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Synthesis and Functions of Neoglycolipids Based on the Glycoblottting Method

(糖鎖捕捉反応を利用した新奇糖脂質の合成と機能)

Doctoral Thesis

2014

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Abbreviations

Ac	Acetyl
AO	Aminooxy
Boc	tert-butylbutoxycarbonyl
Cel	Cellobiose
CG	Ceramide glycanase
CSA	10-camphorsulfonic acid
DCC	<i>N,N</i> -Dicyclohexylcarbodiimide
DCM	Dichloromethane
DHB	Dihydroxybenzoic acid
DIAD	Diisopropyl Azodicarboxylate
DIEA	<i>N,N</i> -diisopropylethylamine
DMF	<i>N,N</i> -dimethylformamide
DMSO	Dimethylsulfoxide
DPPA	Diphenylphosphoryl azide
EEDQ	<i>N</i> -Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline
ESI	Electro spray ionization
Fmoc	9-fluorenylmethylcarbonyl
Fuc	Fucose
Gal	Galactose
Glc	Glucose
GlcNAc	<i>N</i> -acetyl-D-glucosamine

Glu	Glutamic acid
GSLs	Glycosphingolipids
HOBt	1-hydroxybenzotriazole
HSQC	¹ H-detected single quantum coherence spectrum
HRMS	High resolution mass spectrometry
Lac	Lactose
LacCer	Lactosylceramide
Mal	Maltose
MALDI	Matrix Assisted Laser Desorption/Ionization
Me	Methyl
Mel	Melibiose
NMR	Nuclear magnetic resonance
PC-3 cell	Prostate cancer cell
Ph	Phenyl
Phth	Phthaloyl
PG	Protecting Group
PS	Phytosphingosine
Pyr	Pyridine
rEGCase	Recombinant Endo-glycoceramidase
RT, r.t.	Room Temperature
Su	Succinimidyl
TBAF	Tetrabutylammonium Fluoride
TBS	tert-butyldimethylsilyl
TBTA	Tris[(1-benzyl-1H-1, 2, 3-triazol-4-yl)methyl]amine

TEMPO	2,2,6,6-tetramethylpiperidine 1-oxyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TOF	Time of flight
Tr, Trt	Trytyl
Trp	Tryptophan
UV	Ultra violet
Xyl	Xylose
WSC	1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride
Z	Benzyloxycarbonyl

Chapter 1

General Introduction

1-1 Glycolipids

Glycosylation is one of the most important posttranslational modifications of proteins in eukaryotes and this step is essential to modulate a wide range of protein and lipid functions both on the cellular surfaces and within the cells. In mammals, glycans are made up of monosaccharide, such as glucose, galactose, mannose, xylose, *N*-acetylglucosamine, *N*-acetylgalactosamine, fucose, and the negatively charged *N*-acetyl neuraminic acids and glucuronic acids. These nine monosaccharides can give rise to a large number of oligosaccharide structures, which can cause structural diversity mediating biological processes involving an infection, cell adhesion, immunity, differentiation, and quality control of proteins^[1,2] (Fig 1-1).

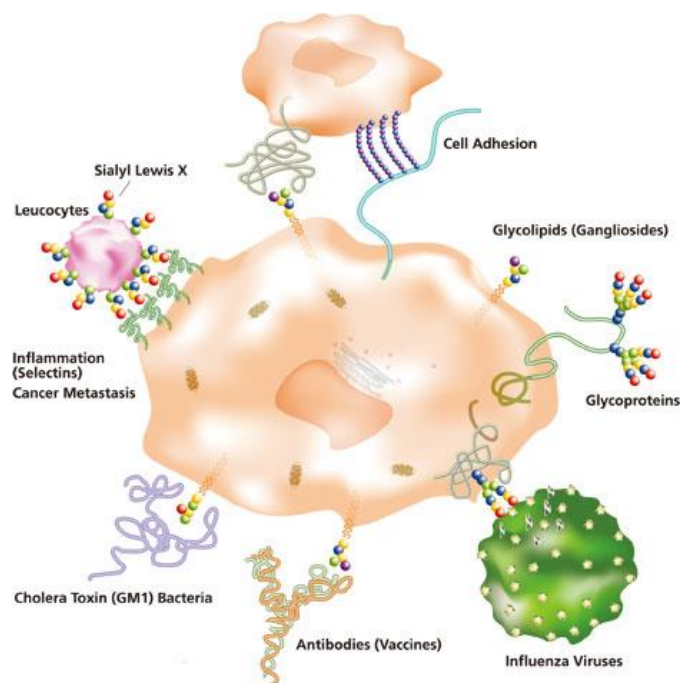


Fig. 1-1. Glycans play various important roles in biological phenomena.

Sphingolipids, a complex and ubiquitous group of membrane lipids in eukaryote^[3], play important roles in diverse biological phenomena, such as cell differentiation, cell–cell interactions, apoptosis, infections, and immune responses^[4]. Glycosylation is the most complex modification process of the sphingolipids to form glycosphingolipids (GSLs) and exert various functions on the cell membrane^[4,5]. Generally, the GSLs are classified to four groups, cerebroside, sulfatide, neutral GSLs, and acidic GSLs. Although the complexity of the GSLs is the source of multiple functions, the variety of the glycan structure make complex to elucidate the function of each structures (Fig. 1-2).

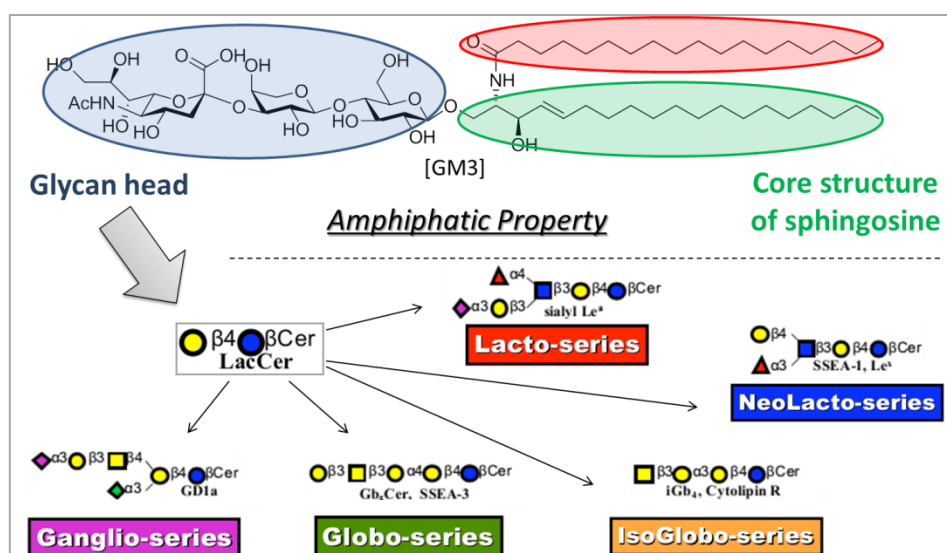


Fig.1-2. Structural feature and glycan diversity of GSLs

Last three decades, many efforts have been paid to synthesize the natural and mimetic glycosphingolipids to elucidate the functions and their application^[5-7]. However, the preparation of GSLs is still skillful and time spending task (Fig. 1-3). One-step chemical ligation is an attractive alternative for such conventional step-wise synthetic strategy to conjugate biomolecules^[8-10].

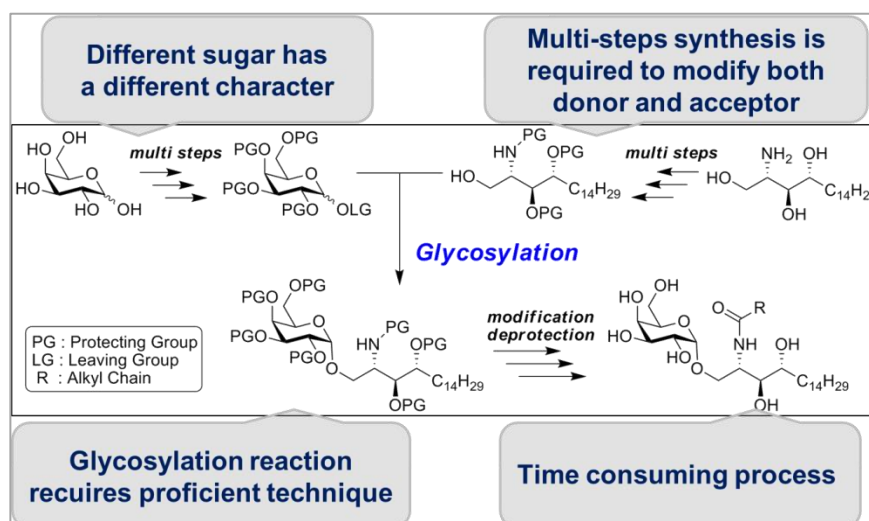


Fig. 1-3. Problems of conventional chemical synthesis of GSLs

1-2 Glycoblotting Method

Oxime formation between aminoxy-functionalized compound and reducing sugars, named glycoblotting^[11], has a potential advantage of rapid preparation of glycan-conjugated compounds without any modification of sugar residue before the conjugation (Fig. 1-4). Our laboratory has been applied this glycoblotting strategy to various glycomic studies such as glycan finger printing^[12], glycan array^[13], and synthesis of glycopeptide library^[14].

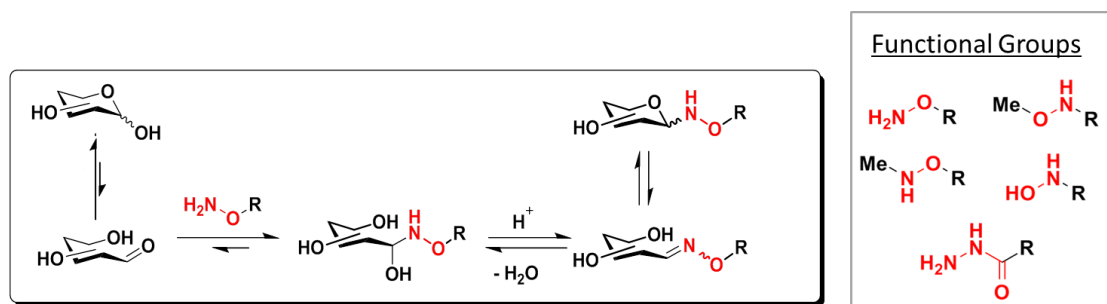


Fig. 1-4. Reaction mechanism and available functional group of glycoblotting

From the point of view to synthesize mimetic compounds of glycoconjugate, the method has great an advantage due to its simplicity. Actually, it has contributed to librarization of various glycoconjugates to date^[15-21] (Fig. 1-5).

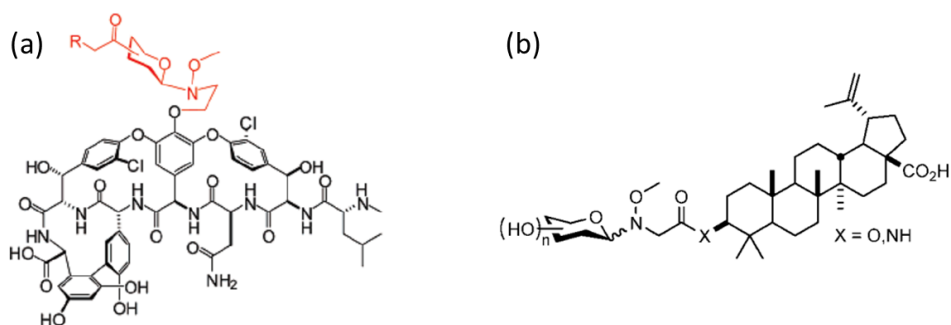


Fig. 1-5. Reported glycoconjugates synthesized by glycoblotting reaction

However, there is no example of glycosphingolipid library constructed by the glycoblotting strategy, although some glycolipid analogues prepared by oxyme formation were reported^[22, 23].

1-3 Objective of Thesis

To elucidate the functions of glycolipids has been demanded for a long time. However, the method for rapid and easy preparation has not been developed although many researchers made efforts.

In this thesis, the author challenged to synthesize functional ceramides which can capture free sugars and construct neoglycolipid library via glycoblotting reaction. In chapter 2, I described design and synthesis of 6 ceramide derivatives having different functional groups. Then, glycoblotting reaction was conducted using them in chapter 3.

Additionally, we tried to evaluate whether synthesized neoglycoside work as a mimetic compound of glycosphingolipids in chapter 4. In chapter 5, further functionalization of neoglycolipids was demonstrated.

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Chapter 2

Design & Synthesis of Functionalized Ceramide Derivatives

2-1 Introduction

Glycolipids, especially glycosphingolipids (GSLs), play an important role not only on cell surface but as a secretion^[1-5]. There is no doubt for the importance to elucidate the functions of glycosphingolipids.

As mentioned in chapter 1, there has been no example of glycosphingolipid library constructed by the glycoblotting strategy although some glycolipid analogues prepared by oxyme formation were reported^[6, 7]. This made me focus on the synthetic study of construction of GSL library using glycoblotting reaction.

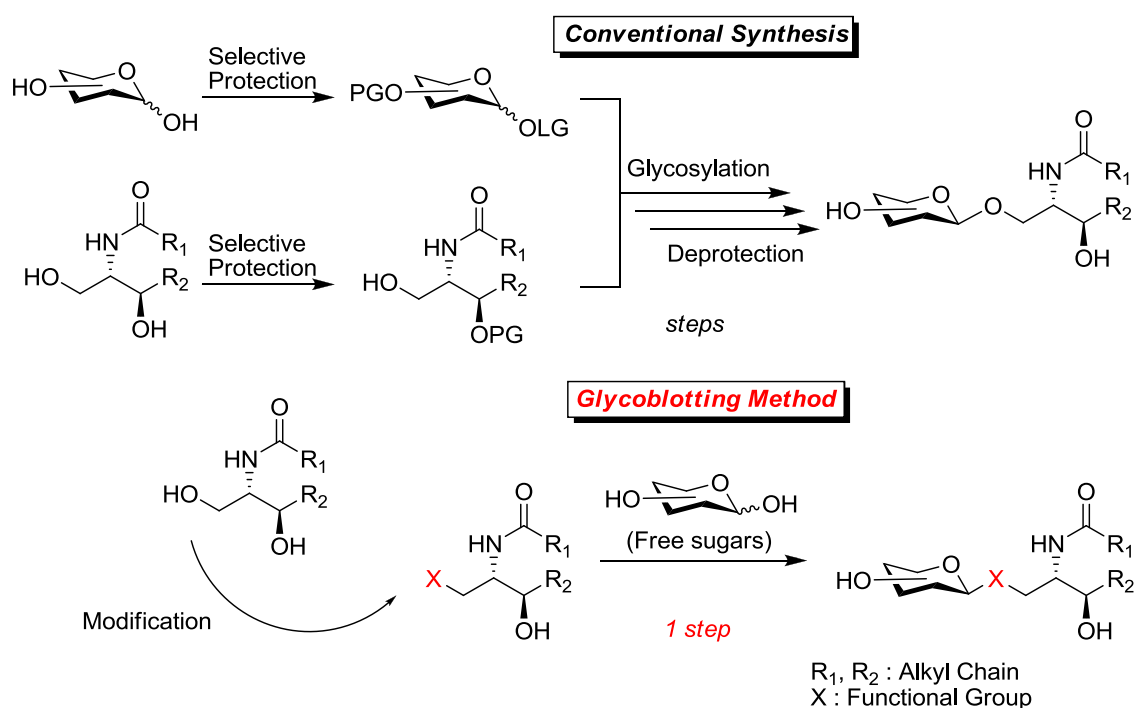


Fig. 2-1-1. Comparison of glycoblotting method with conventional method for synthesis of glycolipid derivatives.

Compared with conventional chemical and chemo-enzymatic synthesis^[8-11] (Fig. 2-1-1), glycoblotting method has greater advantages to synthesize glycoconjugates, where glycosyl linkage can be formed by only mixing free sugar and aglycon with no protection or modification. However, it was inevitable to say that the neoglycosylated bond possibly form several type of linkage (Fig. 2-1-2). To classify them into 2 groups briefly, one of them is ring-open structure and another is ring-closed structure similar to natural glycoconjugates^[12-14]. While this diversity is occasionally troublesome, it can be regulator of bioactivities. That is why some researches tried to conjugate sugars with one aglycon having different functional groups and indicated that activity profiles differed depending on the functional groups if they had the same glycan on their head^[14].

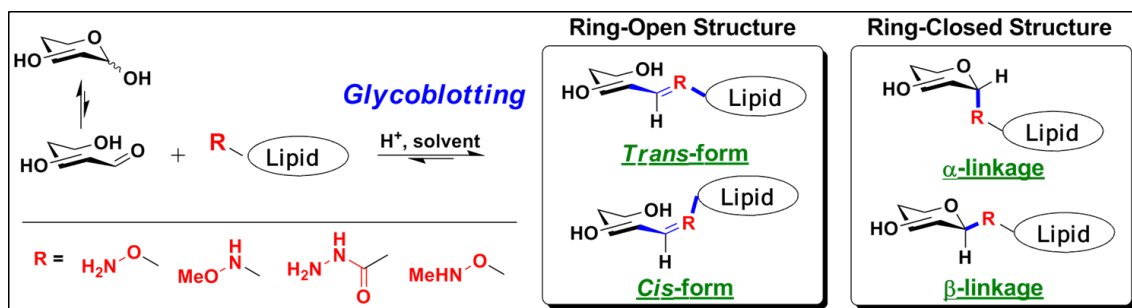


Fig. 2-1-2 Representative structures after glycoblotting reaction

2-2 Results & Discussions

2-2-1 Design and Synthesis of Methoxyamino-Functionalized Ceramide Derivatives **1**

To construct neoglycolipid library, selection of three parts for synthesis of functional ceramide derivative was needed. One of them was the type of functional group and the

others were the structure of based lipid and acyl chain. As the lipid moiety, we chose phytosphingosine (PS) skeleton which is abundant in yeast and plants^[15,16] although there are many kinds of sphingosine structure, D/L erythro- or threosphingosine, lysosphingosine, and so on. That was because various bioactivities of phytosphingolipid derivatives have been reported to date such as induction of apoptosis^[17,18] and activation of immunological responses^[19]. For functionalization of the sphingolipid toward glycoblotting reaction, we firstly chose *O*-methylhydroxylamino group to maintain ring-closed glycan structure^[20-23] and same bond number of natural GSLs after the glycoblotting. As the acyl part, stearic acid which has 18 carbon atoms was selected to be condensed with amine group of sphingosine. However, because of many papers which indicated that the structure of acyl chain affected various biological activities, we considered the acylation step had to be performed later in the total synthesis. Considering that, we conducted retrosynthetic analysis of methoxyaminoderivative **1** starting from abundant phytosphingosine **4** (Fig. 2-2-1). Advantages of this synthetic strategy were clear because of the poor solubility towards common organic solvents of the ceramide derivatives bearing a long *N*-acyl chain and *N*-acyl structure was changeable easily. Besides, the most important advantage is a much similar structure to natural glycoceramide can be expected after glycoblotting reaction (Fig. 2-2-2).

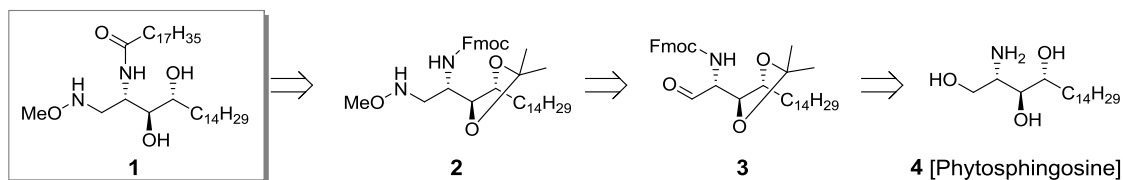


Figure 2-2-1. Design and retrosynthetic analysis of ceramide derivative **1**

containing methoxyamino functional group

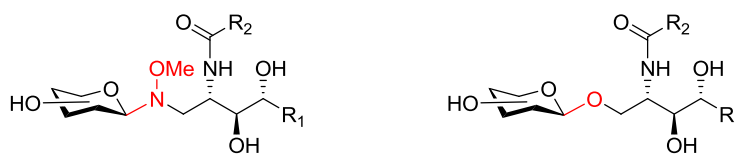
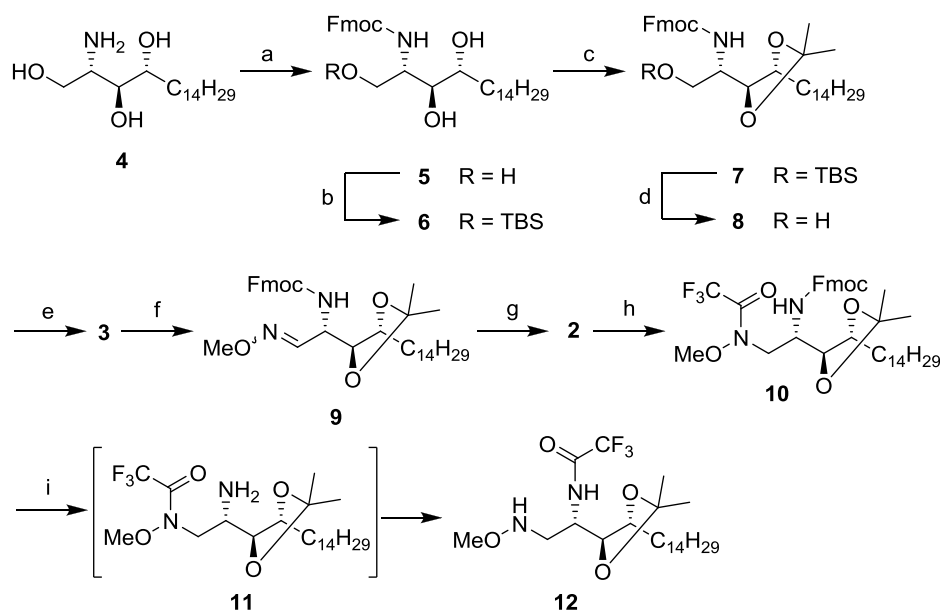


Figure 2-2-2. Comparison of structural similarity between an expected structure of compound **1** after glycoblotting reaction and natural glycosylceramide

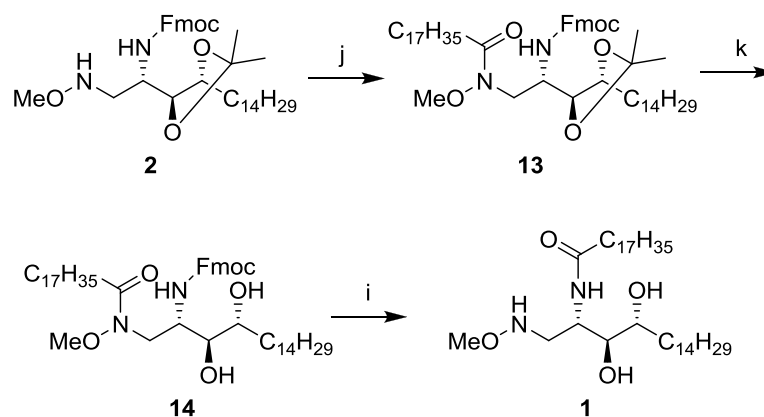
Synthesis of compound **1** was started from commercially available phytosphingosine **4**. As shown in Scheme 2-2-1, amino group of phytosphingosine **4** was protected by Fmoc group at first. Interestingly, this is the first example that Fmoc group was introduced directly to phytosphingosine **4** to give **5** which showed good solubility to organic solvents compared to *N*-acylated one. TBS protection of primary alcohol to give **6** and following isopropyliden protection of secondary alcohol provided the all protected compound **7**. Then, selective deprotection of TBS group by HF-pyr complex gave compound **8**. TEMPO oxidation of the primary hydroxyl group^[24] of **8**, followed by oximization by *O*-methylhydroxylamine gave compound **9**, and reduction by sodium cyanoborohydride in acidic condition^[25] afforded methoxyamino derivative **2**. Then the methoxyamino group of **2** was protected by trifluoroacetyl group to give **10** for selective *N*-acylation with fatty acid. Surprisingly, a complete *N-N'* acyl migration was occurred during Fmoc deprotection process to give compound **12** as sole product.



Scheme 2-2-1. Synthesis of ceramide derivative **1**. Reagents and conditions: a) FmocOSu, THF, RT, 2.5 h, 97%; b) TBSCl, imidazole, THF, RT, 3 h, 98%; c) 2-methoxypropane, CSA, THF, RT, 5 h, 98%; d) HF-pyr, THF, RT, overnight, 95%; e) TEMPO, KBr, *t*BuOCl, NaHCO₃, Na₂CO₃, CH₂Cl₂, 0 °C, 40 min; f) MeONH₃Cl, pyr, THF/MeOH (2:3), 2.5 h, 97% (*E*:*Z* = 8:1, 2 steps); g) NaBH₃CN, THF/AcOH (1:2), 0 °C to RT, 1 h, 51%; h) (CF₃CO)₂O, NaHCO₃, THF, RT, 1 h, 68%; i) piperidine, THF, RT, 3 h, 70%

This unanticipated migration made me deduce that compound **1** could be obtained from fatty acyl modified methoxyamino derivative via this *N*(-OMe) to *N'* acyl migration concurrently with Fmoc deprotection. Although any example of such *N*-*N'* acyl migration for synthetic strategy has never been reported, the occurrence itself was conjectured by Carrasco et al. in the synthesis of a neoglycopeptide by using methoxyamine modified serine residue^[26]. They speculated that the migration was equilibrium reaction and the ratio was dependent on the pH and under basic condition, acyl chain attached to methoxyamine migrated to its α -amino group completely in their

HPLC analysis. This report supported that the basic condition for deprotection of Fmoc group was suitable for my *N-N'* migration strategy. That was why I revised the synthetic scheme (Scheme 2-2-2). By using activated ester reagent^[27], stearoyl group was attached to compound **2** to give compound **13** and TFA treatment gave compound **14**. At the last step, the *N-N'* acyl migration proceeded successfully and immediately after deprotection of Fmoc group by piperidine treatment, to afford desired ceramide derivative **1** in 14 % overall yield.



Scheme 2-2-2. Synthesis of ceramide derivative **1** via acyl migration reaction; j) $\text{H}_{35}\text{C}_{17}\text{COOSu}$, THF/pyr (2:1), 50 °C, 2 d, 82%; k) TFA, $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$ (10:1), RT, 30 min, 57%; i) piperidine, THF, RT, 3 h, 66%

2-2-2 Design of Other Functional Ceramide Derivatives

After completion of the synthesis of compound **1**, I designed other ceramide derivatives based on methoxyamino derivative **1** to compare effects of functional group for neoglycosylation (Fig. 2-2-3).

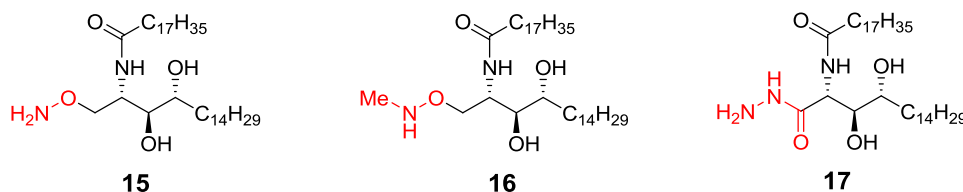


Fig. 2-2-3 Design of other functional ceramide derivatives based on compound **1**

Additionally, we designed two of serine-based ceramide mimics (Fig. 2-2-4). That was because our laboratory had clarified that serine-based glucosylceramide could be a substrate of ceramidoglycanase (CG) and improve skin function^[28]. The result corresponded with my strategy to synthesize mimetics of glycosphingolipid.

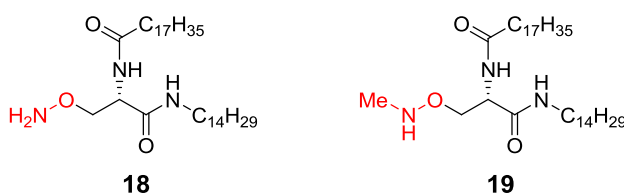
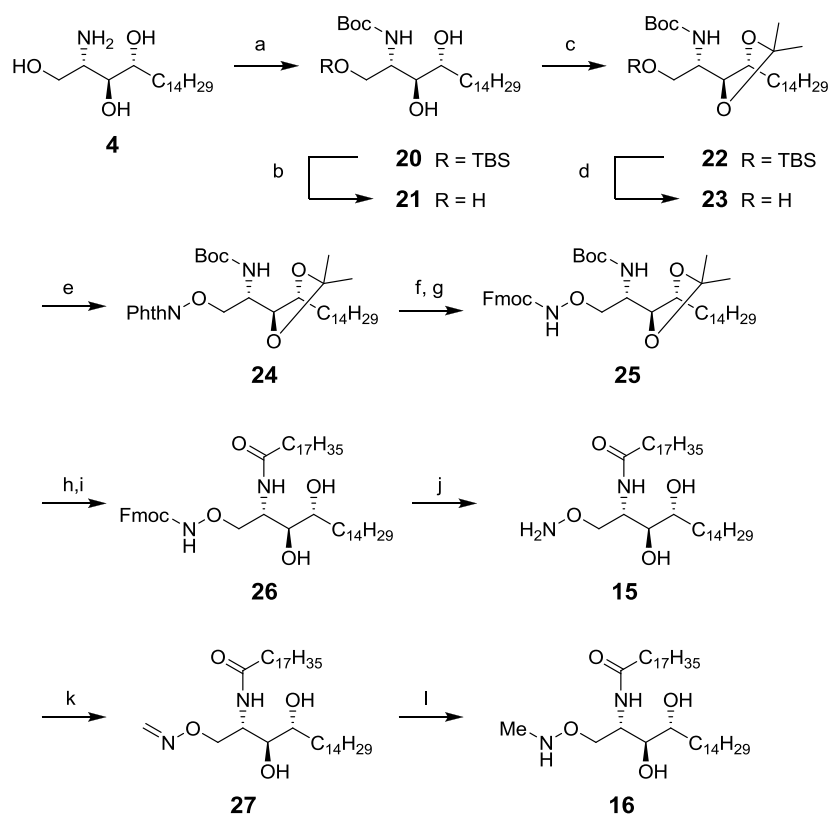


Fig. 2-2-4 Design of serine-based functional ceramide derivatives

2-2-3 Synthesis of Aminoxy-Derivative **15** & Methylaminoxy-Derivative **16**

Synthesis of aminoxy derivative **15** and methylaminoxy derivative **16** was shown in Scheme 2-2-3^[29]. Boc protection to phytosphingosine afforded compound **20** and selective TBS protection was performed at primary hydroxyl group. Remained two hydroxyl groups were capped by isopropyliden in the similar way of Fmoc derivative. After removal of TBS group, Mitsunobu reaction gave N-hydroxyphthalimide-substituted compound **24**. Treatment of MeNH₂ followed by Fmoc protection afforded compound **25**. Boc and isopropyliden groups were removed

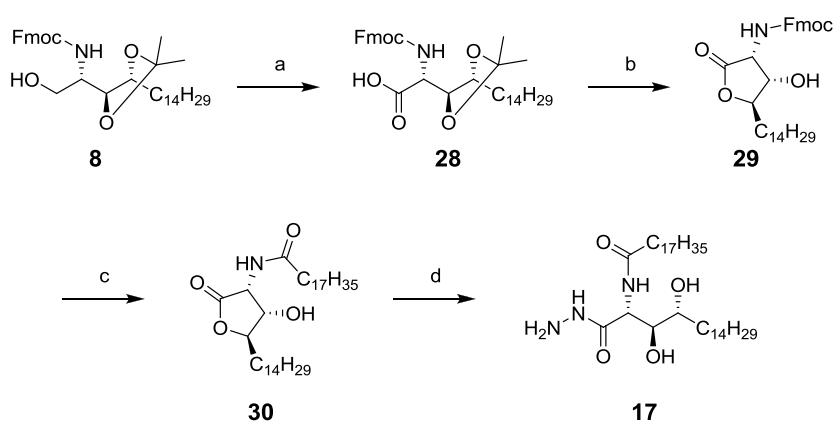
by TFA solution and stearyl group was attached to amino group by using activated reagent. Aminoxy-functionalized compound **15**, which was one of desired ceramide derivatives, was obtained by adding piperidine in 28% total yield. Through the next 2 steps, oximization and reduction, *N*-methylated compound **16** was obtained in 18% overall yield.



Scheme 2-2-3. Synthesis of ceramide derivative **15** and **16**. Reagents and conditions: a) Boc₂O, NaHCO₃, THF, RT, 3.5 h, quantitatively; b) TBSCl, imidazole, DCM, RT, 1 h, 92%; c) 2-methoxypropane, CSA, THF, RT, 2 h, 88%; d) TBAF, THF, RT, 2 h, 90%; e) HONPhth, Ph₃P, DIAD, THF, 50 °C, 9 h, 95%; f) MeNH₂, THF/MeOH, 7 h; g) FmocOSu, THF, RT, 9 h, 82%, 2 steps; h) TFA, DCM, H₂O, RT, 40 min; i) H₃₅C₁₇COOSu, THF, RT, 2 d, 75%, 2 steps; j) piperidine, THF, RT, 6 h, 66%; k) HCHO, DCM, MeOH, AcOH, RT, overnight, 81%; l) NaBH₃CN, AcOH/THF (4:1), RT, 1 h, 80%.

2-2-4 Synthesis of hydrazide-derivative **17**

Hydrazide derivative **17** was synthesized by using Fmoc derivative **8**, which was intermediate for methoxyamino derivative **1**. Long time oxidation of the primary hydroxyl group gave carboxylic acid **28**. TFA treatment removed isopropyliden and caused cyclization which afforded lactone **29**. After removal of Fmoc group by piperidine, stearyl attached compound **30** was obtained. Desired hydrazide **17** was gained by cleavage of lactone bond in 25% overall yield from phytosphingosine **4**.

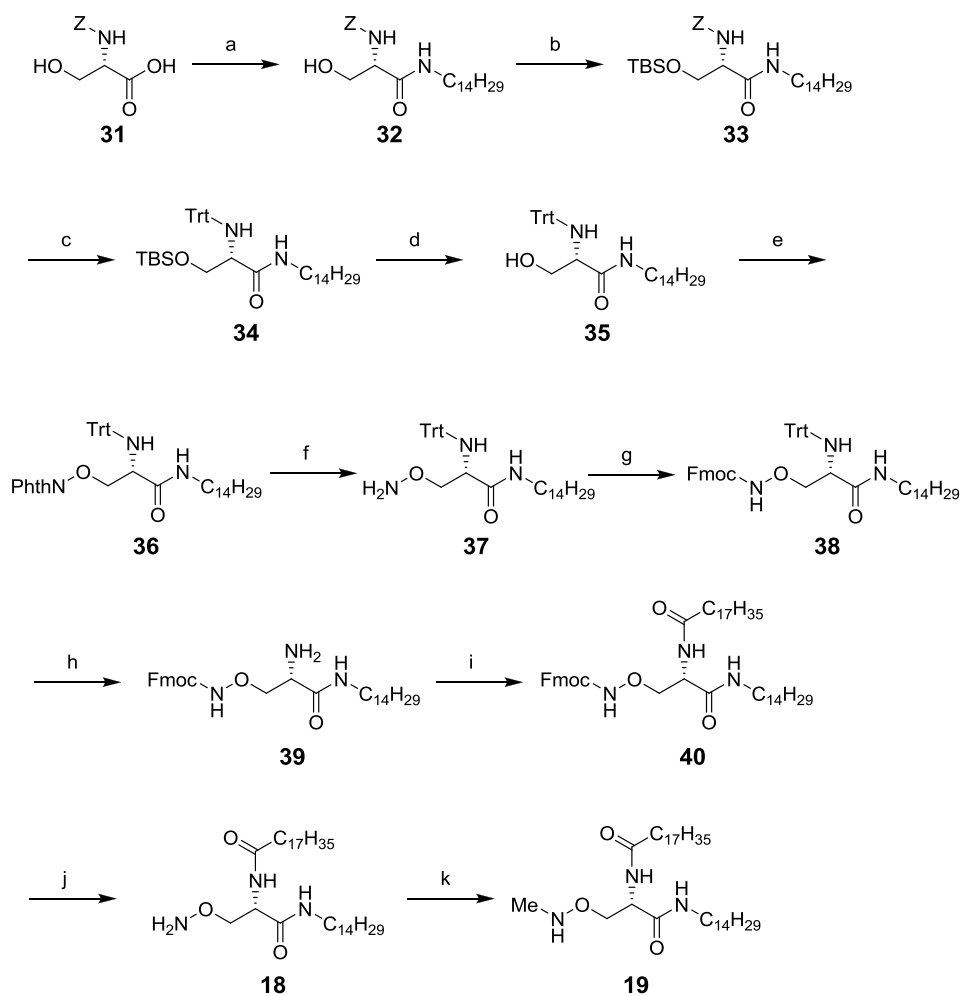


Scheme 2-2-4 Synthesis of hydrazide derivative **17**

Reagents and conditions: a) TEMPO, KBr, *t*BuOCl, NaHCO₃, Na₂CO₃, CH₂Cl₂, 0 °C to RT, overnight, 53%; b) TFA, DCM, H₂O, RT, 3 h, 96%; c) i. piperidine, THF, RT, 30 min; ii. C₁₇H₃₅COOSu, THF, RT, overnight, 60%, 2 steps; d) H₂NNH₂·H₂O, CHCl₃, MeOH, RT, 1.5 h, 91%

2-2-5 Synthesis of Aminoxy-Serine-derivative **18** & Methylaminoxy-Serine-derivative **19**

Serine derivatives were synthesized from Z-serine **31**. Condensation of the starting material with tetradecylamine gave fatty amide **32** and then hydroxyl group was protected by TBS group. Succeeding two steps reaction afforded trityl protected **34**. After removal of TBS group, Mitsunobu reaction^[30] gave *N*-hydroxyphthalimide-substituted compound **36**. Treatment of MeNH₂ followed by Fmoc protection afforded compound **38**. Trityl group was removed by TFA solution and stearoyl group was attached to amino group by using activated reagent. Aminoxy-functionalized compound **18**, which was one of desired ceramide derivatives, was obtained by adding piperidine in 31% overall yield. Through the next 2 steps, oximization and reduction, *N*-methylated compound **19** was obtained in 7% total yield.



Scheme 2-2-5 Synthesis of serine-based functional ceramide derivatives **18**, **19**

Reagents and conditions: a) WSC, HOBT, $\text{H}_2\text{NC}_{14}\text{H}_{29}$, RT, 2 d, 89%; b) TBSCl, imidazole, DCM, RT, overnight, 91%; c) i. H_2 , Pd/C, THF, RT, 5 h; TrtCl, DIEA, THF, RT, 11 h, 86%, 2 steps; d) TBAF, THF, RT, 45 min, 89%; e) HONPhth, Ph_3P , DIAD, THF, 0 °C to 50 °C, 6 h, 84%; f) MeNH_2 , THF/MeOH, 2.5 h, 95%; g) FmocOSu, THF, RT, 2 h, 86%; h) TFA, DCM, MeOH, RT, 1 h, 93%; i) $\text{H}_{35}\text{C}_{17}\text{COOSu}$, THF, RT, 3 d, 86%; j) piperidine, THF, CHCl_3 , MeOH RT, 2 h, 91%; k) i. HCHO, DCM, MeOH, RT, 3 h; ii. NaBH_3CN , TFA, THF, RT, 30 min, 23%, 2 steps.

2-3 Conclusion

In summary, we synthesized 6 ceramide derivatives which were expected to imitate natural glycosphingolipids after glycoblotting reaction. In the synthesis of methoxyamino derivative **1**, we came across an unpredicted acyl migration. This finding motivated us to establish new synthetic pathway toward the targeted methoxyamino-functionalized ceramide **1** using *N-N'* acyl migration as a key reaction. As a result, I have developed an efficient protocol for the synthesis of methoxyamino-functionalized ceramide **1** using a specific *N-N'* acyl migration to allow for the selective modification at *N* α position of the methoxyamino-functionalized phytosphingosine derivatives, notably the precursors of methoxyamino-functionalized ceramide derivatives. This discovery will contribute to construct synthetic strategy of methoxyamino derivative.

2-4 Experimental Section

General Information

All reactions were carried out under a nitrogen atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. Proton and carbon NMR was recorded at 298K with Varian UnityInova 500 MHz (Agilent Inc., USA; ^1H : 500 MHz, ^{13}C : 125 MHz) or Bruker AVANCE DRX 600, equipped with a cryoprobe (Bruker BioSpin Co., Germany; ^1H : 600 MHz, ^{13}C : 150 MHz). Chemical shifts are given in ppm and referenced to internal TMS (δ_{H} 0.00 in CDCl_3), CHCl_3 (δ_{H} 7.26 in CDCl_3), pyridine-*m*-H (δ_{H} 7.22 in d_5 -Pyridine), d_5 -Pyridine-*m*-C (δ_{C} 123.87) or CDCl_3 (δ_{C} 77.00). Assignments in ^1H NMR were made by first-order analysis of the spectra by using ACD/NMR processor software (Advanced Chemistry Development, inc.) and were verified by H–H COSY and HSQC experiments. High/low resolution electrospray ionization mass spectra (ESI-MS) were recorded by JMS-700TZ (JEOL, Japan). TLC was performed on Merck pre-coated plates (20 cm $\times$ 20 cm; layer thickness, 0.25 mm; Silica Gel 60F₂₅₄); spots were visualized by spraying a solution of 90:5:5 (v/v/v) MeOH-*p*-anisaldehyde-concentrated sulfuric acid and heating at 250 °C for ca. 1/2 min, a solution of 95: 5 (v/v) MeOH-concentrated sulfuric acid and heating at 180°C for ca. 1/2 min, and by UV light (256 or 365 nm) when applicable. Column chromatography was performed on Silica Gel N60 (spherical type, particle size 40–50 μm ; Kanto Chemical Industry) with the solvent systems specified, and the ratio of solvent systems was given in v/v. The reaction progress of enzymatic hydrolysis was measured by Park and Johnson method using a microplate reader (SpectraMaxM5, Molecular Devices Co.,

Sunnyvale, CA). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet, br = broad. In addition to those specified above, the following abbreviations, designations and formulas are used throughout the Supporting Information: MeOH = methanol, H₂O = water, EtOAc = ethyl acetate, DCM = dichloromethane, DMF = dimethylformamide, CHCl₃ = Chloroform, Et₃N = triethylamine, NaHCO₃ = sodium bicarbonate, MgSO₄ = magnesium sulfate, aq. = aqueous, sat. = saturated, 1N HCl = 1 normal hydrogen chloride solution

Materials

Phytosphingosine was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Z-serine was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Solvents and other reagents for the chemical syntheses were purchased from Sigma-Aldrich Co., Tokyo Chemical Industry Co., Ltd., and Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and used without further purification.

Experimental procedure for the chemical synthesis of ceramide mimic **1**

(2S, 3S, 4R)-2-[N-(9-fluorenylmethyloxycarbonyl)amino]octadecane-1,3,4-triol (5).

FmocOSu (405 mg, 1.20 mmol) was added to a suspension of phytosphingosine **4** (318 mg, 1.00 mmol) in THF (10 mL), and the mixture was stirred at room temperature for 2.5 h. MeOH was added to the reaction mixture, and the mixture was concentrated to a half volume. After silica gel was added to the mixture, solvent was removed *in vacuo*

completely. The residue was purified by flash column chromatography on silica gel (hexane-EtOAc, 3:2~1:2) to yield the desired compound **5** as a white solid (525 mg, 97%); ^1H NMR (500 MHz, d_5 -pyridine): δ 8.44 (d, J = 8.91 Hz, 1H; NH), 7.86 (d, J = 7.54 Hz, 2H; Ar-*H*), 7.71 (d, J = 7.54 Hz, 2H; Ar-*H*), 7.40 (t, J = 7.20 Hz, 2H; Ar-*H*), 7.26 (m, 2H; Ar-*H*), 4.89 (m, 1H; *H*-2), 4.63 (m, 1H; Fmoc- CH_2a), 4.55-4.59 (m, 2H; *H*-1a, Fmoc- CH_2b), 4.51 (m, 1H; *H*-1b), 4.46 (br.s, 1H; *H*-3), 4.32-4.39 (m, 2H; *H*-4, Fmoc-*CH*), 2.27 (m, 1H; *H*-5a), 1.87-2.03 (m, 2H; *H*-5b, *H*-6a), 1.71 (m, 1H; *H*-6b), 1.35-1.50 (m, 2H; CH_2), 1.17-1.35 (m, 20H; CH_2), 0.87 (t, J = 7.20 Hz, 3H; CH_3); ^{13}C NMR (125 MHz (HSQC), d_5 -pyridine): δ 128.40, 127.84, 126.06, 120.77 (Ar), 77.02 (*C*-3), 73.25 (*C*-4), 67.04 (Fmoc- CH_2), 62.44 (*C*-1), 55.90 (*C*-2), 48.24 (Fmoc-*CH*), 34.23 (*C*-5), 32.49-23.30 (CH_2), 14.65 (CH_3); HRMS (ESI) Calcd. for $\text{C}_{33}\text{H}_{49}\text{NO}_5\text{Na}$ $[\text{M}+\text{Na}]^+$ 562.35084, found 562.35158

(2*S*,3*S*,4*R*)-1-(*tert*-butyldimethylsilyloxy)-2-[*N*-(9-fluorenylmethyloxy-carbonyl)amino]octadecane-3,4-diol (6**).**

To a solution of compound **5** (7.00 g, 13.0 mmol) and imidazole (2.66 g, 39.0 mmol) in THF (130 mL) was added *tert*-butylchlorodimethylsilane (2.94 g, 19.5 mmol). The solution was stirred at room temperature for 3 h. The reaction mixture was diluted with EtOAc, washed with sat. NaHCO_3 aq. and brine, dried over MgSO_4 , and concentrated under reduced pressure. Purification of the crude product by flash column chromatography on silica gel (hexane-EtOAc, 6:1~3:1) yielded compound **6** (8.30 g, 98%) as an amorphous white solid; ^1H NMR (500 MHz, CDCl_3): δ 7.75 (m, 2H; Ar-*H*), 7.56-7.60 (m, 2H; Ar-*H*), 7.38 (t, J = 7.46 Hz, 2H; Ar-*H*), 7.30 (m, 2H; Ar-*H*), 5.53 (d, J

= 8.70 Hz, 1H; NH), 4.38 (m, 2H; Fmoc-CH₂a,b), 4.21 (t, *J* = 7.15 Hz, 1H; Fmoc-CH), 3.93(m, 2H; *H*-1a, *H*-2), 3.79 (m, 1H; *H*-1b), 3.62 (m, 2H; *H*-3, *H*-4), 3.30 (d, *J* = 7.77 Hz, 1H; 4-OH), 2.90 (d, *J* = 6.84 Hz, 1H; 3-OH), 1.69 (m, 1H; *H*-5a), 1.42-1.58 (m, 2H; *H*-5b, *H*-6a), 1.19-1.41 (m, 23H; *H*-6a, CH₂), 0.91 (br.s, 9H; *t*-Bu), 0.88 (t, *J* = 6.84 Hz, 3H; CH₃), 0.11 (d, *J* = 1.55 Hz, 6H; Si-CH₃); ¹³C NMR (125 MHz (HSQC), CDCl₃): δ 127.64, 126.97, 124.98, 119.93 (Ar), 75.96 (C-3), 73.09 (C-4), 66.84 (Fmoc-CH₂), 62.54 (C-1), 52.01 (Fmoc-CH), 47.17 (C-2), 33.37 (C-5), 31.88-29.32 (CH₂), 25.80 (C-6), 25.76 (*t*-Bu), 22.65 (CH₂), 14.08 (CH₃), -5.55, -5.70 (Si-CH₃); HRMS (ESI) Calcd. for C₃₉H₆₃NO₅SiNa [M+Na]⁺ 676.43732, found 676.43409

(2*S*,3*S*,4*R*)-1-(*tert*-butyldimethylsilyloxy-2-[*N*-(9-fluorenylmethyloxy-carbonyl)amino]-3,4-*O*-isopropylidene-octadecane-3,4-diol (7).

Compound **6** (2.48 g, 3.79 mmol) was mixed with 2-methoxypropene (721 μL, 7.58 mmol) in THF (30 mL). CSA (44 mg, 190 μmol) was added and the reaction mixture was stirred at room temperature. After 5 h the reaction was quenched with Et₃N and concentrated under reduced pressure. Purification of the crude product by flash column chromatography on silica gel (hexane-EtOAc, 20:1) yielded compound **7** (2.59 g, 98%) as a colorless oil; ¹H NMR (500 MHz, CDCl₃): δ 7.75 (d, *J* = 7.57 Hz, 2H; Ar-*H*), 7.57 (d, *J* = 7.33 Hz, 2H; Ar-*H*), 7.39 (t, *J* = 7.57 Hz, 2H; Ar-*H*), 7.29 (t, *J* = 7.57 Hz, 2H; Ar-*H*), 5.02 (d, *J* = 9.77 Hz, 1H; NH), 4.38 (d, *J* = 7.08 Hz, 2H; Fmoc-CH₂), 4.23 (t, *J* = 7.08 Hz, 1H; Fmoc-CH), 4.10 (m, 2H; *H*-3, *H*-4), 3.89 (d, *J* = 9.77 Hz, 1H; *H*-1a), 3.83 (br.s, 1H; *H*-2), 3.67 (d, *J* = 8.31 Hz, 1H; *H*-1b), 1.53 (br.s, 2H; *H*-5a,b), 1.42 (s, 3H; (RO)₂CCH₃a), 1.32 (s, 3H; (RO)₂CCH₃b), 1.32-1.19 (m, 24H; CH₂), 0.91 (s, 9H; *t*-Bu),

0.88 (t, $J = 6.97$ Hz, 3H; CH_3), 0.06 (s, 6H; Si-CH_3); ^{13}C NMR (125 MHz (HSQC), CDCl_3): δ 127.65, 126.96, 124.96, 119.94 (Ar), 77.77 (C-3), 75.34 (C-4), 66.79 (Fmoc- CH_2), 62.76 (C-1), 51.33 (C-2), 47.02 (Fmoc-CH), 31.90-22.66 (CH_2), 28.28 ((RO) $_2\text{CCH}_3\text{a}$), 25.91 ((RO) $_2\text{CCH}_3\text{b}$), 25.85 (*t*-Bu), 14.10 (CH_3), -5.49, -5.52 (Si-CH_3); HRMS (ESI) Calcd. for $\text{C}_{42}\text{H}_{67}\text{NO}_5\text{SiNa}$ [$\text{M}+\text{Na}$] $^+$ 716.46862, found 716.46882

(2S,3S,4R)-2-[N-(9-fluorenylmethyloxy-carbonyl)amino]-3,4-O-isopropylidene-octadecan-1,3,4-triol (8).

Compound **7** (9.54 g, 13.7 mmol) was dissolved in THF (80 mL) and was added HF-pyridine (4 mL, 154 mmol). The reaction mixture was stirred vigorously at room temperature overnight then diluted with EtOAc. The solution was washed with 1 N aqueous HCl, sat. NaHCO_3 aq. and brine. The organic phase was dried over MgSO_4 and concentrated under reduced pressure. Purification by precipitation (hexane-EtOAc, 4:1~3:2) gave compound **8** as a white solid (7.57 g, 95%); ^1H NMR (500 MHz, CDCl_3): δ 7.76 (d, $J = 7.64$ Hz, 2H; Ar-*H*), 7.58 (m, 2H, Ar-*H*), 7.40 (m, 2H; Ar-*H*), 7.32 (m, 2H; Ar-*H*), 5.18 (m, 1H; NH), 4.43 (d, $J = 6.72$ Hz, 2H; Fmoc- CH_2), 4.21 (t, $J = 7.03$ Hz, 1H; Fmoc-CH), 4.17 (br.s, 1H; *H*-4), 4.12 (t, $J = 6.11$ Hz, 1H; *H*-3), 3.89 (m, 1H; *H*-1a), 3.82 (br.s, 1H; *H*-2), 3.71 (br.s, 1H; *H*-1b), 2.21 (br.s, 1H; OH), 1.56 (m, 3H; *H*-5a,b, *H*-6a), 1.46 (s, 3H; (RO) $_2\text{CCH}_3\text{a}$), 1.43 (br.s, 1H; *H*-6b), 1.34 (s, 3H; (RO) $_2\text{CCH}_3\text{b}$), 1.33-1.21 (m, 22H; CH_2), 0.88 (t, $J = 7.03$ Hz, 3H; CH_3); ^{13}C NMR (125 MHz (HSQC), CDCl_3): δ 127.70, 127.03, 125.02, 119.97 (Ar), 78.15 (C-3), 77.68 (C-4), 66.80 (Fmoc- CH_2), 63.47 (C-1), 51.58 (C-2), 47.27 (Fmoc-CH), 31.91-22.68 (CH_2), 27.55

((RO)₂CCH₃a), 25.21 ((RO)₂CCH₃b), 14.11 (CH₃); HRMS (ESI) Calcd. for

C₃₆H₅₃NO₅Na [M+Na]⁺ 602.38214, found 602.38025

(2R,3S,4R)-3,4-dihydroxy-2-[N-(9-fluorenylmethyloxy-carbonyl)amino]-3,4-O-isopropylidene-octadecane-1-al (3).

To a solution of compound **8** (1.75 g, 3.02 mmol) and NaHCO₃ (505 mg, 6.04 mmol) in CH₂Cl₂ (20 mL) and H₂O (15 mL), TEMPO (25 mg, 151 μmol), KBr (40 mg, 302 μmol), and Na₂CO₃ (15 mg, 151 μmol) were added at 0°C. The solution was stirred vigorously at 0°C for 5 min then added dropwise *t*-BuOCl (500 μL, 4.53 mmol). After 40 min, the reaction was quenched by MeOH, diluted with EtOAc, and washed with brine. The organic phase was dried over MgSO₄ and concentrated under reduced pressure. Crude product was used in the next reaction without further purification.

Analytical sample was purified by flash column chromatography on silica gel (hexane-EtOAc, 20:1~3:1) and compound **3** was yielded as a white solid; ¹H NMR (500MHz, CDCl₃): δ 9.75 (s, 1H; CHO), 7.76 (d, *J* = 7.49 Hz, 2H; Ar-*H*), 7.60 (m, 2H; Ar-*H*), 7.39 (t, *J* = 7.86 Hz, 2H; Ar-*H*), 7.33 (m, 2H; Ar-*H*), 5.65 (br.s, 1H; NH), 4.51 (m, 1H; *H*-2), 4.40 (m, 3H; *H*-3, Fmoc-CH₂a,b), 4.31 (m, 1H; *H*-4), 4.22 (t, *J* = 7.11 Hz, 1H; Fmoc-CH), 1.77 (m, 2H; *H*-5a,b), 1.60 (m, 1H; *H*-6a), 1.48 (m, 1H; *H*-6b), 1.40 (s, 3H; (RO)₂CCH₃a), 1.38-1.16 (m, 25H; CH₂, (RO)₂CCH₃b), 0.88 (t, *J* = 6.92 Hz, 3H; CH₃); ¹³C NMR (150 MHz (HSQC), CDCl₃): δ 198.13 (CHO), 127.76, 127.10, 125.07, 120.01 (Ar), 78.85 (*C*-3), 77.53 (*C*-4), 67.32 (Fmoc-CH₂), 61.04 (*C*-2), 47.13 (Fmoc-CH), 31.94-22.71 (CH₂, (RO)₂CCH₃a,b), 14.14 (CH₃); HRMS (ESI) Calcd. for C₃₆H₅₁NO₅Na [M+Na]⁺ 600.36649, found 600.36715

**(2S, 3S, 4R)- 2-[N-(9-fluorenylmethyloxycarbonyl)amino]-3,4-O-isopropylidene
-1-[N-(methoxy)imino]octadecane-3,4-diol (9).**

The crude product containing compound **3** was dissolved in THF (10 mL) and MeOH (10 mL). The solution was stirred at room temperature and added another solution of MeONH₃Cl (500 mg, 5.98 mmol) and pyridine (5.0 mL, 6.20 mmol) in MeOH (5 mL). With the progress of reaction, white solid was precipitated and then, the mixture was re-dissolved by diluting with EtOAc after 2.5 h. The solution was washed with sat. NaHCO₃ aq. and brine. The organic phase was dried over MgSO₄ and concentrated under reduced pressure. Purification by precipitation (EtOAc-Hexane) gave a white solid **9** (1.38 g) with a mixture of *E/Z* isomer (8:1, estimated by ¹H NMR). After filtration, the solution was concentrated, purification of the product by flash column chromatography on silica gel (hexane-EtOAc, 20:1~4:1) yielded compound **9** (400 mg) as a white solid. Totally, product **9** was obtained in 97% yield in 2 steps; (*E* conformer): ¹H NMR (500 MHz, CDCl₃): δ 7.76 (d, *J* = 7.64 Hz, 2H; Ar-*H*), 7.60 (m, 2H; Ar-*H*), 7.47 (d, *J* = 3.67 Hz, 1H; N=CH), 7.40 (t, *J* = 7.33 Hz, 2H; Ar-*H*), 7.32 (t, *J* = 7.64 Hz, 2H; Ar-*H*), 5.51 (d, *J* = 8.55 Hz, 1H; NH), 4.49 (m, 1H; *H*-2), 4.42 (m, 1H; Fmoc-CH₂a), 4.35 (m, 1H; Fmoc-CH₂b), 4.24 (m, 3H; *H*-3, *H*-4, Fmoc-CH), 3.88 (s, 3H; OMe), 1.65 (m, 2H; *H*-5), 1.54 (m, 1H; *H*-6a), 1.42 (s, 3H; (RO)₂CCH₃a), 1.40-1.22 (m, 26H; CH₂, (RO)₂CCH₃b), 0.88 (t, *J* = 7.03 Hz, 3H; CH₃); ¹³C NMR (150 MHz (HSQC), CDCl₃): δ 147.37 (N=CH), 127.70, 127.04, 125.14, 119.96 (Ar), 79.14 (C-3), 77.35 (C-4), 67.11 (Fmoc-CH₂), 61.92 (OMe), 51.44 (C-2), 47.18 (Fmoc-CH), 31.93-22.70 (CH₂, (RO)₂CCH₃a,b), 14.12 (CH₃); (*Z* conformer): ¹H NMR (500 MHz, CDCl₃): δ 7.76 (d, *J* = 7.56 Hz, 2H; Ar-*H*), 7.58 (m, 2H; Ar-*H*), 7.40 (t, *J* = 7.37 Hz, 2H; Ar-*H*), 7.32 (m, 2H;

Ar-*H*), 6.82 (d, *J* = 5.67 Hz, 1H; N=CH), 5.33 (d, *J* = 7.94 Hz, 1H; NH), 4.84 (m, 1H; *H*-2), 4.41 (m, 3H; *H*-3, Fmoc-CH₂a,b), 4.22 (t, *J* = 7.18 Hz, 1H; Fmoc-CH), 4.16 (br.s, 1H; *H*-4), 3.90 (s, 3H; OMe), 1.58 (m, 2H; *H*-5a,b), 1.47 (s, 3H; (RO)₂CCH₃a), 1.35 (s, 3H; (RO)₂CCH₃b) 1.50-1.20 (m, 25H; CH₂, *H*-6a,b), 0.88 (t, *J* = 7.18 Hz, 3H; CH₃)
¹³C NMR (150 MHz (HSQC), CDCl₃, 298 K): δ (ppm) = 147.63 (N=CH), 127.71, 127.04, 125.01, 119.98 (Ar), 77.43 (C-3, C-4), 66.96 (Fmoc-CH₂), 62.16 (OMe), 48.06 (C-2), 47.22 (Fmoc-CH), 31.93-22.70 (CH₂, (RO)₂CCH₃a,b), 14.13 (CH₃); HRMS (ESI) Calcd. for C₃₇H₅₄N₂O₅Na [M+Na]⁺ 629.39304, found 629.39096

(2*S*, 3*S*, 4*R*)- 2-[*N*-(9-fluorenylmethyloxycarbonyl)amino]-3,4-*O*-isopropylidene-1-[*N*-(methoxy)amino]octadecane-3,4-diol (2).

NaBH₃CN (157 mg, 2.50 mmol) was added to a suspension of compound **9** (303 mg, 500 μmol) in THF (5 mL) and AcOH (10 mL) at 0°C and temperature was gradually increased to ambient in 1 h. The reaction mixture was diluted with EtOAc and washed with sat. NaHCO₃ aq. (3 times) and brine. The organic phase was dried over MgSO₄, and concentrated under reduced pressure. Purification of the crude product by flash column chromatography on silica gel (CH₂Cl₂-Et₂O, 40:1~30:1, Hexane-EtOAc, 3:1) yielded **2** (155 mg, 51%) as a white solid; ¹H NMR (500 MHz, CDCl₃): δ 7.76 (d, *J* = 7.56 Hz, 2H; Ar-*H*), 7.59 (m, 2H; Ar-*H*), 7.39 (t, *J* = 7.56 Hz, 2H; Ar-*H*), 7.31 (t, *J* = 7.25 Hz, 2H; Ar-*H*), 5.13 (d, *J* = 8.77 Hz, 1H; NH), 4.42 (m, 2H; Fmoc-CH₂a,b), 4.22 (t, *J* = 6.65 Hz, 1H; Fmoc-CH), 4.15 (br.s, 1H; *H*-4), 4.09 (m, 1H; *H*-3), 3.97 (br.s, 1H; *H*-2), 3.51 (s, 3H; OMe), 3.23 (d, *J* = 13.3 Hz, 1H; *H*-1a), 2.97 (dd, *J* = 13.91, 6.65 Hz, 1H; *H*-1b), 1.61 (m, 2H; *H*-5a,b), 1.52 (m, 1H; *H*-6a), 1.43 (s, 3H; (RO)₂CCH₃a), 1.43

(m, 1H; *H*-6b), 1.33 (s, 3H; (RO)₂CCH₃b), 1.37-1.15 (m, H; CH₂), 0.88 (t, *J* = 6.80 Hz, 3H; CH₃); ¹³C NMR (125 MHz (HSQC), CDCl₃): δ 127.68, 127.01, 124.98, 119.95 (Ar), 78.37 (C-3), 77.71 (C-4), 66.64 (Fmoc-CH₂), 61.45 (OMe), 52.92 (C-1), 49.52 (C-2), 47.30 (Fmoc-CH), 31.92-22.69 (CH₂), 28.90 (C-5), 27.33 ((RO)₂CCH₃a), 25.36 ((RO)₂CCH₃b), 14.12 (CH₃); HRMS (ESI) Calcd. for C₃₇H₅₆N₂O₅Na [M+Na]⁺ 631.40869, found 631.40900

(2*S*, 3*S*, 4*R*)- 2-[*N*-(9-fluorenylmethyloxycarbonyl)amino]-3,4-*O*-isopropylidene-1-[*N*-(methoxy)-*N*-(trifluoroacetyl)amino]octadecane-3,4-diol (10**).**

To a suspension of compound **2** (305 mg, 500 μmol) and NaHCO₃ (210 mg, 2.50 mmol) in THF (5 mL) was added trifluoroacetic anhydride (209 μL, 1.50 mmol) and the mixture was stirred at room temperature for 1 hour. After added MeOH, the solution was diluted with EtOAc and washed with sat. NaHCO₃ aq. and brine. The organic phase was dried over MgSO₄, and concentrated under reduced pressure. Purification of the crude product by flash column chromatography on silica gel (hexane-EtOAc, 10:1~5:1) yielded **10** (240 mg, 68%) as colorless oil; ¹H NMR (600 MHz, CDCl₃): δ 7.75 (d, *J* = 7.52 Hz, 2H; Ar-*H*), 7.56 (m, 2H; Ar-*H*), 7.39 (t, *J* = 7.43 Hz, 2H; Ar-*H*), 7.30 (t, *J* = 7.34 Hz, 2H; Ar-*H*), 4.91 (d, *J* = 9.54 Hz, 1H; NH), 4.41 (dd, *J* = 10.64, 6.97 Hz, 1H; Fmoc-CH₂a), 4.30 (m, 1H; Fmoc-CH₂b), 4.15-4.23 (m, 3H; *H*-2, *H*-4, Fmoc-CH), 4.14 (t, *J* = 6.24 Hz, 1H; *H*-3), 4.07 (dd, *J* = 14.67, 9.35 Hz, 1H; *H*-1a), 3.84 (dd, *J* = 15.04, 2.93 Hz, 1H; *H*-1b), 3.75 (s, 3H; OMe), 1.63-1.51 (m, 4H; *H*-5a,b, *H*-6a,b), 1.47 (s, 3H; (RO)₂CCH₃a), 1.34 (s, 3H; (RO)₂CCH₃b), 1.32-1.18 (m, 22H; CH₂), 0.88 (t, *J* = 7.06 Hz, 3H; CH₃); ¹³C NMR (150 MHz (HSQC), CDCl₃): δ 127.45, 126.87, 124.84, 119.70 (Ar),

77.82 (C-3), 77.43 (C-4), 66.98 (Fmoc-CH₂), 62.42 (OMe), 48.86 (C-2), 46.90 (Fmoc-CH), 46.24 (C-1), 31.89-22.33 (CH₂), 29.26 (C-5), 27.38 ((RO)₂CCH₃a), 24.98 ((RO)₂CCH₃b), 14.08 (CH₃); HRMS (ESI) Calcd. for C₃₉H₅₅F₃N₂O₆Na [M+Na]⁺ 727.39099, found 727.39308

(2S,3S,4R)-1-[N-(methoxy)amino]-2-[N-(trifluoroacetyl)-amino]-3,4-O-isopropylidene-octadecane-3,4-diol (12).

Compound **10** (222 mg, 315 μmol) was dissolved in THF (4 mL) and added piperidine (1 mL) at room temperature. The solution was stirred for 3 h and silica gel was added before evaporated completely. The crude product was purified by flash column chromatography on silica gel (hexane-EtOAc, 10:1~3:1) yielded compound **12** (106 mg, 70%) as a white solid; ¹H NMR (600 MHz, CDCl₃): δ 6.95 (d, *J* = 8.62 Hz, 1H; NH), 4.23 (br.s, 1H; *H*-2), 4.16 (m, 2H; *H*-3, *H*-4), 3.49 (s, 3H; OMe), 3.24 (m, 1H; *H*-1a), 3.08 (dd, *J* = 13.94, 6.24 Hz, 1H; *H*-1b), 1.59 (m, 1H; *H*-5a), 1.52 (m, 2H; *H*-5b, *H*-6a), 1.42 (s, 3H; (RO)₂CCH₃a), 1.33 (s, 3H; (RO)₂CCH₃b), 1.36-1.18 (m, 23H), 0.86 (t, *J* = 6.60 Hz, 6H; CH₃); ¹³C NMR (150 MHz (HSQC), CDCl₃): δ 77.37 (C-3, C-4), 61.53 (OMe), 52.04 (C-1), 49.12 (C-2), 31.99-22.35 (CH₂), 28.85 (C-5), 27.08 ((RO)₂CCH₃a), 24.84 ((RO)₂CCH₃b), 14.25 (CH₃); HRMS (ESI) Calcd. for C₂₄H₄₆F₃N₂O₄ [M+H]⁺ 483.34097, found 483.34184

(2S, 3S, 4R)- 2-[N-(9-fluorenylmethyloxycarbonyl)amino]-3,4-O-isopropylidene-1-[N-(methoxy)-N-(octadecanoyl)amino]octadecane-3,4-diol (13).

To a solution of compound **2** (100 mg, 164 μmol) in THF/Pyr (2:1, 15 mL) was added *N*-hydroxysuccinyl octadecanoate (188 mg, 493 μmol) and the mixture was stirred at 50°C for 2 days. After silica gel was added to the mixture, solvent was removed *in vacuo* completely. Purification of the crude product by flash column chromatography on silica gel (hexane-EtOAc, 20:1~10:1) yielded **13** (117 mg, 82%) as a white solid; ^1H NMR (500 MHz, CDCl_3): δ 7.75 (d, J = 7.52 Hz, 2H; Ar-*H*), 7.58 (m, 2H; Ar-*H*), 7.39 (t, J = 7.22 Hz, 2H; Ar-*H*), 7.31 (t, J = 7.52, 2H; Ar-*H*), 5.20 (m, 1H; NH), 4.28 (m, 2H; Fmoc- CH_2), 4.16 (m, 5H; *H*-1a, *H*-2, *H*-3, *H*-4, Fmoc-*CH*), 3.69 (s, 3H; OMe), 3.58 (m, 1H; *H*-1b), 2.39 (m, 2H; $\text{NHCOCH}_2\text{a,b}$), 1.65 (m, 2H; *H*-5a,b), 1.57 (m, 3H; *H*-6a, $\text{NHCOCH}_2\text{CH}_2\text{a,b}$), 1.48 (s, 3H; $(\text{RO})_2\text{CCH}_3\text{a}$), 1.35 (s, 3H; $(\text{RO})_2\text{CCH}_3\text{b}$), 1.41-1.14 (s, 51H; CH_2 , *H*-6b), 0.88 (t, J = 6.92 Hz, 6H; CH_3); ^{13}C NMR (150 MHz (HSQC), CDCl_3): δ 127.46, 126.91, 125.06, 119.74 (Ar), 78.52 (*C*-3), 77.49 (*C*-4), 66.87 (Fmoc- CH_2), 61.70 (OMe), 49.72 (*C*-2), 46.96 (Fmoc-*CH*), 45.68 (*C*-1), 32.19-22.11 (CH_2), 27.01 ($(\text{RO})_2\text{CCH}_3\text{a}$), 24.89 ($(\text{RO})_2\text{CCH}_3\text{b}$), 14.38 (CH_3); HRMS (ESI) Calcd. for $\text{C}_{55}\text{H}_{90}\text{N}_2\text{O}_6\text{Na}$ $[\text{M}+\text{Na}]^+$ 897.66966, found 897.67088

(2*S*,3*S*,4*R*)-2-[*N*-(9-fluorenylmethyloxycarbonyl)amino]-1-[*N*-(methoxy)-*N*-(octadecanoyl)amino]octadecane-3,4-diol (14**).**

To a suspension of compound **13** (525 mg, 600 μmol) in $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$ (10:1, 2.2 mL) was added TFA (8 mL) and the mixture was stirred at room temperature for 30 minutes. The reaction mixture was diluted with EtOAc and washed with H_2O (2 times), sat. NaHCO_3 aq. (3 times) and brine. The organic phase was dried over MgSO_4 , and concentrated under reduced pressure. Purification of the crude product by flash column

chromatography on silica gel (hexane-EtOAc, 10:1~3:1) yielded **14** (285 mg, 57%) as a white solid; ^1H NMR (500 MHz, CDCl_3): δ 7.76 (d, J = 7.59 Hz, 2H; Ar-*H*), 7.58 (d, J = 7.59 Hz, 2H; Ar-*H*), 7.40 (t, J = 7.27 Hz, 2H; Ar-*H*), 7.31 (m, 2H; Ar-*H*), 5.39 (d, J = 8.54 Hz, 1H; NH), 4.39 (m, 2H; Fmoc- CH_2), 4.20 (t, J = 6.96 Hz, 1H; Fmoc-*CH*), 4.14 (m, 1H; *H*-1a), 4.07 (m, 1H; *H*-2), 3.76 (m, 1H; *H*-1b), 3.67 (s, 3H; *OMe*), 3.61 (d, J = 5.38 Hz, 1H; *H*-4), 3.46 (m, 1H; *H*-3), 2.50 (m, 1H; NHCOCH_2a), 2.40 (m, 1H; NHCOCH_2b), 1.65 (m, 3H; *H*-5a, $\text{NHCOCH}_2\text{CH}_2\text{a,b}$), 1.55 (m, 1H; *H*-6a), 1.44 (m, 1H; *H*-5b), 1.38-1.16 (s, 53H; CH_2 , *H*-6b), 0.88 (t, J = 6.64 Hz, 6H; CH_3); ^{13}C NMR (150 MHz (HSQC), CDCl_3): δ 127.36, 126.78, 124.79, 119.65 (Ar), 74.63 (*C*-3), 72.65 (*C*-4), 66.80 (Fmoc- CH_2), 61.32 (*OMe*), 52.45 (*C*-2), 47.06 (Fmoc- CH_2), 45.27 (*C*-1), 33.28-24.21 (CH_2), 14.08 (CH_3); HRMS (ESI) Calcd. for $\text{C}_{52}\text{H}_{86}\text{N}_2\text{O}_6\text{Na}$ [$\text{M}+\text{Na}$] $^+$ 857.63836, found 857.63707

(2*S*, 3*S*, 4*R*)-1-[*N*-(methoxy)amino]-2-[*N*-(octadecanoyl)amino]octadecane-3,4-diol (1).

To a solution of compound **14** (290 mg, 347 μmol) in THF (1.5 mL), piperidine (200 μL) was added and the mixture was stirred at room temperature for 3 hours. After evaporation, purification of the crude product by flash column chromatography on silica gel (toluene-EtOAc, 2:1-1:1-0:1) yielded **1** (140 mg, 66%) as a white solid; ^1H NMR (500 MHz, CDCl_3): δ 6.24 (d, J = 7.82 Hz, 1H; NH), 4.22 (m, 1H; *H*-2), 3.60 (m, 2H; *H*-3, *H*-4), 3.56 (s, 3H; *OMe*), 3.23 (m, 2H; *H*-1a,b), 2.20 (t, J = 7.57 Hz, 2H; $\text{NHCOCH}_2\text{a,b}$), 1.66 (m, 3H; *H*-5a, $\text{NHCOCH}_2\text{CH}_2\text{a,b}$), 1.52 (m, 1H; *H*-6a), 1.44 (m, 1H; *H*-5b), 1.38-1.19 (m, 51H; CH_2 , *H*-6b), 0.88 (t, J = 6.84 Hz, 6H; CH_3); ^{13}C NMR

(150 MHz (HSQC), CDCl₃): δ 76.13 (C-3), 73.20 (C-4), 61.26 (OMe), 51.03 (C-1), 49.78 (C-2), 36.71 (NHCOCH₂), 33.18 (C-5), 31.80-23.24 (CH₂), 13.99 (CH₃); HRMS (ESI) Calcd. for C₃₇H₇₇N₂O₄ [M+H]⁺ 613.58833, found 613.58608

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(2S,3S,4R)-1-phthalimidyloxy-2-[(*tert*-butyloxycarbonyl)amino]-3,4-O-isopropylidene-octadecane-3,4-diol (**24**).

Into a THF solution (150 mL) containing compound **23** (6.58 g, 14.4 mmol), Ph₃P (4.54 g, 17.3 mmol) and *N*-hydroxyphthalimide (2.82 g, 17.3 mmol), DIAD (3.42 mL, 17.3 mmol) was dropped at 0 degree. After 20 min, the reaction mixture was stirred at 50 degree for 9 hours. Then, the solution was evaporated under reduced pressure. Purification by flash column chromatography on silica gel (Hexane-EtOAc, 6:1-4:1) yielded **24** (8.27 g, 95%) as a white solid.; ¹H NMR (500 MHz, CDCl₃): δ 7.84 (m, 2H; Ar-*H*), 7.76 (m, 2H; Ar-*H*), 5.37 (d, *J* = 9.30, 1H; NH), 4.62 (m, 1H; *H*-1a), 4.26 (m, 2H; *H*-1b, *H*-3), 4.16 (dd, *J* = 12.40, 6.51, 1H; *H*-4), 3.92 (m, 1H; *H*-2), 1.60-1.20 (m, 29H; (RO)₂CCH₃b, CH₂), 1.41 (s, 9H; *t*-Bu), 1.38 (s, 3H; (RO)₂CCH₃a), 0.87 (t, *J* = 7.13, 3H; CH₃); ¹³C NMR (150 MHz (HSQC), CDCl₃): δ 134.54 123.53 (Ar) 78.52 (C-1) 77.92 (C-4) 76.28 (C-3) 49.83 (C-2) 31.92-22.69 (CH₂) 28.30 (*t*-Bu) 27.91 ((RO)₂CCH₃a) 25.47 ((RO)₂CCH₃b) 14.11 (CH₃)

(2S,3S,4R)-1-[(9-fluorenylmethylcarbonyl)aminooxy]-2-[(*tert*-butyloxycarbonyl)amino]-3,4-*O*-isopropylidene-octadecane-3,4-diol (25).

Into a THF solution (150 mL) containing compound **24** (1.37 g, 3.0 mmol), MeNH₂ (40% in MeOH, 1.53 mL, 15.0 mmol) was added at room temperature and the reaction mixture was stirred for 7 hours. Then, the solution was diluted with toluene and evaporated under reduced pressure. This procedure was repeated two times to remove the remaining MeNH₂ completely. After evaporaton, the crude product was dissolved in THF (30 mL) and FmocOSu (1.21 g, 3.6 mmol) was added to the solution. Reaction mixture was stirred at room temperature for 9 hours and evaporated under reduced pressure. Purification by flash column chromatography on silica gel (Hexane-EtOAc, 10:1-5:1) yielded **25** (1.71 g, 82%, 2 steps) as a white amorphous solid.

(intermediate)

¹H NMR (600 MHz, CHLOROFORM-*d*) δ ppm 5.54 (br. s, 2 H; ONH₂) 4.62 - 4.71 (m, 1 H; NH) 4.10 - 4.17 (m, 1 H; *H*-4) 3.95 - 4.05 (m, 2 H; *H*-2, *H*-3) 3.80 - 3.88 (m, 1 H; *H*-1a) 3.64 - 3.72 (m, 1 H; *H*-1b) 1.68 – 1.20 (m, 26 H; CH₂) 1.43 (s, 9 H; *t*Bu) 1.42 (s, 3 H; (RO)₂CCH₃a) 1.31 (s, 3 H; (RO)₂CCH₃b) 0.87 (t, *J*=6.60 Hz, 3 H; CH₃); F1 (ppm) 77.92 (*C*-4) 77.48 (*C*-3) 76.12 (*C*-1) 49.20 (*C*-2) 31.89-22.68 (CH₂) 28.35 (*t*-Bu) 27.67 ((RO)₂CCH₃a) 25.59 ((RO)₂CCH₃b) 14.10 (CH₃)

(compound **25**) ¹H NMR (500 MHz, CDCl₃): δ 8.26 (br.s, 1H; ONHFmoc), 7.76 (m, 2H; Ar-*H*), 7.60 (m, 2H; Ar-*H*), 7.40 (m, 2H; Ar-*H*), 7.31 (m, 2H; Ar-*H*), 4.82 (m, 1H; NH), 4.43 (m, 2H; Fmoc-CH₂), 4.27 (t, *J* = 7.37, 1H; Fmoc-CH), 4.14 (m, 2H; *H*-1a, *H*-4), 4.04 (m, 2H; *H*-2, *H*-3), 3.83 (m, 1H; *H*-1b), 1.60-1.20 (m, 26H; CH₂), 1.44 (s, 9H; *t*Bu), 1.42 (s, 3H; (RO)₂CCH₃a), 1.32 (s, 3H; (RO)₂CCH₃b), 0.87 (t, *J* = 7.13, 3H; CH₃); (ppm) 127.79 127.11 125.15 120.00 (Ar) 80.07 (*C*-4) 77.80 (*C*-1) 77.21 (*C*-3)

67.65 (FmocCH₂) 48.86 (C-2) 46.96 (FmocCH) 31.93-22.69 (CH₂) 28.33 (*t*Bu) 27.51 ((RO)₂CCH₃a) 25.42 ((RO)₂CCH₃b) 14.12 (CH₃)

(2*S*,3*S*,4*R*)-1-[(9-fluorenylmethylcarbonyl)aminooxy]-2-[(octadecanoyl)amino]-octadecane-3,4-diol (26**).**

To a suspension of compound **25** (2.37 g, 3.41 mmol) in CH₂Cl₂/H₂O (10:1, 4.5 mL) was added TFA (15 mL) and the mixture was stirred at room temperature for 40 minutes. The reaction solution was neutralized with sat. NaHCO₃ aq. and extracted with CHCl₃ 4 times. The organic phase was dried over MgSO₄ and concentrated under reduced pressure. The crude product was directly used for succeeding reaction without further purification. The product and StearoylOSu were suspended in THF (40 mL) and the mixture was stirred at room temperature for 2 days. The reaction solution was evaporated under reduced pressure completely. After evaporation, obtained white solid was suspended in MeOH and filtrated. The filtercake was dissolved in mixed solvent (CHCl₃/MeOH, 2:1) and silica gel was added to the flask till the solution almost lost fluidity. Remained solvent was removed by evaporation and purification of the crude product by flash column chromatography on silica gel (hexane-EtOAc, 2:1, CHCl₃-*i*PrOH, 20:1) yielded **26** (2.12 g, 75%) as a white solid;

¹H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 7.80 - 7.88 (m, 1 H; Fmoc-NHO) 7.77 (d, *J*=7.41 Hz, 2 H; Fmoc-Ar) 7.56 (d, *J*=7.41 Hz, 2 H; Fmoc-Ar) 7.41 (t, *J*=7.41 Hz, 2 H; Fmoc-Ar) 7.32 (t, *J*=7.41 Hz, 2 H; Fmoc-Ar) 6.57 (br. s, 1 H; NH) 4.49 (d, *J*=7.10 Hz, 2 H; Fmoc-CH₂) 4.17 - 4.26 (m, 3 H; Fmoc-CH, *H*-1a, *H*-2) 4.05 - 4.10 (m, 1 H; *H*-1b) 3.69 - 3.58 (m, 2 H; *H*-3, *H*-4) 2.21 (t, *J*=7.72 Hz, 2 H; NHCOCH₂) 1.97 - 1.18

(m, 56 H; CH_2) 0.88 (t, $J=7.10$ Hz, 6 H; CH_3); F1 (ppm) 127.73 127.27 125.15 120.12 (Ar) 76.50 (C-1) 74.40 (C-3) 73.34 (C-4) 68.18 (Fmoc CH_2) 48.78 (C-2) 45.93 (FmocCH) 36.77-21.88 (CH_2) 14.19 (CH_3)

(2S,3S,4R)-1-aminoxy-2-[(octadecanoyl)amino]-octadecane-3,4-diol (15)

Compound **26** (2.69 g, 3.34 mmol) was dissolved in CH_2Cl_2 (40 mL) and MeOH (10 mL) and piperidine (2.55 mL, 10 eq) was added to the solution at room temperature. After 6 h, solvent was removed by air-drying and white solid was suspended in MeOH. Filtration gave compound **15** (1.02 g, 66%) as a white solid.

^1H NMR (500 MHz, $\text{CHLOROFORM-}d$) δ ppm 6.19 (d, $J=8.43$ Hz, 1 H; NH) 5.55 (br. s, 2 H; ONH_2) 4.26 - 4.33 (m, 1 H; $H-2$) 4.00 (dd, $J=11.73$, 4.76 Hz, 1 H; $H-1a$) 3.87 (dd, $J=11.73$, 4.03 Hz, 1 H; $H-2b$) 3.59 - 3.65 (m, 1 H; $H-4$) 3.53 - 3.59 (m, 1 H; $H-3$) 2.32 (br. s, 1 H; OH) 2.18 - 2.23 (m, 2 H; CH_2) 1.19 - 1.75 (m, 56 H; CH_2) 0.88 (t, $J=6.96$ Hz, 6 H; CH_3); F1 (ppm) 75.18 (C-3) 74.67 (C-1) 73.07 (C-4) 50.92 (C-2) 36.77-21.65 (CH_2) 14.11 (CH_3)

(2S,3S,4R)-1-methylaminoxy-2-[(octadecanoyl)amino]-octadecane-3,4-diol (27)

Compound **15** (1.08 g, 1.80 mmol) was dissolved in CH_2Cl_2 (10 mL) and MeOH (10 mL). Formaldehyde solution (37% in MeOH, 1.0 mL) and AcOH (400 μL) were added to the solution at room temperature and the solution was stirred overnight. Then, solvent was removed by air-drying and white solid was suspended in MeOH. Filtration gave compound **27** (894 mg, 81%) as a white solid.

¹H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 7.06 (d, *J*=7.82 Hz, 1 H; *H*₂C=NOa) 6.50 (d, *J*=7.82 Hz, 1 H; *H*₂C=NOb) 6.05 (br. s, 1 H; NH) 4.46 (dd, *J*=12.70, 5.86 Hz, 1 H; *H*-1a) 4.23 - 4.32 (m, 2 H; *H*-1b, *H*-2) 3.54 - 3.66 (m, 2 H; *H*-3, *H*-4) 3.37 - 3.42 (m, 1 H; OH-3) 2.12 - 2.24 (m, 3 H; OH-4, NHCOCH₂) 1.75-1.18 (m, 56 H) 0.87 (t, *J*=6.84 Hz, 6 H, CH₃); F1 (ppm) 139.00 (*H*₂C=NO) 74.44 (C-3) 73.62 (C-4) 73.17 (C-1) 51.68 (C-2) 36.50-21.88 (CH₂) 14.10 (CH₃)

(2*S*,3*S*,4*R*)-1-methylaminoxy-2-[(octadecanoyl)amino]-octadecane-3,4-diol (16**)**

To a suspension containing compound **27** (122 mg, 200 μmol) in THF (2 mL), NaBH₃CN (38 mg, 600 μmol) and AcOH (8 mL) were added at room temperature. The reaction mixture was stirred for 1 h and neutralized with 4 N NaOH aq.. The solution was diluted with EtOAc and washed with sat. NaHCO₃ 3 times and brine. The organic phase was dried over MgSO₄ and concentrated under reduced pressure. Purification by flash column chromatography on silica gel (Hexane-EtOAc, 2:1-1:1, DCM-THF, 3:1-1:1) yielded **16** (98 mg, 80%) as a white amorphous solid.

¹H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 6.31 - 6.37 (m, 1 H; NH) 4.24 - 4.32 (m, 1 H; *H*-2) 3.99 - 4.05 (m, 1 H; *H*-1a) 3.84 - 3.90 (m, 1 H; *H*-1b) 3.55 - 3.62 (m, 1 H; *H*-4) 3.50 - 3.54 (m, 1 H; *H*-3) 2.73 (s, 3 H; MeNHO) 2.17 - 2.23 (m, 2 H; NHCOCH₂) 1.76 - 1.26 (m, 56 H; CH₂) 0.88 (m, 6 H; CH₃); F1 (ppm) 75.74 (C-3) 74.08 (C-4) 72.42 (C-1) 51.35 (C-2) 39.07 (MeNHO) 37.25-21.98 (CH₂) 14.18 (CH₃)

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HOOC-PS-Fmoc-Isop **28**

To a solution of compound **8** (3.45 g, 5.95 mmol) and NaHCO₃ (1.01 g, 12.1 mmol) in CH₂Cl₂ (40 mL) and H₂O (30 mL), TEMPO (50 mg, 302 μmol), KBr (80 mg, 604 μmol), and Na₂CO₃ (30 mg, 302 μmol) were added at 0°C. The solution was stirred vigorously at 0°C for 5 min then added dropwise *t*-BuOCl (1 mL, 9.06 mmol). After 19 hours, the reaction was quenched by MeOH, diluted with CHCl₃, and washed with brine. The organic phase was dried over MgSO₄ and concentrated under reduced pressure. Purification of the crude product was performed by flash column chromatography on silica gel (hexane-EtOAc, 7:2, CHCl₃-MeOH, 10:1) gave compound **28** (1.89 g, 53%) as a white solid;

¹H NMR (500 MHz, DMSO-*d*₆, 328 K) δ ppm 7.86 (d, *J*=7.57 Hz, 2 H; Fmoc-Ar) 7.75 (br. s, 1 H; NH) 7.66 - 7.70 (m, 2 H; Fmoc-Ar) 7.40 (t, *J*=7.57 Hz, 2 H; Fmoc-Ar) 7.28 - 7.33 (m, 2 H; Fmoc-Ar) 4.30 (dd, *J*=10.01, 6.84 Hz, 1 H; Fmoc-CH₂a) 4.16 - 4.26 (m, 3 H; Fmoc-CH, Fmoc-CH₂b, *H*-3) 4.08 - 4.14 (m, 1 H; *H*-4) 4.01 - 4.08 (m, 1 H; *H*-2) 1.51-1.05 (m, 26 H; CH₂) 1.37 (s, 3 H; (RO)₂CCH₃a) 1.25 (s, 3 H; (RO)₂CCH₃b) 0.85 (t, *J*=7.08 Hz, 3 H; CH₃); F1 (ppm) 127.54 126.82 124.93 119.95 (Ar) 76.91 (C-3) 75.56 (C-4) 65.46 (Fmoc-CH₂) 54.37 (C-2) 46.39 (Fmoc-CH) 31.78-21.49 (CH₂) 27.12 ((RO)₂CCH₃a) 25.15 ((RO)₂CCH₃b) 14.10 (CH₃)

Lactone-Fmoc **29**

To a suspension of compound **28** (310 mg, 522 μmol) in CH₂Cl₂/H₂O (4:1, 2.5 mL) was added TFA (7.5 mL) and the mixture was stirred at room temperature for 3 hours. The reaction mixture was diluted with EtOAc and washed with H₂O (2 times), sat. NaHCO₃ aq. (3 times) and brine. The organic phase was dried over MgSO₄, and

concentrated under reduced pressure. Purification of the crude product by flash column chromatography on silica gel (hexane-EtOAc, 5:1~3:1) yielded **29** (270 mg, 96%) as a white solid;

¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 7.87 (d, *J*=7.34 Hz, 2 H; Fmoc-Ar) 7.75 (br. s, 2 H; Fmoc-Ar) 7.41 (t, *J*=7.03 Hz, 2 H; Fmoc-Ar) 7.29 - 7.36 (m, 2 H; Fmoc-Ar) 5.67 (br. s, 1 H; NH) 4.58 (br. s, 1 H; *H*-2) 4.21 - 4.38 (m, 4 H; Fmoc-CH₂, Fmoc-CH, *H*-4) 4.08 (br. s, 1 H; *H*-3) 1.62 (br. s, 2 H; CH₂) 1.18 - 1.43 (m, 56 H; CH₂) 0.85 (t, *J*=6.73 Hz, 3 H; CH₃); F1 (ppm) 127.50 127.05 125.13 120.00 (Ar) 86.34 (C-4) 70.03 (C-3) 65.72 (Fmoc-CH₂) 53.64 (C-2) 46.75 (Fmoc-CH) 31.77-20.48(CH₂) 13.71 (CH₃)

Lactone-Stearoyl **30**

Compound **29** (162 mg, 300 μmol) was dissolved in DMF (5 mL) and piperidine (150 μL, 1.5 mmol) was added to the solution at room temperature. After 30 minutes, solvent was evaporated with toluene and the crude product was used for the next reaction without further purification. The product and StearoylOSu (300 mg) were suspended in THF (5 mL) and the reaction mixture was stirred at room temperature for 1 day. The solution was concentrated under reduced pressure. The desired product was precipitated in MeOH and filtration gave compound **30** as a white solid (105 mg, 60%, 2 steps).

¹H NMR (500 MHz, pyridine) δ ppm 8.96 (br. s, 1 H; NH) 5.61 - 5.65 (m, 1 H; *H*-2) 4.72 - 4.77 (m, 1 H; *H*-4) 4.69 (d, *J*=5.29 Hz, 1 H; *H*-3) 2.62 - 1.21 (m, 58 H; CH₂) 0.91 (t, *J*=6.53 Hz, 6 H; CH₃); F1 (ppm) 87.55 (C-4) 71.78 (C-3) 52.83 (C-2) 36.38-22.51 (CH₂) 13.99 (CH₃)

H₂NNH-PS-Stearic 17

To a solution of compound 20 (320 mg, 552 μ mol) in CHCl₃/MeOH (2:1, 30 mL), hydrazine monohydrated (1.2 mL) was added. The reaction solution was stirred at room temperature for 1.5 hours and then concentrated by air-drying. The desired product was precipitated in MeOH and filtration gave compound 20 (307 mg, 91%) as a white solid.

¹H NMR (500 MHz, CHLOROFORM-*d*, 328 K) δ ppm 7.90 (br. s, 1 H; H₂NNH) 6.51 (d, *J*=7.57 Hz, 1 H; NH) 4.64 - 4.71 (m, 1 H; *H*-2) 3.87 (br. s, 2 H; H₂NNH) 3.67 (br. s, 1 H; HO-3) 3.51 - 3.61 (m, 2 H; *H*-2) 2.37 (br. s, 1 H; HO-4) 2.23 (t, *J*=7.33 Hz, 2 H; CH₂) 1.82-1.07 (m, 56 H; CH₂) 0.88 (t, *J*=6.35 Hz, 6 H; CH₃); F1 (ppm) 76.24 (C-3) 73.24 (C-4) 51.83 (C-2) 36.07-23.09 (CH₂) 13.69 (CH₃)

Experimental Procedure of Chemical Synthesis of H₂NO-/MeHNO-Ser-derivatives 18, 19

Carbamic acid, *N*-[(1*S*)-1-(hydroxymethyl)-2-oxo-2-(tetradecylamino)ethyl]-, phenylmethyl ester 32

WSC (4.32 g, 22.5 mmol) was added to a DCM solution (200 mL) containing Z-Serine **31** (4.50 g, 18.8 mmol), tetradecylamine (4.80 g, 22.5 mmol) and HOBt·H₂O (3.00 g, 19.6 mmol) at room temperature. The reaction mixture was stirred vigorously for 2 days and then diluted with CHCl₃. The solution was washed with 1 N aqueous HCl, sat. NaHCO₃ aq. and brine. Another CHCl₃ solution was used for extraction from aqueous phases two times. Organic phases were combined, dried over MgSO₄ and concentrated under reduced pressure. Purification by recrystallization (hexane-EtOAc) gave compound **32** as a white solid (6.95 g). Filtrate was evaporated and purification by flash

column chromatography on silica gel (Hexane-EtOAc, 1:1) yielded **32** (315 mg) as a white solid. Totally, 7.27 g of compound **32** was obtained (89%). ; ^1H NMR (500 MHz, CDCl_3): δ 7.36 (m, 5H; Ar-*H*), 6.52 (br.s, 1H; NH-Alkyl), 5.79 (br.s, 1H; NH-Z), 5.14 (s, 2H; PhCH_2), 4.14 (m, 2H; Ser- αH , Ser- βHa), 3.66 (br.s, 1H; Ser- βHb), 3.23 (m, 2H; NHCH_2), 2.93 (br.s, 1H; OH), 1.47 (m, 2H; NHCH_2CH_2), 1.25 (m, 22H; CH_2), 0.88 (t, J = 6.60 Hz, 3H; CH_3);

Carbamic acid, *N*-[(1*S*)-1-(*tert*-butyldimethylsilyloxymethyl)-2-oxo-2-(tetradecylamino)ethyl]-, phenylmethyl ester **33**

To a mixture containing compound **32** (6.95 g, 16.0 mmol) and imidazole (3.27 g, 48.0 mmol) in CH_2Cl_2 (150 mL), TBSCl (3.61 g, 24 mmol) was added at room temperature. The reaction mixture was stirred vigorously overnight and then diluted with EtOAc. The solution was washed with sat. NaHCO_3 aq. and then CHCl_3 solution was used for extraction from aqueous phases. Organic phases were combined, dried over MgSO_4 and concentrated under reduced pressure. Purification by flash column chromatography on silica gel (Hexane-EtOAc, 20:1-5:1) yielded **33** quantitatively as a white solid. ; ^1H NMR (500 MHz, CDCl_3): δ 7.36 (m, 5H; Ar-*H*), 6.44 (br.s, 1H; NH-Alkyl), 5.68 (br.s, 1H; NH-Z), 5.12 (m, 2H; PhCH_2), 4.14 (br.s, 1H; Ser- αH), 4.04 (dd, J = 9.65, 3.51 Hz, 1H; Ser- βHa), 3.61 (br.s, 1H; Ser- βHb), 3.24 (m, 2H; NHCH_2), 1.47 (m, 2H; NHCH_2CH_2), 1.25 (m, 22H; CH_2), 0.89 (m, 12H; CH_3 , *tBu*), 0.09 (m, 6H; Si- CH_3);

Propanamide,

3-*tert*-butyldimethylsilyloxy-*N*-tetradecyl-2-[(triphenylmethyl)amino]-, (2*S*)- (34**)**

To a THF solution (50 mL) containing compound **33** (9.02 g, 16.4 mmol), 10% Pd/C (3.0 g) was added at room temperature. The reaction mixture was stirred vigorously for 5 hours under a 0.25 MPa of H₂ atmosphere and then diluted with CHCl₃. The solid reagent was removed by celite filter and filtrate was evaporated under reduced pressure. Then, the mixture was dissolved in toluene and evaporated under reduced pressure again. This crude product was used directly for the next reaction without any purification steps.

The crude product was dissolved in CH₂Cl₂ (150 mL) and DIEA (5.65 mL). Then TrCl (5.50 g, 19.7 mmol) was added to a solution and the reaction mixture was stirred vigorously for 11 hours at room temperature. EtOAc was used for dilution and the solution was washed with sat. NaHCO₃ aq. and brine. Organic phase was dried over MgSO₄ and concentrated under reduced pressure. Purification by flash column chromatography on silica gel (Hexane-EtOAc, 25:1-5:1) yielded **34** (9.34 g, 86%) as a yellow oil.; ¹H NMR (500 MHz, CDCl₃): δ 7.43 (m, 6H; Ar-*H*), 7.26 (m, 7H; NH-Alkyl, Ar-*H*), 7.20 (m, 3H; Ar-*H*), 3.62 (dd, *J* = 9.52, 2.98 Hz, 1H; Ser-β*Ha*), 3.24 (br.s, 1H; Ser-α*H*), 3.20 (m, 1H; NH-Tr), 3.15 (m, 2H; NHCH₂), 2.49 (dd, *J* = 9.52, 4.46 Hz, 1H; Ser-β*Hb*), 1.47 (m, 2H; NHCH₂CH₂), 1.26 (m, 22H; CH₂), 0.88 (t, *J* = 7.14, 3H; CH₃), 0.81 (s, 1H, *tBu*), -0.09 (m, 3H; Si-CH_{3a}), -0.11 (m, 3H; Si-CH_{3a});

Propanamide, 3-hydroloxy-*N*-tetradecyl-2-[(triphenylmethyl)amino]-, (2*S*)- (35)

To a THF solution (100 mL) containing compound **34** (9.34 g, 14.2 mmol), TBAF solution (1 M in THF, 14.9 mL, 14.9 mmol) was added at room temperature. The solution was stirred vigorously for 45 minutes and evaporated under reduced pressure. Purification by flash column chromatography on silica gel (Toluene-EtOAc, 20:1,

Hexane-EtOAc, 2:1) yielded **35** (6.84 g, 89%) as a yellow oil.; ^1H NMR (500 MHz, CDCl_3): δ 7.41 (m, 6H; Ar-*H*), 7.31 (m, 1H; NH-Alkyl), 7.26 (m, 6H; Ar-*H*), 7.20 (m, 3H; Ar-*H*), 3.52 (dd, $J = 10.64, 3.55$ Hz, 1H; Ser- β Ha), 3.25 (br.s, 1H; Ser- α H), 3.16 (m, 1H; NHCH₂a), 3.05 (m, 1H; NHCH₂b), 2.99 (m, 1H; NH-Tr), 2.66 (dd, $J = 10.64, 5.02$ Hz, 1H; Ser- β Hb), 2.48 (br.s, 1H; OH), 1.43 (m, 2H; NHCH₂CH₂), 1.29 (m, 22H; CH₂), 0.88 (t, $J = 6.80$, 3H; CH₃);

**Propanamide, 3-phthalimidyloxy-*N*-tetradecyl-2-[(triphenylmethyl)amino]-, (2S)-
(36)**

Into a THF solution (100 mL) containing compound **35** (6.37 g, 11.7 mmol), Ph₃P (3.69 g, 14.1 mmol) and *N*-hydroxyphthalimide (2.30 g, 14.1 mmol), DIAD (2.78 mL, 14.1 mmol) was dropped at 0 degree. After 15 min, the reaction mixture was stirred at 50 degree for 6 hours. Then, the solution was evaporated under reduced pressure. Purification by flash column chromatography on silica gel (Hexane-EtOAc, 20:1-6:1) yielded **36** (6.77 g, 84%) as a noncolored viscous oil.; ^1H NMR (500 MHz, CDCl_3): δ 7.74 (m, 2H; Phth-Ar-*H*), 7.66 (m, 2H; Phth-Ar-*H*), 7.61 (m, 1H; NH-Alkyl), 7.47 (d, $J = 8.15$, 6H; Tr-Ar-*H*), 7.16 (t, $J = 7.85$, 6H; Tr-Ar-*H*), 7.07 (t, $J = 7.25$, 3H; Tr-Ar-*H*), 4.33 (d, $J = 9.36$ Hz, 1H; Ser- β Ha), 3.78 (d, $J = 6.95$ Hz, 1H; NH-Tr), 3.37 (m, 2H; Ser- α H, Ser- β Hb), 3.17 (m, 1H; NHCH₂a), 3.02 (m, 1H; NHCH₂b), 1.47 (m, 2H; NHCH₂CH₂), 1.29 (m, 22H; CH₂), 0.89 (t, $J = 6.80$, 3H; CH₃); (^{13}C NMR (125 MHz (HSQC), CDCl_3): δ 171.35, 163.22, 145.44, 134.31, 128.27, 128.24, 127.54, 126.25, 123.17, 79.10, 71.38, 55.45, 39.20, 31.58, 29.35, 29.34, 29.32, 29.31, 29.25, 29.22, 29.06, 29.01, 28.94, 26.53, 22.35, 13.83;

Propanamide, 3-aminooxy-*N*-tetradecyl-2-[(triphenylmethyl)amino]-, (2S)- (37)

To a THF solution (85 mL) containing compound **36** (5.88 g, 8.55 mmol), MeNH₂ (40% in MeOH, 3.32 mL, 30.0 mmol) was added at room temperature and the reaction mixture was stirred for 4 hours. Then, the solution was diluted with toluene and evaporated under reduced pressure. Purification by flash column chromatography on silica gel (Hexane-EtOAc, 3:1-2:1-1:1) yielded **37** (4.45 g, 95%) as a noncolored oil.; ¹H NMR (500 MHz, CDCl₃): δ 7.42 (dd, *J* = 8.25, 0.88, 6H; Ar-*H*), 7.28 (t, *J* = 7.07, 6H; Ar-*H*), 7.21 (m, 4H; NH-Alkyl, Ar-*H*), 5.19 (br.s, 2H; ONH₂), 3.79 (dd, *J* = 10.31, 3.83 Hz, 1H; Ser-β*Ha*), 3.36 (m, 1H; NH-Tr), 3.18 (m, 1H; Ser-α*H*), 3.09 (m, 2H; NHCH₂), 2.72 (dd, *J* = 10.31, 4.12, 1H; Ser-β*Hb*), 1.43 (m, 2H; NHCH₂CH₂), 1.29 (m, 22H; CH₂), 0.88 (t, *J* = 6.92, 3H; CH₃); (¹³C NMR (125 MHz (HSQC), CDCl₃): δ 172.84, 145.70, 128.69, 127.99, 126.81, 76.18, 71.56, 57.66, 39.30, 31.88, 29.65, 29.63, 29.62, 29.61, 29.57, 29.51, 29.31, 26.99, 22.64, 14.08;

Carbamic acid, *N*-

**3-[(2S)-1-oxo-1-tetradecylamino-2-(triphenylmethyl)amino]-propanoxy-,
9H-fluoren-9-ylmethyl ester (38)**

Compound **37** (4.97 g, 8.91 mmol) and Fmoc-OSu (3.61 g, 10.7 mmol) were dissolved in THF (60 mL) and the reaction mixture was stirred at room temperature for 2 hours. Then, the solution was evaporated under reduced pressure. Purification by flash column chromatography on silica gel (Hexane-EtOAc, 10:1-5:1) yielded **38** (5.97 g, 86%) as a white amorphous solid.; ¹H NMR (500 MHz, CDCl₃): δ 7.77 (d, *J* = 7.57, 2H; Fmoc-Ar-*H*), 7.52 (d, *J* = 7.57, 2H; Fmoc-Ar-*H*), 7.42 (m, 9H; NH-Alkyl, Fmoc-Ar-*H*, Tr-Ar-*H*), 7.31 (m, 3H; ONH, Fmoc-Ar-*H*), 7.23 (t, *J* = 7.57, 6H; Tr-Ar-*H*), 7.17 (m,

3H; Tr-Ar-H), 4.43 (dd, $J = 10.01, 6.84$ Hz, 1H; Fmoc-CH₂a), 4.30 (m, 1H; Fmoc-CH₂b), 4.15 (t, $J = 7.33$ Hz, 1H; Fmoc-CH), 3.97 (dd, $J = 10.50, 2.44$ Hz, 1H; Ser-βHa), 3.62 (br.s, 1H; NH-Tr), 3.36 (m, 1H; Ser-αH), 3.16 (m, 1H; NHCH₂a), 3.03 (m, 1H; NHCH₂b), 2.95 (dd, $J = 10.50, 5.37$, 1H; Ser-βHb), 1.43 (m, 2H; NHCH₂CH₂), 1.26 (m, 22H; CH₂), 0.88 (t, $J = 6.72$, 3H; CH₃); (¹³C NMR (125 MHz (HSQC), CDCl₃): δ 157.43, 145.73, 143.39, 141.24, 128.71, 127.94, 127.08, 126.68, 124.99, 120.00, 77.94, 71.64, 67.68, 56.59, 46.78, 39.45, 31.88, 29.59, 29.31, 26.97, 22.67, 14.07;

Carbamic acid, N-3-[(2S)-2-amino-1-oxo-1-tetradecylamino]-propanoxy-, 9H-fluoren-9-ylmethyl ester (39)

To a solution of compound **38** (780 mg, 1.0 mmol) in CH₂Cl₂ (8 mL) and MeOH (1.5 mL), TFA (500 mL) was added and the reaction mixture was stirred at room temperature for 1 hour. Then, the solution was neutralized by sat. NaHCO₃ aq. and extracted with CHCl₃ three times. Organic phase was dried over MgSO₄ and evaporated under reduced pressure. Purification by flash column chromatography on silica gel (Hexane-EtOAc, 3:1, CHCl₃:*i*-PrOH, 40:1) yielded **39** (501 mg, 93%) as a white solid.; ¹H NMR (500 MHz, CDCl₃): δ 7.73 (d, $J = 7.34$, 2H; Ar-H), 7.68 (br.s, 1H; NH-Alkyl), 7.56 (d, $J = 7.34$, 2H; Ar-H), 7.37 (t, $J = 7.64$, 2H; Ar-H), 7.28 (t, $J = 7.34$, 2H; Ar-H), 4.46 (d, $J = 6.72$ Hz, 2H; Fmoc-CH₂), 4.19 (t, $J = 6.72$ Hz, 1H; Fmoc-CH), 4.03 (m, 1H; Ser-βHa), 3.94 (m, 1H; Ser-βHb), 3.53 (m, 1H; Ser-αH), 3.21 (m, 2H; NHCH₂), 1.89 (br.s, 2H; NH₂), 1.47 (m, 2H; NHCH₂CH₂), 1.26 (m, 22H; CH₂), 0.88 (t, $J = 7.03$, 3H; CH₃); (¹³C NMR (125 MHz (HSQC), CDCl₃): δ 172.16, 157.74, 143.34, 143.33, 141.17, 127.70,

127.01, 124.90, 119.88, 78.74, 67.14, 53.52, 46.92, 39.23, 31.81, 29.59, 29.57, 29.55, 29.50, 29.46, 29.37, 29.25, 29.21, 26.84, 22.58, 14.02;

Carbamic acid, *N*-

**3-[(2*S*)-2-octadecanoylamino-1-oxo-1-tetradecylamino]-propanoxy- ,
9H-fluoren-9-ylmethyl ester (**40**)**

Compound **39** (1.78 g, 3.31 mmol) and StearicOSu (1.52 g, 3.97 mmol) were dissolved in THF (30 mL) and the reaction mixture was stirred at room temperature for 3 days. Then, precipitated white solid was collected by filtration (1.78 g) and the filtrate was evaporated under reduced pressure. Purification by flash column chromatography on silica gel (Hexane-EtOAc, 10:1-3.5:1-2:1) yielded **40** (510 mg) as a white solid. Totally, 2.29 g of compound **40** was obtained (86%). ; ¹H NMR (500 MHz, CDCl₃): δ 8.78 (s, 1H; ONH), 7.75 (d, *J* = 7.79, 2H; Ar-*H*), 7.62 (br.s, 1H; NH-Alkyl), 7.56 (dd, *J* = 7.45, 1.02, 2H; Ar-*H*), 7.39 (dd, *J* = 7.45, 2.37, 2H; Ar-*H*), 7.30 (t, *J* = 7.45, 2H; Ar-*H*), 7.03 (br.s, 1H; Ser-NH), 4.66 (m, 1H; Ser-α*H*), 4.44 (m, 2H; Fmoc-CH₂), 4.21 (t, *J* = 7.11 Hz, 1H; Fmoc-CH), 4.16 (dd, *J* = 11.51, 4.74, 1H; Ser-β*Ha*), 3.94 (dd, *J* = 11.17, 7.45, 1H; Ser-β*Hb*), 3.24 (m, 2H; NHCH₂), 2.24 (t, *J* = 7.62, 2H; COCH₂), 1.63 (m, 2H; COCH₂CH₂), 1.47 (m, 2H; NHCH₂CH₂), 1.23 (m, 50H; CH₂), 0.88 (t, *J* = 6.94, 6H; CH₃); (13C NMR (125 MHz (HSQC), CDCl₃): δ 173.75, 169.05, 158.37, 143.29, 143.22, 141.25, 141.24, 127.87, 127.15, 127.12, 124.99, 120.04, 76.19, 67.94, 50.98, 46.86, 39.83, 36.55, 31.90, 29.68, 29.64, 29.62, 29.60, 29.54, 29.49, 29.34, 29.25, 26.84, 25.58, 22.67, 14.09;

Octadecanamide, [1-(aminooxymethyl)-2-oxo-2-(tetradecylamino)ethyl]-, (S) (18)

To a flask containing compound **40** (2.69 g, 3.34 mmol), THF (40 mL) and $\text{CHCl}_3/\text{MeOH}$ (2:1, 10 mL) was added. Although the starting material was not dissolved completely, piperidine (3.30 mL, 33.4 mmol) was added to the reaction mixture and the suspension was stirred at room temperature for 2 hours. Then, the solvent was reduced by air-drying and Et_2O was added for precipitation. Filtration gave compound **18** (1.76 g, 91%) as a white solid. ; ^1H NMR (500 MHz, CDCl_3 , 50 °C): δ 6.44 (d, J = 7.35, 1H; Ser-NH), 6.31 (br.s, 1H; NH-Alkyl), 5.59 (br.s, 2H; ONH_2), 4.70 (dd, J = 13.00, 5.94, 1H; Ser- αH), 3.89 (m, 1H; Ser- βHa), 3.81 (m, 1H; Ser- βHb), 3.24 (m, 2H; NHCH_2), 2.23 (t, J = 7.63, 2H; COCH_2), 1.63 (m, 2H; COCH_2CH_2), 1.49 (m, 2H; NHCH_2CH_2), 1.27 (m, 50H; CH_2), 0.88 (t, J = 6.50, 6H; CH_3); (^{13}C NMR (125 MHz (HSQC), CDCl_3 , 50 °C): δ 173.50, 169.68, 75.42, 52.25, 39.74, 36.69, 31.92, 29.69, 29.65, 29.62, 29.59, 29.55, 29.50, 29.33, 29.27, 26.89, 25.64, 22.66, 14.03;

Octadecanamide, [1-(methyliminooxymethyl)-2-oxo-2-(tetradecylamino)ethyl]-, (S)

^1H NMR (500 MHz, CDCl_3 , 50 °C): δ 7.05 (d, J = 7.82, 1H; $\text{ON}=\text{CH}_2\text{a}$), 6.48 (d, J = 7.82, 1H; $\text{ON}=\text{CH}_2\text{b}$), 6.32 (d, J = 7.57, 1H; Ser-NH), 6.16 (br.s, 1H; NH-Alkyl), 4.66 (dd, J = 12.70, 5.86, 1H; Ser- αH), 4.40 (m, 1H; Ser- βHa), 4.24 (m, 1H; Ser- βHb), 3.25 (m, 2H; NHCH_2), 2.23 (t, J = 7.33, 2H; COCH_2), 1.63 (m, 2H; COCH_2CH_2), 1.48 (m, 2H; NHCH_2CH_2), 1.27 (m, 50H; CH_2), 0.88 (t, J = 6.84, 6H; CH_3);

Octadecanamide, [1-(methylaminooxymethyl)-2-oxo-2-(tetradecylamino)ethyl]-, (S) (19)

^1H NMR (500 MHz, CDCl_3 , 50 $^\circ\text{C}$): δ 6.51 (d, $J = 7.07$, 1H; Ser-NH), 6.31 (br.s, 1H; NH-Alkyl), 4.64 (dd, $J = 12.73$, 5.94, 1H; Ser- αH), 3.92 (m, 4.74, 1H; Ser- βHa), 3.82 (m, 1H; Ser- βHb), 3.25 (dd, $J = 13.01$, 6.79, 2H; NHCH_2), 2.71 (s, 3H; NHMe), 2.24 (t, $J = 7.64$, 2H; COCH_2), 1.64 (m, 2H; COCH_2CH_2), 1.49 (m, 2H; NHCH_2CH_2), 1.27 (m, 50H; CH_2), 0.88 (t, $J = 6.79$, 6H; CH_3);

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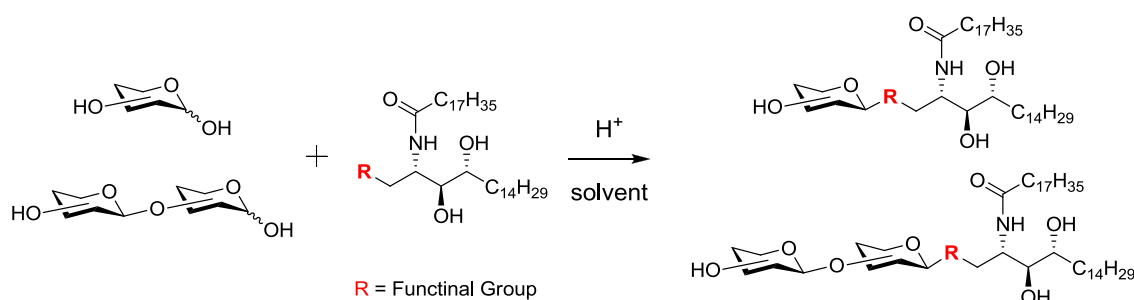
Chapter 3

Glycoblottting Reaction for Construction of Neoglycolipid Library

3-1 Introduction

Glycoblotting reaction^[1-3], also called neoglycosylation, has been used broadly due to its simplicity to conjugate sugars with aglycons. This method focuses on aldehyde group at reducing end of glycan. Aminoxy or hydrazone group can easily capture aldehyde group in acidic condition and the stability of oxime and hydrazone is enough to keep the linkage even though in aqueous solution^[4]. Our laboratory has used this reaction to simply pick up only sugars from biological samples, which include protein, lipid, and so on^[5]. That has enabled to perform simultaneous glycomics rapidly^[6-8]. This technique has been used to immobilize target glycans on the surface of microarray^[9-11] or nanoparticles^[12,13], conjugate glycans with biomolecules^[14], and construct glycoconjugate libraries^[15-20]. In a view of librarization, however, glycosphingolipids have not been a target compound yet^[21, 22].

In this chapter, I tried to construct a neoglycolipid library using functional ceramide to capture free sugars (Scheme 3-1).

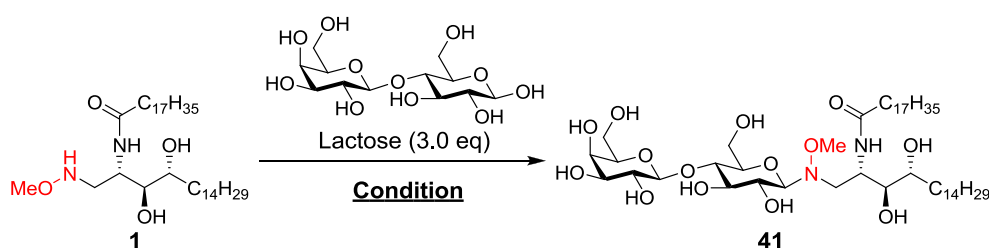


Scheme 3-1. Construction of neoglycolipid library via glycoblotting reaction

3-2 Results and Discussions

3-2-1 Glycoblotting Reaction Using Methoxyamino-Derivative **1**

At first, we tried glycoblotting reaction using methoxyamino derivative **1** and lactose as shown in table 3-2-1. Although the yields were not so high, all reactions afforded the same conjugated product.



Entry	Solvent system	AcOH ^[a] (%)	Yield ^[b] (%)
1	CH ₂ Cl ₂ /MeOH (1:5)	0.5	18
2	CHCl ₃ /MeOH/H ₂ O (10:10:1)	4.5	15
3	CHCl ₃ /MeOH/H ₂ O (25:25:8)	1.7	29

[a] AcOH percentage in total volume

[b] Yields of isolated products after silica gel chromatography

Table 3-2-1. Non-natural lactosylceramide **41** (*N*-LacCer) obtained by one step glycoblotting reaction of ceramide mimic **1**

The structure of the purified product was analyzed by NMR measurement, which included ¹H, ¹³C, COSY, TOCSY, ¹H-¹³C HSQC and HMBC experiments (Fig. 3-2-1). All peaks of HSQC measurement were assigned and the coupling constant of anomeric position at reducing end showed bigger value (8.8 Hz) which was enough to be confirmed as β-glycoside.

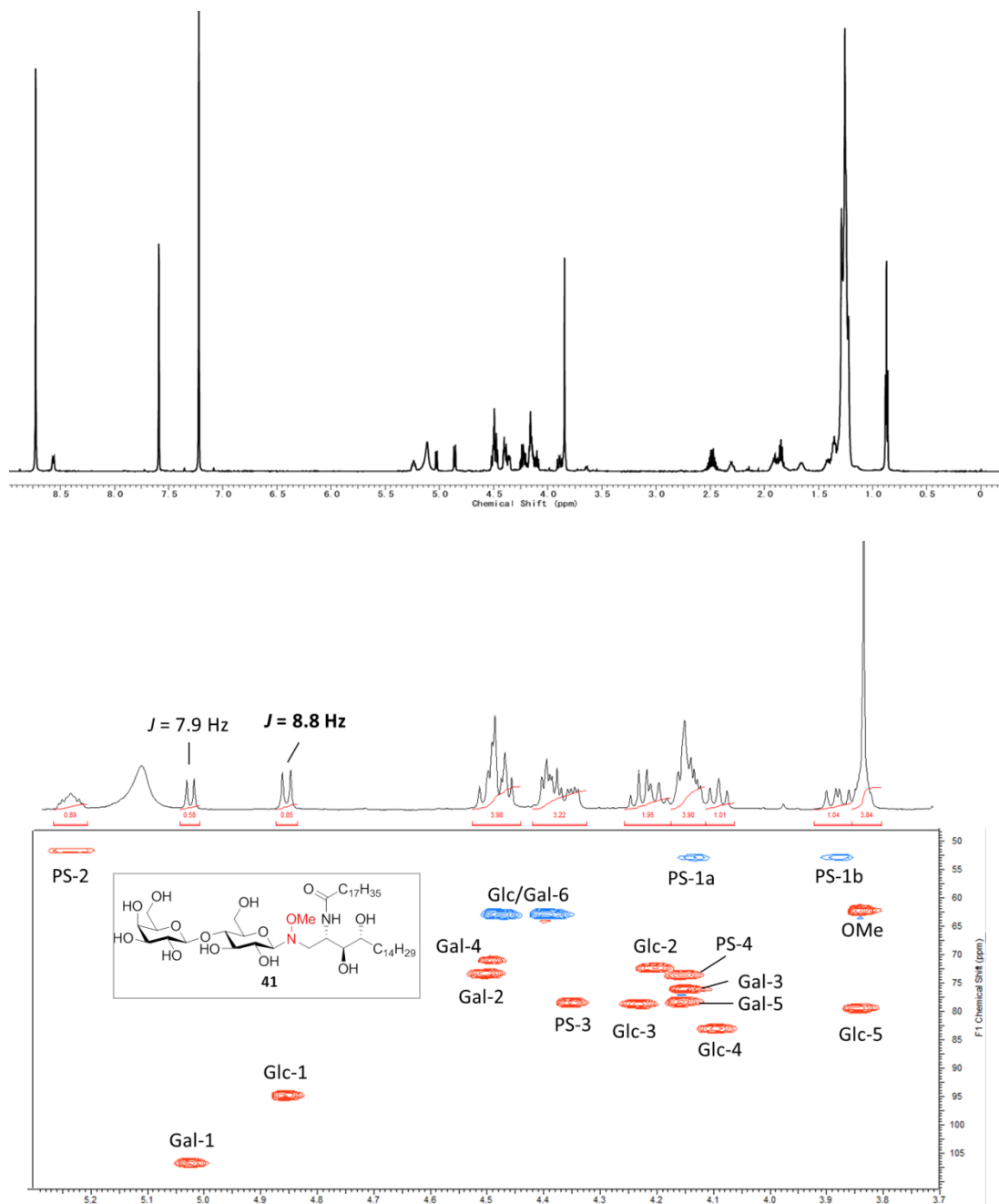


Fig. 3-2-1. NMR spectra of the conjugated product **41**

upper: ^1H spectrum, under: ^1H - ^{13}C HSQC spectrum

(d_5 -pyridine, 300 K)

Then, glycoblotting reaction was performed using other 9 free sugars shown below (Fig. 3-2-2). Di-/tri-saccharides were used under the optimized condition for lactose and monosaccharides under another condition including less H₂O. Though all reactions proceeded, two major products were detected on TLC analysis in case of some monosaccharides.

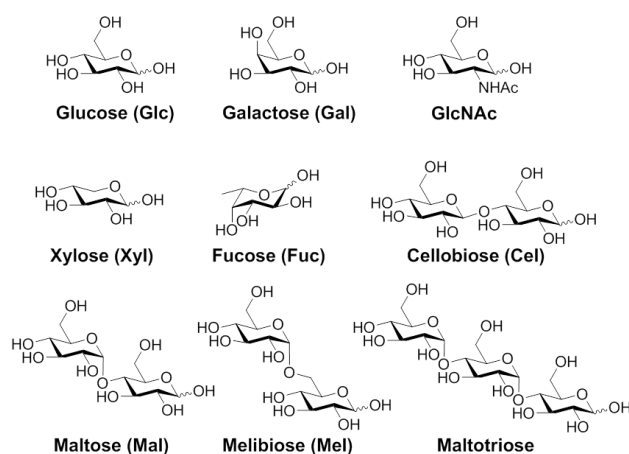


Fig. 3-2-2. Free sugars used for construction of library

To determine the structural difference of two compounds, NMR measurements were performed after acetylation using galactose derivatives. From the results, one of them was confirmed to be β -product as expected (Fig. 3-2-3)

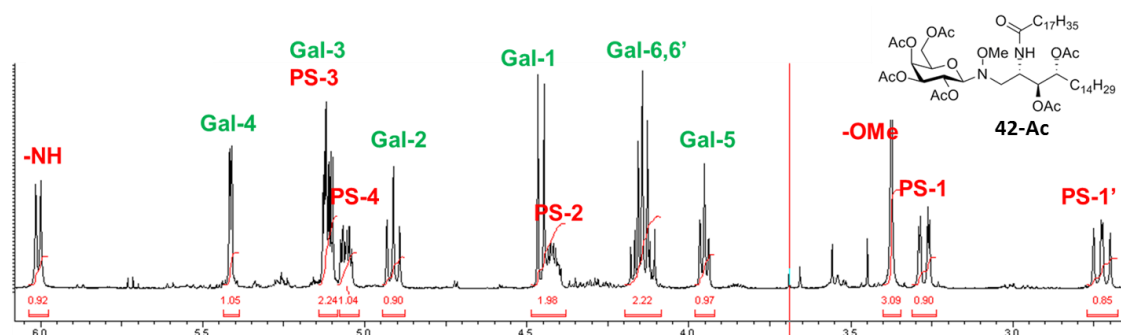


Fig. 3-2-3. ¹H NMR spectrum of acetylated β -product after neoglycosylation using galactose

(CDCl₃, 300 K)

Another one was predicted α -product before the measurement because this type of functional group cannot form ring-open structure. However, the ^1H spectrum suggested it was not α -configured compound (Fig. 3-2-4). Then, we conducted thorough analysis (^1H , ^{13}C , COSY, TOCSY, ^1H - ^{13}C HSQC, HMBC and NOESY experiments) which clarified unexpected structure caused by opening sugar ring and sphingosine cyclizing.

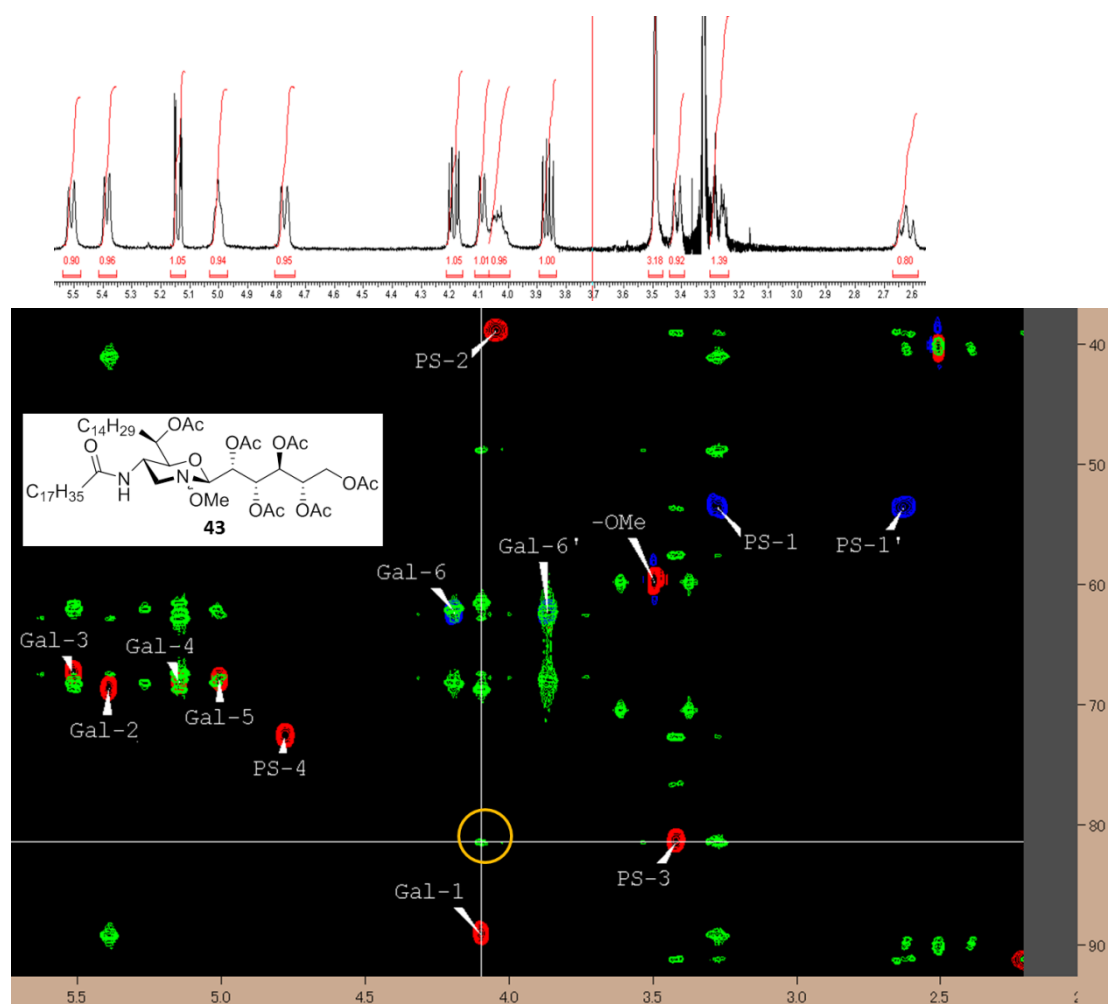


Fig. 3-2-4 HSQC and HMBC spectra of sphingosine-cyclized product **43**

HSQC: Red and Blue, HMBC: Green (d_6 -DMSO, 300 K)

The mechanism how sphingosine-cyclized product was generated was shown in Fig. 3-2-5. Oxime intermediate cannot exist stably due to positive electric charge on nitrogen atom. The instability is canceled by following attack of hydroxyl group at 5th position of sugar molecule normally (pass A). On the contrary, hydroxyl group at 3rd position of sphingosine is also able to work as a nucleophile by forming sphingosine-cyclized compound **43** (pass B).

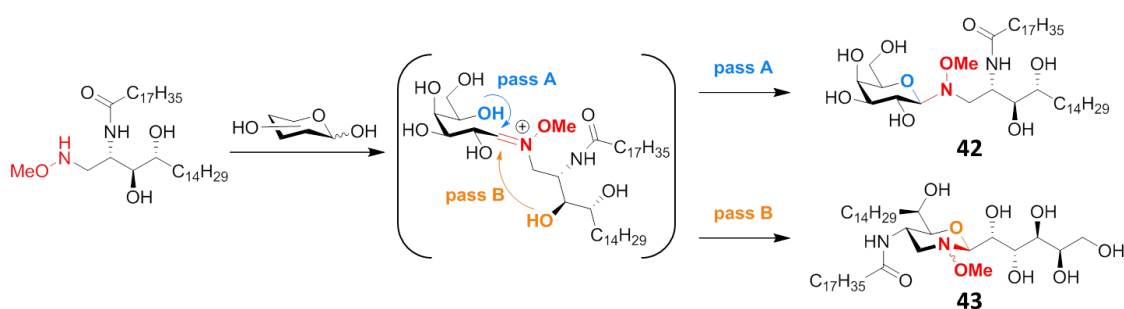


Fig. 3-2-5 Reaction mechanism of generating sphingosine-cyclized product

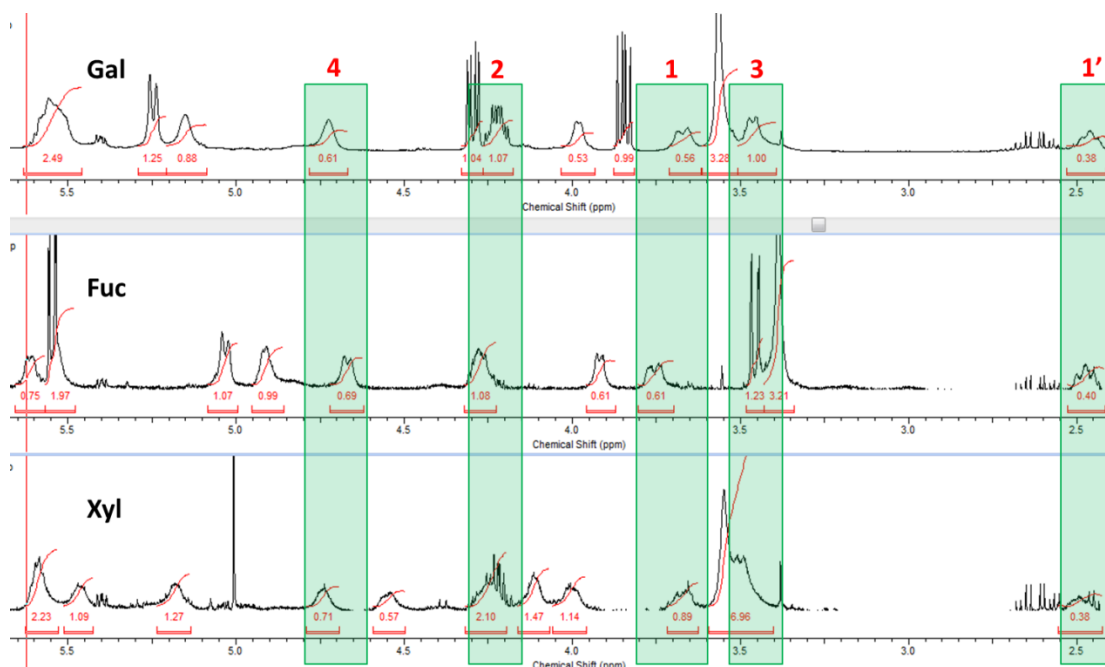


Fig. 3-2-6 Comparison of sphingosine-cyclized products with different sugar

Color bars show ¹H peaks of sphingosine (CDCl₃, 300 K)

In case of other sugars, this type of reaction was also occurred (Fig.3-2-6). The tendency to form cyclized-sphingosine was estimated as Gal > Fuc > Xyl, (Glc) which meant that might relate to the number of axial-configured group. Additionally, 3 reasons:

- byproduct could orient all substituents toward equatorial direction
- acyl chains in byproduct could co-interact with each other as well as desired product
- The longer reaction time, the more byproduct was generated shown below suggested that cyclized-sphingosine may be thermodynamic product in this reaction.

Although separation of the 2 products was not impossible, laborious purification was far from the aim “simple preparation”. That was why we optimized reaction condition to suppress production of the byproduct (Fig. 3-2-7).

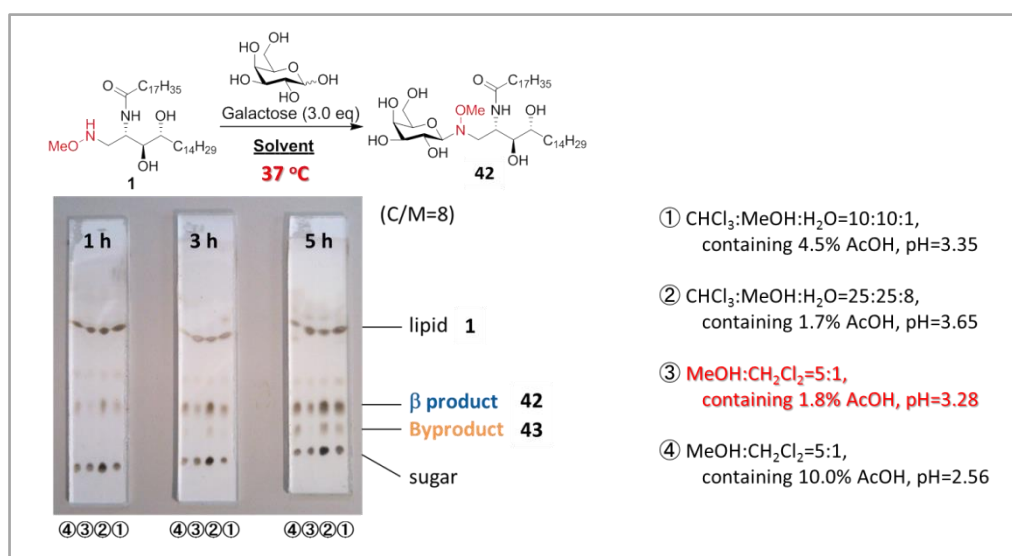


Fig. 3-2-7 Optimization of reaction condition to suppress production of the byproduct

As a result, it was revealed the most suited condition was entry 3 where no sphingosine-cyclized product was detected in 5 hours. That condition was applied to synthesis of other neglycolipids having monosaccharide on their head. As expected, production of the byproduct was suppressed and only β -product was obtained in all reactions. ^1H and ^{13}C NMR data of anomeric position were shown in Table 3-2-2. The results indicated tremendous usability of this strategy for construction of neoglycolipids. Additionally, MALDI-TOF MS spectra of conjugated neoglycolipids are shown in Fig. 3-2-8

	Anomeric H1 δ (ppm)	Anomeric C1 δ (ppm)	J (Hz)	Yield (%)
Galctoside (42)	4.88	94.81	9.2	41
Glucoside (46)	4.94	93.69	8.3	54
Xyloside (45)	4.79	94.90	8.3	76
Fucoside (44)	4.58	95.18	8.8	50
GlcNAc-oside (47)	4.93	94.43	10.1	24
Lactoside (41)	4.86	93.75	8.8	29
Maltoside (48)	4.85	93.65	8.8	30
Cellbioside (49)	4.88	93.79	8.8	40
Mellibioside (50)	4.87	94.23	8.8	28
Maltotrioside (51)	4.92	93.48	8.8	30

Table 3-2-2 ^1H and ^{13}C NMR data of anomeric position at reducing end

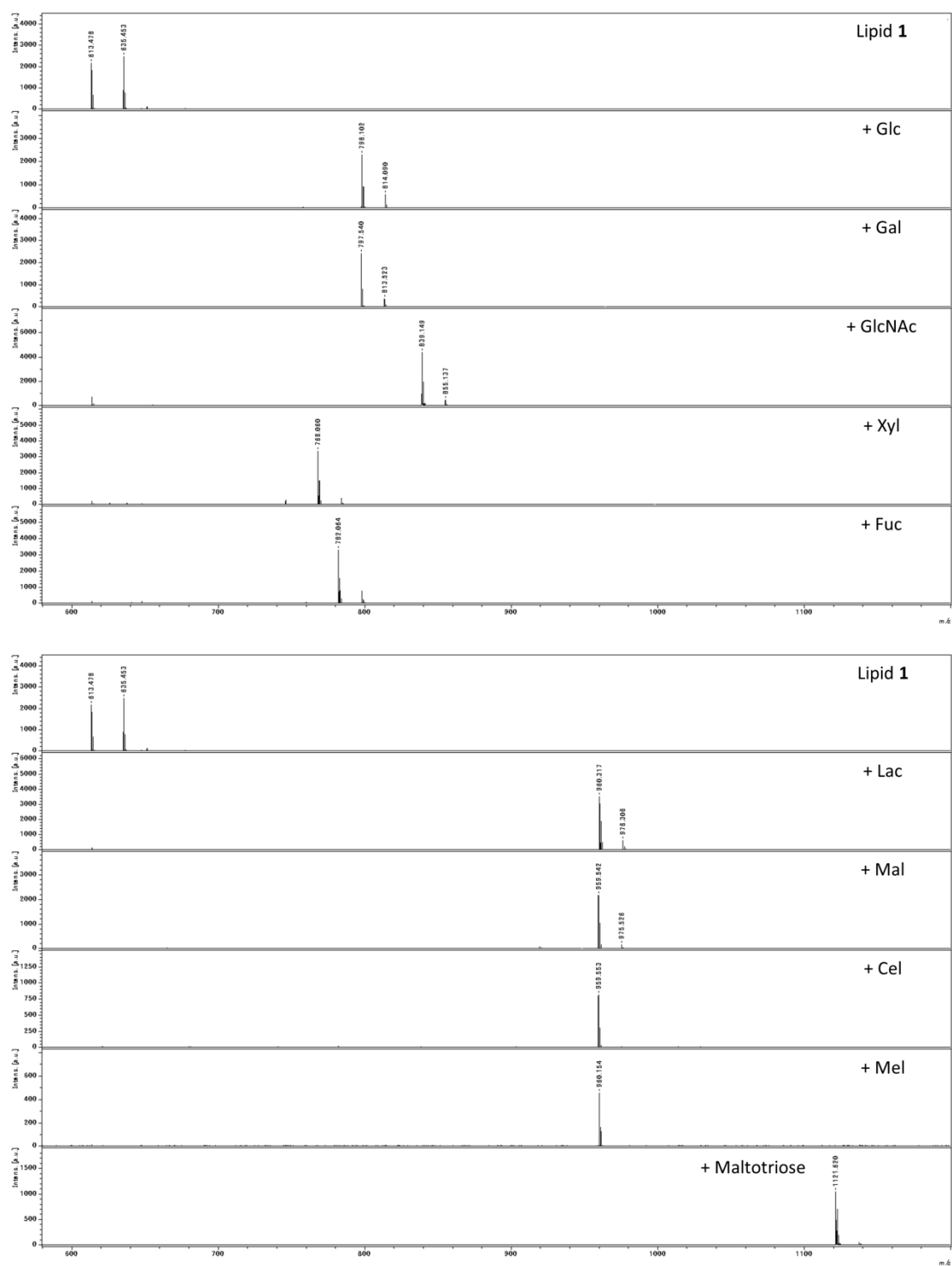
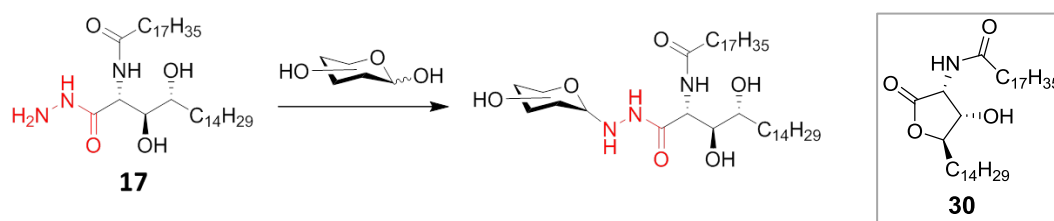


Fig. 3-2-8. MALDI-TOF MS spectra of conjugated products using compound **1**

(DHB, positive)

3-2-2 Glycoblotting Reaction Using Other 5 Derivatives

Next, I used hydrazide derivative **17** as a lipid tag for glycoblotting reaction. The reaction first conducted at 50 °C in $\text{CHCl}_3 : \text{MeOH} : \text{H}_2\text{O} : \text{AcOH} = 10 : 10 : 1 : 1$ system. However, in this condition, lactone compound **30** was major product (Scheme 3-2-3). This result showed hydrazide was decomposed by intramolecular lactonization .



Scheme. 3-2-3. Glycoblotting reaction using hydrazide derivative **17** and decomposed product **30**

Then, I decreased reaction temperature below 40 degree which enabled to obtain neoglycosylated hydrazides. MALDI-TOF MS spectra of conjugated hydrazides were shown in Fig. 3-2-9

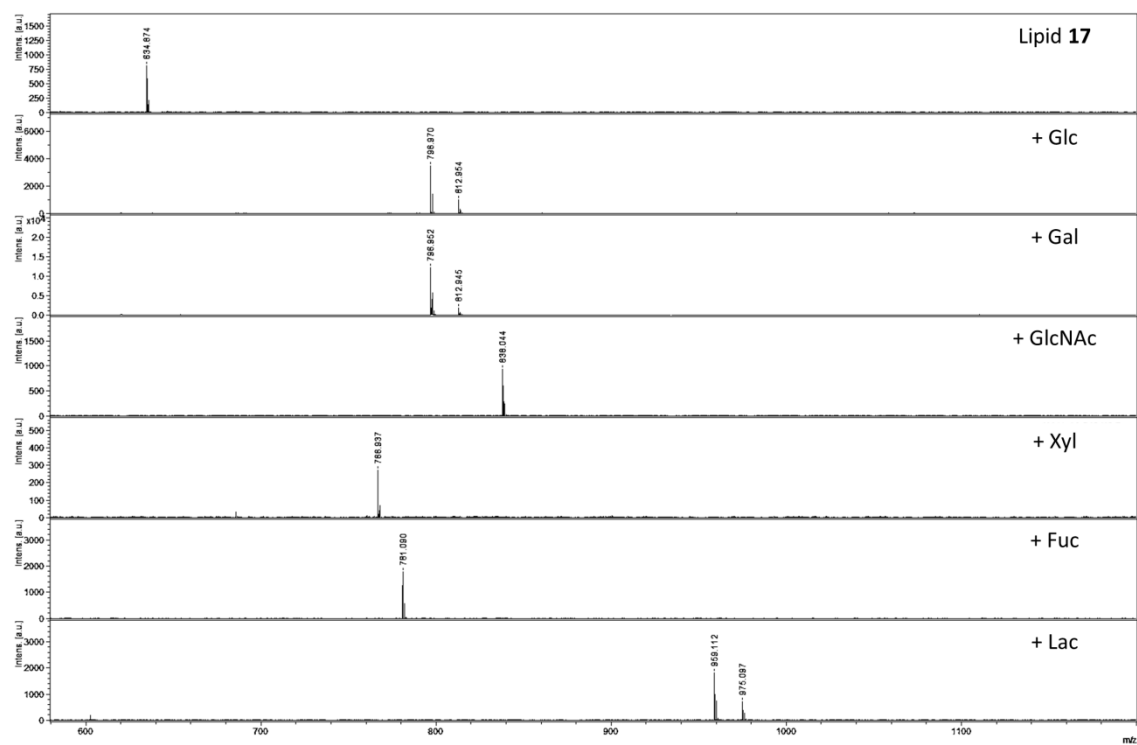
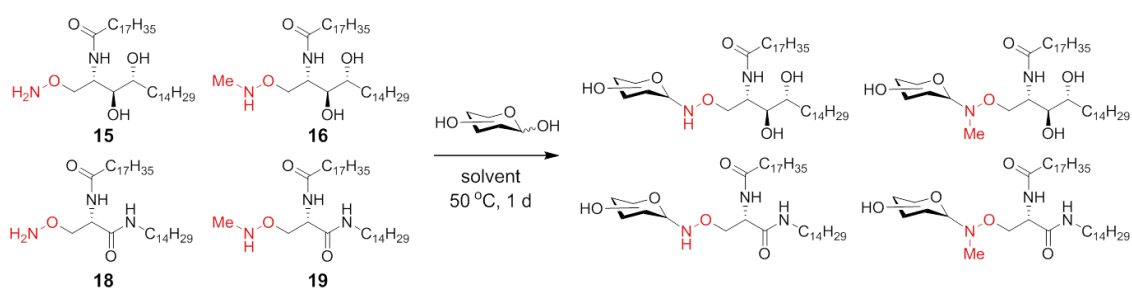


Fig. 3-2-9. MALDI-TOF MS spectra of conjugated products using compound **17**

(DHB, positive)

The other ceramide derivatives were also used for glycoblotting (Scheme 3-2-4). These derivatives afforded neoglycosylated compounds almost without decomposition or yielding byproduct. The yields in case of ceramide derivative **15** were shown in Fig. 3-2-10. Results of each compound are shown in Fig. 3-2-11, 3-2-12, and 3-2-13, respectively. Additionally, methyl substituted aminooxy derivatives **16**, **19** mostly afforded β -product as expected (Fig. 3-2-14).



Scheme 3-2-4. Glycoblotting reaction using ceramide derivative **15**, **16**, **18**, and **19**

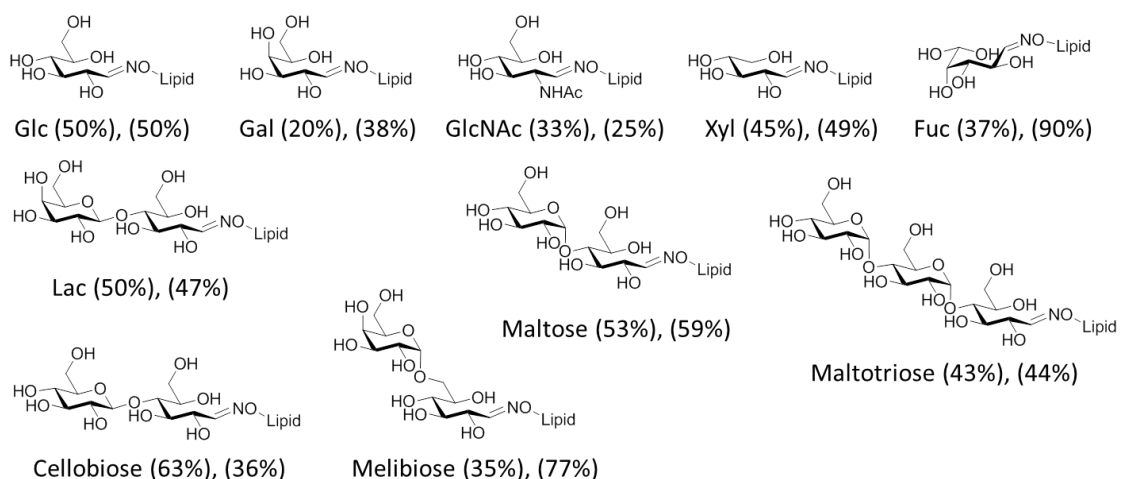


Fig 3-2-10. Yields of neoglycolipids in case of ceramide derivative **15** (left), **16** (right)

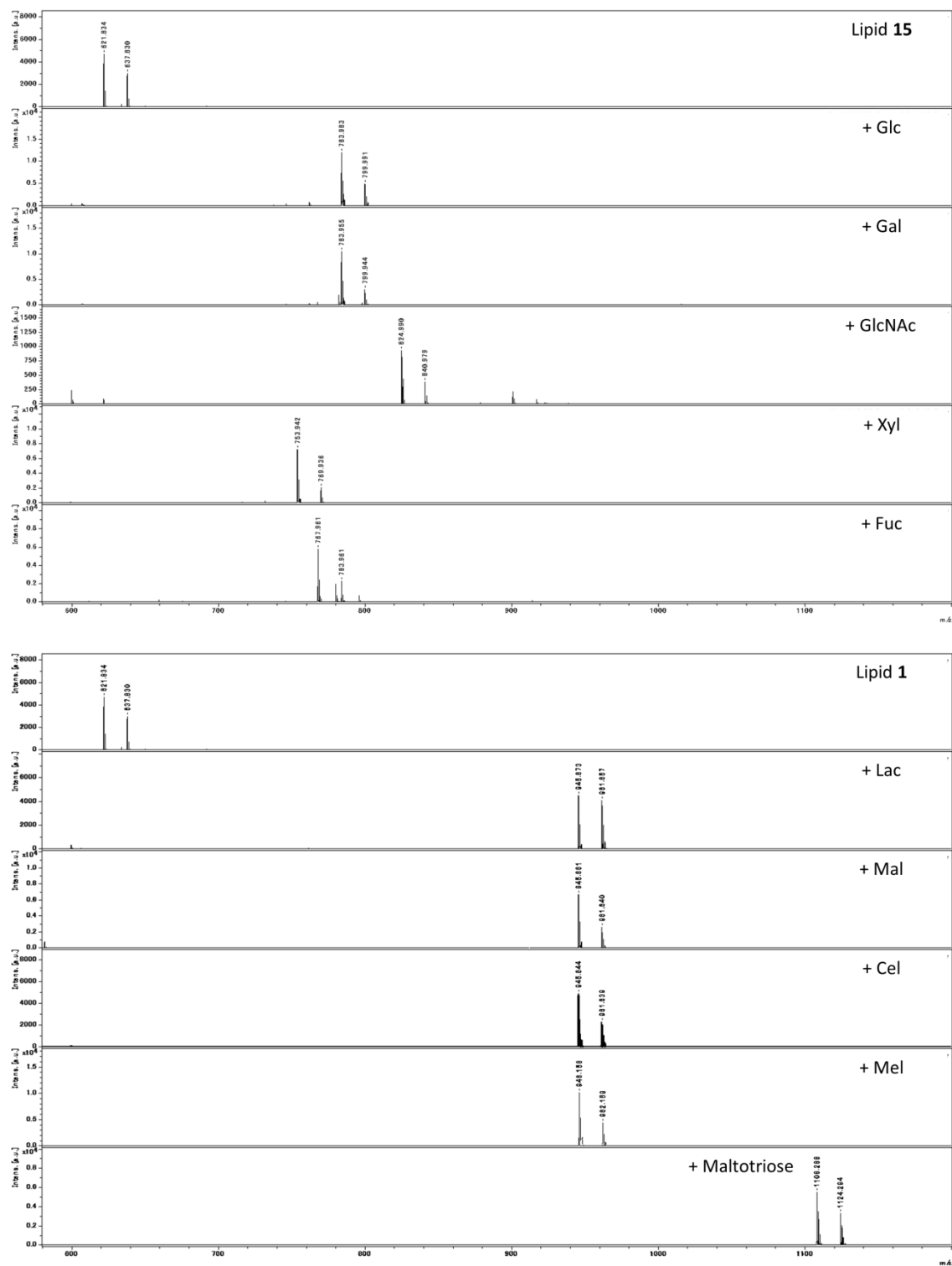


Fig. 3-2-11. MALDI-TOF MS spectra of conjugated products using compound **15**

(DHB, positive)

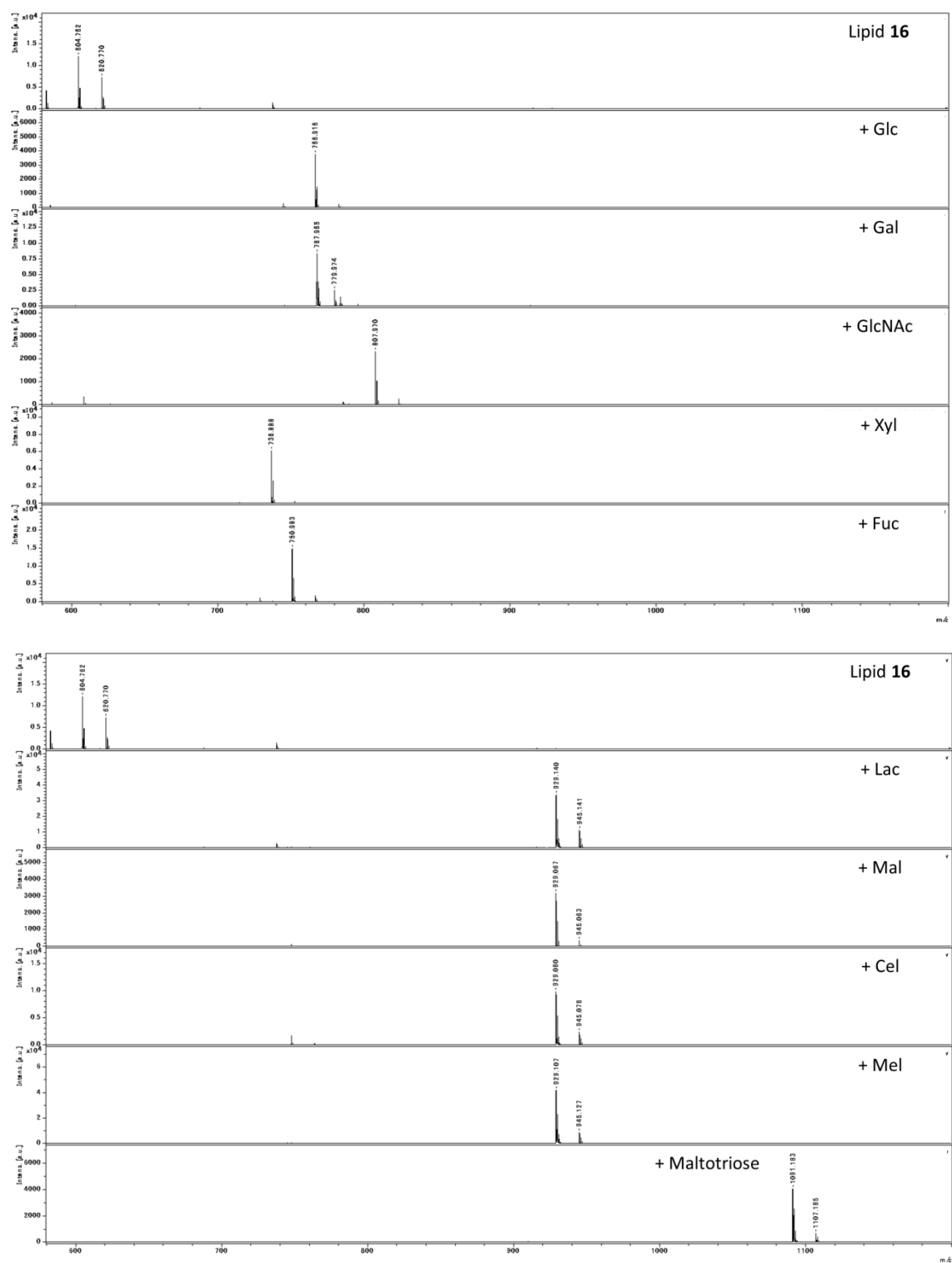


Fig. 3-2-12. MALDI-TOF MS spectra of conjugated products using compound 16

(DHB, positive)

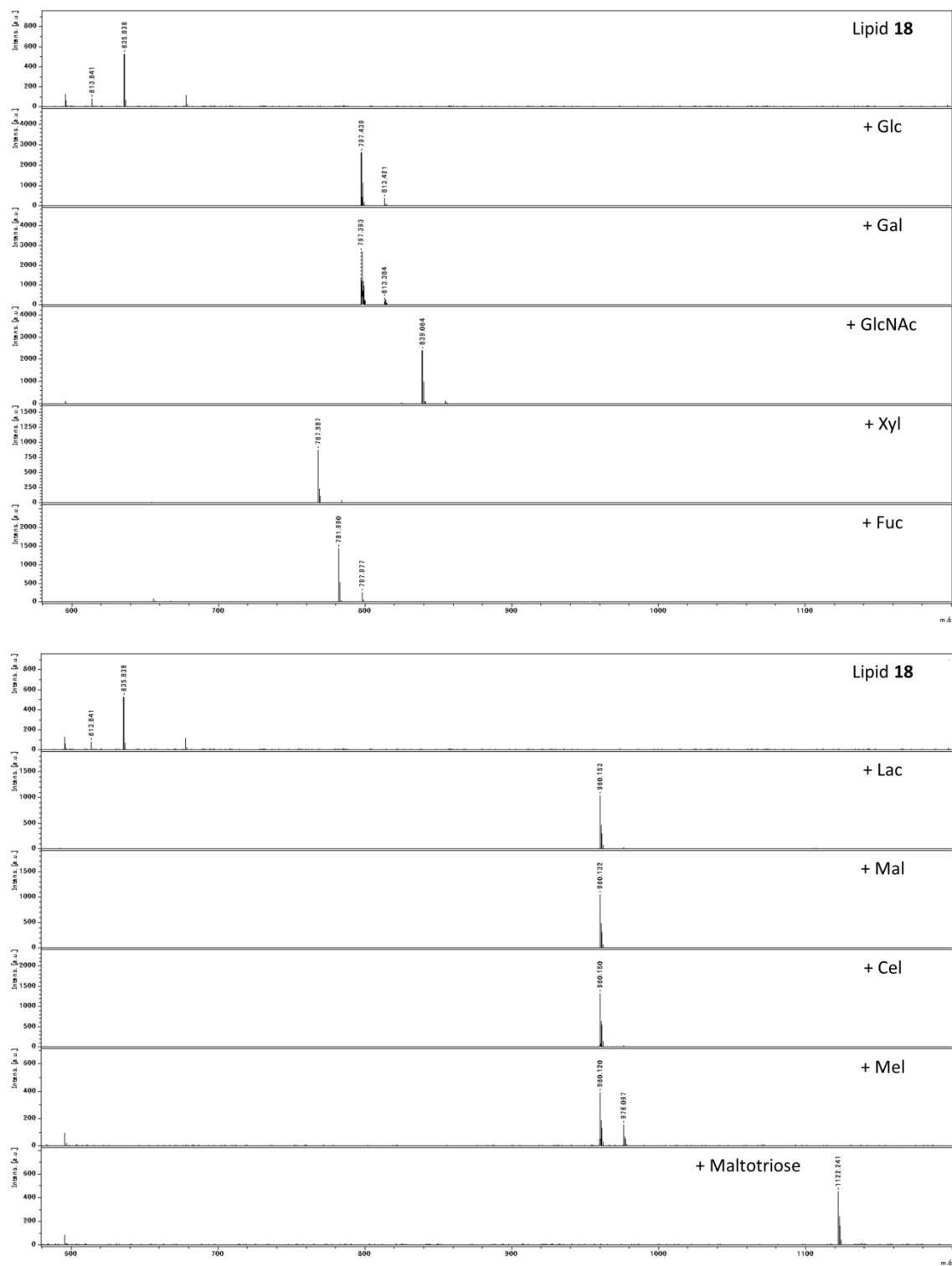


Fig. 3-2-13. MALDI-TOF MS spectra of conjugated products using compound **18**

(DHB, positive)

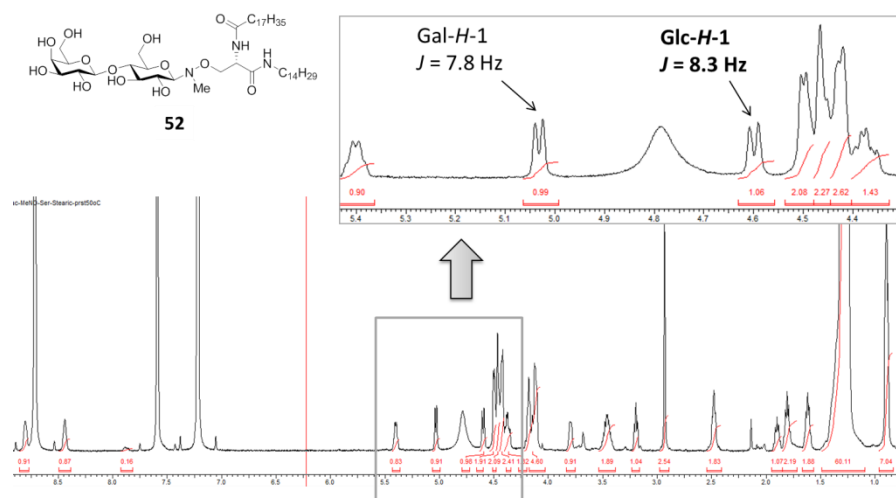


Fig. 3-2-14 ^1H NMR spectrum of β -product **52** (d_5 -pyridine, 300 K)

3-3 Conclusion

In this chapter, I employed glycoblotting reaction to construct neoglycolipid library. In case of methoxyamino derivative **1**, unfortunate side reaction was occurred but optimization of reaction condition enabled to obtain β -product as sole product and construct library. Hydrazide derivative also caused undesired side reaction by self-lactonization. This problem was also overcome by altering condition. Other ceramide derivatives were able to be conjugated with free sugars without any troubles. In this way, neoglycolipid library was successfully constructed by glycoblotting reaction. These results verified the usability of glycoblotting-based method.

3-4 Experimental Section

General Information

All reactions were carried out under a nitrogen atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. Proton and carbon NMR was recorded at 298K with Varian UnityInova 500 MHz (Agilent Inc., USA; ^1H : 500 MHz, ^{13}C : 125 MHz) or Bruker AVANCE DRX 600, equipped with a cryoprobe (Bruker BioSpin Co., Germany; ^1H : 600 MHz, ^{13}C : 150 MHz). Chemical shifts are given in ppm and referenced to internal TMS (δ_{H} 0.00 in CDCl_3), CHCl_3 (δ_{H} 7.26 in CDCl_3), pyridine-*m*-H (δ_{H} 7.22 in d_5 -Pyridine), d_5 -Pyridine (δ_{C} 123.87) or CDCl_3 (δ_{C} 77.00). Assignments in ^1H NMR were made by first-order analysis of the spectra by using ACD/NMR processor software (Advanced Chemistry Development, inc.) and were verified by H–H COSY and HSQC experiments. High/low resolution electrospray ionization mass spectra (ESI-MS) were recorded by JMS-700TZ (JEOL, Japan). TLC was performed on Merck pre-coated plates (20 cm $\times$ 20 cm; layer thickness, 0.25 mm; Silica Gel 60F₂₅₄); spots were visualized by spraying a solution of 90:5:5 (v/v/v) MeOH-*p*-anisaldehyde-concentrated sulfuric acid and heating at 250 °C for ca. 1/2 min, a solution of 95: 5 (v/v) MeOH-concentrated sulfuric acid and heating at 180°C for ca. 1/2 min, and by UV light (256 or 365 nm) when applicable. Column chromatography was performed on Silica Gel N60 (spherical type, particle size 40–50 μm ; Kanto Chemical Industry) with the solvent systems specified, and the ratio of solvent systems was given in v/v. The reaction progress of enzymatic hydrolysis was measured by Park and Johnson method using a microplate reader (SpectraMaxM5, Molecular Devices Co.,

Sunnyvale, CA). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet, br = broad. In addition to those specified above, the following abbreviations, designations and formulas are used throughout the Supporting Information: MeOH = methanol, H₂O = water, EtOAc = ethyl acetate, DCM = dichloromethane, DMF = dimethylformamide, CHCl₃ = Chloroform, Et₃N = triethylamine, NaHCO₃ = sodium bicarbonate, MgSO₄ = magnesium sulfate, aq. = aqueous, sat. = saturated, 1N HCl = 1 normal hydrogen chloride solution

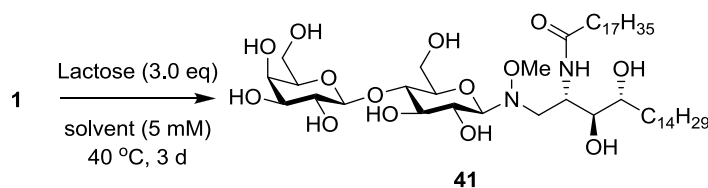
Materials

Solvents and other reagents for the chemical syntheses were purchased from Sigma-Aldrich Co., Tokyo Chemical Industry Co., Ltd., and Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and used without further purification.

Glycoblotting reaction of ceramide mimic 1 with lactose.

To demonstrate the feasibility of the glycoblotting reaction of compound **1** with reducing sugars, some conditions were tested to synthesize *N*-LacCer **41** as indicated in Table 3-4-1.

Table 3-4-1. Glycoblotting reaction using ceramide derivative **1** with lactose



Entry	Solvent system	AcOH ^[a] (%)	Yield ^[b] (%)
1	CH ₂ Cl ₂ /MeOH (1:5)	0.5	18
2	CHCl ₃ /MeOH/H ₂ O (10:10:1)	4.5	15
3	CHCl ₃ /MeOH/H ₂ O (25:25:8)	1.7	29

[a] AcOH percentage in total volume

[b] Yields of isolated products after silica gel chromatography

(2*S*, 3*S*, 4*R*)-1-{ *N*-[β-galactosylpyranosyl-(1→4)-β-glucopyranosyl]
-*N*-(methoxy)amino}-2-[*N*-(octadecanoyl)amino]octadecane-3,4-diol (41).

A suspension of compound **1** (61 mg, 100 μ mol) and lactose (103 mg, 300 μ mol) in various solvent was stirred at 40 $^{\circ}$ C for 3 days (Table 1). Then, silica gel was added to a reaction solution and the mixture was evaporated completely. Purification of the crude product by flash column chromatography on silica gel (CHCl_3 -*i*PrOH, 10:1, CHCl_3 -MeOH, 4:1) yielded **14** as a white solid; ^1H NMR (600 MHz, d_5 -Pyridine) δ 8.56 (d, J = 7.45 Hz, 1H; *NH*), 5.24 (m, 1H; *PS-H-2*), 5.03 (d, J = 7.89 Hz, 1H; *Gal-H-1*), 4.86 (d, J = 8.77 Hz, 1H; *Glc-H-1*), 4.49 (m, 4H; *Glc-H-6a*, *Gal-H-2*, *Gal-H-4*,

Gal-*H*-6b), 4.40 (m, 3H; PS-*H*-3, Glc-*H*-6b, Gal-*H*-6b), 4.35 (dd, $J = 7.45, 3.07$ Hz, 1H; PS-*H*-3), 4.25 (m, 2H; Glc-*H*-2, Glc-*H*-3), 4.15 (m, 4H; PS-*H*-1a, PS-*H*-4, Gal-*H*-3, Gal-*H*-5), 4.10 (t, $J = 9.21$ Hz, 1H; Glc-*H*-4), 3.89 (dd, $J = 14.03, 9.65$ Hz, 1H; PS-*H*-1b), 3.84 (m, 4H; Glc-*H*-5, OMe), 2.49 (m, 2H; NHCOCH₂a,b), 2.30 (m, 1H; PS-*H*-5a), 1.96-1.77 (m, 4H; PS-*H*-5b, PS-*H*-6a, NHCOCH₂CH₂a,b), 1.66 (m, 1H; PS-*H*-6b), 1.46-1.17 (m, 50H; CH₂), 0.87 (t, $J = 7.02$ Hz, 6H; CH₃); ¹³C NMR (150 MHz (HSQC), *d*₅-Pyridine): δ 105.74 (Gal-C-1), 93.75 (Glc-C-1), 82.10 (Glc-C-4), 78.46 (Glc-C-5), 77.70 (Glc-C-3), 77.51 (PS-C-3), 77.37 (Gal-C-5), 75.14 (Gal-C-3), 72.68 (PS-C-4), 72.45 (Gal-C-2), 71.45 (Glc-C-2), 70.03 (Gal-C-4), 62.10 (Glc-C-6, Gal-C-6), 61.50 (OMe), 52.06 (PS-C-1), 50.96 (PS-C-2), 36.94 (NHCOCH₂), 34.81 (PS-C-5), 32.21-22.65 (CH₂), 14.85 (CH₃); HRMS (ESI) Calcd. for C₄₉H₉₆N₂O₁₄Na [M+Na]⁺ 959.67592, found 959.67192

General procedure of glycoblotting reaction

100 mmol (about 60 mg) of ceramide derivative and 5 eq. of sugar were suspended in solvent and the glass bottle was capped tightly. The reaction mixture was stirred vigorously at prescribed temperature. After several hours, the solution was transferred into flask. Solvent was removed to about half volume and silica gel was added to a reaction solution and the mixture was evaporated completely. Purification of the crude product by flash column chromatography on silica gel (CHCl₃-*i*PrOH, 40:1, CHCl₃-MeOH, 3:1, depending on the compound) afforded product as a white solid.

Compound data of conjugated products using compound 1

Acetylated compound 42

^1H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 6.01 (d, $J=7.83$ Hz, 1 H) 5.41 (dd, $J=3.35, 0.84$ Hz, 1 H) 5.09 - 5.14 (m, 2 H) 5.03 - 5.08 (m, 1 H) 4.91 (t, $J=9.78$ Hz, 1 H) 4.46 (d, $J=9.78$ Hz, 1 H) 4.39 - 4.44 (m, 1 H) 4.09 - 4.19 (m, 2 H) 3.93 - 3.98 (m, 1 H) 3.38 (s, 3 H) 3.27 (dd, $J=13.98, 3.07$ Hz, 1 H) 2.73 (dd, $J=13.98, 11.74$ Hz, 1 H) 2.15 (s, 3 H) 2.09 (s, 6 H) 2.04 (s, 3 H) 2.01 (s, 3 H) 1.54 - 1.70 (m, 3 H) 1.20 - 2.23 (m, 58 H) 0.88 (t, $J=6.99$ Hz, 6 H)

Cyclized-sphingosine 43

^1H NMR (600 MHz, DMSO-*d*₆) δ ppm 7.69 (d, $J=9.21$ Hz, 1 H; NH) 5.52 (d, $J=9.87$ Hz, 1 H; Gal-*H*-3) 5.39 (d, $J=8.55$ Hz, 1 H; Gal-*H*-2) 5.15 (d, $J=9.87$ Hz, 1 H; Gal-*H*-4) 5.01 (br. s, 1 H; Gal-*H*-5) 4.78 (d, $J=10.52$ Hz, 1 H; PS-*H*-4) 4.19 (dd, $J=11.84, 4.60$ Hz, 1 H; Gal-*H*-6a) 4.10 (d, $J=8.55$ Hz, 1 H; Gal-*H*-1) 3.99 - 4.07 (m, 1 H; PS-*H*-2) 3.87 (dd, $J=11.84, 6.58$ Hz, 1 H; Gal-*H*-6b) 3.49 (s, 3 H; OMe) 3.42 (d, $J=10.52$ Hz, 1 H; PS-*H*-3) 3.24 - 3.30 (m, 1 H; PS-*H*-1) 2.63 (t, $J=12.50$ Hz, 1 H; PS-*H*-1) 2.10 - 1.20 (m, 58 H) 2.05 - 1.94 (m, 18 H; Ac) 0.85 (t, $J=6.58$ Hz, 6 H)

^{13}C NMR (150 MHz, Pyr, HSQC) δ ppm 89.29 (Gal-*C*-1) 81.57 (PS-*C*-3) 72.77 (PS-*C*-4) 68.81 (Gal-*C*-2) 68.27 (Gal-*C*-4) 68.07 (Gal-*C*-5) 67.46 (Gal-*C*-3) 62.65 (Gal-*C*-6) 59.86 (OMe) 53.74 (PS-*C*-1) 39.07 (PS-*C*-2)

Glc (yield: 54%)

^1H NMR (600 MHz, Pyr) δ ppm 8.60 (d, $J=7.89$ Hz, 1 H; NH) 5.24 - 5.30 (m, 1 H; PS-*H*-2) 4.94 (d, $J=8.33$ Hz, 1 H; Glc-*H*-1) 4.54 (dd, $J=11.40, 2.19$ Hz, 1 H; Glc-*H*-6a) 4.35 (dd, $J=7.45, 3.07$ Hz, 1 H; PS-*H*-3) 4.21 - 4.30 (m, 4 H; Glc-*H*-2, Glc-*H*-3,

Glc-*H*-6b, PS-*H*-1a) 4.14 - 4.19 (m, 1 H; PS-*H*-4) 4.06 - 4.10 (m, 1 H; Glc-*H*-4) 3.94 - 3.98 (m, 1 H; Glc-*H*-5) 3.91 (dd, $J=13.59, 9.65$ Hz, 1 H; PS-*H*-1b) 3.86 (s, 3 H; OMe) 2.55 - 1.12 (m, 58 H) 0.87 (t, $J=7.02$ Hz, 6 H; CH₃)

¹³C NMR (150 MHz, Pyr, HSQC) δ ppm 93.69 (Glc-*C*-1) 80.54 (Glc-*C*-5) 79.85 (Glc-*C*-3) 77.49 (PS-*C*-3) 72.48 (PS-*C*-4) 71.92 (Glc-*C*-2) 71.78 (Glc-*C*-4) 62.88 (Glc-*C*-6) 61.35 (OMe) 52.04 (PS-*C*-1) 50.93 (PS-*C*-2) 37.12 - 14.29 (Alkylchain)

Gal (yield: 41%)

¹H NMR (500 MHz, pyridine) δ ppm 8.50 (d, $J=8.56$ Hz, 1 H; NH) 5.27 (m, 1H; PS-*H*-2) 4.86 (d, $J=9.22$ Hz, 1 H; Gal-*H*-1) 4.60 (t, $J=8.89$ Hz, 1 H; Gal-*H*-2) 4.51 (d, $J=2.96$ Hz, 1 H; Gal-*H*-4) 4.41 - 4.47 (m, 1 H; Gal-*H*-6a) 4.34 - 4.39 (m, 1 H; Gal-*H*-6b) 4.29 - 4.34 (m, 1 H; PS-*H*-3) 4.25 (dd, $J=13.83, 3.29$ Hz, 1 H; PS-*H*-1a) 4.13 - 4.19 (m, 2 H; Gal-*H*-3, ; PS-*H*-4) 4.06 (t, $J=6.26$ Hz, 1 H; Gal-*H*-5) 3.93 (dd, $J=13.50, 9.88$ Hz, 1 H; PS-*H*-1b) 3.83 (s, 3 H; OMe) 2.5 - 1.10 (m, 58 H; CH₂) 0.87 (t, $J=6.59$ Hz, 6 H; CH₃)

GlcNAc (yield: 24%)

¹H NMR (600 MHz, Pyr) δ ppm 9.32 (br. s, 1 H; GlcNAc-NH) 8.24 (br. s, 1 H; PS-NH) 5.22 - 5.29 (m, 1 H; PS-*H*-2) 4.93 (d, $J=10.08$ Hz, 1 H; GlcNAc-*H*-1) 4.57 - 4.64 (m, 1 H; GlcNAc-*H*-2) 4.50 - 4.55 (m, 1 H; GlcNAc-*H*-6a) 4.20 - 4.30 (m, 3 H; GlcNAc-*H*-3, GlcNAc-*H*-6b; PS-*H*-3) 4.02 - 4.15 (m, 3 H; PS-*H*-1ab, PS-*H*-4) 3.99 (t, $J=9.65$ Hz, 1 H; GlcNAc-*H*-4) 3.88 - 3.93 (m, 1 H; GlcNAc-*H*-5) 3.62 (s, 3H; OMe) 2.59 - 1.12 (m, 58 H) 0.88 (t, $J=6.80$ Hz, 6 H)

^{13}C NMR (150 MHz, Pyr, HSQC) δ ppm 94.43 (GlcNAc-C-1) 3.89 80.13
 (GlcNAc-C-5) 77.55 (PS-C-3) 77.32 (GlcNAc-C-3) 72.40 (PS-C-4) 72.16
 (GlcNAc-C-4) 62.79 (GlcNAc-C-6) 60.91 (OMe) 53.88 (GlcNAc-C-2) 51.07 (PS-C-2)
 49.30 (PS-C-1) 36.54-14.27 (Alkylchain) 23.28 (Ac)

Xyl (yield: 76%)

^1H NMR (600 MHz, Pyr) δ ppm 8.54 (d, $J=7.89$ Hz, 1 H; NH) 5.26 - 5.32 (m, 1 H; PS-*H*-2) 4.79 (d, $J=8.33$ Hz, 1 H; Xyl-*H*-1) 4.38 (dd, $J=7.45, 3.51$ Hz, 1 H; PS-*H*-3) 4.31 (dd, $J=11.40, 5.26$ Hz, 1 H; Xyl-*H*-5a) 4.16 - 4.25 (m, 2 H; PS-*H*-1a, PS-*H*-4) 4.06 - 4.16 (m, 3 H; ; Xyl-*H*-2, Xyl-*H*-3, Xyl-*H*-4) 3.84 - 3.91 (m, 4 H; PS-*H*-1b, OMe) 3.64 (t, $J=10.96$ Hz, 1 H; Xyl-*H*-5b) 2.06 – 1.20 (m, 58 H) 0.88 (t, $J=6.80$ Hz, 6 H)

^{13}C NMR (150 MHz, Pyr, HSQC) δ ppm 94.90 (Xyl-C-1) 79.66 (Xyl-C-3) 77.32 (PS-C-3) 72.63 (PS-C-4) 71.69 (Xyl-C-2) 70.99 (Xyl-C-4) 69.11 (Xyl-C-5) 61.38 (OMe) 52.01 (PS-C-1) 51.07 (PS-C-2) 37.01 - 14.27 (Alkylchain)

Fuc (yield: 50%)

^1H NMR (600 MHz, Pyr) δ ppm 8.67 (d, $J=8.33$ Hz, 1 H; NH) 5.21 - 5.28 (m, 1 H; PS-*H*-2) 4.62 (t, $J=9.21$ Hz, 1 H; Fuc-*H*-2) 4.58 (d, $J=8.77$ Hz, 1 H; Fuc-*H*-1) 4.39 (dd, $J=13.59, 4.82$ Hz, 1 H; PS-*H*-1a) 4.31 - 4.36 (m, 1 H; PS-*H*-3) 4.26 - 4.31 (m, 1 H; PS-*H*-4) 4.08 (dd, $J=8.77, 3.07$ Hz, 1 H; Fuc-*H*-3) 4.03 (d, $J=3.07$ Hz, 1 H; Fuc-*H*-4) 3.87 (s, 3 H; OMe) 3.70 - 3.79 (m, 2 H; Fuc-*H*-5, PS-*H*-1b) 2.51 – 1.15 (m, 58 H) 1.49 (d, $J=6.14$ Hz, 3 H; Fuc-*H*-6) 0.88 (t, $J=6.80$ Hz, 6 H; CH_3)

¹³C NMR (150 MHz, Pyr, HSQC) δ ppm 95.18 (Fuc-C-1) 77.65 (PS-C-3) 76.58 (Fuc-C-3) 73.18 (Fuc-C-5) 72.86 (Fuc-C-4) 72.44 (PS-C-4) 68.83 (Fuc-C-2) 61.50 (OMe) 53.74 (PS-C-1) 50.87 (PS-C-2) 37.26 - 14.33 (Alkylchain) 17.77 (Fuc-C-6)

Cellobi (yield: 40%)

¹H NMR (600 MHz, Pyr) δ ppm 8.62 (d, *J*=7.67 Hz, 1 H; NH) 5.22 - 5.27 (m, 1 H; PS-*H*-2) 5.12 (d, *J*=7.89 Hz, 1 H; Glc-B-*H*-1) 4.88 (d, *J*=8.77 Hz, 1 H; Glc-A-*H*-1) 4.54 - 4.59 (m, 1 H; Glc-B-*H*-6a) 4.45 - 4.50 (m, 1 H; Glc-A-*H*-6a) 4.39 - 4.44 (m, 1 H; Glc-A-*H*-6b) 4.34 (dd, *J*=7.24, 3.51 Hz, 1 H; PS-*H*-3) 4.30 (dd, *J*=11.62, 6.36 Hz, 1 H; Glc-B-*H*-6b) 4.11 - 4.26 (m, 7 H; Glc-A-*H*-2, Glc-A-*H*-3, Glc-A-*H*-4, Glc-B-*H*-3, Glc-B-*H*-4, PS-*H*-1a, PS-*H*-4) 4.06 - 4.10 (m, 1 H; Glc-B-*H*-2) 4.01 - 4.06 (m, 1 H; Glc-B-*H*-5) 3.87 - 3.93 (m, 2 H; Glc-A-*H*-5, PS-*H*-1b) 3.85 (s, 3 H; OMe) 2.55-1.15 (m, 58 H; CH₂) 0.87 (t, *J*=6.80 Hz, 6 H; CH₃)

¹³C NMR (150 MHz, Pyr, HSQC) δ ppm 105.00 (Glc-B-C-1) 93.79 (Glc-A-C-1) 81.40 (Glc-A-C-4) 78.52 (Glc-A-C-5, Glc-B-C-5) 78.22 (Glc-B-C-3) 77.89 (Glc-A-C-3) 77.50 (PS-C-3) 74.76 (Glc-B-C-2) 72.58 (PS-C-4) 71.84 (Glc-B-C-4) 71.47 (Glc-A-C-2) 62.56 (Glc-B-C-6) 62.05 (Glc-A-C-6) 61.37 (OMe) 52.04 (PS-C-1) 51.02 (PS-C-2) 37.32 - 14.53 (Alkyl chain)

Malto (yield: 30%)

¹H NMR (600 MHz, Pyr) δ ppm 8.57 (br. s, 1 H; NH) 5.84 (s, 1 H; Glc-B-*H*-1) 5.24 (br. s, 1 H; PS-*H*-2) 4.85 (d, *J*=8.77 Hz, 1 H; Glc-A-*H*-1) 4.44 - 4.61 (m, 4 H; Glc-A-*H*-6a, Glc-B-*H*-3, Glc-B-*H*-4, Glc-B-*H*-6a) 4.27 - 4.40 (m, 4 H; Glc-A-*H*-3, Glc-A-*H*-6b, Glc-B-*H*-6b, PS-*H*-3) 4.09 - 4.26 (m, 6 H; Glc-A-*H*-2, Glc-A-*H*-4, Glc-B-*H*-2,

Glc-B-*H*-5, PS-*H*-1a, PS-*H*-4) 3.79 - 3.91 (m, 4 H; PS-*H*-1b, OMe) 3.75 (br. s, 1 H
Glc-A-*H*-5) 2.54-1.07 (m, 56 H; CH₂) 0.82 - 0.93 (m, 6 H; CH₃)

¹³C NMR (150 MHz, Pyr, HSQC) δ ppm 103.18 (Glc-B-C-1) 93.65 (Glc-A-C-1) 81.63
(Glc-A-C-4) 79.18 (Glc-A-C-3) 78.69 (Glc-A-C-5) 77.55 (PS-C-3) 75.38 (Glc-B-C-3)
75.22 (Glc-B-C-4) 74.39 (Glc-B-C-2) 72.56 (PS-C-4) 71.70 (Glc-B-C-5) 71.22
(Glc-A-C-2) 62.68 (Glc-B-C-6) 62.09 (Glc-A-C-6) 61.44 (OMe) 52.11 (PS-C-1) 50.87
(PS-C-2) 36.82-14.35 (Alkyl chain)

Melibiose (yield: 28%)

¹H NMR (500 MHz, pyridine) δ ppm 5.42 - 5.48 (d, *J*=3.13 Hz, 1 H; Gal-*H*-1) 5.17 -
5.28 (m, 1 H; PS-*H*-2) 4.83 (d, *J*=8.77 Hz, 1 H; Glc-*H*-1) 4.48 - 4.71 (m, 4 H; Gal-*H*-2,
Gal-*H*-3, Gal-*H*-4, Gal-*H*-5) 4.33 - 4.47 (m, 4 H; Glc-*H*-6a, Gal-*H*-6, PS-*H*-3) 4.13 -
4.33 (m, 6 H; Glc-*H*-2, Glc-*H*-3, Glc-*H*-4, Glc-*H*-6b, PS-*H*-1a, PS-*H*-4) 3.90 - 4.07 (m,
2 H; Glc-*H*-5, PS-*H*-1b) 3.84 (s, 3 H; OMe) 2.60-1.04 (m, 58 H; CH₂) 0.81 - 0.94 (m, 6
H; CH₃)

F1 (ppm) 100.52 (Gal-C-1) 94.23 (Glc-C-1) 79.65 (Glc-C-3) 77.81 (Glc-C-5) 77.42
(PS-C-3) 72.78 (Glc-C-4) 72.73 (PS-C-4) 72.50 (Gal-C-5) 71.50 (Glc-C-2) 71.35
(Gal-C-3) 70.91 (Gal-C-4) 70.60 (Gal-C-2) 68.47 (Glc-C-6) 62.60 (Gal-C-6) 61.67
(OMe) 52.67 (PS-C-1) 51.00 (PS-C-2) 36.91-14.34 (Alkyl chain)

Maltotri (yield: 30%)

¹H NMR (600 MHz, Pyr) δ ppm 8.61 (d, *J*=7.89 Hz, 1 H; NH) 5.93 (d, *J*=3.95 Hz, 1 H;
Glc-B-*H*-1) 5.73 (d, *J*=3.51 Hz, 1 H; Glc-C-*H*-1) 5.24 - 5.29 (m, 1 H; PS-*H*-2) 4.92 (d,
J=8.77 Hz, 1 H; Glc-A-*H*-1) 4.65 (t, *J*=9.21 Hz, 1 H; Glc-B-*H*-3) 4.61 (t, *J*=9.21 Hz, 1

H; Glc-C-*H*-3) 4.52 - 4.58 (m, 2 H; Glc-C-*H*-4, Glc-C-*H*-6a) 4.39 - 4.47 (m, 2 H; Glc-B-*H*-6) 4.29 - 4.39 (m, 5 H; Glc-A-*H*-3, Glc-A-*H*-6a, Glc-B-*H*-5, Glc-C-*H*-6b, PS-*H*-3) 4.22 - 4.29 (m, 3 H; Glc-A-*H*-2, Glc-A-*H*-6b, Glc-B-*H*-4) 4.13 - 4.22 (m, 4 H; Glc-C-*H*-2, Glc-C-*H*-5, PS-*H*-1a, PS-*H*-4) 4.07 - 4.13 (m, 2 H; Glc-A-*H*-4, Glc-B-*H*-2) 3.85 - 3.91 (m, 1 H; PS-*H*-1b) 3.79 - 3.85 (m, 4 H; Glc-A-*H*-5, OMe) 2.54-1.19 (m, 58 H; CH₂) 0.87 (t, *J*=6.80 Hz, 6 H; CH₃)

¹³C NMR (150 MHz, Pyr, HSQC) δ ppm 103.04 (Glc-C-C-1) 103.00 (Glc-B-C-1) 93.48 (Glc-A-C-1) 82.14 (Glc-A-C-4) 81.35 (Glc-B-C-4) 79.10 (Glc-A-C-3) 78.68 (Glc-A-C-5) 77.55 (PS-C-3) 75.43 (Glc-C-C-3) 75.22 (Glc-C-C-4) 74.80 (Glc-B-C-3) 74.51 (Glc-C-C-2) 73.88 (Glc-B-C-2) 73.38 (Glc-B-C-5) 72.51 (PS-C-4) 71.80 (Glc-C-C-5) 71.17 (Glc-A-C-2) 62.74 (Glc-C-C-6) 61.99 (Glc-A-C-6) 61.90 (Glc-B-C-6) 61.40 (OMe) 52.14 (PS-C-1) 50.81 (PS-C-2) 36.93 - 14.26 (Alkyl chain)

Compound data of conjugated products using compound **16**

Glc (yield: 68%)

¹H NMR (500 MHz, pyridine) δ ppm 8.85 (d, *J*=8.58 Hz, 1 H; *NH*) 5.26 (br. s, 1 H; PS-*H*-2) 4.69 - 4.78 (m, 1 H; PS-*H*-1a) 4.71 (d, *J*=8.91 Hz, 1 H; Glc-*H*-1) 4.61 (dd, *J*=10.89, 7.26 Hz, 1 H; PS-*H*-1b) 4.51 (d, *J*=10.56 Hz, 1 H; Glc-*H*-6a) 4.29 - 4.39 (m, 2 H; Glc-*H*-6b, PS-*H*-3) 4.10 - 4.24 (m, 4 H; Glc-*H*-2, Glc-*H*-3, Glc-*H*-4, PS-*H*-4) 3.88 (br. s, 1 H; Glc-*H*-5) 2.96 (s, 3 H; MeNO) 2.55-1.07 (m, 58 H; CH₂) 0.87 (t, *J*=6.43 Hz, 6 H; CH₃)

F1 (ppm) 95.17 (Glc-C-1) 80.07 (Glc-C-5) 79.64 (Glc-C-3) 76.50 (PS-C-3) 72.67 (PS-C-4) 72.13 (PS-C-1) 71.78 (Glc-C-2) 71.60 (Glc-C-4) 62.81 (Glc-C-6) 51.82 (PS-C-2) 38.10 (*MeNO*) 36.82-14.11 (Alkyl chain)

Gal (yield: 22%)

¹H NMR (500 MHz, pyridine) δ ppm 8.81 (d, $J=8.55$ Hz, 1 H; *NH*) 5.25 (m, 1 H; PS-*H*-2) 4.69 - 4.74 (m, 2 H; Gal-*H*-1, PS-*H*-1a) 4.59 - 4.66 (m, 1 H; PS-*H*-1b) 4.56 (d, $J=2.63$ Hz, 1 H; Gal-*H*-4) 4.46 - 4.52 (m, 1 H; Gal-*H*-2) 4.40 - 4.44 (m, 1 H; Gal-*H*-6) 4.30 - 4.39 (m, 1 H; PS-*H*-3) 4.18 - 4.25 (m, 1 H; PS-*H*-4) 4.14 (dd, $J=9.21, 3.29$ Hz, 1 H; Gal-*H*-3) 4.02 (t, $J=5.92$ Hz, 1 H; Gal-*H*-5) 2.99 (s, 3 H; *MeNO*) 2.57-1.16 (m, 58 H; CH₂) 0.87 (t, $J=6.74$ Hz, 6 H; CH₃)

F1 (ppm) 95.52 (Gal-C-1) 78.54 (Gal-C-5) 76.45 (Gal-C-3) 76.32 (PS-C-3) 72.85 (PS-C-4) 72.10 (PS-C-1) 70.05 (Gal-C-4) 69.39 (Gal-C-2) 62.23 (Gal-C-6) 51.77 (PS-C-2) 37.85 (*MeNO*) 36.87-14.24 (Alkyl chain)

GlcNAc (yield: 37%)

¹H NMR (500 MHz, pyridine) δ ppm 9.02 (br. s, 1 H; GlcNAc-*NH*) 8.86 (br. s, 1 H; PS-*NH*) 5.15 (m, 1 H; PS-*H*-2) 4.82 - 4.99 (m, 1 H; GlcNAc-*H*-1) 4.43 - 4.75 (m, 4 H; GlcNAc-*H*-2, GlcNAc-*H*-6a, PS-*H*-1) 4.25 - 4.42 (m, 3 H; GlcNAc-*H*-3, GlcNAc-*H*-6b, PS-*H*-3) 4.04 - 4.24 (m, 2 H; GlcNAc-*H*-4, PS-*H*-4) 3.85 (br. s, 1 H; GlcNAc-*H*-5) 3.00 (s, 3 H; *MeNO*) 2.59-1.02 (m, 58 H; CH₂) 2.14 (s, 3 H; *Ac*) 0.76 - 0.97 (m, 6 H; CH₃)

F1 (ppm) 93.55 (GlcNAc-C-1) 80.03 (GlcNAc-C-5) 77.47 (PS-C-3) 76.65 (GlcNAc-C-3) 72.63 (PS-C-4) 72.47 (GlcNAc-C-4) 71.72 (PS-C-1) 62.76

(GlcNAc-C-6) 54.60 (GlcNAc-C-2) 51.86 (PS-C-2) 38.26 (MeNO) 36.84-13.97 (Alkyl chain) 23.49 (Ac)

Xyl (yield: 55%)

¹H NMR (500 MHz, pyridine) δ ppm 8.87 (d, $J=8.59$ Hz, 1 H; NH) 5.22 - 5.28 (m, 1 H; PS-*H*-2) 4.71 - 4.78 (m, 1 H; PS-*H*-1a) 4.62 (d, $J=8.92$ Hz, 1 H; Xyl-*H*-1) 4.58 - 4.65 (m, 1 H; PS-*H*-1b) 4.30 - 4.39 (m, 2 H; Xyl-*H*-5a, PS-*H*-3) 4.18 - 4.26 (m, 1 H; PS-*H*-4) 4.05 - 4.17 (m, 3 H; Xyl-*H*-2, Xyl-*H*-3, Xyl-*H*-4) 3.61 (t, $J=10.24$ Hz, 1 H; Xyl-*H*-5b) 2.96 (s, 3 H; MeNO) 2.55-1.16 (m, 58 H) 0.87 (t, $J=6.60$ Hz, 6 H; CH₃)

F1 (ppm) 95.76 (Xyl-C-1) 79.77 (Xyl-C-3) 76.34 (PS-C-3) 72.58 (PS-C-4) 72.03 (PS-C-1) 71.65 (Xyl-C-2) 70.88 (Xyl-C-4) 68.71 (Xyl-C-5) 51.68 (PS-C-2) 37.93 (MeNO) 36.84-14.06 (Alkyl chain)

Fuc (yield: 49%)

¹H NMR (500 MHz, pyridine) δ ppm 8.77 (d, $J=8.43$ Hz, 1 H; NH) 5.06 (m, 1 H; PS-*H*-2) 4.67 (d, $J=9.16$ Hz, 1 H; Fuc-*H*-1) 4.56 - 4.65 (m, 2 H; PS-*H*-1) 4.34 - 4.44 (m, 2 H; Fuc-*H*-2, PS-*H*-3) 4.18 - 4.25 (m, 1 H; PS-*H*-4) 4.06 - 4.11 (m, 1 H; Fuc-*H*-3) 4.01 - 4.05 (m, 1 H; Fuc-*H*-4) 3.74 - 3.80 (m, 1 H; Fuc-*H*-5) 2.95 (s, 3 H; MeNO) 2.53-1.17 (m, 58 H; CH₂) 1.52 (d, $J=6.23$ Hz, 3 H; Fuc-*H*-6) 0.87 (t, $J=6.78$ Hz, 6 H; CH₃)

F1 (ppm) 95.74 (Fuc-C-1) 76.40 (Fuc-C-3) 76.35 (PS-C-3) 73.01 (Fuc-C-5) 72.83 (PS-C-4) 72.75 (Fuc-C-4) 72.12 (PS-C-1) 69.23 (Fuc-C-2) 52.01 (PS-C-2) 37.76 (MeNO) 36.78-14.32 (Alkyl chain) 17.35 (Fuc-C-6);

Lac (yield: 40%)

¹H NMR (500 MHz, pyridine) δ ppm 8.83 (d, *J*=8.30 Hz, 1 H; NH) 5.21 - 5.28 (m, 1 H; PS-*H*-2) 5.08 (d, *J*=7.82 Hz, 1 H; Gal-*H*-1) 4.72 - 4.78 (m, 1 H; PS-*H*-1a) 4.60 - 4.69 (m, 2 H; Glc-*H*-1, PS-*H*-1b) 4.47 (m, 6 H; Glc-*H*-6, Gal-*H*-2, Gal-*H*-4, Gal-*H*-6) 4.32 - 4.39 (m, 1 H; PS-*H*-3) 4.13 - 4.27 (m, 6 H; Glc-*H*-2, Glc-*H*-3, Glc-*H*-4, Gal-*H*-3, Gal-*H*-5, PS-*H*-4) 3.77 - 3.85 (m, 1 H; Glc-*H*-5) 2.95 (s, 3 H; MeNO) 2.53-1.16 (m, 58 H; CH₂) 0.88 (t, *J*=6.47 Hz, 6 H; CH₃)

F1 (ppm) 105.85 (Gal-C-1) 95.21 (Glc-C-1) 81.99 (Glc-C-4) 78.18 (Glc-C-5) 77.56 (Glc-C-3) 77.46 (Gal-C-5) 76.56 (PS-C-3) 75.36 (Gal-C-3) 72.93 (PS-C-4) 72.41 (PS-C-1) 72.34 (Gal-C-2) 71.48 (Glc-C-2) 70.18 (Gal-C-4) 62.38 (Glc-C-6) 62.14 (Gal-C-6) 52.22 (PS-C-2) 38.19 (MeNO) 36.88-14.35 (Alkyl chain)

Mal (yield: 76%)

¹H NMR (500 MHz, pyridine) δ ppm 8.82 (d, *J*=8.79 Hz, 1 H; NH) 5.87 (d, *J*=3.66 Hz, 1 H; Glc-B-*H*-1) 5.20 - 5.25 (m, 1 H; PS-*H*-2) 4.72 (dd, *J*=10.99, 2.93 Hz, 1 H; PS-*H*-1a) 4.62 (d, *J*=8.79 Hz, 1 H; Glc-A-*H*-1) 4.50 - 4.66 (m, 4 H; Glc-B-*H*-3, Glc-B-*H*-4, Glc-B-*H*-6a, PS-*H*-1b) 4.37 - 4.46 (m, 2 H; Glc-A-*H*-6) 4.31 - 4.37 (m, 2 H; Glc-B-*H*-6b, PS-*H*-3) 4.20 - 4.30 (m, 2 H; Glc-A-*H*-3, Glc-A-*H*-4) 4.12 - 4.20 (m, 4 H; Glc-A-*H*-2, Glc-B-*H*-2, Glc-B-*H*-5, PS-*H*-4) 3.65 - 3.72 (m, 1 H; Glc-A-*H*-5) 2.92 (s, 3 H; MeNO) 2.54-1.17 (m, 58 H; CH₂) 0.87 (t, *J*=6.78 Hz, 6 H; CH₃)

F1 (ppm) 102.94 (Glc-B-C-1) 94.95 (Glc-A-C-1) 80.98 (Glc-A-C-4) 78.77 (Glc-A-C-3) 78.22 (Glc-A-C-5) 76.26 (PS-C-3) 75.11 (Glc-B-C-4) 75.06 (Glc-B-C-3) 74.29 (Glc-B-C-2) 72.10 (PS-C-4) 72.01 (PS-C-1) 71.77 (Glc-B-C-5) 71.44 (Glc-A-C-2)

62.52 (Glc-B-C-6) 61.76 (Glc-A-C-6) 51.76 (PS-C-2) 38.10 (*MeNO*) 36.66-14.09
(Alkyl chain)

Cel (yield: 28%)

¹H NMR (500 MHz, pyridine) δ ppm 8.82 (d, $J=7.82$ Hz, 1 H; *NH*) 5.20 - 5.30 (m, 1 H; PS-*H*-2) 5.17 (d, $J=7.57$ Hz, 1 H; Glc-B-*H*-1) 4.71 - 4.81 (m, 1 H; PS-*H*-1a) 4.65 (m, 2 H; Glc-A-*H*-1, PS-*H*-1b) 4.53 - 4.59 (m, 1 H; Glc-B-*H*-6a) 4.40 - 4.52 (m, 2 H; Glc-A-*H*-6) 4.29 - 4.39 (m, 2 H; Glc-B-*H*-6b, PS-*H*-3) 4.22 (m, 6 H; Glc-A-*H*-2, Glc-A-*H*-3, Glc-A-*H*-4, Glc-B-*H*-3, Glc-B-*H*-4, PS-*H*-4) 4.07 - 4.14 (m, 1 H; Glc-B-*H*-2) 4.00 - 4.07 (m, 1 H; Glc-B-*H*-5) 3.76 - 3.87 (m, 1 H; Glc-A-*H*-5) 2.94 (s, 3 H; *MeNO*) 2.55-1.08 (m, 58 H; *CH*₂) 0.88 (br. s., 6 H; *CH*₃)

F1 (ppm) 105.00 (Glc-B-C-1) 95.03 (Glc-A-C-1) 81.06 (Glc-A-C-4) 78.53 (Glc-B-C-5) 78.11 (Glc-A-C-3, Glc-A-C-5, Glc-B-C-3) 76.69 (PS-C-3) 74.87 (Glc-B-C-2) 72.69 (PS-C-4) 72.32 (PS-C-1) 71.54 (PS-C-4) 71.50 (Glc-A-C-2) 62.45 (Glc-B-C-6) 62.17 (Glc-A-C-6) 52.19 (PS-C-2) 38.23 (*MeNO*) 36.84-14.18 (Alkyl chain)

Melib (yield: 68%)

¹H NMR (500 MHz, pyridine) δ ppm 8.69 (d, $J=8.55$ Hz, 1 H; *NH*) 5.50 (d, $J=3.42$ Hz, 1 H; Gal-*H*-1) 5.11 - 5.18 (m, 1 H; PS-*H*-2) 4.58 - 4.69 (m, 6 H; Glc-*H*-1, Gal-*H*-2, Gal-*H*-4, Gal-*H*-5, PS-*H*-1) 4.51 (dd, $J=10.01, 2.69$ Hz, 1 H; Gal-*H*-3) 4.38 - 4.49 (m, 3 H; Glc-*H*-6a, Gal-*H*-6) 4.29 - 4.38 (m, 2 H; Glc-*H*-6b, PS-*H*-3) 4.17 - 4.24 (m, 1 H; PS-*H*-4) 4.13 (m, 2 H; Glc-*H*-2, Glc-*H*-3) 4.04 - 4.10 (m, 1 H; Glc-*H*-4) 3.86 - 3.94 (m, 1 H; Glc-*H*-5) 2.95 (s, 3 H; *MeNO*) 2.54-1.18 (m, 58 H; *CH*₂) 0.88 (t, $J=6.59$ Hz, 6 H; *CH*₃)

F1 (ppm) 100.96 (Gal-C-1) 95.19 (Glc-C-1) 79.68 (Glc-C-3) 77.94 (Glc-C-5) 76.03 (PS-C-3) 72.80 (PS-C-4) 72.04 (PS-C-1) 71.95 (Glc-C-2) 71.69 (Glc-C-4) 71.59 (Gal-C-3) 71.28 (Gal-C-2, Gal-C-4) 71.15 (Gal-C-5) 68.58 (Glc-C-6) 62.58 (Gal-C-6) 51.68 (PS-C-2) 38.25 (MeNO) 36.81-14.19 (Alkyl chain)

Maltotriose (yield: 53%)

¹H NMR (500 MHz, pyridine) δ ppm 8.81 (d, $J=8.30$ Hz, 1 H; NH) 5.91 (d, $J=3.18$ Hz, 1 H; Glc-C-H-1) 5.78 (d, $J=3.18$ Hz, 1 H; Glc-B-H-1) 5.19 - 5.27 (m, 1 H; PS-H-2) 4.56 - 4.77 (m, 5 H; Glc-A-H-1, Glc-B-H-3, Glc-C-H-3, PS-H-1) 4.49 - 4.56 (m, 2 H; Glc-C-H-4, Glc-C-H-6a) 4.31 - 4.48 (m, 7 H; Glc-A-H-6, Glc-B-H-5, Glc-B-H-6, Glc-C-H-6b, PS-H-3) 4.23 - 4.31 (m, 2 H; Glc-A-H-3, Glc-B-H-4) 4.14 - 4.23 (m, 5 H; Glc-A-H-2, Glc-C-H-4, Glc-C-H-2, Glc-C-H-5, PS-H-4) 4.09 - 4.14 (m, 1 H; Glc-B-H-2) 3.71 - 3.80 (m, 1 H; Glc-A-H-5) 2.93 (s, 3 H; MeNO) 2.54-1.17 (m, 58 H; CH₂) 0.88 (t, $J=6.59$ Hz, 6 H; CH₃)

F1 (ppm) 103.20 (Glc-C-C-1) 103.03 (Glc-B-C-1) 95.28 81.51 (Glc-A-C-4) 81.33 (Glc-B-C-4) 79.13 (Glc-A-C-3) 78.53 (Glc-A-C-5) 76.50 (PS-C-3) 75.28 (Glc-C-C-3) 75.18 (Glc-C-C-4) 74.97 (Glc-B-C-3) 74.53 (Glc-C-C-2) 73.85 (Glc-B-C-2) 73.67 (Glc-B-C-5) 72.50 (PS-C-1) 72.22 (Glc-C-C-5) 72.10 (PS-C-4) 71.41 (Glc-A-C-2) 62.65 (Glc-C-C-6) 62.20 (Glc-A-C-6) 61.92 (Glc-B-C-6) 52.00 (PS-C-2) 38.23 (MeNO) 36.81-14.17 (Alkyl chain)

Compound data of conjugated products using compound 19

Glc (yield: 34%)

^1H NMR (500 MHz, pyridine) δ ppm 9.04 (d, $J=8.06$ Hz, 1 H; NH) 8.64 (t, $J=5.62$ Hz, 1 H; NH) 5.46 - 5.52 (m, 1 H; Ser- $H-\alpha$) 4.72 (d, $J=9.04$ Hz, 1 H; Glc- $H-1$) 4.49 - 4.58 (m, 3 H; Glc- $H-6a$, Ser- $H-\beta$) 4.37 (dd, $J=11.72$, 5.37 Hz, 1 H; Glc- $H-6b$) 4.13 - 4.25 (m, 3 H; Glc- $H-2$, Glc- $H-3$, Glc- $H-4$) 3.88 - 3.95 (m, 1 H; Glc- $H-5$) 3.40 - 3.55 (m, 2 H; CH_2) 2.96 (s, 3 H; MeNO) 2.55-1.15 (m, 56 H; CH_2) 0.86 - 0.92 (m, 6 H; CH_3)
F1 (ppm) 95.28 (Glc-C-1) 80.14 (Glc-C-5) 79.91 (Glc-C-3) 72.68 (Ser-C- β) 71.81 (Glc-C-2, Glc-C-4) 62.88 (Glc-C-6) 53.92 (Ser-C- α) 39.88 (CH_2) 38.19 (MeNO) 36.57-14.17 (Alkyl chain)

Gal (yield: 25%)

^1H NMR (500 MHz, pyridine) δ ppm 8.93 (d, $J=7.82$ Hz, 1 H; NH) 8.56 - 8.61 (m, 1 H; NH) 5.49 - 5.55 (m, 1 H; Ser- $H-\alpha$) 4.70 (d, $J=9.04$ Hz, 1 H; Gal- $H-1$) 4.56 - 4.61 (m, 1 H; Gal- $H-4$) 4.41 - 4.56 (m, 5 H; Gal- $H-2$, Gal- $H-6$, Ser- $H-\beta$) 4.16 (dd, $J=9.04$, 2.69 Hz, 1 H; Gal- $H-3$) 4.04 - 4.09 (m, 1 H; Gal- $H-5$) 3.41 - 3.55 (m, 2 H; CH_2) 3.00 (s, 3 H; MeNO) 2.54-1.15 (m, 56 H; CH_2) 0.89 (t, $J=6.23$ Hz, 6 H; CH_3)
F1 (ppm) 95.67 (Gal-C-1) 78.65 (Gal-C-5) 76.58 (Gal-C-3) 72.56 (Ser-C- β) 70.24 (Gal-C-4) 69.55 (Gal-C-2) 62.31 (Gal-C-6) 53.74 (Ser-C- α) 39.87 (CH_2) 38.04 (MeNO) 36.52-14.22 (Alkyl chain)

Xyl (yield: 38%)

^1H NMR (500 MHz, pyridine) δ ppm 9.02 (d, $J=8.06$ Hz, 1 H; NH) 8.64 (t, $J=5.62$ Hz, 1 H; NH) 5.44 - 5.50 (m, 1 H; Ser- $H-\alpha$) 4.62 (d, $J=8.79$ Hz, 1 H; Xyl- $H-1$) 4.53 (m, 2 H; Ser- $H-\beta$) 4.33 - 4.39 (m, 1 H; Xyl- $H-5a$) 4.11 (m, 3 H; Xyl- $H-2$, Xyl- $H-3$, Xyl- $H-4$)

3.61 - 3.68 (m, 1 H; Xyl-*H*-5b) 3.40 - 3.54 (m, 2 H; *CH*₂) 2.95 (s, 3 H; *MeNO*) 2.54 - 1.17 (m, 56 H; *CH*₂) 0.86 - 0.92 (m, 6 H; *CH*₃)

F1 (ppm) 95.79 (Xyl-*C*-1) 79.81 (Xyl-*C*-3) 72.67 (Ser-*C*-β) 71.76 (Xyl-*C*-2) 71.10 (Xyl-*C*-4) 69.04 (Xyl-*C*-5) 53.73 (Ser-*C*-α) 39.98 (*CH*₂) 37.78 (*MeNO*) 36.52-14.26 (Alkyl chain)

Fuc (yield: 47%)

¹H NMR (500 MHz, pyridine) δ ppm 9.04 (d, *J*=8.06 Hz, 1 H; *NH*) 8.50 - 8.57 (m, 1 H; *NH*) 5.37 - 5.45 (m, 1 H; Ser-*H*-α) 4.73 (d, *J*=9.04 Hz, 1 H; Fuc-*H*-1) 4.49 - 4.56 (m, 1 H; Ser-*H*-βa) 4.42 - 4.49 (m, 1 H; Ser-*H*-βb) 4.35 (t, *J*=8.79 Hz, 1 H; Fuc-*H*-2) 4.09 - 4.15 (m, 1 H; Fuc-*H*-3) 4.03 - 4.08 (m, 1 H; Fuc-*H*-4) 3.79 - 3.87 (m, 1 H; Fuc-*H*-5) 3.38 - 3.53 (m, 2 H; *CH*₂) 2.92 (s, 3 H; *MeNO*) 1.54 (d, *J*=6.11 Hz, 3 H; Fuc-*H*-6) 2.55-1.12 (m, 56 H; *CH*₂) 0.89 (t, *J*=6.35 Hz, 6 H; *CH*₃)

F1 (ppm) 95.71 (Fuc-*C*-1) 76.40 (Fuc-*C*-3) 73.02 (Fuc-*C*-5) 72.71 (Ser-*C*-β) 72.68 (Fuc-*C*-4) 69.16 (Fuc-*C*-2) 54.24 (Ser-*C*-α) 39.72 (*CH*₂) 37.07 (*MeNO*) 36.30-14.22 (Alkyl chain) 17.25 (Fuc-*C*-6)

Lactose (yield: 8%)

¹H NMR (500 MHz, pyridine) δ ppm 9.06 (d, *J*=7.82 Hz, 1 H; *NH*) 8.62 - 8.68 (m, 1 H; *NH*) 5.44 - 5.50 (m, 1 H; Ser-*H*-α) 5.09 (d, *J*=7.82 Hz, 1 H; Gal-*H*-1) 4.63 (d, *J*=8.79 Hz, 1 H; Glc-*H*-1) 4.46 - 4.57 (m, 7 H; Glc-*H*-6, Gal-*H*-2, Gal-*H*-4, Gal-*H*-6a, Ser-*H*-β) 4.40 - 4.46 (m, 1 H; Gal-*H*-6b) 4.18 (m, 5 H; Glc-*H*-2, Glc-*H*-3, Glc-*H*-4, Gal-*H*-3, Gal-*H*-5) 3.79 - 3.86 (m, 1 H; Glc-*H*-5) 3.40 - 3.55 (m, 2 H; *CH*₂) 2.93 (s, 3 H; *MeNO*) 2.55-1.16 (m, 56 H; *CH*₂) 0.88 (t, *J*=6.35 Hz, 6 H; *CH*₃)

F1 (ppm) 106.08 (Gal-C-1) 95.08 (Glc-C-1) 81.98 (Glc-C-4) 78.28 (Glc-C-5) 77.99 (Glc-C-3) 77.50 (Gal-C-5) 75.33 (Gal-C-3) 72.95 (Ser-C- β) 71.77 (Gal-C-2) 71.47 (Glc-C-2) 70.14 (Gal-C-4) 62.32 (Glc-C-6) 62.15 (Gal-C-6) 54.08 (Ser-C- α) 39.88 (CH₂) 38.22 (MeNO) 36.48-14.40 (Alkyl chain)

Maltose (yield: 22%)

¹H NMR (500 MHz, pyridine) δ ppm 9.03 (d, J =8.06 Hz, 1 H; NH) 8.64 (t, J =5.62 Hz, 1 H; NH) 5.92 (d, J =3.91 Hz, 1 H; Glc-B-H-1) 5.44 - 5.50 (m, 1 H; Ser-H- α) 4.61 (d, J =9.04 Hz, 1 H; Glc-A-H-1) 4.50 - 4.59 (m, 4 H; Glc-B-H-3, Glc-B-H-5, Glc-B-H-6a, Ser-H- β) 4.45 (br. s., 2 H; Glc-A-H-6) 4.24 - 4.39 (m, 3 H; Glc-A-H-3, Glc-A-H-4, Glc-B-H-6b) 4.13 - 4.22 (m, 3 H; Glc-A-H-2, Glc-B-H-2, Glc-B-H-4) 3.72 - 3.77 (m, 1 H; Glc-A-H-5) 3.39 - 3.55 (m, 2 H; CH₂) 2.92 (s, 3 H; MeNO) 2.54-1.18 (m, 56 H; CH₂) 0.88 (t, J =6.59 Hz, 6 H; CH₃)

F1 (ppm) 103.28 (Glc-B-C-1) 95.16 (Glc-A-C-1) 81.27 (Glc-A-C-4) 79.11 (Glc-A-C-3) 78.60 (Glc-A-C-5) 75.53 (Glc-B-C-3) 75.21 (Glc-B-C-5) 74.42 (Glc-B-C-2) 72.94 72.12 71.58 (Glc-A-C-2) 62.72 (Glc-B-C-6) 61.94 (Glc-A-C-6) 53.81 (Ser-C- α) 40.03 (CH₂) 38.25 (MeNO) 36.58-14.16 (Alkyl chain)

Melibiose (yield: 21%)

¹H NMR (500 MHz, pyridine) δ ppm 8.96 (d, J =7.82 Hz, 1 H; NH) 8.54 - 8.61 (m, 1 H; NH) 5.51 - 5.57 (m, 1 H; Gal-H-1) 5.42 - 5.49 (m, 1 H; Ser-H- α) 4.60 - 4.71 (m, 4 H; Glc-H-1, Gal-H-2, Gal-H-4, Gal-H-5) 4.40 - 4.56 (m, 6 H; Glc-H-6a, Gal-H-3, Gal-H-6, Ser-H- β) 4.31 - 4.38 (m, 1 H; Glc-H-6b) 4.07 - 4.20 (m, 3 H; Glc-H-2, Glc-H-3,

Glc-*H*-4) 3.92 - 4.00 (m, 1 H; Glc-*H*-5) 3.38 - 3.56 (m, 2 H; CH₂) 2.95 (s, 3 H; MeNO)
 2.57-1.13 (m, 56 H; CH₂) 0.84 - 0.94 (m, 6 H; CH₃)

F1 (ppm) 100.95 (Gal-C-1) 95.13 (Glc-C-1) 79.66 (Glc-C-3) 78.01 (Glc-C-5) 72.74
 (Ser-C-β) 72.61 (Gal-C-5) 71.91 (Gal-C-3) 71.85 (Glc-C-4) 71.61 (Glc-C-2) 71.12
 (Gal-C-4) 70.76 (Gal-C-2) 68.62 (Glc-C-6) 62.63 (Gal-C-6) 53.80 (Ser-C-α) 39.93
 (CH₂) 38.15 (MeNO) 36.57-14.18 (Alkyl chain)

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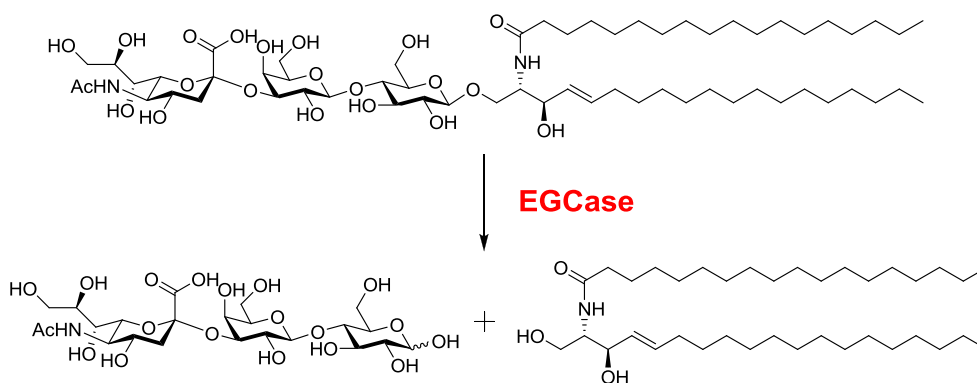
22. Y. Liu, T. Feizi, M. A. Campanero-Rhodes, R. A. Childs, Y. Zhang, B. Mulloy, P. G. Evans, H. M. I. Osborn, D. Otto, P. R. Crocker, and W. Chai, "Neoglycolipid probes prepared via oxime ligation for microarray analysis of oligosaccharide-protein interactions", *Chemistry & Biology*, **2007**, 14, 847-859

Chapter 4

Inhibitory Activity of N-LacCer Derivatives for EGCase II

4-1 Introduction

One of my purposes is to determine whether prepared neoglycolipids work as a mimetic compound of natural glycosphingolipids. For that purpose, my interest was directed to the synthesis and biological characteristics of neoglycosphingolipids having a non-natural N(OMe)-glycosidic linkage between carbohydrate and ceramide. Especially, it seems likely that non-natural GSLs bearing N(OMe)-glycosidic linkage might become promising candidates of the inhibitors against endoglycoceramidases (EGCase) and ceramide glycanases (CGase) that digest specifically major GSLs at O-glycosidic linkage between oligosaccharide moiety and ceramide^[1-6]. (Scheme 4-1-1)



Scheme 4-1-1 EGCase (and CGase) cleaves O-glycosidic linkage between oligosaccharide moiety and ceramide

Among those enzymes, I focused a recombinant EGCase II from *rhodococcus* sp. This enzyme has been most used broadly because mutated products of that have an activity of synthase, which means to conjugate ceramide and glycan part^[7-9]. The activity was verified by X-ray crystallography measurement^[10]. Additionally, Withers et al. also reported the significance of the nitrogen atom at *exo*-anomeric position of the

disaccharide mimetic compound identified as a potent inhibitor for hydrolysis reaction of EGCase II^[11]. Furthermore, X-ray structural study revealed that Glu-233 which is a general acid/base catalytic residue of this enzyme was an essential amino acid residue to interact with the *exo*-nitrogen atom of these compounds.

In this chapter, we focused on 2 compounds shown below and called *N*-LacCer and *O*-LacCer (Fig. 4-1-1)

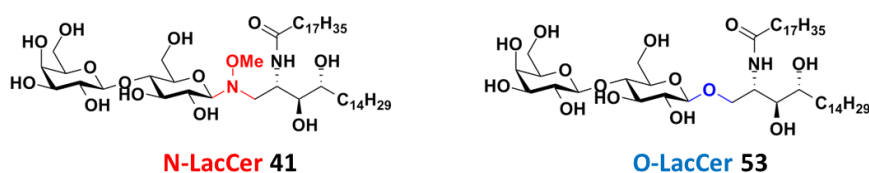
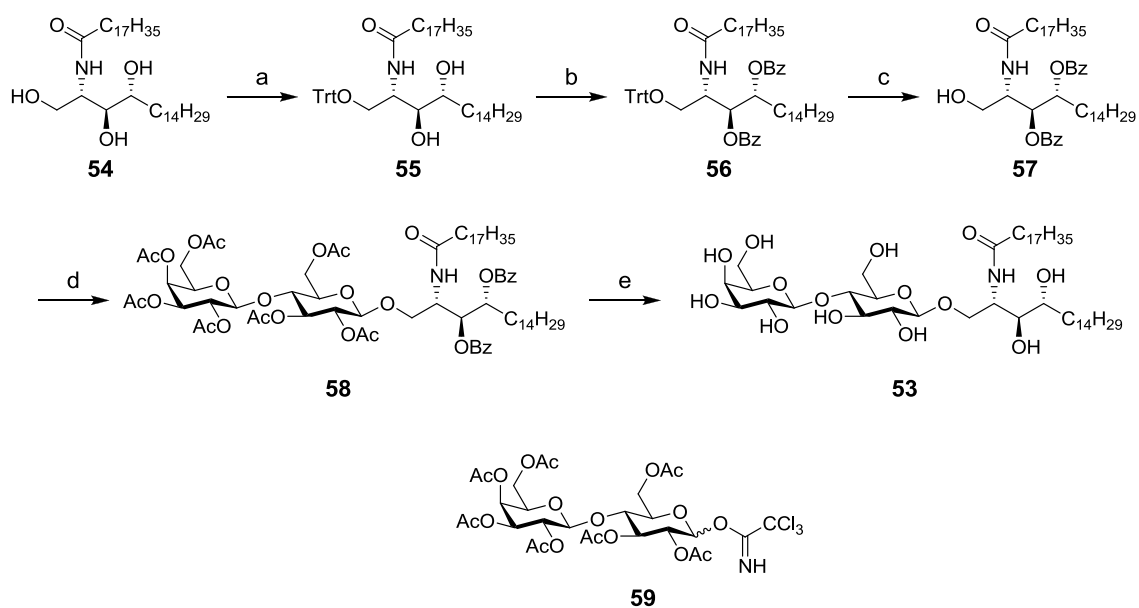


Fig. 4-1-1 Structure of *N*-LacCer **41** and *O*-LacCer **53**

4-2 Results & Discussions

4-2-1 Synthesis of O-LactosylCeramide **53** for a Positive Control

To evaluate the function of neoglycosides correctly, we prepared *O*-glycosyllactosylceramide **53**^[12] which was composed by the same length of phytosphingosine and acyl chain as synthesized neoglycolipids (Scheme 4-2-1). This compound was not confirmed as a substrate of EGCase but expected to be hydrolyzed because of *O*-glycoside.



Scheme 4-2-1. Synthesis of *O*-LacCer **53**. Reagents and conditions: a) TrCl, Pyr, 50 °C, 4 h, 78%; b) BzCl, pyr, CH₂Cl₂, RT, 2 d, 88%; c) HBr-AcOH, CH₂Cl₂, 0 °C, 3 min, 80 %; d) **59**, TMSOTf, CH₂Cl₂, 0 °C, 1 h, 31%; e) NaOMe, CH₂Cl₂, MeOH, RT, 5 h, 95%

4-2-2 Primitive hydrolysis assay

I preliminarily tested the inhibitory effect of *N*-LacCer **41**, methoxyamino derivative having lactoside, on the hydrolysis of *O*-LacCer **53** by a recombinant EGCase II from rhodococcus sp. (Fig. 4-2-1) While *O*-LacCer was hydrolyzed in the presence of the enzyme, *N*-LacCer was remained as the starting form regardless of temperature or the existence of the enzyme. Most interestingly, intact *O*-LacCer was significantly detected even after 8 hours as shown in Entry 5. The result suggested *N*-LacCer worked as an inhibitor to reduce the enzymatic activity. The result indicates an evidence for the inhibitory effect of compound **41** on the hydrolytic activity of this enzyme.

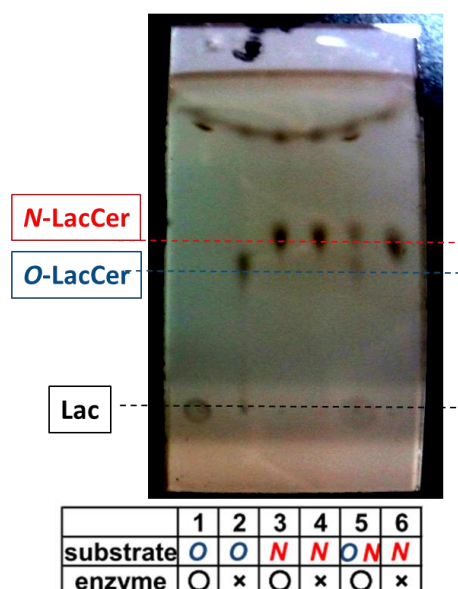
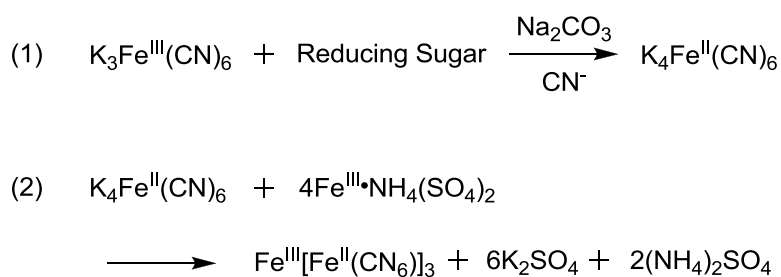


Figure 4-2-1. TLC-based inhibition assay. The spot of each compound and contents of solution were indicated in left figure.

4-2-3 Optimization of Park & Johnson Method to Evaluate Inhibitory Activity

To monitor the inhibitory effect of *N*-LacCer on the hydrolytic activity of recombinant EGCase II, we tested the feasibility of modified Park-Johnson method^[13] for highly sensitive and quantitative analysis of the reducing power of the generated lactose residue from *O*-LacCer as a substrate during the hydrolysis.



Scheme 4-2-2. A principle of colorimetric reaction of Park and Johnson method

Colorimetric method utilizes the reduction ability of free sugars to convert ferricyanide ions into Prussian blue (ferric ferrocyanide) in alkaline solution as indicated in Scheme 4-2-2. Though it was well known that three reagents were needed commonly in the reaction steps, the revised solutions for more sensitive detection was reported recently (T. Ikuma, K. Takeuchi, Y. Takahashi, K. Sagisaka, and T. Takasawa, High sensitive colorimetric method of reducing sugar using ferric iron reagent. *Res. Bull. Obihiro Univ.* **2001**, 22, 109-116). I decided to use this improved protocol and the feasibility in the inhibitory assay for EGCase II activity was tested as follows: (A) 50 mM Na₂CO₃, 10 mM KCN aq., (B) 1.5 mM K₃Fe(CN)₆ aq., and (C) 0.15% (w/v) Fe•NH₄(SO₄)₂, 0.2% (w/v) SDS, 0.03 N H₂SO₄ aq.

I used lactose as a source of reducing sugar and it was dissolved in the buffer for enzymatic reaction at several concentrations (20~120 ng/mL). 20 μ L of each aliquot was injected to a mixture containing 90 μ L of reagent (A) and same volume of reagent (B) in an eppendorf tube. The solution was divided into three portions of 60 μ L in PCR tubes. The tubes were closed strongly by a weight and incubated at 100°C for 20 min. The heat was cooled down in the refrigerator at -4°C for 3 min and then left at room temperature for 10 min. The tubes were opened and added 135 μ L of reagent (C). After shaken by vortex mixer, 150 μ L of each solution was transferred on the 96-wel plate and absorbance at 690 nm was measured by a plate reader at 15 min from adding reagent (C). The result is shown below as a standard curve of lactose. This data was corrected by subtracting the absorbance value of blank area (containing no lactose). We obtained good linearity and error range was too small to be depicted on the graph (Fig.4-2-3). This result indicated that this modified protocol can be used for the quantification of the released lactose from GSLs in the presence of recombinant EGCase.

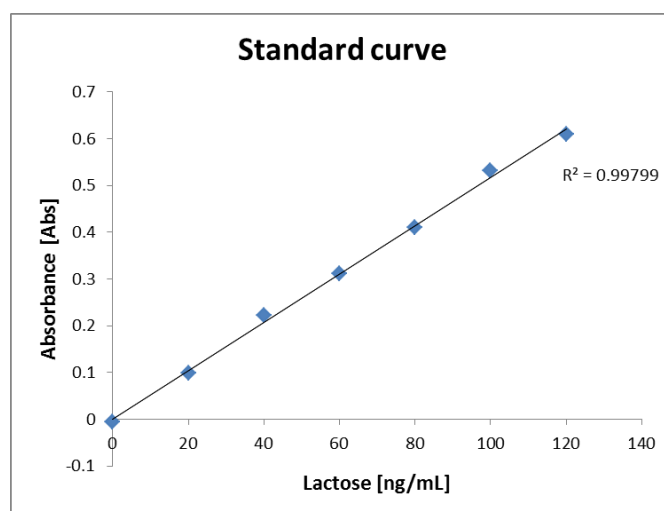
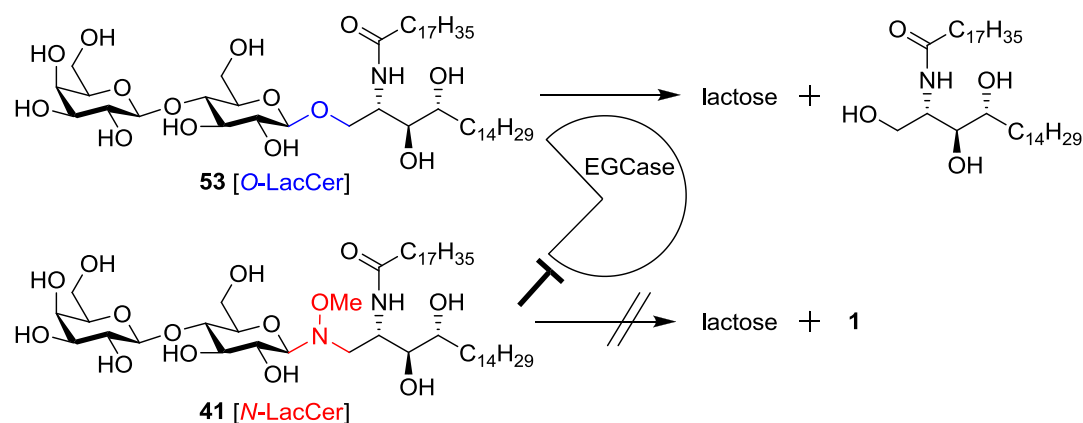


Figure 4-2-3. Standard curve of colorimetric reaction

4-2-4 Inhibition Assay of Recombinant EGCase II



Scheme 4-2-2. A plausible model for the inhibitory effect of *N*-LacCer **41**.

The result of primitive assay suggested that *N*-LacCer inhibited hydrolysis of *O*-LacCer. To determine whether it was true, we first conducted to estimate the k_m value (Fig. 4-2-4).

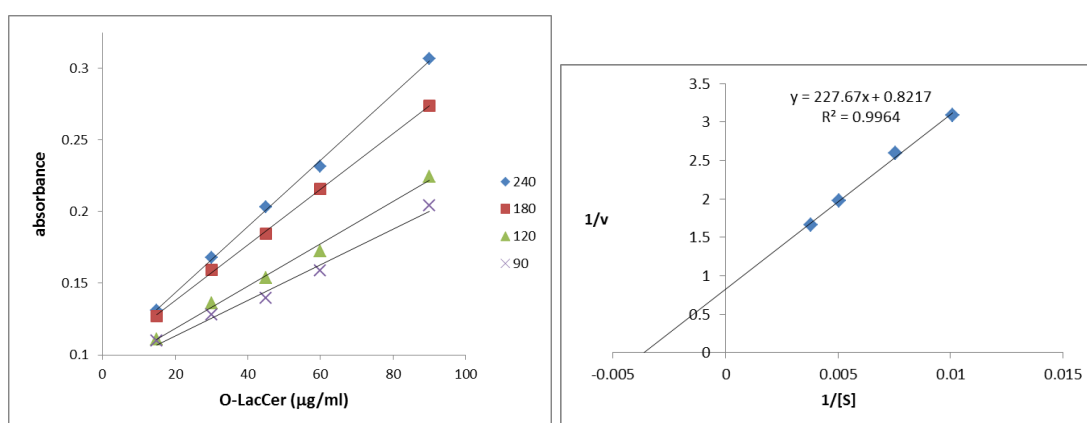


Fig. 4-2-4. Different velocity of hydrolysis dependent on the concentration of *O*-LacCer **53** and estimation of K_m value

K_m value was estimated to be 277 μ M, which was reasonable because the K_m value of asialo GM1 having erythro sphingosine skeleton was reported as 500 μ M previously.

Next, It was demonstrated that hydrolysis of *O*-LacCer **53** (0.45 mM) by EGCase II (12 mU/mL) was reduced significantly in the presence of *N*-LacCer **41** (0.27 mM) the release of lactose from *O*-LacCer **53** in the presence of EGCase II (Fig. 4-2-5). This result clearly indicates that *N*-LacCer **41** interacts directly with EGCase II in a similar manner to *O*-LacCer **53** and appeared to act as a competitive inhibitor. Interestingly, Withers et al. reported the significance of the nitrogen atom at exo-anomeric position of the disaccharide mimetic compound identified as a potent inhibitor of EGCase II. Furthermore, X-ray structural study revealed that Glu-233 which is a general acid/base catalytic residue of this enzyme was an essential amino acid residue to interact with the exo-nitrogen atom of this compound. These results may suggest the importance of a nitrogen atom involved in the β -N(OMe)-glycoside bond of *N*-LacCer **41** for the interaction with Glu-233 residue of EGCase II.

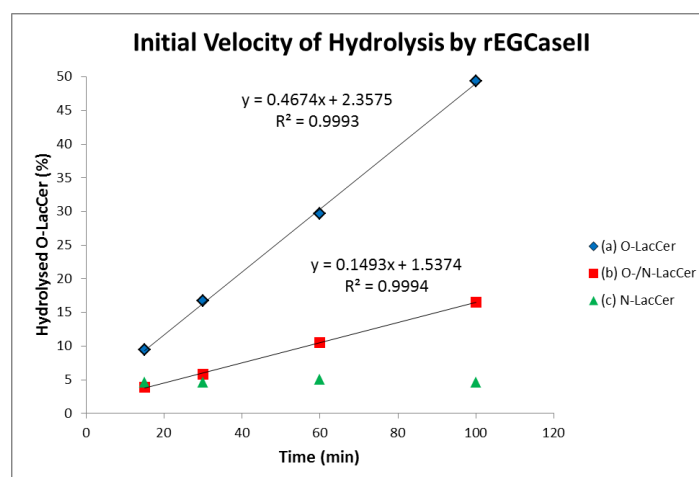


Figure 4-2-5. Inhibitory effect of lactosylceramide mimic **41** (N-LacCer) on the hydrolytic activity of recombinant EGCase II. Initial velocity of the hydrolysis of O-LacCer **53** was determined by quantitating lactose released from O-LacCer using modified Park and Johnson method (Supporting Information). ◆: O-LacCer (450 μ M), ■: O-LacCer (450 μ M) and N-LacCer (270 μ M), ▲: N-LacCer (735 μ M); Reaction condition: 20 mM AcOH buffer (pH 4.7), 0.4% Triton X-100, EGCase II (12 mU/mL), 37°C.

Finally, we tried to estimate the K_i value of N-LacCer **41** for EGCase II. Reactions were performed with different concentration of O-LacCer/N-LacCer. Results showed reaction velocity decreased depending on inhibitor concentration (Fig. 4-2-6) and K_i value was estimated as 96.5 μ M (Fig. 4-2-7) by dixon plot. Result also suggested inhibition form was competitive.

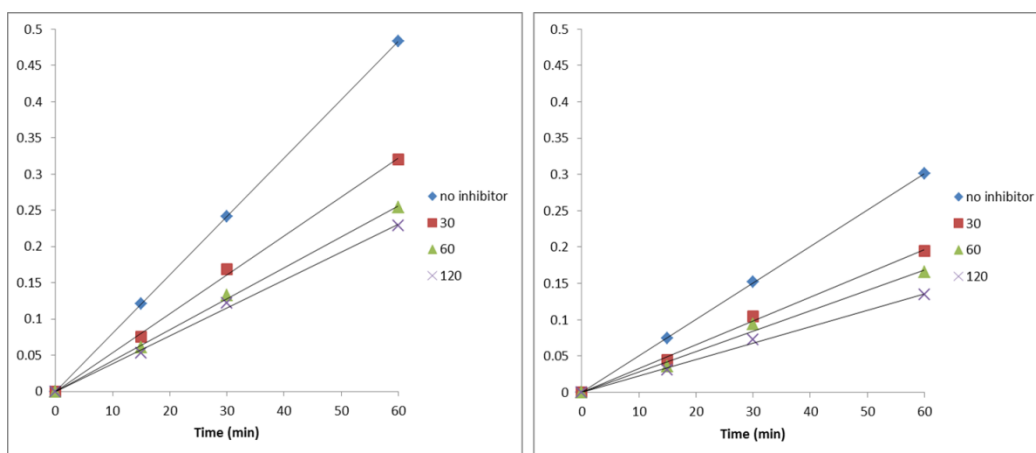


Fig. 4-2-6. Inhibition assay with different concentration

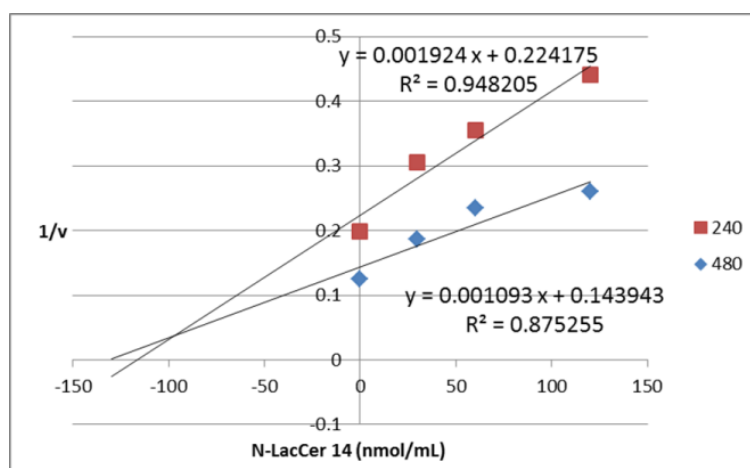


Fig. 4-2-7. Estimation of K_i value

4-3 Conclusion

In this chapter, we evaluate the function of neoglycoside to imitate natural glycosphingolipid using EGCase II. This enzyme could cleave *O*-glycosidic linkage but not non-natural *N*-glycoside bond. It showed that neoglycolipid was not a substrate of the enzyme. However, when the two compounds, *O*-LacCer and *N*-LacCer, were mixed, the velocity of hydrolysis was apparently reduced compared with only *O*-LacCer. It was significantly important because it indicated that *N*-LacCer could be recognized by the enzyme like natural glycosphingolipid. This result enhanced the effectiveness of glycoblotting method for preparation of glycolipids.

4-4 Experimental Section

Synthesis of *O*-LacCer (53).

(2*S*, 3*S*, 4*R*)-2-[*N*-(octadecanoyl)amino]-1-(triphenyl-methyloxy)octadecane-3,4-diol (55).

To a solution of compound **54** (1.51 g, 2.59 mmol) in pyridine (20 mL), trityl chloride (1.44 g, 5.17 mmol) was added and the mixture was stirred for 4 h at 50 °C. Reaction was quenched by MeOH and solvent was removed in vacuo completely after silica gel was added to the mixture. The residue was purified by flash column chromatography on silica gel (hexane-EtOAc, 6:1-3:1) to yield the desired compound **55** as a white amorphous solid (1.67 g, 78%); ¹H NMR (600 MHz, CDCl₃): δ 7.41 (d, *J* = 7.52 Hz, 6H; Ar-*H*), 7.31 (d, *J* = 7.61 Hz, 6H; Ar-*H*), 7.25 (m, 3H; Ar-*H*), 6.07 (d, *J* = 8.44 Hz, 1H; NH), 4.25 (m, 1H; *H*-2), 3.57 (m, 1H; *H*-3), 3.50 (dd, *J* = 9.90, 3.48 Hz, 1H; *H*-1a), 3.38 (br.s, 1H; OH), 3.34 (dd, *J* = 9.90, 4.58 Hz, 1H; *H*-1b), 3.18 (br.s, 1H; OH), 2.36 (br.s, 1H; OH), 2.14 (t, *J* = 7.61 Hz, 2H; NHCOCH₂a,b), 1.67 (m, 1H; *H*-5a), 1.60 (m, 2H; NHCOCH₂CH₂a,b), 1.44 (m, 2H; *H*-5b, *H*-6a), 1.37-1.18 (m, 51H; CH₂, *H*-6b), 0.88 (t, *J* = 6.97 Hz, 6H; CH₃); ¹³C NMR (150 MHz (HSQC), CDCl₃, 298 K): δ (ppm) = 128.15, 127.79, 127.01 (Ar) 75.36 (*C*-3), 73.01 (*C*-4), 62.71 (*C*-1), 50.40 (*C*-2), 36.54 (NHCOCH₂), 32.87-21.67 (CH₂), 13.97 (CH₃); HRMS (ESI) Calcd. for C₅₅H₈₇NO₄Na [M+Na]⁺ 848.65328, found 848.64941

(2S, 3S, 4R)-3,4-(di-O-Benzoyl)-2-[N-(octadecanoyl)amino]-1-(triphenyl-methyloxy)octadecane-3,4-diol (56).

To a solution of compound **55** (826 mg, 1.00 mmol) in pyridine (10 mL), benzoyl chloride (465 μ L, 4.00 mmol) was added dropwise and the mixture was stirred for 2 days at room temperature. It was diluted with EtOAc and washed with 1 N HCl three times, sat. NaHCO₃ aq. and brine. Organic phase was dried over MgSO₄ and concentrated under reduced pressure. Purification of the crude product by flash column chromatography on silica gel (Hexane-EtOAc, 15:1) yielded compound **56** (906 mg, 88%) as colorless oil; ¹H NMR (500 MHz, CDCl₃): 7.96 (d, *J* = 8.18 Hz, 2H; Ar-*H*), 7.89 (d, *J* = 8.18 Hz, 2H; Ar-*H*), 7.53 (m, 2H; Ar-*H*), 7.38 (m, 4H; Ar-*H*), 7.31 (d, *J* = 8.06 Hz, 6H; Ar-*H*), 7.10 (m, 9H; Ar-*H*), 6.10 (d, *J* = 9.53 Hz, 1H; NH), 5.83 (dd, *J* = 9.04, 2.69 Hz, 1H; *H*-3) 5.37 (m, 1H; *H*-4), 4.61 (m, 1H; *H*-2), 3.32 (m, 2H; *H*-1a,b), 2.18 (m, 2H; NHCOCH₂a,b), 1.88 (m, 2H; *H*-5a,b), 1.64 (m, 2H; NHCOCH₂CH₂a,b), 1.45-1.17 (m, 52H; CH₂), 0.87 (t, *J* = 6.96 Hz, 6H; CH₃); ¹³C NMR (150 MHz (HSQC), CDCl₃): δ 132.95, 132.79, 129.66, 128.44, 128.26, 127.67, 126.89 (Ar) 74.08 (C-4), 72.61 (C-3), 61.62 (C-1), 48.51 (C-2), 36.77 (NHCOCH₂), 31.85-22.61 (CH₂), 14.04 (CH₃); HRMS (ESI) Calcd. for C₆₉H₉₅NO₆Na [M+Na]⁺ 1056.70571, found 1056.70205

(2S, 3S, 4R)-3,4-(di-O-Benzoyl)-2-[N-(octadecanoyl)-amino]octadecane-1,3,4-triol (57).

To a solution of compound **56** (713 mg, 689 μ mol) in CH₂Cl₂ (3 mL), HBr-AcOH (270 μ L, 1.38 mmol) was added dropwise and the mixture was stirred for 3 minutes at 0 °C. It was diluted with EtOAc quickly and washed with brine, sat. NaHCO₃ aq. three times

and brine. Organic phase was dried over MgSO_4 and concentrated under reduced pressure. Purification of the crude product by flash column chromatography on silica gel (Hexane-EtOAc, 10:1-4:1) yielded compound **57** (435 mg, 80%) as colorless oil; ^1H NMR (500 MHz, CDCl_3): δ 8.02 (d, J = 8.44 Hz, 2H; Ar-*H*), 7.94 (d, J = 8.29 Hz, 2H; Ar-*H*), 7.60 (m, 1H; Ar-*H*), 7.50 (m, 1H; Ar-*H*), 7.46 (t, J = 8.13 Hz, 2H; Ar-*H*), 7.35 (t, J = 8.13 Hz, 2H; Ar-*H*), 6.78 (d, J = 9.38 Hz, 1H; NH), 5.52 (dd, J = 9.38, 2.50 Hz, 1H; *H*-3) 5.38 (m, 1H; *H*-4), 4.44 (m, 1H; *H*-2), 3.66 (m, 2H; *H*-1a,b), 2.28 (t, J = 7.82 Hz, 2H; NHCOCH_2 a,b), 2.02 (m, 2H; *H*-5a,b), 1.67 (m, 2H; , $\text{NHCOCH}_2\text{CH}_2$ a,b), 1.44 (m, 1H; *H*-6a), 1.40-1.17 (m, 51H; CH_2 , *H*-6b), 0.88 (t, J = 6.96 Hz, 6H; CH_3); ^{13}C NMR (150 MHz (HSQC), CDCl_3): δ 133.55, 132.95, 129.84, 129.59, 128.52, 128.25, (Ar) 74.03 (*C*-4), 73.18 (*C*-3), 61.42 (*C*-1), 49.92 (*C*-2), 36.72 (NHCOCH_2), 31.86-22.62 (CH_2), 14.04 (CH_3); HRMS (ESI) Calcd. for $\text{C}_{50}\text{H}_{81}\text{NO}_6\text{Na}$ $[\text{M}+\text{Na}]^+$ 814.59616, found 814.59354

(2*S*,3*S*,4*R*)-3,4-(di-*O*-Benzoyl)-2-[*N*-(octadecanoyl)-amino]-1-{*O*-[β -2,3,4,6-tetraacetyl-galactosyl-pyranosyl-(1 $\rightarrow$ 4)- β -2,3,6-triacetylglucopyranosyl]}octadecane-1,3,4-triol (58**).**

Compound **57** (276 mg, 379 μmol) and lactose donor **59** (408 mg, 522 μmol) were dissolve in CH_2Cl_2 (5 mL). The mixture was stirred at 0 $^\circ\text{C}$ and TMSOTf (5 mL, 28 μmol) was added dropwise. After 1 hour, the solution was diluted with EtOAc and washed with sat. NaHCO_3 aq. and brine. Organic phase was dried over MgSO_4 and concentrated under reduced pressure. Purification of the crude product by flash column chromatography on silica gel (CH_2Cl_2 -Et $_2\text{O}$, 15:1, Hexane-EtOAc, 10:1-4:1) yielded

compound **58** (165 mg, 31%); ^1H NMR (500 MHz, CDCl_3): δ 8.01 (d, $J = 8.22$ Hz, 2H; Ar-*H*), 7.96 (d, $J = 8.22$ Hz, 2H; Ar-*H*), 7.61 (m, 1H; Ar-*H*), 7.54 (m, 1H; Ar-*H*), 7.47 (t, $J = 8.22$ Hz, 2H; Ar-*H*), 7.40 (t, $J = 8.22$ Hz, 2H; Ar-*H*), 6.14 (d, $J = 9.48$ Hz, 1H; NH), 5.58 (dd, $J = 8.22, 3.48$ Hz, 1H; PS-*H*-3) 5.34 (m, 2H; PS-*H*-4, Gal-*H*-4), 5.11 (t, $J = 9.16$ Hz, 1H; Glc-*H*-3), 5.07 (dd, $J = 10.43, 7.90$ Hz, 1H; Gal-*H*-2), 4.93 (dd, $J = 10.43, 3.48$ Hz, 1H; Gal-*H*-3), 4.80 (dd, $J = 9.48, 7.58$ Hz, 1H; Glc-*H*-2), 4.58 (m, 1H; PS-*H*-2), 4.41 (d, $J = 7.90$ Hz, 2H; Glc-*H*-1, Gal-*H*-1), 4.34 (m, 1H; Glc-*H*-6a), 4.08 (m, 2H; Gal-*H*-6a,b), 3.85 (m, 3H; PS-*H*-1a, Glc-*H*-6b, Gal-*H*-5), 3.70 (m, 2H; PS-*H*-1, Glc-*H*-4), 3.51 (ddd, $J = 9.80, 5.06, 1.90$ Hz, 1H; Glc-*H*-5), 2.24 (s, 2H; $\text{NHCOCH}_2\text{a,b}$), 2.14 (s, 3H; Ac), 2.06 (s, 3H; Ac), 2.02 (s, 3H; Ac), 1.99 (s, 3H; Ac), 1.98 (s, 3H; Ac), 1.95 (s, 3H; Ac), 1.94 (s, 3H; Ac), 1.85 (m, 2H; PS-*H*-5), 1.66 (m, 2H; $\text{NHCOCH}_2\text{CH}_2\text{a,b}$), 1.41-1.16 (m, 52H; CH_2), 0.88 (t, $J = 6.95$ Hz, 6H; CH_3); ^{13}C NMR (150 MHz (HSQC), CDCl_3): δ 133.24, 132.94, 129.67, 129.62, 128.41, 128.26, (Ar) 100.35 (Glc-*C*-1, Gal-*C*-1), 75.80 (Glc-*C*-4), 73.65 (PS-*C*-4), 72.44 (Glc-*C*-5), 72.37 (Glc-*C*-3), 72.31 (PS-*C*-3), 71.49 (Glc-*C*-2), 70.79 (Gal-*C*-3), 70.54 (Gal-*C*-5), 68.75 (Gal-*C*-2), 67.23 (PS-*C*-1), 66.54 (Gal-*C*-4), 61.61 (Glc-*C*-6), 60.55 (Gal-*C*-6), 47.91 (PS-*C*-2), 36.73 (NHCOCH_2), 32.03-22.06 (CH_2), 20.76-20.25 (Ac), 14.50 (CH_3); HRMS (ESI) Calcd. for $\text{C}_{76}\text{H}_{115}\text{NO}_{23}\text{Na}$ [$\text{M}+\text{Na}$] $^+$ 1432.77575, found 1432.77069

(2*S*,3*S*,4*R*)-2-[*N*-(octadecanoyl)amino]-1-{*O*-[β -galactosylpyranosyl-(1 $\rightarrow$ 4)- β -glucopyranosyl]}octadecane-1,3,4-triol (53).

Compound **58** (100 mg, 71 μmol) was dissolved in CH_2Cl_2 (200 mL) and MeOH (1 mL). To the solution, sodium methoxide (1.5 mg, 28 μmol) was added and the mixture

was stirred 5 hours at room temperature. Solvent was completely removed by air drying and the product was precipitated in MeOH. After filtration, white powder of compound **53** (61 mg, 95%) was obtained; ^1H NMR (600MHz, d_5 -Pyridine): δ 8.57 (d, J = 8.77 Hz, 1H; NH), 7.64 (br.s, 1H; Glc-2-OH), 7.52 (d, J = 3.95 Hz, 1H; Gal-2-OH), 6.95 (br.s, 1H; Gal-3-OH), 6.65 (br.s, 1H; Gal-6-OH), 6.58 (br.s, 1H; Gal-4-OH), 6.53 (d, J = 6.14 Hz, 1H; PS-3-OH), 6.45 (br.s, 1H; Glc-6-OH), 6.21 (s, 1H; Glc-3-OH), 5.92 (d, J = 7.23 Hz, 1H; PS-4-OH), 5.16 (m, 1H; PS-*H*-2), 5.01 (Gal-*H*-1, overlapped with H₂O), 4.91 (d, J = 7.89 Hz, 1H; Glc-*H*-1), 4.79 (dd, J = 10.52, 5.70 Hz, 1H; PS-*H*-1a), 4.56-4.43 (m, 5H; Glc-*H*-6a,b, Gal-*H*-2, Gal-*H*-4, Gal-*H*-6a), 4.39 (m, 3H; PS-*H*-1b, PS-*H*-3, Gal-*H*-6b), 4.25 (m, 3H; PS-*H*-4, Glc-*H*-3, Glc-*H*-4), 4.15 (m, 1H; Gal-*H*-3, Gal-*H*-5), 4.04 (t, J = 7.89 Hz, 1H; Glc-*H*-2), 3.84 (m, 1H; Glc-*H*-5), 2.45 (t, J = 7.45 Hz, 2H; NHCOCH₂a,b), 2.24 (m, 1H; PS-*H*-5a), 1.95 (m, 2H; PS-*H*-5b, PS-*H*-6a), 1.82 (m, 2H; NHCOCH₂CH₂a,b), 1.70 (m, 1H; PS-*H*-6b), 1.50-1.19 (m, 50H; CH₂), 0.88 (t, J = 6.58 Hz, 6H; CH₃); ^{13}C NMR (150 MHz (HSQC), d_5 -Pyridine): δ 105.75 (Gal-C-1), 105.22 (Glc-C-1), 81.89 (Glc-C-4), 77.31 (Gal-C-5), 76.72 (Glc-C-3), 76.59 (Glc-C-5), 75.79 (PS-C-3), 75.12 (Gal-C-3), 74.66 (Glc-C-2), 72.67 (PS-C-4), 72.40 (Gal-C-2), 70.55 (PS-C-1), 69.95 (Gal-C-4), 61.95 (Glc-C-6, Gal-C-6), 51.95 (PS-C-2), 37.22 (NHCOCH₂), 33.43-22.66 (CH₂), 14.83 (CH₃); HRMS (ESI) Calcd. for C₄₈H₉₃NO₁₄Na [M+Na]⁺ 930.64937, found 930.64499

A) *TLC-based assay*: 1 mM solutions of the substrate/inhibitor, *O*-LacCer **53** or *N*-LacCer **41**, were prepared by means of the reaction buffer (20 mM AcOH buffer containing 0.4% Triton X-100, pH 4.7). To 98.5 μL of them (*O*-LacCer: Entry 1 and

3, *N*-LacCer: Entry 2, 4, and 6, 1:1 mixture of *O*-LacCer/*N*-LacCer: Entry 5), 2.5 μ L of EGCase solution (Entry 1, 3, and 5) or the buffer (Entry 2, 4, and 6) was added at 37°C (Entry 1-5) or 0°C (Entry 6). Reaction temperature was kept for 8 hours, then TLC analysis was performed ($\text{CHCl}_3/\text{MeOH}$, 3:1) to observe the progress of hydrolysis. Spots were visualized by spraying a solution of 95:5 (v/v) MeOH-concentrated sulfuric acid and heating at 180°C for ca. 1/2 min.

B) *Hydrolysis assay using modified Park-Johnson method for calculation of K_m value:*

240, 180, 120, and 90 $\mu\text{g/mL}$ of *O*-LacCer **53** were incubated with 5 mU/mL of rEGCase at 37°C in the reaction buffer (20 mM AcOH buffer containing 0.4% Triton X-100, pH 4.7) respectively. 20 μ L of aliquots were extracted after 15, 30, 60, and 100 min. The solution was mixed with coloring reagents in PCR tube for quenching the reaction and subjected to the monitoring of the reaction progress.

In the PCR tube, 90 μ L of reagents (A) and (B) described above were mixed respectively. After adding the reaction mixture collected, the solution was divided into three portions in other tubes ($n=3$) and colorimetric reaction was performed as described. The raw data obtained were corrected by using the blank value

C) *Inhibition assay using modified Park-Johnson method:* Before enzymatic the reaction, 900 μM solution of *O*-LacCer **53** and 750 μM solution of *N*-LacCer **41** was prepared by dissolving in the reaction buffer (20 mM AcOH buffer containing 0.4% Triton X-100, pH 4.7) and 3 μ L of enzyme solution was diluted to 10 μ L by the same buffer. Solutions of *O*-LacCer, *N*-LacCer and the reaction buffer were

mixed in eppendorf tubes as shown Table 4-4-1. Then, the reaction was started by adding the enzyme solution at 37°C and 20 µL of aliquots were extracted after 15, 30, 60, and 100 min. The solution was mixed with coloring reagents in PCR tube for quenching the reaction and subjected to the monitoring of the reaction progress.

In the PCR tube, 90 µL of reagents (A) and (B) described above were mixed respectively. After adding the reaction mixture collected, the solution was divided into three portions in other tubes (n=3) and colorimetric reaction was performed as described. The raw data obtained were corrected by using the blank value and plotted in the graph after the unit of the enzyme activity was converted into percentage of the hydrolysis of whole *O*-LacCer in comparison with the standard curve of lactose as shown in Figure 4-2-5.

Entry	a	b	c
<i>O</i> -LacCer	50	50	0
<i>N</i> -LacCer	0	36	98
Buffer	48	12	0
EGCase II	2	2	2
Total (µL)	100	100	100

Table 4-4-1. Composition of the reaction mixture

4-5 References

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Chapter 5

Further Functionalization of Neoglycolipids

5-1 Introduction

Not only for glycolipids, introduction of specific tags or immobilization to research tools are often more useful to discover bioactive compounds or to understand detailed functions than simply synthesizing candidates^[1-8]. (Fig. 5-1-1)

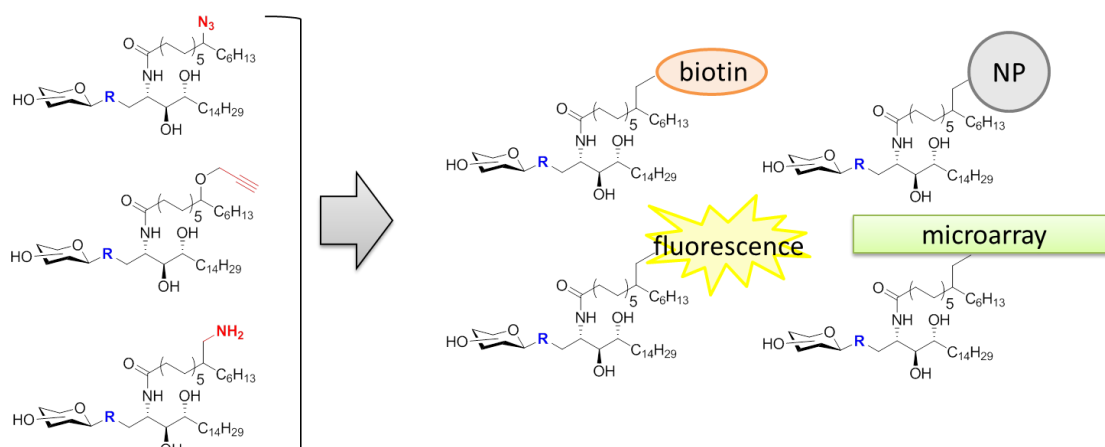


Fig. 5-1-1. Examples of introduction of specific tags and immobilization to research tools

Among many specific reactions, which include glycoblotting reaction of course, I focused on azide-alkyne cyclization^[9, 10] in this chapter. This reaction was reported by R. Huisgen et al^[11], and enables to easily form triazole structure in the presence of Cu catalyst. Additionally, azide-attached compound may keep the original character because of the smallness of azide group compared with other tags, e.g. fluorescent group or biotin. Beltozzi et al. achieved to detect sialic acid, which had been converted from azide-attached N-acetylmannosamine through biosynthesis, at the terminal of glycoprotein^[12- 15]. That is one of the highlights to use azide compound. I tried to apply this approach to analysis of GSLs metabolism^[16, 17]. Understanding the metabolism should be one way to overcome diseases and infections that is why many studies have

been reported. One of the main strategies of glycosphingolipidomics is to use glycolipids labeled by fluorescence^[18, 19]. Fluorescent compounds are incubated with cell culture and detected by combination of HPLC and MS after extraction step. In spite of very high sensitivity, there is a crucial problem that glycosidic linkage between ceramide and sugar at reducing end opt to be cleaved^[20-23]. That caused the ratio of nonglycosylated ceramide to be big. At that point, my neoglycolipids has possibility to show resistance for hydrolysis as in chapter 4. If the resistance can be indicated, usability of neoglycolipids will increase greatly.

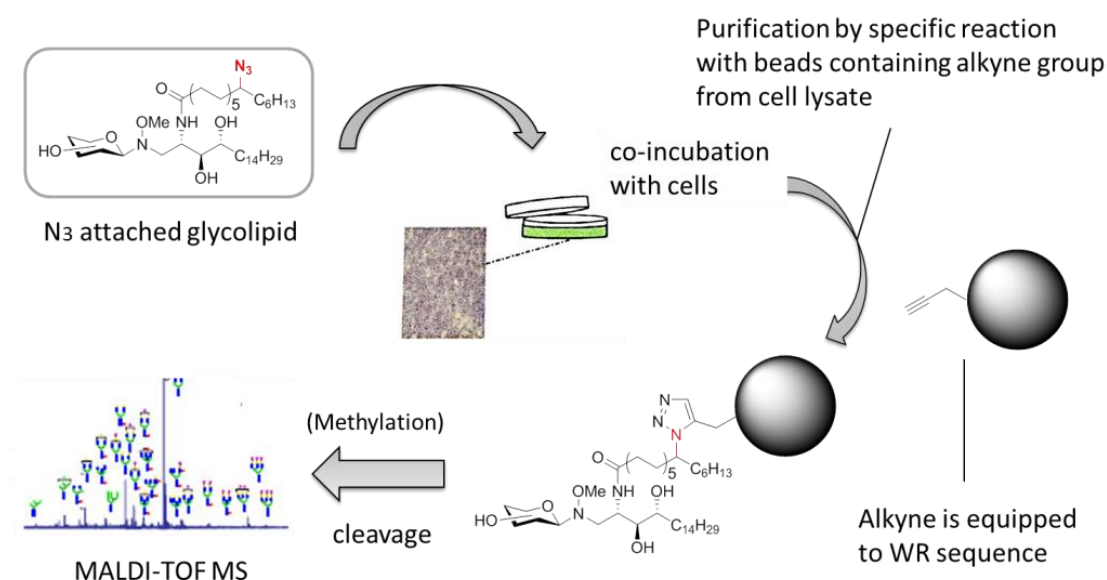


Fig. 5-1-2. Abstract of strategy in this chapter

In chapter 2, several ceramide derivatives were synthesized and the acyl group, which means stearyl, was attached in the latter step for every derivative. That was because acyl group should be exchanged easily. That has great advantage to introduce azide group to ceramide derivatives briefly.

Abstract of this chapter is shown in Fig.5-1-2. Azide group was introduced to neoglycolipids as a form of acyl chain and the azide-attached compounds were incubated with cell. A functional bead displaying alkyne group was used to extract only neoglycolipids from cell culture and MALDI-TOF MS after cleavage would enable to analyze metabolized glycolipids simultaneously.

5-2 Results & Discussions

5-2-1 Synthesis of azide derivatives and alkyne displayed beads

To conduct analysis of GSLs metabolism with azide-alkyne cyclization, I designed several azide-attached compounds and an alkyne-displayed functional bead. (Fig. 5-2-1) As glycolipids, I focused on LacCer, which is a starting glycolipid to be differentiated, and designed 3 compounds. One of them was natural type which had O-glycosidic linkage between ceramide and glucose residue. The others were neoglycolipids containing oxime linkage and N(-OMe) linkage, respectively. Additionally, simple methyl glucoside was designed as a control. The functional bead was designed general resin for Fmoc solid phase peptide synthesis (SPPS), Rink-Amide-ChemMatrix, and alkyne group was introduced to amine group at the terminal of WRGG sequence. The sequence has been used to amplify sensitivity of MS analysis.

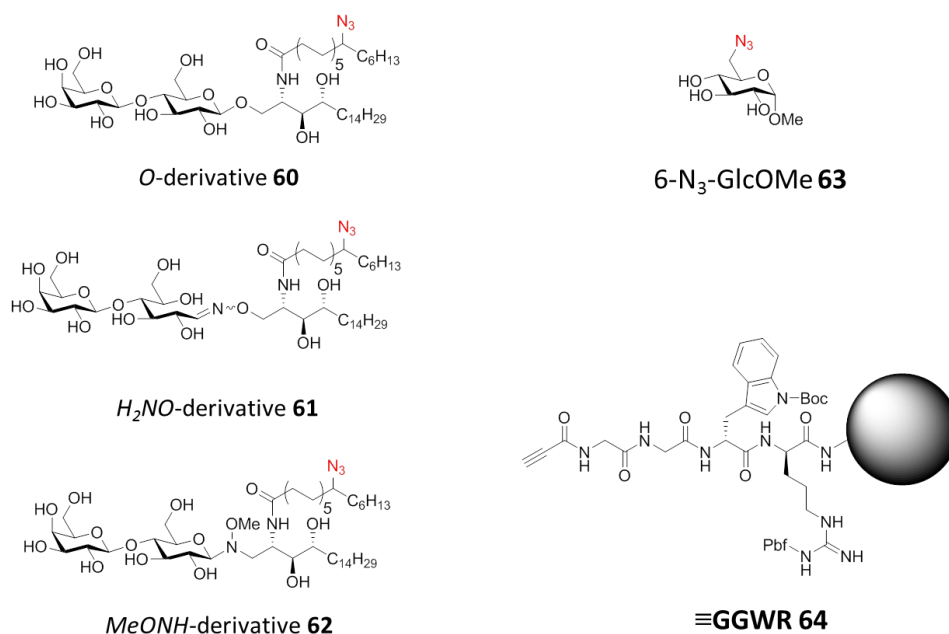
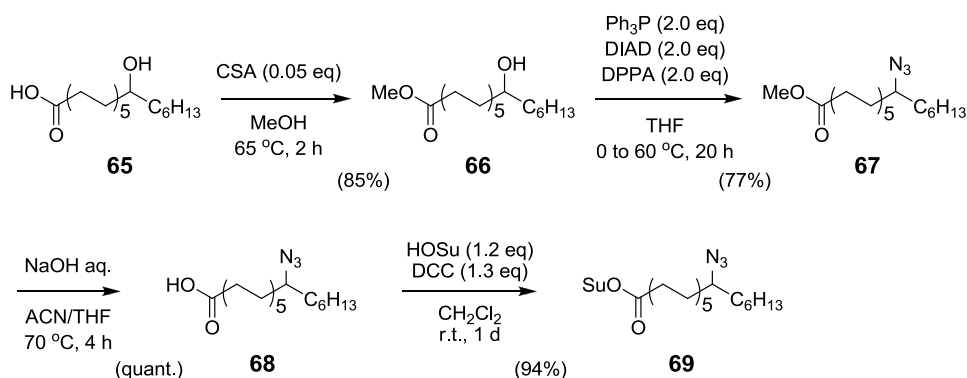


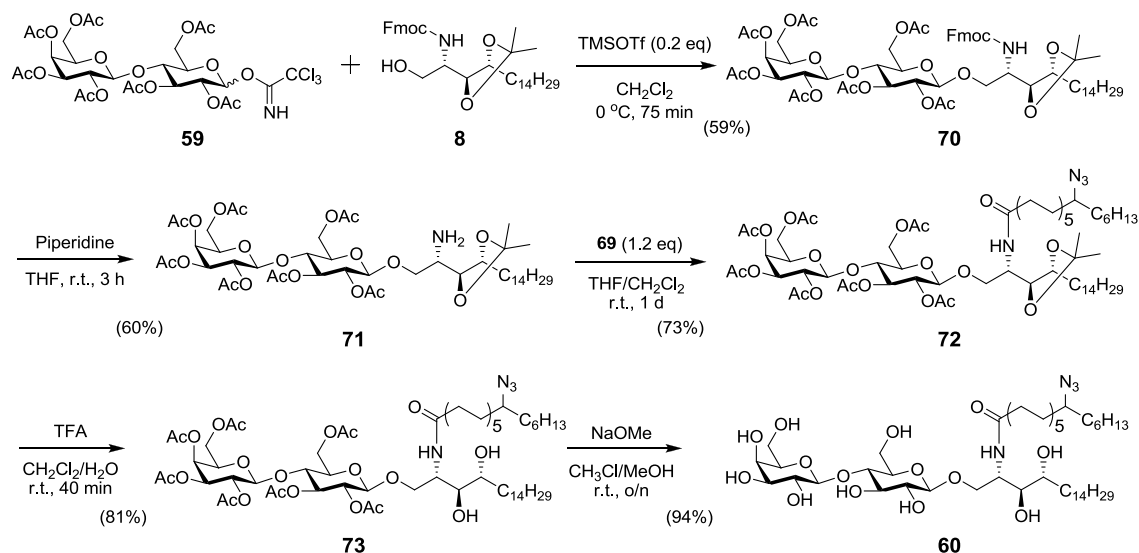
Fig. 5-2-1. Target compounds

At first, I synthesized activated fatty ester containing azide group (Scheme 5-2-1). As the starting material, racemic 12-hydroxy stearic acid **65** was used and esterified in acidic condition. Mitsunobu reaction enabled azide group to be substituted with hydroxyl group in 1 step using DPPA. After hydrolysis reaction in basic condition, *N*-hydroxysuccinimide was condensed with carboxylic acid using DCC to yield desired activated fatty ester **69**.



Scheme 5-2-1. Synthesis of succinimidyl 12-N₃-octadecanoate **69**

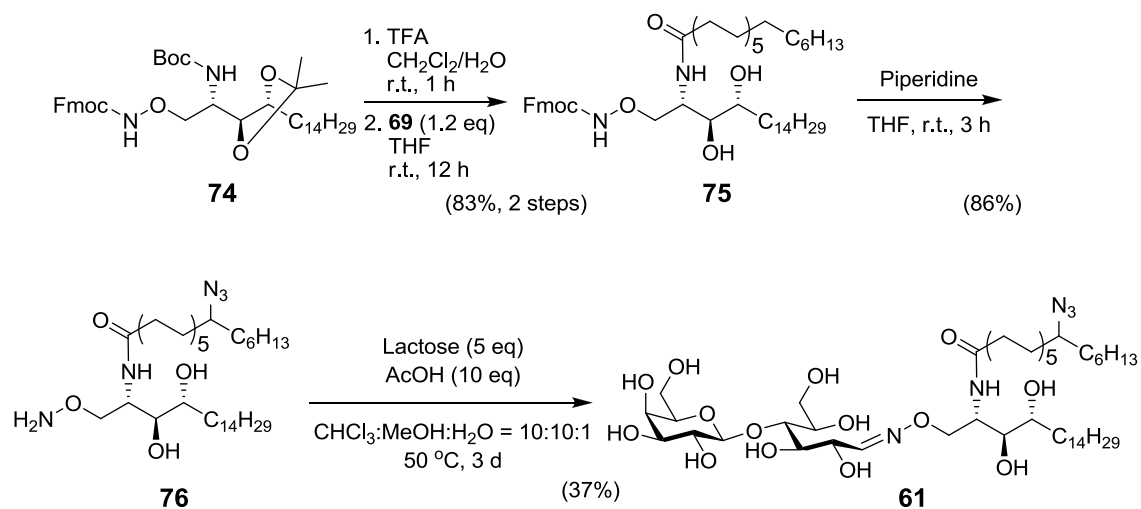
Next, natural type of LacCer containing *O*-glycoside was synthesized (Scheme 5-2-2). Using lactosyl imide **59** in chapter 4 as donor and monohydroxylipid **8** in chapter 2 as acceptor, glycosylation was performed and gave β -product **70**. After removal of Fmoc group by piperidine treatment, compound **69** prepared above was used to attach fatty acyl chain containing azide group to amide group of sphingosine. Isopropiliden group was deprotected by TFA treatment and all acetyl groups were removed under basic condition to afford desired LacCer derivative **60**.



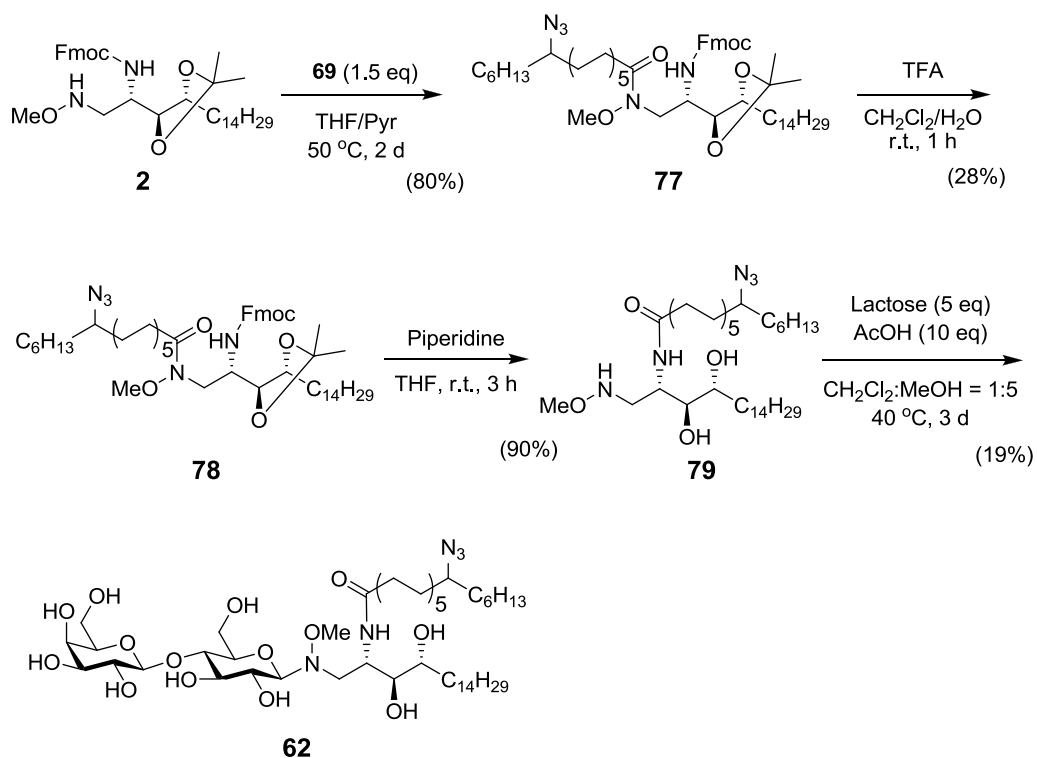
Scheme 5-2-2. Synthesis *O*-LacCer derivative **60**

LacCer derivatives using glycoblotting reaction were synthesized as same way in chapter 2 except activated fatty ester (Scheme 5-2-3, 5-2-4). The results indicated that acyl group of ceramide derivatives were exchanged easily as expected.

Although the racemic structure, 12- N_3 -octadecanoyl group, gave a pair of diastereomers after conjugation with sphingosine, the difference was not detected on TLC analysis and NMR spectra in my synthesis.



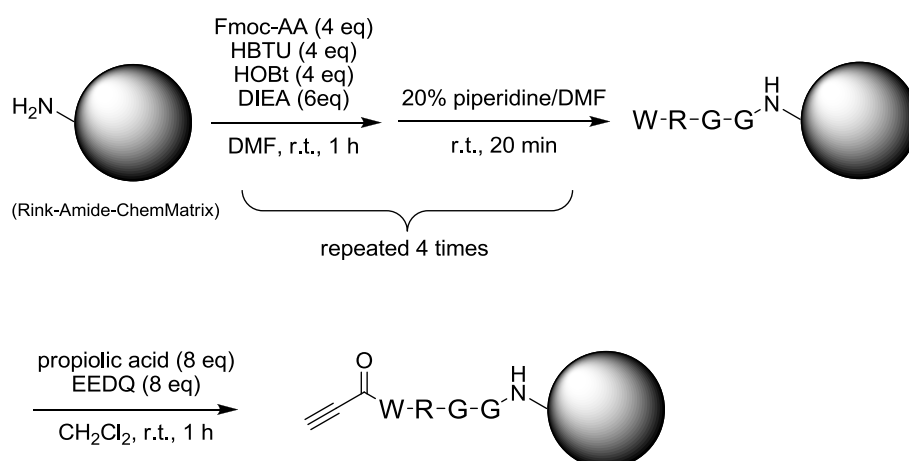
Scheme 5-2-3. Synthesis *H*₂*NO*-LacCer derivative **61**



Scheme 5-2-4. Synthesis *O*-LacCer derivative **62**

Alkyne-displayed bead was synthesized as below (Scheme 5-2-5).

Rink-Amide-ChemMatrix resin was selected and amino acid residues were elongated under general condition of SPPS. At the last step, propiolic acid was condensed with the terminal amino group.



Scheme 5-2-5. Synthesis of alkyne-displayed resin

After synthesis, aliquot of resin was treated by cleavage reagent (TFA : TIS : H₂O = 95 : 2.5 : 2.5) for detection of MALDI-TOF MS. As shown in Fig. 5-2-2, all reactions proceeded completely although the minor peak derived from Boc group attached to Trp residue was also detected.

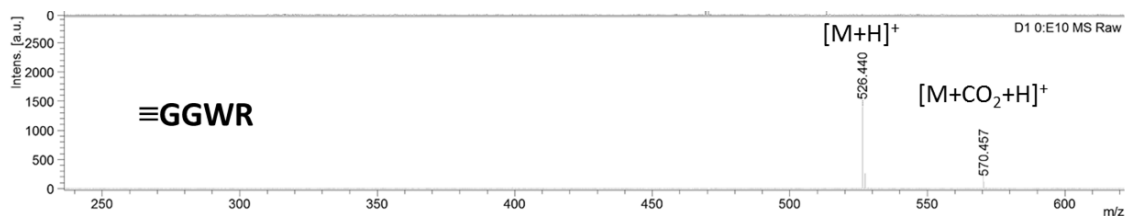


Fig. 5-2-2. MALDI-TOF MS spectrum of cleaved crude compound from synthesized resin (DHB, positive mode)

5-2-2 Click reaction on alkyne-displayed bead

Using synthesized compounds, I tried click reaction on alkyne attached resin. First, the reaction was performed in aqueous solution with detergent but LacCer derivatives were not reacted. Because glucose derivative **63** could form triazole structure in contrast, it was obvious the solubility of glycolipids was problematic (data not shown). Then, solvent system was changed to CHCl₃/MeOH/H₂O system and reaction condition was optimized, which enabled LacCer derivatives to react the alkyne group on resin. However, quantitatively was not enough to discuss yet (Fig. 5-2-3). Further optimization of reaction condition or selection resin was required.

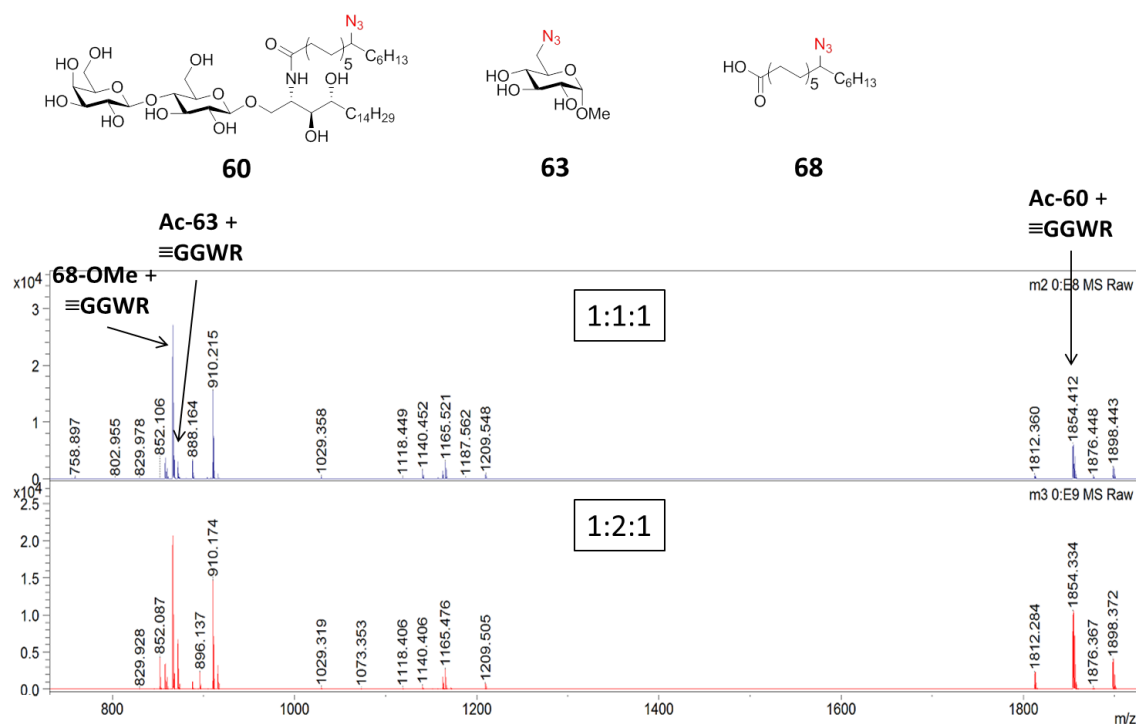


Fig. 5-2-3. MALDI-TOF MS spectra of cleaved crude product from resin after click reaction; Ratios shown in the middle means ratio of concentration of compound **60**, **63**, and **68**

Furthermore, minor peak which was 44 Da bigger than product mass was also detected in a series of experiments (Fig. 5-2-4). Although the peak diminished later, many de-acetylated compounds were detected. This result compelled me to abbreviate acetylation step before cleavage in next experiment.

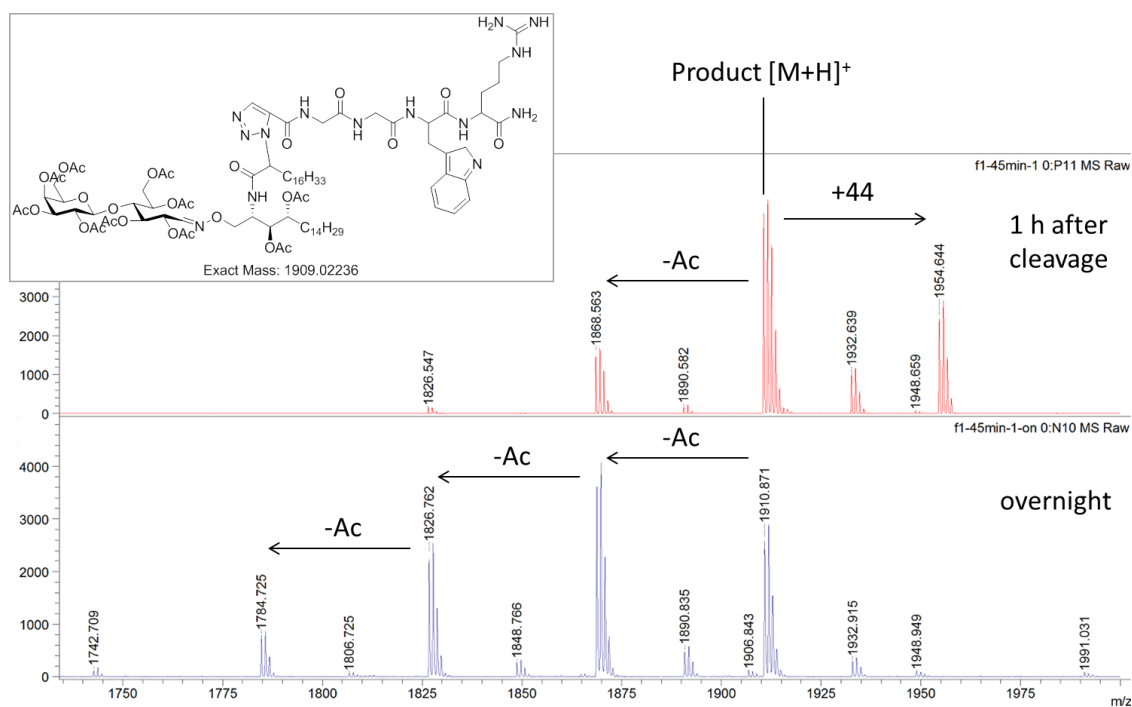


Fig. 5-2-4. Comparison of MALDI-TOF MS spectra after cleavage step
(DHB, positive)

5-2-3 Analysis of GSLs metabolism

Although quantitative analysis had not been developed, I tried to perform glycolipidomics. Synthesized compound **60**, **61**, and **62** were respectively co-incubated with PC-3 cells and compound **63** as a control. After incubation, culture medium was lyophilized. Then, the powder was suspended in CH₂Cl₂/ MeOH solution. Click reaction was performed using clear supernatant liquid under same condition as before except concentration of sodium ascorbate and CuSO₄. After methylation step^[24], MALDI-TOF MS measurement was conducted and the results were shown in Fig. 5-2-5

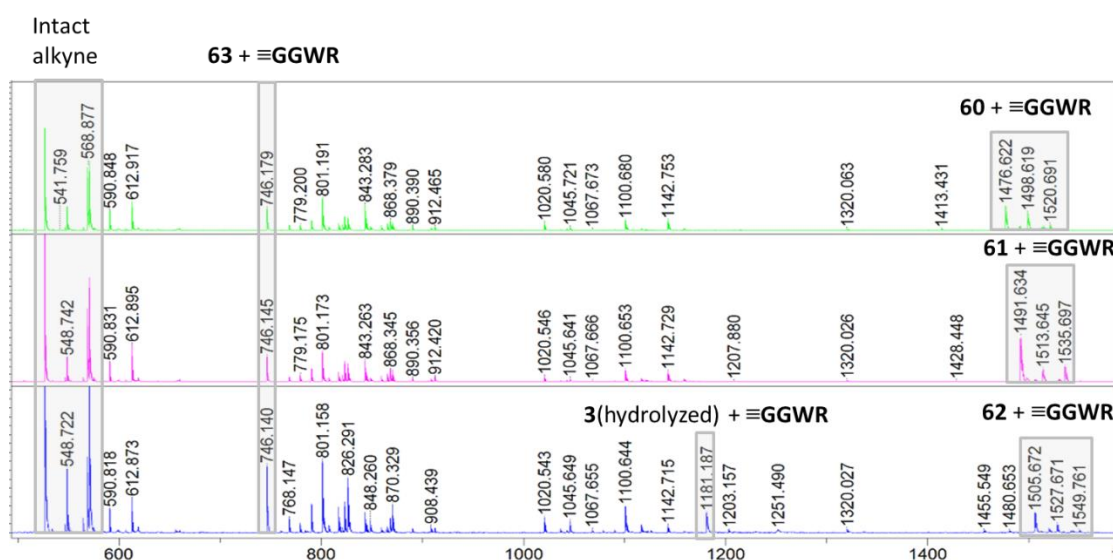


Fig. 5-2-5. MALDI-TOF MS of extracted products (DHB, positive)

Unfortunately, any metabolized product was not detected in each case. To make matters worse, unknown peaks which were certainly derived from resin caused analysis to be difficult and compound **62** was partly hydrolyzed by acid reagent. Those problems will be overcome to choose resin carefully and optimize cleavage condition.

5-3 Conclusion

In this chapter, I tried to conduct further functionalization of neoglycolipids to develop a novel utility value. Azide group was selected as a specific tag and introduced neoglycolipids easily by using fatty acyl group containing azide group. I intended to analyze GSLs metabolism by easy extraction of neoglycolipids through a click reaction. Although metabolized products were not detected in my experiments unfortunately, the strategy of easy extraction itself was indicated to be useful. Metabolized products will be observed to optimize resin, reaction condition for cleavage, and incubation condition.

5-4 Experimental Section

5-4-1 Synthesis of azide derivatives and alkyne displayed beads

Methyl 12-azide-octadecanoate **67**

Into a THF solution (80 mL) containing compound **66** (2.57 g, 8.17 mmol), Ph_3P (4.28 g, 16.3 mmol), and DPPA (3.51 mL, 16.3 mmol), DIAD (3.21 mL, 16.3 mmol) was dropped at 0 degree. After 20 min, the reaction mixture was stirred at 60 degree for 20 hours. Then, the solution was evaporated under reduced pressure. Purification by flash column chromatography on silica gel (Hexane-EtOAc, 40:1) yielded **67** (2.14 g, 77%) as a colorless oil.;

^1H NMR (500 MHz, $\text{CHLOROFORM-}d$) δ ppm 3.65 (s, 3 H; OMe) 3.19 - 3.25 (m, 1 H; N_3CH) 2.29 (t, $J=7.57$ Hz, 2 H; CH_2) 1.65-1.23 (m, 29 H; CH_2) 0.89 (t, $J=6.84$ Hz, 3 H; CH_3)

12-azide-octadecanoic acid 12-HSA- N_3 -OH **68**

4 N NaOH aq. (10 mL) was added to a solution of compound **67** (7.63 g, 22.5 mmol) in THF (20 mL) and ACN (20 mL). The reaction mixture was stirred at 70 degree for 4 hours and neutralized with 1 N HCl aq. CHCl_3 was used 2 times to extract desired compound from aqueous solution. The organic phase was dried over MgSO_4 and concentrated under reduced pressure. Purification by flash column chromatography on silica gel (hexane-EtOAc, 10:1~3:1) gave compound **68** as a colorless oil quantitatively.;

^1H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 3.19 - 3.25 (m, 1 H; N_3CH) 2.35 (t, $J=7.57$ Hz, 2 H; CH_2) 1.67-1.24 (m, 29 H; CH_2) 0.89 (t, $J=6.84$ Hz, 3 H; CH_3)

Succinimidyl 12-azide-octadecanoate 12-HSA- N_3 -OSu **69**

Compound **68** (7.39 g, 22.7 mmol), DCC (6.09 g, 29.5 mmol), and *N*-hydroxysuccinimide (3.14 g, 27.2 mmol) were dissolved in CH_2Cl_2 (100 mL). The reaction mixture was stirred at room temperature for 1 day and diluted with Et_2O . Precipitate was removed by filtration with celite and filtrate was concentrated under reduced pressure. Purification by flash column chromatography on silica gel (hexane-EtOAc, 10:1~3:1) gave compound **69** (9.00 g, 94%) as a white amorphous solid.

^1H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 3.18 - 3.26 (m, 1 H; N_3CH) 2.83 (br. s., 4 H; NCOCH_2) 2.60 (t, $J=7.45$ Hz, 2 H; CH_2) 1.79-1.23 (m, 29 H; CH_2) 0.89 (t, $J=6.59$ Hz, 3 H; CH_3)

AcLac-O-PS-Fmoc-Isop **70**

To a solution of donor **59** (795 mg, 1.02 mmol) and acceptor **8** (393 mg, 679 μmol) in CH_2Cl_2 (10 mL), TMSOTf (25 μL , 136 μmol) was added dropwise at 0 degree. The reaction mixture was stirred at 0 degree for 75 minutes and quenched by sat. NaHCO_3 aq.. CHCl_3 was used 2 times to extract desired compound from aqueous solution. The organic phase was dried over MgSO_4 and concentrated under reduced pressure. Purification by flash column chromatography on silica gel (hexane-EtOAc, 2:1~1.7:1) gave compound **70** (478 mg, 59%) as a white solid

¹H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 7.79 (d, $J=7.32$ Hz, 2 H; Fmoc-Ar) 7.56 - 7.62 (m, 2 H; Fmoc-Ar) 7.41 (t, $J=7.32$ Hz, 2 H; Fmoc-Ar) 7.33 (t, $J=7.32$ Hz, 2 H; Fmoc-Ar) 5.35 (d, $J=3.05$ Hz, 1 H; Gal-*H*-4) 5.20 (t, $J=9.45$ Hz, 1 H; Glc-*H*-3) 5.12 (dd, $J=9.76, 8.23$ Hz, 1 H; Gal-*H*-2) 4.97 (dd, $J=10.37, 3.35$ Hz, 1 H; Gal-*H*-3) 4.90 (t, $J=8.23$ Hz, 1 H; Glc-*H*-2) 4.86 (d, $J=9.15$ Hz, 1 H; NH) 4.48 (d, $J=8.23$ Hz, 3 H; Glc-*H*-6a, Gal-*H*-1, Fmoc-CH₂a) 4.40 (m, 2 H; Glc-*H*-1, Fmoc-CH₂b) 4.18 - 4.24 (m, 1 H; Fmoc-CH) 4.05 - 4.17 (m, 5 H; Glc-*H*-6b, Gal-*H*-6ab, PS-*H*-3, PS-*H*-4) 3.97 - 4.04 (m, 1 H; PS-*H*-1a) 3.87 (t, $J=6.71$ Hz, 2 H; Gal-*H*-5, PS-*H*-2) 3.78 (t, $J=9.45$ Hz, 1 H; Glc-*H*-4) 3.53 - 3.61 (m, 2 H; Glc-*H*-5, PS-*H*-1b) 2.16-1.92 (s, 21 H; Ac) 1.59-1.15 (m, 26 H) 1.41, 1.32 (s, 3 H; CCH₃) 0.89 (t, $J=6.86$ Hz, 3 H; CH₃)

AcLac-O-PS-NH₂-Isop **71**

To a THF solution (5 mL) of compound **70** (470 mg, 392 μ mol), piperidine (1 mL) was added and the reaction mixture was stirred at room temperature for 3 hours. The solution was concentrated under reduced pressure and purification by flash column chromatography on silica gel (hexane-EtOAc, 1:1-1:2, EtOAc) gave compound **71** (230 mg, 60%) as a white solid.

¹H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 5.35 (d, $J=3.39$ Hz, 1 H; Gal-*H*-4) 5.20 (t, $J=9.33$ Hz, 1 H; Glc-*H*-3) 5.11 (dd, $J=10.18, 7.92$ Hz, 1 H Gal-*H*-2) 4.90 - 4.98 (m, 2 H; Glc-*H*-2, Gal-*H*-3) 4.47 - 4.53 (m, 3 H; Glc-*H*-1, Glc-*H*-6a, Gal-*H*-1) 4.05 - 4.17 (m, 4 H; Glc-*H*-6b, Gal-*H*-6ab, PS-*H*-4) 3.87 (t, $J=7.07$ Hz, 1 H; Gal-*H*-5) 3.76 - 3.83 (m, 3 H; Glc-*H*-4, PS-*H*-1a, PS-*H*-3) 3.67 - 3.72 (m, 1 H; PS-*H*-1) 3.58 - 3.63 (m, 1 H; Glc-*H*-5) 2.96 - 3.02 (m, 1 H; PS-*H*-2) 2.15-1.97 (s, 21 H, Ac) 1.60-1.22 (m, 26 H; CH₂) 1.40, 1.30 (s, 6 H; CCH₃) 0.88 (t, $J=6.93$ Hz, 3 H; CH₃)

AcLac-O-PS-HSAN₃-Isop **72**

Compound **71** (230 mg, 236 μ mol) and succinimidyl ester **69** (199 mg, 471 μ mol) were dissolved in CH₂Cl₂ (10 mL) and the reaction mixture was stirred at room temperature for 2 days. The solution was concentrated under reduced pressure and purification by flash column chromatography on silica gel (hexane-EtOAc, 2:1-3:2-1:1) gave compound **72** (230 mg, 73%) as a white solid

¹H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 5.55 (d, *J*=9.04 Hz, 1 H; *NH*) 5.35 (d, *J*=3.42 Hz, 1 H; Gal-*H*-4) 5.19 (t, *J*=9.28 Hz, 1 H; Glc-*H*-3) 5.08 - 5.13 (m, 1 H; Gal-*H*-2) 4.96 (dd, *J*=10.01, 3.18 Hz, 1 H; Gal-*H*-3) 4.88 (t, *J*=8.30 Hz, 1 H; Glc-*H*-2) 4.45 - 4.51 (m, 3 H; Glc-*H*-1, Glc-*H*-6a, Gal-*H*-1) 4.04 - 4.19 (m, 6 H; Glc-*H*-6b, Gal-*H*-6ab, PS-*H*-2, PS-*H*-3, PS-*H*-4) 4.00 (dd, *J*=10.01, 3.91 Hz, 1 H; PS-*H*-1a) 3.87 (t, *J*=6.84 Hz, 1 H; Gal-*H*-5) 3.79 (t, *J*=9.53 Hz, 1 H; Glc-*H*-4) 3.57 - 3.66 (m, 2 H; Glc-*H*-5, PS-*H*-1b) 3.19 - 3.26 (m, 1 H; N₃CH) 2.15-1.97 (s, 21 H; Ac) 2.18-1.21 (m, 57 H; CH₂) 1.41, 1.31 (s, 6 H; CCH₃) 0.86 - 0.92 (m, 6 H; CH₃)

F1 (ppm) 101.03 (Glc-C-1, Gal-C-1) 77.97 (PS-C-3) 76.46 (PS-C-4) 75.96 (Glc-C-4) 72.86 (Glc-C-5) 72.67 (Glc-C-3) 72.04 (Glc-C-2) 71.06 (Gal-C-5) 71.00 (Gal-C-3) 69.13 (Gal-C-2) 69.06 (PS-C-1) 66.54 (Gal-C-4) 63.00 (N₃CH) 61.66 (Glc-C-6) 61.15 (Gal-C-6) 47.97 (PS-C-2) 36.68-14.16 (Alkyl chain) 20.99 (Ac)

AcLac-O-PS-HSAN₃ **73**

To a suspension of compound **72** (185 mg, 144 μ mol) in CH₂Cl₂/H₂O (4:1, 500 μ L) was added TFA (8 mL) and the mixture was stirred at room temperature for 40 minutes. The reaction mixture was diluted with EtOAc and washed with Brine, sat. NaHCO₃ aq. (2

times) and brine. The organic phase was dried over MgSO_4 , and concentrated under reduced pressure. Purification of the crude product by flash column chromatography on silica gel (hexane-EtOAc, 3:2~1:1~2:3) yielded **73** (145 mg, 81%) as a white amorphous solid;

^1H NMR (500 MHz, CHCl_3) δ ppm 6.18 (d, $J=8.30$ Hz, 1 H; *NH*) 5.37 (d, $J=2.44$ Hz, 1 H; Gal-*H*-4) 5.22 (t, $J=9.28$ Hz, 1 H; Glc-*H*-3) 5.13 (t, $J=9.28$ Hz, 1 H; Gal-*H*-2) 5.00 (dd, $J=10.50, 2.93$ Hz, 1 H; Gal-*H*-3) 4.88 (t, $J=8.55$ Hz, 1 H; Glc-*H*-2) 4.55 - 4.60 (m, 1 H; Glc-*H*-6a) 4.49 - 4.55 (m, 2 H; Glc-*H*-1, Gal-*H*-1) 4.18 - 4.24 (m, 1 H; PS-*H*-2) 4.08 - 4.18 (m, 4 H; Glc-*H*-6b, Gal-*H*-6ab, PS-*H*-1a) 3.92 (t, $J=6.59$ Hz, 1 H; Gal-*H*-5) 3.77 - 3.84 (m, 2 H; Glc-*H*-4, PS-*H*-1b) 3.63 - 3.69 (m, 1 H; Glc-*H*-5) 3.56 - 3.62 (m, 1 H; PS-*H*-4) 3.50 - 3.56 (m, 1 H; PS-*H*-3) 3.21 - 3.31 (m, 2 H; N_3CH , *OH*) 2.54 (br. s, 1 H; *OH*) 2.22-1.23 (m, 57 H; CH_2) 2.17-1.99 (s, 21 H; *Ac*) 0.87 - 0.94 (m, 6 H; CH_3)

F1 (ppm) 100.88 (Glc-*C*-1, Gal-*C*-1) 76.07 (Glc-*C*-4) 74.98 (PS-*C*-3) 73.01 (Glc-*C*-5) 72.72 (PS-*C*-4) 72.31 (Glc-*C*-3) 71.73 (Glc-*C*-2) 70.86 (Gal-*C*-3, Gal-*C*-5) 69.41 (PS-*C*-1) 69.11 (Gal-*C*-2) 66.72 (Gal-*C*-4) 63.08 (N_3CH) 61.63 (Glc-*C*-6) 60.54 (Gal-*C*-6) 50.29 (PS-*C*-2) 36.75-14.15 (Alkyl chain) 20.69 (*Ac*)

Lac-O-PS-HSAN₃ 60

Compound **73** (145 mg, 117 μmol) and sodium methoxide (3 mg, 56 μmol) were dissolved in MeOH (5 mL) and CHCl_3 (1 mL) and the reaction mixture was stirred at room temperature for 1 day. The solution was concentrated by air-drying and the desired product was precipitated in MeOH. Filtration gave compound **60** (104 mg, 94%) as a white solid.

^1H NMR (500 MHz, pyridine) δ ppm 8.60 (d, $J=8.77$ Hz, 1 H; NH) 7.63 (br. s, 1 H; Glc-OH-2) 7.51 (d, $J=4.39$ Hz, 1 H; Gal-OH-2) 6.91 - 6.97 (m, 1 H; Gal-OH-3) 6.64 - 6.69 (m, 1 H; Gal-OH-6) 6.57 (d, $J=4.39$ Hz, 1 H; Gal-OH-4) 6.54 (d, $J=6.26$ Hz, 1 H; PS-OH-3) 6.44 - 6.50 (m, 1 H; Glc-OH-6) 6.22 (s, 1 H; Glc-OH-3) 5.95 (d, $J=6.89$ Hz, 1 H; PS-OH-4) 5.16 - 5.20 (m, 1 H; PS-H-2) 5.07 (d, $J=7.83$ Hz, 1 H; Gal-H-1) 4.90 (d, $J=7.83$ Hz, 1 H; Glc-H-1) 4.79 (dd, $J=10.34, 5.64$ Hz, 1 H; PS-H-1a) 4.43 - 4.55 (m, 5 H; Glc-H-6ab, Gal-H-2, Gal-H-4, Gal-H-6a) 4.36 - 4.42 (m, 3 H; Gal-H-6b, PS-H-1b, PS-H-3) 4.21 - 4.29 (m, 3 H; Glc-H-3, Glc-H-4, PS-H-4) 4.11 - 4.17 (m, 2 H; Gal-H-3, Gal-H-5) 4.01 - 4.07 (m, 1 H; Glc-H-2) 3.81 - 3.86 (m, 1 H; Glc-H-5) 3.29 - 3.36 (m, 1 H; N_3CH) 2.45-1.18 (m, 57 H; CH_2) 0.84 - 0.90 (m, 6 H; CH_3)

FmocNHO-PS-HSAN₃ 75

To a suspension of compound **25** (1.53 g, 2.2 mmol) in $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$ (4:1, 2.5 mL), TFA (20 mL) was added and the mixture was stirred at room temperature for 1 hour. The reaction mixture was diluted with CHCl_3 and washed with sat. NaHCO_3 aq. 2 times. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was directly used for succeeding reaction without further purification. The product and compound **69** (1.12 g, 2.64 mmol) were suspended in THF (40 mL) and the mixture was stirred at room temperature for 12 hours. The reaction solution was evaporated under reduced pressure completely. Purification of the crude product by flash column chromatography on silica gel (Toluene-EtOAc, 6:1-2:1) yielded **75** (1.57 g, 83%) as a white amorphous solid;

^1H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 8.18 - 8.26 (m, 1 H; *NHO*) 7.76 (m, 2 H; *Fmoc-Ar*) 7.56 (m, 2 H; *Fmoc-Ar*) 7.41 (m, 2 H; *Fmoc-Ar*) 7.28 - 7.36 (m, 2 H; *Fmoc-Ar*) 6.55 - 6.72 (m, 1 H; *NH*) 4.43 - 4.52 (m, 2 H) 4.21 - 4.30 (m, 2 H) 4.14 - 4.20 (m, 1 H) 4.05 - 4.11 (m, 1 H) 3.65 - 3.70 (m, 1 H) 3.58 - 3.65 (m, 1 H) 3.46 - 3.52 (m, 1 H) 3.17 - 3.26 (m, 1 H; N_3CH) 2.25-1.18 (m, 57 H) 0.84 - 0.93 (m, 6 H)

$\text{H}_2\text{NO-PS-HSAN}_3$ 76

Compound **75** (1.23 g, 1.43 mmol) was dissolved in THF (10 mL) and piperidine (2 mL) was added to the solution at room temperature. After 6 h, solvent was removed by evaporation and purification of the crude product by flash column chromatography on silica gel (Toluene-EtOAc, 2:1-1:2, EtOAc) yielded **76** (783 mg, 86%) as a white amorphous solid;

^1H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 6.22 (d, $J=8.30$ Hz, 1 H; *NH*) 4.28 - 4.33 (m, 1 H) 3.97 - 4.01 (m, 1 H) 3.85 - 3.90 (m, 1 H) 3.59 - 3.64 (m, 1 H) 3.54 - 3.58 (m, 1 H) 3.19 - 3.25 (m, 1 H; N_3CH) 2.23-1.21 (m, 57 H) 0.86 - 0.91 (m, 6 H)

Lac=NO-PS-12- N_3 -octadecane 61

Compound **76** (60 mg, 100 μmol) and lactose (180 mg, 500 μmol) was suspended in $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}/\text{AcOH}$ (25:25:8:1, 5 mL). The reaction mixture was stirred vigorously at 50 degree. After 1 day, the solution was transferred into flask. Solvent was removed to about half volume and silica gel was added to a reaction solution and the mixture was evaporated completely. Purification of the crude product by flash column chromatography on silica gel (CHCl_3 -MeOH, 10:1-4:1) afforded product **61** (35 mg, 37%) as a white solid.

MeON-HSAN₃-PS-Fmoc-Isop **77**

Compound **2** (1.41 g, 2.32 mmol) and compound **69** (1.47 g, 3.48 mmol) were dissolved in CH₂Cl₂ (30 mL) and the reaction mixture was stirred at room temperature for 3 days. The solution was concentrated under reduced pressure and purification of the crude product by flash column chromatography on silica gel (Hexane-EtOAc, 50:1-9:1, EtOAc) yielded **77** (1.70 g, 80%) as a white solid;

¹H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 7.74 (d, *J*=7.57 Hz, 2 H; Fmoc-Ar) 7.58 (t, *J*=6.11 Hz, 2 H; Fmoc-Ar) 7.38 (t, *J*=7.45 Hz, 2 H; Fmoc-Ar) 7.30 (t, *J*=7.57 Hz, 2 H; Fmoc-Ar) 5.26 (d, *J*=7.33 Hz, 1 H; NH) 4.25 - 4.32 (m, 2 H; Fmoc-CH₂) 4.10 - 4.22 (m, 5 H; *H*-1a, *H*-2, *H*-3, *H*-4, Fmoc-CH) 3.68 (s, 3 H; OMe) 3.55 - 3.64 (m, 1 H; *H*-1b) 3.20 (m, 1 H; N₃CH) 2.80 (br. s, 1 H; OH) 2.48-1.15 (m, 57 H; CH₂) 1.48, 1.35 (s, 6 H; CCH₃) 0.86 - 0.91 (m, 6 H; CH₃)

MeONH-PS -HSAN₃ **79**

Compound **78** (477 mg, 544 μmol) was dissolved in THF (6 mL) and piperidine (1.5 mL) was added to the solution at room temperature. After 3 h, solvent was removed by air-drying completely and precipitation was performed by MeOH. Precipitated white solid was separated by filtration and purification of the filtercake by flash column chromatography on silica gel (Toluene-EtOAc, 2:1-1:2, EtOAc) yielded **79** (322 mg, 90%) as a white amorphous solid;

¹H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 6.23 (d, *J*=8.06 Hz, 1 H; NH) 4.18 - 4.26 (m, 1 H; *H*-2) 3.64 - 3.79 (m, 1 H; OH) 3.54 - 3.63 (m, 5 H; *H*-3, *H*-4, OMe) 3.18 - 3.29 (m, 3 H; *H*-1ab, N₃CH) 2.24-1.19 (m, 57 H; CH₂) 0.85 - 0.93 (m, 6 H; CH₃)

Lac-MeON-PS-HAS

Compound **79** (60 mg, 100 μ mol) and lactose (180 mg, 500 μ mol) was suspended in $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}/\text{AcOH}$ (25:25:8:1, 5 mL). The reaction mixture was stirred vigorously at 50 degree. After 1 day, the solution was transferred into flask. Solvent was removed to about half volume and silica gel was added to a reaction solution and the mixture was evaporated completely. Purification of the crude product by flash column chromatography on silica gel (CHCl_3 -MeOH, 10:1-4:1) afforded product **62** (19 mg, 19%) as a white solid.

6-N₃-Glc-OMe 63

Into a THF solution (20 mL) containing compound **6-HO-AcGlc-OMe** (640 mg, 2.00 mmol), Ph_3P (629 mg, 2.40 mmol), and DPPA (517 μ L, 16.3 mmol), DIAD (473 μ L, 2.40 mmol) was dropped at 0 degree. After 20 minutes, the reaction mixture was stirred at room temperature for 32 hours. Then, the solution was evaporated under reduced pressure. Purification by flash column chromatography on silica gel (Hexane-EtOAc, 5:1-2:1) yielded azide compound with some mixtures but the product was used for next reaction without further purification. The product and sodium methoxide were dissolved in MeOH and the reaction mixture was stirred at room temperature for 2 hours. DowX was used for neutralization and removed by filtration. The filtrate was concentrated under reduced pressure and purification of the crude product by flash column chromatography on silica gel (CHCl_3 -MeOH, 30:1-10:1, EtOAc) yielded **63** (310 mg, 71%, 2 steps) as a white amorphous solid

^1H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 4.79 (d, $J=2.69$ Hz, 1 H) 4.53 (br. s, 1 H) 3.99 (br. s, 1 H) 3.68 - 3.76 (m, 2 H) 3.51 - 3.59 (m, 3 H) 3.41 - 3.50 (m, 5 H)

HCC-WRGG-Resine 64

Rink-Amide-ChemMatrix (100 mg, 52 μmol) resin was swelled in filtertube before reaction and washed with DMF. Into the tube, a cocktail of reagents in DMF (574 μL) containing HOBt (208 μmol), HBTU (208 μmol), DIEA (312 μmol), and Fmoc-AA residue (Fmoc-Gly-OH, Fmoc-Trp(Boc)-OH, and Fmoc-Arg(Pbf)-OH, respectively) was added and the reaction mixture was shaken at room temperature for 1 hour. After washing with DMF, 20% piperidine solution in DMF was added and the solution was shaken at room temperature for 20 minutes. These steps were repeated 4 times and then EEDQ (816 μmol) and propiolic acid (816 μmol) in CH_2Cl_2 were added and the mixture was shaken at room temperature for 1 hour. After reaction completed, the resin was washed with DMF and CH_2Cl_2 and stocked in CH_2Cl_2 at room temperature. Characterization of the product was confirmed by MALDI-TOF MS measurement by using aliquot of the resin which was treated by cleavage solution composed of TFA : H_2O : TIS= 95 : 2.5 : 2.5.

5-4-2 Azide-alkyne reaction

In filter tube, the prepared resin (10 mg, 5.2 μmol) was swelled in CH_2Cl_2 before reaction. After CH_2Cl_2 was flushed, reaction solution ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$, 1:3:1, 400 μL) containing compound **60** (280 nmol), compound **63** (280 nmol), compound **68** (280 nmol), sodium ascorbate (200 nmol), Copper sulfate pentahydrate (67 nmol), and TBTA (100 nmol). The mixture was shaken at 50 degree for 2 hours and the resin was washed

with MeOH, CH₂Cl₂/MeOH (1:1), and CH₂Cl₂. Then, Ac₂O/Pyr solution was added and shaken at 40 degree for 1.5 hours. After washing with CH₂Cl₂, CH₂Cl₂ solution of TMS-diazemethane was added and the reaction mixture was shaken at room temperature for 24 hours. After washing with CH₂Cl₂, Characterization of the product was confirmed by MALDI-TOF MS measurement by using aliquot of the resin which was treated by cleavage solution composed of TFA : H₂O: TIS= 95 : 2.5 : 2.5.

5-4-3 Extraction of azide derivatives from culture medium

Cell Culture

PC-3 cells were grown in RPMI-1640 (Sigma) supplemented with 10% (v/v) heat-inactivated fetal bovine serum at 37°C

Co-incubation of prepared compounds with the Cells

To the medium, DMSO solution of compound 60, 61, or 62 were added respectively with compound 63 (Final conc. : 250 µM). Cells were incubated on 60-mm culture dishes for 48 h and scraped with trypsin-EDTA.

Extraction by click reaction

In filter tube, the prepared resin (10 mg, 5.2 µmol) was swelled in CH₂Cl₂ before reaction. After CH₂Cl₂ was flushed, reaction solution (CHCl₃/MeOH/H₂O, 1:3:1, 400 µL) containing 20% of total lyophilized product, sodium ascorbate (1000 nmol), Copper sulfate pentahydrate (1000 nmol), and TBTA (100 nmol). The mixture was shaken at 50 degree for 2 hours and the resin was washed with MeOH, CH₂Cl₂/MeOH (1:1), and CH₂Cl₂. Then, Ac₂O/Pyr solution was added and shaken at 40 degree for 1.5 hours.

After washing with CH_2Cl_2 , CH_2Cl_2 solution of TMS-diazemethane was added and the reaction mixture was shaken at room temperature for 24 hours. After washing with CH_2Cl_2 , Characterization of the product was confirmed by MALDI-TOF MS measurement by using aliquot of the resin which was treated by cleavage solution composed of TFA : H_2O : TIS= 95 : 2.5 : 2.5.

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Chapter 6

Concluding Remarks

Glycosylation is one of the most important posttranslational modifications and performed by template-independent manner providing diversity structure and function into glycoconjugates such as glycoprotein or glycolipid. To elucidate the functions of glycoconjugates deeply, preparation of natural/mimetic compound is important task but the innovative method to synthesize glycosphingolipids has not been developed up to date. In this study, I demonstrated a novel synthetic strategy based on glycoblotting to construct neoglycolipid library.

In chapter 2, we synthesized 6 ceramide derivatives which were expected to imitate natural glycosphingolipids after glycosylation. In the synthesis of methoxyamino derivative **1**, we have developed an efficient protocol using a specific $N\beta \rightarrow N\alpha$ acyl migration to allow for the selective modification at $N\alpha$ position of the methoxyamino-functionalized phytosphingosine derivatives. This discovery will contribute to construct synthetic strategy of methoxyamino derivative.

In chapter 3, we employ glycoblotting reaction to construct neoglycolipid library. In case of methoxyamino derivative, unfortunate side reaction was occurred but optimization of reaction condition enabled to obtain β -product as sole product and construct library. Other ceramide derivatives were able to be conjugated with free sugars, which verified the usability of glycoblotting-based method.

In chapter 4, we evaluate the function of neoglycoside to imitate natural glycoceramide using rEGCase II. This enzyme could cleave *O*-glycosidic linkage but not non-natural *N*-glycoside bond. It showed that neoglycolipid was not a substrate of the enzyme. However, when the two compounds, *O*-LacCer and *N*-LacCer, were mixed, the velocity of hydrolysis was apparently reduced compared with only *O*-LacCer. It was significantly important because it indicated that *N*-LacCer could be recognized by the

enzyme like natural glycosphingolipid. This result enhanced the effectiveness of glycoblotting method for preparation of glycolipids.

In chapter 5, azide group could be attached to functional ceramide derivatives. After co-incubation with cell, ceramide derivatives were easily recovered by specific azide-alkyne cyclization.

This work must accelerate glycolipids/neoglycolipids study.

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Junya Ishida