A Highly Practical Synthesis of Sulfated Lewis X: One-Pot, Two-Step Glycosylation Using "Armed/Disarmed" Coupling and Selective Benzoylation and Sulfation

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In spite of the several attractive biological activities of the Le^x, sLe^x, and sulfated Le^x derivatives, there are still limitations in their practical syntheses. One of the most difficult problems is the many steps required for their syntheses. Therefore, we investigated simple constructions of the Le^x analogs and succeeded in establishing highly practical syntheses of the Le^x and sulfated Le^x analogs. Especially, the "armed/disarmed" method allowed the first synthesis of Le^x derivatives by a one-pot, two-step glycosylation. This synthetic strategy could be an applicable and useful method for oligosaccharide constructions of Le^x, sulfated Le^x, and other derivatives.

Introduction

Recently, some quite interesting biological properties of oligosaccharides have been revealed, and exciting studies have indicated the involvement of selectin−oligosaccharide interactions in various inflammatory diseases.[1] Since Phillips et al.[2] reported that the tetrasaccharide sialyl Lewis x antigenic determinant (sLe^x) is a native ligand for E-selectin, which is a member of the family of cell adhesion molecules, the synthesis of the sLe^x oligosaccharide has attracted the interest of organic chemists, because of the low abundance of sLe^x from natural sources. The total synthesis of sLe^x has been reported by several groups.[3] One of the most attractive strategies for the synthesis of sLe^x incorporates enzymatic and chemoenzymatic approaches,[4] which involve the stereospecific and regiospecific construction of the oligosaccharides. However, these approaches would be mainly limited to the synthesis of the oligosaccharide, because the key enzyme used for the above chemoenzymatic and enzymatic methods is not commercially available. A major disadvantage of the enzymatic methods is that the narrow substrate specificities of the glycosyltransferases impede the synthesis of various modified oligosaccharides.[4b]

On the other hand, recent research has also focused on the construction of Le^x analogs with a sialic acid of sLe^x replaced by more simple functional groups, e.g. sulfuric acid,[5] phosphoric acid,[6] and carboxylic acid.[7] We have found that a 3′-sulfated Le^x analog (**1**) with a branched alkyl chain, 2-tetradecylhexadecyl, was an effective selectin inhibitor both *in vitro* and *in vivo*.[8] In addition, we have investigated the binding mode between compound **1** and E-selectin using the molecular dynamic method and found that the 2-tetradecylhexadecyl group of compound **1** played an important role in the interaction with E-selectin.[9]

It has been reported that the total synthesis of compound **1** involves 17 reaction steps from the starting material, lactose.[5a] There are still limitations for the synthesis of compound **1** at the practical level, because of problems involving the poor selectivity of the benzoylation and the low yield from the eight-steps of glycosylation.[5a] Therefore, to establish a practical synthesis of compound **1**, we focused on the following three points: (1) the optimization of selective benzoylation, (2) a one-pot glycosylation based on the "armed/disarmed" coupling method, and (3) the establishment of selective sulfation. This paper describes the highly practical synthesis for oligosaccharides such as the Le^x and sulfated Le^x deriva-

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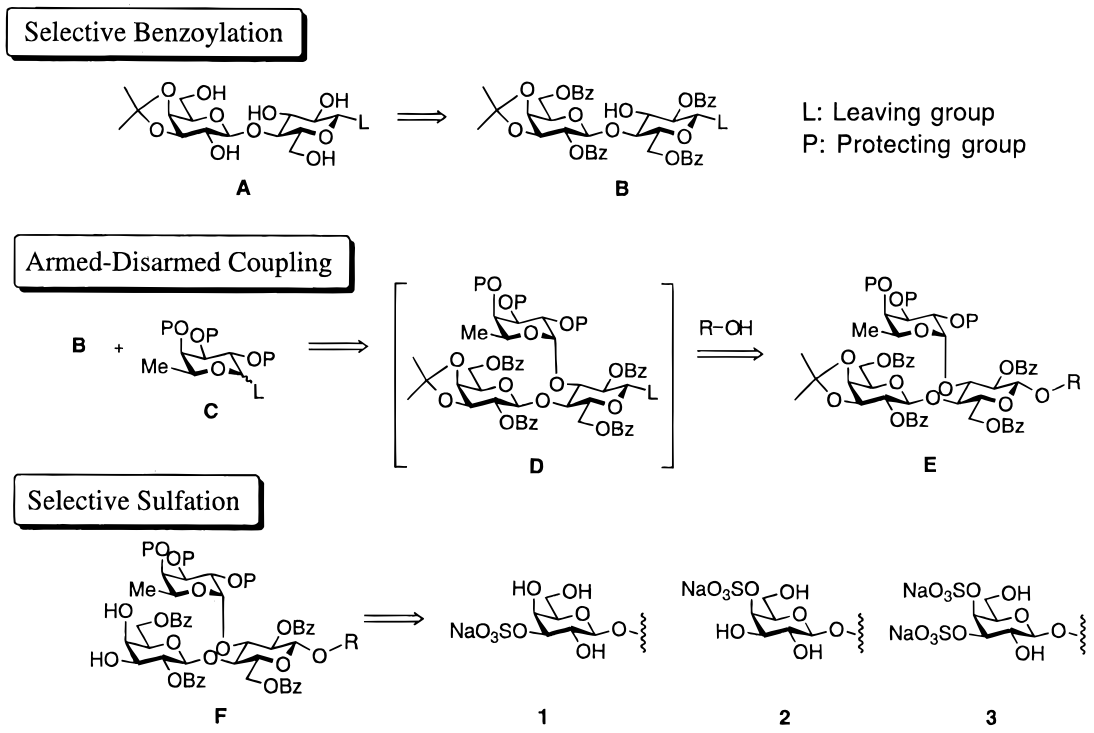
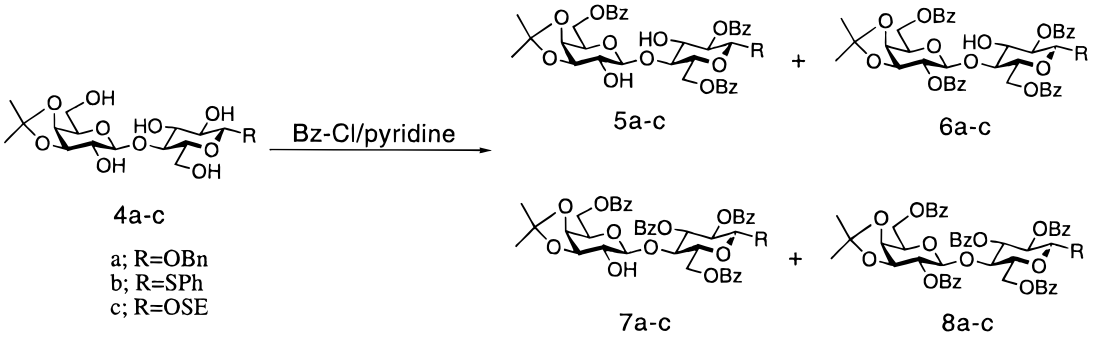
Scheme 1. Synthetic Strategy for Sulfated Le^x Analogs



Scheme 2



tives, which involves a "one-pot" synthesis of two glycosidic linkages with good stereocontrol.

Results and Discussion

Our synthetic strategy for the 3′-sulfated Le^x derivative **1** is to establish methods for the efficient incorporation of the fucose residue, a branched alkyl chain, and the sulfuric acid group to lactose, which is commercially available, as illustrated in Scheme 1.

Selective Benzoylation. 2,6,2′,6′-*O*-Benzoyl lactose derivatives (**6a**−**c**) could be important acceptors for the efficient construction of a Le^x skeleton. Hasegawa et al.[5a] reported that 2,6,2′6′-*O*-benzoyllactose (**6c**, R = OSE; 2-(trimethylsilyl)ethoxy group) was obtained by the benzoylation of the corresponding derivative to a 3′,4′-*O*-isopropylidene derivative (**4c**, R = OSE). In a similar manner to Hasegawa's method, we synthesized a 3′,4′-*O*-isopropylidene derivative (**4a**, R = OBn)[10] from lactose and then tried the benzoylation of **4a**. The desired product **6a** was isolated in a 45% yield together with the byproducts, the 2,6,6′-*O*-benzoyl derivative **5a**, the 2,3,6,6′-*O*-benzoyl derivative **7a**, and the perbenzoyl derivative **8a**. Compound **5a** would be a precursor for compounds **6a** and **7a**, respectively (Scheme 2). The product ratio of **5a**, **6a**, **7a**, and **8a** was 4:10:3:2 from HPLC data.[11] These reaction conditions for the benzoylation are not sufficient to give the 2,6,2′,6′-*O*-benzoyl derivative **6**, selectively. Therefore, improvements in Hasegawa's method[6a] were pursued to obtain the desired compound **6**, selectively. Namely, we studied several reaction conditions, e.g., the solvent effect, the reaction temperature, and the amount of benzoyl chloride, based on Hasegawa's method, which specified the solvent (CH_2Cl_2), the reaction temperature (−40 °C), and the amount of benzoyl chloride (5 equiv).

Table 1 summarizes the optimization studies for the benzoylation of **4a**. In $CHCl_3$, the formation of **7a** was prior to that of **6a**. In THF and toluene, compound **6a** was preferentially produced 20−100-fold more as compared to **7a**; however, the conversion ratio from **5a** to **6a** seemed to be low (run A). Next, the solvents were chosen to be THF and toluene, and then various reaction

(10) ^{1}H NMR data of the compound **4a**; ^{1}H NMR ($CDCl_3$) δ 1.26 (s, 3H), 1.40 (s, 3H), 3.07−3.14 (m, 1H), 3.20−3.37 (m, 4H), 3.54−3.64 (m, 3H), 3.74−3.86 (m, 2H), 3.95−4.0 (t, 1H, J=7.0 Hz), 4.12 (dd, 1H, J = 1.5, 7.1Hz), 4.30 (dd, 2H, J = 8.0, 9.3 Hz), 4.58 (d, 1H, J = 12.2 Hz), 4.83 (d, 1H, J = 12.2 Hz), 7.26−7.41 (m, 5H).

(11) HPLC condition: column (ODS), elution solvent (0.1% TFA: CH_3CN = 1:5), retention time (min); 4.5 for compound **5a**, 5.7 for compound **7a**, 9.0 for compound **6a**, 11.2 for compound **8a**.

Scheme 3

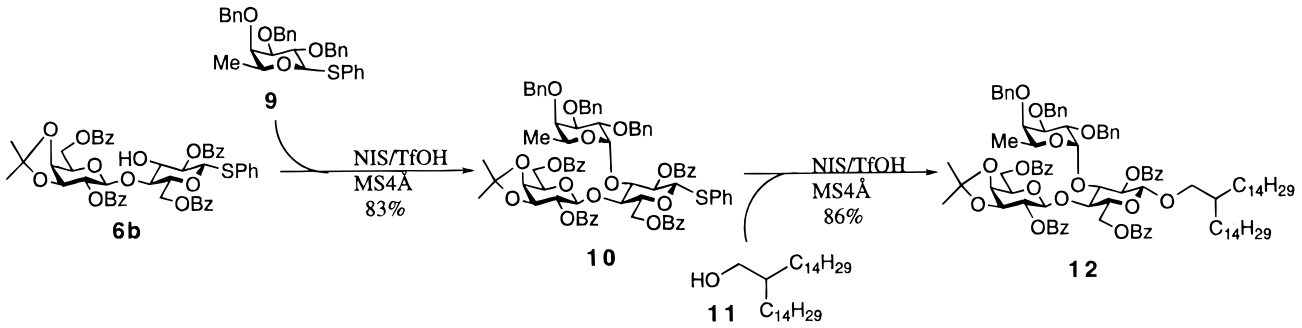


Table 1. Optimization Studies for the Benzoylation of 4a

run	solvent	Bz-Cl (equiv)	temp (°C)	time (h)	product ratio[a] 5a	6a	7a	8a
A[b]	$CHCl_3$	5	−45	3	5.9	1	2.2	0.32
A	THF	5	−45	3	31	1	<0.01	<0.01
A	toluene	5	−45	3	21	1	0.08	<0.01
A	CH_3CN	5	−20	3	19	1	0.33	<0.01
B[c]	THF	5	−45	3	31	1	<0.01	<0.01
B	THF	5	−20	3	5.3	1	0.10	0.01
B	THF	5	0	3	2.5	1	0.10	0.02
B	toluene	5	−45	3	21	1	0.08	<0.01
B	toluene	5	−20	3	5.7	1	0.08	0.02
B	toluene	5	0	3	2.5	1	0.10	0.04
C[d]	THF	5	0	3	2.5	1	0.10	0.02
C	THF	8	0	3	0.57	1	0.18	0.08
C	toluene	5	0	3	1.4	1	0.10	0.04
C	toluene	8	0	3	0.08	1	0.05	0.11

[a] Product ratio was determined by HPLC. HPLC condition: ODS column, 0.1% TFA: CH_3CN = 1:5, see ref 9. [b] Solvent effect. [c] Temperature effect. [d] Amount of effect of benzoyl chloride.

temperatures were individually tested (run B). The ratio of **5a** decreased with increasing reaction temperature in both THF and toluene; however, the ratio of **5a** was still similar to that of **6a**. Then, to accelerate the conversion ratio from **5a** to **6a**, the amount of benzoyl chloride was investigated (run C). As a result, the conversion ratio from **5a** to **6a** increased with increasing benzoyl chloride. Especially, in toluene the desired compound **6a** was obtained selectively. In addition, the reaction of **4a**[10] and **4b**[12] with 8 equiv of benzoyl chloride in toluene at 0 °C afforded the 2,6,2′,6′-*O*-benzoyl derivatives **6a,b** in 73% and 77% yields, respectively.

Glycosylation Using "Armed/Disarmed" Coupling. A "armed/disarmed" principle would be one of the most useful methods for the synthesis of the oligosaccharides, because glycosylation using "armed/disarmed" coupling allows the construction of continuous glycosidic bonds. Fraser-Reid et al.[13] reported that the "armed/disarmed" coupling for glycosylation could have general applicability. There are some examples[14] of glycosylation based on the "armed/disarmed" principle; however, at the present stage little is known concerning the syntheses of the Le^x, sLe^x, and sulfated Le^x derivatives using the "armed/disarmed" method. Therefore, we investigated the practical glycosylation involving the "armed/disarmed" coupling, e.g., fucosylation, followed by the incorporation of an alkyl chain on the 2,6,2′,6′-*O*-benzoyllactose derivative.

Table 2. Glycosylation Using "Armed/Disarmed" Coupling

A. Stepwise Glycosylation

6b (equiv)	**9** (equiv)	temp (°C)	time (h)	**10** (%)[a]
1	1	−20	1	55
1	1.5	−20	1	83
10 (equiv)	**11** (equiv)	temp (°C)	time (h)	**12** (%)[a]
1	2	0	1	53[b]
1	2	−20	1	86

B. One-Pot, Two-Step Glycosylation

6b (equiv)	**9** (equiv)	**11** (equiv)	temp (°C)	time (h)	**12** (%)[a]
1	1.5	2	−20	0.5 + 0.5	66
1	1.5	2	−20	1 + 0.5	80

[a] Isolated yield. [b] Byproduct (**12′**) was obtained together with **12**.

In the present work, we chose a phenyl 2,3,4-tri-*O*-benzyl-thio-β-fucoside (**9**) as the armed donor and a phenyl 2,6,2′,6′-*O*-benzoyl-thio-β-lactoside (**6b**) as the disarmed acceptor, because both compounds were easily prepared and are stable solids (Scheme 3). To produce the desired α-fucoside, the mixture of perbenzylated phenylthiofucoside **9** (1.5 equiv) and **6b** (1 equiv) in chloroform was treated with *N*-iodosuccinimide (NIS, 2.25 equiv) and trifluoromethanesulfonic acid (TfOH, 0.75 equiv) in the presence of 4 Å molecular sieves at −20 °C to isolate the desired α-linked product **10** in an 83% yield (Table 2). This reaction proceeded in a stereospecific manner, because no β-glycoside was isolated. In addition, it was of interest that the glycosyl donor **9** was selectively activated in the presence of the activator, NIS−TfOH. This selective activation is predominantly dependent on the C2 substituents.[13b] Namely, the activation of the phenylthio-fucoside **9**, with a C2 electron-donating group (OCH_2Ph), is prior to that of the phenylthio-lactoside **6b**, with a C2 electron-withdrawing group (OCOPh).

To complete the following glycosidic linkage with a branched alkyl chain (**11**), the glycosylation of the donor

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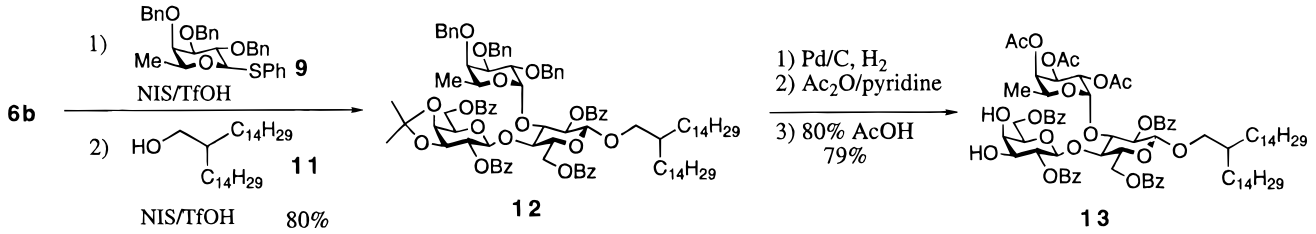
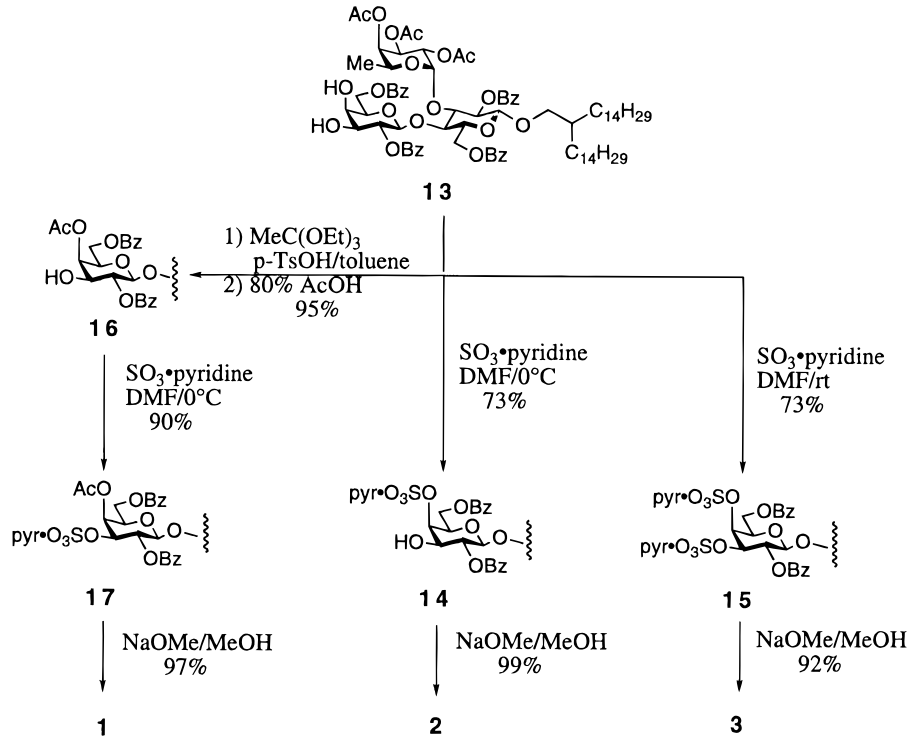
Scheme 4



Scheme 5



10 and the acceptor **11** was investigated. As shown in Table 2, the reaction temperature crucially affected the yield of the desired product **12**. When the glycosylation reaction was carried out at 0 °C, the desired product **12** was isolated in a 53% yield together with the byproduct **12′**, which had cleaved the fucosyl bond from **12**. Thus the trisaccharide **10** was activated with NIS (2 equiv)−TfOH (0.5 equiv) and was added to **11** (2 equiv) in chloroform in the presence of 4 Å molecular sieves at −20 °C. The desired β-linked product **12** was isolated in an 86% yield. This reaction was stereospecifically controlled by the neighboring effect of the C2 benzoyl group of **10** to give only the β-glycoside.

On the other hand, to optimize the glycosylation using the "armed/disarmed" coupling for the construction of the Le^x derivative **12**, a one-pot, two-step glycosylation was investigated. Recently, several methods have been reported to perform sequential glycosylation as a one-pot procedure.[15] Kahne et al.[15a] reported a one-pot glycosylation method that is based on activation of anomeric sulfoxides with Tf_2O or TfOH. Takahashi et al.[15b] reported the synthesis of an elicitor-active hexa-β-D-glucopyranosyl-D-glucitol, based on a one-pot, two-step glycosylation, using a combination of different leaving groups, trichloroacetimidate (-O(CNH)CCl_3) and phenylthio groups (-SPh), and different activators, TMSOTf and NIS−TfOH, respectively. However, there have been few reports concerning the synthesis of Le^x derivatives based on a one-pot, two-step glycosylation using the same leaving group, the SPh group, and the same activator, NIS−TfOH. As a result, the selective activation of the armed donor **9** with NIS (1.5 equiv)-TfOH (0.5 equiv), in the presence of the disarmed acceptor **6b** and 4 Å molecular sieves in chloroform at −20 °C, resulted in the formation of the phenylthio-trisaccharide **10**. To the reaction mixture was added the acceptor **11**, and then another phenylthio group was activated with NIS (2 equiv)−TfOH (0.5 equiv) at the same temperature to give the desired product **12** in an 80% yield, based on the perbenzylfucoside **9**. Hydrogenolysis of the benzyl groups in the product **12** and subsequent acetylation with Ac_2O in pyridine, followed by treatment with 80% AcOH, afforded the 3′,4′-dihydroxyl derivative **13** in a 79% yield (Scheme 4).

Selective Sulfation. Regioselective sulfation has been reported previously.[6] For example, chemical syntheses have involved extensive protection methods to produce a hydroxyl group for sulfation and have used lengthy syntheses. Recently, Flitsch et al.[16] reported the regioselective sulfation of disaccharides using dibutyl-

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stannylene acetals. This methodology could be useful for the selective activation of the 3′-hydroxyl group of a sugar. However, from a practical point of view, a regioselective sulfation without an organometal, such as organotin, would be a more useful method. In addition, the C3 position of the galactosyl residue seems to be relatively reactive as compared to the C4 position of galactose.[17] Therefore, we investigated the regioselective synthesis of a 3′-sulfated Le^x from the 3′,4′-diol trisaccharide **13** with a sulfate reagent, a sulfur trioxide/pyridine complex. When compound **13** was reacted with the sulfur trioxide/pyridine complex in DMF at 0 °C, it was interesting to note that the less reactive C4 hydroxyl group was sulfated to afford the 4′-sulfated Le^x derivative **14** in a 73% yield. This reaction was a regioselective sulfation, because neither the 3′-sulfated Le^x derivative nor the 3′, 4′-disulfated Le^x **15** were isolated. When a similar sulfation of compound **13** was carried out at room temperature, only the 3′,4′-disulfated Le^x **15** was isolated, in a 73% yield. On the other hand, to produce selectively the 3′-sulfated Le^x derivative **17** from **13**, compound **13** was first selectively acetylated at the C4 position according to the previous report,[18] to provide the 4′-acetyl Le^x derivative **16** in a 95% yield. Subsequently, compound **16** was reacted with the sulfur trioxide/pyridine complex in DMF at 0 °C to afford the 3′-sulfated Le^x **17** in a 90% yield. These sulfated derivatives, **17**, **14**, and **15**, were hydrolyzed under basic conditions to afford compounds **1**, **2**, and **3**, respectively.

In conclusion, we succeeded in establishing a highly practical synthesis by the combination of three key reactions: (1) a selective benzoylation, (2) a glycosylation using "armed/disarmed" coupling, and (3) a selective sulfation. Especially, the "armed/disarmed" method allowed the first synthesis of Le^x derivatives by a one-pot, two-step glycosylation. Thus, this synthetic methodology could be an applicable and useful technique for the construction of Le^x and sulfated Le^x derivatives.

Experimental Section

Specific rotations were determined with a Jasco DIP-370 digital polarimeter at 25 °C. Melting points were determined with a Buchi capillary melting point apparatus, Model 535; all melting points are uncorrected. ^{1}H NMR spectra were recorded on a spectrometer with TMS as an internal reference in a solution of $CDCl_3$ and DMSO-d_6. Mass spectra were recorded using a MALDI-TOF Voyager-RP (PerSeptive Biosystems) with a negative mode. Elemental analyses were performed with a Yanagimoto CHN-CORDER MT-3.

Phenyl *O*-(2,6-Di-*O*-benzoyl-3,4-*O*-isopropylidene-*β*-D-galactopyranosyl)-(1−4)-2,6-di-*O*-benzoyl-1-thio-*β*-D-glucopyranoside (6b). To a solution of **4b** (1.0 g, 2.1 mM) and pyridine (15 mL) in toluene (20 mL) was added benzoyl chloride (2.41 g, 16.8 mM), and the mixture was stirred at 0 °C. The course of the reaction was monitored by TLC. After the reaction, methanol was added, and the mixture was concentrated. The residue was dissolved in AcOEt, and the solution was successively washed with water, saturated $NaHCO_3$, 1 M HCl, and water, dried with $MgSO_4$, and then concentrated. The preparative TLC (1:3 AcOEt−cyclohexane, twice) of the residue gave **6b** (1.44 g, 77%) as a coloress solid: $[\alpha]_D$ 14.5° (*c* = 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.35, 1.63 (2s, 6H, Me_2C), 3.6−3.8 (m, 2H), 4.00 (t, 1H, *J* = 8.0Hz, H-3 of Glu), 4.15−4.32 (m, 3H), 4.35−4.50 (m, 3H), 4.57 (d, 1H, *J* = 1.4 Hz, H-4 of Gal), 4.65 (d, 1H, *J* = 8.1 Hz ,H-1 of Glu), 4.72 (d, 1H, *J* = 10.1 Hz, H-1 of Gal), 4.86 (dd, 1H, *J* = 2.7,9.7 Hz), 5.18 (dd, 1H, *J* = 9.2 Hz, H-2 of Gal), 5.34 (t, 1H, H-2 of Glu), 7.00−8.20 (m, 25H, 5Ph). Anal. Calcd for $C_{49}H_{46}O_{14}S$ (890.95): C, 66.06; H, 5.20. Found: C, 65.66 ; H, 5.16.

Benzyl *O*-(2,6-Di-*O*-benzoyl-3,4-*O*-isopropylidene-*β*-D-galactopyranosyl)-(1−4)-2,6-di-*O*-benzoyl-1 -*β*-D-glucopyranoside (6a). According to the synthesis of **6b**, **6a** was isolated in 73% yield: mp 166−168 °C; $[\alpha]_D$ −4.0° (*c* = 0.26, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.34 (s, 3H), 1.62 (s, 3H), 3.55−3.80 (m, 2H), 3.94 (dd, *J* = 8.2, 9.5 Hz, 1H), 4.15−4.30 (m, 3H), 4.35−4.50 (m, 3H), 4.54 (d, *J* = 12.6 z, 1H), 4.66 (d, *J* = 8.1 Hz, 1H), 4.76 (d, *J* = 12.6 Hz, 1H), 5.28 (dd, *J* = 8.1, 9.5 Hz, 1H), 5.34 (t, *J* = 7.5 Hz, 1H), 7.0−8.15 (m, 25H, Ph-H). Anal. Calcd for $C_{50}H_{48}O_{15}$ (888.92): C, 67.56; H, 5.44. Found: C, 67.23 ; H, 5.52.

Phenyl *O*-(2,6-Di-*O*-benzoyl-3,4-*O*-isopropylidene-*β*-D-galactopyranosyl)-(1−4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1−3)]-2,6-di-*O*-benzoyl-1-thio-*β*-D-glucopyranoside (10). To a solution of **6b** (600 mg, 0.67 mM) and **9**[19] (540 mg, 1.03 mM) in $CHCl_3$ (6 mL) was added 4 Å molecular sieves (MS-4Å, 960 mg), and the mixture was stirred for 6 h at room temperature and then cooled to −20 °C. *N*-Iodosuccinimide (NIS, 350 mg) and trifluoromethanesulfonic acid (TfOH, 77 mg) were added to the mixture, and this was stirred for 1 h at −20 °C. The precipitate was filtered off, and the filtrate was successively washed with aqueous $NaHCO_3$, aqueous $Na_2S_2O_3$ and water, dried ($MgSO_4$), and concentrated. Column chromatography (2:7 AcOEt−hexane) of the residue on silica gel gave **10** (730 mg, 83%) as an amorphous mass: $[\alpha]_D$ −3.5° (*c* = 0.17, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.30 (d, 3H, *J* = 6.2 Hz, H-6 of Fuc), 1.31, 1.50 (2s, 6H, Me_2C), 3.7−3.8 (m, 1H, H-4 of Fuc), 3.90 (dd, 1H, *J* = 3.7 Hz, *J* = 10.1 Hz, H-2 of Fuc), 4.00 (t, 1H, *J* = 9.5 Hz, H-3 of Fuc), 4.50 (d, 1H, *J* = 8.8 Hz, H-1 of Glu), 4.67 (d, 1H, *J* = 7.4 Hz, H-1 of Gal), 5.23 (dd, 1H, *J* = 8.7 Hz, H-2 of Gal), 5.39 (d, 1H, H-1 of Fuc), 5.43 (dd, 1H, H-2 of Glc), 6.94−8.20 (m, 40H, 8Ph). Anal. Calcd for $C_{76}H_{74}O_{18}S$ (1307.5): C, 69.82; H, 5.70. Found: C, 69.76 ; H, 5.61.

2-Tetradecylhexadecyl *O*-(2,6-Di-*O*-benzoyl-3,4-*O*-isopropylidene-*β*-D-galactopyranosyl)-(1−4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1−3)]-2,6-di-*O*-benzoyl-*β*-D-glucopyranoside (12). To a solution of **11**[20] (260 mg, 0.59 mM) and **10** (387 mg, 0.30 mM) in $CHCl_3$ (3.3mL) was added 4 Å molecular sieves (MS-4Å, 390 mg), and the mixture was stirred for 8 h at room temperature and then cooled to −20 °C. NIS (200 mg) and TfOH (36 mg) were added to the mixture, and this was stirred for 1 h at −20 °C. The precipitate was filtered off and the filtrate was successively washed with aqueous $NaHCO_3$, aqueous $Na_2S_2O_3$, and water, dried ($MgSO_4$), and concentrated. Column chromatography (1:6 AcOEt−hexane) of the residue on silica gel gave **12** (416mg, 86%) as an amorphous mass: $[\alpha]_D$ +1.9° (*c* = 0.58, $CHCl_3$): ^{1}H NMR ($CDCl_3$) δ 0.75−0.90 (m, 6H, $2MeCH_2$), 1.00−1.50 (m, 59H), 3.07 (dd, 1H, J_{gem} = 9.4 Hz, $J_{1,2}$ = 6.6 Hz, H-1 of lipophilic part), 3.65 (dd, 1H, $J_{1',2}$ = 5.0 Hz, H-1′ of lipophilic part), 3.70−3.85 (m, 2H), 3.91 (dd, 1H, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 10.2 Hz, H-2 of Fuc), 4.22 (dd, 1H, $J_{3,4}$ = 3.7 Hz, H-3 of Fuc), 4.24 (m, 1H, H-3 of Gal), 4.27 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.6 Hz, H-3 of Glc), 4.36 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1 of Glc), 4.49 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1 of Gal), 4.87 (m, 1H, H-5 of Fuc), 5.24 (t, 1H, $J_{1,2} = J_{2,3}$ = 7.7 Hz, H-2 of Gal), 5.40 (dd, 1H, H-2 of Glc), 5.43 (d, 1H, H-1 of Fuc), 6.85−8.20 (m, 35H, 7Ph). Anal. Calcd for $C_{100}H_{130}O_{19}$ (1636.1): C, 73.41; H, 8.01. Found: C, 73.30; H, 7.86.

2-Tetradecylhexadecyl *O*-(2,6-Di-*O*-benzoyl-3,4-*O*-isopropylidene-*β*-D-galactopyranosyl)-(1−4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1−3)]-2,6-di-*O*-benzoyl-*β*-D-glucopyranoside (13′). A solution of **12** (386 mg, 0.24 mM) in methanol (40 mL) and 1,4-dioxane (40 mL) was stirred for 40 h at room temperture in the presence of 10% Pd/C (500 mg) under hydrogen, then filtered, and concentrated. A solution of the residue in pyridine (30 mL) and Ac_2O (15 mL) was

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stirred for 42 h at room temperature. Methanol was added, and the mixture was concentrated. The residue was dissolved in $CHCl_3$ (50 mL), and the solution was successively washed with 2 M HCl and water, dried ($MgSO_4$) and then concentrated. Preparative TLC (1:3 AcOEt−hexane) of the residue gave **13′** (315 mg, 90%) as an amorphous mass: $[\alpha]_D$ −16.7° (*c* = 0.15, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.70−0.95 (m, 6H, $2MeCH_2$), 1.00−1.50 (m, 53H), 1.32 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6 of Fuc), 1.59, 1.64 (2s, 6H, Me_2C), 1.84−2.17 (3s, 9H, 3AcO), 3.00−3.20 (m, 1H), 3.60−3.75 (m, 1H), 3.75−3.90 (m, 1H), 4.35 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1 of Glc), 4.51 (d, 1H, $J_{1,2}$ = 7.4 Hz, H-1 of Gal), 5.26 (dd, 1H, $J_{2,3}$ = 8.6 Hz, H-2 of Gal), 7.20−8.30 (m, 20H, 4Ph). Anal. Calcd for $C_{85}H_{118}O_{22}.2H_2O$ (1527.9): C, 66.82; H, 8.05. Found: C, 66.70; H, 7.63.

2-Tetradecylhexadecyl *O*-(2,6-Di-*O*-benzoyl-*β*-D-galactopyranosyl)-(1−4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1−3)]-2,6-di-*O*-benzoyl-*β*-D-glucopyranoside (13). A solution of **13′** (3.3 g, 2.21 mM) in 80%AcOH was stirred for 4 h at 80 °C and then concentrated. Column chromatography (5:4 AcOEt−hexane) of the residue on silica gel gave **13** (2.81 g, 88%) as an amorphous mass: $[\alpha]_D$ +34.8° (*c* = 0.25, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.75−0.95 (m, 6H, $2MeCH_2$), 1.00−1.45 (m, 53H), 1.29 (d, 3H, $J_{5,6}$ = 6.8 Hz, H-6 of Fuc), 1.89−2.10 (3s, 9H, 3AcO), 2.80 (d, 1H, $J_{3,OH}$ = 9.0 Hz, H- 3-OH), 3.11 (dd, 1H, J_{gem} = 9.6 Hz, $J_{1,2}$ = 6.4 Hz, H-1 of lipophilic part), 3.35−3.80 (m, 5H), 4.37 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1 of Glc), 4.65 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1 of Gal), 4.87(dd,1H, *J* = 11.7 Hz), 5.00−5.15 (m, 2H), 5.15−5.45 (m, 5H), 7.30−8.20 (m, 20H, 4Ph). Anal. Calcd for $C_{82}H_{114}O_{22}$ (1451.8): C, 67.84; H, 7.91. Found: C, 67.47; H, 7.77.

2-Tetradecylhexadecyl *O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-*β*-D-galactopyranosyl)-(1−4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1−3)]-2,6-di-*O*-benzoyl-*β*-D-glucopyranoside (16). To a solution of **13** (2.5 g, 1.7 mM) in toluene (125 mL) was added triethyl orthoacetate (125 mL), and the mixture was stirred for 1 h at room temperature. The solution was neutralized with triethylamine and concentrated. A solution of the residue in 80%AcOH was stirred for 40 min at room temperature and then concentrated. Column chromatography (2:3 AcOEt−hexane) of the residue on silica gel gave **16** (2.5g, 95%) as an amorphous mass: $[\alpha]_D$ −39.9° (*c* = 0.16, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.85−0.91 (m, 6H, $2MeCH_2$), 1.10−1.40 (m, 52H, $26CH_2$), 1.34 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6 of Fuc), 1.87, 1.88, 2.11, 2.25 (4s, 12H, 4AcO), 3.11 (dd, 1H, J_{gem} = 9.5 Hz, $J_{1,2}$ = 6.4 Hz, H-1 of lipophilic part), 3.6−3.8 (m, 2H), 3.85−3.91 (m, 1H, H-3 of Gal), 4.38 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1 of Glc), 4.67 (d, 1H, $J_{1,2}$ = 8.2 Hz, H-1 of Gal), 5.06 (dd, 1H, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 4.0 Hz, H-3 of Fuc), 5.40 (d, 1H, $J_{3,4}$ = 3.6 Hz, H-4 of Gal), 5.47 (d, 1H, $J_{1,2}$ = 2.8 Hz, H-1 of Fuc), 7.30−8.20 (m, 20H, 4Ph). Anal. Calcd for $C_{84}H_{116}O_{23}$ (1493.8): C, 67.54; H, 7.83. Found: C, 67.54; H, 7.72.

2-Tetradecylhexadecyl *O*-(3-*O*-Sulfo-*β*-D-galactopyranosyl)-(1−4)-*O*-[(α-L-fucopyranosyl)-(1−3)]-*β*-D-glucopyranoside Sodium Salt (1). To a solution of **16** (2.41 g, 1.6 mM) in DMF (25 mL) was added sulfur trioxide−pyridine complex (2.6 g, 16 mM), and the mixture was stirred for 1 h at room temperature. The course of the reaction was monitored by TLC. Methanol was added, and the mixture was concentrated at 25 °C to afford **17** (2.4 g, 90%) as an amorphous mass: $[\alpha]_D$ −2.2° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.85−1.31 (m, 58H, 2Me, $26CH_2$), 1.38 (d, 3H, *J* = 6.2 Hz, H-6b), 1.74−2.35 (4s, 12H, 4AcO), 4.37 (d, 1H, *J* = 7.7 Hz, H-1a), 7.09−8.05 (m, 25H, 4Ph, pyridine). To a solution of **17** (2.4 g, 1.6 mM) in MeOH (50 mL) and THF (50 mL) was added sodium methoxide (170 mg), and the mixture was stirred for 24 h at room temperature and then concentrated at 25 °C. Column chromatography (5:4:0.7, $CHCl_3$−MeOH−H_2O) of the residue on Sephadex LH-20 gave **1** (1.42 g, 97%) as an amorphous mass: $[\alpha]_D$ −30° (*c* = 0.8, 1:1, MeOH−$CHCl_3$); ^{1}H NMR (C_5D_5N) δ 0.85−1.31 (m, 58H, 2Me, $26CH_2$), 1.54 (d, 3H, *J* = 6.4 Hz, H-6b), 5.06 (d, 1H, *J* = 2.7 Hz, H-1b), 5.21 (dd, *J* = 10.1, 3.8 Hz, H-3c), 5.41 (d, 1H, *J* = 7.3 Hz, H-1a), 5.48 (d, 1H, *J* = 6.7 Hz, H-1c). The mass spectrum of **1** showed the base peak at *m*/*z* 987 (M − 1 − Na).

2-Tetradecylhexadecyl *O*-(4-*O*-Sulfo-*β*-D-galactopyranosyl)-(1−4)-*O*-[(α-L-fucopyranosyl)-(1−3)]-*β*-D-glucopyranoside Sodium salt (2). According to the similar procedure to the compound **1**, compound **2** (30 mg, 72%) was prepared as a colorless powder: mp 194−195 °C (decomposed); ^{1}H NMR (DMSO-d_6) δ 0.7−0.9 (m, 6H), 1.06 (d, 3H, *J* = 6.4 Hz), 1.1−1.6 (m, 53H), 3.1−3.3 (m, 4H), 3.3−3.8 (m, 12H), 3.96 (d, 1H, *J* = 4.1 Hz), 4.0−4.3 (m, 4H), 4.29 (d, 1H, *J* = 7.8 Hz), 4.42 (d, 1H, *J* = 3.0 Hz), 4.45−4.65 (m, 2H), 4.66 (d, 1H, *J* = 5.9 Hz), 5.0−5.1 (m, 2H), 5.12 (d, 1H, *J* = 3.5 Hz). The mass spectrum of **2** showed the base peak at *m*/*z* 987 (M − 1 − Na)$^-$.

2-Tetradecylhexadecyl *O*-(3,4-*O*-Disulfo-*β*-D-galactopyranosyl)-(1−4)-*O*-[(α-L-fucopyranosyl)-(1−3)]-*β*-D-glucopyranoside Sodium Salt (3). According to the similar procedure to the compound **1**, compound **3** (80 mg, 67%) was prepared as a colorless powder: mp 201−203 °C (decomposed); ^{1}H NMR (DMSO-d_6) δ 0.7−0.9 (m, 6H), 1.06 (d, 3H, *J* = 6.4 Hz), 1.1−1.6 (m, 53H), 3.1−3.3 (m, 3H), 3.3−3.9 (m, 13H), 3.99 (d, 1H, *J* = 6.9 Hz), 4.0−4.3 (m, 3H), 4.4 (d, 1H, *J* = 7.7 Hz), 4.4−4.7 (m, 4H), 5.0 (d, 1H, *J* = 4.9 Hz), 5.1−5.2 (m, 2H). The mass spectrum of **3** showed the base peak at *m*/*z* 1089 (M − 1 − Na)$^-$.

One-Pot, Two-Step Glycosylation. To a solution of **6b** (100 mg, 0.11 mM) and **9** (89 mg, 0.17 mM) in $CHCl_3$ (1 mL) was added 4 Å molecular sieves (160 mg), and the mixture was stirred for 2 h at room temperature and then cooled to −20 °C. NIS (57 mg) and TfOH (8 μL) were added to the mixture, and this was stirred for 30 min at −20 °C. To the reaction mixture was added a solution of **11** (100 mg, 0.23 mM) in $CHCl_3$ (1 mL), NIS (50 mg), and TfOH (5 μL). The reaction mixture was stirred for 30 min at −20 °C. The course of the reaction was monitored by TLC. The precipitate was filtered off and the filtrate was successively washed with aqueous $Na_2S_2O_3$, aqueous $NaHCO_3$, and water, dried with $MgSO_4$, and concentrated. Preparative TLC (AcOEt:hexane = 1:3) of the residue gave **12** (147 mg, 80%) as an amorphous mass.

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