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Carbohydrate Research 339 (2004) 867–872
Carbohydrate RESEARCH

Synthesis and biological activities of 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-β-L-fucopyranoside

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Received 20 November 2003; accepted 20 December 2003

Abstract—An efficient method for the regioselective 3-O-silylation of β-thiofucopyranoside was disclosed. Based on this discovery, we described a high-yielding strategy for the synthesis of the natural core structure of L-fucan and its fully sulfated derivative. The bioassay suggested that 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-β-L-fucopyranoside presented better antitumor activities than that of the free tetramer based on Sarcoma 180 cells and Lewis lung carcinoma model studies.

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

1. Introduction

Sulfated fucans are among the most widely studied of all the sulfated polysaccharides of nonmammalian origin that exhibit biological activities in mammalian systems. Such compounds have been isolated from the cell walls of marine brown algae,[1] the jelly coat from sea urchin eggs,[2] and the sea cucumber body wall.[3] These polyanionic molecules show anticoagulant activity and are potent activators of both antithrombin III and heparin cofactor II.[4] They are inhibitors of retroviral infection, blocking the infection of human cell lines with, for example, HIV, herpes, and cytomegalovirus.[5] They also can act as anti-angiogenic agents and can block selectin-mediated cell–cell binding.[6] The structural components of sulfated fucans necessary for all these biological activities have not yet been determined. However, most of them have a common and simple linear backbone, that of a (1 → 3)-linked α-L-fucopyranoside that differs by specific patterns of sulfation.[7] A comprehensive review article with respect to structures, functions, and biological properties of sulfated fucans has been published very recently.[7b] Curious about the bioactivities of sulfated fucan fragments, we launched a project on the synthesis of a series of sulfated fucoidan oligosaccharides. Here we would like to report the synthesis and antitumor activities of 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.

2. Results and discussion

(1 → 3)-α-L-Fucobioside was previously synthesized by Dejter-Juszynski and Flowers using the Koenigs–Knorr reaction.[8] More recently, a (1 → 3)-α-L-fucopyranosyl trisaccharide derivative was prepared in a moderate yield by reiterative glycosylation using 3,4-di-*O*-benzoyl-2-*O*-benzylfucosyl bromide and regioselective 3-O-fucosylation in the presence of $Hg(CN)_2$ and $HgBr_2$.[9a] A similar strategy was also applied to the preparation of

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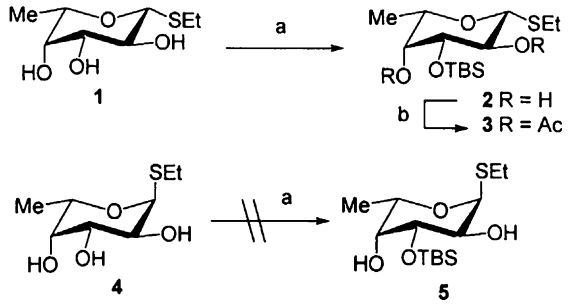
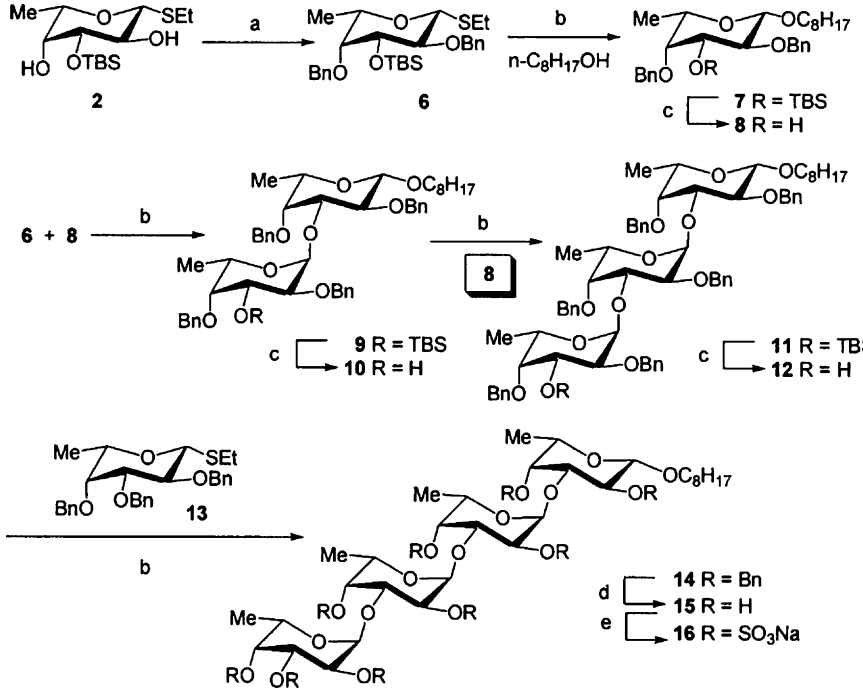
0008-6215/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2003.12.014

(1 → 2)-linked fucopyranosyl oligomers.[9b] It is obvious that a latent 2,4-O-benzylated fucosyl donor, such as thioglycoside **6**, would significantly simplify the synthesis of (1 → 3)-α-L-fucose oligomers, if **6** could be easily prepared. Attempts at regioselective benzylation of alkyl α-L-fucopyranoside afforded 2,4- and 3,4-dibenzyl ethers in the ratio of 3:2.[10] Tin complex-assisted 3-O-allyation of alkyl α-L-fucopyranoside presented a better way to discriminate between the 3-OH and 2,4-OHs, but it is toxic and expensive.[11] We found that direct silylation of ethyl 1-thio-β-L-fucopyranoside (**1**) with *tert*-butyldimethylsilyl chloride (TBSC1) and imidazole in *N*,*N*-dimethylformamide (DMF) at 0 °C gave an excellent yield of monosilylated **2** (92% isolated yield, Scheme 1). Regioselective substitution on C-3 of **2** was further confirmed from its acetylated compound **3**, that is, the chemical shifts of H-2 and H-4 in **3** moved downfield to 5.11 and 5.13 ppm, respectively, from around 3.60 ppm in compound **2**. It is worth noting that ethyl 1-thio-α-L-fucopyranoside (**4**) did not give acceptable yield of **5** under the same reaction conditions, but a mixture of the 2-*O*-silyl and 3-*O*-silyl derivation in a ratio of 2:3.



Scheme 1. Reagents and conditions (yields): (a) TBSC1, DMF, Im, 0 °C (92%); (b) Ac_2O, Pyr (100%).

Encouraged by this basic finding, we envisioned a practical strategy to synthesize the (1 → 3)-linked fucoidan framework, an octyl fucotetroside **15** and its sulfated derivative **16** (Scheme 2). Thus, compound **2** was benzylated with BnBr and NaH in DMF to give **6** in 97% yield. Compound **6** was condensed with 1-octanol in CH_2Cl_2 at −20 °C using *N*-iodosuccinimide/trimethylsilyl trifluoromethanesulfonate (NIS/TMSOTf) as catalysts to give octyl fucoside (**7**), followed by tetrabutylammonium fluoride (TBAF)-catalyzed desilylation affording acceptor **8** in 88% isolated yield for two steps. ^1H NMR analysis surprisingly found that this major fucoside has the β configuration. Doublet (J 7.7 Hz) at δ 4.37 ppm (H-1) clearly indicated a β linkage in **8**. Glycosylation of **6** and **8** as described in the preparation of **7** gave the α linked disaccharide **9** in 89% yield. ^1H–^1H COSY of **9** showed a doublet (J 3.9 Hz) at δ 5.04 ppm confirming the α linkage between the sugar residues. Reiteration of the desilylation with TBAF and glycosylation with **6** transformed disaccharide **9** into trisaccharide acceptor **12** in 59% overall yield. Warning should be given here that trifluoroacetic acid-promoted desilylation on **9** and **11** caused bond breakage between the fucose residues.[12] Final coupling reaction of **12** and thioglycosyl donor **13**[13] furnished the full benzylated



Scheme 2. Reagents and conditions (yields): (a) BnBr, NaH, DMF (97%); (b) NIS, TMSOTf, CH_2Cl_2, −20 °C (97% for **7**; 89% for **9**; 80% for **11**; 80% for **14**); (c) TBAF, THF (89% for **8**; 76% for **10**; 97% for **12**); (d) Pd–C, H_2 1:1 MeOH–EtOAc, 85%; (e) SO_3·Pyr, Pyr; 3 N NaOH (92%).

tetrasaccharide **14** (80%). HMQC assigned four anomeric protons that appeared at δ 4.30 ppm (J 7.4 Hz), 5.07 ppm (J 3.5 Hz), 5.09 ppm (J 3.4 Hz), and 5.10 ppm (J 3.4 Hz), respectively, supporting the structure of compound **14**. Catalytic hydrogenolysis of **14** with H_2 on $Pd(OH)_2$ gave the free tetrasaccharide **15**, which was subsequently sulfated with the sulfur trioxide–pyridine complex in pyridine at 55 °C for 72 h, giving **16** in an excellent yield (92%) after Sephadex LH20 purification and lyophilization of the eluate.

The antitumor activities of the free fucotetroside **15** and the corresponding sulfated derivative **16** were preliminarily tested in vivo according to the method described by Sasaki and Takasuka.[14] Kun-min mice weighing about 20 g and Lewis lung carcinoma cells (1.5×10^6) or Sarcoma-180 cells (5×10^6) were used for the bioassay. Cisplatin® (CDDP) was selected as the positive control in parallel tests. The mice were injected with compound **15** or **16** (daily dose, 2 mg/kg each; iv) 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 volumes were estimated according to the following formula: tumor volume $(cm^3) = 4\pi(xyz)/3$, where x, y, and z are the three perpendicular diameters of the tumor. Inhibition ratio was thus calculated based on tumor volume: $(V_{control} - V_{test})/V_{control}$, where $V_{control}$ is the average tumor volume of saline group, V_{test} is the average tumor volume for the evaluating group. The tumor inhibition ratios for **15**, **16**, and CDDP on Sarcoma-180 cells were 20%, 50%, and 68%, while on Lewis lung carcinoma they were 16%, 48%, and 60%, respectively. It was thus deduced that sulfated fucose oligomers might enhance the antitumor activities.[15] Different structure of fucoidans with specific sulfation patterns are currently under construction, and the related synthesis and bioactivities will be reported in due course.

In conclusion, we have disclosed a simple method for the efficient regioselective 3-O-silylation of β-thiofucoside. Based on this discovery, we have described a high-yielding strategy for the synthesis of the core structure of natural L-fucan and its fully sulfated derivative. The antitumor activities were preliminarily tested in vivo. The results suggested that sulfated analogue **16** presented higher tumor growth inhibition ratios based on studies using Sarcoma-180 cells and Lewis lung carcinoma model studies.

3. Experimental

3.1. General

Optical rotations were determined at 25 °C with a Perkin–Elmer Model 241-Mc automatic polarimeter at $\lambda = 254$ nm. ^{1}H, ^{13}C, ^{1}H–^{1}H 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 or TSP. Mass spectra were measured using MALDITOF or ESIMS with dihydroxybenzoic acid (DHB) as the 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 detector.

3.2. Ethyl 3-*O*-*tert*-butyldimethylsilyl-1-thio-β-L-fucopyranoside (2)

To a solution of compound **1** (1.9 g, 9.13 mmol) in *N*,*N*-dimethylformamide (DMF, 15 mL) was added imidazole (1.595 g, 23.42 mmol) and *tert*-butylchlorodimethylsilane (1.775 g, 11.67 mmol) at 0 °C. The mixture was stirred under these conditions for 30 min and then at room temperature for another 2 h. The reaction mixture was diluted with cold water (50 mL) and extracted with EtOAc (3×50 mL). The organic phase was dried over anhydrous $MgSO_4$ and concentrated. Purification of the residue on a silica gel column (7:1 petroleum ether–EtOAc) gave **2** (2.709 g, 92%) as a syrup; $[\alpha]_D^{25}$ +11° (c 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.24 (d, 1H, J 9.6 Hz, H-1), 3.55–3.66 (m, 4H, H-2, H-3, H-4, H-5), 2.70–2.76 (m, 2H, SCH_2), 1.37 (d, 3H, J 6.4 Hz, H-6), 1.30 (t, 3H, J 7.6 Hz, SCH_2CH_3), 0.92 (s, 9H, $SiC(CH_3)_3$), 0.16, 0.13 (2s, 2×3H, $Si(CH_3)_2$). Anal. Calcd for $C_{14}H_{30}O_4SSi$: C, 52.13; H, 9.38. Found: C, 51.91; H, 9.45.

3.3. Ethyl 2,4-di-*O*-acetyl-3-*O*-*tert*-butyldimethylsilyl-1-thio-β-L-fucopyranoside (3)

Compound **2** (52 mg, 0.16 mmol) was acetylated with Ac_2O (0.5 mL) in pyridine (1 mL) giving **3** as a syrup quantitatively; $[\alpha]_D^{25}$ +36° (c 2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.13 (d, 1H, J 3.2 Hz, H-4), 5.11 (t, 1H, J 9.8 Hz, H-2), 4.36 (d, 1H, J 9.8 Hz, H-l), 3.82 (dd, 1H, J 3.2, 9.8 Hz, H-3), 3.73 (q, 1H, J 6.6 Hz, H-5), 2.68–2.77 (m, 2H, SCH_2), 2.15, 2.08 (2s, 6H, $2CH_3CO$), 1.26 (t, 3H, J 7.6 Hz, SCH_2CH_3), 1.20 (d, 3H, J 6.6 Hz, H-6), 0.82 (s, 9H, $SiC(CH_3)_3$), 0.07, 0.05 (2s, 2×3H, $Si(CH_3)_2$). MALDITOF-MS: calcd for $C_{18}H_{34}O_6SSi$, 406.18; found, 429 $(M+Na)^+$.

3.4. Ethyl 2,4-di-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-1-thio-β-L-fucopyranoside (6)

To a solution of compound **2** (1.114 g, 3,46 mmol) in DMF (15 mL) at 0 °C was added NaH (50% content, 1.040 g, 21.67 mmol) and BnBr (0.90 mL, 7.55 mmol), respectively. The mixture was stirred at 0 °C for 30 min, then at room temperature for 2 h. It was then poured into ice-cold water (50 mL) and extracted with EtOAc (3×50 mL). The organic phase was dried over

anhydrous $MgSO_4$ and concentrated. Purification of the residue by silica gel column chromatography (1:1 petroleum ether–EtOAc) gave **6** (1.678 g, 97%) as a syrup: $[\alpha]_D^{25}$ +10° (*c* 6, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.47–7.28 (m, 10H, Ph), 5.12, 4.78 (2d, 2H, *J* 11.5 Hz, PhCH_2), 4.91, 4.66 (2d, 2H, *J* 10.4 Hz, PhCH_2), 4.42 (d, 1H, *J* 9.3 Hz, H-1), 3.78 (dd, 1H, *J* 2.8, 9.3 Hz, H-3), 3.70 (t, 1H, *J* 9.3 Hz, H-2), 3.57 (dq, 1H *J* 0.5, 6.4 Hz, H-5), 3.49 (dd, 1H, *J* 0.5, 2.8 Hz, H-4), 2.82–2.69 (m, 2H, SCH_2), 1.32 (t, 3H, *J* 7.4 Hz, SCH_2CH_3), 1.23 (d, 3H, *J* 6.4 Hz, H-6), 1.00 (s, 9H, $SiC(CH_3)_3$), 0.17, 0.11 (2s, 2×3H, $Si(CH_3)_2$). Anal. Calcd for $C_{28}H_{42}O_4SSi$: C, 66.89; H, 8.42. Found: C, 67.13; H, 8.36.

3.5. Octyl 2,4-di-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-β-L-fucopyranoside (7)

To a solution of compound **6** (512 mg, 1.02 mmol) and 1-octanol (194 μL, 1.22 mmol) in dry CH_2Cl_2 (3 mL) was added 4 Å molecule sieves (1 g) at −20 °C under a N_2 atmosphere. The mixture was stirred under these conditions for 20 min, then NIS (370 mg, 1.51 mmol) and TMSOTf (19.4 μL, 0.11 mmol) were added. The reaction was monitored by TLC until the starting materials disappeared (ca. 60 min), then the mixture was neutralized with Et_3N (two drops) and concentrated. The residue was purified by silica gel column chromatography (1:1 petroleum ether–EtOAc) to give (569 mg, 97%) as a white foam. This compound was directly used for the next reaction without further characterization.

3.6. Octyl 2,4-di-*O*-benzyl-β-L-fucopyranoside (8)

To a solution of compound **7** (468 mg, 0.82 mmol) in THF (15 mL) was added tetrabutylammonium fluoride monohydrate (TBAF, 1.327 g, 4.21 mmol) at room temperature. The mixture was stirred under these conditions for 2 h, then concentrated and purified by silica gel column chromatography (1:1 petroleum ether–EtOAc) to give **8** (334 mg, 89%) as a white foam. To confirm the structure, compound **8** (30 mg, 0.066 mmol) was acetylated with Ac_2O (0.1 mL) in pyridine (1 mL) affording octyl 3-*O*-acetyl-2,4-di-*O*-benzyl-β-L-fucoside: $[\alpha]_D^{25}$ −40° (c, 0.4, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.26–7.35 (m, 10H, Ph), 4.88, 4.65, 4.62, 4.58 (4d, 4H, *J* 11.8 Hz, PhCH_2), 4.86 (dd, 1H, *J* 3.2, 10.2 Hz, H-3), 4.37 (d, 1H, *J* 7.7 Hz, H-1), 3.97–3.91 (m, 1H, OCH_2), 3,74 (dd, 1H, *J* 7.7, 10.2 Hz, H-2), 3.65 (d, 1H, *J* 3.0 Hz, H-4), 3.60 (q, 1H, *J* 6.4 Hz, H-5), 3.51–3.44 (m, 1H, OCH_2), 1.92 (s, 3H, $COCH_3$), 1.68–1.58 (m, 2H), 1.40–1.22 (m, 10H, CH_2), 1.21 (d, 3H, *J* 6.4 Hz, H-6), 0.87 (t, 3H, *J* 7.0 Hz, CH_3). Anal. Calcd for $C_{28}H_{40}O_5$ for compound **8**: C, 73.65; H, 8.83. Found: C, 73.32; H, 8.89.

3.7. Octyl 2,4-di-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-α-L-fucopyranosyl-(1 → 3)-2,4-di-*O*-benzyl-β-L-fucopyranoside (9)

To a solution of compound **6** (549 mg, 1.09 mmol) and compound **8** (468 mg, 1.03 mmol) in dry CH_2Cl_2 (3 mL) was added 4 Å molecular sieves (1 g) at −20 °C under an N_2 atmosphere. After 20 min, NIS (270 mg, 1.10 mmol) and TMSOTf (15 μL, 0.08 mmol) were added to the above stirred mixture. The reaction mixture was stirred under these conditions for 60 min, quenched by Et_3N (two drops) and concentrated. The residue was purified by silica gel column chromatography (20:1 petroleum ether–EtOAc) to give **9** (819 mg, 89%) as a white foam: $[\alpha]_D^{25}$ −103° (*c* 0.3, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.36–7.18 (m, 20H, Ph), 5.08, 5.02 (2d, 2H, *J* 11.2 Hz, PhCH_2), 5.04 (d, 1H, *J* 3.6 Hz, H-1′), 4.95, 4.76, 4.65, 4,60, 4.55, 4.46 (6d, 6H, *J* 10.7 Hz, PhCH_2), 4.28 (d, 1H, *J* 7.5 Hz, H-1), 4.23 (dd, 1H, *J* 2.8, 9.9 Hz, H-3′), 4.17 (q, 1H, *J* 6.6 Hz, H-5′), 3.94 (dd, 1H, *J* 3.3, 9.9 Hz, H-2′), 3.91–3.87 (m, 1H, OCH_2), 3.71 (dd, 1H, *J* 7.5, 10.0 Hz, H-2), 3.64 (dd, 1H, *J* 2.6, 10.0 Hz, H-3), 3.50 (d, 1H, *J* 2.6 Hz, H-4), 3.45–3.42 (m, 1H, OCH_2), 3.38 (q, 1H, *J* 6.8 Hz, H-5), 3.30 (br d, 1H, *J* 2.8 Hz, H-4′), 1.39–1.23 (m, 15H, CH_2 and H-6′), 0.94 (s, 9H, $SiC(CH_3)_3$), 0.87 (d, 3H, *J* 6.6 Hz, H-6), 0.85 (t, 3H, *J* 7.0 Hz, CH_2CH_3), 0.13, 0.12 (2s, 2×3H, $Si(CH_3)_2$). Anal. Calcd for $C_{54}H_{76}O_9Si$: C, 72.28; H, 8.54. Found: C, 72.66; H, 8.48.

3.8. Octyl 2,4-di-*O*-benzyl-α-L-fucopyranosyl-(1 → 3)-2,4-di-*O*-benzyl-β-L-fucopyranoside (10)

To a solution of compound **9** (800 mg, 0.89 mmol) in THF (5 mL) was added TBAF (890 mg, 0.28 mmol) at room temperature. The mixture was stirred at room temperature until TLC indicated that the reaction was complete. Concentration and purification of the residue by silica gel column chromatography (7:1 petroleum ether–EtOAc) gave **10** (533 mg, 76%) as a white foam: $[\alpha]_D^{25}$ −94° (*c* 1.3, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.35–7.23 (m, 20H, Ph), 5.18 (d, 1H, *J* 3.3 Hz, H-1′), 5.05, 4.52 (2d, 2H, *J* 11.6 Hz, PhCH_2), 4.95, 4.58 (2d, 2H, *J* 10.4 Hz, PhCH_2), 4.71–4.60 (m, 4H, PhCH_2), 4.29 (d, 1H, *J* 7.0 Hz, H-1), 4.17 (q, 1H, *J* 6.5 Hz, H-5′), 4.02 (ddd, 1H, *J* 10.0, 3.0, 6.1 Hz, H-3′), 3.93–3.80 (m, 1H, OCH_2), 3.80 (dd, 1H, *J* 10.1, 3.4 Hz, H-3), 3.75 (dd, 1H, *J* 7.0, 10.1 Hz, H-2), 3.69 (dd, 1H, *J* 10.0, 3.3 Hz, H-2′), 3.56 (d, 1H, *J* 3.4 Hz, H-4), 3.44–3.39 (m, 2H, one proton of OCH_2 and H-5), 3.29 (d, 1H, *J* 3.0 Hz, H-4′), 2.02 (d, 1H, *J* 6.1 Hz, O*H*), 1.65–1.55 (m, 2H, CH_2), 1.40–1.20 (m, 10H, CH_2), 1.14 (d, 3H, *J* 6.3 Hz, H-6),

0.90 (d, 3H, *J* 6.5 Hz, H-6′), 0.85 (t, 3H, *J* 7.0 Hz, CH_2CH_3). Anal. Calcd for $C_{48}H_{62}O_9$: C, 73.63; H, 7.98. Found: C, 73.29; H, 8.06.

3.9. Octyl 2,4-di-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-α-L-fucopyranosyl-(1 → 3)-2,4-di-*O*-benzyl-α-L-fucopyranosyl-(1 → 3)-2,4-di-*O*-benzyl-β-L-fucopyranoside (11)

Compounds **6** (288 mg, 0.57 mmol) and **10** (393 mg, 0.50 mmol) were glycosylated as described in the preparation of compound **9** to give **11** (488 mg, 80%) as a white foam: $[\alpha]_D^{25}$ −76° (*c* 0.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35–7.16 (m, 30H, Ph), 5.10, 5.02 (2d, 2H, *J* 11.6 Hz, PhCH_2), 5,08 (d, 1H, *J* 3.3 Hz, H-1″), 5.06 (d, 1H, *J* 3.4 Hz, H-1′), 5.03, 4.92, 4.79, 4.71, 4.69, 4.64, 4,59, 4.58, 4.49, 4.33 (10d, 10H, *J* 10.9 Hz, PhCH_2), 4.32–4.23 (m, 4H, H-1, H-5′, H-5″, H-3″), 4.07 (dd, 1H, *J* 3.3, 10.4 Hz, H-2″), 4.02 (dd, 1H, *J* 3.4, 10.0 Hz, H-2′), 3.98 (q, 1H, *J* 6.4 Hz, H-5), 3.95–3.87 (m, 1H, OCH_2), 3.72–3.65 (m, 2H, H-3, H-3′), 3.51 (br s, 1H, H-4), 3.46–3.39 (m, 2H, H-2 and one proton of OCH_2), 3.38 (br s, 1H, H-4′), 3.31 (br s, 1H, H-4″), 1.65–1.58 (m, 2H, CH_2), 1.40–1.19 (m, 10H, CH_2), 1.17 (d, 3H, *J* 6.4 Hz, H-6), 1.03 (d, 3H, *J* 6.5 Hz, H-6′), 0.92 (s, 9H, SiC(CH_3)$_3$), 0.85 (t, 3H, *J* 7.0 Hz, CH_3), 0.80 (d, 3H, *J* 6.4 Hz, H-6″), 0.10, 0.08 (2s, 2 × 3H, Si(CH_3)$_2$). Anal. Calcd for $C_{74}H_{98}O_{13}Si$: C, 72.63; H, 8.07. Found: C, 72.97; H, 8.11.

3.10. Octyl 2,4-di-*O*-benzyl-α-L-fucopyranosyl-(1 → 3)-2,4-di-*O*-benzyl-α-L-fucopyranosyl-(1 → 3)-2,4-di-*O*-benzyl-β-L-fucopyranoside (12)

To a solution of compound **11** (308 mg, 0.25 mmol) in THF (2 mL) was added TBAF (890 mg, 0.28 mmol) at room temperature. The mixture was stirred at room temperature until TLC indicated that the reaction was complete. Concentration and purification of the residue by silica gel column chromatography (7:1 petroleum ether–EtOAc) gave **12** (270 mg, 97%) as a white foam: $[\alpha]_D^{25}$ −19° (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$: δ 7.36–7.17 (m, 30H, Ph), 5.19 (d, 1H, *J* 3.4 Hz, H-1″), 5.12 (d, 1H, *J* 3.2 Hz, H-1′), 5.10, 5.02, 4.94 (3d, 3H, *J* 11.3 Hz, PhCH_2), 4.73–4.64 (m, 6H, PhCH_2), 4.57, 4.51, 4.39 (3d, 3H, J 11.3 Hz, PhCH_2), 4.31–4.25 (m, 3H, H-1, H-5′, H-5″), 4.08 (dd, 1H, *J* 3.4, 10.5 Hz, H-2″), 4.04 (ddd, 1H, *J* 3.0, 10.5, 6.3 Hz, H-3″), 4.02 (q, 1H, *J* 6.4 Hz, H-5), 3.95–3.89 (m, 1H, OCH_2), 3.84 (dd, 1H, *J* 3.2, 10.3 Hz, H-2′), 3.75–3.68 (m, 2H, H-3, H-3′), 3.54 (br s, 1H, H-4), 3.46–3.41 (m, 2H, H-2 and one proton of OCH_2), 3.33 (br s, 1H, H-4′), 3.31 (br d, 1H, *J* 3.0 Hz, H-4″), 2.02 (d, *J* 6.3 Hz, O*H*), 1.65–1.56 (m, 2H, CH_2), 1.40–1.20 (m, 10H, CH_2), 1.16 (d, 3H, *J* 6.4 Hz, H-6), 1.03 (d, 3H, *J* 6.7 Hz, H-6″), 0.87 (t, 3H, *J* 7.1 Hz, CH_2CH_3), 0.80 (d, 3H, *J* 6.4 Hz, H-6′). Anal. Calcd for $C_{68}H_{84}O_{13}$: C, 73.62; H, 7.63. Found: C, 73.39; H, 7.57.

3.11. Octyl 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1 → 3)-2,4-di-*O*-benzyl-α-L-fucopyranosyl-(1 → 3)-2,4-di-*O*-benzyl-α-L-fucopyranosyl-(1 → 3)-2,4-di-*O*-benzyl-β-L-fucopyranoside (14)

Glycosylation of compounds **13** (139 mg, 0.29 mmol) and **12** (270 mg, 0.24 mmol) as described in the preparation of **9** gave **14** (295 mg, 80%) as a white foam: $[\alpha]_D^{25}$ −90° (*c* 0.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35–7.10 (m, 45H, Ph), 5.12 (d, 1H, *J* 3.4 Hz, H-1″), 5.10 (d, 1H, *J* 3.4 Hz, H-1′), 5.09 (d 1H, *J* 11.4 Hz, PhCH_2), 5.07 (d, 1H, *J* 3.2 Hz, H-1‴), 5.05 (d, 1H, *J* 11.4 Hz, PhCH_2), 4.92, 4.90, 4.80 (3d, 3H, *J* 11.6 Hz, PhCH_2), 4.70–4.64 (m, 5H, PhCH_2), 4.62, 4.60, 4.58, 4.56, 4.54, 4.40, 4.37 (7d, 7H, PhCH_2), 4.31–4.26 (m, 3H, H-1, H-5, H-5′), 4.20 (q, 1H, *J* 6.3 Hz, H-5‴), 4.15–4.09 (m, 3H, H-2‴, H-3′, H-3″), 4.04 (dd, 1H, *J* 3.5, 10.2 Hz, H-2″), 3.95 (q, 1H, *J* 6.5 Hz, H-5″), 3.94–3.85 (m, 2H, H-2″, one proton of OCH_2), 3.71–3.65 (m, 2H, H-3, H-3‴), 3.53 (br s, 1H, H-4‴), 3.47–3.40 (m, 2H, one proton of OCH_2, H-2), 3.35–3.30 (m, 3H, H-4, H-4′, H-4″), 1.64–1.56 (m, 2H), 1.40–1.20 (m, 10H, CH_2), 1.18 (d, 3H, *J* 6.4 Hz, H-6), 0.96 (d, 3H, *J* 7.6 Hz, H-6′), 0.90–0.82 (m, 6H, CH_2CH_3, H-6″), 0.80 (d, 3H, *J* 6.4 Hz, H-6‴); ^{13}C NMR (100 MHz, $CDCl_3$): δ 139.02, 138.92, 138.90, 138.82, 138.65, 138.56, 138.31, 138.13, 138.07, 103.98 (C-1), 96.23 (C-1‴), 95.02 (C-1′), 94.87 (C-1″), 79.47, 78.67, 78.43, 77.32, 77.01, 76.50, 76.30, 76.00, 75.62, 75.53, 75.18, 74.86, 74.78 (3 C), 74.39, 74.30, 74.18 (2 C), 73.94, 72.46, 70.12 (C-5‴), 69.91, 66.80 (C-5″), 66.50 (C-5), 66.23 (C-5′), 31.79, 29.73, 29.40, 29.20, 26.14, 22.66, 16.78 (C-6), 16.48 (C-6′), 16.43 (C-6‴), 16.25 (C-6″), 14.10. Anal. Calcd for $C_{95}H_{112}O_{17}$: C, 74.78; H, 7.40. Found: C, 75.16; H, 7.46.

3.12. Octyl α-L-fucopyranosyl-(1 → 3)-α-L-fucopyranosyl-(1 → 3)-α-L-fucopyranosyl-(1 → 3)-β-L-fucopyranoside (15)

To a solution of compound **14** (212 mg, 0.14 mmol) in 1:1 MeOH–EtOAc (20 mL) was added 20% $Pd(OH)_2$ on charcoal (209 mg, 0.14 mmol). The mixture was bubbled with H_2 under the flow rate of 100 mL/min for 70 h. The reaction mixture was filtered, and the filtrate was concentrated to give **15** (84 mg, 85%) as a white foam; $[\alpha]_D^{25}$ +5° (*c* 0.4, H_2O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.00 (br s, 3H, H-1′, H-1″, H-1‴), 4.25 (br s, 4H, H-5, H-5′, H-5″, H-5‴), 3.94–3.85 (m, 8H, H-4, H-4′, H-4″, H-4‴, H-1, H-3‴, H-2, OCH_2), 3.26 (br s, 3H, H-3, H-3′, H-3″), 3.57–3.25 (m, 4H, H-2′, H-2″, H-2‴, OCH_2), 1.55 (br s, 2H), 1.20–1.13 (m, 22H), 0.79 (br s, 3H); ^{13}C NMR (100 MHz, D_2O): δ 102.85 (C-1), 95.70 (C-1″, C-1‴), 95.44 (C-1′), 77.80 (C-3), 74.97 (C-3′, C-3″), 71.91 (C-4‴), 70.45 (C-3‴, OCH$_2$), 69.46 (C-4), 68.74 (C-2‴), 68.52 (C-2′, C-4′, C-4″), 68.03 (C-2″), 67.62 (C-5‴), 66.95

(C-5), 66.47 (C-2, C-5′, C-5″), 31.50, 29.06, 28.93 (2C), 25.43, 22.34, 15.84, 15.59, 15.48, 15.33 (4 C-6), 13.70; ESIMS (negative-ion): calcd for $C_{32}H_{58}O_{17}$, 714.37; found, 713.3 $(M-H)^+$.

3.13. 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-β-L-fucopyranoside (16)

To a solution of compound **15** (83 mg, 0.12 mmol) in pyridine (4 mL) was added sulfur trioxide–pyridine complex (530 mg, 3.26 mmol). The mixture was stirred at 55 °C for 72 h, then cooled to 4 °C to generate a precipitate. The pyridine phase was sucked off, and the precipitate was dissolved in water (1 mL). Sodium hydroxide (3 N) was added until pH 10. The water phase was washed with CH_2Cl_2 (2 × 1 mL), then loaded on to a Sephadex LH20 column and eluted with water. The desired fractions were combined and freeze dried to give **16** (174 mg, 92%) as an amorphous white solid; $[\alpha]_D^{25}$ −75° (*c* 0.5, H_2O); ^1H NMR (400 MHz, $CDCl_3$): δ 5.30 (br s, 3H, H-1′, 1″, 1‴), 4.90–4.85 (m, 5H, H-3‴, H-4,4′, 4″, 4‴), 4.76 (d, 1H, *J* 6.7 Hz, H-1), 4.56–4.45 (m, 8H, H-2, 2′, 2″, 2‴, H-5, 5′, 5″, 5‴), 4.30–4.18 (m, 3H, H-3, 3′, 3″), 4.05–3.91 (m, 1H, OCH_2), 3.80–3.70 (m, 1H, OCH_2), 1.54–1.49 (m, 2H), 1.30–1.10 (m, 22H, H-6 and CH_2), 0.79 (t, 3H, *J* 7.2 Hz, CH_3); Selected ^{13}C NMR (100 MHz, D_2O): δ 103.80, 99.71 (2C), 97.54 (4C-1). Anal. Calcd for $C_{32}H_{49}Na_9O_{44}S_9 \cdot 3H_2O$: C, 22.76; H, 3.26; S, 17.07. Found: C, 22.39; H, 3.32; S, 17.18.

Acknowledgements

This work was supported by NNSF of China (Project 20372081, 30330690) and RCEES of CAS. The authors thank Professor Huimin Lu (Division of Toxicology, CDC of China) for performing the mice tests.

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