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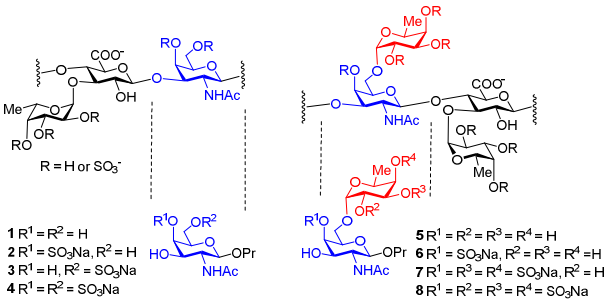

Synthesis and NMR analysis of model compounds related to fucosylated chondroitin sulfates: GalNAc and Fuc(1→6)GalNAc derivatives

Dmitry Z. Vinnitskiy, Nadezhda E. Ustyuzhanina,* Andrey S. Dmitrenok, Alexander S. Shashkov, and Nikolay E. Nifantiev*

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Abstract. Unsubstituted and 6-*O*-α-L-fucosylated propyl 2-acetamido-2-deoxy-β-D-galactopyranosides and their selectively *O*-sulfated (both in GalNAc and Fuc units) derivatives were synthesized as model compounds representing the fragments of fucosylated chondroitin sulfates (FCS) from sea cucumbers. Per-*O*-acetylated 2-deoxy-2-*N*-phthalimido-D-glucopyranose was used as a key precursor for the preparation of all 2-acetamido-2-deoxy-D-galactopyranoside containing products. Attempts at 6-*O*-glycosylation of propyl 3-*O*-benzoyl-2-deoxy-2-*N*-phthalimido-D-galactoside by 2-*O*-benzyl-3,4-di-*O*-chloracetyl-L-fucosyl trichloracetimidate in the presence of TMSOTf gave a 1:1 mixture of the corresponding α- and β-isomeric disaccharides, while the use of structurally related fucosyl bromide donor with promotion by Bu_4NBr led to the formation of desired α-isomeric disaccharide exclusively. Selective removal of orthogonal *O*-protections permitted subsequent *O*-sulfation both at the GalNAc and Fuc units. Further removal of blocking groups yielded the target products which were systematically studied by ^{1}H and ^{13}C NMR spectroscopy in order to determine the spectral effects of *O*-sulfation and α-L-fucosylation needed for the development of computer assisted structural analysis of natural FCS.

Keywords: fucosylated chondroitin sulfate; oligosaccharide; synthesis; glycosylation; NMR; spectral glycosylation effect; spectral sulfation effect

1. Introduction

Fucosylated chondroitin sulfates (FCS) from sea cucumbers attract attention due to their high level of anticoagulant, antithrombotic, antitumor, anti-inflammatory, and other types of biological activity [1-4]. The backbone of FCS is built of the repeating disaccharide units →3)-β-D-GalNAc-(1→4)-β-D-GlcA-(1→ bearing α-L-fucosyl branches (Fig. 1). Structural variations of FCS from different sea cucumber species are determined by the number and position of branches, as well as by the pattern of *O*-sulfation of all monosaccharide units. It is noteworthy that biological effect depends on these structural features of the polysaccharide [3-6]. For the two last decades selectively *O*-sulfated α-L-fucosyl residues attached to *O*-3 of the GlcA unit (see unit **A**, Fig. 1) were considered to be the main type of branches in FCS [1, 5-8]. However, recently the presence of α-L-fucosyl branches linked to *O*-6 of N-acetyl-galactosamine units (see unit **II**, Fig. 1) was found in the structure of FCS from *Actinopyga mauritiana* [9].

Here, we conduct systematic syntheses of oligosaccharides related to essential structural motifs of FCS. These compounds are required for subsequent biological studies to assess the structures of pharmacophore fragments in FCS [10-11]. In addition, the same compounds are indispensable models for NMR studies to determine spectral effects of glycosylation and sulfation which are needed for the development of computer assisted analysis of natural FCS [12-13].

Previously we performed the synthesis of oligosaccharides built of 3-*O*-fucosylated glucuronic acid [14]. In this communication we report the synthesis and NMR analysis of mono- and disaccharides **1**-**8** related to GalNAc and Fuc(1→6)GalNAc fragments of FCS (Fig. 1).

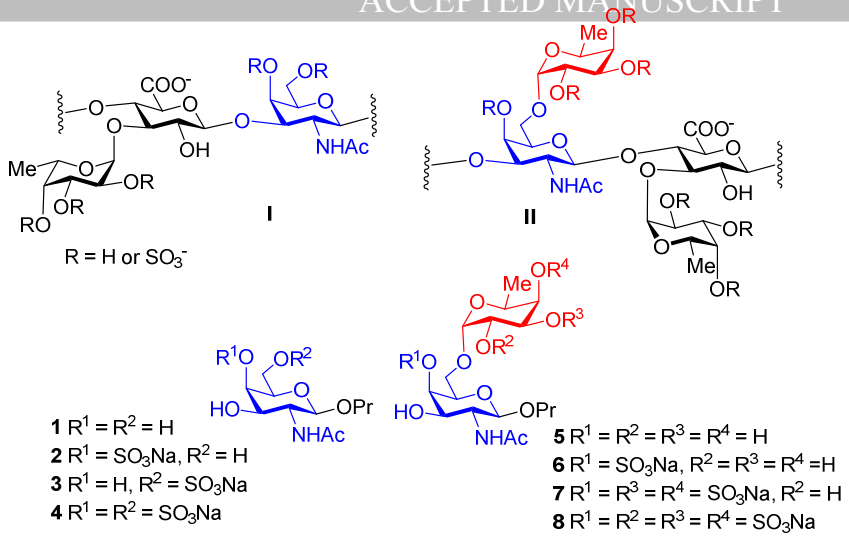


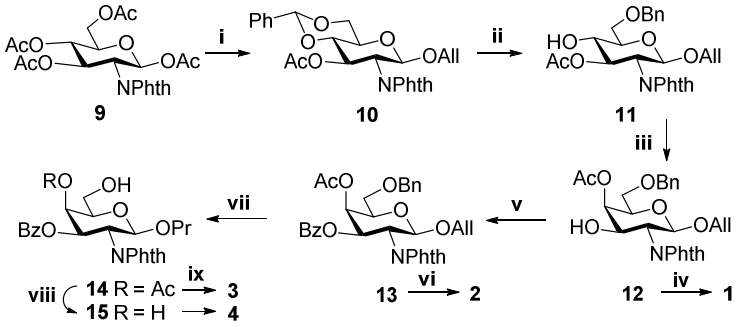
Figure 1. Key repeating units **I** and **II** of fucosylated chondroitin sulfates and synthetic mono- and disaccharides **1**-**8** related to GalNAc and Fuc(1→6)GalNAc fragments.

2. Results and Discussion

2.1. Synthesis

The synthesis of all N-acetyl-D-galactosamine derivatives was started from per-*O*-acetylated 2-deoxy-2-phthalimido-D-glucose **9** [15]. Its treatment with allyl alcohol in the presence of $BF_3{\cdot}Et_2O$ gave the respective allyl glycoside, which was *O*-deacetylated under acidic conditions and further transformed to 3-*O*-acetyl-4,6-di-*O*-benzylidene derivative **10** in overall yield of 64% (Scheme 1). Regioselective reductive opening of the benzylidene ring in **10** under the treatment with $BH_3{\cdot}Me_3N$ and $AlCl_3$ in THF gave 6-O-benzylated glycoside **11** in a yield of 90%. Presence of the benzyl group at *O*-6 was confirmed by the low field shift of the *C*-6 signal in the ^{13}C NMR spectrum (δ 70.2 ppm). Transformation of compound **11** with *gluco*-configuration into *galacto*-derivative **12** was performed *via* intermediate formation of 4-triflate of **11**. Intramolecular attack of the 3-*O*-acetyl group on the triflate group at *C*-4 led to the dioxalenium cation, which was opened in the presence of water with the formation of 4-*O*-acetylated product **12**. The presence of the acetyl group at *O*-4 in **12** was confirmed by the low field shift of the *H*-4 signal in the ^1H NMR spectrum (δ 5.44 ppm). *Galacto*-configuration of **12** was evidenced by the

characteristic value of $J_{3\text{-}4}$ constant (3.4 Hz). Deprotection of **12** accompanied by reduction of the allyl group into the propyl one and followed by N-acetylation led to the target monosaccharide **1**.

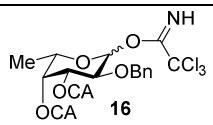
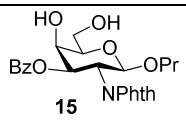
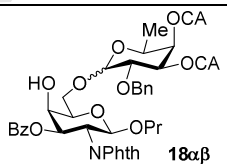
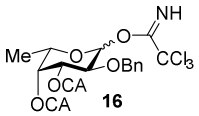
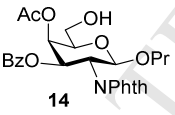
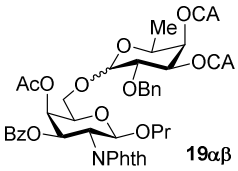
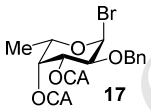
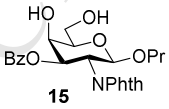
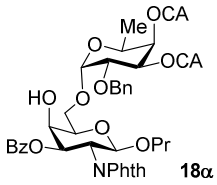


Scheme 1. **i:** 1. AllOH, $BF_3 \cdot Et_2O$, DCM, 2. AcCl/MeOH, DCM 3. $PhC(OMe)_2$, CSA, DMF, 4. AcCl, Py, DCM, 64%; **ii:** $BH_3 \cdot NMe_3$, $AlCl_3$, H_2O, THF, 90%; **iii:** 1. Tf_2O, Py, DCM, -50 °C, 2. H_2O, DCM, 50 °C, 94%; **iv:** 1. $Pd(OH)_2/C$, EtOAc, MeOH, 2. $N_2H_4 \cdot H_2O$, EtOH, 90 °C, 3. Ac_2O, $NaHCO_3$, MeOH, H_2O, 56%; **v:** BzCl, Py, DCM, 91%; **vi:** 1. AcCl/MeOH, DCM 2. $Py \cdot SO_3$, DMF, 3. $Pd(OH)_2/C$, EtOAc, MeOH, 4. $N_2H_4 \cdot H_2O$, EtOH, 90 °C, 5. Ac_2O, $NaHCO_3$, MeOH, H_2O, 48%; **vii:** $Pd(OH)_2/C$, EtOAc, MeOH, 89%; **viii:** AcCl/MeOH, DCM, 96%; **ix:** 1. $Py \cdot SO_3$, DMF, 2. $N_2H_4 \cdot H_2O$, EtOH, 90 °C, 3. Ac_2O, $NaHCO_3$, MeOH, H_2O, 52% for **3**, 48% for **4**.

Benzoylation of **12** gave glycoside **13** bearing the orthogonal 4-*O*-acetyl and 6-*O*-benzyl groups. Acidic *O*-deacetylation of **13** followed by sulfation, deprotection and, finally N-acetylation, led to the target 4-*O*-sulfated monosaccharide **2** in an overall yield of 48%. Hydrogenolysis of **13** in the presence of $Pd(OH)_2/C$ gave propyl glycoside **14** bearing free OH-group at *C*-6. *O*-Sulfation of **14** and subsequent deprotection and N-acetylation produced 6-*O*-sulfated **3** in a yield of 52%. Similarly, *O*-deacylation of **14** (→**15**, 96%) and subsequent sulfation, saponification and N-acetylation gave 4,6-di-*O*-sulfated propyl glycoside **4**. The position of the sulfate groups in compounds **2**-**4** was confirmed by characteristic low field values of the corresponding proton and carbon chemical shifts in the ^{1}H and ^{13}C NMR spectra (Table 2).

For the synthesis of disaccharides **5-8** the glycosylation of diol **15** by 2-*O*-benzyl-3,4-di-*O*-chloroacetyl-fucosyltrichloroacetimidate **16** [14] in the presence of TMSOTf was studied first. Previously, 3,4-di-*O*-acyl-fucosyltrichloroacetimidates were shown to be efficient α-glycosylating agents due to remote participation of acyl groups at O-3 and/or O-4 which favour nucleophilic attack from the α-side [14, 16-18]. In spite of previous observations, the reaction of **15** with **16** proceeded without any stereoselectivity and led to a 1:1 mixture of α- and β-isomers **18αβ** (Table 1). Their ratio was determined by the integration of H-6(α-Fuc) and H-6(β-Fuc) signals in the ^{1}H NMR spectrum.

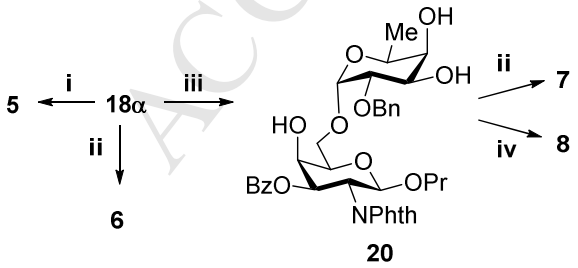
Table 1. Results of glycosylation.

Donor	Acceptor	Conditions	Products	Yield	α:β
 **16**	 **15**	TMSOTf, -50 °C, DCM, 30 min	 **18αβ**	51%	1:1
 **16**	 **14**	TMSOTf, -50 °C, DCM, 30 min	 **19αβ**	78%	2:1
 **17**	 **15**	Bu_4NBr, rt, DCM, 7 days	 **18α**	56%	only α

Fucosylation under similar conditions of 6-hydroxy derivative **14** which can be regarded as a less reactive acceptor was slightly more efficient and led to a 2:1 mixture of α- and β-isomers **19αβ** (Table 1). Their ratio was determined by the integration of H-1(α-Fuc) and H-1(β-Fuc) signals in the ^{1}H NMR spectrum. Surprisingly, the best result of

glycosylation was achieved with the use of the less active fucosyl bromide donor **17** bearing the same pattern of blocking groups as that in imidate **16**. The coupling of compounds **15** and **17** under Lemieux conditions [19] in the presence of Bu_4NBr proceeded more slowly than that with trichloroacetimidate **16** (7 days instead of 30 min, Table 1) but with stereospecific formation of the desired α-isomer **18α**. α-Configuration of the formed glycosidic bond was confirmed by the characteristic value of the $J_{1'\text{-}2'}$ constant (3.4 Hz).

Disaccharide **18α** was further transformed into the target products **5**-**8**. Thus, the deprotection and N-acetylation of **18α** gave non-sulfated disaccharide **5**, while the sulfation of **18α** by the $Py\cdot SO_3$ complex followed by deprotection and N-acetylation led to 4-*O*-sulfated disaccharide **6**. Selective removing of chloroacetyl groups in **18α** by thiourea in the presence of collidine gave triol **20** (92%). It was then subjected to per-*O*-sulfation followed by further deprotection and N-acetylation to give 4,3',4'-tri-*O*-sulfated disaccharide **7**. Hydrogenolysis of **20** followed by per-*O*-sulfation, deprotection and N-acetylation led to 4,2',3',4'-tetra-*O*-sulfated disaccharide **8**. Similarly to monosaccharide sulfates **2**-**4**, the location of the sulfate groups in compounds **6**-**8** was confirmed by the characteristic low field values of the corresponding proton and carbon chemical shifts in the ^1H and ^{13}C NMR spectra (Table 2).



Scheme 2. **i:** 1. $Pd(OH)_2/C$, EtOAc, MeOH, 2. $N_2H_4\cdot H_2O$, EtOH, 90 °C, 3. Ac_2O, $NaHCO_3$, MeOH, H_2O, 51%. **ii:** 1. $Py\cdot SO_3$, DMF, 2. $Pd(OH)_2/C$, EtOAc, MeOH, 3. $N_2H_4\cdot H_2O$, EtOH, 90 °C, 4. Ac_2O, $NaHCO_3$, MeOH, H_2O, 42% for **6**, 37% for **7**. **iii:** Thiourea, 2,4,6-collidine, MeOH,

60 °C, 92%. **iv:** 1. $Pd(OH)_2/C$, EtOAc, MeOH, 2. $Py \cdot SO_3$, DMF, 3. $N_2H_4 \cdot H_2O$, EtOH, 90 °C, 4. Ac_2O, $NaHCO_3$, MeOH, H_2O, 47%.

2.2. NMR analysis

All synthesized mono- and disaccharides **1**-**8** were characterized by ^1H and ^{13}C NMR spectroscopy (Table 2) in order to determine the corresponding spectral effects of sulfation and glycosylation, which could be applied to the analysis of complicated NMR spectra of FCS which can be illustrated by the analysis of the structure of natural sulfated polysaccharide fucoidan from *Fucus distichus* [13].

The effects of sulfation or glycosylation on protons ($\Delta\delta H_i$) and carbons ($\Delta\delta C_i$) were calculated according to typical equations: $\Delta\delta Hi = \delta Hi(A) - \delta Hi(B)$ and $\Delta\delta Ci = \delta Ci(A) - \delta Ci(B)$ [10, 20, 21], where A was the sulfated or glycosylated product and B was the corresponding non-sulfated or non-glycosylated product, respectively (Table 3). Thus, for the calculation of sulfation effects in the case of monosaccharides **2**-**4**, galactosaminide **1** was accounted as reference B, while for disaccharides **6**-**8** it was compound **5**. It was found that the carbon α-effects of sulfation ΔδCi were always positive and amounted to a value of 6.1-8.2 ppm, while the β-effects were usually negative and did not exceed the value of -2.8 ppm. The same trend was observed previously for oligosaccharides related to fucoidan fragments [20-22]. The proton α-effects of sulfation ΔδHi were also positive, and the value of ΔδH4 was significantly higher than that of ΔδH6. In contrast to the carbon β-effects, the values of proton β-effects were positive and could amount to +0.35 ppm. This could be explained by the negative inductive effect of the sulfate group on neighbouring protons.

The influence of the sulfate group at O-2 of the fucosyl residue on the NMR spectra was determined by comparing the chemical shifts of the respective signals of compounds **7** and **8** (Table 3, last entry). In addition to the potent α-effect (ΔδH-2 = +0.54 ppm, ΔδC-2 = +6.4 ppm), significant β-effects on H-1 and C-1 atoms (ΔδH-1 = +0.27

ppm, ΔδC-1 = -2.7 ppm) were determined. The later observation could be helpful for identification of the signals of Fuc2S(1→6) residue in the anomeric region of the NMR spectra of fucosylated chondroitin sulfates.

The proton and carbon effects of 6-O-glycosylation in the spectra of disaccharides **6**-**8** were calculated with the use of spectral data for 4-O-sulfated galactosaminide **2** as reference B. The values ΔδC6 were shown to be significant indicating the potent α-effect of glycosylation comparable with the α-effect of sulfation (Table 3). This makes impossible identification of 6-O-sulfated or 6-O-fucosylated GalNAc units only on the basis of chemical shift values of C-6 signals in the NMR spectra of natural FCS. The β-effects of glycosylation on ^{13}C (negative) and ^{1}H (positive) were also comparable with the corresponding spectral β-effects of sulfation.

Notably, the introduction of substituents both at O-4 and O-6 of GalNAc causes a distinction of usually equal CH_2 protons of GalNAc (see the data for **4** and **6**-**8**). Overall, the parent NMR chemical shifts of model compounds **1**-**8** together with the calculated sulfation and fucosylation effects forms a useful data set which could be applied for the analysis of NMR spectra of natural FCS.

Table 2. Data of ^{1}H and ^{13}C NMR spectra (δ, ppm) of **1**-**8** (bold numerals indicate positions of sulfates).

Residue	H-1 (C-1)	H-2 (C-2)	H-3 (C-3)	H-4 (C-4)	H-5 (C-5)	H-6 (C-6)
1 GalNAc	4.49 (103.0)	3.90 (53.9)	3.74 (72.5)	3.96 (69.3)	3.70 (76.5)	3.81 (62.4)
2 GalNAc	4.54 (103.4)	3.90 (54.5)	3.90 (72.0)	**4.71** (**77.5**)	3.82 (76.2)	3.83 (62.5)
3 GalNAc	4.49	3.91	3.75	4.00	3.93	**4.22**

	(103.0)	(53.9)	(72.4)	(69.0)	(74.0)	(**68.5**)
4 GalNAc	4.52	3.89	3.89	**4.70**	4.05	**4.19**; **4.31**
	(102.7)	(54.1)	(71.4)	(**77.0**)	(74.0)	(**69.3**)
5 GalNAc	4.49	3.90	3.72	3.96	3.88	3.82; 3.88
	(103.0)	(53.9)	(72.4)	(69.5)	(75.0)	(69.0)
Fuc	4.97	3.80	3.86	3.81	4.10	1.21
	(100.7)	(69.3)	(71.0)	(73.2)	(68.2)	(16.9)
6 GalNAc	4.56	3.90	3.90	**4.74**	4.00	3.84; 3.94
	(102.6)	(54.1)	(72.4)	(**77.3**)	(74.3)	(69.2)
Fuc	4.97	3.78	3.90	3.81	4.15	1.21
	(100.9)	(69.8)	(70.8)	(73.6)	(68.5)	(16.6)
7 GalNAc	4.55	3.92	3.92	**4.74**	4.05	3.90; 4.00
	(102.6)	(53.9)	(72.4)	(**77.3**)	(74.6)	(69.7)
Fuc	5.08	4.00	**4.62**	**4.90**	4.30	1.21
	(100.7)	(67.4)	(**76.6**)	(**80.0**)	(67.7)	(16.9)
8 GalNAc	4.53	3.90	3.90	**4.78**	4.01	3.88; 4.00
	(103.0)	(53.9)	(72.0)	(**77.5**)	(74.8)	(69.0)
Fuc	5.35	**4.54**	**4.72**	**4.94**	4.30	1.21
	(98.0)	(**73.8**)	(**73.8**)	(**80.3**)	(67.5)	(16.9)

Table 3. ^{1}H and ^{13}C NMR spectral effects of sulfation and glycosylation (Δδ, ppm; bold numerals indicate positions of substituents).

Compound	O-Sulfation effects						Glycosylation effects	
and Residue	ΔδH-1	ΔδH-2	ΔδH-3	ΔδH-4	ΔδH-5	ΔδH-6	ΔδH-5	ΔδH-6
	(ΔδC-1)	(ΔδC-2)	(ΔδC-3)	(ΔδC-4)	(ΔδC-5)	(ΔδC-6)	(ΔδC-5)	(ΔδC-6)
2 GalNAc	0.05	0	0.16	**0.75**	0.12	0.02	n/a*	n/a
	(0.4)	(0.6)	(-0.5)	(**8.2**)	(-0.3)	0.1	n/a	n/a

3 GalNAc	0	0	0.01	0.04	0.23	**0.41**	n/a	n/a
	(0)	(0)	(-0.2)	(-0.3)	(-2.5)	(**6.1**)	n/a	n/a
4 GalNAc	0.03	-0.01	0.15	**0.74**	0.35	**0.38; 0.50**	n/a	n/a
	(-0.3)	(0.2)	(-1.1)	(**7.7**)	(-2.5)	(**6.9**)	n/a	n/a
6 GalNAc	0.07	0	0.18	**0.78**	0.12	0.02; 0.06	0.18	**0.01; 0.11**
	(-0.4)	(0.2)	(0)	(**7.8**)	(-0.7)	(0.2)	(-1.9)	(**6.7**)
Fuc	0	0.02	0.04	0	0.05	0	n/a	n/a
	(0.2)	(0.05)	(0.2)	(0.4)	(-0.3)	(0.3)	n/a	n/a
7 GalNAc	0.06	0.02	0.2	**0.78**	0.17	0.08; 0.12	0.23	**0.09; 0.19**
	(0.4)	(0)	(0)	(**7.8**)	(-0.4)	(0.7)	(-1.6)	(**7.2**)
Fuc	0.11	0.2	**0.76**	**1.09**	0.2	0	n/a	n/a
	(0)	(-1.9)	(**5.6**)	(**6.8**)	(-0.8)	(0)	n/a	n/a
8 GalNAc	0.04	0	0.18	**0.82**	0.13	0.02; 0.06	0.19	**0.07; 0.19**
	(0)	(0)	(-0.4)	(**8.0**)	(-0.2)	(0)	(-1.4)	(**6.5**)
Fuc	0.38	**0.74**	**0.86**	**1.13**	0.2	0	n/a	n/a
	(-2.7)	(**4.5**)	(**2.8**)	(**7.1**)	(-0.8)	(0)	n/a	n/a
Fuc**	0.27	**0.54**	0.1	0.04	0	0	n/a	n/a
	(-2.7)	(**6.4**)	(-2.8)	(0.3)	(-0.2)	(0)	n/a	n/a

*n/a – not applicable.

** $\Delta\delta$Hi = δHi (**8**) – δHi (**7**) and $\Delta\delta$Ci = δCi (**8**) – δCi (**7**)

3. Conclusions

The synthesis of the selectively sulfated and non-sulfated derivatives of propyl 2-acetamido-2-deoxy-D-galactopyranoside and 6-*O*-α-L-fucosyl-2-acetamido-2-deoxy-D-galactopyranoside was performed. Monosaccharides **1**-**4** were prepared from per-O-acetylated N-phthalimido-D-glucosamine with inversion of C-4 configuration. For the synthesis of disaccharides **5**-**8**, two types of glycosyl donors were studied in the glycosylation reaction. Application of 2-O-benzyl-3,4-di-O-chloracetyl-L-fucopyranosyl trichloracetimidate (**16**) in the presence of TMSOTf gave a mixture of α- and β-isomeric disaccharides in a ratio of 1:1. To increase α-stereoselectivity of the reaction respective fucosyl bromide **17** bearing equal blocking groups was applied under promotion with

Bu_4NBr. It can be supposed that the switching the mechanism of the reaction from S_N1 towards S_N2 led to exclusive formation of required α-isomeric glycosylation product. Developed approach was shown to be effective and could be applied for the synthesis of more complicated oligosaccharides related to FCS.

All the synthesized mono- and disaccharides were characterized by ^1H and ^{13}C NMR spectroscopy to determine corresponding sulfation and glycosylation effects in their spectra. Further conformational analysis of these oligosaccharides using nOe experiments and theoretical modeling (quantum mechanics and molecular dynamics [20,21]) are in progress to explain above reported NMR characteristics and to assess 3D shape FCS chains.

4. Experimental

4.1. General procedures

Dimethylformamide (DMF, ≥99.5%) and Py (≥99.5%) were purchased from Sigma-Aldrich. Dichloromethane (CH_2Cl_2) was successively distilled from diethanolamine, P_2O_5 and CaH_2 under argon. Analytical thin-layer chromatography (TLC) was performed on Silica Gel 60 F_{254} aluminium sheets (Merck), and visualization was accomplished using UV light or by charring at ~150 °C with 10% (v/v) H_3PO_4 in ethanol. Liquid column chromatography was performed on Silica Gel 60, 40-63 μm (Merck). Gel chromatography was performed on the Sephadex G-15 column (2×60 cm) by elution with water at a flow rate of 1 mL/min and TSK HW-40S by elution with 0.1N $AcOH_{(aq.)}$ at a flow rate of 0.5 mL/min. ^1H and ^{13}C NMR spectra were recorded on Bruker AV400 and AV600 spectrometers at 303 K in $CDCl_3$ or D_2O. Signal assignment in ^1H and ^{13}C NMR spectra were made using COSY, TOCSY, ROESY and ^1H–^{13}C HSQC techniques. High-resolution mass spectra were acquired by electrospray ionization on a MicrOTOF II

(Bruker Daltonics) instrument. Optical rotation values were measured using a JASCO P-2000 polarimeter at ambient temperature in solvents specified.

4.2. Standard procedures.

A - acidic O-deacetylation. A solution of AcCl (10% v/v) in MeOH was added to a solution of saccharide in DCM. The reaction mixture was kept overnight, then neutralized with NEt_3 and concentrated *in vacuo*. Purification by flash column chromatography was applied if necessary.

B – hydrogenolysis. The catalyst 10% $Pd(OH)_2/C$ was added to a stirring solution of saccharide in an EtOAc/MeOH (1:1) mixture, and the flask was filled with H_2. The mixture was vigorously stirred under H_2 (1 atm) at rt until full consumption of the starting material and then filtered through a celite layer. The catalyst was carefully washed with EtOAc/MeOH, and the combined filtrates were concentrated *in vacuo*. Purification by flash column chromatography was applied if necessary.

C – N,O-deacylation. Hydrazine monohydrate (0.5 mL) was added to a solution of saccharide in EtOH (5mL). The reaction mixture was kept at 90 °C for 3 h and then concentrated *in vacuo*. The residue was purified by gel chromatography on the TSK HW-40S gel with 0.1N $AcOH_{(aq.)}$ elution followed by lyophilization to give free amine.

D - N-acetylation. Ac_2O and $NaHCO_3$ were added to a stirring solution of free amine in MeOH/H_2O (1:1). The reaction mixture was stirred at rt overnight and then concentrated *in vacuo*. The residue was purified by gel chromatography on the Sephadex G-15 gel with water elution followed by lyophilization to give N-acetylated amine.

E – O-sulfation: $Py \cdot SO_3$ complex (5 eq. for each OH group) was added to a solution of saccharide in DMF. The reaction mixture was kept at rt until the reaction was completed (TLC) and then quenched with aqueous $NaHCO_3$ and evaporated twice with water. The residue was dissolved in a minimal amount of water, and then MeOH was added to

precipitate inorganic salts. The solids were filtered off and washed with MeOH, and the filtrate was concentrated *in vacuo*. Purification by flash column chromatography was applied if necessary.

4.3. Synthesis of monosaccharide derivatives 1-4.

4.3.1. Allyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (10)

Allyl alcohol (1.5 mL, 21 mmol) was added to a stirring solution of tetraacetate **9** (5 g, 10.5 mmol) in DCM (30 mL). The mixture was cooled to 0 °C, and $BF_3 \cdot Et_2O$ was added dropwise. The reaction mixture was stirred at rt overnight, then neutralized with NEt_3 (5 mL, 35 mmol), diluted with EtOAc (250 mL), and washed with H_2O (500 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was dissolved in DCM (30 mL) and treated according to general procedure A with a 10% solution of AcCl in MeOH (10 mL).Then CSA (300 mg, 10 mg/mL) and benzaldehyde dimethyl acetal (2.4 mL, 16 mmol) were added to the stirring solution of the crude product in DMF (30 mL). The reaction mixture was stirred at rt until the starting material converted to the product (1 h, TLC control, Tol–EtOAc 2:1, R_f=0.5), then diluted with EtOAc (250 mL), and washed with H_2O (500 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, Tol–EtOAc, 30:1→10:1) to give 3.15 g of the intermediate. The latter was dissolved in DCM (30 mL), and then Py (0.7 mL, 8.7 mmol) and acetyl chloride (0.55 ml, 7.8 mmol) were added. The reaction mixture was kept at rt until full consumption of the starting material (15 min, TLC control) and then diluted with EtOAc (250 mL) and washed with H_2O (500 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, Tol–EtOAc, 50:1→40:1) to give monosaccharide **10** (3.24 g, 64% overall), $[\alpha]_D$ –0.2° (*c* 1, EtOAc). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.87 (br s, 2H, Phth), 7.74 (m, 2H, Phth), 7.46 (m, 2H, Ph), 7.36 (m, 3H, Ph), 5.90 (dd, 1H, $J_{2\text{-}3}$=10.3 Hz, $J_{3\text{-}4}$=9.0 Hz, *H*-3), 5.75–5.62 (m, 1H, All), 5.55 (s, 1H, PhC*H*), 5.48 (d, 1H, $J_{1\text{-}2}$=8.4

Hz, *H*-1), 5.15 (dd, 1H, J=17.2 Hz, J=1.6 Hz, All), 5.06 (dd, 1H, J=10.5 Hz, J=1.3 Hz, All), 4.42 (dd, 1H, J=10.5 Hz, J_{5-6}=4.8 Hz, *H*-6), 4.33 (dd, 1H, J_{1-2}=8.4 Hz, J_{2-3}=10.3 Hz, *H*-2), 4.29 (ddt, 1H, J=12.9 Hz, J=5.1 Hz, J=1.5 Hz, All), 4.05 (ddt, 1H, J=13.1 Hz, J=6.2 Hz, J=1.3 Hz, All), 3.89–3.84 (m, 1H, *H*-6'), 3.79 (t, 1H, J_{3-4}=J_{4-5}=9.2 Hz, H-4), 3.74 (td, 1H, J_{4-5}=9.6 Hz, J_{5-6}=4.7 Hz, H-5), 1.90 (s, 3H, Ac). ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.2 (Ac), 134.3 (Phth), 133.2 (All), 129.2 (Ph), 128.2 (Ph), 126.2 (Ph), 123.6 (Phth), 117.9 (All), 101.7 (Ph*C*H), 97.8 (*C*-1), 79.3 (*C*-4), 70.4 (All), 69.7 (*C*-3), 68.7 (*C*-6), 66.1 (*C*-5), 55.3 (*C*-2), 20.6 (Ac). HRMS (ESI): Calcd m/z for $[M+K]^+$ $C_{26}H_{25}NO_8$ 518.1212, found 518.1202.

4.3.2. Allyl 3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (11)

Borane trimethylamine complex (1 g, 13.3 mmol) was added to a stirring solution of monosaccharide **10** (1.6 g, 3.33 mmol) in THF (15 mL). Then the mixture was cooled with an ice bath, and anhydrous $AlCl_3$ (2.7 g, 20 mmol) was added slowly. After its total dissolution H_2O (120 µL, 6.66 mmol) was added. The reaction mixture was stirred at rt until the full consumption of the starting material (TLC control, 45 min). Then the mixture was diluted with EtOAc (150 mL) and washed with $NaHCO_3$ (200 mL) and H_2O (200 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, Tol–EtOAc, 10:1→5:1) to give monosaccharide **11** (1.44 g, 90%), $[\alpha]_D$ –9° (*c* 1, EtOAc). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.84 (dd, 2H, J=5.4 Hz, J=3.1 Hz, Phth), 7.71 (dd, 2H, J=5.5 Hz, J=3.0 Hz, Phth), 7.36 (d, 2H, J=4.4 Hz, Ph), 7.30–7.21 (m, 3H, Ph), 5.77–5.67 (m, 1H, All), 5.65 (dd, 1H, J_{2-3}=10.8 Hz, J_{3-4}=8.8 Hz, *H*-3), 5.40 (d, 1H, J_{1-2}=8.4 Hz, *H*-1), 5.15–5.10 (m, 1H, All), 5.05–5.00 (m, 1H, All), 4.65 (d, 1H, J=12.0 Hz, PhC*H*H'), 4.61 (d, 1H, J=12.0 Hz, PhCH*H*'), 4.29–4.24 (m, 1H, *H*-2), 4.07–4.02 (m, 1H, All), 3.86 – 3.78 (m, 3H, *H*-6, *H*-4), 3.73 (dt, 1H, J_{4-5}=9.4 Hz, J_{5-6}=4.6 Hz, *H*-5), 3.05 (br s, 4-O*H*), 1.92 (s, 3H, Ac). ^{13}C NMR (150 MHz, $CDCl_3$): δ 171.2 (Ac), 134.2 (Phth), 133.5 (All), 128.5 (Bn), 127.9 (Bn), 127.7 (Bn), 123.5 (Phth), 117.5 (All), 97.2 (*C*-1), 74.2 (*C*-5), 73.8 (Ph*C*H_2), 73.7 (*C*-3), 71.5 (*C*-4), 70.2 (*C*-6), 70.0

(All), 54.6 (*C*-2), 20.7 (Ac). HRMS (ESI): Calcd m/z for $[M+Na]^+$ $C_{26}H_{27}NO_8$ 504.1629, found 504.1625.

4.3.3. Allyl 4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-galactopyranoside (12)

Py (1 mL, 12 mmol) was added to a stirring solution of **11** (1.44 g, 3.0 mmol) in DCM (15 mL) under argon protection. Then the mixture was cooled to -30 °C, and Tf_2O (1 mL, 6 mmol) was added dropwise under argon protection. The reaction mixture was stirred while allowing to be warmed from -30 °C to 10 °C over 1 h. TLC control showed full consumption of the starting material. Then H_2O (4 mL) was added, and the reaction mixture was stirred at 50 °C for 2 days. Then the mixture was diluted with EtOAc (150 mL) and washed with H_2O (300 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, Tol–EtOAc, 8:1→4:1) to give monosaccharide **12** (1.35 g, 94%), $[\alpha]_D$ –25° (*c* 1, EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82 (dd, 2H, J=5.4 Hz, J=3.1 Hz, Phth), 7.70 (dd, 2H, J=5.5 Hz, J=3.0 Hz, Phth), 7.40–7.22 (m, 5H, Ph), 5.78–5.63 (m, 1H, All), 5.44 (d, 1H, $J_{3\text{-}4}$=3.4 Hz, *H*-4), 5.26 (d, 1H, $J_{1\text{-}2}$=8.5 Hz, *H*-1), 5.12 (dd, 1H, J=17.2 Hz, J=1.6 Hz, All), 5.04 (dd, 1H, J=10.4 Hz, J=1.4 Hz, All), 4.65 (dd, 1H, $J_{2\text{-}3}$=11.1 Hz, $J_{3\text{-}4}$=3.5 Hz, *H*-3), 4.59 (d, 1H, J=11.9 Hz, PhC*H*H'), 4.49 (d, 1H, J=11.9 Hz, PhCH*H*'), 4.40 (dd, 1H, $J_{2\text{-}3}$=11.1 Hz, $J_{1\text{-}2}$=8.5 Hz, *H*-2), 4.29 (ddt, 1H, J=13.0 Hz, J=5.1 Hz, J=1.5 Hz, All), 4.04 (ddt, 1H, J=13.0 Hz, J=6.3 Hz, J=1.3 Hz, All), 3.96 (t, 1H, $J_{5\text{-}6}$=6.6 Hz, *H*-5), 3.66–3.57 (m, 2H, *H*-6), 2.13 (s, 3H, Ac). ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.6 (Ac), 134.1 (Phth), 133.5 (All), 128.5 (Bn), 127.8 (2xBn), 123.5 (Phth), 117.6 (All), 97.6 (*C*-1), 73.6 (Ph*C*H_2), 72.5 (*C*-5), 70.3 (*C*-4), 70.0 (All), 68.2 (*C*-6), 67.8 (*C*-3), 54.5 (*C*-2), 20.9 (Ac). HRMS (ESI): Calcd m/z for $[M+Na]^+$ $C_{26}H_{27}NO_8$ 504.1629, found 504.1627.

4.3.4. Allyl 3-*O*-benzoyl-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-galactopyranoside (13)

Py (0.25 mL, 3.0 mmol) and benzoyl chloride (0.26 ml, 2.2 mmol) were added to a solution of **12** (700 mg, 1.45 mmol) in DCM (8 mL). The reaction mixture was kept at rt until full consumption of the starting material (6 h, TLC control), diluted with EtOAc (100 mL), and washed with H_2O (200 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, Tol–EtOAc, 50:1→30:1) to give monosaccharide **13** (780 mg, 91%), $[\alpha]_D$ 34° (*c* 1, EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.84–7.76 (m, 4H, Phth, Bz), 7.71–7.62 (m, 2H, Phth), 7.52–7.43 (m, 1H, Bz), 7.39–7.23 (m, 7H, Bz, Bn), 5.96 (dd, 1H, $J_{2\text{-}3}$=11.4 Hz, $J_{3\text{-}4}$=3.4 Hz, *H*-3), 5.73 (d, 1H, $J_{3\text{-}4}$=4.4 Hz, *H*-4), 5.79–5.67 (m, 1H, All), 5.48 (d, 1H, $J_{1\text{-}2}$=8.5 Hz, *H*-1), 5.15 (dd, 1H, J=17.2 Hz, J=1.6 Hz, All), 5.06 (dd, 1H, J=10.4 Hz, J=1.4 Hz, All), 4.76 (dd, 1H, $J_{2\text{-}3}$=11.4 Hz, $J_{1\text{-}2}$=8.5 Hz, *H*-2), 4.60 (d, 1H, J=12.0 Hz, PhC*H*H'), 4.48 (d, 1H, J=12.0 Hz, PhCH*H*'), 4.33 (ddt, 1H, J=12.9 Hz, J=5.1 Hz, J=1.5 Hz, All), 4.16–4.06 (m, 2H, *H*-5, All), 3.69–3.57 (m, 2H, *H*-6), 2.07 (s, 3H, Ac). ^{13}C NMR (101 MHz, $CDCl_3$): δ 134.2 (Phth), 133.3 (All), 133.2 (Bz), 129.6 (Bz), 128.4 (Bz, Bn), 127.9 (Bn), 127.8 (Bn), 123.6 (Phth), 117.9 (All), 97.5 (*C*-1), 73.5 (Ph*C*H_2), 72.4 (*C*-5), 70.2 (All), 69.1 (*C*-3), 67.8 (*C*-6), 67.4 (*C*-4), 51.7 (*C*-2), 20.6 (Ac). HRMS (ESI): Calcd m/z for $[M+NH_4]^+$ $C_{33}H_{31}NO_9$ 603.2337, found 603.2338.

4.3.5. Propyl 3-*O*-benzoyl-4-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranoside (14)

Hydrogenolysis of **13** (560 mg, 0.95 mmol) in EtOAc (4 mL) and MeOH (4 mL) with 10% $Pd(OH)_2$/C (250 mg) was performed as described in the general procedure B for 1 h. Purification by flash column chromatography (silica gel, Tol–EtOAc, 8:1→4:1) gave product **14** (420 mg, 89%), $[\alpha]_D$ 47° (*c* 1, EtOAc). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.88–7.77 (m, 4H, Phth, Bz), 7.72–7.65 (m, 2H, Phth), 7.53–7.46 (m, 1H, Bz), 7.37–7.31 (m, 2H, Bz), 5.99 (dd, 1H, $J_{2\text{-}3}$=11.4 Hz, $J_{3\text{-}4}$=3.5 Hz, *H*-3), 5.65 (d, 1H, $J_{3\text{-}4}$=3.5 Hz, *H*-4), 5.47 (d, 1H, $J_{1\text{-}2}$=8.5 Hz, *H*-1), 4.78 (dd, 1H, $J_{2\text{-}3}$=11.4, $J_{3\text{-}4}$=8.5 Hz, *H*-2), 4.03 (dd, 1H, $J_{5\text{-}6}$=7.1 Hz, $J_{5\text{-}6'}$=6.6 Hz, *H*-5), 3.89–3.78 (m, 2H, Pr, *H*-6), 3.64 (dd, $J_{6\text{-}6'}$=11.8 Hz, $J_{5\text{-}6'}$=6.6 Hz, *H*-6'), 3.45 (dt, 1H, J=9.7 Hz, J=6.9 Hz, Pr), 2.18 (s, 3H,

Ac), 1.55–1.42 (m, 2H, Pr), 0.69 (t, 3H, J=7.4 Hz, Pr). ^{13}C NMR (150 MHz, $CDCl_3$): δ 171.1 (Bz), 165.1 (Ac), 134.2 (Phth), 133.4 (Bz), 129.6 (Bz), 128.5 (Bz), 123.4 (Phth), 98.6 (*C*-1), 73.7 (*C*-5, 71.8 (Pr), 69.0 (*C*-3), 67.8 (*C*-4), 60.8 (*C*-6), 51.9 (*C*-2), 22.6 (Pr), 20.7 (Ac), 10.1 (Pr). HRMS (ESI): Calcd m/z for $[M+Na]^+$ $C_{26}H_{27}NO_9$ 520.1578, found 520.1575.

4.3.6. Propyl 3-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-galactopyranoside (15)

Monosaccharide **14** (350 mg, 0.70 mmol) was dissolved in DCM (4 mL) and treated according to the general procedure A with a 10% solution of AcCl in MeOH (2 mL). Purification by flash column chromatography (silica gel, Tol–EtOAc, 4:1→2:1) gave product **15** (305 mg, 96%), $[\alpha]_D$ 54° (*c* 1, EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.91 (d, 2H, J=8.3 Hz, Bz), 7.80–7.75 (m, 2H, Phth), 7.66 (dd, 2H, J=5.6 Hz, J=2.9 Hz, Phth), 7.52–7.43 (m, 1H, Bz), 7.34 (t, 2H, J=7.8 Hz, Bz), 5.82 (dd, 1H, J_{2-3}=11.4 Hz, J_{3-4}=3.2 Hz, *H*-3), 5.41 (d, 1H, J_{1-2}=8.5 Hz, *H*-1), 4.84 (dd, 1H, J_{2-3}=11.4 Hz, J_{1-2}=8.5 Hz, *H*-2), 4.45 (d, 1H, J_{3-4}=3.2 Hz, *H*-4), 4.08–3.95 (m, 2H, *H*-6), 3.90–3.76 (m, 2H, *H*-5, Pr), 3.43 (dt, 1H, J=9.7 Hz, J=6.8 Hz, Pr), 1.53–1.38 (m, 2H, Pr), 0.68 (t, 3H, J=7.4 Hz, Pr). ^{13}C NMR (101 MHz, $CDCl_3$): δ 165.5 (Bz), 134.2 (Phth), 133.4 (Bz), 129.8 (Bz), 128.5 (Bz), 123.5 (Phth), 98.6 (*C*-1), 74.1 (*C*-5), 71.6 (*C*-3, Pr), 67.7 (*C*-4), 62.6 (*C*-6), 51.3 (*C*-2), 22.6 (Pr), 10.1 (Pr). HRMS (ESI): Calcd m/z for $[M+Na]^+$ $C_{24}H_{25}NO_8$ 478.1472, found 478.1469.

4.3.7. Propyl 2-acetamido-2-deoxy-β-D-galactopyranoside (1)

Monosaccharide **12** (40 mg, 0.068 mmol) was successively treated according to general procedures B [EtOAc (1mL), MeOH (1 mL), 10% $Pd(OH)_2$/C (20 mg), 1 h, flash column chromatography (silica gel, Tol–EtOAc, 4:1→2:1)], C [$N_2H_4 \cdot H_2O$ (0.5 mL), EtOH (5 mL)], and D [Ac_2O (0.4 mL, $NaHCO_3$ (340 mg), H_2O (2 mL), MeOH (2 mL)] to give **1** (10 mg, 56%), $[\alpha]_D$ 5° (*c* 1, H_2O). ^{1}H NMR (400 MHz, D_2O): δ 4.44 (d, 1H, J_{1-2}=8.5 Hz, *H*-1), 3.92 (d, 1H, J_{3-4}=3.2 Hz, *H*-4), 3.90–3.63 (m, 6H, *H*-2, Pr, *H*-6, *H*-3, *H*-5), 3.54 (dt, 1H, J=10.1 Hz, J=6.5 Hz, Pr),

2.02 (s, 3H, Ac), 1.60–1.48 (m, 2H, Pr), 0.86 (t, 3H, J=7.4 Hz, Pr). ^{13}C NMR (101 MHz, D_2O): δ 176.2 (Ac), 103.0 (*C*-1), 76.5 (*C*-5), 73.6 (Pr), 72.5 (*C*-3), 69.3 (*C*-4), 62.4 (*C*-6), 53.9 (*C*-2), 23.6 (Pr) 23.5 (Ac), 11.0 (Pr). HRMS (ESI): Calcd m/z for $[M+Na]^+$ $C_{11}H_{21}NO_6$ 286.1261, found 286.1254.

4.3.8. Propyl 4-*O*-sulfo-2-deoxy-2-phthalimido-β-D-galactopyranoside sodium salt (2)

Monosaccharide **13** (56 mg, 0.095 mmol) was successively treated according to general procedures A [DCM (2 mL, 10% solution of AcCl in MeOH (2 mL), flash column chromatography (silica gel, Tol–EtOAc, 10:1→4:1)], E [Py·SO_3 (76 mg, 0.48 mmol), DMF (2 mL), 1 h], B [EtOAc (1mL), MeOH (1 mL), 10% $Pd(OH)_2$/C (30 mg), overnight], C [$N_2H_4 \cdot H_2O$ (0.5 mL), EtOH (5 mL)], and D [Ac_2O (0.4 mL, $NaHCO_3$ (340 mg), H_2O (2 mL), MeOH (2 mL)] to give product **2** (17 mg, 48%), $[\alpha]_D$ –1.5° (*c* 1, H_2O). ^{1}H NMR (600 MHz, D_2O): δ 4.68 (d, 1H, $J_{3\text{-}4}$=2.0 Hz, *H*-4), 4.54–4.47 (m, 1H, *H*-1), 3.89–3.77 (m, 6H, *H*-2, *H*-3, *H*-5, *H*-6, Pr), 3.56 (dt, 1H, J=10.2 Hz, J=6.6 Hz, Pr), 2.03 (s, 3H, Ac), 1.59–1.49 (m, 2H, Pr), 0.86 (t, 3H, J=7.4 Hz, Pr). ^{13}C NMR(150 MHz, D_2O): 176.8 (Ac), 103.4 (*C*-1), 77.8 (*C*-4).76.3 (*C*-5), 74.3 (Pr), 72.1 (*C*-3), 63.0 (*C*-6), 55.0 (*C*-2), 24.3 (Ac), 24.1 (Pr), 11.6 (Pr). HRMS (ESI): Calcd m/z for $[M-Na]^-$ $C_{11}H_{20}NO_9S$ 342.0864, found 342.0852.

4.3.9. Propyl 6-*O*-sulfo-2-deoxy-2-phthalimido-β-D-galactopyranoside sodium salt (3)

Monosaccharide **14** (40 mg, 0.08 mmol) was successively treated according to general procedures E [Py·SO_3 (65 mg, 0.4 mmol), DMF (2 mL), 1 h], C [$N_2H_4 \cdot H_2O$ (0.5 mL), EtOH (5 mL)], and D [Ac_2O (0.4 mL, $NaHCO_3$ (340 mg), H_2O (2 mL), MeOH (2 mL)] to give product **3** (15 mg, 52%), $[\alpha]_D$ 2.4° (*c* 1, H_2O). ^{1}H NMR (400 MHz, D_2O): δ 4.46 (d, 1H, $J_{1\text{-}2}$=8.5 Hz, *H*-1), 4.25–4.14 (m, 2H, *H*-6), 3.98 (d, 1H, $J_{3\text{-}4}$=3.3 Hz, *H*-4), 3.93–3.87 (m, 2H, *H*-2, *H*-5), 3.87–3.79 (m, 1H, Pr), 3.73 (dd, 1H, $J_{2\text{-}3}$=10.8 Hz, $J_{3\text{-}4}$=3.3 Hz, *H*-3), 3.56 (dt, 1H, J=10.2 Hz, J=6.5 Hz, Pr), 2.02 (s, 3H, Ac), 1.61–1.48 (m, 2H, Pr), 0.86 (t, 3H, J=7.4 Hz, Pr). ^{13}C NMR (101 MHz,

D_2O): δ 176.2 (Ac), 103.0 (*C*-1), 74.0 (*C*-5), 73.8 (Pr), 72.3 (*C*-3), 69.0 (*C*-4), 68.5 (*C*-4), 53.8 (*C*-2), 23.6 (Pr), 23.5 (Ac), 11.0 (Pr). HRMS (ESI): Calcd m/z for $[M-Na]^-$ $C_{11}H_{20}NO_9S$ 342.0864, found 342.0864.

4.3.10. Propyl 4,6-di-*O*-sulfo-2-deoxy-2-phthalimido-β-D-galactopyranoside sodium salt (4)

Monosaccharide **15** (77 mg, 0.169 mmol) was successively treated according to general procedures E [$Py \cdot SO_3$ (270 mg, 1.7 mmol), DMF (2 mL), 2 h], C [$N_2H_4 \cdot H_2O$ (0.5 mL), EtOH (5 mL)], and D [Ac_2O (0.4 mL, $NaHCO_3$ (340 mg), H_2O (2 mL), MeOH (2 mL)] to give **4** (39 mg, 48%). $[\alpha]_D$ 6° (*c* 1, H_2O). ^1H NMR (600 MHz, D_2O): δ 4.67 (d, 1H, J_{3-4}=1.4 Hz, *H*-4), 4.50–4.46 (m, 1H, *H*-1), 4.27 (dd, 1H, $J_{6-6'}$=11.5 Hz, J_{5-6}=3.5 Hz, H-6), 4.16 (dd, 1H, $J_{6-6'}$=11.5 Hz, $J_{5-6'}$=8.4 Hz, *H*-6'), 4.03–3.99 (m, 1H, *H*-5), 3.85–3.82 (m, 2H, *H*-2, *H*-3), 3.81 (dt, 1H, J=10.2 Hz, J=6.3 Hz, Pr), 3.54 (dt, 1H, J=10.2 Hz, J=6.6 Hz, Pr), 1.98 (s, 3H, Ac), 1.51 (dd, 2H, J=13.9 Hz, J=6.6 Hz, Pr), 0.82 (t, 3H, J=7.4 Hz, Pr). ^{13}C NMR (151 MHz, D2O): δ 176.2 (Ac), 102.7 (*C*-1), 77.0 (*C*-4), 73.8 (Pr), 73.5 (*C*-5), 71.4 (*C*-3), 69.2 (*C*-6), 54.1 (*C*-2), 23.6 (Ac), 23.5 (Pr), 11.0 (Pr). HRMS (ESI): Calcd m/z for $[M-Na]^-$ $C_{11}H_{19}NO_{12}S_2Na$ 444.0252, found 444.0252.

4.4. Synthesis of disaccharide derivatives 5-8.

4.4.1. 2-*O*-benzyl-3,4-di-*O*-chloroacetyl-α-L-fucopyranose bromide (17).

0.1 M TMSOTf in DCM (250 μL) was added to a stirred solution of donor 16 (565 mg, 1.02 mmol) in wet DCM (7 mL), and the mixture was stirred for 30 min. Then the mixture was neutralized with Et_3N and evaporated *in vacuo*. The residue was purified by flash column chromatography (silica gel, eluent: toluene/EtOAc = 10:1→2:1). The resulting hemiacetal was dissolved in DCM (5 mL), tetrabromomethane (815 mg, 2.46 mmol) and triphenylphosphine (645 mg, 2.46 mmol) were added, and the mixture was stirred at rt for 30 min and then purified by column chromatography (eluent: toluene/EtOAc = 50:1→30:1) to give bromide **17** (410 mg, 85%), $[\alpha]_D$ –70° (*c* 1, EtOAc). ^1H NMR (400 MHz, $CDCl_3$): δ 7.39–7.29 (m, 5H, Bn), 6.10 (t,

1H, $J_{1\text{-}2}$=4.5 Hz, *H*-1), 5.46–5.33 (m, 2H, *H*-3, *H*-4), 4.72–4.66 (m, 2H, PhC*HH*'), 4.51–4.40 (m, 1H, *H*-5), 4.11 (d, 1H, J=5.8 Hz, C*H*H'Cl), 4.06 (dd, 1H, $J_{2\text{-}3}$=10.2 Hz, $J_{1\text{-}2}$=3.9 Hz, *H*-2), 3.97 (d, 1H, J = 1.3 Hz, CH*H*'Cl), 3.92–3.80 (m, 1H, C*H*H'Cl), 3.79–3.75 (m, 1H, CH*H*'Cl), 1.20 (d, 3H, $J_{5\text{-}6}$=6.6 Hz, *H*-6). ^{13}C NMR (101 MHz, $CDCl_3$): δ 167.1 (CA), 128.6 (Bn), 128.3 (Bn), 128.0 (Bn), 93.3 (*C*-1), 73.0 (Bn), 72.5 (*C*-2), 72.1 (*C*-4), 71.50 (*C*-3), 67.5 (*C*-5), 40.4 (CA), 24.9 (CA), 15.60 (*C*-6). HRMS (ESI): Calcd m/z for $[M+NH_4]^+$ $C_{17}H_{19}BrCl_2O_6$ 486.0080, found 486.0082.

4.4.2. Propyl 6-O-(2-*O*-benzyl-3,4-di-*O*-chloroacetyl-L-fucopyranosyl)-3-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-galactopyranoside (18αβ)

0.1 M TMSOTf in DCM (30 μL) was added to a mixture of glycosyl donor **16** (61 mg, 0.11 mmol), glycosyl acceptor **15** (42 mg, 0.09 mmol), and molecular sieves 4 Å (100 mg) in DCM (1 mL) at -50 °C under argon protection. The mixture was stirred for 30 min, then neutralized with Et_3N, and subjected to column chromatography (eluent: toluene/EtOAc = 20:1→5:1) to give glycosylation product 18αβ (39 mg, 0.046 mmol, 51%) as a mixture of isomers (α:β=1:1). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.94–7.89 (m, 4H, Bz^{α}, Bz^{β}), 7.81–7.79 (m, 4H, $Phth^{\alpha}$, $Phth^{\beta}$), 7.69–7.65 (m, 4H, $Phth^{\alpha}$, $Phth^{\beta}$), 7.53–7.47 (m, 2H, Bz^{α}, Bz^{β}), 7.39–7.15 (m, 14H, Bz^{α}, Bz^{β}, Bn^{α}, Bn^{β}), 5.87 (dd, 1H, $J_{2\text{-}3}$=11.3 Hz, $J_{3\text{-}4}$=3.2 Hz, *H*-3 ($GalN^{\alpha}$)), 5.83 (dd, 1H, $J_{2\text{-}3}$=11.4 Hz, $J_{3\text{-}4}$=3.2 Hz, *H*-3 ($GalN^{\beta}$)), 5.41–5.33 (m, 4H, *H*-1 ($GalN^{\alpha}$), *H*-1 ($GalN^{\beta}$), *H*-3 (Fuc^{α}), *H*-4 (Fuc^{α})), 5.26 (d, 1H, $J_{3\text{-}4}$=3.4 Hz, *H*-4 (Fuc^{β})), 5.07 (dd, 1H, $J_{2\text{-}3}$=10.1 Hz, $J_{3\text{-}4}$=3.4 Hz, *H*-3 (Fuc^{β})), 4.88 (d, 1H, $J_{1\text{-}2}$=3.7 Hz, *H*-1 (Fuc^{α})), 4.85–4.76 (m, 3H, *H*-2 ($GalN^{\alpha}$), *H*-2 ($GalN^{\beta}$), PhC*H*H'$^{\beta}$), 4.72–4.60 (m, 3H, PhCH*H*'$^{\beta}$, PhCH_2^{α}), 4.55 (d, 1H, $J_{1\text{-}2}$=7.7 Hz, *H*-1 ($GalN^{\beta}$)), 4.40 (d, 2H, $J_{3\text{-}4}$=2.9 Hz, *H*-4 ($GalN^{\alpha}$), *H*-4 ($GalN^{\beta}$)), 4.24 (q, 1H, $J_{5\text{-}6}$=6.5 Hz, *H*-5 (Fuc^{α})), 4.18–4.09 (m, 5H, *H*-6 ($GalN^{\beta}$), CA^{α}, CA^{β}), 4.06–3.97 (m, 4H, *H*-5 ($GalN^{\alpha}$), *H*-5 ($GalN^{\beta}$), *H*-6' ($GalN^{\beta}$), *H*-6 ($GalN^{\alpha}$)), 3.96–3.90 (m, 4H, CA^{α}, CA^{β}), 3.89–3.83 (2H, m, *H*-6' ($GalN^{\alpha}$), *H*-2 (Fuc^{α})), 3.81–3.76 (m, 3H, *H*-5 (Fuc^{β}), Pr^{α}, Pr^{β}), 3.73 (dd, 1H, $J_{6\text{-}6'}$=10.1 Hz, $J_{5\text{-}6}$=5.2 Hz, *H*-6'($GalN^{\alpha}$)), 3.64 (dd, 1H, $J_{2\text{-}3}$=10.1 Hz, $J_{1\text{-}}$

$_2$=7.7 Hz, *H*-2 (Fuc$^{\beta}$)), 3.42–3.37 (m, 2H, Pr$^{\alpha}$, Pr$^{\beta}$), 1.50 – 1.39 (m, 4H, Pr$^{\alpha}$, Pr$^{\beta}$), 1.26 (d, 3H, J_{5-6}=6.4 Hz, *H*-6 (Fuc$^{\beta}$)), 1.15 (d, 3H, J_{5-6}=6.6 Hz, H-6 (Fuc$^{\alpha}$)), 0.68 (t, 6H, J=7.4 Hz, Pr$^{\alpha}$, Pr$^{\beta}$). ^{13}C NMR (151 MHz, $CDCl_3$): δ 134.1 (Phth$^{\alpha}$, Phth$^{\beta}$), 133.4 (Bz$^{\alpha}$, Bz$^{\beta}$), 129.9 (Bz$^{\alpha}$), 129.8 (Bz$^{\beta}$), 128.5 (2xBn$^{\alpha}$, 2xBn$^{\beta}$), 128.3 (Bz$^{\alpha}$, Bz$^{\beta}$), 127.9 (Bn$^{\beta}$), 127.8 (Bn$^{\alpha}$), 123.4 (Phth$^{\alpha}$, Phth$^{\beta}$), 103.6 (*C*-1 (Fuc$^{\beta}$)), 98.6 (*C*-1 (GalN$^{\beta}$)), 98.5 (*C*-1 (GalN$^{\alpha}$)), 97.9 (*C*-1 (Fuc$^{\alpha}$)), 75.9 (*C*-2 (Fuc$^{\beta}$)), 74.9 (Bn$^{\beta}$)), 74.2 (*C*-3 (Fuc$^{\beta}$)), 73.2 (*C*-4 (Fuc$^{\alpha}$), Bn$^{\alpha}$), 72.9 (*C*-2 (Fuc$^{\alpha}$)), 72.6 (*C*-5 (GalN$^{\beta}$)), 72.5 (*C*-5 (GalN$^{\alpha}$)), 72.4 (*C*-4 (Fuc$^{\beta}$)), 71.8 (*C*-3 (Fuc$^{\alpha}$)), 71.5 (*C*-3 (GalN$^{\beta}$)), 71.4 (Pr$^{\alpha}$, Pr$^{\beta}$),71.3 (*C*-3 (GalN$^{\alpha}$)), 68.7 (*C*-5 (Fuc$^{\beta}$)), 67.9 (*C*-6 (GalN$^{\beta}$)), 67.2 (*C*-4 (GalN$^{\beta}$)), 67.0 (*C*-6 (GalN$^{\alpha}$)), 66.6 (*C*-4 (GalN$^{\alpha}$)), 64.3 (*C*-5 (Fuc$^{\alpha}$)), 51.4 (*C*-2 (GalN$^{\alpha}$), *C*-2 (GalN$^{\beta}$)), 40.5 (CA$^{\alpha}$, CA$^{\beta}$), 40.4 (CA$^{\alpha}$, CA$^{\beta}$), 22.6 (Pr$^{\alpha}$, Pr$^{\beta}$), 16.1 (*C*-6 (Fuc$^{\beta}$)), 15.8 (*C*-6 (Fuc$^{\alpha}$)), 10.1 (Pr$^{\alpha}$, Pr$^{\beta}$). HRMS (ESI): Calcd m/z for $[M+Na]^+$ $C_{41}H_{43}Cl_2NO_{14}$ 866.1953, found 866.1957.

4.4.3. Propyl 6-*O*-(2-*O*-benzyl-3,4-di-*O*-chloroacetyl-L-fucopyranosyl)-3-*O*-benzoyl-4-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranoside (19αβ).

0.1 M TMSOTf in DCM (100 μL) was added to a mixture of glycosyl donor **16** (240 mg, 0.43 mmol), glycosyl acceptor **14** (180 mg, 0.36 mmol), and molecular sieves 4 Å (300 mg) in DCM (3 mL) at -50 °C under argon protection. The mixture was stirred for 30 min, then neutralized with Et_3N, and purified by column chromatography (eluent: toluene/EtOAc = 20:1→10:1) to give glycosylation product **19αβ** (250 mg, 78%) as a mixture of isomers (α:β=2:1). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.92 (d, 2H, J=8.3 Hz, Bz$^{\beta}$), 7.86–7.76 (m, 6H, Bz$^{\alpha}$, Phth$^{\alpha}$, Phth$^{\beta}$), 7.70–7.63 (m, 4H, Phth$^{\alpha}$, Phth$^{\beta}$), 7.53–7.43 (m, 2H, Bz$^{\alpha}$, Bz$^{\beta}$), 7.40–7.22 (m, 14H, Bz$^{\alpha}$, Bz$^{\beta}$, 5xBn$^{\alpha}$, 5xBn$^{\beta}$), 6.01–5.94 (m, 1H, *H*-3 (GalN$^{\alpha}$)), 5.85–5.81 (m, 1H, *H*-3 (GalN$^{\beta}$)), 5.71 (d, 1H, J_{3-4}=3.6 Hz, *H*-4 (GalN$^{\alpha}$)), 5.64 (d, 1H, J_{3-4}=3.5 Hz, *H*-4 (GalN$^{\beta}$)), 5.50–5.37 (m, 3H, *H*-1 (GalN$^{\alpha}$), *H*-1 (GalN$^{\beta}$), *H*-3 (Fuc$^{\alpha}$)), 5.35–5.31 (m, 1H, *H*-4 (Fuc$^{\alpha}$)), 5.25 (d, 1H, J_{3-4}=3.4 Hz, *H*-4 (Fuc$^{\beta}$)), 5.04 (dd, 1H, J_{2-3}=10.1 Hz, J_{3-4}=3.4 Hz, *H*-3 (Fuc$^{\beta}$)), 4.89 (d, 1H, J_{1-2}=3.7 Hz, *H*-1 (Fuc$^{\alpha}$)), 4.83–4.72 (m, 3H, *H*-2 (GalN$^{\alpha}$), *H*-2 (GalN$^{\beta}$), PhC*H*H'$^{\beta}$), 4.71–4.63 (m, 2H, PhCH_2^{α}), 4.58 (d, 1H, J=11.9

Hz, PhCH*H*'$^{\beta}$), 4.49 (d, 1H, $J_{1\text{-}2}$=7.7 Hz, *H*-1 (Fuc$^{\beta}$)), 4.19–4.08 (m, 6H, *H*-5 (GalN$^{\alpha}$), *H*-5 (Fuc$^{\alpha}$), CA$^{\alpha}$, CA$^{\beta}$), 4.04–4.00 (m, 2H, *H*-5 (GalN$^{\beta}$), *H*-6 (GalN$^{\beta}$)), 3.97–3.91 (m, 4H, CA$^{\alpha}$, CA$^{\beta}$), 3.89–3.76 (m, 6H, *H*-2 (Fuc$^{\alpha}$), *H*-6' (GalN$^{\beta}$), *H*-5 (Fuc$^{\beta}$), *H*-6 (GalN$^{\alpha}$), Pr$^{\alpha}$, Pr$^{\beta}$), 3.67 (dd, 1H, $J_{6\text{-}6'}$=10.5 Hz, $J_{5\text{-}6}$=6.4 Hz, *H*-6' (GalN$^{\alpha}$)), 3.61 (dd, 1H, $J_{2\text{-}3}$=10.2 Hz, $J_{1\text{-}2}$=7.6 Hz, *H*-2 (Fuc$^{\beta}$)), 3.48–3.37 (m, 2H, Pr$^{\alpha}$, Pr$^{\beta}$), 2.17 (s, 3H, Ac$^{\beta}$), 2.16 (s, 3H, Ac$^{\alpha}$), 1.53–1.37 (m, 4H, Pr$^{\alpha}$, Pr$^{\beta}$), 1.26 (d, 3H, $J_{5\text{-}6}$=6.7 Hz, *H*-6 (Fuc$^{\beta}$)), 1.12 (d, 3H, $J_{5\text{-}6}$=6.6 Hz, *H*-6 (Fuc$^{\alpha}$)), 0.74–0.60 (m, 6H, Pr$^{\alpha}$, Pr$^{\beta}$). **^{13}C NMR** (151 MHz, $CDCl_3$): δ 167.2 (Bz), 165.0 (Ac), 134.2 (Phth$^{\alpha}$, Phth$^{\beta}$), 133.3 (Bz$^{\alpha}$, Bz$^{\beta}$), 129.8 (Bn$^{\beta}$), 129.6 (Bz$^{\alpha}$, Bz$^{\beta}$), 128.5 (Bn$^{\alpha}$), 128.4 (Bz$^{\alpha}$, Bz$^{\beta}$), 128.3 (Bn$^{\beta}$), 128.0 (Bn$^{\alpha}$, Bn$^{\beta}$), 127.7 (Bn$^{\alpha}$), 123.4 (Phth$^{\alpha}$, Phth$^{\beta}$), 103.4 (*C*-1 (Fuc$^{\beta}$)), 98.6 (*C*-1 (GalN$^{\beta}$)), 98.5 (*C*-1 (GalN$^{\alpha}$)), 98.2 (*C*-1 (Fuc$^{\alpha}$)), 75.8 (*C*-2 (Fuc$^{\beta}$)), 74.7 (Bn$^{\beta}$), 74.0 (*C*-3 (Fuc$^{\beta}$)), 73.7 (*C*-5 (GalN$^{\beta}$)), 73.2 (*C*-4 (Fuc$^{\alpha}$)), 73.0 (*C*-2 (Fuc$^{\alpha}$)), 72.9 (Bn$^{\alpha}$), 72.3 (*C*-4 (Fuc$^{\beta}$)), 72.2 (*C*-5 (GalN$^{\alpha}$)), 71.7 (Pr$^{\beta}$), 71.6 (Pr$^{\alpha}$), 71.5 (*C*-3 (Fuc$^{\alpha}$)), 69.0 (*C*-3 (GalN$^{\alpha}$)), 68.6 (*C*-5 (Fuc$^{\beta}$)), 67.8 (*C*-4 (GalN$^{\beta}$)), 67.6 (*C*-4 (GalN$^{\alpha}$)), 67.3 (*C*-6 ($GalN_{\alpha}$), *C*-3 (GalN$^{\beta}$)), 67.2 (*C*-6 (GalN$^{\beta}$)), 64.4 (*C*-5 (Fuc$^{\alpha}$)), 51.9 (*C*-2 (GalN$^{\beta}$)), 51.8 (*C*-2 (GalN$^{\alpha}$)), 40.5 (CA$^{\alpha}$), 40.4 (CA$^{\beta}$), 22.6 (Pr$^{\alpha}$, Pr$^{\beta}$), 20.6 (Ac$^{\alpha}$, Ac$^{\beta}$), 16.1 (*C*-6 (Fuc$^{\beta}$)), 15.9 (*C*-6 (Fuc$^{\alpha}$)), 10.1 (Pr$^{\alpha}$, Pr$^{\beta}$). HRMS (ESI): Calcd m/z for $[M+K]^+$ $C_{43}H_{45}Cl_2NO_{15}$ 924.1798, found 924.1794.

4.4.4. Propyl 6-O-(2-*O*-benzyl-3,4-di-*O*-chloroacetyl-α-L-fucopyranosyl)-3-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-galactopyranoside (18α)

TBAB (65 mg, 0.15 mmol) was added to a mixture of glycosyl donor **16** (260 mg, 0.55 mmol) and glycosyl acceptor **15** (200 mg, 0.44 mmol) in DCM (4 mL). The mixture was kept for 7 days and then subjected to column chromatography (eluent: toluene/EtOAc = 20:1→2:1) to give a mixture of **15** and **16** (230 mg) and glycosylation product **18α** (104 mg, 56%), $[\alpha]_D$ –44° (*c* 1, EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 (d, 1H, J=8.3 Hz, Bz), 7.80 (dd, 2H, J=5.2 Hz, J=3.3 Hz, Phth), 7.67 (dd, 2H, J=5.6 Hz, J=2.9 Hz, Phth), 7.54–7.46 (m, 1H, Bz), 7.42–7.22 (m, 7H, Bz, Bn), 5.87 (dd, 1H, $J_{2\text{-}3}$=11.3 Hz, $J_{3\text{-}4}$=3.2 Hz, *H*-3 (GalN)), 5.40 (d, 1H, $J_{1\text{-}2}$=8.5 Hz, *H*-1

(GalN)), 5.39–5.28 (m, 2H, *H*-3 (Fuc), *H*-4 (Fuc)), 4.88 (t, 1H, $J_{1\text{-}2}$=4.4 Hz, *H*-1 (Fuc)), 4.81 (dd, 1H, $J_{2\text{-}3}$=11.3 Hz, $J_{1\text{-}2}$=8.5 Hz, *H*-2 (GalN)), 4.76–4.65 (m, 2H, PhCH_2), 4.41 (br s, 1H, *H*-4 (GalN)), 4.24 (q, 1H, $J_{5\text{-}6}$=5.9 Hz, *H*-5 (Fuc)), 4.12 (d, 2H, J=5.6 Hz, CA), 4.06–3.97 (m, 2H, *H*-5 (GalN), *H*-6 (GalN)), 3.96 – 3.70 (m, 5H, CA, *H*-2 (Fuc), Pr, *H*-6' (GalN)), 3.40 (dt, 1H, J=9.6 Hz, J=6.8 Hz, Pr), 2.61 (br s, 1H,4-O*H* (GalN)), 1.50–1.38 (m, 2H, Pr), 1.15 (d, 3H, $J_{5\text{-}6}$=6.5 Hz, *H*-6 (Fuc)), 0.67 (t, 3H, J=7.4 Hz, Pr). ^{13}C NMR (101 MHz, $CDCl_3$): δ 167.3 (Bz), 165.3 (CA), 134.1 (Phth), 133.4 (Bz), 129.9 (Bz), 128.5 (2xBn), 128.5 (Bz), 128.1 (Bn), 123.5 (Phth), 98.5 (*C*-1 (GalN)), 97.9 (*C*-1 (Fuc)), 73.30 (Ph*C*H_2), 73.0 (*C*-2 (Fuc)), 72.7 (*C*-4 (Fuc)), 72.4 (*C*-5 (GalN)), 72.0 (*C*-3 (Fuc)), 71.3 (*C*-3 (GalN), Pr), 67.1 (*C*-4 (GalN)), 67.0 (*C*-6 (GalN)), 64.3 (*C*-5 (Fuc)), 51.3 (*C*-2 (GalN)), 40.5 (CA), 25.1 (CA), 22.6 (Pr), 15.8 (*C*-6 (Fuc)), 10.2 (Pr). HRMS (ESI): Calcd m/z for $[M+Na]^+$ $C_{41}H_{43}Cl_2NO_{14}$ 866.1953, found 866.1955.

4.4.5. Propyl 6-O-(2-*O*-benzyl-α-L-fucopyranosyl)-3-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-galactopyranoside (20).

A mixture of disaccharide **18α** (35 mg, 0.041 mmol), 2,4,6-collidine (28 μL, 0.207 mmol), and thiourea (50 mg, 0.62 mmol) in MeOH (4 mL) was refluxed for 24 h at 60 °C, then cooled, and taken to dryness. The residue was purified by flash column chromatography (silica gel, eluent: toluene/EtOAc = 3:1→1:1) to give **21** (26.5 mg, 92%), $[\alpha]_D$ –28° (*c* 1, EtOAc). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.92 (d, 2H, J=8.3 Hz, Bz), 7.82–7.77 (m, 2H, Phth), 7.67 (dd, 2H, J=5.5, J=3.0 Hz, Phth), 7.51–7.45 (m, 1H, Bz), 7.38–7.30 (m, 7H, Bz, Bn), 5.82 (dd, 1H, $J_{2\text{-}3}$=11.3 Hz, $J_{3\text{-}4}$=3.2 Hz, *H*-3 (GalN)), 5.38 (d, 1H, $J_{1\text{-}2}$=8.5 Hz, *H*-1 (GalN)), 4.85 (dd, 1H, $J_{2\text{-}3}$=11.3 Hz, $J_{1\text{-}2}$=8.5 Hz, *H*-2 (GalN)), 4.82 (d, 1H, $J_{1\text{-}2}$=3.6 Hz, *H*-1 (Fuc)), 4.67 (d, 1H, J=12.0 Hz, PhC*H*H'), 4.64 (d, 1H, J=12.0 Hz, PhCH*H*'), 4.43 (d, 1H, $J_{3\text{-}4}$=3.2 Hz, *H*-4 (GalN)), 4.05–3.98 (m, 3H, *H*-5 (Fuc), *H*-3 (Fuc), *H*-6 (GalN)), 3.92 (t, 1H, $J_{5\text{-}6}$=5.3 Hz, *H*-5 (GalN)), 3.81 (d, 1H, $J_{3\text{-}4}$=3.4 Hz, *H*-4 (Fuc)), 3.78 (dt, 1H, J=9.7 Hz, J=6.5 Hz, Pr), 3.72 (dd, 1H, $J_{2\text{-}3}$=9.8 Hz, $J_{1\text{-}2}$=3.6 Hz, *H*-2 (Fuc)), 3.64 (dd, 1H, $J_{6\text{-}6'}$=10.5 Hz, $J_{5\text{-}6}$=5.0 Hz, *H*-6' (GalN)), 3.40 (dt, 1H, J=9.7 Hz, J=6.8 Hz,

Pr), 1.50–1.38 (m, 2H, Pr), 1.27 (d, 3H, J_{5-6}=6.6 Hz, *H*-6 (Fuc)), 0.68 (t, 3H, J=7.4 Hz, Pr). ^{13}C NMR (151 MHz, $CDCl_3$): δ 165.5 (Bz), 134.1 (Phth), 133.3 (Bz), 129.8 (Bz), 128.6 (Bn), 128.4 (Bz, Bn), 128.1 (Bn), 123.4 (Phth), 98.6 (*C*-1 (GalN)), 97.2 (*C*-1 (Fuc)), 76.5 (*C*-2 (Fuc)), 72.9 (Ph*C*H_2) , 72.5 (*C*-5 (GalN)), 71.6 (*C*-4 (Fuc)), 71.5 (*C*-3 (GalN)), 71.3 (Pr), 69.4 (*C*-3 (Fuc)), 67.4 (*C*-4 (GalN)), 67.2 (*C*-6 (GalN)), 65.8 (*C*-5 (Fuc)), 51.3 (*C*-2 (GalN)), 22.6 (Pr), 16.1 (*C*-6 (Fuc)), 10.1 (Pr). HRMS (ESI): Calcd m/z for $[M+Na]^+$ $C_{37}H_{41}NO_{12}$ 714.2521, found 714.2515.

4.4.6. Propyl 6-*O*-(α-L-fucopyranosyl)-2-acetamido-2-deoxy-β-D-galactopyranoside (5)

Disaccharide **18α** (36 mg, 0.043 mmol) was successively treated according to general procedures B [EtOAc (1mL), MeOH (1 mL), 10% $Pd(OH)_2$/C (30 mg), 3 h], C [$N_2H_4 \cdot H_2O$ (0.5 mL), EtOH (5 mL)] and D [Ac_2O (0.4 mL, $NaHCO_3$ (340 mg), H_2O (2 mL), MeOH (2 mL)] to give **5** (9 mg, 51%), $[\alpha]_D$ –25° (*c* 1, H_2O). ^{1}H NMR (600 MHz, D_2O): δ 4.96 (d, 1H, J_{1-2}=3.9 Hz, *H*-1 (Fuc)), 4.48 (d, 1H, J_{1-2}=8.5 Hz, *H*-1 (GalN)), 4.10 (q, 1H, J_{5-6}=6.7 Hz, *H*-5 (Fuc)), 3.96 (d, 1H, J_{3-4}=3.3 Hz, *H*-4 (GalN)), 3.92–3.77 (m, 8H, *H*-6 (GalN), *H*-6’ (GalN), *H*-2 (GalN), *H*-5 (GalN), *H*-3 (Fuc), *H*-4 (Fuc), Pr, *H*-2 (Fuc)), 3.74 (dd, 1H, J_{2-3}=10.8 Hz, J_{3-4}=3.3 Hz, *H*-3 (GalN)), 3.56 (dt, 1H, J=10.0 Hz, J=6.6 Hz, Pr), 2.05 (s, 3H, Ac), 1.60–1.52 (m, 2H, Pr), 1.24 (d, 1H, J_{5-6}=6.6 Hz, H-6 (Fuc)), 0.88 (t, 3H, J=7.4 Hz, Pr). ^{13}C NMR(151 MHz, $CDCl_3$): δ 103.0 (*C*-1 (GalN)), 100.7 (*C*-1 (Fuc)), 74.9 (*C*-5 (GalN)), 73.7 (Pr), 73.1 (*C*-4 (Fuc)), 72.4 (*C*-3 (GalN)), 70.9 (*C*-3 (Fuc)), 69.4 (*C*-2 (Fuc)), 69.3 (*C*-4 (GalN)), 69.0 (*C*-6 (GalN)), 68.2 (*C*-5 (Fuc)), 53.7 (*C*-2 (GalN)), 23.6 (Ac), 23.5 (Pr), 16.8 (*C*-6 (Fuc)), 11.0 (Pr)). HRMS (ESI): Calcd m/z for $[M+Na]^+$ $C_{17}H_{31}NO_{10}$ 432.1840, found 432.1828.

4.4.7. Propyl 6-*O*-(α-L-fucopyranosyl)-4-*O*-sulfo-2-deoxy-2-acetyl-β-D-galactopyranoside sodium salt (6).

Disaccharide **18α** (20 mg, 0.024 mmol) was successively treated according to general procedures E [$Py \cdot SO_3$ (20 mg, 0.12 mmol), DMF (2 mL), 1 h, flash column chromatography (silica gel,

DCM–MeOH, 10:1→2:1)], B [EtOAc (1mL), MeOH (1 mL), 10% $Pd(OH)_2/C$ (30 mg), 2 days], C [$N_2H_4 \cdot H_2O$ (0.5 mL), EtOH (5 mL)] and D [Ac_2O (0.4 mL, $NaHCO_3$ (340 mg), H_2O (2 mL), MeOH (2 mL)] to give **6** (5 mg, 42%), $[\alpha]_D$ –48° (*c* 1, H_2O). ^{1}H NMR (600 MHz, D_2O): δ 4.95 (d, 1H, $J_{1\text{-}2}$=3.9 Hz, *H*-1 (Fuc)), 4.71 (s, 1H, *H*-4 (GalN), srp), 4.56–4.50 (m, 1H, *H*-1 (GalN)), 4.11 (q, 1H, $J_{5\text{-}6}$=6.6 Hz, *H*-5 (Fuc)), 3.98 (dd, 1H, $J_{5\text{-}6'}$=8.3 Hz, $J_{5\text{-}6}$=3.9 Hz, H-5 (GalN)), 3.91 (dd, 1H, $J_{6\text{-}6'}$=11.7 Hz, $J_{5\text{-}6}$=3.9 Hz, *H*-6 (GalN)), 3.88–3.78 (m, 6H, *H*-2 (GalN), *H*-3 (GalN), *H*-3 (Fuc), Pr, *H*-6' (GalN), *H*-4 (Fuc)), 3.75 (dd, 1H, $J_{2\text{-}3}$=10.4 Hz, $J_{1\text{-}2}$=3.9 Hz, *H*-2 (Fuc)), 3.55 (dt, 1H, J=10.0 Hz, J=6.6 Hz, Pr), 2.03 (s, 3H, Ac), 1.59–1.51 (m, 2H, Pr), 1.22 (d, 3H, $J_{5\text{-}6}$=6.6 Hz, *H*-6 (Fuc)), 0.86 (t, 3H, J=7.4 Hz, Pr). ^{13}C NMR (151 MHz, D_2O): δ 176.0 (Ac), 102.6 (*C*-1 (GalN)), 100.9 (*C*-1 (Fuc)), 77.3 (*C*-4 (GalN)), 74.3 (*C*-5 (GalN)), 73.6 (Pr), 73.0 (*C*-4 (Fuc)), 71.3 (*C*-3 (GalN)), 70.8 (*C*-3 (Fuc)), 69.3 (*C*-2 (Fuc)), 69.2 (*C*-6 (GalN)), 68.0 (*C*-5 (Fuc)), 54.1 (*C*-2 (GalN)), 23.4 (Pr), 23.3 (Ac), 16.7 (*C*-6 (Fuc)), 10.8 (Pr). HRMS (ESI): Calcd m/z for $[M\text{-}Na]^-$ $C_{17}H_{30}NO_{13}S$ 488.1443, found 488.1436.

4.4.8. Propyl 6-*O*-(3,4-di-*O*-sulfo-α-L-fucopyranosyl)-4-*O*-sulfo-2-deoxy-2-acetyl-β-D-galactopyranoside sodium salt (7)

Disaccharide **20** (16 mg, 0.023 mmol) was successively treated according to general procedures E [$Py \cdot SO_3$ (55 mg, 0.35 mmol), DMF (2 mL), 1h, flash column chromatography (silica gel, DCM – MeOH, 10:1→2:1)], B [EtOAc (1mL), MeOH (1 mL), 10% $Pd(OH)_2/C$ (30 mg), 1 day], C [$N_2H_4 \cdot H_2O$ (0.5 mL), EtOH (5 mL)] and D [Ac_2O (0.4 mL, $NaHCO_3$ (340 mg), H_2O (2 mL), MeOH (2 mL)] to give **7** (6.2 mg, 37%), $[\alpha]_D$ –66° (*c* 1, H_2O). ^{1}H NMR (600 MHz, D_2O): δ 4.93 (d, 1H, $J_{1\text{-}2}$=3.9 Hz, *H*-1 (Fuc)), 4.76 (d, 1H, $J_{3\text{-}4}$=3.0 Hz, *H*-4 (Fuc)), 4.59 (d, 1H, $J_{3\text{-}4}$=2.8 Hz, *H*-4 (GalN)), 4.46 (dd, 1H, $J_{2\text{-}3}$=10.4 Hz, $J_{3\text{-}4}$=3.0 Hz, *H*-3 (Fuc)), 4.41 (d, 1H, $J_{1\text{-}2}$=7.8 Hz, *H*-1 (GalN)), 4.14 (q, 1H, $J_{5\text{-}6}$=6.6 Hz, *H*-5 (Fuc)), 3.90 (dd, 1H, $J_{5\text{-}6}$=8.6 Hz, $J_{5\text{-}6'}$=3.3 Hz, *H*-5 (GalN)), 3.87–3.82 (m, 2H, *H*-2 (Fuc), H-6 (GalN)), 3.79–3.70 (m, 4H, *H*-2 (GalN), *H*-3 (GalN), *H*-6' (GalN), Pr), 3.45 (dt, 1H, J=10.1 Hz, J=6.6 Hz, Pr), 1.91 (s, 3H, Ac), 1.44 (dd, 2H, J=13.9

Hz, J=6.7 Hz, Pr), 1.17 (d, 3H, $J_{5\text{-}6}$=6.6 Hz, *H*-6 (Fuc)), 0.75 (t, 3H, J=7.4 Hz, Pr). **^{13}C NMR** (151 MHz, D_2O): δ 102.6 (*C*-1 (GalN)), 100.7 (*C*-1 (Fuc)), 80.2 (*C*-4 (Fuc)), 77.3 (*C*-4 (GalN)), 76.6 (*C*-3 (Fuc)), 74.6 (*C*-5 (GalN)), 73.6 (Pr), 71.3 (*C*-3 (GalN)), 69.6 (*C*-6 (GalN)), 67.7 (*C*-5 (Fuc)), 67.4 (*C*-2 (Fuc)), 54.1 (*C*-2 (GalN)), 23.3 (Pr, Ac), 17.3 (*C*-6 (Fuc)), 10.8 (Pr). HRMS (ESI): Calcd m/z for $[M\text{-}2Na]^{2-}$ $C_{17}H_{28}NO_{19}S_3Na$ 334.5163, found 334.5154.

4.4.9. Propyl 6-*O*-(2,3,4-tri-*O*-sulfo-α-L-fucopyranosyl)-4-*O*-sulfo-2-acetamido-2-deoxy-β-D-galactopyranoside sodium salt (8)

Disaccharide **20** (26 mg, 0.037 mmol) was successively treated according to general procedures B [EtOAc (1mL), MeOH (1 mL), 10% $Pd(OH)_2/C$ (40 mg), 2 h], E [$Py\cdot SO_3$ (120 mg, 0.75 mmol), DMF (2 mL), 2 h], C [$N_2H_4\cdot H_2O$ (0.5 mL), EtOH (5 mL)] and D [Ac_2O (0.4 mL, $NaHCO_3$ (340 mg), H_2O (2 mL), MeOH (2 mL)] to give **8** (14.3 mg, 47%), $[\alpha]_D$ –54° (*c* 1, H_2O). ^{1}H NMR (600 MHz, D_2O): δ 5.43 (d, 1H, $J_{1\text{-}2}$=3.7 Hz, *H*-1 (Fuc)), 5.03 (d, 1H, $J_{3\text{-}4}$=3.1 Hz, *H*-4 (Fuc)), 4.86 (d, 1H, $J_{3\text{-}4}$=2.2 Hz, *H*-4 (GalN))), 4.81 (dd, 1H, $J_{2\text{-}3}$=10.6 Hz, $J_{3\text{-}4}$=3.1 Hz, *H*-3 (Fuc)), 4.65–4.59 (m, 2H, *H*-2 (Fuc), *H*-1 (GalN)), 4.39 (q, 1H, $J_{5\text{-}6}$=6.6 Hz, *H*-5 (Fuc)), 4.12–4.06 (m, 2H, *H*-5 (GalN), *H*-6 (GalN)), 4.00–3.92 (m, 4H, *H*-2 (GalN), *H*-3 (GalN), *H*-6’ (GalN), Pr), 3.66 (dt, 1H, J=10.1 Hz, J=6.5 Hz, Pr), 2.13 (s, 3H, Ac), 1.69–1.62 (m, 2H, Pr), 1.40 (d, 3H, $J_{5\text{-}6}$=6.6 Hz, H-6 (Fuc)), 0.97 (t, 3H, J=7.4 Hz, Pr). ^{13}C NMR (151 MHz, D_2O): δ 176.0 (Ac), 102.6 (*C*-1 (GalN)), 98.6 (*C*-1 (Fuc)), 80.4 (*C*-4 (Fuc)), 77.5 (*C*-4 (GalN)), 74.4 (*C*-5 (GalN)), 73.8 (*C*-3 (Fuc)), 73.6 (Pr), 73.5 (*C*-2 (Fuc)), 71.4 (*C*-3 (GalN)), 69.0 (*C*-6 (GalN)), 67.6 (*C*-5 (Fuc)), 54.1 (*C*-2 (GalN)), 23.5 (Pr), 23.3 (Ac), 17.2 (*C*-6 (Fuc), 10.9 (Pr). HRMS (ESI): Calcd m/z for $[M\text{-}Na]^-$ $C_{17}H_{27}NO_{22}S_4Na_3$ 793.9606, found 793.9611.

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- Selectively sulfated derivatives of GalNAc and α-Fuc-(1→6)-GalNAc were synthesized.
- Fucosyl bromide was applied for stereoselective 6-*O*-α-L-fucosylation.
- NMR effects of sulfation and fucosylation were calculated.
- Obtained NMR-data are vital for the development of computer assisted analysis of natural FCS.