, CH₂Cl₂/MeOH: 9/1); **¹H NMR (250 MHz, CDCl₃/CD₃OD: 3/1)** δ (ppm) = 8.10-8.06 (m, 2H, H-Ar), 7.81-7.71 (m, 2H, H-Ar), 7.68-7.61 (m, 3H, H-Ar), 7.52-7.43 (m, 4H, H-Ar), 7.36-7.34 (m, 1H, H-Ar), 5.22 (dd, ³J₂₋₃ = ³J₃₋₄ = 9.5 Hz, 1H, H-3 GlcA), 5.12 (dd, ³J₄₋₅ = 9.5 Hz, 1H, H-4 GlcA), 5.00 (dd, ³J₁₋₂ = 8.0 Hz, 1H, H-2 GlcA), 4.88 (ABq, 2H, CH₂NAP), 4.70 (d, 8.0 Hz, 1H, H-1 GlcA), 4.70 (d, ³J₁₋₂ = 8.0 Hz, 1H, H-1 GalN), 4.66-4.58 (m, 2H, H-6a, H-6b GalN), 4.15 (d, ³J₃₋₄ = 3.0 Hz, 1H, H-4 GalN), 4.09 (d, ³J₄₋₅ = 10.0 Hz, 1H, H-5 GlcA), 4.11-4.08 (m, 1H, H-3 GalN), 4.02 (t, ³J₂₋₃ = 8.0 Hz, 1H, H-2 GalN), 3.86 (t, ³J₅₋₆ = 6.0 Hz, 1H, H-5 GalN), 3.68 (s, 3H, CO₂Me), 3.35 (bs, 1H, OH), 2.02, 2.01 (2s, 9H, 3 OAc); **¹³C NMR (100MHz, CDCl₃/CD₃OD: 3/1)** δ (ppm) = 174.2, 174.1, 173.8 (3C, OAc), 171.3, 170.6 (OBz and C-6 GlcA), 161.9 (q, ²J_{C-F} = 37 Hz, (C=O)CF₃), 138.1, 137.2, 137.0, 136.9 (4C, C-Ar), 133.7, 133.5, 132.4, 132.1, 131.6, 131.5, 130.8, 130.0, 129.9, 129.6 (10C, CH-Ar), 119.8 (q, ¹J_{C-F} = 288 Hz, CF₃), 105.0 (C-1 GlcA), 101.9 (C-1 GalN), 82.3 (C-5 GlcA), 76.2 (C-5 GalN), 75.9 (C-3 GlcA), 75.6 (C-3 GalN), 74.8 (C-2 GlcA), 74.1 (CH₂ (NAP)), 73.3 (C-4 GlcA), 71.7 (C-4 GalN), 67.5 (C-6 GalN), 56.8 (CO₂Me), 56.5 (C-2 GalN), 24.2, 24.1, 23.8 (3C, CH₃, OAc); **¹⁹F NMR (376.5 MHz, CDCl₃/CD₃OD: 3/1)** δ (ppm) = -72.0 (CF₃); **IR (neat)** $\tilde{\nu}$ (cm⁻¹) = 3491, 3297 (N-H and O-H), 2939 (C_{sp3}-H), 1739 1723, 1701 (C=O), 1566 (C=C), 1379 (C_{sp3}-H), 1222 (C-O), 1166 (C-CF₃), 816 (C_{sp2}-H), 709 (C_{sp2}-H); **HRMS (ESI⁺):** *m/z* calcd for C₃₉H₄₀F₃NNaO₁₆ : 858.2193 [M+Na]⁺; found : 858.2191.

4.14. 2-Naphthylmethyl *O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-(1→3)-sodium-6-benzoyl-2-deoxy-2-trifluoroacetamido-4-*O*-sulfonato-β-D-galactopyranoside (**9**)

Sulfur trioxide trimethylamine complex (125 mg, 897 μmol, 15 equiv.) was added to a solution of disaccharide **8** (50 mg, 59.8 μmol, 1.00 equiv.) in dry DMF (0.5 mL, 0.11 M). The reaction mixture was

stirred for 48h at 70°C then sulfur trioxide trimethylamine complex was added again (83 mg, 598 μmol , 10 equiv.) and the reaction then was heated for 24h. The reaction was cooled down to rt, quenched by addition of methanol (2 mL) and stirred 30 min. The crude mixture was directly purified by Sephadex LH-20 (DCM/MeOH 1/1) then by Sephadex SP C25 [Na⁺] (DCM/MeOH/H₂O: 9/5/1) to give the desired product **9** (44 mg, 78%) as a white solid.

R_f = 0.33 (DCM/MeOH 8/1); $[\alpha]_D^{20}$ = -32.5 (*c* 0.90, CH₂Cl₂/MeOH/H₂O: 5/5/1); **¹H NMR (400 MHz, DMSO-*d*₆)** δ (ppm) = 9.19 (d, ³ $J_{\text{NH-2}}$ = 9.0 Hz, 1H, N-H), 8.07-7.29 (m, 12H, H-Ar), 5.21 (dd, ³ J_{2-3} = ³ J_{3-4} = 9.0 Hz, 1H, H-3 GlcA), 5.04 (dd, ³ J_{4-5} = 9.5 Hz, 1H, H-4 GlcA), 4.91-4.82 (m, 2H, H-1, H-2 GlcA), 4.77 (ABq, 2H, CH₂NAP), 4.61 (d, ³ J_{3-4} = 3.0 Hz, 1H, H-4 GalN), 4.57 (d, ³ J_{5-6} = 6.0 Hz, 2H, H-6 GalN), 4.30 (d, ³ J_{1-2} = 8.0 Hz, 1H, H-1 GalN), 4.28 (d, ³ J_{4-5} = 10.0 Hz, 1H, H-5 GlcA), 4.08-4.01 (m, 1H, H-2 GalN), 3.98 (d, ³ J_{2-3} = 10.0 Hz, 1H, H-3 GalN), 3.81 (t, 1H, H-5 GalN), 3.64 (s, 3H, CO₂Me), 1.97, 1.93, 1.89 (3s, 9H, 3 OAc); **¹³C NMR (100 MHz, DMSO-*d*₆)** δ (ppm) = 170.0, 169.7, 169.5 (3C, OAc), 167.9, 166.0 (OBz and C-6 GlcA), 156.9 (q, ² $J_{\text{C-F}}$ = 37 Hz, (C=O)CF₃), 135.1, 133.8, 133.1, 133.0 (4C, C-Ar), 130.4, 129.8, 129.2, 128.3, 128.0, 127.9, 126.7, 126.6, 126.5, 126.1 (10C, CH-Ar), 116.5 (q, ¹ $J_{\text{C-F}}$ = 288 Hz, CF₃), 100.4 (C-1 GlcA), 99.1 (C-1 GalN), 75.7 (C-3 GalN), 73.3 (C-4 GalN), 73.1 (C-5 GalN), 72.6 (C-3 GlcA), 71.6 (C-5 GlcA), 71.4 (C-2 GlcA), 69.6 (CH₂ (NAP)), 69.4 (C-4 GlcA), 65.0 (C-6 GalN), 53.1 (CO₂Me), 52.3 (C-2 GalN), 20.7, 20.6, 20.5 (3C, CH₃, OAc); **¹⁹F NMR (376.5 MHz, DMSO-*d*₆)** δ (ppm) = -74.4 (CF₃); **IR (neat)** $\tilde{\nu}$ (cm⁻¹) = 3310 (N-H), 2958 (C_{sp3}-H), 1745, 1705 (C=O), 1559 (C=C), 1372 (C_{sp3}-H), 1226 (C-O), 1167 (C-CF₃); **HRMS (ESI):** *m/z* calcd for C₃₉H₃₉F₃NO₁₉S : 914.1497 [M-Na⁺]; found : 914.1792.

4.15. 2-Naphthylmethyl O-(sodium- β -D-glucopyranosyluronate)-(1 \rightarrow 3)-sodium-2-acetamido-2-deoxy-4-O-sulfonato- β -D-galactopyranoside (**10**)

A cold solution of LiOH 1M (250 μL , 250 μmol , 5.0 equiv./CO₂Me) and H₂O₂ 30% (123 μL) was added to a solution of disaccharide **9** (50 mg, 59.8 μmol , 1.00 equiv.) in THF/H₂O 3/1 (2 mL) at 0°C then the reaction mixture was stirred at rt for 24h. It was then diluted with a solution of methanol/water 3/1 (6.5 mL) and NaOH 4M was added (1 mL, 2.96 mmol, 15 equiv./ester). The reaction mixture was stirred for 48h at rt, then neutralised till pH = 7 with Amberlite IR 120 [H⁺] resin, filtrated, evaporated under reduced pressure and the crude mixture was used without any further purification.

Triethylamine (90 μL , 0.64 mmol, 13 equiv.) and acetic anhydride (100 μL , 0.99 mmol, 20 equiv.) was added to a solution a previously crude in MeOH/H₂O 4/1 (5 mL) for 2h at rt. Amberlite IR 120 [H⁺] resin was added to the reaction mixture until pH = 5, then the reaction mixture was filtrated, concentrated and co-evaporated with water 2 times under reduced pressure. Then NaOH 1M was added to the mixture until pH = 10, followed by a purification using Sephadex LH-20. The product was then eluted from a column of DOWEX 50WX4-Na⁺ and finally purified using Reveleris[®] column chromatography on

C-18 reverse phase (H₂O/MeOH 100/0 to H₂O/MeOH 9/1) to give the deprotected dissaccharide **10** as a sodium salt (26.9 mg, 82%).

$R_f = 0.48$ (AE/MeOH/H₂O 4/2/1); $[\alpha]_D^{20} = -11.1$ (c 1.00, H₂O); $^1\text{H NMR}$ (400 MHz, D₂O) δ (ppm) = 7.93-7.43 (m, 7H, H-Ar), 4.88 (ABq, 2H, CH₂NAP), 4.76-4.70 (m, 1H, H-4 GalN), 4.54 (d, $^3J_{1-2} = 8.0$ Hz, 1H, H-1 GalN), 4.33 (d, $^3J_{1-2} = 8.0$ Hz, 1H, H-1 GlcA), 4.03-4.93 (m, 2H, H-2, H-3 GalN), 3.78-3.74 (m, 3H, H-5, H-6 GalN), 3.53 (d, $^3J_{4-5} = 10.0$ Hz, 1H, H-5 GlcA), 3.45 (dd, $^3J_{3-4} = 9.0$ Hz, 1H, H-4 GlcA), 3.45-3.35 (t, $^3J_{2-3} = 9.0$ Hz, 1H, H-3 GlcA), 3.27 (t, 1H, H-2 GlcA), 1.77 (s, 3H, NH-Ac); $^{13}\text{C NMR}$ (100 MHz, D₂O) δ (ppm) = 175.8, 174.5 (C-6 GlcA and NH-Ac), 134.4, 132.9, 132.8 (3C, C-Ar), 128.4, 127.9, 127.7, 127.5, 126.7, 126.6, 126.4 (10C, CH-Ar), 103.1 (C-1 GlcA), 100.0 (C-1 GalN), 76.4, 76.3 (C-4 GalN and C-5 GlcA), 75.1 (C-3 GlcA), 74.5 (2C, C-3 and C-5 GalN), 72.5 (C-2 GlcA), 71.8 (C-4 GlcA), 71.6 (CH₂-NAP), 61.1 (C-6 GalN), 51.9 (C-2 GalN), 22.2 (CH₃, NH-Ac); IR (neat) $\tilde{\nu}$ (cm⁻¹) = 3390 (O-H and N-H), 2891 (C_{sp3}-H), 1605 (C=O), 1414 (C_{sp3}-H); HRMS (ESI): m/z calcd for C₂₅H₂₉NNa₂O₁₅S : 307.5637 [M-2Na]²⁻; found : 307.5634.

4.16. 2-[2-(2-azidoethoxy)ethoxy]ethyl O-(sodium- β -D-glucopyranosyluronate)-(1 \rightarrow 3)-2-acetamido-2-deoxy- β -D-galactopyranoside (**11b**)

A cold solution of LiOH 1M (267 μ L, 267 μ mol, 5.0 equiv.) and H₂O₂ 30% (130 μ L) was added to a solution of disaccharide **7b** (40 mg, 53 μ mol, 1.00 equiv.) in THF/H₂O: 3/1 (4 mL) at 0°C then rt. After 24h, the reaction mixture was diluted by addition of a solution of methanol/water 4/1 (5 mL) and NaOH 4M was added (800 μ L, 5.08 mmol, 60.0 equiv.). The reaction mixture was stirred for 48h at rt, then neutralised at pH = 7 with Amberlite IR 120 [H⁺] resin, filtrated, evaporated under reduced pressure and the crude mixture was used without any further purification.

Triethylamine (96 μ L, 0.689 mmol, 13.0 equiv.) and acetic anhydride (106 μ L, 1.06 mmol, 20 equiv.) were added to a solution of the crude disaccharide in MeOH/H₂O: 4/1 (5 mL) for 2h at rt. Amberlite IR 120 [H⁺] resin was added to the reaction mixture until pH = 5, then the reaction mixture was filtrated, concentrated and co-evaporated with water 2 times under reduced pressure. Then NaOH 1M was added to the mixture until pH = 10, followed by a purification using Sephadex LH-20 (water) to give the fully deprotected dissaccharide **11b** as a white salt (11.9 mg, 41%).

$R_f = 0.39$ (EA/MeOH/H₂O 4/2/1); $[\alpha]_D^{20} = -17.0$ (c 0.94, H₂O); $^1\text{H NMR}$ (400 MHz, D₂O) δ (ppm) = 4.48 (d, $^3J_{1-2} = 8.5$ Hz, 1H, H-1 GalN), 4.44 (d, $^3J_{1-2} = 8.0$ Hz, 1H, H-1 GlcA), 4.11 (d, $^3J_{3-4} = 3.0$ Hz, 1H, H-4 GalN), 3.98-3.93 (m, 2H, H-2 GalN, (PEG₃) CH₂a), 3.77 (dd, $^3J_{2-3} = 11.0$ Hz, 1H, H-3 GalN), 3.74-3.61 (m, 13H, (PEG₃) CH₂b, 4 CH₂ (PEG₃ (CH₂)), H-5 GlcA, H-5 GalN, H-6 GalN), 3.45-3.38 (m, 4H, CH₂ (PEG₃ (CH₂)), H-3 GlcA, H-4 GlcA), 3.27 (t, $^3J_{2-3} = 8.5$ Hz, 1H, H-2 GlcA), 1.96 (s, 3H, NH-Ac); $^{13}\text{C NMR}$ (100 MHz, D₂O) δ (ppm) = 174.7 ((C=O)-Ac), 104.1 (C-1 GlcA), 101.3 (C-1 GalN), 80.2 (C-3 GalN), 76.1 (C-5 GalN or C-5 GlcA), 75.3 (C-3 GlcA or C-4 GlcA), 74.9 (C-5 GalN or C-5 GlcA), 72.2 (C-2 GlcA), 71.8 (C-3 GlcA or C-4 GlcA), 69.7 69.6, 69.2, 68.8, 61.0 (6C, PEG₃ (CH₂), C-6 GalN), 67.8 (C-4 GalN), 51.2 (C-2 GalN), 50.1 (PEG₃

(CH₂), 22.3 (NH-Ac); **IR (neat)** $\tilde{\nu}$ (cm⁻¹) = 3323 (N-H and O-H), 2914, 2877 (C_{sp3}-H), 2111 (N₃), 1606 (C=O), 1413, 1372, 1255 (C_{sp3}-H), 1029 (C-OH); **HRMS (ESI)**: *m/z* calcd for C₂₀H₃₃N₄O₁₄ : 553.1999 [M-Na]⁻; found : 553.2000.

4.17. 3-Azidopropyl O-(sodium-β-D-glucopyranosyluronate)-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranoside (11d)

A cold solution of LiOH 1M (1.62 mL, 1.623mm, 8.5 equiv.) and H₂O₂ 30% (1.91 mL) was added to a solution of disaccharide **7d** (128.8 mg, 191 μmol, 1.00 equiv.) in THF (9.5 mL, 0.02M) at 0°C then rt. After 24h, the reaction mixture was diluted with methanol/water 4/1 (27 mL) and NaOH 4M was added (4.78 mL, 19.1 mmol, 100 equiv.). The reaction mixture was stirred for 48h at rt, then neutralised at pH = 7 with Amberlite IR 120 [H⁺] resin, filtrated, evaporated under reduced pressure and the crude mixture was used without any further purification.

Triethylamine (345 μL, 2.48 mmol, 13.0 equiv.) and acetic anhydride (382 μL, 20.0 mmol, 20 equiv.) were added to a solution of previous crude disaccharide in MeOH/H₂O: 4/1 (27 mL) and the reaction mixture was stirred at rt for 2h. Amberlite IR 120 [H⁺] resin was added to the reaction mixture until pH = 5, then the reaction mixture was filtrated, concentrated and co-evaporated with water 2 times under reduced pressure. Then NaOH 1M was added to the mixture until pH = 10, followed by a purification using Sephadex LH-20 (water) to give the deprotected dissaccharide **11d** as a sodium salt (52.9 mg, 55%).

R_f = 0.32 (AE/MeOH/H₂O 4/2/1); [α]_D²⁰ = -14.0 (c 0.98, H₂O); **¹H NMR (400 MHz, D₂O)** δ (ppm) = 4.42-4.45 (m, 2H, H-1 GalN, H-1 GlcA), 4.11 (d, ³J₃₋₄ = 2.5 Hz, 1H, H-4 GalN), 3.91-3.96 (m, 2H, H-2, O-CH₂a), 3.62-3.78 (m, 3H, H-3, H-6a GalN), 3.60-3.65 (m, 3H, H-5 GlcA, H-5 GalN, O-CH₂b), 3.40-3.46 (m, 2H, H-3, H-4 GlcA), 3.26-3.34 (m, 3H, H-2 GlcA, CH₂-N₃), 1.97 (s, 3H, NH-Ac), 1.71-1.85 (m, 2H, -CH₂-); **¹³C NMR (100 MHz, D₂O)** δ (ppm) = 181.5 ((C=O)Ac), 175.5 (C-6 GlcA), 104.1, 101.4 (C-1 GalN, C-1 GlcA), 80.1 (C-3 GalN), 76.1 (C-5 GalN), 75.3 (C-3 or C-4 GlcA), 74.9 (C-5 GlcA), 72.7 (C-2 GlcA), 71.8 (C-3 or C-4 GlcA), 67.7 (C-4 GalN), 67.1 (O-CH₂), 61.1 (C-6 GalN), 51.2 (C-2 GalN), 47.8 (CH₂-N₃), 28.1 (-CH₂-), 23.3 (CH₃, OAc); **IR (neat)** $\tilde{\nu}$ (cm⁻¹) = 3386 (N-H and OH), 2924 (C_{sp3}-H), 2100 (N₃), 1746 (C=O), 1370 (C_{sp3}-H), 1220 (C-N), 1036 (C-O); **HRMS (ESI)**: *m/z* calcd for C₁₇H₂₇N₄NaO₁₂ : 503.1595 [M+H]⁺; found : 503.1591.

Acknowledgements

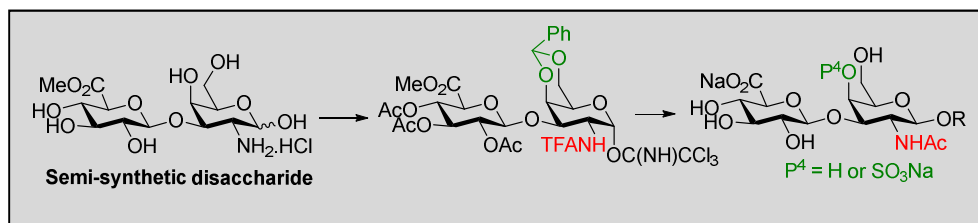
We thank Chloé Jolly and Zoé Leteur for their help during synthesis of starting material. We gratefully thank the CNRS and Université d'Orléans (funding for PB) for the support of this work. We also thank the projects MISTIC FEDER-FSE (2014-2020-EX003242), CHemBio (FEDER-FSE 2014-2020-EX003677), Techsab (FEDER-FSE 2014-2020-EX011313), the RTR Motivhealth (2019-00131403) and the Labex

programs SYNORG (ANR-11-LABX-0029) and IRON (ANR-11-LABX-0018-01) for their financial support of ICOA, UMR 7311, University of Orléans, CNRS.

References

- [1] Kjellen, U.; Lindahl, U. *Annu. Rev. Biochem.* **1991**, *60*, 443-475.
- [2] a) Sugahara, K.; Mikami, T.; Uyama, T.; Mizuguchi, S.; Nomura, K.; Kitagawa, H. *Curr. Opin. Struct. Biol.* **2003**, *13*, 612-620; b) Deepa, S. S.; Umehara, Y.; Higashiyama, S.; Itoh, N.; Sugahara, K. *J. Biol. Chem.* **2002**, *277*, 43707-43716; c) Kawashima, H.; Atarashi, K.; Hirose, M.; Hirose, J.; Yamada, S.; Sugahara, K.; Miyasaka J. *Biol. Chem.* **2002**, *277*, 12921-12930; d) Clark, J. S.; Bishop, P. N.; Day, A. J. *Front. Immunol.* **2013**, *4*, 412.
- [3] Lopin, C.; Jacquinet, J.-C. *Angew. Chem. Int. Ed.* **2006**, *45*, 2574-2578.
- [4] a) Vibert, A.; Lopin-Bon, C.; Jacquinet, J.-C. *Chem. Eur. J.* **2009**, *15*, 9561-9578. b) Jacquinet, J.-C.; Vibert, A.; Lopin-Bon, C. *Chem. Eur. J.* **2009**, *15*, 9579-9595. c) Vibert, A.; Lopin-Bon, C.; Jacquinet, J.-C. *Eur. J. Org. Chem.* **2011**, 4183-4204; d) Jacquinet, J.-C.; Lopin-Bon, C. *Carbohydr. Res.* **2015**, *402*, 35-43.
- [5] a) Wolfrom, M. L.; Conigliaro, P. J. *Carbohydr. Res.* **1969**, *11*, 63-76; b) Hartman, M. C. T.; Coward, J. K. *J. Am. Chem. Soc.* **2002**, *124*, 10036-10053; c) Bongat, A. F. G.; Demchenko, A. V. *Carbohydr. Res.* **2007**, *342*, 374-406; d) Myska, H.; Bednarczyk, D.; Najder, M.; Kaca, W. *Carbohydr. Res.* **2003**, *338*, 133-141.
- [6] a) Solera, C.; Macchione, G.; Maza, S.; Kayser, M.; Carzana, F.; de Paz, J. L.; Nieto, P. M. *Chem. Eur. J.* **2016**, *22*, 2356-2369; b) Macchione, G.; Maza, S.; Kayser, M.; de Paz, J. L.; Nieto, P. M. *Eur. J. Org. Chem.* **2014**, 3868-3884; c) Domínguez-Rodríguez, P.; Reina, J. J.; Gil-Caballero, S.; Nieto, P. M.; de Paz, J. L.; Rojo, J. *Chem. Eur. J.* **2017**, *47*, 11338-11345
- [7] Schmidt, R.R. *Angew. Chem.* **1986**, *98*, 213; *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 212-235.
- [8] Bélot, F.; Jacquinet, J.-C. *Carbohydr. Res.* **2000**, *325*, 93– 106.
- [9] Lucas, H; Basten, J. E. M.; van Dinther, Th. G.; Meuleman, D. G.; van Aelst, S. F.; van Boeckel, A. A. *Tetrahedron* **1990**, *46*, 8207-8228.

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New chondroitin disaccharide bearing a *N*-trifluoroacetyl group and a benzylidene acetal was prepared on a gram-scale from a semi-synthetic disaccharide. Stereoselective glycosylation could be achieved and allowed the formation of various non sulfated and 4-sulfated disaccharides. The deprotection of the TFA moiety was validated in the presence of aglycons functionalized with azides groups which was not possible with the TCA group used so far.