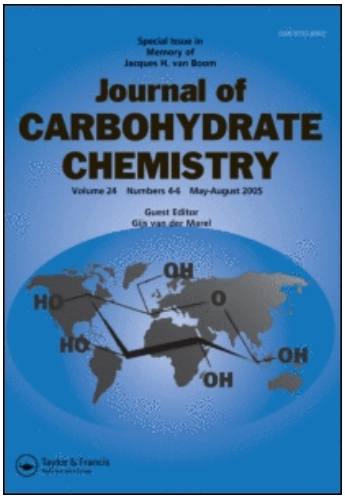

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Stereoselective Synthesis of Di- and Trisaccharide Fucoidan Fragments Bearing α-D-Glucuronic Acid Residue

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Stereoselective Synthesis of Di- and Trisaccharide Fucoidan Fragments Bearing α-D-Glucuronic Acid Residue*

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The first stereoselective synthesis of disaccharide α-D-GlcA-(1 $\rightarrow$ 2)-α-L-Fuc-OPr (**1**), trisaccharide (1 $\rightarrow$ 3)-α-L-Fuc-[α-D-GlcA-(1 $\rightarrow$ 2)]-α-L-Fuc-OPr (**3**), and their selectively *O*-sulfated derivatives **2** and **4** bearing sulfo-groups at O(4) of the fucose units has been performed. Compounds **1**–**4** represent the fragments of the chain of the fucoidan from *Cladosiphon okamuranus* brown seaweed. Glucuronylation by a series of selectively *O*-acetylated glucuronyl bromides was studied to obtain the target products. It has been found that 3-*O*-acetylated donor **6** is the most efficient agent for α-glycoside bond formation that can be connected with intramolecular remote participation of 3-*O*-acetyl group favoring α-stereoselectivity.

Keywords Fucoidan, *Cladosiphon okamuranus* brown seaweed, fucosylation, glucuronylation, *O*-sulfation

INTRODUCTION

Brown seaweed polysaccharide fucoidans have been shown to be active anticoagulant agents[2–5] as well as effective inhibitors of angiogenesis development, P- and L-selectin-mediated inflammation, and some other biological processes (see papers[3–5] and references therein). The backbones of fucoidans consist mainly of partially sulfated α-L-fucose residues, but the exact structure

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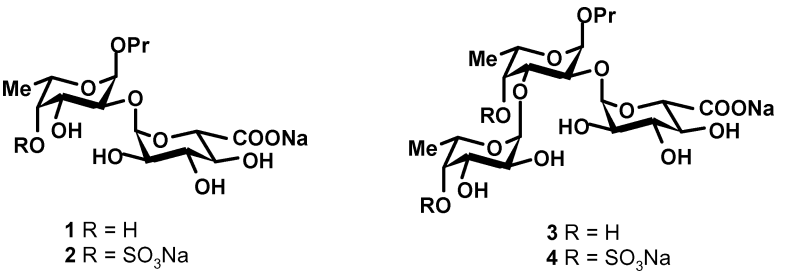


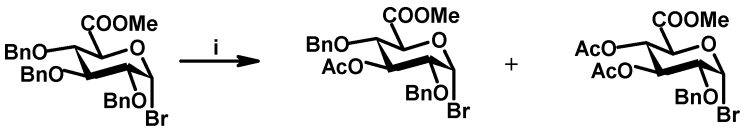
Figure 1: Structures of studied ogliosaccharides.

of these polysaccharides could not be determined in all details because of irregularity and heterogeneity of their chains. Structural features of fucoidans such as the types of glycoside bonds, pattern of sulfation, presence of nonfucose monosaccharide residues, branched fragments, and other characteristics depend on algae species. Recently we demonstrated that the fucoidans from different brown seaweeds could vary dramatically in the profile of their biological activity.[5] Thus, the polysaccharide from *Cladosiphon okamuranus* effectively inhibits inflammation but not blood coagulation processes, whereas fucoidan from *Laminaria saccharina* possesses both anti-inflammatory and anticoagulant activities. These two polysaccharides have similar backbones built up of $(1 \rightarrow 3)$-linked α-L-fucose residues, but differ in their branching parts. Thus, the fucoidan from *L. saccharina* contains α-L-fucose or sulfo-group as a substituent at O(2) of the fucose residues in the main chain,[6] whereas the fucoidan from *C. okamuranus* bears the $(1 \rightarrow 2)$-α-D-glucuronic acid unit as a substituent.[7]

To determine the structural features of fucoidans responsible for their spatial organization and biological properties, we carry out systematic synthesis, NMR, and conformational analysis of fucoidan fragments and study their biological activity.[8,9] In this communication we report the first regio- and stereoselective synthesis of nonsulfated and selectively *O*-sulfated di- and trisaccharides **1–4** (Fig. 1) related to the fragments of fucoidan from *C. okamuranus*.

RESULTS AND DISCUSSION

The most difficult aspect in the synthesis of target compounds **1–4** was the stereoselective introduction of α-D-glucuronic residue. In order to choose an efficient glucuronosyl donor for this process, model glycosylation of allyl 3,4-*O*-isopropylidene-α-L-fucopyranoside (**8**)[10] with fully benzylated (**5**) and partially *O*-acetylated 2-*O*-benzylglucuronosyl bromides (**6**, **7**) was studied. Based on our previous results,[11–13] we anticipated that the remote stereocontrolling effect of the 3-*O*-acetyl group would favor α-glycoside formation.



5 6 7

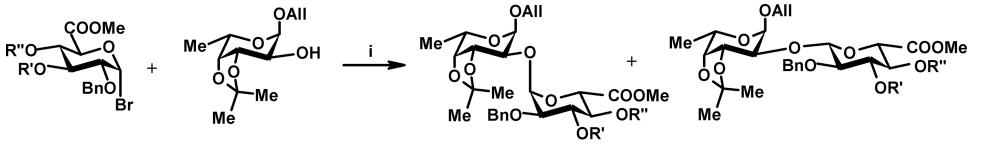
Scheme 1. (i) HBr, AcOH, Ac_2O, CH_2Cl_2, 1.5h.

Compounds **6** and **7** were prepared in yields of 59% and 25%, respectively, by treatment of fully benzylated precursor **5**[14] with HBr in acetic acid in the presence of Ac_2O (Sch. 1). 3-*O*-Acetylated and 3,4-di-O-acetylated glucuronosyl bromides **6** and **7** were easily separated by column chromatography. The presence of the acetyl group at O(3) in **6** was confirmed by the downfield shift of the corresponding H(3) signal (5.58 ppm) in the ^{1}H NMR spectrum, while the low-field signals for H(3) and H(4) (5.50 and 5.10 ppm, respectively) in the spectrum of **7** confirmed its 3,4-di-O-acetylation.

Glycosylation of compound **8** with fully benzylated bromide **5** in the presence of AgOTf gave a mixture of α- and β-disaccharides **9** and **10** in a ratio of 2:1 and total yield of 89% (Sch. 2). The isomers were easily separated by column chromatography on silica gel. The configuration of the glycoside bond formed was confirmed by corresponding characteristic coupling constant values $J_{1,2}$ (3.5 Hz for α-isomer, 7.8 Hz for β-isomer) in the ^{1}H NMR spectra.

Glucuronylation of fucoside **8** by selectively *O*-acetylated bromides **6** and **7** proceeded more α-stereoselectively. Thus, 3-*O*-acetylated bromide **6** produced α-linked disaccharide **11** exclusively, whereas glycosylation with 3,4-di-*O*-acetylated bromide **7** under the same conditions was less stereoselective and gave a mixture of α- and β-isomers **12** and **13** in a ratio of 5:1.

We assumed that the higher α-stereoselectivity in glycosylation with acetylated donors **6** and **7** as compared to fully benzylated donor **5** is connected with the remote participation of the 3-*O*-acetyl group resulting in the formation of the stabilized glycosyl cation **II** (Fig. 2). Nucleophilic attack on this cation is favored from the α-side, while in the case of the nonstabilized intermediate **I** from fully benzylated donor **5**, it is possible both from α- and β-sides. Efficiency of remote participation of the 3-*O*-acetyl group was



5 R' = R" = Bn
6 R' = Ac, R" = Bn
7 R' = R" = Ac

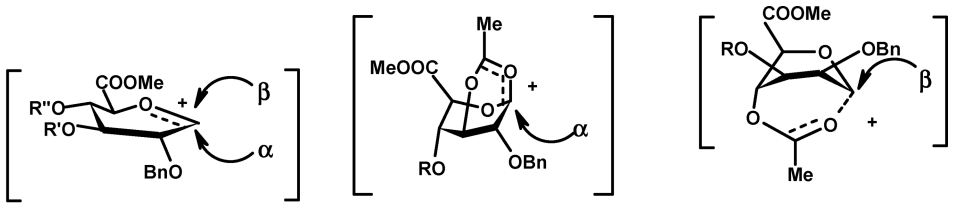
8

9 R' = R" = Bn
11 R' = Ac, R" = Bn
12 R' = R" = Ac

10 R' = R" = Bn

13 R' = R" = Ac

Scheme 2. (i) AgOTf, CH_2Cl_2, -30°C.



"non-stabilized" cation **I** (could be generated from **6**-**8**)

"stabilized" cation **II** (could be generated from **7** and **8**)

"stabilized" cation **III** (could be generated from **8**)

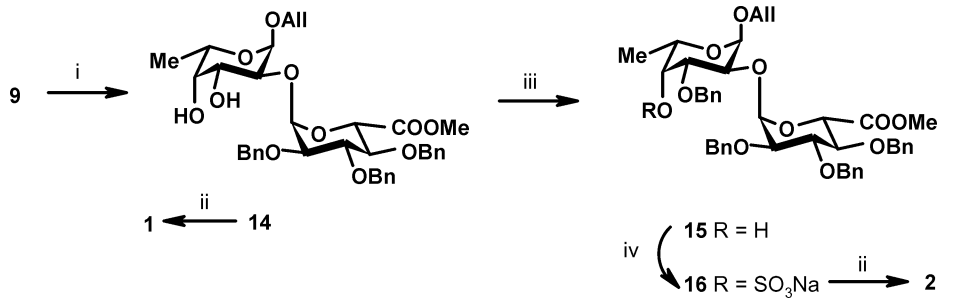
Figure 2 Delocalization of positive charge in the glycosyl cation **I** via intramolecular participation of acetyl group at O(3) (cation **II**) and O(4) (cation **III**).

confirmed by molecular calculations[13]; a similar stereo-effect has been observed earlier in the reactions with fucosyl,[1,11] glucosyl,[12,13] xylosyl,[13,15] and mannuronosyl[16] donors.

3,4-Di-*O*-acetylated bromide **7** is a less α-selective glycosylating agent than mono-3-*O*-acetylated bromide **6** (Sch. 2). This could be connected with the existence of the equilibrium between two sorts of the stabilized cationic intermediates **II** and **III** (Fig. 2). The interaction of the latter with a glycosyl acceptor proceeds preferentially from the β-side to give a β-linked disaccharide. Similar stereocontrolling effects of *O*-acetyl groups were observed in glycosylation by selectively 3-*O*- and 4-*O*-acetylated di-*O*-benzyl-α-D-mannuronosyl thioglycosides.[16] The possibility of the formation of the intermediates of type **II** and **III** and the comparison of the efficiencies of both stabilization effects as well as of possible participation of a methoxycarbonyl substituent[17] at C(5) are under investigation in this laboratory by computer calculations according to the described procedure[11] with the use of special series of selectively substituted glucuronic acid derivatives and will be reported elsewhere.

Removal of the isopropylidene group in compound **9** by acidic hydrolysis gave quantitatively corresponding diol **14**, which was subjected to catalytic hydrogenolysis accompanied by the reduction of the allyl to the propyl group to give the target disaccharide **1** in 61% yield (Sch. 3). To synthesize its sulfated derivative **2**, diol **14** was subjected to regioselective 3-*O*-benzylation using the stannylene procedure[18] to give the disaccharide **15** in a yield of 84%. Its sulfation with the $Py{\cdot}SO_3$ complex and further catalytic hydrogenolysis followed by saponification of the ester group in the compound **16** gave disaccharide **2**. The presence of a sulfo-group at O(4) in this compound was confirmed by a characteristic downfield chemical shift of the H(4) signal (3.85 $\rightarrow$ 4.64 ppm) in the comparison of the ^{1}H NMR spectra for **1** and **2**.

Disaccharides **11** and **12** are convenient synthetic blocks for assembling larger oligosaccharides carrying glycosylated glucuronic acid. Particularly, such structural motif represented by the terminal α-L-Fuc*f* -(1 $\rightarrow$ 4)-α-D-GlcA*p*

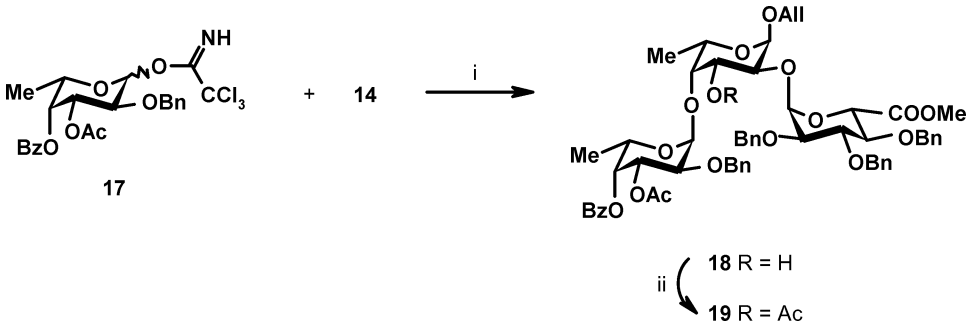


Scheme 3. (i) CF_3COOH_{aq}, CH_2Cl_2; (ii) (a) H_2, Pd/C; (b) 0.4 M NaOH; (iii) Bu_2SnO, toluene; BnBr, Bu_4NBr; (iv) Py·SO_3, DMF, Py, Amberlite (Na^+).

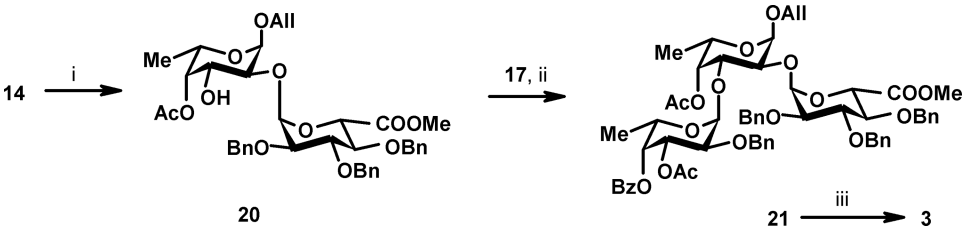
disaccharide fragment was recently found[19] in the fucoidan from the brown alga *Chordaria flagelliformis*.

Diol **14** was chosen as glycosyl acceptor block for the synthesis of trisaccharides **3** and **4**. It was supposed that this compound could be regioselectively 3-*O*-glycosylated with the use of one equivalent of fucosyl donor **17**[1] due to the difference in the reactivity of equatorial and axial OH-groups at C(3) and C(4). However, this reaction afforded only unexpected 4-*O*-fucosylated trisaccharide **18** (Sch. 4). This result could be explained by decreasing of the reactivity of the equatorial hydroxyl group at C(3) in diol **14** due to electronic and steric effects of the neighboring 2-*O*-glucuronosyl residue. The structure of **18** was confirmed by ^{1}H NMR spectroscopy after its transformation into corresponding acetylated derivative **19**; the downfield location of the signal for H(3) (5.42 ppm) revealed the presence of the free OH-group at C(3) in **18**.

To avoid the $(1\rightarrow 4)$-glycoside bond formation, diol **14** was converted into 4-*O*-acetylated derivative **20**; its glycosylation with fucosyl donor **17** provided the desired $(1\rightarrow 3)$-α-linked trisaccharide **21** in 78% yield (Sch. 5). The α-configuration of the glycoside bond formed was confirmed by the characteristic coupling constant value $J_{1'',2''}$ (3.5 Hz) in the ^{1}H NMR spectrum of **21**.



Scheme 4. (i) 0.1 M TMSOTf, CH_2Cl_2, –30°C; (ii) AcCl, Py, CH_2Cl_2.

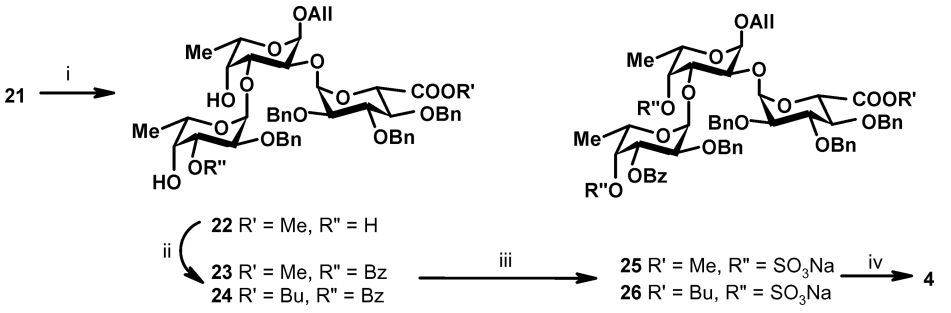


Scheme 5. (i) $CH_3C(OEt)_3$, TsOH, CH_2Cl_2; $AcOH_{aq}$, CH_2Cl_2; (ii) 0.1 M TMSOTf, CH_2Cl_2, –30°C; (iii) (a) H_2, Pd/C; (b) 0.4 M NaOH.

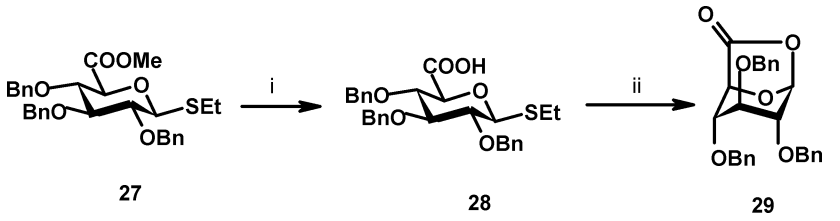
Catalytic hydrogenolysis and saponification of acyl groups in compound **21** gave the target trisaccharide **3** in yield of 79%.

For the synthesis of disulfated trisaccharide **4**, all acyl groups in the compound **21** were saponified, and the carboxylic moiety of the uronic acid was esterified with excess of CH_2N_2 to give corresponding methyl uronate **22** bearing three hydroxyl groups (Sch. 6). Subsequent stannylene activation and regioselective benzoylation of the equatorial hydroxyl group in compound **22** afforded 3″-*O*-benzoylated trisaccharide **23**. In the course of the reaction the formation of butyl uronate **24** was observed. Compounds **23** and **24** were separated by column chromatography and characterized by ^{1}H NMR and mass-spectral data. Sulfation of the mixture of uronates **23** and **24** and further deprotection of products **25** and **26** gave the target trisaccaride **4** in a yield of 70%. The presence of the sulfate groups at C(4) and at C(4″) in compound **4** was confirmed by the downfield chemical shifts of the H(4) and H(4″) signals to 4.87 ppm and 4.58 ppm, respectively, in the ^{1}H NMR spectrum.

Additionally, we studied the possibility of the use of *O*-benzylated glucuronic lactone **29** as a glucuronosyl donor. It was synthesized from ethyl thioglycoside **27**[20] by transformation into acid **28** followed by its intramolecular cyclization in 92% yield (Sch. 7). The structure of **29** was confirmed by ^{1}H NMR



Scheme 6. (i) LiOH, EtOH; CH_2N_2, CH_2Cl_2, (ii) Bu_2SnO, toluene; BzCl; (iii) SO_3·Py, DMF, Py, Amberlite (Na^+); (iv) (a) H_2, Pd/C; (b) 0.4 M NaOH.



Scheme 7. (i) LiOH, EtOH; (ii) NIS, TfOH, CH_2Cl_2.

data. Particularly, the $^1C_4$ conformation of the pyranose ring was confirmed by small (<1 Hz) coupling constant values $J_{1,2}$, $J_{2,3}$, $J_{3,4}$, and $J_{4,5}$.

Unfortunately, we failed to involve lactone **29** into the glycosylation reaction. The use of a number of Lewis acids ($SnCl_4$, TMSOTf, $BF_3{\cdot}Et_2O$, $TiCl_4$) or TfOH as promoters at –30°C led only to destruction of the acceptor **8** after a few hours without activating lactone **29,** which remained unchanged in the reaction mixtures.

CONCLUSIONS

The first regio- and stereoselective synthesis of di- and trisaccharides **1–4** related to the fucoidan from *Cladosiphon okamuranus* algae has been performed. The efficiency of a series of glucuronosyl donors was compared to select the better agent for stereoselective α-glycoside bond formation. It was shown that the presence of an acetyl group at O(3) in the glucuronosyl donor strongly favored the formation of the desired α-isomer. The results of conformational analysis and studies of biological activity of compounds synthesized will be published elsewhere.

EXPERIMENTAL

TLC was performed on Silica Gel 60 F_{254} (Merck) with detection by charring with H_3PO_4. Column chromatography was performed on Silicagel 60–200 μm (Fluka). Gel chromatography was performed on a Sephadex G-10 column (2 × 20 cm) by elution with water at a flow rate of 1 mL/min. Optical rotations were determined with a Jasco DIP-360 digital polarimeter at 26 to 30°C. All solvents used for syntheses were purified according to conventional procedures.[21] NMR spectra for protected derivatives were recorded on Bruker WM-250 and AM-300 spectrometers at 303 K. NMR spectra for oligosaccharides **1–4** were recorded in D_2O on a Bruker DRX-500 spectrometer. Gradient-enhanced 2D gCOSY, gNOESY, and gHSQC experiments as well as TOCSY experiments were used for resonance assignment. Mass spectra of the compounds synthesized were recorded on a Finnigan LCQ mass spectrometer.

Methyl 3-*O*-acetyl-2,4-di-*O*-benzyl-α-D-glucopyranosyluronate bromide (6) and Methyl 3,4-di-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranosyluronate bromide (7)

To a solution of glucuronosyl bromide **5** (600 mg, 1.1 mmol) in CH_2Cl_2(5 mL), 0.01 M solution of HBr in acetic acid (0.5 mL) and Ac_2O (0.1 mL) were added at 0°C. The mixture was kept for 1.5 h at 0°C, then diluted with CH_2Cl_2(50 mL) and washed with saturated aqueous solution of $NaHCO_3$ (50 mL) and water (50 mL). The organic layer was separated and concentrated, and column chromatography of the residue gave bromide **6** (324 mg, 59%) and bromide **7** (122 mg, 25%).

Data for bromide **6**: R_f = 0.5 (Tol:EtOAc, 7:1), ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.02 (s, 3H, CH_3), 3.50 (dd, 1H, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 3.75 (s, 3H, CH_3), 3.82 (t, 1H, $J_{3,4}$=$J_{4,5}$= 9.5 Hz, H-4), 4.44–4.70 (m, 5H, H-5, 2CH_2Ph), 5.58 (t, 1H, H-3), 6.31 (d, 1H, H-1), 7.20–7.40 (m, 10H, 2Ph). ESI $[M+Na]^+$ 515.1.

Data for the bromide **7**: R_f = 0.3 (Tol:EtOAc, 7:1), ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.02 (s, 6H, 2CH_3), 3.58 (dd, 1H, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 9.7 Hz, H-2), 3.73 (s, 3H, CH_3), 4.50–4.65 (m, 3H, H-5, CH_2Ph), 5.10 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, H-4), 5.50 (t, 1H, H-3), 6.31 (d, 1H, H-1), 7.20–7.40 (m, 5H, Ph). ESI $[M+Na]^+$ 467.0.

Allyl 3,4-*O*-isopropylidene-2-*O*-(methyl 2,3,4-tri-*O*-benzyl-α- and β-D-glucopyranosyl uronate)-α-L-fucopyranosides (9 and 10)

A solution of glucuronosyl bromide **5** (28 mg, 0.052 mmol) and allyl fucoside **8** (13 mg, 0.052 mmol) in CH_2Cl_2 (2 mL) was stirred with molecular sieves (4Å) (200 mg) under Ar for 30 min. Then AgOTf (15 mg, 0.057 mmol) was added at –30°C, and the mixture was stirred for 15 min at –30°C, and then Et_3N (0.1 ml) was added. The sieves and solid were filtered off through a Celite pad, and the filtrate was washed with 1 M $Na_2S_2O_3$solution (50 mL) and water (50 mL). The organic layer was concentrated, and column chromatography of the residue gave disaccharides **9** (23 mg, 61%) and **10** (10 mg, 28%).

Data for compound **9**: $[\alpha]_D$ –26° (EtOAc, c = 1), R_f = 0.7 (Toluene:EtOAc 3:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (d, 3H, $J_{5,6}$ = 5.7 Hz, 3H-6), 1.37 and 1.55 (2s, 6H, 2CH_3), 3.61 (dd, 1H, $J_{1',2'}$ = 3.5 Hz, $J_{2',3'}$ = 9.8 Hz, H-2′), 3.70 (s, 3H, CH_3), 3.72–3.76 (m, 2H, H-2, H-4′), 4.04–4.70 (m, 13H, H-3, H-4, H-5, H-3′, H-5′, 3CH_2Ph, CH_2 = CHCH_2O), 4.95 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1), 5.20 and 5.37 (m, 2H, CH_2 = CHCH_2O), 5.26 (d, 1H, H-1′), 5.95 (m, 1H, CH_2 = C*H*CH_2O), 7.20–7.45 (m, 15H, 3Ph).

Anal. Calcd for $C_{40}H_{48}O_{11}$: C, 68.16; H, 6.86. Found: C, 68.19; H, 6.90.

Data for compound **10**: $[\alpha]_D$ –64° (EtOAc, = 1); R_f = 0.6 (Toluene:EtOAc, 3:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (d, 3H, $J_{5,6}$ = 5.7 Hz, 3H-6), 1.37 and 1.57 (2s, 6H, 2CH_3), 3.60 (dd, 1H, $J_{1',2'}$ = 7.8 Hz, $J_{2',3'}$ = 9.8 Hz, H-2′), 3.70 (s, 3H, CH_3), 4.00–4.70 (m, 15H, H-2, H-3, H-4, H-5, H-3′, H-4′, H-5′, 3CH_2Ph,

CH_2 =CHCH_2O), 4.58 (d, 1H, $J_{1',2'}$ = 7.8 Hz, H-1′), 4.98 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 5.24 and 5.37 (m, 2H, CH_2 = CHCH_2O), 5.81 (m, 1H, CH_2 = C*H*CH_2O), 7.20–7.48 (m, 15H, 3Ph).

Allyl 3,4-*O*-isopropylidene-2-*O*-(methyl 3-O-acetyl-2,4-di-*O*-benzyl-α-D-glucopyranosyl uronate)-α-L-fucopyranoside (11)

Glycosylation of allyl fucoside **8** (10 mg, 0.041 mmol) with glucuronosyl bromide **6** (20 mg, 0.041 mmol) as described for the synthesis of compounds **9** and **10** gave disaccharide **11** (21 mg, 80%): $[\alpha]_D$ –22° (EtOAc, c = 1), R_f = 0.4 (Toluene:EtOAc 4:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32 (d, 3H, $J_{5,6}$ = 5.7 Hz, 3H-6), 1.37 and 1.50 (2s, 6H, 2CH_3), 1.98 (s, 3H, CH_3), 3.52 (dd, 1H, $J_{1',2'}$ = 3.5 Hz, $J_{2',3'}$ = 9.8 Hz, H-2′), 3.70 (s, 3H, CH_3), 3.74–3.78 (m, 2H, H-2, H-4′), 4.05–4.70 (m, 10H, H-3, H-4, H-5, H-5′, 2CH_2Ph, CH_2 = CHCH_2O), 4.88 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 5.20 and 5.35 (m, 2H, CH_2 = CHCH_2O), 5.30 (d, 1H, H-1′), 5.56 (t, 1H, $J_{3',4'}$ = 9.8 Hz, H-3′), 5.59 (m, 1H, CH_2 =C*H*CH_2O), 7.20–7.40 (m, 10H, 2Ph). ESI $[M+Na]^+$ 679.4.

Allyl 3,4-*O*-isopropylidene-2-*O*-(methyl 3,4-di-*O*-acetyl-2-*O*-benzyl-α- and β-D-glucopyranosyl uronate)-α-L-fucopyranosides (12 and 13)

Glycosylation of allyl fucoside **8** (16 mg, 0.067 mmol) with glucuronyl bromide **7** (30 mg, 0.067 mmol) as described for the synthesis of compounds **9** and **10** gave a mixture of disaccharides **12** and **13** in a ratio of 5:1 (35 mg, 85%). The ratio of the isomers was established by NMR data of the mixture. R_f = 0.25 (Toluene:EtOAc, 4:1).

Data for compound **12**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (d, 3H, $J_{5,6}$ = 5.7 Hz, 3H-6), 1.37 and 1.55 (2s, 6H, 2CH_3), 2.00 and 2.02 (2s, 6H, 2CH_3), 3.60 (dd, 1H, $J_{1',2'}$ = 3.5 Hz, $J_{2',3'}$ = 9.8 Hz, H-2′), 3.71 (s, 3H, CH_3), 3.75 (dd, 1H, $J_{1,2}$ = 3.4 Hz, $J_{2,3}$ = 9.8 Hz, H-2), 3.90–4.20 (m, 4H, H-4, H-5, CH_2 = CHCH_2O), 4.39 (dd, 1H, $J_{3,4}$ = 5.9 Hz, H-3), 4.46 (d, 1H, H-5′), 4.65 (q, 2H, CH_2 = CHCH_2O), 4.90 (d, 1H, H-1), 5.05 (t, 1H, $J_{3',4'}$ = 9.8 Hz, H-4′), 5.20 and 5.37 (m, 2H, CH_2 = CHCH_2O), 5.25 (d, 1H, H-1′), 5.50 (t, 1H, H-3′), 5.95 (m, 1H, CH_2 =C*H*CH_2O), 7.20–7.45 (m, 5H, Ph).

Data for compound **13**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (d, 3H, $J_{5,6}$ = 5.7 Hz, 3H-6), 1.37 and 1.55 (2s, 6H, 2CH_3), 1.91 and 2.00 (2s, 6H, 2CH_3), 3.55 (dd, 1H, $J_{1',2'}$ = 7.5 Hz, $J_{2',3'}$ = 9.8 Hz, H-2′), 3.71 (s, 3H, CH_3), 3.75 (dd, 1H, $J_{1,2}$ = 3.4 Hz, $J_{2,3}$ = 9.8 Hz, H-2), 3.90–4.20 (m, 4H, H-4, H-5, CH_2 =CHCH_2O), 4.39 (dd, 1H, $J_{3,4}$ = 5.9 Hz, H-3), 4.46 (d, 1H, H-5′), 4.60 (d, 1H, H-1′), 4.65 (q, 2H, CH_2 =CHCH_2O), 4.95 (d, 1H, H-1), 5.05 (t, 1H, $J_{3',4'}$ = 9.8 Hz, H-4′), 5.20 and 5.37 (m, 2H, CH_2 = CHCH_2O), 5.50 (t, 1H, H-3′), 5.80 (m, 1H, CH_2 = C*H*CH_2O), 7.20–7.45 (m, 5H, Ph). ESI $[M+Na]^+$ 631.2.

Allyl 2-*O*-(methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosyluronate)-α-L-fucopyranoside (14)

To a solution of the disaccharide **9** (95 mg, 0.135 mmol) in H_2Cl_2 (2 mL), 90% aqueous solution of CF_3COOH (0.1 mL) was added. The mixture was kept for 15 min, then concentrated and coevaporated with toluene (10 mL). Flash chromatography of the residue gave diol **14** (81 mg, 90%): $[\alpha]_D$ –16° (c = 1, EtOAc), R_f = 0.3 (Toluene:EtOAc, 2:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (d, 3H, $J_{5,6}$ = 5.6 Hz, 3H-6), 3.60–3.65 (m, 2H, H-2, H-2′, H-4′), 3.70 (s, 3H, CH_3), 3.86 (t, 1H, $J_{3',4'}$ = 9.5 Hz, H-4′), 3.99 (q, 1H, H-5), 4.06–4.16 (m, 4H, H-3, H-3′, CH_2 =CHCH_2O), 4.47 (d, 1H, $J_{4',5'}$ = 10.1 Hz, H-5′), 4.63–4.93 (m, 8H, H-1, H-1′, 3CH_2Ph), 5.20 and 5.35 (m, 2H, CH_2 =CHCH_2O), 5.98 (m, 1H, CH_2 =CHCH_2O), 7.20–7.40 (m, 15H, 3Ph).

Anal. Calcd for $C_{37}H_{44}O_{11}$: C, 66.85; H, 6.67. Found: C, 66.86; H, 6.70.

Allyl 3-*O*-benzyl-2-*O*-(methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosyluronate)-α-L-fucopyranoside (15)

To a solution of diol **14** (30 mg, 0.045 mmol) in toluene (5 mL), Bu_2SnO (12 mg, 0.050 mmol) was added. The mixture was refluxed with azeotropic removal of water for 45 min to the volume of 1 mL and then cooled to 25°C. BnBr (5 μL, 0.05 mmol) and Bu_4NBr (16 mg, 0.05 mmol) were added, and the mixture was kept for 1 h at 25°C. Column chromatography of the mixture gave the disaccharide **15** (29 mg, 84%): R_f = 0.4 (Toluene:EtOAc, 3:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24 (d, 3H, $J_{5,6}$ = 5.6 Hz, 3H-6), 3.48 (dd, 1H, $J_{1',2'}$ = 3.6 Hz, $J_{2',3'}$ = 9.5 Hz, H-2′), 3.70–3.75 (m, 4H, H-2, H-4, H-5, H-4′), 3.70 (s, 3H, CH_3), 3.90 (dd, 1H, $J_{3,4}$ = 9.5 Hz, H-3), 4.06–4.16 (m, 3H, H-3′, CH_2 =CHCH_2O), 4.53 (d, 1H, $J_{4',5'}$ = 10.1 Hz, H-5′), 4.63–4.93 (m, 8H, 4CH_2Ph), 5.01 (d, 1H, $J_{1,2}$ = 3.3 Hz, H-1), 5.09 (d, 1H, H-1′), 5.20 and 5.45 (m, 2H, CH_2 =CHCH_2O), 5.98 (m, 1H, CH_2 = CHCH_2O), 7.20–7.40 (m, 20H, 4Ph). ESI $[M+Na]^+$ 777.3.

Allyl 3-*O*-acetyl-2-*O*-(methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl uronate)-4-*O*-(2-*O*-benzyl-3-*O*-acetyl-4-*O*-benzoyl-α-L-fucopyranosyl)-α-L-fucopyranoside (19)

A solution of fucosyl trichloroacetimidate **17** (13 mg, 0.024 mmol) and diol **14** (16 mg, 0.024 mmol) in CH_2Cl_2 (2 mL) was stirred with molecular sieves (4Å) (200 mg) under Ar for 30 min. Then 0.1 M solution of TMSOTf in CH_2Cl_2 (10 μl) was added at –40°C. The mixture was stirred for 1 h at –30°C, then quenched with Et_3N (0.1 mL), filtered through Celite, and concentrated. Column chromatography of the residue gave trisaccharide **18**, which was further treated with a mixture of AcCl (0.1 mL) and Py (0.1 mL). The mixture was kept for 10 min and then evaporated with toluene (5 mL). Column chromatography

of the residue gave compound **19** (20 mg, 74%): $R_f = 0.5$ (Toluene:EtOAc, 4:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.07 (d, 3H, $J_{5'',6''} = 5.6$ Hz, 3H-6″), 1.31 (d, 3H, $J_{5,6} = 5.6$ Hz, 3H-6), 1.98 (s, 3H, CH_3), 3.63 (dd, 1H, $J_{1',2'} = 3.8$ Hz, $J_{2',3'} = 9.5$ Hz, H-2′), 3.70 (s, 3H, CH_3), 3.74 (t, 1H, $J_{3',4'} = 9.5$ Hz, H-4′), 3.8 (m, 3H, H-2, H-4, H-5), 4.02 (dd, 1H, $J_{1'',2''} = 3.4$ Hz, $J_{2'',3''} = 9.6$ Hz, H-2″), 4.07 (t, 1H, H-3′), 4.08–4.16 (m, 2H, CH_2 =CHCH_2O), 4.50 (d, 1H, $J_{4',5'} = 9.6$ Hz, H-5′), 4.59 (q, 1H, H-5″), 4.63–4.93 (m, 8H, 4CH_2Ph), 5.04 (d, 1H, H-1′), 5.10 (2d, 2H, H-1, H-1″), 5.20 and 5.35 (m, 2H, CH_2 =CHCH_2O), 5.42 (d, 1H, H-3), 5.54 (dd, 1H, H-3″), 5.63 (d, 1H, H-4″), 5.99 (m, 1H, CH_2 =C*H*CH_2O), 7.20–8.00 (m, 25H, 5Ph). ESI $[M+Na]^+$ 1111.4.

Allyl 4-*O*-acetyl-2-*O*-(methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosyluronate)-α-L-fucopyranoside (20)

To a solution of disaccharide **14** (100 mg, 0.15 mmol) in CH_2Cl_2 (3 mL), $CH_3C(OEt)_3$ (0.1 mL) and TsOH (15 mg) were added. The mixture was kept for 1 h, and then 80% aqueous AcOH (0.5 mL) was added. After 30 min the solution was evaporated with toluene (10 mL). Column chromatography of the residue gave compound **20** (95 mg, 90%): $[\alpha]_D$ –13° (EtOAc, = 1), $R_f = 0.2$ (Toluene:EtOAc, 4:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.14 (d, 3H, $J_{5,6} = 5.6$ Hz, 3H-6), 2.22 (s, 3H, CH_3), 3.55 (dd, 1H, $J_{1',2'} = 3.6$ Hz, $J_{2',3'} = 9.5$ Hz, H-2′), 3.69 (dd, 1H, $J_{1,2} = 3.5$ Hz, $J_{2,3} = 9.6$ Hz, H-2), 3.70 (s, 3H, CH_3), 3.73 (t, 1H, $J_{3',4'} = 9.5$ Hz, H-4′), 4.06 (t, 1H, H-3′), 4.08–4.16 (m, 3H, H-5, CH_2 =CHCH_2O), 4.23 (dd, 1H, H-3′), 4.43 (d, 1H, $J_{4',5'} = 10.1$ Hz, H-5′), 4.63–4.93 (m, 7H, H-1′, 3CH_2Ph), 4.97 (d, 1H, H-1), 5.20 and 5.35 (m, 2H, CH_2 =CHCH_2O), 5.32 (d, 1H, H-4), 5.98 (m, 1H, CH_2 =C*H*CH_2O), 7.20–7.40 (m, 15H, 3Ph). ESI $[M+Na]^+$ 729.3.

Allyl 4-*O*-acetyl-2-*O*-(methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosyluronate)-3-*O*-(2-*O*-benzyl-3-*O*-acetyl-4-*O*-benzoyl-α-L-fucopyranosyl)-α-L-fucopyranoside (21)

Glycosylation of disaccharide **20** (100 mg, 0.142 mmol) with fucosyl trichloroacetimidate **17** (78 mg, 0.144 mmol) as described for the preparation of compound **18** gave trisaccharide **21** (134 mg, 87%): $[\alpha]_D$ –160° (EtOAc, =1), $R_f = 0.4$ (Toluene:EtOAc, 5:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.12 (d, 3H, $J_{5,6} = 5.6$ Hz, 3H-6), 1.19 (d, 3H, $J_{5'',6''} = 5.6$ Hz, 3H-6″), 1.98 (s, 3H, CH_3), 3.51 (dd, 1H, $J_{1',2'} = 3.7$ Hz, $J_{2',3'} = 9.5$ Hz, H-2′), 3.68 (t, 1H, $J_{3',4'} = 9.5$ Hz, H-4′), 3.70 (s, 3H, CH_3), 3.75 (dd, 1H, $J_{1,2} = 3.2$ Hz, $J_{2,3} = 9.5$ Hz, H-2), 3.83 (t, 1H, H-3′), 4.01 (dd, 1H, $J_{1'',2''} = 3.5$ Hz, $J_{2'',3''} = 9.6$ Hz, H-2″), 4.03 (d, 1H, H-4), 4.08–4.16 (m, 2H, CH_2 =CHCH_2O), 4.41 (dd, 1H, H-3), 4.47 (d, 1H, H-1′), 4.49 (d, 1H, $J_{4',5'} = 9.6$ Hz, H-5′), 4.63–4.93 (m, 8H, 4CH_2Ph), 4.98 (d, 1H, H-1), 5.01 (q, 1H, H-5″), 5.31 (d, 1H, H-1″), 5.20 and 5.35 (m, 2H, CH_2 =CHCH_2O), 5.45 (d,

1H, H-4), 5.60 (d, 1H, H-4″), 5.71 (dd, 1H, H-3″), 5.99 (m, 1H, CH_2 =C*H*CH_2O), 7.20–8.00 (m, 25H, 5Ph). ESI $[M+Na]^+$ 1111.3.

Allyl 2-*O*-(methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl uronate)-3-*O*-(2-*O*-benzyl-α-L-fucopyranosyl)-α-L-fucopyranoside (22)

Trisaccharide **21** (100 mg, 0.092 mmol) was dissolved in a mixture of EtOH (5 mL), THF (5 mL), and H_2O (1 mL) and the solution obtained was treated with 2 N LiOH (10 mL). After stirring for 12 h, (5 mL) and 2 N NaOH (5 mL) were added and the mixture was vigorously stirred for another 24 h. Then the mixture was diluted with $CHCl_3$ (100 mL) and washed with 1 M HCl (50 mL) and water (50 mL). The organic layer was separated and concentrated. The crude residue was dissolved in CH_2Cl_2 (2 mL) and 1.2 M solution of CH_2N_2 in Et_2O (0.5 mL) was added. An excess of CH_2N_2 was removed by Ar current. Flash chromatography of the residue gave methyl uronate **22** (62 mg, 75%): $[\alpha]_D$ –80° ($CHCl_3$, = 1); R_f = 0.3 (Toluene:EtOAc, 2:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (d, 3H, $J_{5'',6''}$ = 5.6 Hz, 3H-6″), 1.32 (d, 3H, $J_{5,6}$ = 5.6 Hz, 3H-6), 3.55 (dd, 1H, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 3.64 (dd, 1H, $J_{1',2'}$ = 3.7 Hz, $J_{2',3'}$ = 9.5 Hz, H-2′), 3.68 (t, 1H, $J_{3',4'}$ = 9.5 Hz, H-4′), 3.70 (s, 3H, CH_3), 3.73 (m, 2H, H-4, H-4″), 3.86 (m, 2H, H-3′, H-2″), 3.90 (q, 1H, H-5), 3.95 (dd, 1H, H-3), 4.10–4.16 (m, 3H, H-3″, CH_2 =CHC*H*$_2$O), 4.57 (m, 2H, H-5′, H-5″), 4.63–4.93 (m, 8H, 4C*H*$_2$Ph), 4.84 (d, 1H, H-1′), 4.95 (d, 1H, H-1), 5.08 (d, 1H, H-1″), 5.20 and 5.38 (m, 2H, C*H*$_2$ =CHCH_2O), 6.00 (m, 1H, CH_2 =C*H*CH_2O), 7.20–8.00 (m, 20H, 4Ph).

Anal. Calcd for $C_{50}H_{60}O_{15}$: C, 66.65; H, 6.71. Found: C, 66.68; H, 6.70.

Allyl 2-*O*-(methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl uronate)-3-*O*-(2-*O*-benzyl-3-*O*-benzoyl-α-L-fucopyranosyl)-α-L-fucopyranoside (23) and Allyl 2-*O*-(butyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl uronate)-3-*O*-(2-*O*-benzyl-3-*O*-benzoyl-α-L-fucopyranosyl)-α-L-fucopyranoside (24)

To a solution of compound **22** (43 mg, 0.048 mmol) in toluene (5 mL), Bu_2SnO (18 mg, 0.072 mmol) was added. The mixture was refluxed with azeotropic removal of water for 1 h to the volume of 1 mL and then cooled to 25°C. BzCl (6 μL, 0.05 mmol) was added and the mixture was kept at 60°C for 1 h. Column chromatography of the residue gave compound **23** (30 mg, 62%) and compound **24** (7 mg, 15%).

Data for compound **23**: R_f = 0.6 (Toluene:EtOAc, 2:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22 (d, 3H, $J_{5'',6''}$ = 5.6 Hz, 3H-6″), 1.30 (d, 3H, $J_{5,6}$ = 5.6 Hz, 3H-6), 3.54 (dd, 1H, $J_{1',2'}$ = 3.7 Hz, $J_{2',3'}$ = 9.5 Hz, H-2′), 3.70–3.74 (m, 6H, H-4, H-4′, H-4″, CH_3), 3.84 (dd, 1H, $J_{1,2}$ = 3.3 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 3.90 (q, 1H,

H-5), 3.97 (t, 1H, H-3′), 4.10–4.16 (m, 3H, H-3, $CH_2=CHCH_2O$), 4.59 (d, 1H, H-5′), 4.63–4.93 (m, 9H, H-5″, $4CH_2Ph$), 4.76 (d, 1H, H-1′), 4.92 (d, 1H, H-1″), 5.04 (d, 1H, H-1), 5.21 and 5.38 (m, 2H, $CH_2=CHCH_2O$), 5.64 (dd, 1H, H-3″), 6.00 (m, 1H, $CH_2=CHCH_2O$), 7.20–8.00 (m, 20H, 4Ph). ESI $[M+Na]^+$ 1027.4.

Data for compound **24**: R_f = 0.7 (Toluene:EtOAc, 2:1). ESI $[M+Na]^+$ 1069.2.

Ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside Uronic Acid (28)

To a solution of ethyl thioglycoside **27** (50 mg, 0.096 mmol) in CH_2Cl_2 (2 mL) and EtOH (2 mL), 2 M aqueous solution of LiOH (0.5 mL) was added. The mixture was kept for 1 h at 25°C, and then MeOH (4 mL) and Amberlite (H^+) were added to pH = 7. The resin was filtered off, and the solution was evaporated with toluene (5 mL). Column chromatography of the residue gave acid **28** (40 mg, 82%): R_f = 0.4 (CH_2Cl_2:EtOH, 10:1). ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.35 (t, 3H, CH_3), 2.79 (m, 2H, CH_2), 3.49 (t, 1H, $J_{1,2} = J_{2,3} = 9.9$ Hz, H-2), 3.71 (t, 1H, $J_{3,4} = 9.9$ Hz, H-3), 3.87 (t, 1H, $J_{4,5} = 9.9$ Hz, H-4), 3.96 (d, 1H, H-5), 4.57 (d, 1H, H-1), 4.65–5.01(m, 6H, $3CH_2Ph$), 7.15–7.40 (m, 15H, 3Ph). ESI $[M+H]^+$ 509.2, $[M+Na]^+$ 531.2.

2,3,4-Tri-*O*-benzyl-β-D-glucopyranurono-1,6-lactone (29)

A solution of acid **28** (20 mg, 0.039 mmol) in CH_2Cl_2(2 mL) was stirred with molecular sieves (4Å) (150 mg) under Ar for 30 min. Then NIS (10 mg, 0.04 mmol) and TfOH (0.03 mmol, 3 μL) were added to the mixture at –5°C. After stirring for 10 min at –5°C, Et_3N (0.1 mL) was added. The sieves were filtered off through a Celite pad, and the filtrate was washed with 1 M $Na_2S_2O_3$ solution (50 mL) and water (50 mL). The organic layer was concentrated, and column chromatography of the residue gave the lactone **29** (16 mg, 92%): R_f = 0.6 (Toluene:Acetone, 10:1), ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.50 (s, 1H, H-2), 3.66 (s, 1H, H-4), 3.70 (s, 1H, H-3), 4.30–4.44 (m, 2H, CH_2Ph), 4.52 (s, 1H, H-5), 4.58–4.70 (m, 4H, $2CH_2Ph$), 5.80 (s, 1H, H-1), 7.20–7.40 (m, 15H, 3Ph). ESI $[M+Na]^+$ 469.2.

Propyl 2-*O*-(α-D-glucopyranosyl Uronic Acid)-α-L-fucopyranoside, Sodium Salt (1)

A mixture of diol **14** (50 mg, 0.075 mmol) and 10% Pd/C (20 mg) in EtOAc (3.5 mL) and MeOH (3.5 mL) was stirred under H_2 atmosphere for 2 h. Then the catalyst was filtered off through a Celite pad, and the solution was concentrated. The residue was dissolved in distilled water (1 mL) and 2 N NaOH

(0.5 ml) was added. The mixture was kept at 25°C for 2 h, and then the disaccharide **1** (25 mg, 82%) was isolated by gel chromatography on Sephadex G-10: $[\alpha]_D$ –27° (H_2O, = 1). ^1H NMR (500 MHz, D_2O) δ 0.92 (t, 3H, $CH_3CH_2CH_2O$), 1.21 (d, 3H, $J_{5,6}$ = 5.3 Hz,3H-6), 1.67 (m, 2H, $CH_3CH_2CH_2O$), 3.43 and 3.63 (2m, 2H, $CH_3CH_2CH_2O$), 3.45 (t, 1H, $J_{3',4'}$ = $J_{4',5'}$ = 9.3 Hz,H-4′), 3.58 (dd, 1H, $J_{1',2'}$ = 3.7 Hz, $J_{2',3'}$ = 9.3 Hz, H-2′), 3.73 (t, 1H, H-3′), 3.82 (dd, 1H, $J_{1,2}$ = 3.9 Hz, $J_{2,3}$ = 9.1 Hz, H-2), 3.85 (d, 1H, $J_{3,4}$ = 3.3 Hz, H-4), 4.00 (d, 1H, H-5′), 4.12 (dd, 1H, H-3), 4.20 (q, 1H, H-5), 5.02 (d, 1H, H-1), 5.10 (d, 1H, H-1′). HRESIMS: *m/z* $[M+Na]^+$ Calcd for $C_{15}H_{25}Na_2O_{11}$: 427.119. Found: 427.117.

Propyl 4-*O*-sulfonato-2-*O*-(α-D-glucopyranosyl Uronic Acid)-α-L-fucopyranoside, Disodium Salt (2)

To a solution of disaccharide **15** (40 mg, 0.057 mmol) in DMF (0.5 mL) and pyridine (0.5 mL), Py·SO_3 complex (45 mg, 0.285 mmol) was added, and the mixture was kept at 40°C for 1 h. Then MeOH (5 mL), $NaHCO_3$ (100 mg), and Amberlite IR-120 (Na^+) were added and the mixture was stirred for 2 h. Resin and solid were filtered off, and the filtrate was concentrated. Flash chromatography of the residue ($CHCl_3$:MeOH, 30:1) on silica gel gave disaccharide **16**. Catalytic hydrogenolysis and subsequent saponification of the ester group in compound **16** as described for the preparation of **1** gave disaccharide **2** (26 mg, 70%): ^1H NMR (500 MHz, D_2O) δ 0.92 (t, 3H, $CH_3CH_2CH_2O$), 1.28 (d, 3H, $J_{5,6}$ = 5.3 Hz,3H-6), 1.67 (m, 2H, $CH_3CH_2CH_2O$), 3.43 and 3.63 (2m, 2H, $CH_3CH_2CH_2O$), 3.45 (t, 1H, $J_{3',4'} = J_{4',5'}$ = 9.3 Hz,H-4′), 3.58 (dd, 1H, $J_{1',2'}$ = 3.7 Hz, $J_{2',3'}$ = 9.3 Hz, H-2′), 3.73 (t, 1H, H-3′), 3.85 (dd, 1H, $J_{1,2}$ = 3.9 Hz, $J_{2,3}$ = 9.1 Hz, H-2), 4.00 (d, 1H, H-5′), 4.12 (dd, 1H, H-3), 4.20 (q, 1H, H-5), 4.64 (d, 1H, $J_{3,4}$ = 3.1 Hz, H-4), 5.04 (d, 1H, H-1), 5.14 (d, 1H, H-1′). HRESIMS: *m/z* $[M+Na]^+$ Calcd for $C_{15}H_{24}Na_3O_{14}S$: 529.058. Found: 529.057.

Propyl 2-*O*-(α-D-glucopyranosyl Uronic Acid)-3-*O*-(α-L-fucopyranosyl)-α-L-fucopyranoside, Sodium Salt (3)

Catalytic hydrogenolysis of the disaccharide **21** (45 mg, 0.041 mmol) and subsequent saponification of the ester group in the product as described for the preparation of the compound **1** gave trisaccharide **3** (18 mg, 79%): $[\alpha]_D$ –92° (H_2O, = 1). ^1H NMR (500 MHz, D_2O) δ 0.91 (t, 3H, $CH_3CH_2CH_2O$), 1.21 (d, 3H, $J_{5'',6''}$ = 5.4 Hz,3H-6″), 1.24 (d, 3H, $J_{5,6}$ = 5.2 Hz,3H-6), 1.67 (m, 2H, $CH_3CH_2CH_2O$), 3.44 and 3.61 (2m, 2H, $CH_3CH_2CH_2O$), 3.48 (t, 1H, $J_{3',4'}$ = $J_{4',5'}$ = 9.3 Hz,H-4′), 3.59 (dd, 1H, $J_{1',2'}$ = 3.7 Hz, $J_{2',3'}$ = 9.3 Hz, H-2′), 3.68 (t, 1H, H-3′), 3.79 (d, 1H, $J_{3'',4''}$ = 3.4 Hz, H-4″), 3.81 (dd, 1H, $J_{1'',2''}$ = 3.4 Hz, $J_{2'',3''}$ = 9.1 Hz, H-2″), 3.94 (dd, 1H, H-3″), 4.01 (d, 1H, H-5′), 4.03 (dd, 1H, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 9.3 Hz, H-2), 4.07 (q, 1H, H-5), 4.08 (d, 1H, $J_{3,4}$ = 3.6 Hz, H-4), 4.13 (dd, 1H, 1H, H-3), 4.49 (d, 1H, H-5″), 5.04 (d, 1H, H-1), 5.16 (d, 1H, H-1′),

5.12 (d, 1H, H-1″). HRESIMS: *m/z* $[M+Na]^+$ Calcd for $C_{21}H_{35}Na_2O_{15}$: 573.177. Found: 573.178.

Propyl 4-*O*-Sulfonato-2-*O*-(α-D-glucopyranosyl Uronic Acid)-3-*O*-(4-*O*-sulfonato-α-L-fucopyranosyl)-α-L-fucopyranoside, Trisodium Salt (4)

Sulfation of the mixture of methyl and butyl uronates **23** (29 mg, 0.029 mmol) and **24** (6 mg, 0.006 mmol) and further catalytic hydrogenolysis and saponification of acyl groups in **25** and**26** as described for the preparation of compound **2** gave trisaccharide **4** (18 mg, 70%): $[\alpha]_D$ –67° (H_2O, = 0.75), ^1H NMR (500 MHz, D_2O) δ 0.91 (t, 3H, $CH_3CH_2CH_2O$), 1.29 (d, 3H, $J_{5,6} = 5.4$ Hz,3H-6), 1.31 (d, 3H, $J_{5'',6''} = 5.2$ Hz,3H-6″), 1.67 (m, 2H, $CH_3CH_2CH_2O$), 3.43 and 3.61 (2m, 2H, $CH_3CH_2CH_2O$), 3.49 (t, 1H, $J_{3',4'} = J_{4',5'} = 9.4$ Hz,H-4′), 3.65 (dd, 1H, $J_{1',2'} = 3.7$ Hz, $J_{2',3'} = 9.4$ Hz, H-2′), 3.68 (t, 1H, H-3′), 3.86 (dd, 1H, $J_{1'',2''} = 3.4$ Hz, $J_{2'',3''} = 9.1$ Hz, H-2″), 4.03 (dd, 1H, $J_{1,2} = 3.5$ Hz, $J_{2,3} = 9.3$ Hz, H-2), 4.08 (dd, 1H, H-3″), 4.10 (d, 1H, H-5′), 4.18 (q, 1H, H-5), 4.31 (dd, 1H, $J_{3,4} = 3.6$ Hz, H-3), 4.58 (d, 1H, H-4″), 4.78 (q, 1H, H-5″), 4.87 (d, 1H, H-4), 5.11 (d, 1H, H-1), 5.16 (d, 1H, H-1′), 5.30 (d, 1H, H-1″). HRESIMS: *m/z* $[M+Na]^+$ Calcd for $C_{21}H_{33}Na_4O_{21}S_2$: 777.058. Found: 777.057.

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