Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 339 (2004) 2083–2090
Carbohydrate RESEARCH

Synthesis and biological activities of octyl 2,3-di-*O*-sulfo-α-L-fucopyranosyl-(1 → 3)-2-*O*-sulfo-α-L-fucopyranosyl-(1 → 4)-2,3-di-*O*-sulfo-α-L-fucopyranosyl-(1 → 3)-2-*O*-sulfo-α-L-fucopyranosyl-(1 → 4)-2,3-di-*O*-sulfo-β-L-fucopyranoside

Yuxia Hua,[a] Yuguo Du,[a,*] Gangli Yu[b] and Shidong Chu[b]

[a]*Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, PO Box 2871, Beijing 100085, PR China*
[b]*Institute of Marine Drug and Food, Ocean University of China, Qingdao 266003, China*

Received 27 April 2004; accepted 5 June 2004
Available online 7 July 2004

Abstract—Octyl 2,3-di-*O*-sulfo-α-L-fucopyranosyl-(1 → 3)-2-*O*-sulfo-α-L-fucopyranosyl-(1 → 4)-2,3-di-*O*-sulfo-α-L-fucopyranosyl-(1 → 3)-2-*O*-sulfo-α-L-fucopyranosyl-(1 → 4)-2,3-di-*O*-sulfo-β-L-fucopyranoside, a fucosyl pentasaccharide with a regular structure resembling the repeating unit of a natural sulfated fucan, was chemically synthesized using a convergent '2 + 3' strategy. Regioselective 3-*O*-silylation of β-thiofucopyranoside and AgOTf-catalyzed glycosylation of the protected glycosyl trichloroacetimidate facilitated a one-pot trisaccharide synthesis. The synthesized target compound showed good antitumor activity in vivo, and promising anticoagulant activity in vitro.
© 2004 Elsevier Ltd. All rights reserved.

Keywords: Regioselective silylation; Fucan; Sulfated oligosaccharide; Antitumor activity; Glycosylation

1. Introduction

Sulfated fucans, which are commonly called fucoidans, have been isolated mainly from brown algae,[1] including *Fucus vesiculosus*,[2] *Sargassum stenophyllum*,[3] and *Cladosiphon okamuranus*.[4] Sulfated fucans are among the most widely studied of all the sulfated polysaccharides of nonmammalian origin that exhibit biological activities in mammalian systems. These polyanionic molecules usually have a linear repeating unit, that of (1 → 3)-linked β-L-fucopyranoside that differs by specific patterns of sulfation.[5–7] Recently, sulfated fucans were also isolated from marine invertebrates and characterized.[2,3] These animal sulfated fucans consist of (1 → 3) and/or (1 → 4)-linked fucosyl linear backbones that are partially substituted at C-2 and/or C-4 with sulfate groups.[3] In terms of biological effects on mammalian systems, sulfated fucans show anticoagulant activity and are potent activators of both antithrombin III and heparin cofactor II.[8] They are inhibitors of sperm–egg interaction,[9] and of retroviral infection, blocking the infection of human cell lines with, for example, HIV, herpes, and cytomegalovirus.[10] They also can act as anti-angiogenic agents and can block cell–cell binding mediated by P- or L-Selectin.[11] The structural components of sulfated fucan necessary for all these biological activities have not been determined yet. Curious about the bioactivities of sulfated fucan fragments, we launched a project on the synthesis of a series of sulfated fucose-containing oligosaccharides.

In our previous report,[12] we synthesized octyl 2,3,4-tri-*O*-sulfo-α-L-fucopyranosyl-(1 → 3)-2,4-di-*O*-sulfo-α-L-fucopyranosyl-(1 → 3)-2,4-di-*O*-sulfo-α-L-fucopyranosyl-(1 → 3)-2,4-di-*O*-sulfo-α-L-fucopyranoside, and the corresponding bioassay suggested that this oversulfated

* Corresponding author. Tel.: +86-10-62914475; fax: +86-10-629235-63; e-mail: duyuguo@mail.rcees.ac.cn

0008-6215/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2004.06.006

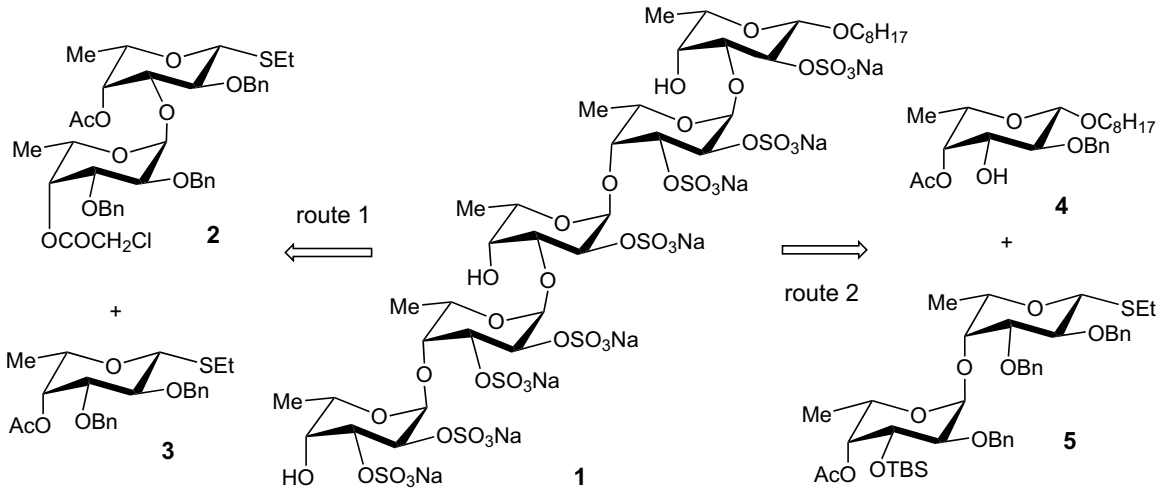


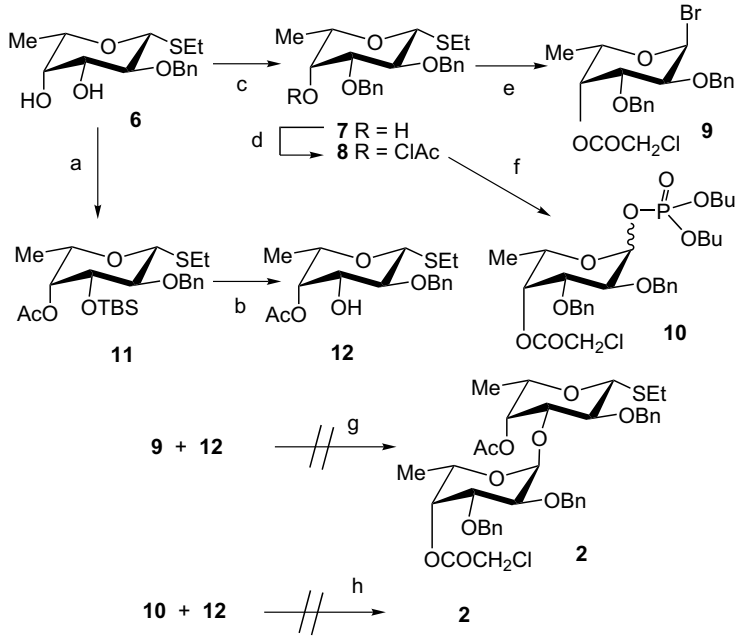
Figure 1. Two proposed strategies for the synthesis of target **1**.

tetrasaccharide presented better antitumor activities than that of its unsulfated counterpart based on Sarcoma 180 and Lewis lung carcinoma model studies. In our continuing work on this project, we disclose the synthesis and biological activities of a natural fucose pentasaccharide[2] having a specific sulfate pattern, that is, octyl 2,3-di-*O*-sulfo-α-L-fucopyranosyl-(1 → 3)-2-*O*-sulfo-α-L-fucopyranosyl-(1 → 4)-2,3-di-*O*-sulfo-α-L-fucopyranosyl-(1 → 3)-2-*O*-sulfo-α-L-fucopyranosyl-(1 → 4)-2,3-di-*O*-sulfo-β-L-fucopyranoside (Fig. 1).

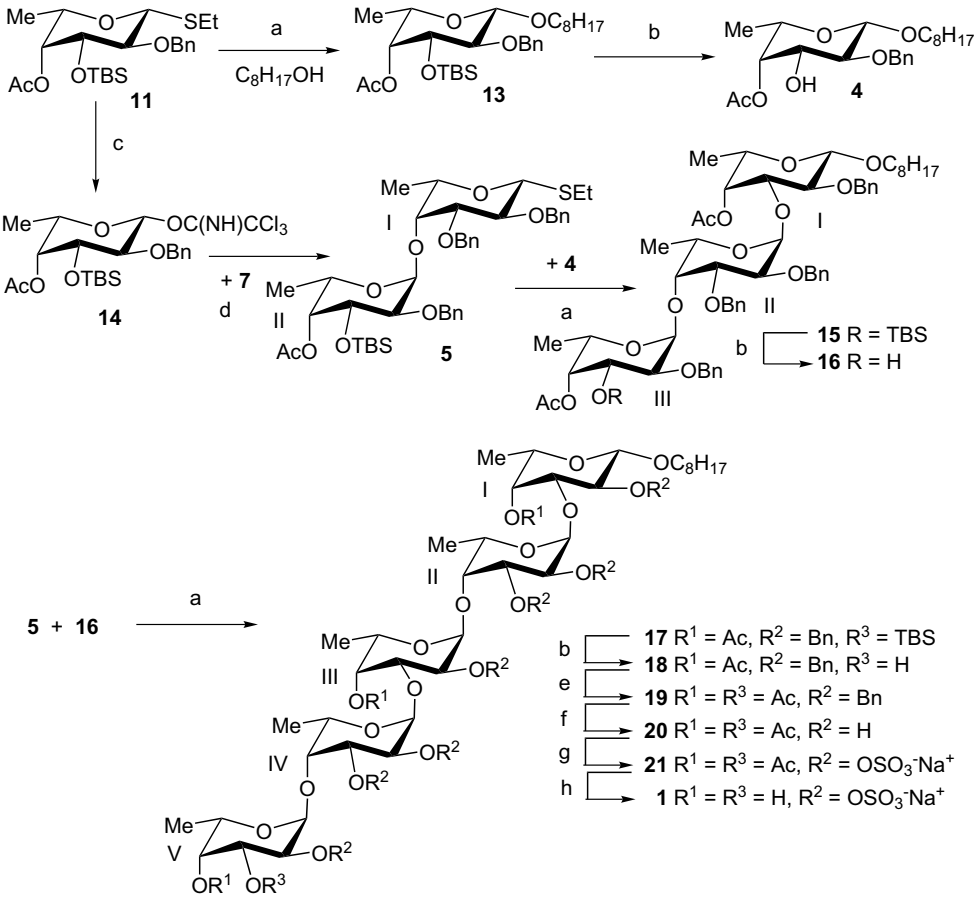
2. Results and discussion

Retrosynthetic analysis of sulfate pentafucosyl oligosaccharide **1** reasonably gave two possible routes toward the target. In the first route, a (1 → 3)-linked disaccharide **2** was selected as the basic building block. Coupling **2** with octanol, removal of chloroacetyl (ClAc) from the 4-OH, followed by glycosylation with **2**, removing chloroacetyl again (→ a tetrasaccharide), and a final step of attaching **3**[13] to the tetrasaccharide would finish the total synthesis of **1**. In the second route, a (1 → 4)-linked disaccharide **5** was used as the building block. Coupling **5** with **4** would give a trisaccharide, which was desilylated on the 3-OH, and glycosylated with **5** again, would obtain the backbone of the target **1**. In both routes, benzyl protection groups were planned to mask intending sulfate positions. As we have already successfully synthesized a (1 → 3)-linked fucosyl tetrasaccharide, we initially thought route 1 would be familiar to us.[12] To this end (Scheme 1), ethyl 2-*O*-benzyl-1-thio-β-L-fucopyranoside (**6**)[13] was transformed into **8** in 77% overall yield via tin complex-assisted 3-*O*-benzylation (→**7**) and 4-OH chloroacetylation with chloroacetic anhydride in dry pyridine. Treatment of **8** with bromine in CH_2Cl_2 gave bromide **9** as a mixture, which was directly used as a donor for the next reaction. Convergently, **6** was silylated with *tert*-butyldimethylsilyl chloride (TBSCl) and imidazole in *N*,*N*-dimethylformamide (DMF) at 0 °C to give the 3-*O*-silylated product, which was further acetylated with acetic anhydride in pyridine, giving **11** in 84% yield. Hydrogen fluoride–pyridine assisted desilylation of **11** gave acceptor **12** in an excellent yield (91%). ^{1}H NMR spectrum of **12** showed H-3 and H-4 at δ 3.79 ppm (J = 9.3, 3.5 Hz) and δ 5.10 ppm (J = 3.5, 1.0 Hz), respectively, indicating the correct position for all protecting groups. It should be noted that tetrabutylammonium fluoride (TBAF)-catalyzed desilylation of **11** gave partial acetyl group migrated byproduct. Glycosylation of bromide **9** and thioglycoside **12** in CH_2Cl_2 with promotion by silver trifluoromethanesulfonate (AgOTf) gave complicated TLC results. Changing the solvent to ether or toluene, and adding a small amount of lutidine to the reaction, did not improve the results. We thought it would be helpful if we were to use the more stable phosphate **10** instead of unstable **9**. Thus, compound **8** was condensed with dibutyl phosphate in CH_2Cl_2 at −20 °C, using *N*-iodosuccinimide/trimethylsilyl trifluoromethanesulfonate (NIS/TMSOTf) as catalysts, to give an anomeric phosphate mixture **10**. Unfortunately, exhaustive efforts in the glycosylation of **10** and **12** under various conditions were fruitless.

Since route 1 was troublesome, we next focused our attention on route 2 as described in Scheme 2. Compound **11** was glycosylated with 1-octanol under standard reaction conditions, followed by HF–pyridine promoted desilylation to afford acceptor **4** in 76% yield over two steps. To prepare donor **14**, **11** was treated with *N*-bromosuccinimide (NBS) in (1:9) water–acetone at rt for 10 min, then transformed into trichloroacetim-



Scheme 1. Reagents and conditions (yields): (a) TBSCl, DMF, Im, 0 °C → rt, overnight; Ac_2O, Pyr, rt, 4 h (84%); (b) HF–Pyr, 0 °C → rt, overnight (91%); (c) Bu_2SnO, MeOH, reflux; BnBr, TBAI, DMF (89%); (d) $ClAc_2O$, Pyr (87%); (e) Br_2, CH_2Cl_2; (f) Bu_2HPO_4, NIS, TMSOTf (90%); (g) AgOTf, CH_2Cl_2; (h) TMSOTf, CH_2Cl_2.



Scheme 2. Reagents and conditions (yields): (a) NIS, TMSOTf, CH_2Cl_2, −20 °C (90% for **13**; 62% for **15**; 75% for **17**); (b) HF–Pyr (84% for **4**; 74% for **16**); (c) NBS, 1:9 H_2O–acetone; Cl_3CCN, DBU, CH_2Cl_2 (85%); (d) AgOTf, Et_2O, 0 °C, 50 min (82%); (e) Ac_2O, Pyr (79% from **17** to **19**); (f) Pd–C, H_2, 1:1 MeOH–EtOAc, 80%; (g) SO_3·Pyr, Pyr, 55 °C; 3 N NaOH; (h) NaOMe, MeOH (60% from **20**).

idate with trichloroacetonitrile and 1,8-di-azabicyclo-[5.4.0]undec-7-ene (DBU) in dry methylene chloride, affording the product in 85% yield over two steps. AgOTf-catalyzed glycosylation[14] of **14** and **7** in dry ether gave an excellent yield of disaccharide **5** (82% isolated yield), which could be directly used as a donor to react with **4** in the presence of NIS/TMSOTf in a one-pot reaction, affording trisaccharide **15** (62%). ^1H–^1H COSY of **5** showed a doublet ($J = 3.4$ Hz) at δ 5.03 ppm, while two characteristic α H-1s of **15** appeared at δ 4.80 ppm ($J = 3.4$ Hz) and δ 4.97 ppm ($J = 3.5$ ppm), respectively, confirming the α-linkage between sugar residues. Interestingly, TMSOTf-catalyzed the same reaction in CH_2Cl_2 gave much lower yield due to the quick decomposition of **14**. To avoid acetyl migration during desilylation on C-3, **15** was treated with HF–pyridine giving the trisaccharide acceptor **16** in a yield of 74%. Condensation of **5** and **16** as described in the preparation of **15** furnished pentasaccharide **17** in 75% yield. Removal of the TBS group from **17** with HF–pyridine (→18), followed by acetylation with Ac_2O in pyridine (→19), hydrogenolysis with H_2 on $Pd(OH)_2$ (→20), sulfation with sulfur trioxide·pyridine complex in pyridine at 55 °C (→21), and the final deacetylation with NaOMe in MeOH complete the synthesis of target compound **1**. HMQC spectra assigned five anomeric protons at δ 4.29 ppm ($J = 7.8$ Hz), 4.94 ppm ($J = 3.3$ Hz), 5.21 ppm ($J = 3.3$ Hz), 5.29 ppm ($J = 3.2$ Hz) and 5.31 ppm ($J = 3.1$ Hz), respectively, supporting the structure of compound **1**. After desalting on an AKTA–FPLC chromatography system, compound **1** was directly used for the following bioassays.

The antitumor activity of **1** was preliminarily tested in vivo according to the method described by Sasaki and Takasuka.[15] Kun-min mice weighing about 20 g and Sarcoma-180 cells (5×10^6) were used for the bioassay. Cisplatin® (CDDP) was selected as the positive control in the parallel tests. The mice were injected with compound **1** (daily dose, 1 mg/kg each; i.v.) from day 3 to 14 after inoculation with the tumor cells, while CDDP was given every other day. Control mice were injected with saline alone. The tumor inhibition ratios for **1** and CDDP on S_{180} were 45% and 65%, respectively. The anti-Xa activity of compound **1** was also measured according to the standard method,[16] and it inhibited factor Xa at $IC_{50} = 60$ μM (or $K_i = 33$ μM) based on the protease inhibition model in vitro. Preliminary testing of the cytotoxicity of compound **1** was carried out using a procedure described by Connolly et al.[17] Compound **1** showed no activity towards human hepatocellular carcinoma BEL-7402 cells. It is thus deduced that sulfated fucose oligomers might enhance the antitumor activities by acting as immunostimulants in vivo.

In conclusion, we have successfully synthesized a fucosyl pentasaccharide having specific pattern of sulfation using convergent '2 + 3' strategy. Regioselective 3-*O*-silylation of β-thiofucoside and AgOTf-promoted glycosylation using a Schmidt donor facilitated a one-pot trisaccharide preparation. The synthesized target compound **1** showed good antitumor activity in vivo, and promising anticoagulant activity in vitro.

3. Experimental

3.1. General

Optical rotations were determined at 25 °C with a Perkin–Elmer Model 241-Mc automatic polarimeter at the sodium D-line. ^1H, ^{13}C, ^1H–^1H COSY, and HMQC NMR spectra were recorded with ARX 400 spectrometers for solutions in $CDCl_3$ or D_2O. Chemical shifts are given in ppm downfield from internal Me_4Si. Mass spectra were measured using MALTITOF-MS with dihydroxybenzoic acid (DHB) as matrix. Thin-layer chromatography (TLC) was performed on silica gel HF_{254} with detection by charring with 30% (v/v) H_2SO_4 in MeOH or in some cases by a UV lamp.

3.2. Ethyl 2,3-di-*O*-benzyl-1-thio-β-L-fucopyranoside (7)

Compound **6** (2.44 g, 8.20 mmol) and dibutyltin oxide (2.25 g, 8.86 mmol) were pre-dried in one flask under vacuum for 3 h, then dissolved in MeOH (35 mL). The mixture was stirred under reflux for 3 h, concentrated to dryness, then suspended in toluene (80 mL). To the above mixture was added benzyl bromide (1.05 mL, 8.81 mmol) and Bu_4NI (3.04 g, 8.20 mmol). The reaction was carried out at 60 °C for 24 h, at the end of which time TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. Concentration of the reaction mixture, and purification of the residue by column chromatography (4:1 petroleum ether–EtOAc) gave syrupy **7** (2.82 g, 89%).[13]

3.3. Ethyl 2,3-di-*O*-benzyl-4-*O*-chloroacetyl-1-thio-β-L-fucopyranoside (8)

To a solution of **7** (1.16 g, 2.99 mmol) in pyridine (5 mL) was added chloroacetic anhydride (714 mg, 3.80 mmol). The mixture was stirred at rt for 6 h, and then co-evaporated with toluene to dryness. The residue was purified by silica gel column chromatography (9:1 petroleum ether–EtOAc), affording **8** (1.21 g, 87%) as a syrup: $[\alpha]_D^{25}$ −21 (*c* 1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): 1.24 (d, 3H, *J* 6.4 Hz, H-6), 1.31 (t, 3H, *J* 7.2 Hz, CH_3), 2.71–2.79 (m, 2H, SCH_2), 3.55 (t, 1H, *J* 9.6 Hz, H-2), 3.64 (dd, 1H, *J* 3.3, 9.6 Hz, H-3), 3.70 (dq, 1H, *J* 6.4, 1.0 Hz, H-5), 4.20 (s, 2H, $ClCH_2CO$), 4.45 (d, 1H, *J* 9.6 Hz, H-1), 4.54, 4.72, 4.74, 4.82 (4 d, 4H, *J*

10.1 Hz, PhCH_2), 5.43 (dd, 1H, *J* 3.3, 1.0 Hz, H-4), 7.25–7.40 (m, 10H, Ph). Anal. Calcd for $C_{24}H_{29}ClO_5S$: C, 61.99; H, 6.29. Found: C, 62.27; H, 6.32.

3.4. Dibutyl 2,3-di-*O*-benzyl-4-*O*-chloroacetyl-β-L-fucopyranosyl 1-phosphate (10)

To a cooled (−20 °C) solution of compound **8** (1.05 g, 2.26 mmol) and dibutyl phosphate (470 μL, 2.52 mmol) in dry CH_2Cl_2 (10 mL) was added *N*-iodosuccinimide (830 mg, 3.39 mmol) and TMSOTf (41 μL, 0.23 mmol). The mixture was stirred for 30 min under an N_2 atmosphere, quenched by Et_3N (two drops), and concentrated. The residue was purified by silica gel column chromatography (3:1 petroleum ether–EtOAc) to give syrupy **10** as an α,β mixture (1.24 g, 90%); Selected peaks for the β isomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85, 0.92 (2t, 6H, *J* 7.4 Hz, 2 CH_3), 1.17 (d, 3H, *J* 6.5 Hz, H-6), 1.23–1.68 (m, 8H), 3.79–3.82 (m, 1H), 3.92–4.05 (m, 5H), 4.15 (s, 2H, ClCH_2CO), 4.26 (q, 1H, *J* 6.5 Hz), 4.58, 4.72 (2 d, 2H, *J* 11.5 Hz), 4.74 (s, 2H), 5.45 (dd, 1H, *J* 1.0, 3.2 Hz), 5.83 (dd, 1H, *J* 3.5, 6.7 Hz), 7.25–7.34 (m, 10H). Anal. Calcd for $C_{30}H_{42}ClO_9P$: C, 58.77; H, 6.91. Found: C, 58.91; H, 6.83.

3.5. Ethyl 4-*O*-acetyl-2-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-1-thio-β-L-fucopyranoside (11)

To a mixture of compound **6** (1.41 g, 4.73 mmol) in *N*,*N*-dimethylformamide (10 mL) was added imidazole (0.90 g, 11.22 mmol) and TBSCl (1.01 g, 5.70 mmol) at 0 °C. The mixture was stirred under these conditions for 30 min, then overnight at room temperature. The reaction mixture was diluted with water (100 mL) and extracted with EtOAc (3 × 100 mL). The organic phase was dried over anhyd $MgSO_4$ and concentrated. Purification of the residue by column chromatography (8:1 petroleum ether–EtOAc), followed by acetylation with Ac_2O in pyridine, afforded **11** (1.81 g, 84%) as a syrup: $[\alpha]_D^{25}$ −25 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.04, 0.08 (2 s, 2 × 3H, Si(CH_3)$_2$), 0.88 (s, 9H, SiC(CH_3)$_3$), 1.18 (d, 3H, *J* 6.6 Hz, H-6), 1.31 (t, 3H, *J* 7.6 Hz, CH_3CH$_2$S), 2.16 (s, 3H, CH_3CO), 2.70–2.81 (m, 2H, CH$_3$CH_2S), 3.48 (t, 1H, *J* 9.3 Hz, H-2), 3.66 (q, 1H, *J* 6.6 Hz, H-5), 3.76 (dd, 1H, *J* 9.3, 3.5 Hz, H-3), 4.45 (d, 1H, *J* 9.3 Hz, H-1), 4.72, 4.83 (2 d, 2 × 1H, *J* 10.2 Hz, PhCH_2), 5.10 (d, 1H, J 3.5 Hz, H-4), 7.28–7.45 (m, 5H, Ph). Anal. Calcd for $C_{23}H_{38}O_5SSi$: C, 60.75; H, 8.42. Found: C, 60.82; H, 8.33.

3.6. Ethyl 4-*O*-acetyl-2-*O*-benzyl-1-thio-β-L-fucopyranoside (12)

To a solution of **11** (1.62 g, 3.57 mmol) in pyridine (40 mL) was added HF–Pyridine (5 mL) at 0 °C. The mixture was stirred at room temperature overnight, poured into aq $NaHCO_3$ (100 mL), and extracted with EtOAc (3 × 80 mL). The combined EtOAc extracts were sequentially washed with 5% aq HCl (3 × 200 mL), aq $NaHCO_3$ (3 × 200 mL), brine (3 × 200 mL), and then dried over anhyd Na_2SO_4 and concentrated. The crude product was subjected to column chromatography with 3:1 petroleum ether–EtOAc as eluent, giving **12** as a white solid (1.10 g, 91%): $[\alpha]_D^{25}$ −23 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.20 (d, 3H, *J* 6.6 Hz, H-6), 1.33 (t, 3H, *J* 7.4 Hz, CH_3CH$_2$S), 2.16 (s, 3H, CH_3CO), 2.77 (m, 2H, CH$_3$CH_2S), 3.48 (t, 1H, *J* 9.5 Hz, H-2), 3.68 (dq, 1H, *J* 6.6, 1.0 Hz, H-5), 3.79 (dd, 1H, *J* 9.3, 3.5 Hz, H-3), 4.45 (d, 1H, *J* 9.5 Hz, H-1), 4.72, 4.83 (2 d, 2 × 1H, *J* 10.2 Hz, PhCH_2), 5.10 (dd, 1H, *J* 3.5,1.0 Hz, H-4), 7.35 (m, 5H, Ph). Anal. Calcd for $C_{17}H_{24}O_5S$: C, 59.98; H, 7.11. Found: C, 59.78; H, 7.20.

3.7. Octyl 4-*O*-acetyl-2-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-β-L-fucopyranoside (13)

To a solution of compound **11** (1.51 g, 3.33 mmol) and 1-octanol (800 μL, 5.04 mmol) in dry CH_2Cl_2 (10 mL) was added 4 Å molecular sieves (1 g). The mixture was stirred at −20 °C for 20 min under an N_2 atmosphere, then *N*-iodosuccinimide (1.223 g, 4.99 mmol) and TMSOTf (50 μL, 0.28 mmol) were added. The mixture was stirred under these conditions for another 30 min, quenched by Et_3N (two drops), and concentrated. The residue was purified by silica gel column chromatography (10:1 petroleum ether–EtOAc) to give **13** as a white foam (1.56 g, 90%): $[\alpha]_D^{25}$ −48 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.02, 0.08 (2 s, 2 × 3H, Si(CH_3)$_2$), 0.86 (s, 9H, SiC(CH_3)$_3$), 0.84–0.90 (m, 12H, SiC(CH_3)$_3$, CH_3C$_7$H$_{14}$O), 1.22 (d, 3H, *J* 6.6 Hz, H-6), 1.32–1.63 (m, 12H, OCH$_2$C$_6$H$_{12}$CH$_3$), 2.14 (s, 3H, CH_3CO), 3.40–3.50 (m, 2H, H-2 and one proton of OCH_2), 3.65 (dq, 1H, *J* 6.6, 1.0 Hz, H-5), 3.71 (dd, 1H, *J* 9.4, 3.7 Hz, H-3), 3.91–3.97 (m, 1H, one proton of OCH_2), 4.34 (d, 1H, *J* 7.8 Hz, H-1), 4.55, 4.55 (2 d, 2 × 1H, *J* 10.8 Hz, PhCH_2), 5.10 (dd, 1H, *J* 3.7, 1.0 Hz, H-4), 7.29–7.37 (m, 5H, Ph). Anal. Calcd for $C_{29}H_{50}O_6Si$: C, 66.63; H, 9.64. Found: C, 66.71; H, 9.69.

3.8. Octyl 4-*O*-acetyl-2-*O*-benzyl-β-L-fucopyranoside (4)

Removal of the TBS group from compound **13** (1.31 g, 2.51 mmol) as described in the preparation of **12** gave **4** as a white solid (860 mg, 84%): $[\alpha]_D^{25}$ −35 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, 3H, *J* 7.0 Hz, CH_3C$_7$H$_{14}$O), 1.21 (d, 3H, *J* 6.6 Hz, H-6), 1.29–1.69 (m, 12H, OCH$_2$C$_6$H$_{12}$CH$_3$), 2.14 (s, 3H, CH_3CO), 3.46–3.53 (m, 2H, H-2 and one proton of OCH_2), 3.67 (dq, 1H, *J* 6.6 Hz, H-5), 3.75 (dd, 1H, *J* 9.7, 3.2 Hz, H-3), 3.94–3.98 (m, 1H, one proton of OCH_2), 4.37 (d, 1H, *J* 7.8 Hz, H-1), 4.81, 5.01 (2 d, 2 × 1H, *J* 11.2 Hz, PhCH_2), 5.18 (d,

1H, *J* 3.2 Hz, H-4), 7.29–7.35 (m, 5H, Ph). Anal. Calcd for $C_{23}H_{36}O_6$: C, 67.62; H, 8.88. Found: C, 67.47; H, 8.94.

3.9. 4-*O*-Acetyl-2-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-β-L-fucopyranosyl trichloroacetimidate (14)

To a solution of compound **11** (775 mg, 1.71 mmol) in (1:9) H_2O–CH_3COCH_3 (40 mL) was added *N*-bromosuccinimide (610 mg, 3.42 mmol) at 0 °C. The mixture was vigorously stirred under these conditions for 10 min, then diluted with CH_2Cl_2 and washed with aq $NaHCO_3$ (3 × 100 mL). The organic phase was dried over anhyd Na_2SO_4 and concentrated under diminished pressure giving a white residue(670 mg). To a solution of the above residue in dry CH_2Cl_2 (5 mL) was added CCl_3CN (490 μL, 4.89 mmol) and DBU (75 μL, 0.50 mmol) under an N_2 atmosphere, and the mixture was then stirred for 3 h under these conditions. Concentration of the reaction mixture and purification of the residue by column chromatography (6:1 petroleum ether–EtOAc) gave **14** (810 mg, 85% over two steps) as a syrup: $[\alpha]_D^{25}$ −34 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): *δ*. 01, 0.08 (2 s, 2 × 3H, Si(CH_3)$_2$), 0.86 (s, 9H, SiC(CH_3)$_3$), 2.15 (s, 3H, CH_3CO), 3.75 (t, 1H, *J* 7.8 Hz, H-2), 3.83–3.88 (m, 2H, H-3, H-5), 4.73, 4.92 (2 d, 2 × 1H, *J* 10.8 Hz, PhCH_2), 5.12 (d, 1H, *J* 2.4 Hz, H-4), 5.77 (d, 1H, *J* 7.8 Hz, H-1), 7.25–7.35 (m, 5H, Ph), 8.64 (s, 1H, N*H*). MALDITOF-MS: Calcd for $C_{23}H_{34}Cl_3NO_6Si$, *m*/*z* 553; found: *m*/*z* 576 $(M+Na)^+$.

3.10. Ethyl 4-*O*-acetyl-2-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-α-L-fucopyranosyl-(1 → 4)-2,3-di-*O*-benzyl-1-thio-β-L-fucopyranoside (5)

To a solution of **7** (500 mg, 1.29 mmol) and **14** (786 mg, 1.42 mmol) in dry Et_2O (5 mL) was added 4 Å molecular sieves (1 g) at 0 °C under an N_2 atmosphere. The mixture was stirred in a dark room for 20 min, then AgOTf (313 mg, 1.42 mmol) was added. The reaction mixture was stirred under these conditions for 30 min, quenched by Et_3N (one drop), and concentrated. The residue was purified by silica gel column chromatography (15:1 petroleum ether–EtOAc) giving **5** (824 mg, 82%) as a white foam: $[\alpha]_D^{25}$ −59 (*c* 2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 0.04, 0.11 (2 s, 2 × 3H, Si(CH_3)$_2$), 0.85 (s, 9H, SiC(CH_3)$_3$), 0.88, 0.92 (2 d, 2 × 3H, *J* 6.6 Hz, H-6^I, H-6^{II}), 1.28 (t, 3H, *J* 7.6 Hz, CH_3CH$_2$S), 2.14 (s, 3H, CH_3CO), 2.60–2.82 (m, 2H, CH$_3$CH_2S), 3.45 (dd, 1H, *J* 9.7, 2.7 Hz, H-3^{II}), 3.49 (q, 1H, *J* 6.6 Hz, H-5^I), 3.68 (dd, 1H, *J* 9.3, 3.5 Hz, H-3^I), 3.71 (t, 1H, *J* 9.3 Hz, H-2^I), 3.78 (d, 1H, *J* 2.3 Hz, H-4^I), 4.21 (dd, 1H, *J* 9.7, 3.4 Hz, H-2^{II}), 4.31 (d, 1H, *J* 9.3 Hz, H-1^I), 4.47 (q, 1H, *J* 6.6 Hz, H-5^{II}), 4.63, 4.71, 4.74, 4.76, 4.84, 4.91 (6 d, 6 × 1H, *J* 10.4 Hz, PhCH_2), 5.03 (d, 1 H, *J* 3.4 Hz, H-1^{II}), 5.10 (d, 1 H, *J* 2.7 Hz, H-4^{II}), 7.25–7.40 (m, 15H, Ph). Anal. Calcd for $C_{43}H_{60}O_9SSi$: C, 66.12; H, 7.74. Found: C, 66.40; H, 7.63.

3.11. Octyl 4-*O*-acetyl-2-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-α-L-fucopyranosyl-(1 → 4)-2,3-di-*O*-benzyl-α-L-fucopyranosyl-(1 → 3)-4-*O*-acetyl-2-*O*-benzyl-β-L-fucopyranoside (15)

To a mixture of **4** (373 mg, 0.91 mmol) and **5** (709 mg, 0.91 mmol) in dry CH_2Cl_2 (5 mL) was added 4 Å molecular sieves (1 g). The mixture was stirred at −20 °C under an N_2 atmosphere for 20 min, then NIS (370 mg, 1.51 mmol) and TMSOTf (19.4 μL, 0.11 mmol) were added. The reaction mixture was stirred under these conditions for 30 min, quenched by Et_3N (two drops), and concentrated. The residue was purified by silica gel column chromatography (10:1 petroleum ether–EtOAc) to give **15** (639 mg, 62%) as white foam: $[\alpha]_D^{25}$ −109 (*c* 0.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 0.04, 0.06 (2 s, 2 × 3H, Si(CH_3)$_2$), 0.77 (d, 1H, *J* 6.5 Hz, H-6^{II}), 0.84 (s, 9H, SiC(CH_3)$_3$), 0.86 (s, 3H, CH_3), 0.94 (d, 3H, *J* 6.5 Hz, H-6^I), 1.19 (d, 3H, *J* 6.5 Hz, H-6^{III}), 1.23–1.66 (m, 12H, OCH$_2$C$_6$H$_{12}$CH$_3$), 1.95, 2.10 (2 s, 2 × 3H, CH_3CO), 3.43 (br s, 1H, H-3^{II}), 3.50 (q, 1H, *J* 7.0 Hz, OCH_2), 3.52 (dd, 1H, *J* 7.7 Hz, H-2^I), 3.56–3.62 (m, 2H, one proton of OCH_2H-5^{II}), 3.79 (br s, H-2^{II}, H-4^{II}), 3.81 (dd, 1H, *J* 9.9, 3.4 Hz, H-3^{III}), 3.93–3.98 (m, 2H, H-3^I and H-5^I), 4.06 (dd, 1H, *J* 9.9, 3.4 Hz, H-2^{III}), 4.26 (q, 1H, *J* 6.7 Hz, H-5^{III}), 4.35 (d, 1H, *J* 7.7 Hz, H-1^I), 4.80 (d, 1H, *J* 3.4 Hz, H-1^{III}), 4.47, 4.57, 4.64, 4.66, 4.67, 4.68, 4.70, 4.73 (8 d, 8 × 1H, *J* 10.8 Hz, PhCH_2), 4.97 (d, 1H, *J* 3.5 Hz, H-1^{II}), 5.11 (br s, 1H, H-4^I), 5.31 (d, 1H, *J* 3.4 Hz, H-4^{III}), 7.12–7.40 (m, 20H, Ph). Anal. Calcd for $C_{64}H_{90}O_{15}Si$: C, 68.18; H, 8.05. Found: C, 68.29; H, 7.98.

3.12. Octyl 4-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl-(1 → 4)-2,3-di-*O*-benzyl-α-L-fucopyranosyl-(1 → 3)-2-*O*-benzyl-4-*O*-acetyl-β-L-fucopyranoside (16)

Removal of the TBS group from compound **15** (380 mg, 0.34 mmol) as described in the preparation of **12** gave **16** (251 mg, 74%) as a syrup: $[\alpha]_D^{25}$ −131 (*c* 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): 0.75 (d, 3H, *J* 6.5 Hz, H-6^{II}), 0.85 (t, 3H, *J* 7.0 Hz, OC$_7$H$_{14}$CH_3), 0.93 (d, 3H, *J* 6.5 Hz, H-6^I), 1.19 (d, 3H, *J* 6.5 Hz, H-6^{III}), 1.32–1.67 (m, 12H, OCH$_2$C$_6$H$_{12}$CH$_3$), 1.97, 2.10 (2 s, 2 × 3H, CH_3CO), 3.43 (br s, 1H, H-3^{II}), 3.48–3.55 (m, 1H, OCH_2), 3.57 (dd, 1H, *J* 8.0, 9.7 Hz, H-2^I), 3.62–3.67 (m, 2H, OCH_2C$_7$H$_{15}$, H-5^{II}), 3.75 (dd, 1H, *J* 10.3, 3.2 Hz, H-3^{III}), 3.79 (br s, 2H, H-2^{II}, H-4^{II}), 3.93–3.99 (m, 2H, H-3^I, H-5^I), 4.03 (dd, 1H, *J* 10.3, 3.4 Hz, H-2^{III}), 4.28 (q, 1H, *J* 6.6 Hz, H-5^{III}), 4.35 (d, 1H, *J* 7.8 Hz, H-1^I), 4.86 (d, 1H, *J* 3.4 Hz, H-1^{III}), 4.39, 4.54, 4.61, 4.67, 4.70, 4.71, 4.73, 4.90 (8 d, 8 × 1H, *J* 10.7 Hz, PhCH_2), 5.06 (d, 1H, *J*

2.2 Hz, H-4^{I}), 5.08 (s, 1H, H-1^{II}), 5.28 (d, 1H, *J* 3.2 Hz, H-4^{III}), 7.12–7.40 (m, 20H, Ph). Anal. Calcd for $C_{58}H_{76}O_{15}$: C, 68.75; H, 7.56. Found: C, 68.49; H, 7.66.

3.13. Octyl 4-*O*-acetyl-2-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-α-L-fucopyranosyl-(1 → 4)-2,3-di-*O*-benzyl-α-L-fucopyranosyl-(1 → 3)-4-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl-(1 → 4)-2,3-di-*O*-benzyl-α-L-fucopyranosyl-(1 → 3)-4-*O*-acetyl-2-*O*-benzyl-β-L-fucopyranoside (17)

Coupling of **5** (170 mg, 0.23 mmol) and **16** (200 mg, 0.20 mmol) as described in the preparation of **15** gave **17** (260 mg, 75%) as a white foam; $[\alpha]_D^{25}$ −37 (*c* 0.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.04, 0.08 (2 s, 2×3H, $Si(CH_3)_2$), 0.73 (d, 3H, *J* 6.5 Hz, H-6^{II}), 0.77 (d, 3H, *J* 6.6 Hz, H-6^{IV}), 0.84–0.87 (m, 12H, $CH_3SiC(CH_3)_3$), 0.93 (d, 3H, *J* 6.6 Hz, H-6^{I}), 1.13 (d, 3H, *J* 6.5 Hz, H-6^{V}), 1.19 (d, 3H, *J* 6.5 Hz, H-6^{III}), 1.23–1.63 (m, 12H, $OCH_2C_6H_{12}CH_3$), 1.88, 1.90, 2.10 (3 s, 3×3H, CH_3CO), 3.43 (d, 1H, H-3^{II}), 3.48–3.51 (m, 2H, H-3^{IV} and OCH_2), 3.57 (dd, 1H, *J* 7.8, 9.7 Hz, H-2^{I}), 3.62–3.66 (m, 2H, H-3^{III}, OCH_2), 3.73–3.97 (m, 10H, H-2^{II}, H-2^{III}, H-2^{IV}, H-3^{V}, H-5^{II}, H-5^{IV}, H-5^{I}, H-4^{II}, H-4^{IV}), 4.11 (dd, 1H, *J* 3.5, 9.7 Hz, H-3^{I}), 4.18 (dd, 1H, *J* 3.0, 9.8 Hz, H-3^{III}), 4.21 (q, 1H, *J* 6.6 Hz, H-5^{V}), 4.31 (q, 1H, *J* 6.7 Hz, H-5^{III}), 4.35 (d, 1H, *J* 7.8 Hz, H-1^{I}), 4.41–4.74 (m, 13H, $PhCH_2$), 4.80 (d, 1H, *J* 3.5 Hz, H-1^{III}), 4.83 (d, 1H, *J* 3.3 Hz, H-1^{V}), 4.89 (d, 1H, *J* 10.5 Hz, $PhCH_2$), 4.99 (br d, 1H, *J* 3.5 Hz, H-4^{I}), 5.11 (d, 1H, *J* 2.7 Hz, H-1^{II}), 5.20 (d, 1H, *J* 3.4 Hz, H-1^{IV}), 5.24 (br d, 1H, *J* 2.3 Hz, H-4^{III}), 5.30 (d, 1H, *J* 3.4 Hz, H-4^{V}), 7.15-7.40 (m, 35H, Ph). Anal. Calcd for $C_{99}H_{130}O_{24}Si$: C, 68.65; H, 7.56. Found: C, 68.89; H, 7.61.

3.14. Octyl 3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl-(1 → 4)-2,3-di-*O*-benzyl-α-L-fucopyranosyl-(1 → 3)-4-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl-(1 → 4)-2,3-di-*O*-benzyl-α-L-fucopyranosyl-(1 → 3)-4-*O*-acetyl-2-*O*-benzyl-β-L-fucopyranoside (19)

Removal of the TBS group from compound **17** (220 mg, 0.13 mmol) as described in the preparation of **4** gave syrupy **18** (180 mg), which was acetylated with Ac_2O (2 mL) in pyridine (4 mL), gave **19** (166 mg, 79% for two steps) as a syrup: $[\alpha]_D^{25}$ −18 (*c* 0.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.72 (d, 6H, *J* 6.5 Hz, 2 H-6), 0.85 (t, 3H, $OC_7H_{14}CH_3$), 0.96, 1.13, 1.19 (3 d, 3×3H, *J* 6.5 Hz, 3 H-6), 1.32–1.67 (m, 12H, $OCH_2C_6H_{12}CH_3$), 1.85, 1.90, 1.96, 2.08 (4 s, 4×3H, 4 CH_3CO), 3.43 (d, 1H, *J* 2.3 Hz, H-3^{II}), 3.51–3.55 (m, 2H, H-3^{IV}, one proton of OCH_2), 3.57 (dd, 1H, *J* 7.6, 9.2 Hz, H-2^{I}), 3.61 (q, 1H, *J* 6.4 Hz, H-5^{II}), 3.70–3.85 (m, 6H, one proton of OCH_2H-2^{II}, H-2^{IV}, H-5^{I}, H-5^{IV}, H-2^{III}), 3.87 (dd, 1H, *J* 3.7, 9.4 Hz, H-2^{V}), 3.91–4.00 (m, 4H, H-4^{II}, H-4^{IV}), H-5^{III}, H-5^{V}), 4.16 (dd, 2H, *J* 2.8, 9.4 Hz, H-3^{I}, H-3^{III}), 4.35 (d, 1H, *J* 7.6 Hz, H-1^{I}), 4.40–4.70 (m, 13H, $PhCH_2$), 4.78 (d, 1H, *J* 3.5 Hz, H-1^{III}), 4.79 (d, 1H, *J* 4.4 Hz, H-1^{IV}), 4.89 (d, 1H, *J* 10.9 Hz, $PhCH_2$), 5.11 (d, 1H, *J* 3.1 Hz, H-1^{II}), 5.17–5.5.22 (m, 3H, H-4^{I}, H-4^{III}, H-1^{V}), 5.29 (dd, 1H, *J* 9.4, 1.9 Hz, H-3^{V}), 5.30 (d, 1H, *J* 1.9 Hz, H-4^{V}), 7.18–7.45 (m, 35H, Ph). MALDITOF-MS: Calcd for $C_{95}H_{118}O^{25}$, *m*/*z* 1658.8; found: *m*/*z* 1680.5 $(M+Na)^+$, 1696.5 $(M+K)^+$.

3.15. Octyl 3,4-di-*O*-acetyl-α-L-fucopyranosyl-(1 → 4)-α-L-fucopyranosyl-(1 → 3)-4-*O*-acetyl-α-L-fucopyranosyl-(1 → 4)-α-L-fucopyranosyl-(1 → 3)-4-*O*-acetyl-β-L-fucopyranoside (20)

To a solution of compound **19** (152 mg, 0.09 mmol) in 1:1 MeOH–EtOAc (30 mL) was added 20% $Pd(OH)_2$-on-charcoal (70 mg, 0.05 mmol). The mixture was bubbled with H_2 at a flow rate of 100 mL/min for 70 h. The reaction mixture was filtered, and the filtrate was concentrated to give **20** (75 mg, 80%) as a white foam: $[\alpha]_D^{25}$ −57 (*c* 0.8, H_2O); ^{1}H NMR (400 MHz, CD_3OD): δ 0.88 (t, 3H, *J* 7.1 Hz, $OC_7H_{14}CH_3$), 1.01, 1.04, 1.13, 1.16, 1.28 (5 d, 5×3H, *J* 6.5 Hz, 5 H-6), 1.27–1.64 (m, 12H, $OCH_2C_6H_{12}CH_3$), 1.97, 2.10, 2.12 (3 s, 4×3H, 4CH_3CO), 3.51–3.92 (m, 11H), 4.05 (dd, 1H, *J* 3.3, 10.4 Hz, H-2^{V}), 4.08 (q, *J* 9.4 Hz, H-5), 4.28 (d, 1H, *J* 8.0 Hz, H-1^{I}), 4.36 (q, 1H, *J* 6.6 Hz, H-5), 4.68 (q, 1H, *J* 6.6 Hz, H-5), 4.81 (q, 1H, *J* 6.6 Hz, H-5), 4.91 (d, 1H, *J* 4.0 Hz, H-1), 4.93 (d, 1H, *J* 3.8 Hz, H-1), 4.98 (d, 1H, *J* 4.0 Hz, H-1), 5.01 (d, 1H, *J* 3.8 Hz, H-1), 5.15 (dd, 1H, *J* 3.3, 10.4 Hz), 5.21, 5.28, 5.33 (3 d, 3×1H, *J* 3.3 Hz, 3 H-4). MALDITOF-MS: Calcd for $C_{46}H_{76}O_{25}$, *m*/*z* 1028; found: *m*/*z* 1051.3 $(M+Na)^+$.

3.16. Octyl 3,4-di-*O*-acetyl-2-*O*-sulfo-α-L-fucopyranosyl-(1 → 4)-2,3-di-*O*-sulfo-α-L-fucopyranosyl-(1 → 3)-4-*O*-acetyl-2-*O*-sulfo-α-L-fucopyranosyl-(1 → 4)-2,3-di-*O*-sulfo-α-L-fucopyranosyl-(1 → 3)-4-*O*-acetyl-2-*O*-sulfo-β-L-fucopyranoside (1)

To a solution of compound **20** (65 mg, 0.06 mmol) in pyridine (4 mL) was added sulfur trioxide·pyridine complex (322 mg, 1.98 mmol). The mixture was stirred at 55 °C for 72 h, then cooled to 4 °C to generate a precipitate that was filtered and dissolved in water (1 mL). Aq. 3 N NaOH was added to the mixture until pH 10. The water phase was washed with CH_2Cl_2 (2×10 mL), then loaded onto a Sephadex LH-20 column and eluted with water. The desired fractions were combined and freeze dried to give **21**, which was directly dissolved into ammonia-saturated MeOH (50 mL). The mixture was stirred at room temperature for 3 days, and concentrated to dryness. To remove the salt in the mixture, the residue was dissolved in water (1 mL) and passed through a 0.22-μm syringe filter. The filtrate was loaded on a Superdex-8482 column (XK16/60 cm, Amersham Biosciences, Sweden) and eluted with doubly deionized

H_2O at 0.8 mL/min with detection by an RI detector. The desired fractions were combined and freeze dried to give 1 (60 mg, 60%) as an amorphous white solid; $[\alpha]_D^{25}$ −75 (*c* 0.5, H_2O); ^{13}C NMR (600 MHz, D_2O): δ 100.9, 98.4, 98.2, 94.4, 93.1, 79.1, 79.0, 75.6, 75.5, 73.3, 72.9, 72.0, 71.9, 71.8, 70.5, 70.1, 69.0, 67.4, 67.3, 67.1, 66.9, 66.8, 66.7, 62.0, 30.6, 29.8, 28.3, 28.0, 27.9, 24.4, 21.6, 15.1, 15.0, 14.8, 13.0. Anal. Calcd for $C_{38}H_{61}Na_7O_{42}S_7 \cdot 2H_2O$: C, 28.32; H, 4.04; S, 13.91. Found: C, 28.24; H, 4.10; S, 13.78.

Acknowledgements

This work was supported by National Basic Research Program of China (2003CB415001) and NNSF of China (Project 20372081 and 30330690). The authors thank Dr. Ye Zhang (Medical cell biology, Beijing University) for performing partial bioassay.

References

1. Percival, E.; McDowell, R. H. *Chemistry and Enzymology of Marine Algal Polysaccharides*; Academic: New York, 1967; pp 157–175.
2. Chevolot, L.; Mulloy, B.; Ratiskol, J.; Foucault, A.; Colliec-Jouault, S. *Carbohydr. Res.* **2001**, *330*, 529–535.
3. Duarte, M. E. R.; Cardoso, M. A.; Noseda, M. D.; Cerezo, A. S. *Carbohydr. Res.* **2001**, *333*, 281–293.
4. Nagaoka, M.; Shibata, H.; Kimura-Takagi, I.; Hashimoto, S.; Kimura, K.; Makino, T.; Aiyama, R.; Ueyama, S.; Yokokura, T. *Glycoconjugate J.* **1999**, *16*, 19–26.
5. Mulloy, B.; Ribeiro, A.-C.; Alves, A.-P.; Vieira, R. P.; Mourão, P. A. S. *J. Biol. Chem.* **1994**, *269*, 22113–22123.
6. Alves, A.-P.; Mulloy, B.; Diniz, J. A.; Mourão, P. A. S. *J. Biol. Chem.* **1997**, *272*, 6965–6971.
7. Pereira, M. S.; Mulloy, B.; Mourão, P. A. S. *J. Biol. Chem.* **1999**, *274*, 7656–7667.
8. (a) Sinniger, V.; Tapon-Bretaudiere, J.; Millieu, C.; Muller, D.; Jozefonvicz, J.; Fischer, A. M. *J. Chromatogr.* **1992**, *615*, 215–223; (b) Pereira, M. S.; Vilela-Silva, A.-C. E. S.; Valente, A.-P.; Mourão, P. A. S. *Carbohydr. Res.* **2002**, *337*, 2231–2238.
9. Glabe, C. G.; Grabel, L. R.; Vacquier, V. D.; Rosen, S. D. *J. Cell. Biol.* **1982**, *94*, 123–128.
10. (a) McClure, M. O.; Moore, J. P.; Blanc, D. F.; Scotting, P.; Cook, G. M.; Keynes, R. J.; Weber, J. N.; Davies, D.; Weiss, R. A. *AIDS Res. Hum. Retroviruses* **1992**, *8*, 19–26; (b) Yoshida, T.; Hatanaka, K.; Uryu, T.; Kaneko, Y.; Suzuki, E.; Miyano, H.; Mimura, T.; Yoshida, O.; Yamamoto, N. *Macromolecules* **1990**, *23*, 3717–3722.
11. (a) Hahnenberger, R.; Jakobson, A. M. *Glycoconjugate J.* **1991**, *8*, 350–353; (b) Khoo, K.-H.; Sarda, S.; Xu, X.; Caulfield, J. P.; McNeil, M. R.; Homans, S. W.; Morris, H. R.; Dell, A. *J. Biol. Chem.* **1995**, *270*, 17114–171232; (c) Foxall, C.; Watson, S. R.; Dowbencko, D.; Lasky, L. A.; Kiso, M.; Hasegawa, A.; Asa, D.; Brandaley, B. K. *J. Cell. Biol.* **1992**, *117*, 895–902.
12. Hua, Y.; Gu, G.; Du, Y. *Carbohydr. Res.* **2004**, 867–872.
13. Mukherjee, A.; Palcic, M. M.; Hindsgaul, O. *Carbohydr. Res.* **2000**, *326*, 1–21.
14. Wei, G.; Gu, G.; Du, Y. *J. Carbohydr. Chem.* **2003**, *22*, 385–394.
15. Sasaki, T.; Takasuka, N. *Carbohydr. Res.* **1976**, *47*, 99–104.
16. Hames, B. D.; Hooper, N. M.; Houghton, J. D. *Instant Notes in Biochemistry*; Bios Scientific Publishers Ltd: Oxford, 1997; pp 65–69.
17. Connolly, D. T.; Knight, M. B.; Harakas, N. K.; Wittwer, A. J.; Feder, J. *Anal. Biochem.* **1986**, *152*, 136–143.