Pergamon

Bioorganic & Medicinal Chemistry 6 (1998) 587–593

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis of Sialyl Lewis X Pentasaccharide Analogue for High-throughput Screening of Selectin Blockers

Takao Kiyoi,[a] Yoshimasa Inoue,[b] Hiroshi Ohmoto,[b] Masahiro Yoshida,[a] Makoto Kiso[c] and Hirosato Kondo[a,*]

[a]*Department of Medicinal Chemistry, Kanebo, New Drug Research Laboratories, 1-5-90 Tomobuchi-Cho, Miyakojima-Ku, Osaka 534, Japan*
[b]*Department of Molecular Biology, Kanebo, New Drug Research Laboratories, 1-5-90 Tomobuchi-Cho, Miyakojima-Ku, Osaka 534, Japan*
[c]*Department of Applied Bioorganic Chemistry, Gifu University, Gifu 501-11, Japan*

Received 23 October 1997; accepted 22 December 1997

Abstract—We have developed an effective synthesis of sLe^x pentasaccharide glycolipid analogue **2**. As a part of application of sLe^x pentasaccharide glycolipid **2** synthesized here, we have investigated the construction of a high-throughput screening system for discovery of selectin blockers. As a result, it was found that compound **2** was a useful ligand for in vitro ELISA assay and could be an important material for high-throughput screening of selectin blockers.

Introduction

Selectins are a family of cell adhesion molecules which participate in the initial phase of adhesion between leukocytes and endothelial cells, and play an important role in leukocyte infiltration into tissue in inflammatory reactions.[1] Recent studies have indicated that the ligand recognized by selectins was a carbohydrate,[2] e.g. sialyl Lewis X (sLe^x), and a selectin-carbohydrate interaction occurred at the very early stage of inflammatory reaction called 'rolling'.[3] Selectins are expressed on a variety of cell surfaces. For example, E-selectin is expressed on vascular endothelial cells during inflammation.[4] P-selectin is expressed on platelets and vascular endothelial cells,[5] and L-selectin is an adhesion molecule expressed on leukocytes.[6] The role of selectins in diseases has been gradually evident through the several experiments using mAb[7] and/or knockout mice[8] of selectins. In addition, recently it has been reported that selectins played an important role in angiogenesis and a selectin blocker inhibited angiogenesis in vitro and in vivo.[9] Such a integral event in vivo makes the selectins attractive targets for the therapy of these diseases.

In a series of study of selectin blockers, we have recently reported a useful in vitro ELISA assay[10] for high-throughput screening of selectin blockers, and using this ELISA assay system, we have already discovered some potent selectin blockers in vitro and in vivo.[11] For the effective research of selectin blockers, one of the most important things is to construct in vitro ELISA assay system which involves an inhibitory activities of selectin blockers toward sLe^x-selectin binding. However, our ELISA assay system reported previously[10] has some points to improve, because a synthetic sLe^x pentasaccharide linked to natural ceramide (**1**) necessary for ELISA assay is difficult and expensive to produce, at least for now. Especially, the sLe^x pentasaccharide and natural ceramide require several complicated and expensive synthesis steps. Therefore, to establish more practical in vitro ELISA assay system we should overcome the issue, how easy to make sLe^x pentasaccharide analog. For a start, we focused on the synthesis of simpler sLe^x glycolipid analog. Namely, we selected a 3-tridecylhexadecyl group as an alternative to a ceramide. The chain length of this group seems to be slight shorter than the natural ceramide, and it has not yet been reported whether the natural ceramide of sLe^x glycolipid could be replaced by a simple alkyl chain such as 3-tridecylhexadecyl group.

By the way, the 3-tridecylhexadecyl group is commercially available and would be quite readily introduced to the anomeric position of sLe^x.[12] Therefore, the

*Corresponding author.

0968-0896/98/$19.00
PII: S0968-0896(98)00003-0

replacement of natural ceramide to simpler chain such as 3-tridecylhexadecyl group would remarkably support the efficient ELISA assay system, especially for the high throughput screening. Our goal is an effective construction method of sLe^x pentasaccharide and a new finding of ceramide analogue instead of the natural ceramide.

In this paper, we describe the effective synthesis of 3-tridecylhexadecyl-sLe^x pentasaccharide **2**, which would be easier to make than sLe^x pentasaccharide linked to natural ceramide **1**, and the comparison of the ligand avidity of compounds **1** and **2** using the known selectin blocker.

Results and Discussion

Chemistry

We have already reported[13] the synthesis of sLe^x pentasaccharide **3**, which involves the quantitative construction of a tetrasaccharide, NeuAcα(2→3) Galβ(1→4) GlcNAcβ(1→3) GalβOEt, and an effective incorporation of fucose moiety toward the tetrasaccharide. As a part of the application of this synthetic method, we investigated a synthesis of sLe^x pentasaccharide glycolipid analogue **2**, which would be a useful material for in vitro ELISA assay.

As shown in Scheme 1, GlcNAcβ(1→3)GalβOSE acceptor **6** was obtained from disaccharide **5**[14] by the combination of protection and/or deprotection of the hydroxyl groups according to the established methods, i.e. 4-methoxybenzylation of the free hydroxyl group and selective cleavage of 4′,6′-*O*-benzylidene moiety. The glycosylation of **6** with the NeuAcα(2→3)GalβSMe donor **7**[13] in the presence of dimethyl(methylthio) sulfonium triflate[15] (DMTST) as a promoter gave the tetrasaccharide **8** in 77% yield. Removal of the 4-methoxybenzyl (MPM) group of **8** gave the tetrasaccharide **9** quantitatively, which was glycosylated with a fucose donor **10**[13] to provide the sLe^x pentasaccharide **11** in an 82% yield.

We next investigated the incorporation of a branched alkyl chain to the sLe^x pentasaccharide skeleton. As shown in Scheme 2, the hydrogenolysis of the benzyl groups of **11** in the presence of a catalytic amount of 10% Pd-C, followed by the acetylation of free hydroxyl groups in the presence of acetic anhydride and pyridine afforded a compound **12** in a moderate yield. The removal of 2-(trimethylsilyl)ethyl (SE) group of **12** with trifluoroacetic acid was followed by the treatment with trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in chloroform at 0 °C to afford the glycosyl donor **13** in a good yield. The glycosylation of 3-tridecylhexadecanol **14**[12] with the trichloroacetimidate donor **13**, in dichloromethane in the presence of boron trifluoride etherate, gave exclusively the β-glycoside **15**, in a 74% yield, because of the neighboring effect of the C-2 acetyl group of donor **13**. Finally, the compound **15** was transformed quantitatively, by the removal of the protecting groups, into the desired compound **2**.

Biological activities: Investigation of in vitro ELISA assay using 3-tridecylhexadecyl-sLe^x pentasaccharide 2

According to our previous paper,[10] we established the ELISA inhibition assay system (Figure 1; Schematic diagram) using the selectin-Ig chimera and 3-tridecylhexadecyl- sLe^x pentasaccharide **2**, and evaluated the known selectin blocker **4** (Figure 2). As shown in Table 1, compound **4** gave the similar IC_{50} values toward E-selectin/ ligands **1** and/or E-selectin/ligand **2** bindings (14 μM for ligand **1** and 16 μM for ligand **2**). In addition, against the P- and L-selectins, each sLe^x pentasaccharide **1** and **2** gave the quite similar IC_{50} values toward **4**, 46 μM, 49 μM for P-selectin and 22 μM, 21 μM for L-selectin, respectively. As shown in Table 1, both compounds **1** and **2** had very closed ligand avidity toward E-, P-, and L-selectins binding in spite of having a different chains, natural ceramide and artificial branched alkyl chain, respectively. Namely, although the chain length of 3-tridecylhexadecyl group is a little bit shorter than that of natural ceramide, it was proved that 3-tridecylhexadecyl group was enough length for coating on the well and did not affect the selectin binding.

In conclusion, we succeeded in an effective synthesis of sLe^x glycolipid analogue **2**. In addition, we have constructed an ELISA assay system using the sLe^x glycolipid **2** and evaluated inhibitory activities of the known selectin blocker. As a result, it was found that compound **2** could also be a useful ligand for the in vitro ELISA assay and our ELISA assay system constructed here was a convenient evaluation system for discovery

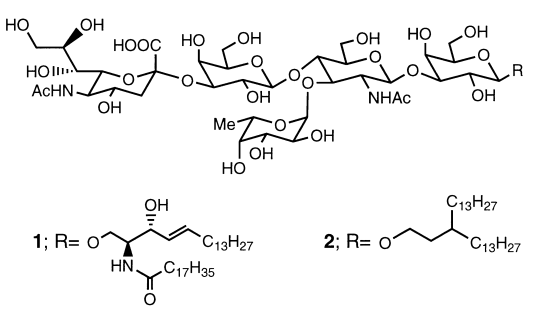
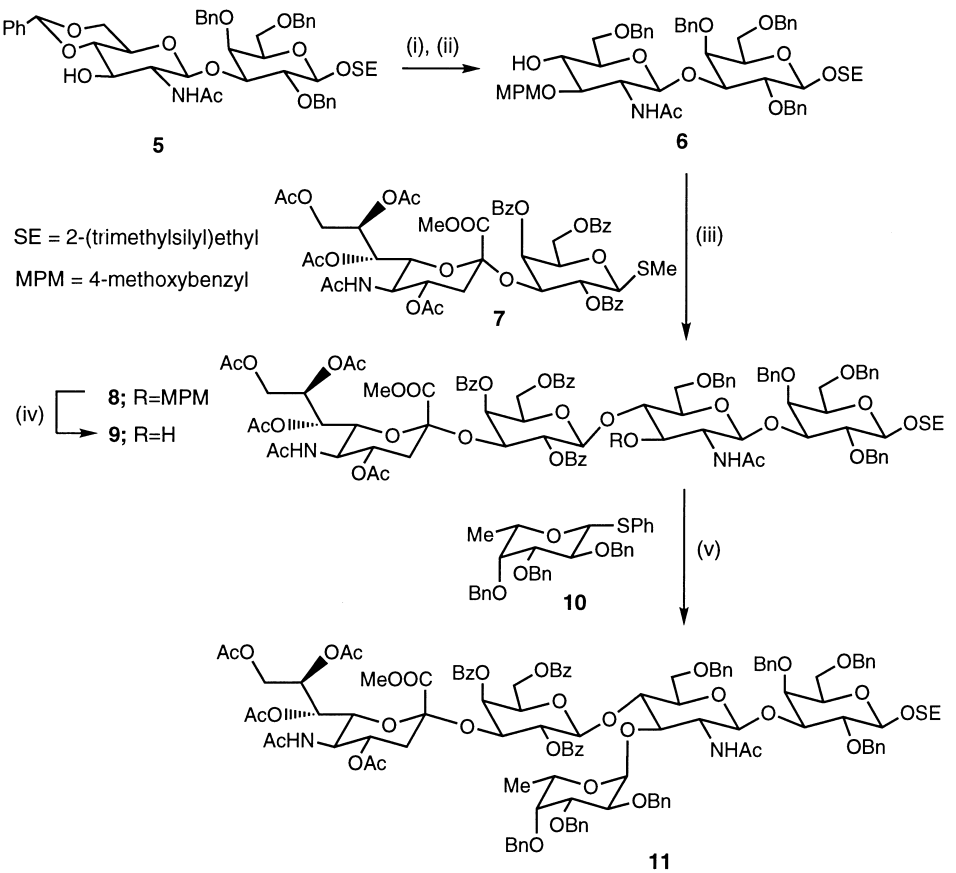
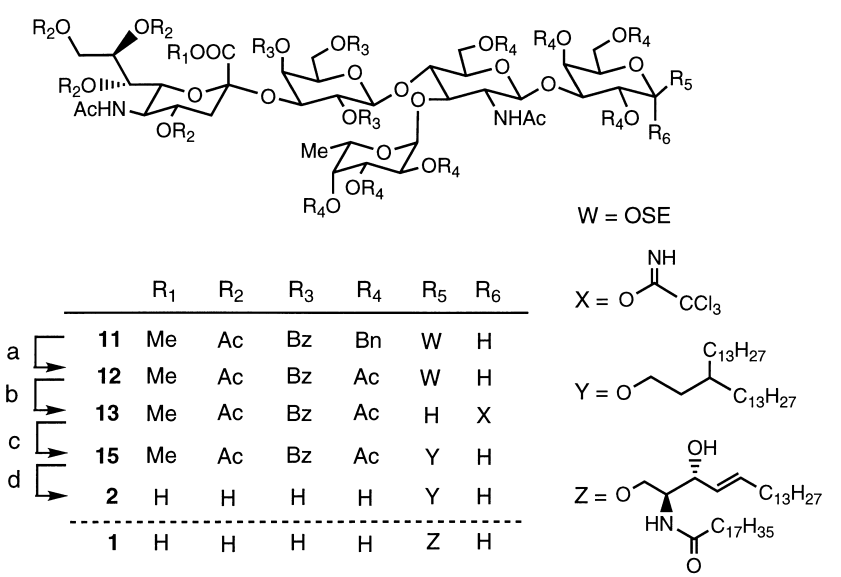


Chart 1.



Scheme 1. Reagents and conditions: (i) NaH, MPMCl, DMF (100%); (ii) $NaBH_3CN$/HCl (53%); (iii) DMTST, CH_2Cl_2 (77%); (iv) $Ce(NH_4)_2(NO_3)_6$, CH_3CN (100%); (v) NIS/TfOH, CH_2Cl_2 (82%).



		R_1	R_2	R_3	R_4	R_5	R_6
a	**11**	Me	Ac	Bz	Bn	W	H
b	**12**	Me	Ac	Bz	Ac	W	H
c	**13**	Me	Ac	Bz	Ac	H	X
d	**15**	Me	Ac	Bz	Ac	Y	H
	2	H	H	H	H	Y	H
	1	H	H	H	H	Z	H

Scheme 2. Reagents: (a) H_2/10%Pd/C, Ac_2O/pyridine, DMAP (75%); (b) (i) CF_3CO_2H, $CHCl_3$ (93%), (ii) CCl_3CN/DBU, $CHCl_3$ (85%); (c) 3-tridecylhexadecanol (**14**), BF_3/OEt_2, CH_2Cl_2 (74%); (d) NaOMe, MeOH (100%).

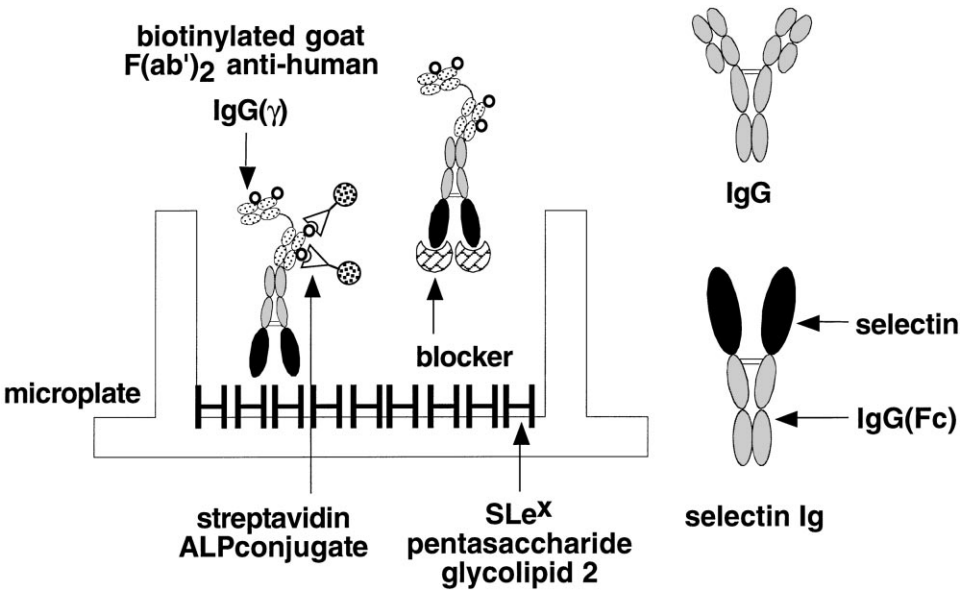


Figure 1. Schematic diagram of ELISA inhibition assay using selectin-Ig chimera. For details see Experimental.

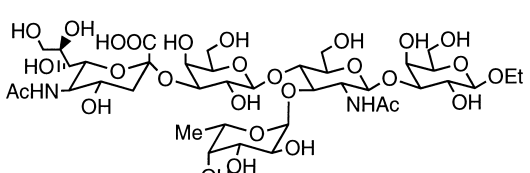
of selectin blockers. These findings indicate that sLe^x glycolipid **2** could be an important material for high-throughput screening of selectin blockers.

Experimental

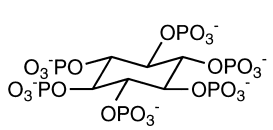
In vitro ELISA assay of E-, P-, and L-selectin - sLe^x binding

The construction of the selectin-immunoglobulin was carried out according to the previous paper.[2f]

A solution of sLe^x pentasaccharide ceramide **1** or 1-(3-tridecyl)hexadecyl sLe^x pentasaccharide **2**, respectively, in a 1:1 mixture of methanol and distilled water, was pipetted into microtiter plate wells (96 wells, Falcon PRO-BIND) at 100 pmol/50 μL/well, and was adsorbed by evaporating the solvent. The wells were blocked with 5% BSA (bovine serum albumin) −1 mM $CaCl_2$/50 mM imidazole buffer (pH 7.2) for 1 h at room temperature, and washed three times with 50 mM imidazole buffer (pH 7.2).



3



4

Figure 2. Selectin blockers (**3**,**4**).

Separately, the following were added to 1% BSA −1 mM $CaCl_2$/50 mM imidazole buffer (pH 7.2): (a) 1:500 dilution of biotinylated goat $F(ab')_2$ anti-human IgG(γ) (BIOSOURCE); (b) 1:500 dilution of streptavidin-alkaline phosphatase (Zymed Lab Inc.); and (c) 10 μg/ml E-selectin IgG chimera, 20 μg/ml P-selectin IgG chimera, or 5 μg/ml L-selectin IgG chimera to form a complex. The test compound (**4**) was dissolved in distilled water at 10 mM and diluted by 1% BSA −1 mM $CaCl_2$/50 mM imidazole buffer (pH 7.2) to final concentrations at 200, 100, 50, 25, 12.5, 6.25, 3.13, and 1.56 μM, respectively. Reactant solutions were prepared by incubating 30 μL of this solution at each concentration with 30 μL of the above complex solution for 30 min at room temperature. This reactant solution was then added to the above microtiter wells at 50 μL/well and incubated at 37 °C for 45 min. The wells were washed three times with 50 mM imidazole buffer (pH 7.2) and distilled water respectively, followed by addition of p-nitrophenylphosphate (1 mg/mL) and 0.01% of $MgCl_2$ in 1 M diethanolamine (pH 9.8) at 50 μL/well. The reactant mixture was developed for 120 min at room temperature and absorbance at 405 nm was

Table 1. Blocking activity of compound **4** using the glycolipids **1** and **2**

	IC_{50}, μM					
	E-selectin		P-selectin		L-selectin	
Compd/glycolipids	**1**	**2**	**1**	**2**	**1**	**2**
4	14	16	46	49	22	21

measured. Percent binding was calculated by the following equation:

$$\% \text{ Binding} = (X - C/A - C) \times 100$$

wherein X is the absorbance of wells containing the test compound at each concentration, C is the absorbance of wells not containing the selectin-Ig and test compound, and A is the absorbance of control wells not containing the test compound. The results of inhibitory activities are presented in Table 1 as IC_{50} values. The number of replicates is two.

2-(Trimethylsilyl)ethyl-*O*-[2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-(4-methoxybenzyl)-β-D-glucopyranosyl]-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (6). To a solution of 2-(trimethylsilyl)ethyl *O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside(**5**)[14] (925 mg, 1.10 mmol) in DMF (4.0 mL) was added a suspension of sodium hydride in oil (44 mg, 60% of sodium hydride by weight). The mixture was stirred for 20 min at 0 °C, 4-methoxybenzyl chloride (194 μL, 1.43 mmol) was add dropwise, and stirring was continued for 4 h at room temperature. Moreover, a suspension of sodium hydride in oil (22 mg, 60% of sodium hydride by weight) was add to the reaction mixture, and stirring was continued for 6 h at room temperature. The reaction mixture was cooled to 0 °C, and methanol (2 mL) and ethyl acetate (20 mL) were added, then the mixture was washed with water, and extracted with ethyl acetate. The extract was washed with brine, dried (Na_2SO_4), and concentrated. Column chromatography (chloroform) of the residue on silica gel gave 2-(trimethylsilyl)ethyl *O*-[2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(4-methoxybenzyl)-β-D-glucopyranosyl]-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (1.07 g, 100%). To a solution of the latter compound (1.07 g, 1.10 mmol) in THF (15 mL) was added 4 Å molecular sieves (powder, 1.0 g), and the mixture was stirred for 2 h at room temperature, and sodium cyanoborohydride (992 mg) was added portionwise. After the reagent had dissolved, 1.0 M hydrogen chloride in ether was added dropwise at 0 °C until the evolution of gas ceased. The mixture was neutralized with triethylamine and filtered, the residue was washed with methanol and the combined filtrate and washings were concentrated. Column chromatography (2:1 hexane:ethyl acetate, gradient elution to 1:2) of the residue on silica gel gave **6** (561 mg, 53%): ^{1}H NMR($CDCl_3$, 250 MHz) δ; −0.01 (s, 9H, $SiMe_3$), 0.95–1.05 (m, 2H, CH_2Si), 1.55 (s, 3H, NHAc), 3.80 (s, 3H, OMe), 4.79 (d, J=9 Hz, 1H, CH_2Ph), 4.85 (d, J=8 Hz, 1 H, H-1, GlcNAc), 4.90 (d, J=12 Hz, 1 H, CH_2Ph), 5.06 (d, J=12 Hz, 2H, CH_2Ph), 6.86 (d, J=9 Hz, 2H, MPM), 7.21 (d, J=9 Hz, 2H, MPM), 7.25–7.45 (m, 20H, aromatic H). M.S. (m/e) $(M+Na)^+$: 986.

2-(Trimethylsilyl)ethyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-(4-metboxybenzyl)-β-D-glucopyranosyl]-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (8). The compound **6** (150 mg, 0.156 mmol) and methyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (**7**) (201 mg, 0.202 mmol) were dissolved in dry CH_2C1_2 (3.0 mL) and stirred over 4 Å molecular sieves (powder, 400 mg) at room temperature for 15 h. The mixture was then cooled to 0 °C, and the mixture of DMTST (281 mg, 1.089 mmol) and 4 Å molecular sieves (powder, 212 mg) was added. The mixture was stirred for 24 h at 7 °C. To the reaction mixture, methanol (1.0 mL), triethylamine (1.0 mL) and ethyl acetate (10 mL) were added, then the mixture was filtered and washed with water. The aqueous layer was extracted with ethyl acetate and the organic layer was washed with brine, and dried over anhydrous $MgSO_4$, then the solvent was removed in vacuo. The residue was subjected to chromatography (5:1 toluene:acetone, gradient elution to 4:1) to afford **8** (229 mg, 77% yield) as a white solid: ^{1}H NMR($CDCl_3$, 250 MHz) δ; −0.02 (s, 9H, $SiMe_3$), 0.97 (t, J=9 Hz, 2H, CH_2Si), 1.54 (s, 3H, NHAc), 1.56 (s, 3H, NHAc), 1.67 (t, J=12 Hz, 1H, Neu-3a), 1.79 (s, 3H, OAc), 1.92 (s, 3H, OAc), 1.98 (s, 3H, OAc), 2.15 (s, 3H, OAc), 2.46 (dd, J=5, 13 Hz, 1H, Neu-3e), 3.67 (s, 3H, CO_2Me), 3.83 (s, 3H, OMe), 5.08 (d, J=8 Hz, 1H, GlcNAc H-1), 5.23 (dd, J=3, 10 Hz, 1H, Neu, H-7), 5.60–5.73 (m, 1H, Neu, H-8), 7.10–8.30 (m, 35H, aromatic H). M.S. (m/e) $(M+Na)^+$: 1935.

2-(Trimethylsilyl)ethyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-6-*O*-benzy]-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (9). To a solution of **8** (407 mg, 0.213 mmol) in a mixture of acetonitrile (4.0 mL) and water (0.5 mL) was added cerium (IV) diammonium nitrate (282 mg, 0.516 mmol). The solution was stirred at room temperature for 4 h, then to the mixture ethyl acetate (20 mL) was added and washed with water. The aqueous layer was extracted with ethyl acetate and the

combined organic layer was washed with saturated aqueous $NaHCO_3$, water, and brine, and dried over $MgSO_4$, then the solvent was removed in vacuo. Column chromatography (1:1 hexane:ethyl acetate, gradient elution to 1:4) of the residue on silica gel gave **9** (385 mg, 1 00%): ^{1}H NMR($CDCl_3$, 250 MHz) δ; −0.03 (s, 9H, $SiMe_3$) 0.92–1.00 (m, 2H, CH_2Si), 1.55 (s, 3H, NHAc), 1.58 (s, 3H, NHAc), 1.63 (t, J = 12 Hz, 1H, Neu-3a), 1.79 (s, 3H, OAc), 1.91 (s, 3H, 0Ac), 2.03 (s, 3H, OAc), 2.19 (s, 3H, OAc), 2.46 (dd, J = 5, 13 Hz, 1H, Neu-3e), 3.87 (s, 3H, CO_2Me), 5.00 (d, J = 10 Hz, 1H, GlcNAc H- 1), 5.22 (dd, J = 3, 10 Hz, 1H, Neu, H-7), 5.60–5.72 (m 1H, Neu, H-8), 7.00–8.20 (m, 35H, aromatic H). M.S. (m/e) $(M + Na)^+$: 1813.

2-(Trimethylsilyl)ethyl-*O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-*O*-(2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (11). To a solution of **9** (403 mg, 0.225 mmol) and phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside (**10**) (237 mg, 0.450 mmol) in toluene (4.0 mL) and dichloromethane (2.0 mL), was added 4 Å molecular sieves (powder, 500 mg), and the mixture was stirred for 13 h at room temperature. *N*-Iodosuccinimide (151 mg, 0.675 mmol) and trifluoromethanesulfonic acid (3 μL, 0.034 mmol) were added to the mixture at −20 °C, and the stirring was continued for 4 h at −15 °C. Moreover, **10** (237 mg), *N*-iodosuccinirnide (151 mg), and trifluoromethanesulfonic acid (3 μL) were added to the mixture at −15 °C, and the mixture was stirred for 3 h at −15 °C. Ethyl acetate was added to the mixture, the insoluble materials were filtered off, and the filtrate was washed with saturated aqueous $NaHCO_3$. The aqueous layer was extracted with ethyl acetate and the organic layer were combined, washed with saturated sodium thiosulfate solution, water, and brine, and dried ($MgSO_4$). Column chromatography (2:1 hexane : ethyl acetate, gradient elution to 1:4) on silica gel gave **11** (406 mg, 82%): ^{1}H NMR($CDCl_3$, 25O MHz) δ; −0.01 (s, 9H, $SiMe_3$), 0.98 (t, J = 9 Hz, 2H, CH_2Si), 1.14 (d, J = 6 Hz, 3H, Fuc H-6), 1.58 (s, 3H, NHAc), 1.61 (s, 3H, NHAc), 1.70 (t, J = 12 Hz, 1H, Neu-3a), 1.80 (s, 3H, OAc), 1.92 (s, 3H, OAc), 1.97 (s, 3H, OAc), 2.15 (s, 3H, OAc), 2.43 (dd, J = 4, 12 Hz, 1H, Neu-3e), 3.84 (s, 3H, CO_2Me), 4.57 (d, J = 13 Hz, 1H, CH_2Ph), 4.84 (d, J = 12 Hz, 1H, CH_2Ph), 5.00 (d, J = 8 Hz, 1H, GlcNAc, H-1), 5.26 (d, J = 2 Hz, 1H, Fuc, H-1), 5.40–5.50 (m, 1 H, Neu , H-7), 5.60–5.75 (m, 1 H, Neu, H-8), 7.10–8.30 (M 50H, aromatic H). M.S. (m/e) $(M + Na)^+$: 2230.

2-(Trimethylsilyl)ethyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-*O*-(2-acetamido-6-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (12). A solution of **11** (370 mg, 0.168 mmol) in ethanol (30 mL) and acetic acid (15 mL) was hydrogenolyzed in the presence of 10 % Pd-C (400 mg) for 20 h at 50 °C, then filtered and concentrated. The residue was acetylated with acetic anhydride (4.0 mL)-pyridine (8.0 mL) for 3 h in the presence of DMAP (2.0 mg) at 40 °C. The product was purified by chromatography (1: 2 hexane : ethyl acetate, gradient elution to ethyl acetate) on silica gel to give **12** (236 mg, 75%): ^{1}H NMR($CDCl_3$, 250 MHz) δ; −0.01 (s, 9H, $SiMe_3$), 0.85–0.95 (m, 2H, CH_2Si), 1.20 (d, J = 7 Hz, 3H, Fuc H-6), 1.56 (s, 3H, NHAc), 1.90 (s, 3H, 0Ac), 1.93 (s, 3H, OAc), 2.00 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.08 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.12 (s, 3H, OAc), 2.13 (s, 3H, OAc), 2.41 (dd, J = 4, 12 Hz, 1 H, Neu-3e), 3.81 (s, 3H, CO_2Me), 5.30–5.45 (m 1 H, Neu , H-7), 5.55–5.70 (m, 1 H, Neu, H-8), 7.25–8.30 (m, 15H, aromatic H).

***O*-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2- nonulopyranosylonate)-(2→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-*O*-(2-acetamido-6-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-acetyl-α-D-galactopyranosy1 trichloroacetimidate (13).** To a solution of **12** (96 mg, 0.51 mmol) in chloroform (0.8 mL) was added trifluoroacetic acid (0.6 mL) at 0 °C, and the mixture was stirred for 90 min at room temperature and concentrated. Column chromatography (1:4; hexane : ethyl acetate, gradient elution to ethyl acetate) on silica gel gave *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-*O*-(2-acetamido-6-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-acetyl-D-galactopyranose (85 mg, 93%). To a mixture of this pentasaccharide (58 mg, 0.033 mmol) and trichloroacetonitrile (0.1 mL) in chloroform (1.0 mL) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 5.2 mg) at 0 °C, and the mixture was stirred for 100 min at the same temperature, then concentrated. Column chromatography (1:2; hexane:ethyl acetate, gradient elution to ethyl acetate) on silica gel afforded **13** (53 mg, 85%). ^{1}H NMR($CDCl_3$, 250 MHz) δ; 1.21 (d, J = 7 Hz, 3H, Fuc- 6), 1.58 (s, 3H, NAc), 1.78 (s, 3H, NAc), 1.85-2.15 (11s, 33H, OAc), 2.41 (dd, J = 5, 13 Hz, 1H, Neu-3e), 3.81 (s, 3H, MeO), 5.55–5.70 (m, 1H, Neu-8), 6.50 (d, J = 4 Hz, 1H, Gal-1), 7.40–8.20 (m 15H, aromatic H), 8.61 (s, 1H, C=NH). M.S. (m/e) $(M + Na)^+$: 1938.

3-Tridecylhexadecyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-*O*-(2-acetamido-6-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (15). To a solution of **13** (25 mg, 0.013 mmol) and 3-tridecylhexadecanol (**14**, 28 mg) in dichloromethane (0.3 mL) was added 4 Å molecular sieves type AW300 (powder, 56 mg), and the mixture was stirred for 4 h at room temperature, then cooled to 0 °C. Boron trifluoride etherate (8 mg) was added, and the mixture was stirred for 18 h at 0 °C and then filtered. The insoluble material was washed with ethyl acetate, and the combined filtrate and washings were washed with saturated aqueous $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated. Column chromatography (hexane: ethyl acetate 1: 1, gradient elution to ethyl acetate) on silica gel gave **15** (21 mg, 74%): $[\alpha]_D$ −10° (*c* 0.59, $CHCl_3$); ^1H NMR($CDCl_3$, 25O MHz) δ; 0.89 (t, $J=7$ Hz, 6H, CH_3), 1.15–1.40 (m 48H), 1.21 (d, $J=7$ Hz, 3H, Fuc-6), 1.78 (s, 3H, NAc), 1.82 (s, 3H, NAc), 1.90 (s, 3H, OAc), 1.93 (s, 3H, OAc), 2.00 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.08 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.12 (s, 3H, OAc), 2.41 (dd, $J=4$, 13 Hz, 1H, Neu-3e), 3.81 (s, 3H, $COOCH_3$), 5.60–5.70 (m, 1H, Neu-8), 7.40–8.20 (m, 15H, aromatic H). M.S. (m/e) $(M+Na)^+$: 1793.

3-Tridecylhexadecyl *O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-*O*-(β-D-galactopyranosyl)-(1→4)-*O*-[(α-L-fucopyranosyl)-(1→3)]-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside (2). To a solution of **15** (46 mg, 0.021 mmol) in methanol (10 mL) was added sodium methoxide (8 mg), and the mixture was stirred for 14 h at 50 °C, and water (1.0 mL) was added. The solution was stirred for 24 h at 50 °C, then treated with DOWEX (5OW-X8, H^+ form) resin and filtered. The resin was washed with methanol, and combined filtrate and washings were concentrated. Column chromatography (5:4 chloroform: methanol) of the residue on Sephadex LH-20 gave **2** (29 mg, 100%) as an amorphous mass: $[\alpha]_D$ −15° (*c* 0.49,1 : 1; $CHCl_3$-MeOH); ^1H NMR(CD_3OD, 250 MHz) δ; 0.90 (t, $J=6$ Hz, 6H, CH_3), 1.17 (d, $J=7$ Hz, 3H, Fuc-6), 1.20–1.40 (m, 48H), 1.89 (t, $J=12$ Hz, 1H, Neu-3a), 1.98 (s, 3H, NAc), 2.00 (s, 3H, NAc), 2.78 (dd, $J=4$, 13 Hz, 1H, Neu-3e), 4.22 (d, $J=7$ Hz, 1H,), 4.52 (d, $J=8$ Hz, 1H,), 5.06 (d, $J=4$ Hz, 1H, Fuc- 1). M.S. (m/e) $(M+Na)^+$: 1413.

References

1. (a) Halhnan, R.; Jutila, M. A.; Smith, C. W.; Anderson, D. C.; Kishimoto, T. K.; Butcher, E. C. *Biochem. Biophys. Res. Commun.* **1991**, *174*, 236. (b) Lawrence, M. B.; Springer, T. A. *Cell* **1991**, *65*, 859. (c) Watson, S. R.; Fennie, C.; Laskey, L. A. *Nature* **1991**, *349*, 164. (d) Mayades, T. N.; Johnson, R. C.; Rayburn, H.; Hynes, R. O.; Wagner, D. D. *Cell* **1993**, *74*, 541.

2. (a) Phillips, M. L.; Nudelman, E.; Gaeta, F. C. A.; Perez, M.; Singhal, A. K.; Hakomori, S.-T.; Paulson, J. C. *Science* **1990**, *250*, 1130. (b) Walz, G.; Aruffo, A.; Kolanus, W.; Bevilacqua, M.; Seed, B. *Science* **1990**, *250*, 1132–1134. (c) Watson, S. A.; Imai, Y.; Fennie, C; Geoffroy, J. S.; Rosen, S. D.; Lasky, L. A. A. *J. Cell Biol.* **1990**, *110*, 2221. (d) Aruffo, A.; Kolanus, W.; Walz, G.; Fredman, P.; Seed, B. *Cell* **1991**, *67*, 35. (e) Erbe, D. V.; Watson, S. R.; Presta, L. G.; Wolitzky, B. A.; Foxall, C.; Brandley, B. K.; Lasky, L. A. *J. Cell. Biol.* **1993**, *120*(5), 1227. (f) Faxall, C.; Watson, S. R.; Dowbenko, D.; Fennie, C.; Lasky, L. A.; Kiso, M.; Hasegawa, A.; Brandley, B. K. *J. Cell Biol.* **1992**, *117*, 895.

3. Tedder, T.; Steeber, D.; Chen, A.; Engel, P. *FASEB J.* **1995**, *9*, 866.

4. Bevilacqua, M. P.; Stengelin, S.; Gimbrone, M. A. *Science* **1989**, *243*, 1160.

5. Isenberg, W. M.; MeEver, R. P.; Shuman, M. A.; Bainton, D. F. *Blood Cells* **1986**, *12*, 191.

6. Butcher, E. C. *Cell* **1991**, *67*, 1033.

7. (a) Mulligan, M. S.; Polley, M. J.; Bayer, R. J.; Nunn, M. F.; Paulson, L. C.; Ward, P. A. *J. Clin. Invest.* **1992**, *90*, 1600. (b) Weyrich, A. S.; Ma, X.; Lefer, D. J.; Albertine, K. H.; Lefer, A. M. *J. Clin. Invest.* **1993**, *91*, 2620. (c) Winn, R. K.; Liggitt, D.; Vedder, N. B.; Paulson, L. C.; Harlan, J. M. *J. Clin. Invest.* **1993**, *92*, 2042. (d) Chen, L. Y.; Nichols, W. M.; Hendricks, L. B.; Yang, B. C.; Mehta, J. L. *Cardiovasc. Res.* **1994**, *28*, 1414.

8. Frenette, P. S.; Wagner, D. D. *Thrombosis and Haemostasis* **1997**, *78*, 60.

9. Nguyen, M.; Eilber, F. R.; Defrees, S. *Biochem. Biophys. Res. Commun.* **1996**, *228*, 716.

10. Ohmoto, H.; Nakamura, K.; Inoue, T.; Kondo, N.; Inoue, Y.; Yoshino, K.; Kondo, H.; Ishida, H.; Kiso, M.; Hasegawa, A. *J. Med. Chem.* **1996**, *39*, 1339.

11. (a) Wada, Y.; Saito, T.; Matsuda, N.; Ohmoto, H.; Yoshino, K.; Ohashi, M.; Kondo, H.; Ishida, H.; Kiso, M.; Hasegawa, A. *J. Med. Chem.* **1996**, *39*, 2055. (b) Tsukida, T.; Yoshida, M.; Kurokawa, K.; Nakai, Y.; Achiha, T.; Kiyoi, T.; Kondo, H. *J. Org. Chem.* **1997**, *62*, 6976. (c) Tsukida, T.; Hiramatsu, Y.; Tsujishita, H.; Kiyoi, T.; Yoshida, M.; Kurokawa, K.; Modyama, S.; Ohmoto, H.; Wada, Y.; Saito, T.; Kondo, H. *J. Med. Chem.* **1997**, *40*, 3534.

12. Hasegawa, A.; Suzuki, N.; Ishida, H.; Kiso, M.; *J. Carbhydr. Chem.* **1996**, *15*, 623.

13. Kiyoi, T.; Nakai, Y.; Kondo, H.; Ishida, H.; Kiso, M.; Hasegawa, A. *Bioorg. Med. & Chem.* **1996**, *4*, 1167.

14. Hasegawa, A.; Ando, T.; Kameyama, A.; Kiso, M. *J. Carbohydr. Chem.* **1992**, *11*, 645.

15. (a) Fügedi, R; Garegg, P. L. *Carbohydrate Res.* **1986**, *149*, C9. (b) Murase, T.; Ishida, H.; Kiso, M.; Hasegawa, A. *Carbohydrate Res.* **1989**, *188*, 71. (c) Rovenscroft, M.; Roberts, R. M. G.; Tillett, J. G. *J. Chem. Soc. Perkin Trans. II* **1982**, 1569.