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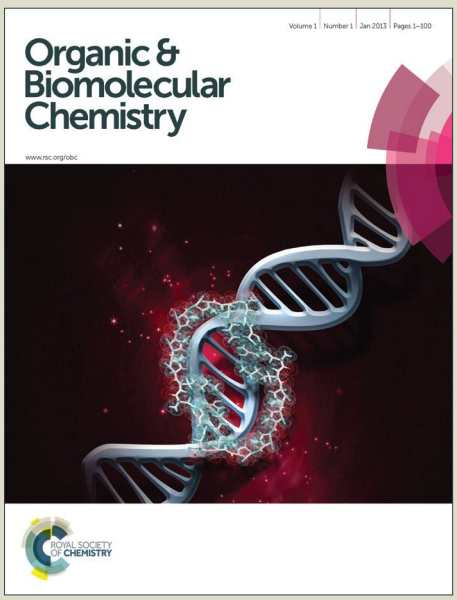





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# Systematic synthesis of low-molecular weight fucoidan derivatives and their effect on cancer cells

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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Low-molecular weight type I and II fucoidan derivatives with different sulfation patterns were designed and systematically synthesized from the corresponding common key intermediate and their anti-proliferative activities and apoptosis-inducing activities against human breast cancer MCF-7 and human cervical epithelioid carcinoma HeLa cells were evaluated. Our results demonstrated that one of the type II fucoidan derivatives, **9**, effectively reduced the number of viable MCF-7 and HeLa cells in a dose-dependent manner without causing cytotoxicity toward normal WI-38 cells, and that the anti-proliferative activity of **9** was comparable to that of fucoidan **2** isolated from *Fucus vesiculosus*. Moreover, it was found that both **2** and **9** exhibited similar apoptosis-inducing activities through activation of caspase-8 and -9 on MCF-7 and HeLa cells, respectively.

## Introduction

Fucoidan is a class of sulfated, fucose-rich polysaccharides found in the fibrillar cell walls and intracellular spaces of brown algae. The chemical structures and compositions of fucoidans are diverse and vary depending on the algal species, and factors such as cultivation conditions, habitat, harvesting time, and the method used to isolate the fucoidans. In general, fucoidans are classified into two groups.[1] One group (type I) includes the fucoidans from, for example, *Cladosiphon okamuranus* and *Chorda filum,* whose central chains are composed of (1→3)-linked α-L-fucopyranosyl residues. The second group (type II) comprises fucoidans isolated from, for example, *Ascophyllum nodosum* and *Fucus vesiculosus,* whose central chains are composed of repeating (1→3)- and (1→4)-linked α-L-fucopyranosyl residues (Fig. 1). Fucoidans exhibit a wide range of biological activities such as anti-inflammatory, anticoagulant, and antitumor activities.[1,2] In particular, the antitumor activity of fucoidans has attracted much attention following reports that fucoidans inhibit lymphoma[3] and mouse breast cancer[4] tumor growth and show anti-angiogenesis activities against Lewis lung carcinoma and B16 melanoma[5] *in vivo*. *In vitro* studies have led to several mechanisms being postulated for the observed antitumor activities.[6] For example, it was recently reported that type I fucoidan **1**, isolated from *C. okamuranus*, significantly inhibited the growth of peripheral blood mononuclear cells of adult T-cell leukemia patients and human T-cell leukemia virus type 1-infected T-cell lines, but not of normal peripheral blood mononuclear cells.[7] In addition, type II fucoidan **2**, isolated from *F. vesiculosus*, was found to induce apoptosis in human HS-Sultan cells[8] and human breast cancer MCF-7 cells.[9] Thus, fucoidans hold promise as potential antitumor agents causing few side effects. However, the structural complexity, heterogeneity, and uniformity of the sulfation patterns of fucoidans has hampered understanding of the relationship between their molecular structure and biological activity, obstructing the development of medicinal applications. Despite several attempts to generate low-molecular weight fucoidans (LMWFs) from natural sources by free radical,[10] chemical hydrolysis[2d] and enzymatic hydrolysis,[2a] it remains difficult to reproducibly obtain structurally uniform and pure LMWFs. Several studies of chemically synthesized homogeneous and structurally well-defined oligofucosides with different sulfation patterns have been reported,[11] but

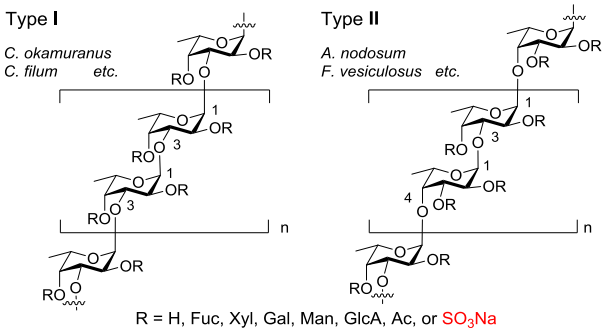


**Fig. 1** Two types (I and II) of homofucose chains in brown seaweed fucoidans.

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*Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x*

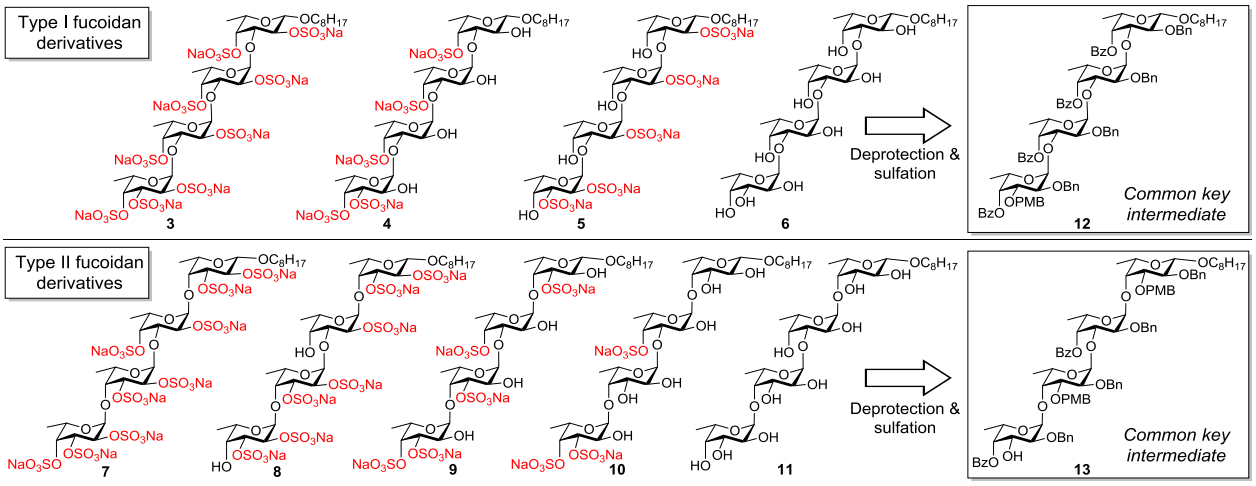


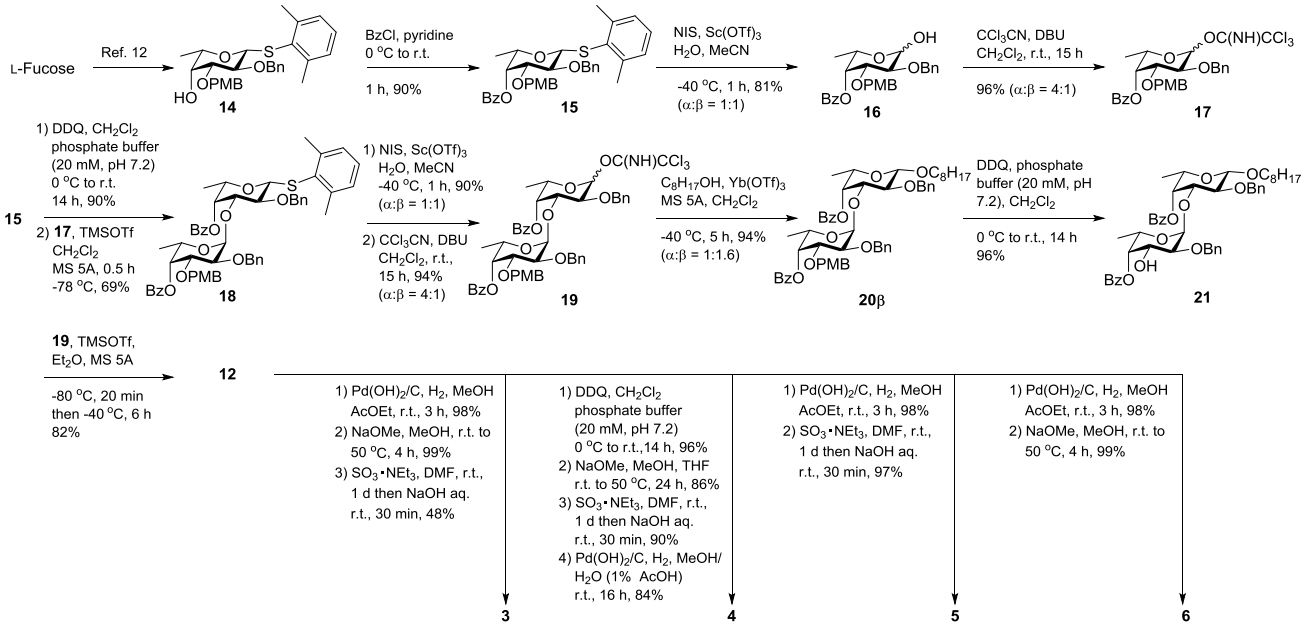

**Fig. 2** Chemical structures of the fucoidan derivatives **3**-**11** and the common key intermediates **12** and **13**.

detailed structure-activity relationships and the mode of action of the antitumor properties of fucoidans have yet to be elucidated. Here, the design and systematic synthesis of type I and II fucoidan derivatives with different sulfation patterns are described and their antitumor activities against MCF-7 and HeLa cells are evaluated.

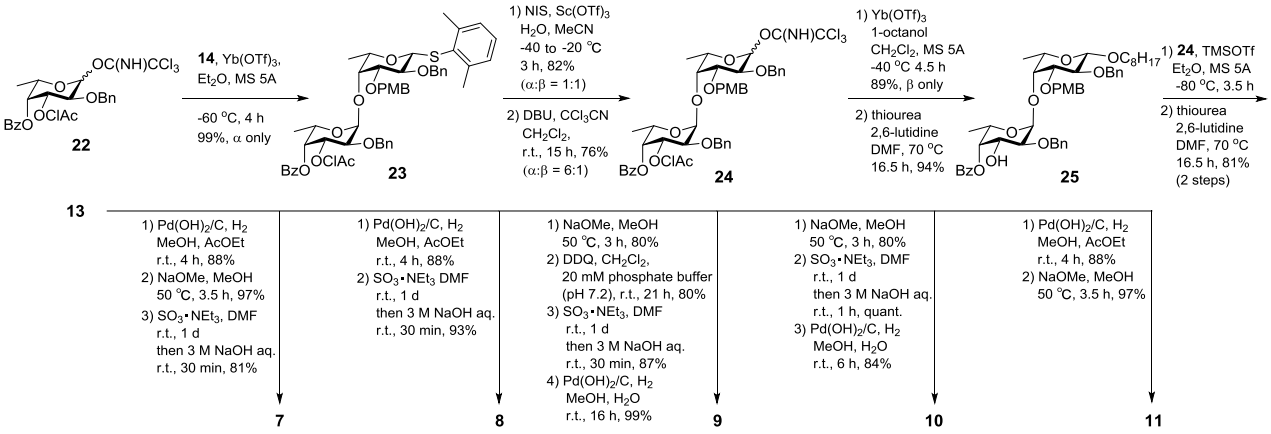
## Results and discussion

Our designed low-molecular weight fucoidan derivatives are shown in Fig. 2: type I fucoidan derivatives **3**-**6** with different sulfation patterns (2,4-*O*-sulfated type **3**,[11a] 4-*O*-sulfated type **4**, 2-*O*-sulfated type **5** and non-sulfated type **6**[11a]), and type II fucoidan derivatives **7**-**11**[12] with different sulfation patterns (2,3,4-*O*-sulfated type **7**, 2,3-*O*-sulfated type **8**, 3,4-*O*-sulfated type **9**, 4-*O*-sulfated type **10** and non-sulfated type **11**). We envisaged that an orthogonal deprotection strategy utilizing the common key intermediates **12** and **13** with three types of protecting groups (benzyl (Bn), benzoyl (Bz) and *p*-methoxybenzyl (PMB)) at appropriate positions would support effective systematic synthesis by minimizing the number of synthetic steps and affording sufficient quantities of oligofucosides for biological assays.

As shown in Scheme 1, we first synthesized the key intermediate **12** and type I fucoidan derivatives **3**-**6**. Thioglycoside **14**[12] was prepared from L-fucose in 8 steps. Benzoylation of **14**, followed by hydrolysis of **15** using NIS/Sc(OTf)$_3$ and introduction of a trichloroacetimidate group, provided monosaccharide donor **17** in high overall yield. Deprotection of the PMB group in **15**, followed by chemoselective glycosylation with **17** using a catalytic amount of TMSOTf in $CH_2Cl_2$, provided **18**. The carbohydrate chain was elongated by converting **18** to a disaccharide donor and acceptor pair (**19** and **21**). Hydrolysis of **18** using NIS/Sc(OTf)$_3$,




**Scheme 1** Synthetic scheme for **12** and type I fucoidan derivatives **3**-**6**.




**Scheme 2** Synthetic scheme for **13** and type II fucoidan derivatives **7**-**11**.

followed by introduction of a trichloroacetimidate group, provided disaccharide donor **19** in high yield. Compound **19** was condensed with 1-octanol using $Yb(OTf)_3$ in $CH_2Cl_2$ at −40 °C for 5 h to afford octyl fucoside **20**.[13] Deprotection of the PMB group in **20**β using DDQ provided **21** in high yield. Glycosylation of **21** with **19** using TMSOTf as an activator in $Et_2O$ at −80 to −40°C provided the desired key intermediate **12** as a single isomer in 82% yield. Next, the designed sulfated and non-sulfated tetrafucosides **3**-**6** were synthesized from the common intermediate **12**. Deprotection of the Bn and PMB groups in **12** under hydrogenolysis conditions, followed by methanolysis, provided the non-sulfated **6**. Sulfation of **6** using $SO_3$•$NEt_3$ complex in DMF gave the 2,4-*O*-sulfated tetrasaccharide **3**. Alternatively, deprotection of the Bn and PMB groups in **12**, followed by sulfation and saponification, provided 2-*O*-sulfated tetrasaccharide **5**. Finally, deprotection of the PMB group in **12**, followed by methanolysis, sulfation, and hydrogenolysis, afforded the desired 4-*O*-sulfated tetrafucoside **4**. Similarly, key intermediate **13** and type II fucoidan derivatives **7**-**11** were synthesized as shown in Scheme 2. In brief, chemoselective glycosylation of **14** with **22**[11d] using a catalytic amount of $Yb(OTf)_3$ provided the disaccharide **23** as a single isomer in 99% yield. Use of the 2,6-dimethylphenylthio group[14] in **14** was critical for increasing the chemical yield: when the ethyl thioglycoside was used as an acceptor instead of **14**, the ethylthio group was activated and the aglycon transfer reaction proceeded as a side reaction. Next, hydrolysis of the 2,6-dimethylphenylthio group in **23**, followed by introduction of a trichloroacetimidate group, provided disaccharide donor **24**. Glycosylation of 1-octanol with **24** using $Yb(OTf)_3$, followed by deprotection of the ClAc group, afforded disaccharide glycosyl acceptor **25**.[13] The β configuration of **25** was confirmed by $^1$H-NMR analysis ($J_{1,2}$=7.8 Hz). Glycosylation of **25** with **24** using TMSOTf as an activator in $Et_2O$ at −80 °C, followed by deprotection of the ClAc group, provided the desired key intermediate **13**. Next, designed sulfated and non-sulfated tetrafucosides **7**-**11** were synthesized from the common intermediate **13**. Deprotection of the Bn and PMB groups in **13** under hydrogenolysis conditions, followed by methanolysis, provided the non-sulfated **11**. Sulfation of **11** using $SO_3$•$NEt_3$ complex gave the 2,3,4-*O*-sulfated tetrasaccharide **7**. Alternatively, deprotection of the Bn and PMB groups in **13**, followed by sulfation and methanolysis, provided 2,3-*O*-sulfated tetrasaccharide **8**; in addition, methanolysis of **13**, followed by sulfation and hydrogenolysis, provided 4-*O*-sulfated tetrasaccharide **10**. Finally, methanolysis of **13**, followed by deprotection of the PMB groups with DDQ, sulfation, and hydrogenolysis, afforded the desired 3,4-*O*-sulfated tetrafucoside **9**.

With the designed tetrafucosides in hand, the effects of **3**-**11** on the proliferation of MCF-7 and HeLa cells were examined using the MTT assay. The cells were treated with different doses (10-800 μM) for 96 h. The results of type I fucoidan derivatives **3**-**6** are shown in Figs 3a and b. When MCF-7 cells were treated with **3**-**6**, all derivatives were found to effectively and significantly reduce the number of viable MCF-7 cells in a dose-dependent manner; of these, 2,4-*O*-sulfated

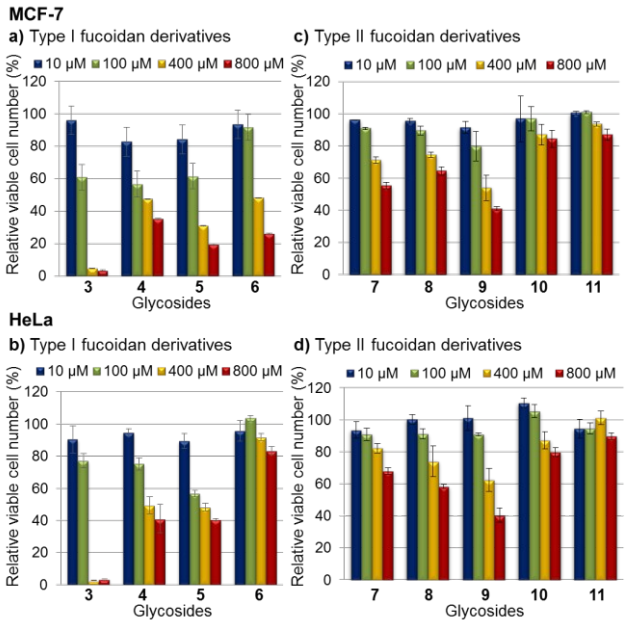



**Fig. 3** Effects of a) **3**-**6** and c) **7**-**11** on MCF-7 cell proliferation, and effects of b) **3**-**6** and d) **7**-**11** on HeLa cell proliferation. Cells were seeded into 96-well plates ($1\times10^3$ cells well$^{-1}$). After 24 h, compounds were added at the indicated concentrations and the cells were incubated for 96 h at 37 °C in 5% $CO_2$ in air. MTT reagent was then added to each well, and the cells were incubated for up to 3 additional hours. The absorbance of each well was read at 540 nm using a plate reader.

type **3** showed the highest anti-proliferation activity (Fig. 3a). When HeLa cells were treated with **3**-**6**, non-sulfated **6** provided only low anti-proliferation activity even at 800 μM, whereas derivatives **3**-**5** showed anti-proliferation activities similar to those observed in MCF-7 cells (Fig. 3b). The results obtained using type II fucoidan derivatives **7**-**11** are shown in Figs 3c and d. Treatment of MCF-7 cells with **7**-**11** showed that 4-*O*-sulfated type **10** and non-sulfated **11** provided only low anti-proliferative activities, whereas 2,3,4-*O*-sulfated type **7**, 2,3-*O*-sulfated type **8**, and 3,4-*O*-sulfated type **9** effectively reduced the number of MCF-7 cells in a dose-dependent manner, with **9** showing the highest anti-proliferative activity (Fig. 3c). Similar results were obtained when HeLa cells were treated with **7**-**11** (Fig. 3d). These results provide the first demonstration that homogeneous and structurally-defined sulfated tetrafucosides show anti-proliferative activity against MCF-7 and HeLa cells. Moreover, these results suggest that slight differences both in the sugar backbone chain and in the pattern of sulfate groups on **3**-**11** affect their anti-proliferative activities.

Since treatment of MCF-7 and/or HeLa cells with 800 μM of compounds **3**-**6** or **9** for 96 h resulted in less than 50% survival, we examined the cytotoxicity of these active compounds by conducting MTT assays using normal human lung fibroblast WI-38 cells. The results are summarized in Fig. 4. Compounds **3**-**6**, with a type I backbone, significantly reduced the number of WI-38 cells in a dose-dependent manner, whereas 3,4-*O*-sulfated type **9**, with a type II backbone, exhibited neither cytotoxicity nor anti-proliferative activity against WI-38 cells. These results clearly indicate that a 3,4-*O*-sulfated oligofucoside structure with a type II backbone exhibits selective anti-proliferative activity against human cancer cells.

Next, to elucidate the minimum carbohydrate chain-length required for anti-proliferative activity, 3,4-*O*-sulfated type disaccharide **26** and hexasaccharide **27**, both with type II backbones, were designed and synthesized as shown in Scheme 3. Deprotection of the Bz groups in **25**, followed by deprotection of the PMB group, sulfation, and hydrogenolysis, gave the 3,4-*O*-sulfated type disaccharide **26**. Glycosylation of tetrasaccharide acceptor **12** with disaccharide donor **19** using a catalytic amount of TMSOTf in $Et_2O$ provided **29** as a single isomer in 94% yield. The desired hexasaccharide **27** was

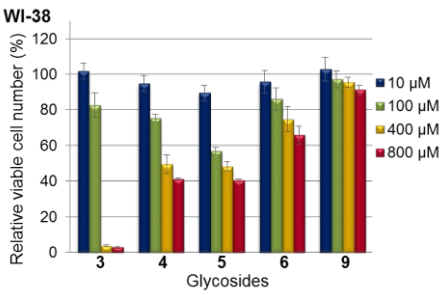
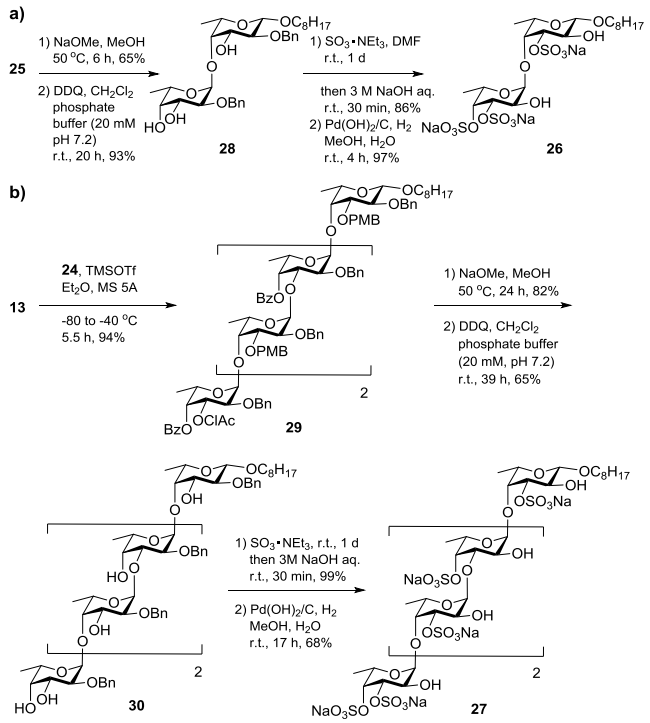



**Fig. 4** Effects of **3**-**6** and **9** on WI-38 cell proliferation. WI-38 cells were seeded into 96-well plates ($2.5\times10^3$ cells $well^{-1}$). After 24 h, compounds were added at the indicated concentrations and the cells were incubated for 96 h at 37 °C in 5% $CO_2$ in air. MTT reagent was then added to each well, and the cells were incubated for up to 3 additional hours. The absorbance of each well at 540 nm was read using a plate reader.




**Scheme 3** Synthetic scheme of 3,4-*O*-sulfated type disaccharide **26** and hexasaccharide **27**.

obtained by the following 4 steps from **29**: 1. deprotection of the ClAc and Bz groups; 2. deprotection of the PMB groups; 3. sulfation; 4. deprotection of the Bn groups. We examined the anti-proliferative activities of **26** and **27** using MCF-7 cells and the MTT assay under the same conditions as described above. As shown in Fig. 5a, the disaccharide **26** exhibited lower anti-proliferative activity than the tetrasaccharide **9** or the hexasaccharide **27**, and **9** showed slightly higher activity than **27**. These results clearly indicate that **9** is the minimum structure required for anti-proliferative activity against MCF-7 cells. When HeLa cells were used in the MTT assay, the disaccharide **26** exhibited a slight dose-dependent effect, whereas the tetrasaccharide **9** showed the highest anti-proliferative activity of the three compounds (Fig. 5b).

Since the tetrasaccharide **9** exhibited selective and effective anti-proliferative activity against human cancer cells, we conducted MTT assays using **9** and fucoidan **2** (600 kDa,

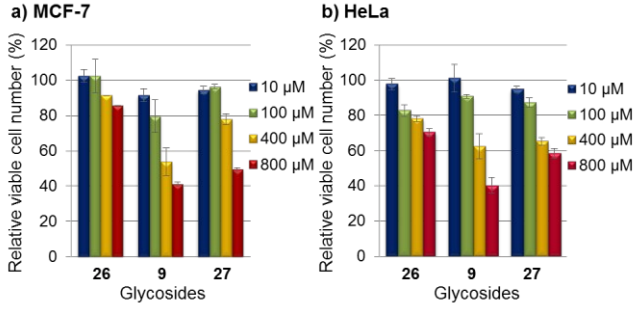



**Fig. 5** Effects of **26**, **9**, and **27** on a) MCF-7 and b) HeLa cell proliferation. Cells were seeded into 96-well plates ($1\times10^3$ cells $well^{-1}$). After 24 h, compounds were added at the indicated concentrations and the cells were incubated for 96 h at 37 °C in 5% $CO_2$ in air. MTT reagent was then added to each well, and the cells were incubated for up to 3 additional hours. The absorbance of each well at 540 nm was read using a plate reader.

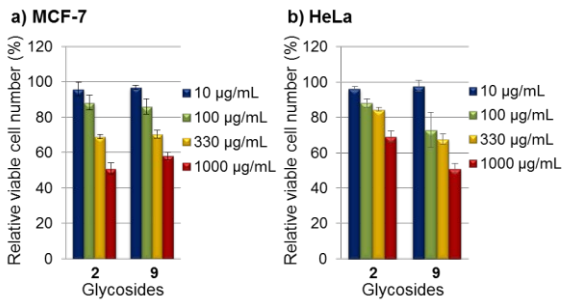



**Fig. 6** Effects of **2** and **9** on a) MCF-7 and b) HeLa cell proliferation. Cells were seeded into 96-well plates ($1\times10^3$ cells well$^{-1}$). After 24 h, compounds were added at the indicated concentrations and the cells were incubated for 96 h at 37 °C in 5% $CO_2$ in air. MTT reagent was then added to each well, and the cells were incubated for up to 3 additional hours. The absorbance of each well at 540 nm was read using a plate reader.

isolated from *F. vesiculosus*, and also having the type II backbone) to compare their anti-proliferative activities against MCF-7 and HeLa cells. The cells were treated with different doses (10-1000 μg mL$^{-1}$) for 96 h (Fig. 6). Interestingly, the anti-proliferative activities of **2** against both types of cancer cells were similar to that of **9**, suggesting that the 3,4-*O*-sulfated oligofucoside structure with a type II backbone chain is a key component for fucoidans exhibiting anti-proliferative activity against human cancer cells.

We next determined whether micelle formation by **9**, which possesses a C8 alkyl chain at the reducing end, is related to its anti-proliferative activity. The critical micelle concentration (cmc) value of **9** was determined using a fluorescent probe, *N*-phenyl-1-naphthylamine (NPN).[15] As shown in Fig. 7, the cmc value of **9** was 1.9-2.1 mM, indicating that micelle formation by **9** is not required to induce its anti-proliferative activity against MCF-7 and HeLa cells.

To determine whether the fucoidan **2** and the derivative **9** could induce apoptotic cell death in MCF-7 and HeLa cells, we evaluated the morphology of the nucleus in individual cells by staining with Hoechest 33342[16] after treatment with **2** or **9**. As shown in Fig. 8, treatment of MCF-7 and HeLa cells with **2** or **9** induced chromatin condensation, visualized as intense blue-white fluorescence within the cell nucleus (Figs 8b, c, g and h). In addition, exposure of cells to **2** or **9** together with Z-VAD-

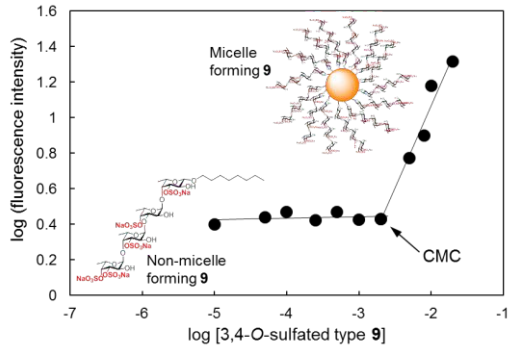



**Fig. 7** Determination of the cmc of **9** with 1 μM NPN in phosphate buffered saline. Fluorescence intensity of NPN is plotted as a function of the concentration of **9**. The intersection of the two straight lines indicates the cmc.

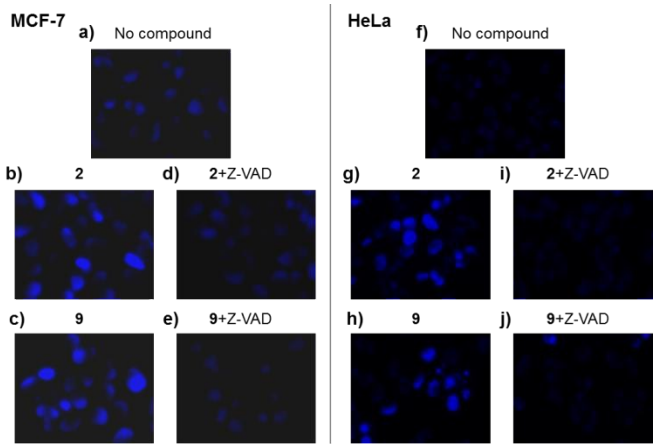



**Fig. 8** The degree of apoptosis represented as fluorescent images of the cell nucleus by fluorescence microscopy. a-e) MCF-7 or f-j) HeLa cells were seeded into 12-well plates ($3\times10^4$ cells well$^{-1}$). After 24 h, the cells were treated with a & f) no compound, b & g) **2** (1000 μg mL$^{-1}$), c & h) **9** (1000 μg mL$^{-1}$), d & i) **2** (1000 μg mL$^{-1}$) and Z-VAD (10 μM), and e & j) **9** (1000 μg mL$^{-1}$) and Z-VAD (10 μM) for 48 h at 37 °C in 5% $CO_2$ in air. The cells were stained with the DNA-specific fluorescent dye, Hoechst 33342.

fmk[8] (Z-VAD), a general caspase inhibitor, significantly decreased the number of apoptotic cells, as shown in Figs 8d, e, I, and j. These results clearly indicate that **2** and **9** induce caspase-dependent apoptosis in both MCF-7 and HeLa cells.

Finally, we evaluated the activation of caspase-8 and 9 to confirm the involvement of caspases in **2**- or **9**-induced apoptosis in MCF-7 and HeLa cells. Caspase-8 and 9 are essential for executing the major extrinsic and intrinsic pathways of apoptosis, respectively.[17] MCF-7 and HeLa cells were treated with **2** or **9** (330 μg mL$^{-1}$) for 1-4 d and the results are summarized in Fig. 9. Exposure of MCF-7 cells to these compounds resulted in no detectable cleavage of caspase-9, whereas the active fragments of caspase-8 (43 and 41 kDa) were clearly observed after treatment with **2** or **9** (Figs 9a and b). Caspase-8 plays a crucial role in apoptosis triggered by the interaction of ligand with integrins[18] or death receptors such as Fas, the tumor necrosis factor (TNF) receptor, and TNF-related apoptosis-inducing ligand receptor.[19] Thus, these results may suggest that not only the naturally occurring fucoidan **2**, but also the low-molecular weight derivative **9**, interact with target receptor proteins on the MCF-7 cell membrane. Interestingly, when HeLa cells were exposed to these compounds, no cleavage of caspase-8 was detected, whereas the active fragments of caspase-9 (37 and 35 kDa) were clearly observed after treatment with **2** or **9** (Figs 9c and d). Caspase-9 activation is initiated by the release of cytochrome c from mitochondria, caused by stress signaling (JNK/SAPK).[20] Hence, these results may suggest that both **2** and **9** play an important role in activation of the stress signaling pathways. In addition, these results provide the first demonstration that a homogeneous and structurally-defined sulfated tetrafucoside epitope can be sufficient for the induction of apoptosis in MCF-7 and HeLa cells through activation of caspase-8 and -9, respectively.

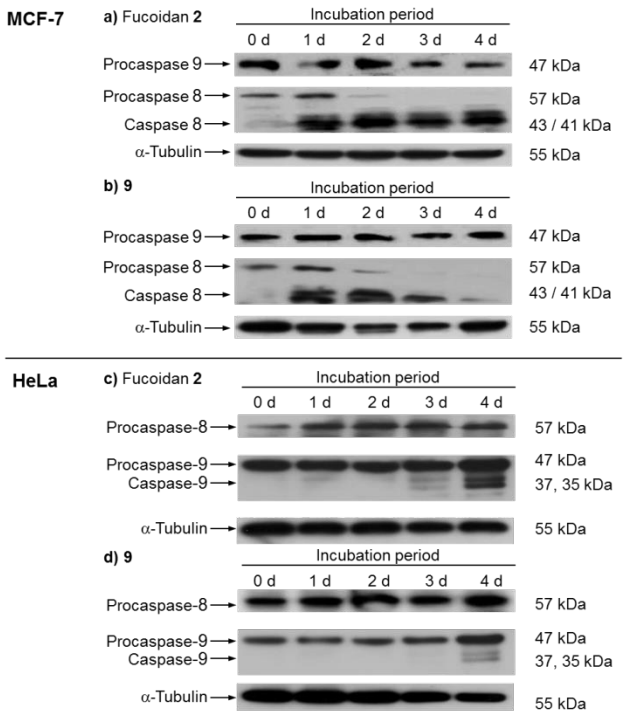



**Fig. 9** Western blot analysis of the effect of fucoidan **2** *(F. vesiculosus)* and **9** on protein levels in a & b) MCF-7 and c & d) HeLa cells. Cells were treated with each compound for 0-4 d at 37 °C and in 5% $CO_2$ in air. Each sample was analyzed using tricine-SDS-PAGE and immunoblotting with appropriate monoclonal antibodies. α-Tubulin levels are shown as the protein loading control.

## Conclusions

In conclusion, our designed type I and II fucoidan derivatives **3**-**11** with different sulfation patterns were systematically synthesized from the common key intermediates **12** (type I) and **13** (type II). Synthesized type I fucoidan derivatives showed anti-proliferative activities against both cancer cells and normal WI-38 cells. In contrast, one type II sulfated tetrafucoside, 3,4-*O*-sulfated type **9**, effectively reduced the number of MCF-7 and HeLa cells in a dose-dependent manner without causing cytotoxicity towards normal WI-38 cells. Furthermore, the tetrafucoside epitope was found to be a key structural requirement for high anti-proliferative activity. Moreover, the fucoidan **2** and **9** induced apoptosis in MCF-7 and HeLa cells through activation of caspase-8 and -9, respectively. We anticipate that the results presented here will contribute to the development of sulfated oligosaccharide-based antitumor agents exhibiting fewer side effects than current therapeutics. The identification of the target protein of **9** is now under investigation in our laboratories.

## Experimental section

Experimental details of the previously reported compounds **7**-**11**, **13** and **23**-**30**,[12] and NMR spectra of all of the compounds were provided in the supporting information.

### General methods for the chemical syntheses

NMR spectra were recorded on a JEOL ECA-500 (500 MHz for $^1$H, 125 MHz for $^{13}$C) spectrometer. $^1$H NMR data are reported as follows; chemical shift in parts per million (ppm) downfield or upfield from tetramethylsilane ($\delta$ 0.00), $CD_3OD$ ($\delta$ 3.31), $C_6D_6$ ($\delta$ 7.16) or $CDCl_3$ ($\delta$ 7.26), integration, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, d = quartet, and m = multiplet) and coupling constants (Hz). $^{13}$C chemical shifts are reported in ppm downfield or upfield from $CDCl_3$ ($\delta$ 77.1), $CD_3OD$ ($\delta$ 49.0), $C_6D_6$ ($\delta$ 128.06) or acetone-$d_6$ ($\delta$ 29.8). ESI-TOF Mass spectra were measured on a Waters LCT premier XE. Melting points were determined on a micro hot-stage (Yanako MP-S3) and were uncorrected. Optical rotations were measured on a JASCO P-2200 polarimeter. Silica gel TLC and column chromatography were performed using Merck TLC 60F-254 (0.25 mm) plates and Silica Gel 60 N (spherical, neutral, 63-210 μm) (Kanto Chemical Co., Inc.), respectively. Gel filtration chromatography separations were performed using Sephadex LH-20 (GE Healthcare). Air- and/or moisture-sensitive reactions were carried out under an argon atmosphere using oven-dried glassware. In general, organic solvents were purified and dried using appropriate procedures, and evaporations and concentrations were carried out under reduced pressure below 30 °C, unless otherwise noted.

### Synthesis of the common key intermediate 12

**2,6-Dimethylphenyl 4-*O*-benzoyl-2-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-1-thio-β-L-fucopyranoside (15)**. To a solution of **14** (8.5 g, 17.1 mmol) in pyridine (57.2 mL) was dropwisely added BzCl (12.0 mL, 51.3 mmol) at 0 °C. After the mixture was stirred for 1 h, the reaction was quenched with saturated $NH_4Cl$ aq. (5 mL). The resultant mixture was extracted with EtOAc (50 mL×3), and then the extracts were washed with brine (200 mL), dried over $Na_2SO_4$ and concentrated in *vacuo*. The residue was subjected to silica gel column chromatography (4/1 *n*-hexane/EtOAc) to give **15** (9.05 g, 15.4 mmol, 90% yield). White solid; $R_f$ 0.40 (2/1 *n*-hexane/EtOAc); m.p. 110-111 °C; $[\alpha]^{28}_D$ −75.9° (*c* 1.0, $CHCl_3$); $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$ 8.17 (2H, d, *J* = 8.0 Hz, Ar-H), 7.61 (1H, m, Ar-H), 7.51 (2H, m, Ar-H), 7.39 (2H, m, Ar-H), 7.35-7.26 (3H, m, Ar-H), 7.22-7.08 (5H, m, Ar-H), 6.77 (2H, m, Ar-H), 5.56 (1H, br-d, *J* = 3.5 Hz, H-4), 4.93 and 4.84 (2H, ABq, *J* = 10.5 Hz, Ar$CH_2$), 4.74 and 4.46 (2H, ABq, *J* = 11.0 Hz, Ar$CH_2$), 4.41 (1H, d, $J_{1,2}$ = 9.5 Hz, H-1), 3.76 (3H, s, OMe), 3.70 (1H, d, $J_{1,2}$ = 9.5 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 3.66 (1H, dd, $J_{2,3}$ = 9.5, $J_{3,4}$ = 3.5 Hz, H-3), 3.58 (1H, br-q, *J* = 6.6 Hz, H-5), 2.59 (6H, s, SPh$Me_2$), 1.17 (3H, d, $J_{5,6}$ = 6.6 Hz, H-6); $^{13}$C-NMR (125 MHz, $CDCl_3$) $\delta$ 159.4, 144.4, 138.4, 132.3, 129.9, 129.5, 128.8, 128.3, 128.2, 128.0, 127.7, 113.9×2, 90.2, 82.8, 78.0, 76.0, 73.7, 71.9, 69.4, 55.2, 22.6×2, 16.5; HRMS (ESI-TOF) *m/z* 599.2464 (599.2467 calcd. for $C_{36}H_{39}O_6S$, $[M+H]^+$).

**4-*O*-Benzoyl-2-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-L-fucopyranose (16).** To a solution of **15** (6.0 g, 10.3 mmol) in MeCN (205 mL) were added $H_2O$ (1.85 mL, 103 mmol), NIS (9.23 g, 41.2 mmol) and $Sc(OTf)_3$ (505 mg, 1.03 mmol) at −40 °C. After the mixture was stirred for 1 h, the reaction was quenched with saturated $Na_2S_2O_3$ aq./saturated $NaHCO_3$ aq.

(1/1, v/v, 100 mL). The resultant mixture was extracted with EtOAc (150 mL×3), and then the extracts were washed with brine (300 mL), dried over $Na_2SO_4$ and concentrated in *vacuo*. The residue was subjected to silica gel column chromatography (2/1 *n*-hexane/EtOAc) to give **16** (4.00 g, 8.34 mmol, 81% yield). White foam; $R_f$ 0.24 (2/1 *n*-hexane/EtOAc); $^1$H-NMR (500 MHz, $CDCl_3$) α isomer: $\delta$ 8.08 (2H, m, Ar-H), 7.63-7.25 (10H, m, Ar-H), 6.80 (2H, m, Ar-H), 5.63 (1H, br-d, $J$ = 2.5 Hz, H-4), 5.31 (1H, d, $J_{1,2}$ = 3.5 Hz, H-1), 4.90-4.48 (4H, m, Ar$CH_2$×2), 4.43 (1H, br-q, $J$ = 6.5 Hz, H-5), 4.02 (1H, dd, $J_{2,3}$ = 7.0, $J_{3,4}$ = 2.5 Hz, H-3), 3.89 (1H, dd, $J_{1,2}$ = 3.5, $J_{2,3}$ = 7.0 Hz, H-2), 3.77 (3H, s, OMe), 2.93 (1H, m, OH), 1.20 (3H, d, $J_{5,6}$ = 6.5 Hz, H-6); β isomer: $\delta$ 8.14 (2H, m, Ar-H), 7.63-7.25 (10H, m, Ar-H), 6.80 (2H, m, Ar-H), 5.57 (1H, br-d, $J$ = 3.0 Hz, H-4), 4.90-4.48 (4H, m, Ar$CH_2$×2), 4.76 (1H, d, $J$ = 7.5 Hz, H-1), 3.81 (1H, br-q, $J$ = 6.5 Hz, H-5), 3.68 (1H, dd, $J_{2,3}$ = 7.5, $J_{3,4}$ = 3.0 Hz, H-3), 3.77 (3H, s, OMe), 3.63 (1H, dd, $J_{1,2}$ = 7.5, $J_{2,3}$ = 7.5 Hz, H-2), 3.10 (1H, m, OH), 1.28 (3H, d, $J_{5,6}$ = 6.5 Hz, H-6); $^{13}$C-NMR (125 MHz, $CDCl_3$) $\delta$ 166.3, 166.2, 159.1×2, 138.5, 138.0, 130.1, 130.0, 129.9, 129.8, 129.7, 129.6, 129.5, 128.3×2, 128.2, 128.0, 127.8, 127.6, 113.6×2, 97.2, 92.1, 79.8, 79.1, 75.7, 75.3, 75.1, 73.6, 71.4, 71.2, 71.1, 70.2, 69.5, 65.1, 55.1×2, 16.5, 16.3; HRMS (ESI-TOF) *m/z* 479.2060 (479.2070 calcd. for $C_{28}H_{31}O_7$, $[M+H]^+$).

**4-*O*-Benzoyl-2-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-L-fucopyranosyl trichloroacetimidate (17).** To a solution of **16** (2.28 g, 4.76 mmol) in $CH_2Cl_2$ (79 mL) were added $CCl_3CN$ (4.78 mL, 14.3 mmol) and DBU (0.21 mL, 1.43 mmol) at room temperature. After the mixture was stirred for 14 h, the reaction was concentrated in *vacuo*. The residue was subjected to silica gel column chromatography (2/1 *n*-hexane/EtOAc, 1% $NEt_3$) to give **17** (2.92 g, 4.57 mmol, 96% yield (α/β = 4/1)). White foam; $R_f$ 0.58(α), 0.46(β) (2/1 *n*-hexane/EtOAc, 1% $NEt_3$); $^1$H-NMR (500 MHz, $CDCl_3$) α isomer: $\delta$ 8.58 (1H, s, OC(N*H*)$CCl_3$), 8.07 (2H, m, Ar-H), 7.61 (1H, m, Ar-H), 7.46 (2H, m, Ar-H), 7.30-7.21 (7H, m, Ar-H), 6.76 (2H, m, Ar-H), 6.54 (1H, d, $J_{1,2}$ = 3.5 Hz, H-1), 5.66 (1H, br-d, $J$ = 3.5 Hz, H-4), 4.79-4.56 (4H, ABq, $J$ = 11.5 Hz, Ar$CH_2$×2), 4.34 (1H, br-q, $J$ = 6.5 Hz, H-5), 4.13 (1H, dd, $J_{1,2}$ = 3.5, $J_{2,3}$ = 7.5 Hz, H-2), 4.08 (1H, dd, $J_{2,3}$ = 7.5, $J_{3,4}$ = 3.5 Hz, H-3), 3.77 (3H, s, OMe), 1.21 (3H, d, $J_{5,6}$ = 6.5 Hz, H-6); β isomer: $\delta$ 8.66 (1H, s, OC(N*H*)$CCl_3$), 8.15 (2H, m, Ar-H), 7.60 (1H, m, Ar-H), 7.49 (2H, m, Ar-H), 7.29-7.22 (7H, m, Ar-H), 6.78 (2H, m, Ar-H), 5.81 (1H, d, $J_{1,2}$ = 8.5 Hz, H-1), 5.60 (1H, br-d, $J$ = 3.0 Hz, H-4), 4.88 and 4.79 (2H, ABq, $J$ = 10.5 Hz, Ar$CH_2$), 4.76 and 4.52 (2H, ABq, $J$ = 11.5 Hz, Ar$CH_2$), 3.97-3.89 (2H, m, H-2, 5), 3.78 (1H, br-d, $J$ = 3.0 Hz, H-3), 3.77 (3H, s, OMe), 1.30 (3H, d, $J_{5,6}$ = 6.5 Hz, H-6); $^{13}$C-NMR (125 MHz, $CDCl_3$) $\delta$ 166.1, 161.2, 159.1, 138.3, 133.2, 130.0, 129.9, 129.8, 129.7, 129.6, 128.4, 128.2, 127.4, 113.6×2, 95.2, 91.4, 75.0, 74.6, 73.1, 71.4, 71.0, 68.0, 55.2×2, 16.3; HRMS (ESI-TOF) *m/z* 622.1148 (622.1166 calcd. for $C_{30}H_{31}NO_7Cl_3$, $[M+H]^+$).

**2,6-Dimethylphenyl 4-*O*-benzoyl-2-*O*-benzyl-1-thio-β-L-fucopyranoside (S1).** To a solution of **15** (18.0 mg, 30.9 μmol) in $CH_2Cl_2$/20 mM phosphate buffer (pH 7.2) (1/1, v/v, 1.8 mL) were added DDQ (21.0 mg, 92.7 μmol) at 0 °C. The mixture was stirred for 16 h, the reaction was quenched with saturated $NaHCO_3$ aq. (500 μL). The resultant mixture was extracted with $CHCl_3$ (5 mL×3), and then the extracts were washed with brine (15 mL), dried over $Na_2SO_4$ and concentrated in *vacuo*. The residue was subjected to silica gel column chromatography (12/1 PhMe/acetone) to give **S1** (13.3 mg, 27.8 μmol, 90% yield). White foam; $R_f$ 0.40 (2/1 *n*-hexane/EtOAc); $[\alpha]^{28}_D$ −7.9° (*c* 1.0, $CHCl_3$); $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$ 8.12 (2H, d, $J$ = 6.5 Hz, Ar-H), 7.62 (1H, t, $J$ = 6.5 Hz, Ar-H), 7.50 (2H, t, $J$ = 7.0 Hz, Ar-H), 7.44 (2H, t, $J$ = 6.0 Hz, Ar-H), 7.35 (2H, m, Ar-H), 7.30 (1H, m, Ar-H), 7.18-7.13 (3H, m, Ar-H), 5.39 (1H, br-d, $J$ = 3.5 Hz, H-4), 5.15 and 4.81 (2H, ABq, $J$ = 11.0 Hz, Ar$CH_2$), 4.38 (1H, d, $J_{1,2}$ = 9.5 Hz, H-1), 3.85 (1H, m, H-3), 3.68 (1H, dd, $J_{1,2}$ = 9.5 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 3.61 (1H, br-q, $J$ = 6.0 Hz, H-5), 2.62 (6H, s, SPh$Me_2$), 1.15 (3H, d, $J_{5,6}$ = 6.0 Hz, H-6); $^{13}$C-NMR (125 MHz, $CDCl_3$) $\delta$ 166.8, 144.3, 138.0, 133.2, 132.0, 130.0, 129.6, 129.0, 128.5, 128.4, 128.3, 128.1, 128.0, 89.9, 79.1, 75.7, 74.1, 73.4, 73.0, 22.6×2, 16.5; HRMS (ESI-TOF) *m/z* 479.1883 (479.1892 calcd. for $C_{28}H_{31}O_5S$, $[M+H]^+$).

**2,6-Dimethylphenyl 4'-*O*-benzoyl-2'-*O*-benzyl-3'-*O*-(*p*-methoxybenzyl)-α-L-fucopyranosyl-(1'→3)-4-*O*-benzoyl-2-*O*-benzyl-1-thio-β-L-fucopyranoside (18).** To a solution of **17** (2.3 g, 3.69 mmol) and **S1** (884 mg, 1.85 mmol) in $CH_2Cl_2$ (80.5 mL) was added MS 5A (100 wt% to **17**) at room temperature. After being stirred at the same temperature for 1 h, the reaction mixture was cooled to −78 °C, and then TMSOTf (33.4 μL, 0.37 mmol) was dropwisely added to the reaction mixture. After the mixture was stirred for 0.5 h, the reaction was quenched with saturated $NaHCO_3$ aq. (4 mL). The resultant mixture was filtered through Celite. The resultant mixture was extracted with $CHCl_3$ (120 mL×3), and then the extracts were washed with brine (240 mL), dried over $Na_2SO_4$ and concentrated in *vacuo*. The residue was subjected to silica gel column chromatography (29/1 PhMe/$Et_2O$) to give **18** (1.20 g, 1.28 mmol, 69% yield). White foam; $R_f$ 0.63 (19/1 PhMe/$Et_2O$); $[\alpha]^{27}_D$ −144.5° (*c* 1.0, $CHCl_3$); $^1$H-NMR (500 MHz, $C_6D_6$) $\delta$ 8.28-8.19 (4H, m, Ar-H×2), 7.40-6.96 (21H, m, Ar-H), 6.70 (2H, d, $J$ = 8.5 Hz, Ar-H), 5.59 (1H, br-d, $J$ = 3.0 Hz, H-4), 5.57 (1H, d, $J_{1',2'}$ = 3.0 Hz, H-1'), 5.36 (1H, br-d, $J$ = 3.5 Hz, H-4'), 5.30 and 4.68 (2H, ABq, $J$ = 11.0 Hz, Ar$CH_2$), 4.70 and 4.53 (2H, ABq, $J$ = 11.0 Hz, Ar$CH_2$), 4.70 and 4.34 (2H, ABq, $J$ = 11.0 Hz, Ar$CH_2$), 4.44 (1H, d, $J_{1,2}$ = 10.0 Hz, H-1), 4.40 (1H, br-q, $J$ = 6.5 Hz, H-5'), 4.18 (1H, dd, $J_{1',2'}$ = 3.0 Hz, $J_{2',3'}$ = 7.5 Hz, H-2'), 4.12 (1H, dd, $J_{2',3'}$ = 7.5 Hz, $J_{3',4'}$ = 3.0 Hz, H-3'), 4.06 (1H, dd, $J_{1,2}$ = 10.0 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 3.97 (1H, dd, $J_{2,3}$ = 10.0, $J_{3,4}$ = 3.5 Hz, H-3), 3.13 (3H, s, OMe), 2.78 (1H, br-q, $J$ = 6.5 Hz, H-5), 2.73 (6H, s, SPh$Me_2$), 1.08 (3H, d, $J_{5',6'}$ = 6.5 Hz, H-6'), 0.99 (3H, d, $J_{5,6}$ = 6.5 Hz, H-6); $^{13}$C-NMR (125 MHz, $C_6D_6$) $\delta$ 166.6, 166.3, 159.7, 144.7, 139.2. 139.0, 133.1, 132.9×2, 131.0, 130.9, 130.5, 130.3, 130.2, 129.8, 129.4, 128.7×2, 128.5, 128.3, 127.7, 127.4, 114.1×2, 94.4, 91.1, 78.5, 76.5, 76.3, 76.1, 74.9, 73.5, 73.1×2, 73.0, 71.9×2, 71.8, 69.3, 65.5, 54.7, 23.0×2, 16.8, 16.3; HRMS (ESI-TOF) *m/z* 939.3756 (939.3778 calcd. for $C_{56}H_{59}O_{11}S$, $[M+H]^+$).

**4'-*O*-Benzoyl-2'-*O*-benzyl-3'-*O*-(*p*-methoxybenzyl)-β-L-fucopyranosyl-(1'→3)-4-*O*-benzoyl-2-*O*-benzyl-L-fucopyranoside (S2).** To a solution of **18** (7.50 g, 7.99 mmol) in MeCN (161 mL) were added $H_2O$ (1.44 mL, 79.9 mol), NIS (7.19 g, 32.0 mmol) and $Sc(OTf)_3$ (393 mg, 0.80 mmol) at −40 °C. After the mixture was stirred for 1 h, the reaction was quenched with saturated $Na_2S_2O_3$ aq./saturated $NaHCO_3$ aq.

(1/1, v/v, 100 mL). The resultant mixture was extracted with EtOAc (200 mL×3), and then the extracts were washed with brine (600 mL), dried over $Na_2SO_4$ and concentrated in *vacuo*. The residue was subjected to silica gel column chromatography (2/1 n-hexane/EtOAc) to give **S2** (5.88 g, 7.19 mmol, 90% yield ($\alpha/\beta$ = 1/1)). White foam; $R_f$ 0.39 (1/1 *n*-hexane/EtOAc); $^1$H-NMR (500 MHz, $C_6D_6$) $\delta$ 8.24-8.19 (4H, m, Ar-H), 7.33-6.80 (18H, m, Ar-H), 6.70 (2H, m, Ar-H), 5.72 (1/2H, d, *J* = 3.0 Hz), 5.66 (1H, br-d, *J* = 2.5 Hz), 5.61 (1H, d, *J* = 3.7 Hz), 5.58-5.55 (3/2H, m), 5.26 (1/2H, m), 5.10 (1/2H, ABq, *J* = 10.5 Hz), 4.83-4.70 (2H, m), 4.65-4.42 (9/2H, m), 4.29-4.22 (2H, m), 4.20-4.18 (1H, m), 4.10-4.01 (1/2H, m), 3.95-3.88 (1H, m), 3.20 (3H, s, OMe), 1.19-1.11 (6H, m, H-6, 6'); $^{13}$C-NMR (125 MHz, $C_6D_6$) $\delta$ 166.6×2, 166.5×3, 159.7, 159.6, 139.3, 139.2×2, 138.6, 133.1, 133.0×2, 131.0, 130.9, 130.7, 130.5, 130.4, 130.2×2, 129.9, 128.7×2, 128.4, 127.4×2, 114.0×2, 98.5, 94.6, 94.4, 91.8, 80.5, 77.7, 76.6, 76.4, 76.0, 75.8, 75.2, 74.9, 74.1, 73.1×2, 73.0, 72.2×2, 71.9×2, 70.9, 70.5, 69.7, 69.3, 54.7, 54.6, 16.7, 16.6, 16.4, 16.3; HRMS (ESI-TOF) *m/z* 819.3393 (819.3381 calcd. for $C_{48}H_{51}O_{12}$, $[M+H]^+$).

**4'-*O*-Benzoyl-2'-*O*-benzyl-3'-*O*-(*p*-methoxybenzyl)-α-L-fucopyranosyl-(1'→3)-4-*O*-benzoyl-2-*O*-benzyl-β-L-fucopyranosyl trichloroacetimidate (19).** To a solution of **S2** (6.70 g, 8.18 mmol) in $CH_2Cl_2$ (235 mL) were added $CCl_3CN$ (8.20 mL, 81.8 mmol) and DBU (0.380 mL, 2.73 mmol) at room temperature. After the mixture was stirred for 16 h, the reaction was concentrated in *vacuo*. The residue was subjected to silica gel column chromatography (2/1 *n*-hexane/EtOAc, 1% $NEt_3$) to give **19** (7.41 g, 7.69 mmol, 94% yield ($\alpha/\beta$ = 4/1)). White foam; $R_f$ 0.57($\alpha$), 0.27($\beta$) (2/1 *n*-hexane/EtOAc 1% $NEt_3$); $^1$H-NMR (500 MHz, $C_6D_6$) $\alpha$ isomer: $\delta$ 8.51 (1H, s, OC(N*H*)$CCl_3$), 8.23-8.15 (4H, m, Ar-H), 7.32-7.05 (15H, m, Ar-H), 6.94 (3H, m, Ar-H), 6.67 (2H, d, *J* = 3.5 Hz, Ar-H), 5.75 (1H, br-d, *J* = 2.0 Hz, H-4'), 5.66 (1H, d, $J_{1,2}$ = 3.5 Hz, H-1), 5.62 (1H, br-d, *J* = 2.0 Hz, H-4), 4.88 and 4.56 (2H, ABq, *J* = 11.2 Hz, Ar$CH_2$), 4.81 and 4.51 (2H, ABq, *J* = 10.9 Hz, Ar$CH_2$), 4.61 (1H, dd, $J_{1',2'}$ = 3.4, $J_{2',3'}$ = 6.5 Hz, H-2'), 4.60 (1H, d, $J_{1',2'}$ = 3.5 Hz, H-1'), 4.58 (1H, dd, $J_{2',3'}$ = 6.5, $J_{3',4'}$ = 3.5 Hz, H-3'), 4.45 (1H, br-q, *J* = 6.0 Hz, H-5), 4.30 (1H, dd, $J_{2,3}$ = 6.4, $J_{3,4}$ = 2.0 Hz, H-3), 4.26 (1H, dd, $J_{1,2}$ = 3.4, $J_{2,3}$ = 6.4 Hz, H-2), 4.25-4.16 (2H, ABq, *J* = 11.0 Hz, Ar$CH_2$), 4.12 (1H, br-q, *J* = 6.0 Hz, H-5'), 3.20-3.18 (3H, s, OMe), 1.08 (6H, m, H-6, 6'). β isomer: $\delta$ 8.69 (1H, s, OC(N*H*)$CCl_3$), 8.23-8.16 (4H, m, Ar-H), 7.32-7.05 (15H, m, Ar-H), 6.95 (3H, m, Ar-H), 6.82-6.80 (2H, m, Ar-H), 6.11 (1H, d, $J_{1,2}$ = 8.0 Hz, H-1), 5.54-5.50 (3H, m, H-4, 1', 4'), 4.85 and 4.51 (2H, ABq, *J* = 11.2 Hz, Ar$CH_2$), 4.71 and 4.45 (2H, ABq, *J* = 10.9 Hz, Ar$CH_2$), 4.55 and 4.39 (2H, ABq, *J* = 10.5 Hz, Ar$CH_2$), 4.53 (1H, br-q, *J* = 6.5 Hz, H-5), 4.29-4.13 (2H, m, H-2', 3'), 4.32 (1H, dd, $J_{1,2}$ = 8.0, $J_{2,3}$ = 8.0 Hz, H-2), 4.10 (1H, dd, $J_{2,3}$ = 8.0, $J_{3,4}$ = 3.5 Hz, H-3), 3.20-3.18 (4H, m, OMe, H-5'), 1.11 (3H, d, $J_{5,6}$ = 6.5 Hz, H-6), 1.08 (3H, m, H-6'); $^{13}$C-NMR (125 MHz, $C_6D_6$) $\delta$ 166.4×2, 161.4, 159.7, 139.3, 138.5, 133.1, 132.9, 131.0, 130.5, 130.2×2, 129.9, 128.7, 128.3, 127.4, 114.1, 114.0, 95.5, 94.1, 92.0, 76.5, 75.4, 74.8, 73.2, 73.1, 72.0, 71.8, 70.3, 69.9, 69.6, 65.5×2, 54.6×2, 16.4, 16.3; HRMS (ESI-TOF) *m/z* 962.2457 (962.2477 calcd. for $C_{50}H_{51}NO_{12}Cl_3$, $[M+H]^+$).

**Octyl 4'-*O*-benzoyl-2'-*O*-benzyl-3'-*O*-(*p*-methoxybenzyl)-α-L-fucopyranosyl-(1'→3)-4-*O*-benzoyl-2-*O*-benzyl-L-fucopyranoside (20).** To a solution of **19** (2.40 g, 2.49 mmol) and octanol (1.95 mL, 12.5 mmol) in $CH_2Cl_2$ (36 mL) was added MS 5A (100 wt% to **19**) at room temperature. After being stirred at the same temperature for 1 h, the reaction mixture was cooled to −40 °C, and then $Yb(OTf)_3$ (1.24 g, 1.99 mmol) was added to the reaction mixture. After the mixture was stirred for 5 h, the reaction was quenched with saturated $NaHCO_3$ aq. (4.0 mL). The resultant mixture was filtered through Celite. The resultant mixture was extracted with $CHCl_3$ (80 mL×3), and then the extracts were washed with brine (240 mL), dried over $Na_2SO_4$ and concentrated in *vacuo*. The residue was subjected to silica gel column chromatography (14/1 PhMe/EtOAc) to give **20** (2.15 g, 2.34 mmol, 94% yield ($\alpha/\beta$ = 1/1.6)). White foam; $R_f$ 0.53($\alpha$), 0.51($\beta$) (12/1 PhMe/EtOAc); $\alpha$ isomer: $[\alpha]^{26}_D$ −155.5° (*c* 1.0, $CHCl_3$); β isomer: $[\alpha]^{29}_D$ −141.2° (*c* 1.0, $CHCl_3$); $^1$H-NMR (500 MHz, $C_6D_6$) $\alpha$ isomer: $\delta$ 8.23 (4H, m, Ar-H), 7.39-7.03 (18H, m, Ar-H), 6.60 (2H, m, Ar-H), 5.80 (1H, br-d, *J* = 2.5 Hz, H-4), 5.68-5.65 (2H, m, H-1', H-4'), 5.20 (1H, d, $J_{1,2}$ = 4.0 Hz, H-1), 4.78 and 4.46 (2H, ABq, *J* = 11.0 Hz, Ar$CH_2$), 4.72 and 4.58 (2H, ABq, *J* = 12.0 Hz, Ar$CH_2$), 4.68-4.55 (4H, m, H-3, H-5', Ar$CH_2$), 4.31 (1H, dd, $J_{2',3'}$ = 6.5, $J_{3',4'}$ = 3.5 Hz, H-3'), 4.22 (1H, dd, $J_{1,2}$ = 3.5, $J_{2,3}$ = 6.5 Hz, H-2), 4.18 (1H, dd, $J_{1',2'}$ = 3.5, $J_{2',3'}$ = 6.5 Hz, H-2'), 3.92 (1H, br-q, *J* = 6.5 Hz, H-5), 3.65 (1H, m, -OC$H_2$$CH_2$-), 3.34 (1H, m, -OC$H_2$$CH_2$-), 3.19 (3H, s, OMe), 1.58 (2H, m, -$CH_2$C$H_2$$CH_2$-), 1.40-1.13 (16H, m, H-6, 6', -$CH_2$C$H_2$$CH_2$-), 0.91 (3H, t, *J* = 7.5 Hz, -$CH_2$C$H_3$); $^{13}$C-NMR (125 MHz, $C_6D_6$) $\delta$ 166.6, 166.4, 159.6, 139.4, 139.2, 132.8×2, 130.1×2, 130.3, 130.2, 129.9, 128.8, 128.7, 128.4, 128.3, 127.3, 114.0×2, 97.9, 94.4, 76.6, 75.8, 75.3, 73.1, 72.7, 72.2, 71.8, 71.1, 70.7, 68.6, 65.6, 65.4, 54.6, 32.3, 30.1, 29.8×2, 26.7, 23.1, 16.6, 16.4, 14.4; β isomer: $\delta$ 8.23 (4H, m, Ar-H), 7.37-7.03 (18H, m, Ar-H), 6.93 (2H, m, Ar-H), 5.64 (1H, br-d, *J* = 2.5 Hz, H-4), 5.60 (1H, d, $J_{1',2'}$ = 3.0 Hz, H-1'), 5.56 (1H, br-d, *J* = 3.0 Hz, H-4'), 5.26 and 4.72 (2H, ABq, *J* = 11.0 Hz, Ar$CH_2$), 4.84 and 4.49 (2H, ABq, *J* = 10.5 Hz, Ar$CH_2$), 4.76 and 4.60 (2H, ABq, Ar$CH_2$), 4.61 (1H, m, H-5'), 4.42 (1H, d, $J_{1,2}$ = 7.0 Hz, H-1), 4.26 (1H, dd, $J_{2',3'}$ = 7.0, $J_{3',4'}$ = 3.0 Hz, H-3'), 4.20 (1H, dd, $J_{1',2'}$ = 3.0, $J_{2',3'}$ = 7.0 Hz, H-2'), 4.11-4.02 (3H, m, H-2, 3, -OC$H_2$$CH_2$-), 3.50 (1H, m, -OC$H_2$$CH_2$-), 3.20 (3H, s, OMe), 3.11 (1H, br-q, *J* = 7.0 Hz, H-5), 1.72-1.56 (2H, m, -OC$H_2$C$H_2$-), 1.45-1.20 (10H, m, -$CH_2$C$H_2$$CH_2$-), 1.16-1.10 (6H, m, H-6, 6'), 0.88 (3H, t, *J* = 6.9 Hz, -$CH_2$C$H_3$); $^{13}$C-NMR (125 MHz, $C_6D_6$) $\delta$ 166.7, 166.4, 159.7, 139.1, 132.9×2, 131.1, 131.0×2, 130.5, 130.3, 130.2, 129.9, 128.7, 128.6×2, 128.5, 128.4, 114.0×2, 104.4, 94.7, 79.3, 76.6, 75.9, 74.9, 74.2, 73.1, 72.2, 71.9, 69.9, 69.5, 69.4, 65.4, 54.6, 32.2, 30.3, 29.8, 29.7, 26.7, 23.1, 16.7, 16.4, 14.4; HRMS (ESI-TOF) *m/z* 931.4672 (931.4633 calcd. for $C_{56}H_{67}O_{12}$, $[M+H]^+$).

**Octyl 4'-*O*-benzoyl-2'-*O*-benzyl-α-L-fucopyranosyl-(1'→3)-2-*O*-benzyl-4-*O*-benzoyl-β-L-fucopyranoside (21).** To a solution of **20β** (2.31 g, 2.48 mmol) in $CH_2Cl_2$/20 mM phosphate buffer (pH 7.2) (1/1, v/v, 231 mL) were added DDQ (1.69 g, 7.44 mmol) at 0 °C. After the mixture was stirred for 16 h, the reaction was quenched with saturated $NaHCO_3$ aq. (10 mL). The resultant mixture was extracted with $CHCl_3$ (150 mL×3), and then the extracts were washed with brine (450 mL), dried

over $Na_2SO_4$ and concentrated in *vacuo*. The residue was subjected to silica gel column chromatography (12/1 PhMe/acetone) to give **21** (1.93 g, 2.38 mmol, 96%). White foam; $R_f$ 0.29 (12/1 PhMe/acetone); $[\alpha]^{27}_D$ −197.6° (*c* 1.0, $CHCl_3$); $^1$H-NMR (500 MHz, $C_6D_6$) $\delta$ 8.20-8.13 (4H, m, Ar-H), 7.37-7.32 (2H, m, Ar-H), 7.21-6.80 (14H, m, Ar-H), 5.65 (1H, br-d, *J* = 3.0 Hz, H-4), 5.60 (1H, br-d, *J* = 3.0 Hz, H-4'), 5.43 (1H, d, $J_{1',2'}$ = 1.5 Hz, H-1'), 5.20 and 4.56 (2H, ABq, *J* = 11.0 Hz, Ar$CH_2$), 4.76 and 4.59 (2H, ABq, *J* = 10.5 Hz, Ar$CH_2$), 4.58 (1H, br-q, *J* = 6.5 Hz, H-5'), 4.41 (1H, d, $J_{1,2}$ = 7.5 Hz, H-1), 4.38 (1H, br-d, *J* = 1.5 Hz, H-2'), 4.07-4.02 (4H, m, H-2, 3, 3', -OC$H_2$CH$_2$-), 3.51 (1H, m, -OC$H_2$CH$_2$-), 3.11 (1H, br-q, *J* = 6.5 Hz, H-5), 1.72-1.58 (2H, m, -OCH$_2$C$H_2$-), 1.45-1.20 (10H, m, -CH$_2$C$H_2$CH$_2$-), 1.15 (3H, d, $J_{5,6}$ = 6.5 Hz, H-6), 1.11 (3H, d, $J_{5',6'}$ = 6.5 Hz, H-6'), 0.90 (3H, t, *J* = 6.9 Hz, -CH$_2$C$H_3$); $^{13}$C-NMR (125 MHz, $C_6D_6$) $\delta$ 166.7, 166.6, 139.1, 138.8, 133.1, 132.9, 130.9, 130.3, 130.2, 128.7, 128.6×2, 128.5, 128.4×2, 128.2, 127.6, 104.5, 93.5, 79.0, 75.8, 74.6, 74.0, 72.4, 69.9, 69.4, 69.3, 68.6, 65.5, 32.2, 30.2, 29.8, 29.7, 26.6, 23.1, 16.7, 16.3, 14.4; HRMS (ESI-TOF) *m/z* 811.4040 (811.4057 calcd. for $C_{48}H_{59}O_{11}$, $[M+H]^+$).

**Octyl 4'''-*O*-benzoyl-3'''-*O*-(*p*-methoxybenzyl)-2'''-*O*-benzyl-α-L-fucopyranosyl-(1'''→3'')-4''-*O*-benzoyl-2''-*O*-benzyl-α-L-fucopyranosyl-(1''→3')-4'-*O*-benzoyl-2'-*O*-benzyl-α-L-fucopyranosyl-(1'→3)-4-*O*-benzoyl-2-*O*-benzyl-β-L-fucopyranoside (12).** To a solution of **19** (2.0 g, 2.07 mmol) and **21** (842 mg, 1.04 mmol) in $Et_2O$ (60 mL) was added MS 5A (100 wt% to **19**) at room temperature. After being stirred at the same temperature for 1 h, the reaction mixture was cooled to −80 °C, and then TMSOTf (40.0 μL, 207 μmol) was added to the reaction mixture. After the mixture was stirred for 20 min, the mixture was warmed to −40 °C. After the reaction was stirred for 6 h, the reaction was quenched with $Et_3N$ (1 mL). The resultant mixture was filtered through Celite. The resultant mixture was extracted with EtOAc (40.0 mL×3), and then the extracts were washed with brine (120 mL), dried over $Na_2SO_4$ and concentrated in *vacuo*. The residue was subjected to silica gel column chromatography (20/1 PhMe/EtOAc) to give **12** (1.37 g, 1.70 mmol, 82% yield). White foam; $R_f$ 0.72 (14/1 PhMe/EtOAc); $[\alpha]^{26}_D$ −218.6°(*c* 1.0, $CHCl_3$); $^1$H-NMR (500 MHz, $CDCl_3$) δ 8.12-7.89 (8H, m, Ar-H), 7.59-6.94 (34H, m, Ar-H), 6.64 (2H, m, Ar-H), 5.60 (1H, br-d, *J* = 3.0 Hz, H-4), 5.25 (1H, br-d, *J* = 1.7 Hz, H-4' or 4'' or 4'''), 5.22 (1H, br-d, *J* = 1.7 Hz, H-1' or 1'' or 1'''), 5.18 (1H, d, *J* = 3.5 Hz, H-1' or 1'' or 1'''), 5.16 (2H, d, *J* = 3.5 Hz, H-1' or 1'' or 1'''), 5.08 and 4.65 (2H, ABq, *J* = 10.5 Hz, Ar$CH_2$), 5.03-5.00 (2H, m, H-4' or 4'' or 4'''×2), 4.59 and 4.32 (2H, ABq, *J* = 11.5 Hz, Ar$CH_2$), 4.53 and 4.26 (2H, ABq, *J* = 11.5 Hz, Ar$CH_2$), 4.50 (1H, d, $J_{1,2}$ = 7.5 Hz, H-1), 4.45 and 4.30 (2H, ABq, *J* = 13.5 Hz, Ar$CH_2$), 4.41 and 4.19 (2H, ABq, *J* = 11.0 Hz, Ar$CH_2$), 4.34-4.31 (2H, m, H-2' or 2'' or 2''', H-3' or 3'' or 3'''), 4.23 (1H, br-q, *J* = 6.5 Hz, H-5), 4.14 (1H, br-d, *J* = 3.0 Hz, H-3' or 3'' or 3'''), 4.10 (1H, br-q, *J* = 6.5 Hz, H-5' or 5'' or 5'''), 4.05-4.00 (2H, m, H-5' or 5'' or 5''', -OC$H_2$CH$_2$-), 3.96-3.89 (3H, m, H-3, H-2' or 2'' or 2''' ×2, H-3' or 3'' or 3'''), 3.81-3.77 (2H, m, H-2, H-5' or 5'' or 5'''), 3.66 (3H, s, OMe), 3.61-3.57 (1H, m, -OC$H_2$CH$_2$-), 1.70-1.69 (2H, m, -OCH$_2$C$H_2$-), 1.44-1.20 (13H, m, H-6' or 6'' or 6''', -CH$_2$C$H_2$CH$_2$-), 0.90-0.80 (12H, m, H-6', 6'', 6''', -CH$_2$C$H_3$), 0.69 (3H, d, $J_{5,6}$ = 6.5 Hz, H-6); $^{13}$C-NMR (125 MHz, $CDCl_3$) $\delta$ 166.5, 166.3, 166.2, 158.7, 138.5, 138.3, 138.2, 137.7, 132.8×2, 130.3, 130.0×2, 129.9×2, 129.8, 129.7, 129.2, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.5, 127.4, 127.2, 126.8, 113.3×2, 103.9, 93.6, 92.8, 92.6, 78.3, 75.7, 75.5, 73.9, 73.8, 72.8, 72.6, 72.3, 72.1, 71.3, 70.8, 70.4, 70.3, 69.9, 69.7, 69.4, 68.9, 65.0, 64.7, 64.4, 55.0, 31.8, 29.7, 29.4, 29.2, 26.1, 22.6, 16.5, 16.0, 15.9, 15.8, 14.1; HRMS (ESI-TOF) *m/z* 1611.7188 (1611.7254 calcd. for $C_{96}H_{107}O_{22}$, $[M+H]^+$).

**Synthesis of the type I fucoidan derivatives 3-6.**

**Octyl 4'''-*O*-benzoyl-α-L-fucopyranosyl-(1'''→3'')-4''-*O*-benzoyl-α-L-fucopyranosyl-(1''→3')-4'-*O*-benzoyl-β-L-fucopyranosyl-(1'→3)-4-*O*-benzoyl-L-fucopyranoside (S3).** To a solution of **12** (102.5 mg, 63.6 μmol) in MeOH/EtOAc (1/1, v/v, 10.3 mL) was added $Pd(OH)_2$/C (102.5 mg, 100 wt%) under H2 atmosphere at room temperature. After being stirred for 3 h, the reaction mixture was filtered through Celite, and then the filtrate was concentrated in *vacuo*. The residue was subjected to silica gel column chromatography (8/1 $CHCl_3$/MeOH) to give **S3** (66.7 mg, 62.3 μmol, 98% yield). White solid; $R_f$ 0.45 (8/1 $CHCl_3$/MeOH); m.p. 140-141 °C; $[\alpha]^{26}_D$ −155.8° (c 1.0, $CHCl_3$); $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$ 8.18-8.05 (8H, m, Ar-H), 7.60-7.40 (16H, m, Ar-H), 5.52 (1H, br-d, *J* = 3.0 Hz, H-4), 5.38 (1H, br-d, *J* = 2.0 Hz, H-4' or 4'' or 4'''), 5.37 (1H, br-d, *J* = 2.0 Hz, H-4' or 4'' or 4'''), 5.34 (1H, m, H-4' or 4'' or 4'''), 5.20 (1H, d, *J* = 4.0 Hz, H-1' or 1'' or 1'''), 4.96 (1H, br-d, *J* = 2.0 Hz, H-1' or 1'' or 1'''), 4.95 (1H, br-d, *J* = 3.5 Hz, H-1' or 1'' or 1'''), 4.49 (1H, br-q, *J* = 3.5 Hz, H-5' or 5'' or 5'''), 4.40 (1H, br-q, *J* = 3.5 Hz, H-5' or 5'' or 5'''), 4.38 (1H, d, $J_{1,2}$ = 10.0 Hz, H-1), 4.33 (1H, br-q, *J* = 3.5 Hz, H-5' or 5'' or 5'''), 4.15 (1H, dd, *J* = 3.2, 10.3 Hz, H-3' or 3'' or 3'''), 4.06 (1H, dd, *J* = 3.5, 10.6 Hz, H-3' or 3'' or 3'''), 3.90-3.73 (9H, m, H-2, 3, 5, 2', 2'', 2''', H-3' or 3'' or 3'''×2 and -OC$H_2$CH$_2$-), 3.60 (1H, m, -OC$H_2$CH$_2$-), 3.01 (1H, m, -OH), 3.00 (1H, d, *J* = 10.5 Hz, OH), 2.75 (1H, d, *J* = 10.5 Hz, OH), 2.69 (1H, m, OH), 1.72-1.69 (2H, m, -OCH$_2$C$H_2$-), 1.40-1.24 (13H, m, H-6, -CH$_2$C$H_2$CH$_2$-), 1.18 (3H, d, *J* = 6.6 Hz, H-6' or 6'' or 6'''), 1.11 (3H, d, *J* = 6.6 Hz, H-6' or 6'' or 6'''), 0.89 (6H, m, H-6' or 6'' or 6''', -CH$_2$C$H_3$); $^{13}$C-NMR (125 MHz, $CDCl_3$) $\delta$ 167.6, 167.5, 167.3, 166.5, 133.7, 133.6, 133.1, 130.2, 129.9, 129.8, 129.4, 129.2×2, 129.1, 128.6, 128.3, 103.0, 100.3, 99.5, 98.5, 81.7, 73.5, 72.9, 72.4, 71.7, 70.5, 69.7, 69.6, 69.3, 67.8, 66.2, 65.8, 65.7, 31.8, 29.5, 29.4, 29.2, 25.9, 22.6, 16.3, 16.1, 15.8, 14.1; HRMS (ESI-TOF) *m/z* 1131.4757 (1131.4801 calcd. for $C_{60}H_{75}O_{21}$, $[M+H]^+$).

**Octyl α-L-fucopyranosyl-(1'''→3'')-α-L-fucopyranosyl-(1''→3')-α-L-fucopyranosyl-(1'→3)-β-L-fucopyranoside (6).** To a solution of **S3** (36.6 mg, 32.4 μmol) in MeOH (1.80 mL) was added 28% NaOMe in MeOH (0.121 mL, 648 μmol) and then the resultant mixture was stirred at 50 °C for 4 h. After cooling to room temperature, the reaction was quenched with Amberlite IR 120 ($H^+$) form. The resultant suspension was filtered, the filtrate was concentrated in *vacuo*. The residue was subjected to silica gel chromatography ($CHCl_3$/MeOH 3/1) to give **6** (22.4 mg, 32.1 μmol, 99% yield). White solid; $R_f$ 0.20 (3/1 $CHCl_3$/MeOH); m.p. 153-154 °C; $[\alpha]^{25}_D$ +5.0° (*c* 1.0, $H_2O$); $^1$H-NMR (500 MHz, $D_2O$) $\delta$ 4.90-4.87 (3H, m, H-1', 1'', 1'''),

4.32-4.26 (3H, m, H-5', 5'', 5''' ), 4.15 (1H, d, $J_{1,2}$ = 7.0 Hz, H-1), 3.90-3.68 (11H, m, H-4, 2', 2'', 2''', 3', 3'', 3''', 4', 4'', 4''', -OC$H_2$CH$_2$-), 3.60-3.40 (4H, m, H-2, 3, 5, -OC$H_2$CH$_2$-), 1.57-1.51 (2H, m, -OCH$_2$C$H_2$-), 1.13-1.11 (22H, m, H-6, 6', 6'', 6''', -CH$_2$C$H_2$CH$_2$-), 0.82 (3H, t, $J$ = 7.0 Hz, -CH$_2$C$H_3$); $^{13}$C-NMR (125 MHz, D$_2$O) $\delta$ 102.2, 95.2, 95.1, 95.0, 77.3, 74.5, 74.3, 71.5, 70.3, 70.1, 69.0, 68.4, 68.1, 67.6, 67.2, 66.5, 66.2, 66.1, 66.0×2, 30.7, 28.4, 28.1×2, 24.7, 21.7, 15.1, 14.9, 14.8, 13.0; HRMS (ESI-TOF) *m/z* 715.3787 (715.3752 calcd. for $C_{32}H_{59}O_{17}$, $[M+H]^+$).

**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 (3).** To a solution of **6** (30.4 mg, 42.5 μmol) was in DMF (1.52 mL) was added $SO_3$•$NEt_3$ (1.04 g, 5.74 mmol) at room temperature. After the reaction mixture was stirred for 1 d, 3M NaOH aq. (516 μL, 11.5 mmol) was added to the reaction mixture and the mixture was stirred for 0.5 h. And then, the resultant mixture was subjected to reverse phase silica gel column chromatography (100/0 to 0/100 $H_2O$/MeOH) to give **3** (31.7 mg, 20.4 μmol, 48% yield). White solid; $R_f$ 0.25 (10/10/3 $CHCl_3$/MeOH/$H_2O$); m.p. >300 °C ; $[\alpha]^{25}_D$ −73.9° (*c* 0.5, $H_2O$); $^1$H-NMR (500 MHz, D$_2$O) $\delta$ 5.40 (1H, d, $J$ = 3.5 Hz, H-1' or 1'' or 1'''), 5.36-5.35 (2H, m, H-1' or 1'' or 1'''×2), 5.24 (1H, br-d, $J$ = 3.7 Hz, H-4 or 4' or 4'' or 4'''), 4.91-4.70 (6H, m, H-1, 3, H-4 or 4' or 4'' or 4'''×3, H-5' or 5'' or 5'''), 4.60 (1H, m, H-3' or 3'' or 3'''), 4.54-4.47 (4H, m, H-2', 2'', 2''', H-3' or 3'' or 3'''), 4.38-4.33 (2H, m, H-3' or 3'' or 3''', H-5' or 5'' or 5'''), 4.29-4.21 (3H, m, H-2, H-3' or 3'' or 3''', H-5' or 5'' or 5'''), 4.11 (1H, m, H-5), 3.71-3.69 (1H, m, -OC$H_2$CH$_2$-), 3.55 (1H, m, -OC$H_2$CH$_2$-), 1.59-1.57 (2H, m, -OCH$_2$C$H_2$-), 1.43 (3H, d, $J$ = 6.0 Hz, H-6' or 6'' or 6'''), 1.29-1.15 (19H, m, H-6, H-6' or 6'' or 6'''×2, -CH$_2$C$H_2$CH$_2$-), 0.83 (3H, m, -CH$_2$C$H_3$); $^{13}$C-NMR (125 MHz, D$_2$O) $\delta$ 104.9, 97.2, 97.1, 96.1, 84.6, 84.2, 83.1, 79.4, 79.2, 79.0, 75.3, 73.5×2, 73.1, 72.7, 71.9, 71.8, 67.6, 67.1, 66.7, 66.3, 30.7, 28.1, 28.0, 27.9, 24.8, 21.6, 16.5, 15.6, 15.4×2, 13.1; HRMS (ESI-TOF) *m/z* 1608.8350 (1608.8322 calcd. for $C_{32}H_{49}O_{44}Na_8S_9$, $[M-Na]^-$).

**Octyl 2''',3'''-di-*O*-sulfo-α-L-fucopyranosyl-(1'''→3'')-2''-*O*-sulfo-α-L-fucopyranosyl-(1''→3')-2'-*O*-sulfo-α-L-fucopyranosyl-(1'→3)-2-*O*-sulfo-β-L-fucopyranoside (5).** To a solution of **S3** (25.3 mg, 22.4 μmol) was in DMF (1.25 mL) was added $SO_3$•$NEt_3$ (304 mg, 1.68 mmol) at room temperature. After the reaction mixture was stirred for 1 d, 3M NaOH aq. (168 μL, 6.72 mmol) was added to the reaction mixture and the mixture was stirred for 0.5 h. And then, the resultant mixture was subjected to reverse phase silica gel column chromatography (100/0 to 30/70 $H_2O$/MeOH) to give **5** (26.9 mg, 21.7 μmol, 97% yield). White solid; $R_f$ 0.75 (10/10/3 $CHCl_3$/MeOH/$H_2O$); m.p. >300 °C; $[\alpha]^{28}_D$ −98.2° (*c* 1.0, $H_2O$); $^1$H-NMR (500 MHz, D$_2$O) $\delta$ 5.37-5.34 (3H, m, H-1', 1'', 1'''), 4.70 (1H, dd, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 3.5 Hz, H-3' or 3'' or 3'''), 4.58-4.49 (6H, m, H-1, 2', 2'', 2''', H-5' or 5'' or 5'''×2), 4.43 (1H, br-q, $J$ = 6.5 Hz, H-5' or 5'' or 5'''), 4.33 (1H, dd, $J_{1,2}$ = 10.0 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 4.21-4.15 (2H, m, H-3' or 3'' or 3''', H-4' or 4'' or 4'''), 4.12-4.09 (3H, m, H-3' or 3'' or 3''', H-4' or 4'' or 4'''×2), 4.02 (1H, br-d, $J$ = 3.5 Hz, H-4), 3.86-3.76 (3H, m, H-3, 5, -OC$H_2$CH$_2$-), 3.68 (1H, m, -OC$H_2$CH$_2$-), 1.65-1.56 (2H, m, -OCH$_2$C$H_2$-), 1.38-1.23 (22H, m, H-6, 6', 6'', 6''', -CH$_2$C$H_2$CH$_2$-), 0.85 (3H, t, $J$ = 7.0 Hz, -CH$_2$C$H_3$). $^{13}$C-NMR (125 MHz, D$_2$O) $\delta$ 101.0, 94.8, 94.5, 92.9, 75.8, 75.5, 74.6, 73.8, 73.2, 72.7, 72.6, 71.9, 70.6, 70.2, 70.1, 69.0, 68.8, 67.1, 66.3, 66.2, 66.0, 30.7, 28.4, 28.1, 28.0, 24.6, 21.6, 15.1, 14.9×2, 13.1; HRMS (ESI-TOF) *m/z* 1201.0745 (1201.0714 calcd. for $C_{32}H_{53}O_{32}Na_4S_5$, $[M-Na]^-$).

**Octyl 4'''-*O*-benzoyl-2'''-*O*-benzyl-α-L-fucopyranosyl-(1'''→3'')-4''-*O*-benzoyl-2''-*O*-benzyl-α-L-fucopyranosyl-(1''→3')-4'-*O*-benzoyl-2'-*O*-benzyl-α-L-fucopyranosyl-(1'→3)-4-*O*-benzoyl-2-*O*-benzyl-β-L-fucopyranoside (S4).** To a solution of **12** (31.2 mg, 19.4 μmol) in $CH_2Cl_2$/20 mM phosphate buffer (pH 7.2) (1/1, v/v, 3.12 mL) were added DDQ (13.2 mg, 58.2 μmol) at 0 °C. After the mixture was stirred for 14 h at room temperature, the reaction was quenched with saturated $NaHCO_3$ aq. (2 mL). And then the mixture was extracted with $CHCl_3$ (5 mL×3), and then the extracts were washed with brine (20 mL), dried over $Na_2SO_4$ and concentrated in *vacuo*. The residue was subjected to silica gel column chromatography (9/1 PhMe/EtOAc) to give **S4** (28 mg, 18.2 μmol, 97% yield); White foam; $R_f$ 0.43 (7/1 PhMe/EtOAc); $[\alpha]^{29}_D$ −233.2° (c 1.0, $CHCl_3$); $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$ 8.11 (2H, d, $J$ = 7.0 Hz, Ar-H), 7.94-7.86 (6H, m, Ar-H×3), 7.60-6.96 (32H, m, Ar-H), 5.61 (1H, br-d, $J$ = 3.0 Hz, H-4), 5.26 (2H, m, H-1'or 1'' or 1''', H-4' or 4'' or 4'''), 5.19-5.14 (3H, m, H-1'or 1'' or 1'''×2, H-4' or 4'' or 4'''), 5.07 and 4.63 (2H, ABq, $J$ = 11.0 Hz, ArC$H_2$), 4.81 (1H, br-d, $J$ = 3.0 Hz, H-4' or 4'' or 4'''), 4.59 and 4.23 (2H, ABq, $J$ = 10.5 Hz, ArC$H_2$), 4.57 and 4.30 (2H, ABq, $J$ = 11.0 Hz, ArC$H_2$), 4.50-4.09 (5H, m, H-1, H-3' or 3'' or 3'''×2, H-5' or 5'' or 5'''×2), 4.08-4.00 (2H, m, H-5' or 5'' or 5''', -OC$H_2$CH$_2$-), 3.94 (3H, m, H-3, H-2' or 2'' or 2'''×2), 3.88-3.77 (3H, m, H-2, 5, H-3' or 3'' or 3'''), 3.63-3.57 (2H, m, H-2' or 2'' or 2''', -OC$H_2$CH$_2$-), 1.75-1.65 (2H, m, -OCH$_2$C$H_2$-), 1.44-1.20 (13H, m, H-6' or 6'' or 6''', -CH$_2$C$H_2$CH$_2$-), 0.90-0.80 (9H, m, H-6' or 6'' or 6'''×2, -CH$_2$C$H_3$), 0.66 (3H, d, $J_{5,6}$ = 6.0 Hz, H-6); $^{13}$C-NMR (125 MHz, $CDCl_3$) $\delta$ 166.5, 166.4, 166.3, 166.2, 138.3, 138.0, 137.8, 137.7, 133.1, 133.0, 133.0, 132.9, 130.0, 129.8, 129.8, 129.8, 129.7, 129.7, 128.5, 128.3, 128.2, 128.2, 128.1, 128.0, 127.9, 127.8, 127.6, 127.5, 127.4, 127.3, 103.9, 93.7, 92.5, 91.5, 78.4, 75.7, 75.3, 74.2, 74.0, 73.4, 72.8, 72.7, 72.6, 71.5, 70.4, 69.7, 69.4, 69.0, 67.6, 65.1, 64.7, 64.5, 31.8, 29.8, 29.7, 29.4, 29.2, 26.2, 22.6, 16.6, 16.0, 15.9, 15.8, 14.1; HRMS (ESI-TOF) *m/z* 1491.6609 (1491.6679 calcd. for $C_{88}H_{99}O_{21}$, $[M+H]^+$).

**Octyl 2'''-*O*-benzyl-α-L-fucopyranosyl-(1'''→3'')-2''-*O*-benzyl-α-L-fucopyranosyl-(1''→3')-2'-*O*-benzyl-α-L-fucopyranosyl-(1'→3)-2-*O*-benzyl-β-L-fucopyranoside (S5).** To a solution of **S4** (95.8 mg, 64.2 μmol) in MeOH/THF (1/1, v/v, 9.58 mL) was added 28% NaOMe in MeOH (743 μL, 86.4 mM) and then the resultant mixture was stirred at 50 °C for 24 h. After cooling to room temperature, the reaction was quenched with Amberlite IR 120 (H+) form. The resultant suspension was filtered, the filtrate was concentrated in *vacuo*. The residue was subjected to silica gel chromatography (4/1 PhMe/acetone) to give **S5** (58.7 mg, 54.6 μmol, 85%). White solid; $R_f$ 0.70 (10/1 $CHCl_3$/MeOH); m.p. 240-241 °C; $[\alpha]^{29}_D$ −119.6° (c 1.0, $CHCl_3$); $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$ 7.39-7.21 (16H, m, Ar-H), 6.85-6.80 (4H, m, Ar-H), 4.93 and 4.58 (2H, ABq, $J$ = 11.0 Hz, ArC$H_2$), 4.84 (1H, d, $J$ = 4.0 Hz, H-1' or 1'' or 1'''), 4.79 (1H, br-d, $J$ = 3.5 Hz, H-4 or 4' or 4'' or 4'''), 4.74 (1H, br-d,

J = 3.5 Hz, H-4 or 4' or 4'' or 4'''), 4.71-4.55 (6H, ABq, ArC$H_2$×3), 4.32 (1H, d, $J_{1,2}$ = 8.0 Hz, H-1), 4.11 (1H, br-q, $J$ = 7.0 Hz, H-5 or 5' or 5'' or 5'''), 3.97-3.91 (4H, m, H-3 or 3' or 3'' or 3''', H-4 or 4' or 4'' or 4''', H-5 or 5' or 5'' or 5''', H-2' or 2'' or 2'''), 3.85-3.80 (3H, m, H-3 or 3' or 3'' or 3''', H-5 or 5' or 5'' or 5''', H-2' or 2'' or 2'''), 3.77-3.72 (2H, m, H-3 or 3' or 3'' or 3'''×2), 3.67-3.56 (4H, m, H-2, H-1' or 1'' or 1'''×2, H-4 or 4' or 4'' or 4''', -OC$H_2$CH$_2$-), 3.57-3.44 (3H, m, H-3 or 3' or 3'' or 3''', H-5 or 5' or 5'' or 5''', -OC$H_2$CH$_2$-), 1.68-1.60 (2H, m, -OCH$_2$C$H_2$-), 1.40-1.20 (13H, m, H-6 or 6' or 6'' or 6''', -CH$_2$C$H_2$CH$_2$-), 1.16 (6H, m, H-6 or 6' or 6'' or 6'''×2), 1.00 (3H, d, $J$ = 7.0 Hz, H-6 or 6' or 6'' or 6'''), 0.89 (3H, t, $J$ = 6.9 Hz, -CH$_2$C$H_3$); $^{13}$C-NMR (125 MHz, $CDCl_3$) $\delta$ 138.8, 137.7, 137.4, 128.8, 128.6, 128.5, 128.4, 128.4, 128.3, 128.2, 128.1, 127.9, 127.5, 103.7, 93.9, 93.7, 93.6, 77.6, 76.3, 75.0, 74.9, 74.8, 74.6, 74.5, 74.3, 73.8,71.9, 70.1, 69.8, 69.6, 68.1, 67.8, 67.5, 66.1, 65.6, 65.2, 31.8, 30.9, 29.7, 29.6, 29.3, 29.2, 26.1, 22.6, 16.4, 16.1, 15.9, 15.8, 14.0; HRMS (ESI-TOF) *m/z* 1075.5670 (1075.5630 calcd. for $C_{60}H_{83}O_{17}$, $[M+H]^+$).

**Octyl 2'''-*O*-benzyl-3''',4'''-di-*O*-sulfo-α-L-fucopyranosyl-(1'''→3'')-2''-*O*-benzyl-4''-*O*-sulfo-α-L-fucopyranosyl-(1''→3')-2'-*O*-benzyl-4'-*O*-sulfo-α-L-fucopyranosyl-(1'→3)-2-*O*-benzyl-4-*O*-sulfo-β-L-fucopyranoside (S6).** To a solution of **S5** (28.6 mg, 26.6 μmol) was in DMF (1.43 mL) was added $SO_3$•$NEt_3$ (362 mg, 2.00 mmol) at room temperature. After the reaction mixture was stirred for 1 d, 3M NaOH aq. (133 μL, 4.00 mmol) was added to the reaction mixture and the mixture was stirred for 0.5 h. And then, the resultant mixture was subjected to reverse phase silica gel column chromatography (100/0 to 30/70 $H_2O$/MeOH) to give **S6** (42.0 mg, 26.4 μmol, 99% yield). White solid; $R_f$ 0.70 (10/10/3 $CHCl_3$/MeOH/$H_2O$); m.p. 200-201 °C; $[\alpha]^{26}_D$ −123.9° (c 1.0, $H_2O$); $^1$H-NMR (500 MHz, $D_2O$) $\delta$ 7.58-7.55 (4H, m, Ar-H), 7.49-7.30 (16H, m, Ar-H), 5.48 (1H, br-dd, $J$ = 3.5 Hz, H-1' or 1'' or 1'''), 5.41 (1H, d, $J$ = 3.5 Hz, H-1' or 1'' or 1'''), 5.20 (1H, d, $J$ = 4.0 Hz, H-1' or 1'' or 1'''), 4.97 and 4.56 (2H, ABq, $J$ = 10.5 Hz, ArC$H_2$), 4.93 and 4.61 (2H, ABq, $J$ = 10.5 Hz, ArC$H_2$), 4.86-4.73 (4H, m, H-4, H-3' or 3'' or 3''', ArC$H_2$), 4.70-4.54 (5H, m, H-4', 4'', 4''', ArC$H_2$), 4.48 (1H, d, $J_{1,2}$ = 7.5 Hz, H-1), 4.28-4.20 (4H, m, H-3' or 3'' or 3''', H-5 or 5' or 5'' or 5''' ×2), 4.14-4.07 (2H, m, H-5 or 5' or 5'' or 5''', H-3' or 3'' or 3'''), 3.98-3.90 (3H, m, H-2' or 2'' or 2'''×2, -OC$H_2$CH$_2$-), 3.85 (1H, dd, $J_{2,3}$ = 7.5, $J_{3,4}$ = 3.5 Hz, H-3), 3.79-3.71 (2H, m, H-2' or 2'' or 2''', H-5' or 5'' or 5'''), 3.68-3.61 (2H, m, H-2, -OC$H_2$CH$_2$-), 1.63-1.57 (2H, m, -OCH$_2$C$H_2$-), 1.40-1.13 (22H, m, H-6 or 6' or 6'' or 6'''×3 and -CH$_2$C$H_2$CH$_2$-), 0.88 (3H, m, H-6 or 6' or 6'' or 6'''), 0.80 (3H, t, $J$ = 6.5 Hz, -CH$_2$C$H_3$); $^{13}$C-NMR (125 MHz, $D_2O$) $\delta$ 137.6, 137.4, 128.6, 128.3, 128.2, 128.1×2, 128.0, 127.6, 127.5, 102.6, 93.6, 93.4, 93.2, 78.9, 77.4, 76.3, 74.8, 74.5, 74.4, 74.1, 73.0, 72.3, 72.2, 71.1, 70.9, 70.1, 69.6, 66.5, 65.8, 65.6, 30.9, 28.6, 28.3×2, 25.1, 21.8, 15.9×2, 15.6, 15.3, 13.3; HRMS (ESI-TOF) *m/z* 1561.2611 (1561.2592 calcd. for $C_{60}H_{77}O_{32}Na_4S_5$, $[M-Na]^-$).

**Octyl 3''',4'''-di-*O*-sulfo-α-L-fucopyranosyl-(1'''→3'')-4''-*O*-sulfo-α-L-fucopyranosyl-(1''→3')-4'-*O*-sulfo-α-L-fucopyranosyl-(1'→3)-4-*O*-sulfo-β-L-fucopyranoside (4).** To a solution of **S6** (10 mg, 35.7 μmol) in MeOH/$H_2O$ (1/1, v/v, 1% AcOH, 8.00 mL) was added $Pd(OH)_2$/C (84.8 mg, 300 wt%) under $H_2$ atmosphere at room temperature. After being stirred for 16 h, the reaction mixture was filtered through Celite, and then the filtrate was concentrated in *vacuo*. The residue was subjected to reverse phase silica gel column chromatography (100/0 to 30/70 $H_2O$/MeOH) to give **4** (5.6 mg, 30.0 μmol, 84% yield). White solid; $R_f$ 0.54 (10/10/3 $CHCl_3$/MeOH/$H_2O$); m.p. >300 °C; $[\alpha]^{26}_D$ −98.8° (*c* 1.0, $H_2O$); $^1$H-NMR (500 MHz, $D_2O$) $\delta$ 5.15 (1H, d, $J$ = 4.0 Hz, H-1' or 1'' or 1'''), 5.11 (1H, d, $J$ = 3.8 Hz, H-1' or 1'' or 1'''), 5.10 (1H, d, $J$ = 4.3 Hz, H-1' or 1'' or 1'''), 4.88 (1H, br-d, $J$ = 2.9 Hz, H-4' or 4'' or 4'''), 4.82-4.76 (2H, m, H-4' or 4'' or 4'''×2), 4.68 (1H, br-d, $J$ = 2.9 Hz, H-4), 4.61 (1H, dd, $J$ = 2.5, $J$ = 8.5 Hz, H-3' or 3'' or 3'''), 4.54-4.46 (4H, m, H-1, H-5' or 5'' or 5'''×3), 4.08-4.01 (2H, m, H-3' or 3'' or 3'''×2), 3.91-3.85 (6H, m, H-5, H-2' or 2'' or 2'''×3, H-3' or 3'' or 3''', -OC$H_2$CH$_2$-), 3.77 (1H, dd, $J_{2,3}$ = 8.0, $J_{3,4}$ = 2.9 Hz, H-3), 3.68-3.57 (2H, m, H-2, -OC$H_2$CH$_2$-),1.63-1.57 (2H, m, -OCH$_2$C$H_2$-), 1.35-1.20 (22H, m, H-6, 6', 6'', 6''', -CH$_2$C$H_2$CH$_2$-), 0.85 (3H, t, $J$ = 6.9 Hz, -CH$_2$C$H_3$); $^{13}$C-NMR (125 MHz, $D_2O$) $\delta$ 102.3, 98.3, 98.1, 97.2, 79.2, 79.0, 78.9, 78.8, 78.1, 75.9, 75.2, 75.1, 70.5, 69.4, 68.7, 68.1, 66.8, 66.7, 66.2×2, 66.1, 30.7, 28.3, 28.0×2, 24.6, 22.8, 21.6, 19.6, 15.5, 15.2, 15.1, 13.0; HRMS (ESI-TOF) *m/z* 1201.0735 (1201.0714 calcd. for $C_{32}H_{53}O_{32}Na_4S_5$, $[M-Na]^-$).

**Materials and methods for biological assay**

Fucoidan isolated from *F. vesiculosus* (ca 600 kDa) was purchased from Elicityl (Crolles, France). Antibodies to caspase-8 (1C12) and -9 were purchased from Cell Signaling Technology, Inc. (Beverly, MA). An antibody to α-tublin (DM1A) and horseradish peroxidase (HRP)-conjugated donkey anti-rabbit IgG (sc-2313) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). HRP-conjugated anti-mouse IgG (NA931A) was purchased from GE healthcare. The caspase inhibitor, Z-VAD-fmk was purchased from Promega Coporation, Madison, WI, USA. Hoechst 33342 was purchased from Dojindo Laboratories. Fetal bovine serum (FBS) was purchased from MP Biomedicals. Calf serum (CS) was purchased from Biowest (Miami, USA). The human breast cancer MCF-7, the human cervical epithelioid carcinoma HeLa and the human normal diploid fibroblast WI-38 were provided by the RIKEN BRC through the National Bio-Resource Project of the MEXT, Japan.

**Cell culture**

Human breast cancer MCF-7 and human normal diploid fibroblast WI-38 were grown at 37 °C in 5% $CO_2$ in air in DMEM medium supplemented with phenol red, L-glutamine (2 mM), penicillin (100 units mL$^{-1}$), kanamycin (100 μg mL$^{-1}$) and 10% FBS. Human cervical epithelioid carcinoma HeLa was grown at 37 °C in 5% $CO_2$ in air in DMEM medium supplemented with phenol red, L-glutamine (2 mM), penicillin (100 units mL$^{-1}$), kanamycin (100 μg mL$^{-1}$) and 5% CS.

**MTT assay**

MCF-7 cells or WI-38 cells were seeded at 1.0×10$^3$ cells well$^{-1}$ or 2.5×10$^3$ cells well$^{-1}$ in 96-well in 10% FBS DMEM, respectively. After 24 h, samples were incubated with the indicated concentration of compounds. Cells were then kept for 96 h at 37 °C and in 5% $CO_2$ in air, and then MTT reagent

was added to each well and cells were incubated for up to 3 additional hours at 37 °C. The absorbance at a single wavelength of 540 nm was read on a plate reader SAFIRE (TECAN). HeLa cells were seeded at $1.0\times10^3$ cells $well^{-1}$ in 96-well in 5% CS DMEM. After 24 h, samples were incubated with the indicated concentration of compounds. Cells were then kept for 96 h at 37 °C and in 5% $CO_2$ in air, and then MTT reagent was added to each well and cells were incubated for up to 3 additional hours at 37 °C. The absorbance at a single wavelength of 540 nm was read on a plate reader SAFIRE (TECAN).

**Morphology of nucleus**

MCF-7 and HeLa cells were cultured on $\phi$18 mm micro cover glass (Matsunami Glass Industrial, Ltd.) in 12-well plate ($3.0\times10^4$ cells $well^{-1}$) in the presence of each compond (1000 μg $mL^{-1}$) or vehicle with or without caspase inhibitor, Z-VAD-fmk (10 μM), for 48 h. And then, the cells were washed three times with PBS and fixed with 4% paraformaldehyde phosphate buffer solution for 15 min at room temperature. After removing paraformaldehyde, cells were washed three times with PBS and blocked with 2% BSA phosphate buffer solution for 30 min at room temperature. After removing BSA, cells were stained with Hoechst 33342 for 10 min. The nucleus morphological changes were observed by inverted fluorescence microscope (EVOS FL Cell Imaging System; Life Technologies).

**Immunoblotting**

MCF-7 and HeLa cells ($4.0\times10^5$ cells) were plated on 100 mm dishes. After 24 h, samples were incubated with each compound (330 μg $mL^{-1}$). The cells were then kept for 1-4 d at 37 °C and in 5% $CO_2$ in air. After the incubation time, medium was collected and then, adherent cells were scraped with rubber policeman and centrifuged for 5 min at 3000 rpm at 4 °C. The pellet was then resuspended in 100 μL lysis buffer (25 mM Tris-HCl (pH 7.6), 150 mM NaCl, 0.1% SDS, 1% triton X-100, 1 mM EDTA (pH 8.0), 1% sodium deoxycholate) containing protease inhibitor cocktail (NAKARAI TESQUE Inc.) and homogenized with ULTRA SONIC HOMOGENIZER UH-50 (SMT Co., Ltd.). The lysate was centrifuged for 10 min at 14000 rpm at 4 °C. Equal amounts of protein were separated by SDS-PAGE in 10% polyacrylamide gels. Proteins were transferred onto nitrocellulose membranes Hybond™–ECL (GE Healthcare). Membranes were blocked with Tris-buffered saline-0.1% Tween 20 (TBST) containing 5% BSA or 5% non-fat dry milk for 30 min at room temperature and membranes were incubated with appropriately diluted primary antibodies at 4 °C overnight. After washing five times with TBST, the blots were incubated with horseradish peroxidase-conjugated specific secondary antibody for 2 h at 4 °C and then again washed five times. Then the complexes were visualized in Medical Film Processer FPM100 (Fujifilm Co.) using the enhanced chemiluminescence reagents Immobilon™ Western (Millipore Co.). The following primary antibodies were used for detection of the specific bands: caspase-8, caspase-9, and $\alpha$-tubulin. The following secondary antibodies were used for detection of the specific bands: HRP-conjugated anti-mouse IgG and HRP-conjugated anti-rabbit IgG.

**Formation of 9 assembly**

The cmc value of **9** was determined with a fluorescent probe, *N*-phenyl-1-naphthylamine (NPN). **9** was suspended in PBS containing 1 μM NPN with a vortex mixer and diluted to 10 μM to 20 mM. Fluorescence intensity was measured on a fluorescence spectrophotometer FP-6500 (JASCO Co.) using a 5 mm cuvette at 25 °C. In the presence of **9** assemblies, the fluorescence emission has a maximum at 450 nm upon

## Acknowledgements

We wish to thank Prof. Dr. Siro Simizu, Faculty of Science and Technology, Keio University, for helpful discussions regarding biological assays. This research was supported in part by the MEXT-supported Program for the Strategic Research Foundation at Private Universities, 2012-2016, from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT) and by the Mizuho Foundation for the Promotion of Sciences.

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