Synthesis of Sialyl Lewis X-Polysaccharide Conjugates

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Sialyl Lewis X (SLe^X) is well known as a ligand of the cell adhesion molecule E-selectin which is specifically expressed at inflammatory lesion sites. We have synthesized several SLe^X–polysaccharide conjugates and examined their potential for drug delivery to inflammatory lesions. The *AUC* (area under the blood concentration–time curve) 0—24 h of SLe^X–CMCht (1), SLe^X–CMPul (2) and SLe^X–DSH (3) at the inflammatory lesion was about 60-, 300-, and 30-fold higher than that of the monovalent SLe^X (7), respectively. Moreover, 1 showed 2-fold higher accumulation in the inflammatory lesion than SLN–CMCht (4), and 2 showed 2.5-fold higher accumulation than SLN–CMPul (5).

Key words sialyl Lewis X; E-selectin; carboxymethyl chitosan; carboxymethyl pullulan; *N*-desulfated heparin; active-targeting drug delivery system

Carbohydrate recognition has been shown to be involved in various biological processes and should be useful for an active-targeting DDS (Drug Delivery System). However, carbohydrate recognition has not yet been applied to targeting delivery to tissues or lesions, except for a galactose ligand for the asialoglycoprotein receptor expressed in liver.[2,3] Also, there are disadvantages for targeting delivery using galactose derivatives. For example, in the case of hepatic tumors, anti-tumor drugs modified with galactose derivatives could not be delivered selectively to tumor sites because the asialoglycoprotein receptor is expressed throughout the whole liver containing non-tumor sites. Therefore, it is not a tissue-specific but a lesion-specific active-targeting DDS that is required.

Recently, attention has been directed to cell adhesion molecules called selectins which are involved in various aspects of immune cell trafficking. We have focused on the interaction between E-selectin and sialyl Lewis X (SLe^X, Neu5Acα2→3Galβ1→4(Fucα1→3)GlcNAc).[4] E-selectin is expressed on the endothelial cells stimulated by cytokines, such as IL-1β or TNF-α at inflammatory sites, and plays an important role in the transport of neutrophils to inflammatory sites. The tetrasaccharide SLe^X is distributed on the surface of neutrophils, and has been shown to be a ligand recognized by E-selectin. As the interaction between SLe^X and E-selectin is essential for the initial stage of neutrophil infiltration of the inflammatory site, SLe^X and its derivatives, which block this interaction, should be useful as new anti-inflammatory agents.[5]

SLe^X and its derivatives may be effective homing devices for active-targeting DDS to inflammatory lesions because E-selectin is only expressed on such lesions. Thus, DDS using SLe^X derivatives as homing devices may be effective for drug delivery not to normal tissue but directly to inflammatory lesions. However, to the best of our knowledge, there have been no examples using SLe^X as a homing device for DDS. The reason may be that the affinity of carbohydrate for their protein has been shown to be relatively weak and carbohydrates are generally sensitive to glycosidase *in vivo*. Moreover, oligosacharides such as SLe^X should be rapidly filtered at the glomerulus because of its high hydrophilicity and low molecular weight.[6] One way to solve these problems would be to support SLe^X on a macromolecule[7] which would stabilize the sugar moiety and enhance multivalent interaction of SLe^X with E-selectin expressed at the inflammatory lesion. In our research laboratories, we have studied DDS using liposomes,[3] polypeptides,[8] or polysaccharides[9,10] as carriers and have already reported that some polysaccharides of molecular size above 70 kDa, such as carboxymethylchitosan (CMCht)[9] and carboxymethylpullulan (CMPul),[10] are useful as carriers for a passive-targeting DDS to tumors. The doxorubicin–CMPul conjugate dramatically enhances the therapeutic index of the antitumor effects.[10] This type of conjugate is highly biocompatible and is retained in the circulating blood.[11] Moreover, these conjugates have been found to accumulate in tissues with enhanced vascular permeability such as tumor and inflammatory sites, where E-selectin is expressed, and thus, a synergic effect can be expected between SLe^X and polysaccharides.

These findings prompted us to synthesize SLe^X–polysaccharide (CMCht and CMPul) conjugates and evaluate their usefulness as an active-targeting device for delivery to inflammatory lesions.

The sulfated polysaccharide heparin, or *N*-desulfated heparin (DSH), is an important drug used to prevent cardiovascular diseases and it has been reported to have many unique biological activities.[12] Since these polysaccharides are also expected to be useful as drug carriers of DDS, because of their price and ease of acquisition, we synthesized a conjugate of SLe^X with *N*-desulfated heparin (DSH) and evaluated

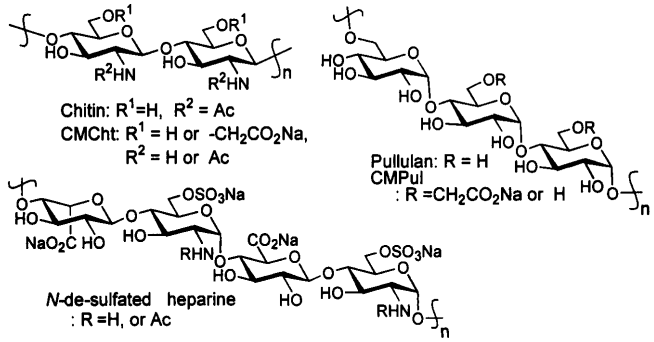

Fig. 1. Structure of Polysaccharides

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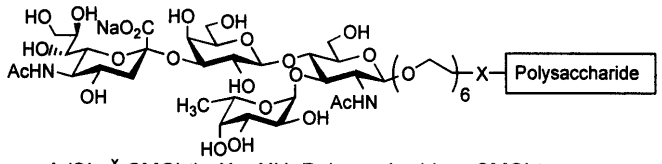
its pharmacokinetics.

We also synthesized SLN(Neu5Acα2→Galβ1→4Glc-NAc)–polysaccharide conjugates as the negative control. SLN is a trisaccharide without the fucose moiety of SLe^X and it has been reported not to support E-selectin-mediated adhesion.[13]

Synthesis of SLe^X–Polysaccharide Conjugates It was planned to prepare a SLe^X–CMCht conjugate (**1**) by *N*-alkylation using the amino group of CMCht and the bromo group of **6**. The synthesis of SLe^X–bromide (**6**)[14] is described in Chart 1. The bromo alcohol (**8**) was prepared in two steps from commercially available hexaethyleneglycol [i) MsCl/Py., ii) LiBr/2-butanone, 40%]. 2-Methyl-4,5-(3,4,6-tri-*O*-acetyl-2-deoxy-a-D-glucopyrano)-2-oxazoline (**9**),[15] which is readily available from *N*-acetyl-D-glucosamine, was reacted with bromo alcohol (**8**) in the presence of TMSOTf to give a β-glycoside (**10**, 80%), which was converted to the glycosyl acceptor (**11**) in two steps [i) NaOMe/MeOH, ii) $PhCH(OMe)_2$/CSA/DMF (65%)]. Compound **11** was glycosylated with thioglycoside (**12**)[16] using $Me_2SSMe\cdot OTf$[17] in CH_2Cl_2 at 0 °C to afford the disaccharide **13**, as a mixture of α- and β-glycosides (95%, $\alpha:\beta=15:1$, separation of the α- and β-glycosides was difficult). By means of the regioselective ring opening reaction of benzylidene acetal using $NaBH_3CN$–HCl,[18] **13** was converted to the 6-*O*-benzyl ether with the 4-hydroxy unsubstituted compound **14** (77%). However, $NaBH_3CN$ is a hazardous chemical and difficult to use safely on a large scale, so we investigated the ring opening reaction using alkylsilane derivatives[19] in place of $NaBH_3CN$ as a reducing reagent and found Et_3SiH–TfOH to be useful (83%). On the other hand, when $PhBCl_2$ was used instead of TfOH, 4,6-*O*-benzylidene acetal was converted to the corresponding 4-*O*-benzyl ether with the 6-hydroxy unsubstituted compound in excellent yield, and regioselectivity was complete. Our improved method has proved to be useful[20] not only for this substrate but other carbohydrates having the 4,6-*O*-benzylidene ring and therefore should be useful for the synthesis of various oligosaccharides.



1 (SLe^X-CMCht) : X = NH, Polysaccharide = CMCht
2 (SLe^X-CMPul) : X = NHCO, Polysaccharide = CMPul
3 (SLe^X-DSH) : X = NH, Polysaccharide = DSH

Fig. 2. Structure of SLe^X–Polysaccharide Conjugates

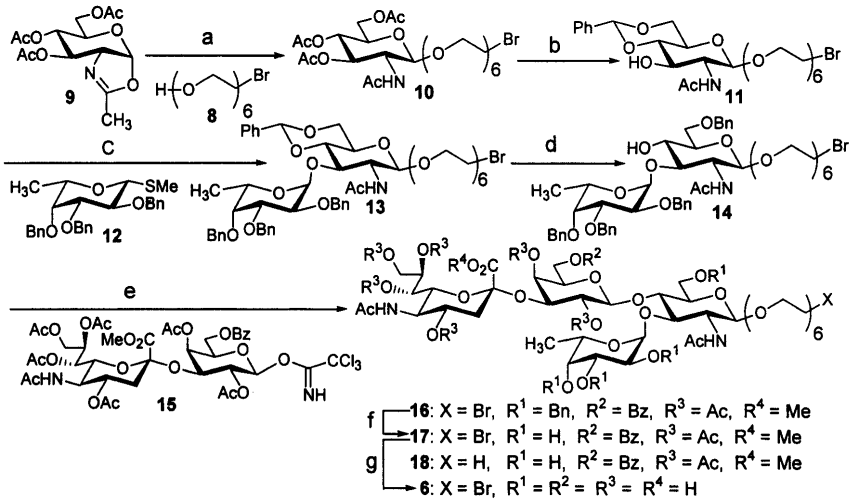
Glycosylation of **14** with sialyl-galactose imidate (**15**)[21] in the presence of $BF_3\cdot OEt_2$ led to the tetrasaccharide **16** (61%), which was hydrogenated with 10% Pd–C in MeOH to afford **17** (73%) and **18** (16%). When this hydrogenation was carried out for a long period (over *ca.* 12 h), **18** was obtained as a major product. Deacylation of **17** followed by saponification of the methyl ester group gave the bromide (**6**, 93%).

CMCht was prepared from chitin according to a known procedure,[9] and had the following characteristics: M.W., about 100 kDa; degrees of substitution (ds) of *N*-acetyl group, 0.47; ds of amino group, 0.45; ds of carboxymethyl group, 0.7.

By means of *N*-alkylation of CMCht with bromide (**6**) in 0.5% $NaHCO_3$–H_2O for 160 h at 60 °C, a SLe^X–CMCht conjugate (**1**) was obtained after dialysis against distilled water (M.W. cut: 12000—13000). The SLe^X content of this conjugate was determined by the resorcinol–HCl method for the quantitative analysis of sialic acid,[22] and the result was 33 wt% (the ds of SLe^X was 0.17 per glucosamine residue of CMCht).

The SLe^X–CMPul conjugate (**2**) was prepared by condensation of CMPul and the SLe^X derivative (**21**) with an amino group. The synthesis is described in Chart 2. Treatment of **14** with NaN_3 afforded **19** (93%), which was coupled with imidate **15** ($BF_3\cdot OEt_2/CH_2Cl_2$) to give **20** (52%). Hydrogenation of **20** with 10% Pd–C in THF–HCl gave amine **21** (87%).

Condensation of amine **21** with CMPul[10] (M.W. about 190 kDa, ds of carboxymethyl group was 0.6 per glucose residue), which was prepared from commercially available pullulan by carboxymethylation (aq. $NaOH/ClCH_2CO_2H$)



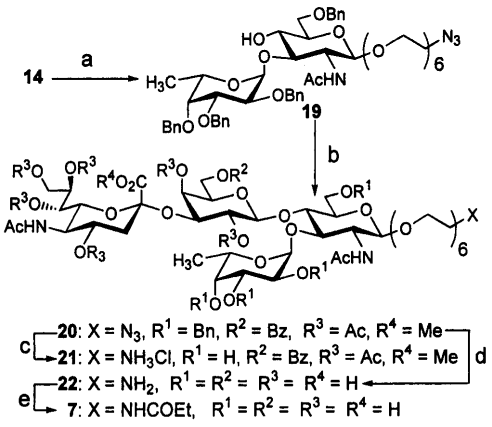
a) TMSOTf / $C_2H_4Cl_2$ / 50°C (80%) b) 1) NaOMe / MeOH / rt, 2) $PhCH(OMe)_2$ / CSA / DMF (65%)
c) Me_2SSMe•OTf / CH_2Cl_2 / 0°C (95%, $\alpha:\beta$ =15:1) d) Et_3SiH / TfOH / CH_2Cl_2 / MS4A / -78°C (83%)
e) BF_3•OEt_2 / CH_2Cl_2 / MS4A / 0°C (61%) f) Pd-C / H_2 / MeOH / rt (**17**:73%, **18**:16%)
g) 1) NaOMe / MeOH / rt, 2) aq.NaOH / rt (90%)

Chart 1

using 1-ethoxy-carbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) in DMF–H_2O at 40 °C, and subsequent deprotection with 1 N aq. NaOH, gave a SLe^X–CMPul conjugate (**2**). The SLe^X content of **2** was determined to be 31 wt% by the same method used for **1**.[22] (The degree of substitution of SLe^X was 0.13 per glucose residue of CMPul.) ^{1}H-NMR spectroscopy showed no benzoyl or *O*-acetyl signals confirming deprotection by 1 N aq. NaOH to be complete.

The SLe^X–DSH conjugate (**3**) was prepared by *N*-alkylation as for the SLe^X–CMCht conjugate (**1**).

Commercially available *N*-de-sulfated heparin (DSH, M.W. about 40 kDa) was treated with $NaBH_4$ to reduce the terminal hemiacetal group, and this polysaccharide was reacted with bromide (**6**) in 0.5% $NaHCO_3$–H_2O at 60 °C, to afford a SLe^X–DSH conjugate (**3**, SLe^X contents of **3**: 24 wt%).



a) NaN_3 / DMF (93%) b) **15** / BF_3•OEt_2 / CH_2Cl_2 / MS4A / 0°C (52%) c) Pd-C / H_2 / THF-HCl / rt (87%)
d) 1) NaOMe / MeOH / rt, 2) aq.NaOH / rt, 3) Pd-C / H_2 / MeOH / *p*TsOH•H_2O (72%) e) $EtCO_2Su$ / MeOH / NMM (79%)

Chart 2

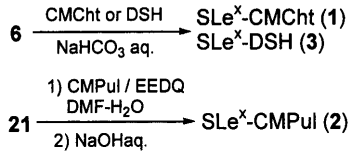
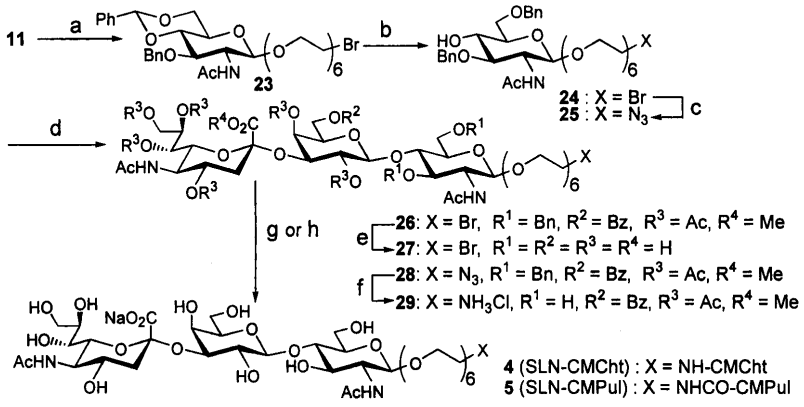


Chart 3

A SLN–CMCht conjugate (**4**) and a SLN–CMPul conjugate (**5**) were synthesized according to a procedure similar to that described for **1** and **2** (**4**, ds=0.17, **5**, ds=0.13, Chart 4).

Accumulations of SLe^X–Polysaccharide Conjugates (1—3) and Monomeric SLe^X (7) in Inflammatory Lesions To examine the pharmacokinetics of **1**—**5** and **7**, radiolabelled versions of compounds **1**—**5** and **7** were prepared. CMCht derivatives (**1**, **4**) and SLe^X–DSH (**3**) were radiolabelled using *N*-succinimidyl [2,3-^{3}H]-propionate, and CMPul derivatives (**2**, **5**) were radiolabeled using [^{3}H]-glycine and EEDQ (radioactivity: 5—15 mCi/mg). Radiolabelled monovalent SLe^X (**7**) was prepared from **22** using *N*-succinimidyl [2,3-^{3}H]-propionate. In order to evaluate the ability to actively target the inflammatory lesion *in vivo*, we used arachidonic acid-induced ear edema in mice, a model of acute-phase inflammatory disease. First, SLe^X–polysaccharide conjugates (**1**—**3**) and monovalent SLe^X (**7**) were compared with respect to their accumulation in inflammatory lesions (dose: 1 mg/mg, i.v.). The plasma concentration of monovalent SLe^X (**7**) decreased rapidly and could not be detected one hour later, and the concentration in the inflamed ear remained low. On the other hand, SLe^X–polysaccharide conjugates (**1**—**3**) were retained in blood circulation as expected, and subsequent maked enhancement of accumulation in the targeted lesion was observed. The *AUC* (area under the blood concentration–time curve) 0—24 h of macromolecules **1**, **2**, and **3** at the inflammatory lesion was about 60-, 300-, and 30-fold higher than that of the monovalent SLe^X (**7**), respectively. The *AUC* 0—24 h of **3** in plasma was lower than that of **1** or **2**. Thus, compound **3** underwent glomerular filtration before arriving at the inflammatory lesion faster than **1** or **2**, and the accumuation of **3** in the inflammatory lesion was considered to be lower. Reduced accumulation of CMCht conjugate **1** compared with **2** was due to the degradation of CMCht by lysozyme *in vivo*. These findings show that supporting SLe^X on polysaccharide is an effective way to enhance accumulation in inflammatory lesions.

Secondly, the accumulation of SLe^X–polysaccharide conjugates (**1**, **2**) in inflammatory lesions was compared with that of SLN–polysaccharide conjugates (**4**, **5**). Compound **1**



a) $BnBr$ / BaO / $Ba(OH)_2$•$8H_2O$ / DMF / rt (85%) b) Et_3SiH / TfOH / CH_2Cl_2 / MS4A / -78°C (87%)
c) NaN_3 / DMF (98%) d) **15** / BF_3•OEt_2 / CH_2Cl_2 / MS4A / 0°C (**26**: 72%, **28**:48%)
e) 1) Pd-C / H_2 / MeOH / rt, 2) NaOMe / MeOH / rt, 3) aq.NaOH / rt (85%) f) Pd-C / H_2 / THF-HCl / rt (73%) g) CMCht / **27** / aq.$NaHCO_3$ / 60°C h) 1) CMPul / **29** / EEDQ / DMF-H_2O / rt 2) aq.NaOH

Chart 4

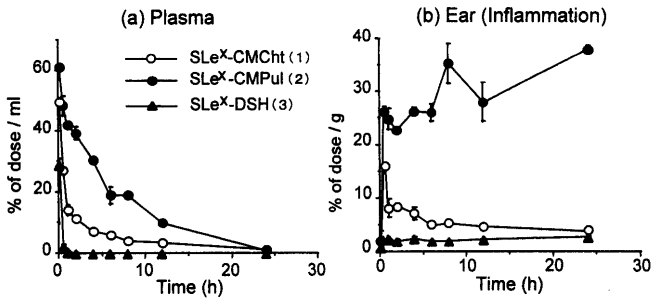


Fig. 3. Concentration–Time Profile of SLe^X–Polysaccharide Conjugates
Data represents mean±S.D. of three mice.

showed a 2-fold higher accumulation in the inflammatory lesion than **3**, and **2** showed a 2.5-fold higher accumulation than **4**. These differences in distribution are thought to be due to differences in the ability to bind to E-selectin.

These results indicate that the interaction between SLe^X and E-selectin could be applied to targeting DDS, and SLe^X–polysaccharide conjugates was useful as drug carriers to inflammatory lesions.

SLe^X–CMPul (**2**) showed a 16-fold higher accumulation in the spleen than SLN–CMPul (**4**). Although the reason is not clear, the accumulation dose not involve E-selectin because it was observed in non-treated normal mice. These are interesting results because the pharmacokinetics of macromolecules such as CMPul change markedly simply following changes in the sugar residues in the macromolecule.

In conclusion, we established a method of synthesizing SLe^X–polysaccharide conjugates, and these conjugates have been shown to be a useful novel system for active-targeting DDS to inflammatory lesions. In the future, we plan to introduce an anti-inflammatory agent to our system and evaluate the subsequent therapeutic effects.

Experimental

All melting points were determined on a Yanagimoto micromelting point apparatus and were not corrected. ^{1}H-NMR spectra were measured on a Varian VXR-500S (500 MHz) spectrometer, unless otherwise specified. Chemical shifts (δ) are given in ppm relative to tetramethylsilane as an internal standard. IR spectra were measured on a Shimadzu FT-IR-4300. Optical rotations were determined with a Perkin-Elmer 430 polarimeter. FAB-Mass spectra were recorded on a Hitachi M-90 instrument.

17-Bromo-3,6,9,12,15-pentaoxa-1-heptadecanol (8) To a stirred solution of hexaethyleneglycol (23.8 g, 84.3 mmol) in CH_2Cl_2 (200 ml) were added Et_3N (14.1 ml, 101 mmol), and methanesulfonylchloride (6.52 ml, 84.3 mmol) at 0 °C. After being stirred at 0 °C for 1 h, the mixture was diluted with CH_2Cl_2, washed with 2% aqueous citric acid and saturated aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*.

The crude mesylate was dissolved in 2-butanone (300 ml), and then LiBr (36.6 g, 422 mmol) was added . After stirring for 1 h under reflux, the mixture was diluted with CH_2Cl_2, washed with saturated brine, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography [SiO_2 500 g, CH_2Cl_2 : MeOH=20 : 1] to give **8** (11.8 g, 40%) as a colorless oil. IR ($CHCl_3$) cm^{-1}: 3500. ^{1}H-NMR ($CDCl_3$) δ: 3.48 (2H, t, J=6.3 Hz), 3.60—3.63 (2H, m), 3.64—3.70 (8H, m), 3.70—3.75 (2H, m), 3.82 (2H, t, J=6.3 Hz).

17-Bromo-3,6,9,12,15-pentaoxaheptadecyl 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (10) To a stirrred mixture of **9**[15] (1.85 g, 5.62 mmol), **8** (1.56 g, 4.52 mmol) and powdered molecular sieve 4 Å (MS4A, 1.2 g) in 1,2-dichloroethane (12 ml), was added trimethylsilyl trifluomethanesulfonate (835 μl, 4.30 mmol) at room temperature, and the stirring was continued for 1.5 h at 50 °C. The mixture was cooled to room temperature, and then triethylamine (1.40 ml, 10.0 mmol) was added. After stirring for 10 min at room temperature, the resultant mixture was filtered, and the filtrate poured into CH_2Cl_2. The organic phase was washed with water, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography [SiO_2 150 g, toluene : acetone : MeOH=500 : 300 : 8] to give **10** (2.45 g, 80%) as a colorless syrup. $[\alpha]_D^{27}$ −14.7° (c=1.18, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 3622, 1747, 1678. ^{1}H-NMR ($CDCl_3$) δ: 1.97, 2.09 (each 3H, s), 2.01 (6H, s), 3.48 (2H, t, J=6.3 Hz), 3.58—3.75 (18H, m), 3.76—3.86 (2H, m), 3.81 (2H, t, J=6.3 Hz), 3.90 (1H, m), 4.10 (1H, m, H-2 of GlcNAc), 4.17 (1H, dd, J=12.2, 2.4 Hz, H-6 of GlcNAc), 4.26 (1H, dd, J=12.2, 4.6 Hz, H-6 of GlcNAc), 4.79 (1H, d, J=8.5 Hz, H-1 of GlcNAc), 5.10—5.11 (2H, m, H-3, 4 of GlcNAc), 6.61 (1H, d, J=9.3 Hz, NH). *Anal.* Calcd for $C_{26}H_{44}NO_{14}Br \cdot 0.5H_2O$: C, 45.69; H, 6.64; N, 2.05. Found: C, 45.63; H, 6.68; N, 2.29.

17-Bromo-3,6,9,12,15-pentaoxaheptadecyl 2-Acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (11) To a stirred solution of **10** (13.0 g, 19.3 mmol) in MeOH (30 ml) at room temperature was added 28% sodium methoxide in MeOH (0.3 ml) and the stirring was continued at room temperature for 30 min. The reaction mixture was neutralized with Dowex 50W×8 (H^+ form) and filtered. The filtrate was concentrated *in vacuo*.

The residue was dissolved in *N*,*N*′-dimethylformamide (50 ml), and then were added benzaldehyde dimethyl acetal (10.9 ml), and 10-camphor sulfonic acid (125 mg). After stirring for 3 h at 55 °C under reduced pressure (45 mmHg), the mixture was neutralized with anion exchange resin (AG-1(OH^-)) and filtered. The filtrate was concentrated *in vacuo*, and the crude product was purified by silica gel column chromatography (SiO_2 150 g, toluene : acetone : MeOH=20 : 30 : 1) to give **11** (7.57 g, 65%) as a colorless syrup. $[\alpha]_D^{28}$ −55.4° (c=1.04, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 3352, 1666. ^{1}H-NMR ($CDCl_3$) δ: 2.07 (3H, s), 3.44 (2H, t, J=6.2 Hz), 3.45 (1H, m, H-5 of GlcNAc), 3.57—3.72 (17H, m), 3.75 (2H, t, J=6.2 Hz), 3.77—3.94 (5H, m), 4.33 (1H, dd, J=10.5, 4.9 Hz, H-6 of GlcNAc), 4.75 (1H, d, J=8.1 Hz, H-1 of GlcNAc), 5.57 (1H, s), 7.15 (1H, br d, J=6.3 Hz, NH), 7.32—7.38 (3H, m), 7.47—7.52 (2H, m). *Anal.* Calcd for $C_{27}H_{42}NO_{11}Br$: C, 50.95; H, 6.65; N, 2.20. Found: C, 51.07; H, 6.70; N, 2.20.

17-Bromo-3,6,9,12,15-pentaoxaheptadecyl *O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (13) To a solution of **11** (636 mg, 1.00 mmol) and **12**[15] (697 mg, 1.50 mmol) in CH_2Cl_2 (20 ml), was added powdered MS4A (10 g), and the mixture was stirred for 2 h at room temperature. Dimethyl(methylthio)sulfonium triflate (DMTST, 1.16 g, 4.50 mmol) was added to the stirred mixture at 0 °C, and stirring was continued for 30 min at 0 °C. MeOH (2 ml), and Et_3N (1 ml) were added, and then the resultant mixture was filtered. The filtrate was diluted with CH_2Cl_2, washed with H_2O, dried over $MgSO_4$, and concentrated *in vacuo*.

The crude product was chromatographed on a silica gel column (SiO_2 70 g, CH_2Cl_2 : MeOH=50 : 1) to give **13** (896 mg, 95%) as an inseparable mixture of anomers (α : β=15 : 1). IR ($CHCl_3$) cm^{-1}: 1677. ^{1}H-NMR ($CDCl_3$) δ: 0.82 (3H, d, J=6.3 Hz, H-6 of Fuc), 1.75 (3H, s), 3.45 (2H, t, J=6.3 Hz), 3.45—3.52 (2H, m), 3.56 (1H, m, H-4 of Fuc), 3.58—3.70 (20H, m), 3.72—3.80 (2H, m), 3.78 (2H, t, J=6.3 Hz), 3.94 (1H, dd, J=10.3, 2.7 Hz, H-3 of Fuc), 4.04 (1H, dd, J=10.3, 3.7 Hz, H-2 of Fuc), 4.11 (1H, q, J=6.3 Hz, H-5 of Fuc), 4.22 (1H, dd, J=9.5, 9.5 Hz, H-3 of GlcNAc), 4.33 (1H, dd, J=10.5, 4.9 Hz, H-6 of GlcNAc), 4.57, 4.71, 4.79, 4.91 (each 1H, d, J=11.7 Hz), 4.70, 4.78 (each 1H, d, J=11.5 Hz), 4.92 (1H, d, J=8.3 Hz, H-1 of GlcNAc), 5.17 (1H, d, J=3.7 Hz, H-1 of Fuc), 5.50 (1H, s), 6.05 (1H, d, J=8.1 Hz, NH), 7.24—7.39 (18H, m), 7.42—7.45 (2H, m). *Anal.* Calcd for $C_{54}H_{74}NO_{15}Br$: C, 61.35; H, 7.05; N, 1.32. Found: C, 61.41; H, 6.79; N, 1.19.

17-Bromo-3,6,9,12,15-pentaoxaheptadecyl *O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (14) Powdered MS4A (1 g) was placed in a 20 ml flask, and dried at 140 °C over 4 h under vacuum (*ca.* 0.1 mmHg). After cooling to room temperature, **13** (210 mg, 0.20 mmol), and CH_2Cl_2 (3.5 ml) were added. After stirring for 1 h at room temperature, the mixture was cooled to −78 °C, and then Et_3SiH (112 μl, 0.70 mmol) and TfOH (46 μl, 0.60 mmol) were added, successively. After stirring for 1 h at −78 °C, Et_3N (1 ml) and MeOH (1 ml) were added, successively, and the mixture was diluted with $CHCl_3$, washed with aqueous $NaHCO_3$, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by a silica gel column (20 g, $CHCl_3$: MeOH=100 : 1) to give **14** (175 mg, 83 %) as a colorless syrup. $[\alpha]_D^{27}$ −50.8° (c=0.51, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 3631, 3450, 1674. ^{1}H-NMR ($CDCl_3$+D_2O) δ: 1.14 (3H, d, J=6.3 Hz, H-6 of Fuc), 1.66 (3H, s), 3.45 (2H, t, J=6.3 Hz), 3.41—3.52 (3H, m), 3.57—3.84 (23H, m), 3.78 (2H, t, J=6.3 Hz), 3.95 (1H, m), 3.97 (1H, m, H-3 of Fuc), 4.06 (1H, dd, J=10.3, 3.7 Hz, H-2 of Fuc), 4.13 (1H, q, J=6.3 Hz, H-5 of Fuc), 4.58, 4.62 (each 1H, d, J=12.2 Hz), 4.61, 4.95 (each 1H, d, J=11.2 Hz), 4.67, 4.75, 4.79, 4.81 (each 1H, d, J=11.7 Hz), 4.84 (1H, d, J=8.3 Hz, H-1 of GlcNAc), 4.97

(1H, d, J=3.7 Hz, H-1 of Fuc), 6.14 (1H, d, J=7.8 Hz, NH), 7.25—7.41 (20H, m). *Anal.* Calcd for $C_{54}H_{72}NO_{15}Br$: C, 61.47; H, 6.87; N, 1.32. Found: C, 61.27; H, 6.75; N, 1.20.

17-Bromo-3,6,9,12,15-pentaoxaheptadecyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (16) To a solution of **14** (1.07 g, 1.01 mmol) and **15**[19] (400 mg, 0.406 mmol) in CH_2Cl_2 (6 ml), was added powdered MS4A (2 g), and the mixture was stirred for 2 h at room temperature. To the stirred mixture, $BF_3 \cdot OEt_2$ (100 ml, 0.812 mmol) was added at 0 °C, and the stirring was continued for 2 h at 0 °C. The reaction mixture was filtered, and the filtrate was diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (SiO_2 120 g, CH_2Cl_2 : MeOH=30 : 1) to give **16** (460 mg, 61%) as a colorless powder. $[\alpha]_D^{28}$ −34.0° (c=0.63, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 1744, 1688. ^{1}H-NMR ($CDCl_3$) δ: 1.10 (3H, d, J=6.3 Hz, H-6 of Fuc), 1.71 (1H, dd, J=12.4, 12.0 Hz, H-3 of Sia), 1.85, 1.89, 1.96, 1.97, 2.01, 2.07, 2.09, 2.22 (each 3H, s), 2.56 (1H, dd, J=12.4, 4.6 Hz, H-3 of Sia), 3.46 (2H, t, J=6.3 Hz), 3.48—3.67 (21H, m), 3.73—3.87 (5H, m), 3.75 (3H, s), 3.79 (2H, t, J=6.3 Hz), 3.87 (1H, dd, J=10.3, 2.4 Hz, H-3 of Fuc), 3.91 (1H, dd, J=10.0, 4.7 Hz), 3.94 (1H, dd, J=7.3, 7.1 Hz, H-5 of Gal), 3.98 (1H, dd, J=12.4, 5.6 Hz, H-9 of Sia), 4.01 (1H, m), 4.04 (1H, m, H-5 of Sia), 4.05—4.10 (2H, m), 4.17 (1H, dd, J=11.0, 7.3 Hz, H-6 of Gal), 4.20 (1H, m, H-5 of Fuc), 4.24 (1H, dd, J=11.0, 7.1 Hz, H-6 of Gal), 4.30 (1H, dd, J=12.4, 2.7 Hz, H-9 of Sia), 4.44, 4.57, 4.75, 4.77 (each 1H, d, J=12.2 Hz), 4.63, 4.94 (each 1H, d, J=11.7 Hz), 4.67 (1H, dd, J=10.0, 3.7 Hz, H-3 of Gal), 4.70, 4.81 (each 1H, d, J=12.9 Hz), 4.74 (1H, d, J=7.3 Hz, H-1 of GlcNAc), 4.81 (1H, d, J=7.8 Hz, H-1 of Gal), 4.90 (1H, ddd, J=12.0, 10.3, 4.7 Hz, H-4 of Sia), 4.97 (1H, dd, J=10.0, 8.1 Hz, H-2 of Gal), 5.04 (1H, br d, H-4 of Gal), 5.06 (1H, br d, NH), 5.21 (1H, d, J=3.7 Hz, H-1 of Fuc), 5.37 (1H, dd, J=9.3, 2.7 Hz, H-7 of Sia), 5.69 (1H, ddd, J=9.3, 5.6, 2.7 Hz, H-8 of Sia), 6.23 (1H, m, NH), 7.22—7.36 (20H, m), 7.37 (2H, m), 7.50 (1H, m), 7.97 (2H, m). *Anal.* Calcd for $C_{91}H_{117}N_2O_{35}Br \cdot H_2O$: C, 57.62; H, 6.32; N, 1.47. Found: C, 57.64; H, 6.32; N, 1.55.

17-Bromo-3,6,9,12,15-pentaoxaheptadecyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside (17) and 3,6,9,12,15-Pentaoxaheptadecyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside (18) To a solution of **16** (200 mg) in tetrahydrofuran (15 ml) was added Pd–C (10%, 100 mg), and then hydrogenation was carried out for 24 h at room temperature. The mixture was filtered, and the filtrate was concentrated *in vacuo*. The crude product was purified by preparative thin-layer chromatography (CH_2Cl_2 : MeOH=8 : 1) to give **17** (118 mg, 73%), and **18** (25 mg, 16%).

17: Colorless powder. $[\alpha]_D^{27}$ −53.1° (c=0.66, $CHCl_3$). IR (KBr) cm^{-1}: 3450, 1749, 1665. ^{1}H-NMR ($CDCl_3+D_2O$) δ: 1.27 (3H, d, J=6.6 Hz, H-6 of Fuc), 1.71 (1H, dd, J=12.7, 12.0 Hz, H-3 of Sia), 1.85, 2.01, 2.04, 2.06, 2.11, 2.24 (each, 3H, s), 2.14 (6H, s), 2.59 (1H, dd, J=12.7, 4.6 Hz, H-3 of Sia), 3.48 (2H, t, J=6.3 Hz), 3.60—3.73 (25H, m), 3.77 (3H, s), 3.81 (2H, t, J=6.3 Hz), 3.85 (1H, m), 3.90—4.15 (7H, m), 4.23 (1H, dd, J=11.0, 6.8 Hz, H-6 of Gal), 4.42—4.38 (2H, m), 4.43 (1H, dd, J=12.4, 2.9 Hz, H-9 of Sia), 4.63 (1H, dd, J=10.3, 3.4 Hz, H-3 of Gal), 4.66 (1H, d, J=5.1 Hz, H-1 of GlcNAc), 4.75 (1H, d, J=8.1 Hz, H-1 of Gal), 4.89 (1H, ddd, J=12.0, 10.7, 4.6 Hz, H-4 of Sia), 4.98 (1H, dd, J=10.3, 8.1 Hz, H-2 of Gal), 5.05 (1H, d, J=3.9 Hz, H-1 of Fuc), 5.06 (1H, d, J=2.7 Hz, H-4 of Gal), 5.33 (1H, dd, J=9.3, 2.7 Hz, H-7 of Sia), 5.61 (1H, m), 7.48 (2H, m), 7.59 (1H, m), 8.04(2H, m). *Anal.* Calcd for $C_{63}H_{93}N_2O_{35}Br \cdot H_2O$: C, 49.25; H, 6.23; N, 1.82. Found: C, 49.15; H, 6.40; N, 1.71.

18: Colorless powder. $[\alpha]_D^{28}$ −56.5° (c=0.34, $CHCl_3$). IR (KBr) cm^{-1}: 3452, 1749, 1663. ^{1}H-NMR ($CDCl_3+D_2O$) δ: 1.21 (3H, t, J=7.1 Hz), 1.26 (3H, d, J=6.6 Hz, H-6 of Fuc), 1.72 (1H, dd, J=12.7, 12.0 Hz, H-3 of Sia), 1.86, 2.01, 2.05, 2.06, 2.11, 2.14, 2.14, 2.24 (each, 3H, s), 2.59 (1H, dd, J=12.7, 4.4 Hz, H-3 of Sia), 3.53 (2H, q, J=7.1 Hz), 3.57—3.75 (23H, m), 3.76—3.87 (3H, m), 3.78 (3H, s, $-OCH_3$), 3.89—4.16 (7H, m), 4.23 (1H, dd, J=11.2, 6.8 Hz, H-6 of Gal), 4.39 (1H, dd, J=11.0, 6.8 Hz, H-6 of Gal), 4.42 (1H, dd, J=12.5, 2.9 Hz, H-9 of Sia), 4.39 (1H, m), 4.63 (1H, dd, J=10.3, 3.4 Hz, H-3 of Gal), 4.66 (1H, d, J=4.9 Hz, H-1 of GlcNAc), 4.74 (1H, d, J=8.3 Hz, H-1 of Gal), 4.89 (1H, m, H-4 of Sia), 4.98 (1H, dd, J=10.3, 8.3 Hz, H-2 of Gal), 5.04—5.07 (2H, m, H-4 of Gal, H-1 of Fuc), 5.33 (1H, dd, J=9.1, 2.7 Hz, H-7 of Sia), 5.61 (1H, m), 7.48 (2H, m), 7.59 (1H, m), 8.04 (2H, m). *Anal.* Calcd for $C_{63}H_{94}N_2O_{35}\ H_2O$: C, 51.92; H, 6.64; N, 1.92. Found: C, 51.72; H, 6.70; N, 1.74.

17-Bromo-3,6,9,12,15-pentaoxaheptadecyl *O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside (6) To a solution of **17** (220 mg) in MeOH (5.0 ml) was added 3% sodium methoxide (200 ml), and the mixture was stirred for 30 min at room temperature. The reaction mixture was neutralized with Dowex 50W×8 (H^+), the mixture filtered, and the filtrate concentrated *in vacuo*.

The residue was dissolved in 0.1 N NaOH aq. (3.0 ml), and then the mixture was stirred for 10 min at room temperature. The reaction mixture was neutralized with Dowex 50W×8 (H^+), the mixture filtered, and the filtrate concentrated *in vacuo*.

The crude product was chromatographed on a column of Sephadex LH-20 (50 g, MeOH) to give **6** (150 mg, 90%) as a colorless powder. $[\alpha]_D^{27}$ −39.4° (c=0.66, MeOH). IR (KBr) cm^{-1}: 3460, 1653. ^{1}H-NMR (CD_3OD) δ: 1.16 (3H, d, J=6.6 Hz, H-6 of Fuc), 1.85 (1H, dd, J=13.3, 11.5 Hz, H-3 of Sia), 1.97, 2.00 (each 3H, s), 2.80 (1H, dd, J=12.4, 4.4 Hz, H-3 of Sia), 3.45—3.41 (2H, m), 3.50 (1H, dd, J=9.0, 1.2 Hz, H-6 of Sia), 3.51 (2H, t, J=6.1 Hz), 3.56 (1H, dd, J=9.5, 7.8 Hz, H-2 of Gal), 3.60—3.79 (28H, m), 3.81 (2H, t, J=6.1 Hz), 3.83—3.95 (8H, m), 4.00 (1H, dd, J=12.2, 3.7 Hz), 4.03 (1H, dd, J=9.5, 2.9 Hz, H-3 of Gal), 4.50 (1H, d, J=7.6 Hz), 4.52 (1H, d, J=7.8 Hz), 4.83—4.85 (1H, m, H-5 of Fuc), 5.03 (1H, d, J=3.9 Hz, H-1 of Fuc). *Anal.* Calcd for $C_{43}H_{75}N_2O_{28}Br \cdot 3H_2O$: C, 42.96; H, 6.79; N, 2.33. Found: C, 43.11; H, 6.84; N, 2.52.

Synthesis of a SLe^X–CMCht Conjugate (1) To a solution of CMCht (40 mg, M.W.: 100 kDa) in 0.5% aq. $NaHCO_3$ (3 ml) were added **20** (276 mg, 0.24 mmol), and powdered $NaHCO_3$ (20 mg). After stirring for 160 h at 60 °C, the reaction mixture was poured into 99.5% EtOH (35 ml) and the entire mixture centrifuged. The precipitate was successively washed with 95% EtOH, acetone, and Et_2O, then dried *in vacuo* to give almost pure **1**. After dissolving the product in H_2O (10 ml), the solution was dialyzed using membrane tubing (M.W. cut off; 12000—13000, Spectra) against deionized H_2O (10000 ml) for 12 h, and lyophilized to give a SLe^X–CMCht conjugate (**1**, 47 mg).

It was confirmed by a GPC analysis that low M.W. molecules derived from **6** were completely excluded from **1**, and the structure of **1** was confirmed by ^{1}H-NMR.

17-Azido-3,6,9,12,15-pentaoxaheptadecyl *O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (19) To a solution of **14** (750 mg) in *N*,*N*′-dimethylformamide (3 ml) was added sodium azide (92.5 mg, 1.42 mmol). After stirring for 1 h at 70 °C, the mixture was diluted with CH_2Cl_2, washed with saturated brine, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography [SiO_2 150 g, CH_2Cl_2 : MeOH=100 : 1] to give **19** (674 mg, 93%) as a colorless syrup. $[\alpha]_D^{26}$ −31.0° (c=1.07, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 3450, 2106, 1674. ^{1}H-NMR ($CDCl_3+D_2O$) δ: 1.14 (3H, d, J=6.3 Hz, H-6 of Fuc), 1.66 (3H, s), 3.36 (2H, t, J=5.0 Hz), 3.43—3.52 (3H, m), 3.56—3.76 (23H, m), 3.77—3.85 (2H, m), 3.95 (1H, m), 3.97 (1H, m, H-3 of Fuc), 4.06 (1H, dd, J=10.2, 3.4 Hz, H-2 of Fuc), 4.13 (1H, q, J=6.6 Hz, H-5 of Fuc), 4.58, 4.62 (each 1H, d, J=12.2 Hz), 4.61, 4.95 (each 1H, d, J=11.5 Hz), 4.67, 4.75, 4.79, 4.81 (each 1H, d, J=11.7 Hz), 4.84 (1H, d, J=8.5 Hz, H-1 of GlcNAc), 4.98 (1H, d, J=3.4 Hz, H-1 of Fuc), 6.13 (1H, d, J=7.6 Hz, NH), 7.26—7.41 (20H, m). *Anal.* Calcd for $C_{54}H_{72}N_4O_{15}$: C, 63.76; H, 7.13; N, 5.50. Found: C, 63.65; H, 7.06; N, 5.48.

17-Azido-3,6,9,12,15-pentaoxaheptadecyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (20) Compound **19** was converted to **20** (52%) using the procedure described for **16**. Colorless powder. $[\alpha]_D^{28}$ −27.9° (c=0.55, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 2108, 1744, 1688. ^{1}H-NMR ($CDCl_3$) δ: 1.10 (3H, d, J=6.3 Hz, H-6 of Fuc), 1.71 (1H, dd, J=12.7, 12.0 Hz, H-3 of Sia), 1.85, 1.89, 1.96, 1.97, 2.01, 2.07, 2.08, 2.22 (each 3H, s), 2.56 (1H, dd, J=12.7, 4.6 Hz, H-3 of Sia), 3.37 (2H, t, J=5.1 Hz), 3.48—3.65 (21H, m), 3.75 (3H, s), 3.79—3.87 (4H, m), 3.87 (1H, dd, J=10.3, 2.7 Hz, H-3 of Fuc), 3.90 (1H, m), 3.98 (1H, dd, J=12.4, 5.6 Hz, H-9 of Sia), 4.01 (1H, m), 4.04 (1H, m, H-5 of Sia), 4.08 (1H, dd, J=10.0, 3.7 Hz, H-2 of Fuc), 4.08 (1H, m), 4.16 (1H, dd, J=11.0, 7.3 Hz, H-6 of Gal), 4.23 (1H, q, J=6.6 Hz, H-5 of Fuc), 4.24 (1H, dd, J=11.0, 6.8 Hz, H-6 of Gal),

4.29 (1H, dd, J=12.4, 2.7 Hz, H-9 of Sia), 4.36, 4.48, 4.61 (each 1H, d, J=12.0 Hz), 4.67 (1H, dd, J=10.0, 3.7 Hz, H-3 of Gal), 4.44, 4.58, 4.70, 4.75, 4.77, 4.81 (each 1H, d, J=12.0 Hz), 4.63, 4.92 (each 1H, d, J=11.7 Hz), 4.74 (1H, d, J=7.3 Hz, H-1 of GlcNAc), 4.81 (1H, d, J=8.1 Hz, H-1 of Gal), 4.90 (1H, ddd, J=12.0, 10.5, 4.6 Hz, H-4 of Sia), 4.97 (1H, dd, J=10.0, 8.1 Hz, H-2 of Gal), 5.04 (1H, d, J=3.7 Hz, H-4 of Gal), 5.07 (1H, d, J=10.3 Hz, NH), 5.22 (1H, d, J=3.7 Hz, H-1 of Fuc), 5.37 (1H, dd, J=9.3, 2.7 Hz, H-7 of Sia), 5.59 (1H, ddd, J=9.3, 5.6, 2.7 Hz, H-8 of Sia), 6.25 (1H, br.s, NH), 7.16—7.49 (22H, m), 7.50 (1H, m), 7.97 (2H, m). *Anal.* Calcd for $C_{91}H_{117}N_5O_{35}\cdot H_2O$: C, 58.79; H, 6.45; N, 3.76. Found: C, 58.50; H, 6.46; N, 3.69.

17-Amino-3,6,9,12,15-pentaoxaheptadecyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside, Hydrochloride Salt (21) To a solution of **20** (200 mg) in MeOH (15 ml) were added Pd–C (10%, 150 mg) and 1 N HCl (330 μl), then hydrogenation was carried out at 3.5×10^4 kg/m^2 (50 psi) for 12 h. The mixture was filtered, and the filtrate concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (SiO_2 45 g, CH_2Cl_2 : MeOH : H_2O=65 : 35 : 10 (lower phase)) to give **21** (140 mg, 87%) as colorless powder. $[\alpha]_D^{27}$ −58.3° (c=0.52, $CHCl_3$). IR (KBr) cm^{-1}: 3430, 1749, 1663. ^{1}H-NMR (CD_3OD) δ: 1.27 (3H, d, J=6.3 Hz, H-6 of Fuc), 1.53 (1H, dd, J=12.4, 12.2 Hz, H-3 of Sia), 1.81, 1.97, 1.99, 2.04, 2.07, 2.08, 2.16, 2.26 (each 3H, s), 2.58 (1H, dd, J=12.4, 4.6 Hz, H-3 of Sia), 3.22 (1H, ddd, J=13.7, 10.0, 5.1 Hz), 3.22 (1H, ddd, J=13.7, 6.1, 4.6 Hz), 3.42 (1H, m), 3.62—3.76 (22H, m), 3.74 (3H, s), 3.79 (5H, m), 3.85 (1H, m), 3.89 (1H, dd, J=12.0, 4.9 Hz, H-6 of GlcNAc), 3.95 (1H, dd, J=10.5, 10.5 Hz, H-5 of Sia), 3.99—4.09 (4H, m), 4.15 (1H, dd, J=10.5, 8.5 Hz), 4.41 (1H, dd, J=12.7, 2.9 Hz, H-9 of Sia), 4.45 (1H, d, J=8.5 Hz, H-1 of GlcNAc), 4.52 (1H, dd, J=10.5, 5.9 Hz, H-6 of Gal), 4.81—4.86 (1H, m, H-5 of Fuc), 4.87 (1H, m, H-4 of Sia), 4.91 (1H, d, J=8.3 Hz, H-1 of Gal), 4.98 (1H, dd, J=10.0, 8.3 Hz, H-2 of Gal), 5.06 (1H, d, J=3.9 Hz, H-1 of Fuc), 5.17 (1H, d, J=3.4 Hz, H-4 of Gal), 5.37 (1H, dd, J=9.3, 2.7 Hz, H-7 of Sia), 5.60 (1H, ddd, J=9.3, 5.6, 2.9 Hz, H-8 of Sia), 7.51 (2H, t-like), 7.63 (1H, t-like), 8.05 (2H, d-like). FAB-MS: 1454 (M+H), 1476 (M+Na). *Anal.* Calcd for $C_{63}H_{94}N_3O_{35}Cl\cdot 1.5H_2O$: C, 49.92; H, 6.45; N, 2.77; Cl, 2.34. Found: C, 49.88; H, 6.58; N, 3.08; Cl, 2.08.

17-Amino-3,6,9,12,15-pentaoxaheptadecyl *O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside (22) To a solution of **20** (185 mg, 0.100 mmol) in MeOH (4 ml) was added 28% sodium methoxide (400 μl), and the mixture was stirred for 30 min at room temperature. The reaction mixture was neutralized with Dowex 50W×8 (H^+), the mixture was filtered, and then the filtrate concentrated *in vacuo*.

The residue was dissolved in a solution of 0.1 N aq. NaOH (4 ml) and 1,4-dioxane (2 ml), and then the mixture was stirred for 10 min at room temperature. The reaction mixture was neutralized with Dowex 50W×8 (H^+), the mixture was filtered, and then the filtrate concentrated *in vacuo*.

The crude product was chromatographed on a column of Sephadex LH-20 (70 g, MeOH), to give tetrabenzyl ether (139 mg, 94%) as a colorless powder. $[\alpha]_D^{26}$ −46.9° (c=0.33, MeOH). IR (KBr) cm^{-1}: 3377, 2110, 1655. ^{1}H-NMR (CD_3OD) δ: 1.15 (3H, d, J=6.6 Hz, H-6 of Fuc), 1.83 (1H, t-like, H-3 of Sia), 2.00, 2.01 (each 3H, s), 2.83 (1H, m, H-3 of Sia), 3.21 (1H, dd, J=7.8, 3.4 Hz, H-6 of Sia), 3.33 (2H, t, J=5.1 Hz), 3.50—3.56 (4H, m), 3.59—3.70 (25H, m), 3.73—4.01 (13H, m), 4.00 (1H, dd, J=10.0, 2.7 Hz, H-3 of Fuc), 4.04—4.11 (2H, m), 4.16 (1H, dd, J=9.3, 9.3 Hz, H-3 of GlcNAc), 4.44 (1H, d, J=8.3 Hz, H-1 of GlcNAc or Gal), 4.47 (1H, d, J=7.6Hz, H-1 of GlcNAc or Gal), 4.56 (1H, d, J=11.0 Hz), 4.55, 4.57 (each 1H, d, J=12.0 Hz), 4.75 (1H, d, J=11.7 Hz), 4.78—4.88 (5H, m), 5.35 (1H, d, J=3.7 Hz, H-1 of Fuc), 7.22—7.44 (20H, m).

To a solution of tetrabenzyl ether (30 mg, 20.6 mmol) in MeOH (10 ml) was added Pd–C (10%, 60 mg), and 0.1 M p-TsOH–MeOH solution (210 ml, 21.0 mmol), then hydrogenation was carried out at 3.5×10^4 kg/m^2 (50 psi) for 24 h. The mixture was filtered, and the filtrate concentrated *in vacuo*. The crude product was chromatographed on a column of Sephadex LH-20 (50 g, MeOH), to give **22** (17 mg, 72% from **22**) as colorless powder. ^{1}H-NMR (CD_3OD) δ: 1.16 (3H, d, J=6.6 Hz, H-6 of Fuc), 1.71 (1H, m, H-3 of Sia), 1.98, 2.01 (each 3H, s), 2.89 (1H, dd, J=12.4, 4.2 Hz, H-3 of Sia), 3.41—3.45 (2H, m), 3.50 (1H, dd, J=9.0, 1.2 Hz, H-6 of Sia), 3.51 (2H, t, J=6.1 Hz), 3.56 (1H, dd, J=9.5, 7.8 Hz, H-2 of Gal), 3.60—3.79 (28H, m), 3.81 (2H, t, J=6.1 Hz), 3.83—3.95 (8H, m), 4.00 (1H, dd, J=12.2, 3.7 Hz), 4.03 (1H, dd, J=9.5, 2.9 Hz, H-3 of Gal), 4.50 (1H, d, J=8.5 Hz), 4.51 (1H, d, J=7.8 Hz), 5.05 (1H, d, J=3.9 Hz, H-1 of Fuc).

3,6,9,12,15-Pentaoxa-17-propionylamino-heptadecyl *O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside (7) Compound **22** (6 mg, 5.54 mmol) was dissolved in 20 mM *N*-succinimidyl propionate–MeOH solution (560 μl, 11.2 mmol), and then 0.1 M *N*-methyl morpholine–MeOH solution (168 μl, 16.8 mmol) was added. After stirring for 12 h at room temperature, the reaction mixture was concentrated. The residue was dissolved in H_2O (3 ml), and the solution was treated with Dowex 50W×8 (H^+ form). The resultant solution was filtrated, and the filtrate concentrated. The crude product was purified by silica gel column chromatography (SiO_2 1 g, CH_2Cl_2 : MeOH : H_2O= 6 : 4 : 1), and gel filtration chromatography (Sephadex LH-20, 20 g, MeOH) to give **7** (5 mg, 79%) as a colorless powder. $[\alpha]_D^{27}$ −26.7° (c=0.20, MeOH). IR (KBr) cm^{-1}: 3400, 1650. ^{1}H-NMR (CD_3OD) δ: 1.12 (3H, t, J=7.6 Hz), 1.15 (3H, d, J=6.6 Hz, H-6 of Fuc), 1.71 (1H, m, H-3 of Sia), 1.96, 2.01 (each 3H, s), 2.21 (2H, q, J=7.6 Hz), 2.87 (1H, dd, J=12.5, 3.9 Hz, H-3 of Sia), 3.36 (2H, t, J=5.6 Hz), 3.42—3.48 (3H, m), 3.52—3.74 (27H, m), 3.54 (2H, t, J=5.6 Hz), 3.75 (1H, dd, J=11.5, 7.1 Hz, H-6 of Gal), 3.82—3.96 (9H, m), 4.00 (1H, dd, J=12.0, 3.7 Hz), 4.04 (1H, dd, J=9.8, 3.2 Hz, H-3 of Gal), 4.50 (1H, d, J=8.3 Hz, H-1 of GlcNAc or H-1 of Gal), 4.50 (1H, d, J=7.8 Hz, H-1 of GlcNAc or H-1 of Gal), 4.82 (1H, m, H-5 of Fuc), 5.03 (1H, d, J=3.9 Hz, H-1 of Fuc). *Anal.* Calcd for $C_{46}H_{81}N_3O_{26}\cdot 2.5H_2O$: C, 46.62; H, 7.31; N, 3.55. Found: C, 46.32; H, 7.51; N, 3.40.

Synthesis of a SLe^X–CMPul Conjugate (2) To a solution of CMPul (50 mg) in H_2O (2 ml) were added *N*,*N*′-dimethyl formamide (2 ml), **21** (184 mg, 0.124 mmol) and 2-ethoxy-1-ethoxycarbonyl-1,2-dihyroquinoline (EEDQ, 610 mg). After stirring for 160 h at 40 °C, the reaction mixture was concentrated. The residue was dissolved in 1 N NaOH (7 ml), and the mixture was stirred for 12 h at room temperature. The resultant mixture was poured into 99.5% EtOH (35 ml) and the entire mixture centrifuged. The precipitate was successively washed with 95% EtOH, acetone, and Et_2O, and dried *in vacuo* to give almost pure **2**. After dissolving the product in H_2O (10 ml), the solution was dialyzed using membrane tubing (M.W. cut off: 12000—13000 Spectra) against deionized H_2O (10000 ml) for 12 h, and lyophilized to give a SLe^X–CMPul conjugate (**2**, 57 mg).

It was confirmed by GPC Analysis that low M.W. molecules derived from **21**, were completely excluded from **2**, and the structure of **2** was confirmed by ^{1}H-NMR.

Synthesis of a SLe^X–DSH Conjugate (3) To a solution of DSH (300 mg, M.W.: 40 kDa, Sigma Co. Ltd) in H_2O (20 ml) was added $NaBH_4$ (100 mg). After stirring for 19 h at room temperature, the solution was adjusted to pH 5.0 with AcOH at 0 °C. After stirring for 5 min at 0 °C, the solution was adjusted to pH 8.5 with 1 N NaOH at room temperature, and then the reaction mixture was poured into 99.5% EtOH (35 ml) and the entire mixture was centrifuged. The precipitate was successively washed with 95% EtOH, acetone, and Et_2O, and dried *in vacuo* to give a DSH derivative which was reduced the terminal hemiacetal group (278 mg).

To a solution of the DSH derivative (40 mg) in 0.5% aq. $NaHCO_3$ (3 ml) were added **6** (276 mg, 0.24 mmol), and powdered $NaHCO_3$ (20 mg). After stirring for 160 h at 60 °C, the reaction mixture was poured into 99.5% EtOH (35 ml) and the entire mixture was centrifuged. The precipitate was successively washed with 95% EtOH, acetone, and Et_2O, and dried *in vacuo* to give almost pure **3**. After dissolving the product in H_2O (10 ml), the solution was dialyzed using membrane tubing (M.W. cut off: 12000—13000 Spectra) against deionized H_2O (10000 ml) for 12 h, and lyophilized to give a SLe^X–DSH conjugate (**3**, 47 mg).

It was confirmed by a GPC analysis that low M.W. molecules derived from **6**, were completely excluded from **3**, and the structure of **3** was confirmed by ^{1}H-NMR.

17-Bromo-3,6,9,12,15-pentaoxaheptadecyl 2-Acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (23) To a solution of **11** (240 mg, 0.377 mmol) in *N*,*N*′-dimethylformamide (3 ml) were added BaO (127 mg, 0.754 mmol), $Ba(OH)_2\cdot 8H_2O$ (24 mg, 0.0754 mmol) and benzyl bromide (90 μl). After stirring for 12 h at 55 °C, MeOH (3 ml) and 28% NaOMe–MeOH (150 μl) were added and the stirring was contined for 20 min. The mixture was diluted with CH_2Cl_2, washed with saturated brine, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (SiO_2 30 g, CH_2Cl_2 : MeOH= 50 : 1) to give **23** (233 mg, 85%) as a colorless syrup. $[\alpha]_D^{27}$ −13.4° (c=1.03, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 3460, 3340, 1674. ^{1}H-NMR ($CDCl_3$) δ: 1.95 (3H, s), 3.44 (2H, t, J=6.3 Hz), 3.73—3.46 (22H, m), 3.77 (2H, t, J= 6.3 Hz), 3.80 (1H, m), 3.90 (1H, m), 4.05 (1H, dd, J=9.8, 9.5 Hz, H-3 of GlcNAc), 4.35 (1H, dd, J=10.5, 5.1 Hz, H-6 of GlcNAc), 4.66, 4.90 (each

1H, d, J=12.0 Hz), 4.95 (1H, d, J=8.1 Hz, H-1 of GlcNAc), 5.57 (1H, s), 6.36 (1H, d, J=8.1 Hz, NH), 7.22—7.42 (8H, m), 7.47—7.51 (2H, m). *Anal.* Calcd for $C_{34}H_{48}NO_{11}Br$: C, 56.19; H, 6.65; N, 1.92. Found: C, 56.18; H, 6.56; N, 1.81.

17-Bromo-3,6,9,12,15-pentaoxaheptadecyl 2-Acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (24) Compound **23** was converted to **24** (87%) using the procedure described for **14**. Colorless syrup. $[\alpha]_D^{24}$ −14.8° (c=1.11, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 3460, 3350, 1674. ^{1}H-NMR ($CDCl_3$) δ: 1.95 (3H, s), 2.76 (1H, br s, OH), 3.45 (2H, t, J=6.3 Hz), 3.51 (1H, m), 3.57—3.71 (19H, m), 3.77 (2H, t, J=6.3 Hz), 3.72—3.80 (5H, m), 3.90 (1H, m), 4.56, 4.61 (each 1H, d, J=12.0 Hz), 4.71, 4.77 (each 1H, d, J=11.5 Hz), 4.81 (1H, d, J=8.3 Hz, H-1 of GlcNAc), 6.36 (1H, d, J=7.3 Hz, NH), 7.24—7.38 (10H, m). *Anal.* Calcd for $C_{34}H_{50}NO_{11}Br$: C, 56.04; H, 6.92; N, 1.92. Found: C, 55.89; H, 6.99; N, 1.84.

17-Azido-3,6,9,12,15-pentaoxaheptadecyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (25) Compound **24** was converted to **25** (98%) using the procedure described for **19**. Colorless syrup. $[\alpha]_D^{26}$ −14.3° (c=1.02, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 2108, 1674. ^{1}H-NMR ($CDCl_3$) δ: 1.90 (3H, s), 2.15 (1H, d, J=2.2 Hz, OH), 3.36 (2H, t, J=5.1 Hz), 3.51 (1H, dt, J=9.5, 4.9 Hz), 3.57—3.71 (22H, m), 3.72—3.80 (4H, m), 3.89 (1H, m), 4.57, 4.61 (each 1H, d, J=12.2 Hz), 4.71, 4.77 (each 1H, d, J=11.5 Hz), 4.82 (1H, d, J=8.3 Hz, H-1 of GlcNAc), 6.33 (1H, d, J=7.3 Hz, NH), 7.37—7.26 (10H, m). *Anal.* Calcd for $C_{34}H_{50}N_4O_{11}$: C, 59.12; H, 7.30; N, 8.11. Found: C, 59.21; H, 7.29; N, 8.01.

17-Bromo-3,6,9,12,15-pentaoxaheptadecyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (26) Compound **24** was converted to **26** (72%) using the procedure described for **16**. Colorless powder. $[\alpha]_D^{24}$ −21.8° (c=0.38, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 3300, 1751, 1662. ^{1}H-NMR ($CDCl_3$) δ: 1.72 (1H, dd, J=12.7, 12.0 Hz, H-3 of Sia), 1.85, 1.96, 1.96, 2.01, 2.11, 2.22 (each 3H, s), 2.07 (6H, s), 2.58 (1H, dd, J=12.7, 4.7 Hz, H-3 of Sia), 3.45 (2H, t, J=6.3 Hz), 3.56—3.70 (22H, m), 3.75 (3H, s), 3.79 (2H, t, J=6.3 Hz), 3.84 (1H, t-like, H-5 of Gal), 3.88—3.95 (4H, m), 3.97 (1H, dd, J=12.2, 6.1 Hz, H-9 of Sia), 4.04 (1H, t-like), 4.05 (1H, q-like, H-5 of Sia), 4.11 (1H, dd, J=11.0, 7.1 Hz, H-6 of Gal), 4.16 (1H, dd, J=11.0, 6.6 Hz, H-6 of Gal), 4.32 (1H, dd, J=12.4, 2.4 Hz, H-9 of Sia), 4.51, 4.59 (each 1H, d, J=12.0 Hz), 4.66 (1H, dd, J=10.3, 3.7 Hz, H-3 of Gal), 4.67, 4.77 (each 1H, d, J=11.5 Hz), 4.71 (1H, d, J=6.1 Hz, H-1 of GlcNAc), 4.85 (1H, d, J=7.8 Hz, H-1 of Gal), 4.88 (1H, ddd, J=12.0, 10.5, 4.7 Hz, H-4 of Sia), 5.03 (1H, br d, H-4 of Gal), 5.04 (1H, br d, NH), 5.04 (1H, dd, J=10.3, 8.1 Hz, H-2 of Gal), 5.36 (1H, dd, J=9.3, 2.7 Hz, H-7 of Sia), 5.60 (1H, ddd, J=9.3, 6.1, 2.9 Hz, H-8 of Sia), 6.30 (1H, br d, NH), 7.18—7.34 (10H, m), 7.38 (2H, m), 7.53 (1H, m), 7.96 (2H, m). *Anal.* Calcd for $C_{71}H_{95}N_2O_{31}Br\ H_2O$: C, 54.30; H, 6.22; N, 1.78. Found: C, 54.41; H, 6.22; N, 1.81.

17-Bromo-3,6,9,12,15-pentaoxaheptadecyl *O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (27) Compound **26** was converted to **27** (85%) using the procedure described for **6** and **17**. Colorless powder. $[\alpha]_D^{24}$ −12.2° (c=1.02, MeOH). IR (KBr) cm^{-1}: 3446, 1735, 1655. ^{1}H-NMR (CD_3OD) δ: 1.87 (1H, t-like, H-3 of Sia), 1.98, 2.00 (each 3H, s), 2.79 (1H, dd, J=12.9, 4.2 Hz, H-3 of Sia), 3.40 (1H, m), 3.50 (1H, m, H-6 of Sia), 3.51 (2H, t, J=6.1 Hz), 3.55—3.95 (36H, m), 3.81 (2H, t, J=6.1 Hz), 4.05 (1H, dd, J=9.8, 2.9 Hz, H-3 of Gal), 4.45 (1H, d, J=7.8 Hz, H-1 of Gal), 4.50 (1H, d, J=8.3 Hz, H-1 of GlcNAc). *Anal.* Calcd for $C_{37}H_{65}N_2O_{24}Br\cdot 2.5H_2O$: C, 42.45; H, 6.74; N, 2.68. Found: C, 42.46; H, 6.82; N, 2.93.

17-Azido-3,6,9,12,15-pentaoxaheptadecyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (28) Compound **25** was converted to **28** (48%) using the procedure described for **20**. Colorless powder. $[\alpha]_D^{24}$ −20.9° (c=1.05, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 2100, 1744, 1682. ^{1}H-NMR ($CDCl_3$) δ: 1.72 (1H, dd, J=12.7, 12.3 Hz, H-3 of Sia), 1.96, 2.07 (each 6H, s), 1.85, 2.01, 2.10, 2.22 (each 3H, s), 2.58 (1H, dd, J=12.7, 4.6 Hz, H-3 of Sia), 3.37 (2H, t, J=5.1 Hz), 3.56—3.70 (22H, m), 3.75 (3H, s), 3.78 (2H, t, J=6.3 Hz), 3.84 (1H, t-like, H-5 of Gal), 3.86—3.95 (4H, m), 3.97 (1H, dd, J=12.7, 6.1 Hz, H-9 of Sia), 4.03—4.04 (1H, m), 4.05 (1H, q-like, H-5 of Sia), 4.11 (1H, dd, J=11.0, 7.3 Hz, H-6 of Gal), 4.16 (1H, dd, J=11.0, 6.8 Hz, H-6 of Gal), 4.31 (1H, br d, H-9 of Sia), 4.51, 4.59 (each 1H, d, J=12.0 Hz), 4.66 (1H, dd, J=10.3, 3.7 Hz, H-3 of Gal), 4.67, 4.77 (each 1H, d, J=11.5 Hz), 4.71 (1H, d, J=6.1 Hz, H-1 of GlcNAc), 4.85 (1H, d, J=7.8 Hz, H-1 of Gal), 4.89 (1H, ddd, J=12.0, 10.7, 4.6 Hz, H-4 of Sia), 5.00—5.09 (3H, m, H-2,4 of Gal, NH), 5.36 (1H, dd, J=9.3, 2.7 Hz, H-7 of Sia), 5.60 (1H, ddd, J=9.3, 6.1, 2.7 Hz, H-8 of Sia), 6.32 (1H, m, NH), 7.18—7.35 (10H, m), 7.38 (2H, m), 7.53 (1H, m), 7.96 (2H, m). *Anal.* Calcd for $C_{71}H_{95}N_5O_{31}\ H_2O$: C, 55.64; H, 6.38; N, 4.57. Found: C, 55.46; H, 6.47; N, 4.44.

7-Amino-3,6,9,12,15-pentaoxaheptadecyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside, Hydrochloride salt (29) Compound **28** was converted to **29** (73%) using the procedure described for **21**. Colorless powder. $[\alpha]_D^{26}$ −3.4° (c=1.04, $CHCl_3$). IR (KBr) cm^{-1}: 3480, 3420, 1743, 1688. ^{1}H-NMR (CD_3OD) δ: 1.54 (1H, dd, J=12.5, 12.0 Hz, H-3 of Sia), 1.81, 1.97, 1.97, 2.03, 2.07, 2.12, 2.14, 2.31 (each 3H, s), 2.58 (1H, dd, J=12.4, 4.6 Hz, H-3 of Sia), 3.11 (1H, ddd, J=13.2, 5.9, 3.4 Hz), 3.22 (1H, ddd, J=13.7, 7.1, 3.9 Hz), 3.41 (1H, m), 3.52—3.83 (25H, m), 3.80 (3H, s), 3.89 (1H, d-like, H-6 of Sia), 3.93 (1H, m), 3.97 (1H, dd, J=10.3, 10.3 Hz, H-5 of Sia), 3.99 (1H, dd, J=12.2, 5.9 Hz, H-9 of Sia), 4.05 (1H, m), 4.17 (1H, t-like, H-5 of Gal), 4.30 (1H, dd, J=11.2, 7.3 Hz, H-6 of Gal), 4.36 (1H, dd, J=11.2, 5.8 Hz, H-6 of Gal), 4.39 (1H, dd, J=12.7, 2.7 Hz, H-9 of Sia), 4.40 (1H, d, J=8.5 Hz, H-1 of GlcNAc), 4.73 (1H, dd, J=10.3, 3.2 Hz, H-3 of Gal), 4.85 (1H, d, J=8.3 Hz, H-1 of Gal), 4.87 (1H, m, H-4 of Sia), 5.00 (1H, dd, J=10.0, 8.3 Hz, H-2 of Gal), 5.17 (1H, d, J=3.2 Hz, H-4 of Gal), 5.36 (1H, dd, J=9.5, 2.4 Hz, H-7 of Sia), 5.63 (1H, ddd, J=9.3, 5.9, 2.7 Hz, H-8 of Sia), 7.48 (2H, t-like), 7.62 (1H, t-like), 8.07 (2H, d-like).

Syntheses of Radiolabeled 1 and Radiolabeled 3 To a solution of a SLe^x–CMCht conjugate (**1**, 2 mg) in 1% aq. $NaHCO_3$ (200 μl) was added 100 μl (0.98 nmol) *N*-succinimidyl [2,3-^{3}H] propionate-toluene solution (100 μCi, 1 mCi/ml, Amersham International plc.). After stirring for 24 h at room temperature, the reaction mixture was poured into 99.5% EtOH (1.4 ml) and the entire mixture centrifuged. The precipitate was washed with 95% EtOH (1.4 ml), and dissolved in H_2O. The resultant solution was applied to a PD-10 column (M.W. cut off: 5000, Pharmacia-LKB, Uppsala, Sweden) equibrated with saline and the void fraction was pooled. Radioactivity was determined to be 5 μCi/mg by liquid scintillation counting.

Radiolabeled **3** was prepared by a similar procedure to that used for radiolabeled **1**.

Synthesis of Radiolabeled 2 To a SLe^X–CMPul conjugate (**2**, 2 mg) in a reaction vessel were added 300 μl [2-^{3}H] glycine–water solution (300 μCi, 1 mCi/ml, Amersham International plc.), and 200 μl 1% (w/v) 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ)-*N*,*N*′-dimethylformamide solution. The reaction mixture was stirred for 12 h at room temperature and applied to a PD-10 column (M.W. cut off: 5000, Pharmacia-LKB, Uppsala, Sweden) equilibrated with saline and the void fraction was pooled. Radioactivity was determined to be 15 μCi/mg by liquid scintillation counting.

Radiolabeled **4** and radiolabeled **5** were prepared by a similar procedure to that used for radiolabeled **1** and radiolabeled **2**, respectively.

Synthesis of Radiolabeled 7 To a solution of **22** (2 mg) in H_2O (400 μl) were added 400 μl (3.92 nmol) *N*-succinimidyl [2,3-^{3}H] propionate–toluene solution (400 μCi, 1 mCi/ml, Amersham International plc.), 20 mM non-labelled *N*-succinimidyl propionate–H_2O solution (187 μl, 3.92 mmol), and 0.1 M *N*-methyl morpholine–H_2O solution (56 μl, 5.61 μmol). After stirring for 20 h at room temperature, the reaction mixture was washed with $CHCl_3$ to remove the remaining unreacted *N*-succinyl propionate and *N*-succinimidyl [2,3-^{3}H] propionate, and the H_2O phase was treated with Dowex 50W×8 (H^+ form). The resultant solution was purified by gel filtration chromatography on Bio-Gel P-2 (1×50 cm) equilibrated with 50 mM pyridine/AcOH (pH 5.0) to give radiolabeled **7**. The radioactivity and fucose content were measured by liquid scintillation counting and Gibbon's method, respectively.

Tissue Distribution Experiment Using the Mouse Ear Edema Model Male ICR mice (27—33 g body weight; age 5—6 weeks) were obtained from Japan SLC, Inc., and allowed free access to food and water (standard laboratory chow). Mice were anesthetized with diethyl ether. ^{3}H-labeled monovalent SLe^X (**7**) or oligosaccharide (SLe^X or SLN)–polysaccharide conjugates were administered intravenously at a dose of 365 nmol/kg with respect to the concentration of saccharide. Immediately after administration, arachidonic acid (Sigma Chemical Co.) in acetone (1 mg/20 μl) was applied to both surfaces of the right ear. At various intervals after administration, the mice were anesthetized again and exsanguinated through the heart or femoral artery. The lung, spleen, kidney, liver, right ear, and left ear were then excised, rinsed with saline, and weighed. After drying the plasma and tissues had been dried on combustion cones (Parkard Instrument Co., Inc.) at room temperature, the ^{3}H in each sample was collected as ^3H_2O by the

combustion method (Automatic Sample Combustion System, Aloka ASC-113, Tokyo, Japan). The ^{3}H radioactivity was measured with a liquid scintillation counter (Aloka LSC-350) using a liquid scintillation cocktail (Aquasol-II, New England Nuclear Research).

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References and Notes

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