Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 4013–4022

BIOORGANIC & MEDICINAL CHEMISTRY

Facile Synthesis of Stable Lipid Analogues Possessing a Range of Alkyl Groups: Application to Artificial Glycolipids

Yasuo Azefu,[a] Hitoshi Tamiaki,[a,*] Reiko Sato[b] and Kazunori Toma[b]

[a]*Department of Bioscience and Biotechnology, Faculty of Science and Engineering, Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan*

[b]*The Noguchi Institute, Itabashi, Tokyo 173-0003, Japan*

Received 6 June 2002; accepted 3 July 2002

Abstract—Efficient preparation of lipid analogues is described in which various long alkoxy chains and 2-hydroxyethyl group were covalently linked with benzoic acid derivatives. An α-mannopyranosyl group was stereoselectively introduced by the conventional imidate method into the terminal hydroxy group without any alternation of other moieties in a molecule. The resulting new glycoconjugates acted as models of natural glycolipids for protein-carbohydrate interactions.

Introduction

Carbohydrates exist on a cell surface as a part of the glycoconjugates, such as glycolipids and glycoproteins. Numerous cellular recognition processes are triggered by specific interactions of carbohydrates with proteins including lectins. Such carbohydrates play important roles in many biological communications including fertilization, cell signaling, pathogen identification, and inflammatory response.[1,2] The great affinity and specificity are ascribable to clustering of binding events into multivalent arrays, while the attractive force at the constitutive monovalent interactions is relatively weak.[3,4] Both molecular structures of hydrophilic and hydrophobic moieties affect the clustering of the glycoconjugates in a cell membrane. To elucidate the biological events at the molecular level, many types of structurally defined glycoconjugates are necessary and should be systematically investigated. The synthetic glycoconjugates have to be prepared in large quantities as chemically pure materials, because the naturally occurring glycoconjugates are scarce and often structurally heterogeneous.

There are many problems in preparing naturally occurring glycolipids: (a) difficulty in synthesizing the stereo- and regiocontrolled compounds; (b) instability of the compounds for reduction, isomerization, racemization and/or hydrolysis; and (c) difficulty in detection of the compounds usually lacking any useful chromophores. For example, sphingolipid-type compounds as the lipid segment in a designed glycolipid could be prepared only with great difficulty because of complex synthetic routes.[5] Several groups have already reported synthetic lipids/glycolipids,[6] but to our knowledge there have been limited systematic preparation of lipid models and further development into synthetic glycolipids.[7] Complicated syntheses of glycolipids that possess a variety of lengths or numbers at hydrophobic chains prevent us from systematically investigating their functions.

Here we report on synthesis of lipid and glycolipid models possessing long alkoxy groups as hydrophobic tails and benzoic acid derivatives as the linker. Multihydroxylated benzoic acids allow various numbers and lengths of alkyl groups to be adopted as the tails of lipids, which resemble fatty acid portions of natural ones. A linker module with the aromatic ring could be successively and selectively introduced without any alternation of alkoxy chains. In the solution phase, we can employ well-established reactions for the glycosyl bond formation, which is capable of controlling stereochemistry of anomeric configurations without any difficulty. The synthetic conjugates are easily separated from reaction mixtures and conventionally purified due to their chemical stabilities, appropriate hydrophobicities

*Corresponding author. Tel.: +81-77-566-1111; fax: +81-77-561-2659; e-mail: tamiaki@se.ritsumei.ac.jp

0968-0896/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0968-0896(02)00266-3

and aromatic chromophores possessing a relatively large absorption at 254 nm. Moreover, we report specific recognition of lectin with the carbohydrate moiety of the synthetic glycolipids on a membrane, indicating that the artificial glycolipids can be good functional models of natural glycolipids.

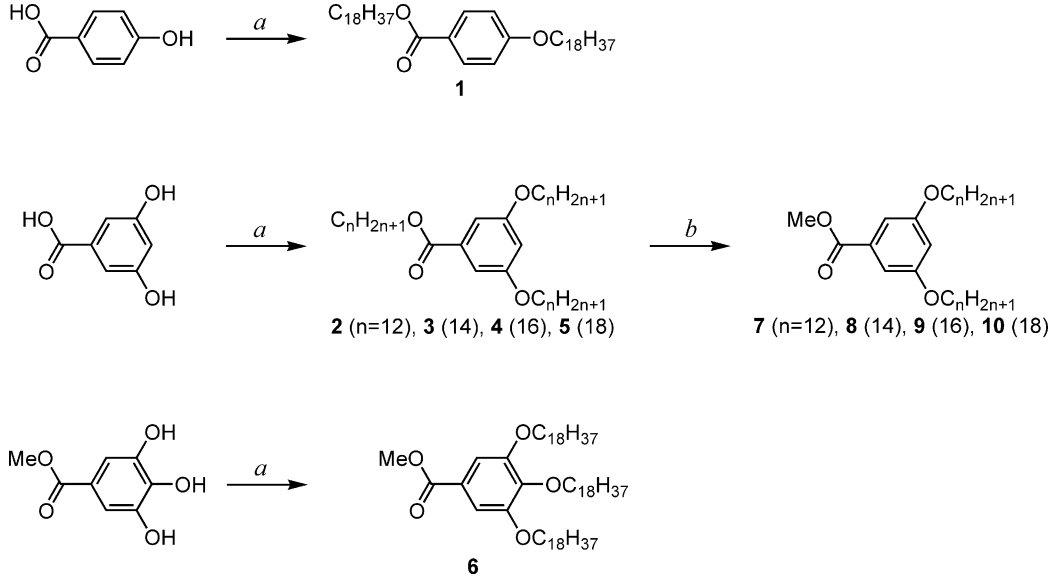
Results and Discussion

The lipid components that can link a carbohydrate residue were prepared as follows. Long alkyl chains were introduced into phenolic hydroxy groups of commercially available benzoic acid derivatives by slight modification of previously reported procedures,[8] in which the free carboxyl group in a molecule was esterified simultaneously (Scheme 1). The etherification and esterification with a series of 1-bromoalkane in the presence of potassium carbonate in dimethylformamide (DMF) smoothly proceeded to give alkoxybeonzoates **1**–**6** possessing various numbers and lengths of alkyl chains in high yields.

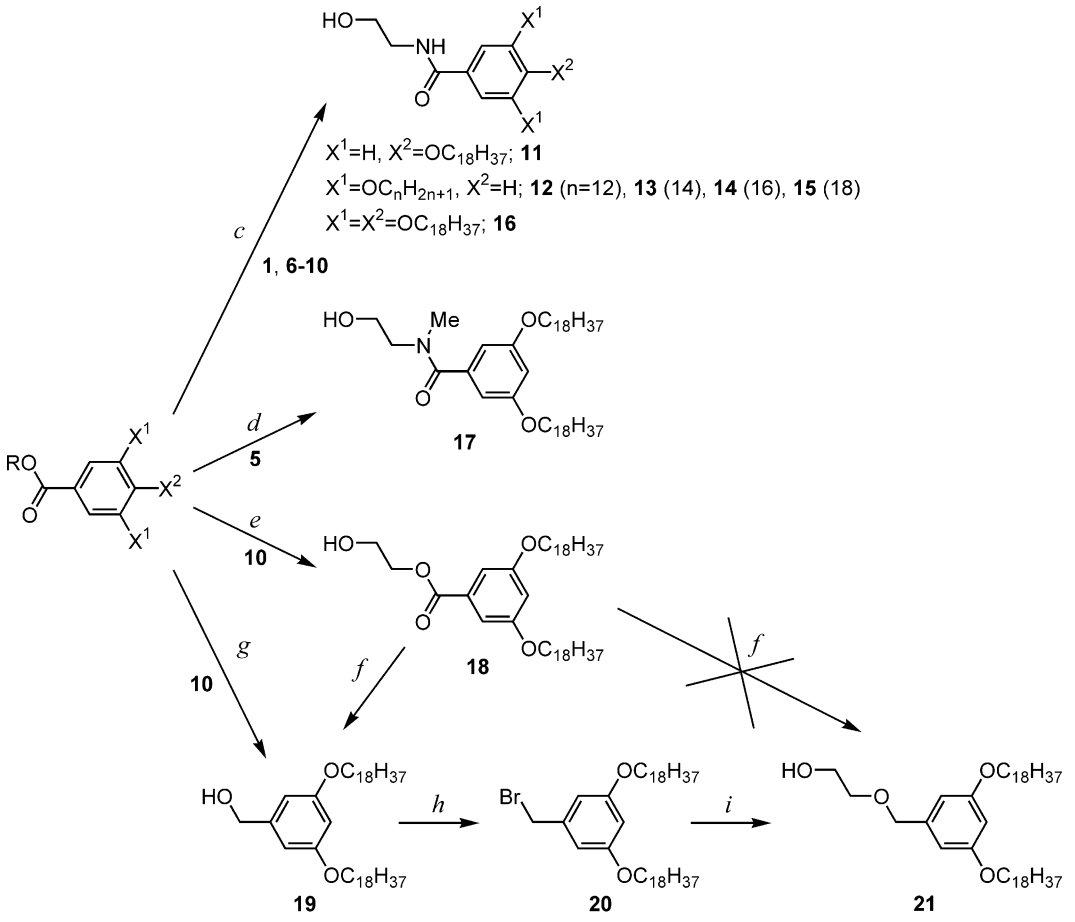
Aminolysis of esters **1** and **6**–**10** by aminoethanol in DMF and of ester **5** by neat *N*-methylaminoethanol efficiently afforded the corresponding primary amides **11**–**16** and secondary amide **17**, respectively (Scheme 2). Treatment of **1** and **6**–**10** with neat aminoethanol gave none of the desired amide products, but recovered the starting esters. Use of DMF as a polar solvent was necessary for the aminolysis by aminoethanol. The amino group in neat aminoethanol is less reactive to the carbonyl group because of intra- and intermolecular hydrogen bonding with amino and hydroxyl groups. DMF molecules would break the hydrogen bonding and then the amino group would increase the nucleophilicity to react with the benzoates. Enhanced solubility of the benzoates in DMF rather than in aminoethanol would also accelerate the aminolysis. In addition, double chain benzoates **2**–**5** possessing a long alkyl ester had to be changed to the corresponding methyl esters **7**–**10** before the reaction with aminoethanol, because the long alkyl ester was unreactive due to their high steric hindrance around the carbonyl group and the low solubility in aminoethanol and DMF as the mixed solvents. NMR spectra of the resulting amides **11**–**17** in $CDCl_3$ at 293 K show that primary amides **11**–**16** were exclusively *trans* conformers around the CO–NH bond, but that secondary amide **17** was a mixture of *cis*/*trans* conformers (1:5) in the solution.

Acid catalyzed alcoholysis of **10** by 1,2-dihydroxyethane in 1,2-dimethoxyethane gave the corresponding 2-hydroxyethyl benzoate (**18**) in a high yield. The ester **18** is applicable for a lipid analogue but not suitable as a precursor of glycolipid in the present study because of its instability in removing benzoyl groups as a protecting group of reactive hydroxyl moieties on a carbohydrate (see below). Treatment of ester **18** with 1 M solution of borane-tetrahydrofuran (BH_3·THF) complex in THF did not afford the reductive product **21**, but predominantly gave benzyl alcohol (**19**). The desired **21**, having a benzyl ether bond as a spacer tolerant of the above ester-cleavage, was efficiently prepared from **10** by a series of reactions: reduction of the methyl ester with $LiAlH_4$ to **19**, bromination of the benzyl alcohol with triphenylphosphine and carbon tetrabromide to **20** and Williamson ether synthesis of the bromide with 1,2-dihydroxyethane to **21**. These three linkages, amide, methylamide and ether bonds, in the synthetic lipid analogues were resistant to the following glycosylation using a conventional imidate method and the successive cleavage of benzoyl protecting groups on the carbohydrate moiety. All the new lipids **11**–**18** and **21** were easily purified by flash column chromatography (FCC) with silica gel and recrystallization, and fully characterized by their ^1H NMR, FAB-MS and/or FT-IR spectra.

The introduction of a carbohydrate moiety to the hydroxyl group of the above synthetic lipids was achieved as shown in Scheme 3. Glycosylation of lipid analogues **11**–**17** and **21** with 2,3,4,6-tetra-*O*-benzoyl-α-



Scheme 1. Synthesis of precursors for lipid analogues: *a*. $C_nH_{2n+1}Br$, K_2CO_3, DMF, 74–88%; *b*. MeONa/MeOH, CH_2Cl_2, >96%.



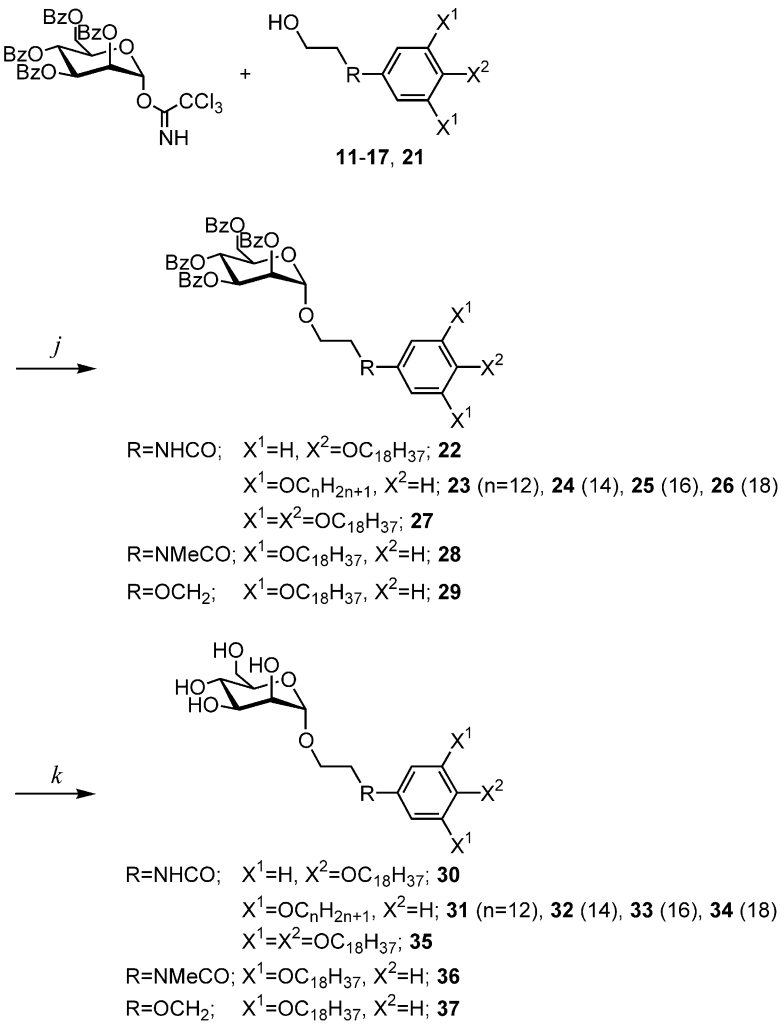
Scheme 2. Synthesis of lipid analogues: *c*. $HOCH_2CH_2NH_2$, DMF, 77–94%; *d*. $HOCH_2CH_2NMeH$, 90%; *e*. $HOCH_2CH_2OH$, $MeOCH_2CH_2OMe$, concd H_2SO_4, 88%; *f*. BH_3·THF, THF, 82%; *g*. $LiAlH_4$, THF, 97%; *h*. CBr_4, PPh_3, CH_2Cl_2, 90%; *i*. $HOCH_2CH_2OH$, NaH, THF, 73%.

D-mannopyranosyl trichloroacetimidate, prepared from a commercially available D-mannopyranoside by reported procedures,[9] in the presence of a catalytic amount of trimethylsilyl trifluoromethanesulfonate (TMSOTf) gave protected mannolipids **22**–**29** moderately after purification by silica gel column chromatography with ethyl acetate–hexane. Irrespective of the number and length of alkyl chains at the hydrophobic moiety, the glycosylation occurred stereoselectively to give the α-anomer exclusively from their ^{1}H NMR analyses. This glycosylation could be applicable to other saccharides; for example, use of β-D-galacto- and α/β-D-glucopyranosides instead of the above mannopyranoside gave the corresponding glycoconjugates.[10]

Deprotection of all four benzoate groups in **22**–**29** using sodium methoxide cleanly afforded the free-OH products **30**–**37**. After silica gel column chromatography with methanol–$CHCl_3$, these glycolipid analogues were recrystallized from methanol to yield pure materials as a white solid. The solids **30**–**37** were so stable that they could be stored at room temperature for a long period (>1 year) without any alteration. All the new glycolipids (**22**–**37**) in both protected and unprotected forms were fully characterized by ^{1}H NMR, H–H COSY and FAB-MS spectra. Compounds **28** and **36**, having methyl amide linkage, were a mixture of *cis*/*trans* conformers (1:3 and 1:5, respectively) in $CDCl_3$ at 293 K.

Interaction of the synthesized glycolipid analogues with lectins was examined by a commercially available surface plasmon resonance (SPR) instrument (BIACORE 3000) using a fluid ligand-layer formed on the surface of the sensor chip, HPA chip. A gold surface of HPA is modified with octadecyl groups attached through thioethers. An additional self-assembled monolayer (SAM) composed of 20% synthetic glycolipid and phosphatidylcholine from egg yolk (EPC) was generated upon the hydrophobic chip surface.[11]

The binding amounts of proteins including lectins on the above surface modified with mannolipid **34** were evaluated by SPR analysis as shown in Figure 1. Injection of proteins in an aqueous buffer solution increased the SPR response by binding the proteins on the surface and changing the bulk refractive index.[4] The response shows that concanavalin A (Con A), which is one of the lectins that binds specifically to an α-mannosyl residue,[12] had considerably higher affinity for the artificial monolayer than did β-galactose specific-binding ricin (RCA_{120})[12] and bovine serum albumin (BSA) lacking specific affinity with any carbohydrates. The responses by ricin and BSA were mainly ascribable to bulk refractive index changes. These results indicate that the binding events of Con A specifically occurred at the interface of the aqueous solution with the hydrophilic surface and that the α-mannosyl residues were present



Scheme 3. Synthesis of glycolipid analogues possessing the α-D-mannopyranosyl residue: *j*. TMSOTf, CH_2Cl_2; *k*. MeONa/MeOH. The overall yields of **30**–**37** based on consumed **11**–**17** and **21** were 26–78%.

on the artificial membrane surface. The other glycolipid analogues showed a similar behavior to **34**. Therefore, the present artificial glycoconjugates (**30**–**37**) are promising as good models of natural glycolipids.

Conclusion

In the synthetic lipid molecules, the 2-hydroxyethyl group and a series of alkyl chains were connected with hydroxylated benzoic acid derivatives at both ends. Number and length of the alkyl chains could control hydrophobicity of the lipid analogues. The number of alkyl chains actually depended on the starting material: 4-hydroxybenzoic acid, 3,5-dihydroxybenzoic acid and methyl gallate (methyl 3,4,5-trihydroxybenzoate) were converted into lipid analogues possessing a single alkyl chain, double chains and triple chains, respectively. Carbon number of bromoalkane can define the length of alkyl chains: using a series of bromoalkane afforded the corresponding analogues, which have dodecyl, tetradecyl, hexadecyl or octadecyl groups at the hydrophobic chains. Structurally determined pure lipid analogues can be systematically and easily synthesized in this way. A successive conventional imidate method gave a series of glycolipid analogues in an adequate yield. Moreover, these compounds can be stored at the solid state for one year or longer without any alteration.

The glycolipid analogues behaved as a membrane component with EPC on the SPR surface. The increased response to Con A over the other proteins demonstrated that the lipid-like membrane containing the analogues functioned similarly as a biomembrane. These analogues will contribute elucidating the biological functions of glycolipids on a cell surface.

Experimental

General

All materials were obtained from commercial suppliers and used as provided. Solvents were distilled by standard protocols. N_2 or Ar gas was used after drying by $CaCl_2$. All reactions were monitored by TLC on 0.25 mm precoated Merck silica gel 60 F_{254} and visualized with ultraviolet irradiation (254 nm) or 5% H_2SO_4–ethanol stain. All melting points were measured with a Yanagimoto micro melting point apparatus and

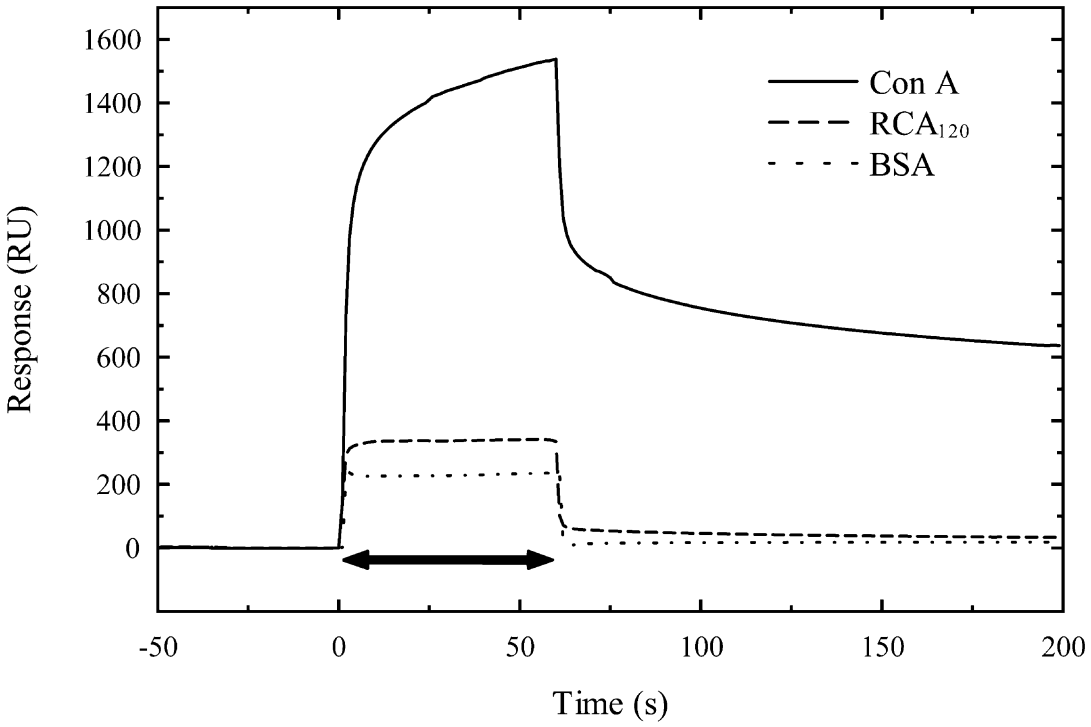


Figure 1. Titration of proteins on mannolipid-EPC surface: aqueous buffer solutions of proteins (20 μM in HBS-N buffer) were injected for 60 s (inset arrow) over a mannolipid **34** and EPC (1:4) monolayer on an octadecyl-coated Au chip. Solid, dashed and dotted lines are responses for Con A, RCA_{120} and BSA, respectively.

were uncorrected. ^1H NMR spectra were recorded on a Bruker AC-300 spectrometer (300 MHz) at 293 K (unless another specific description is given); δ-values are expressed in parts per million relative to $CHCl_3$ (7.26 ppm) or CD_2HCOCD_3 (2.04 ppm) as an internal reference. Infrared absorption spectra were measured with a Shimadzu FTIR-8600 spectrophotometer. FAB-MS spectrum data were collected on a JEOL JMS-GCmateIIR using *m*-nitrobenzyl alcohol as a matrix. SPR measurements were performed on a BIACORE 3000 operated using the version 3.1.1 software.

General synthetic procedures

Ether synthesis for 1–6. A mixture of 1-bromoalkane and K_2CO_3 in distilled DMF was heated to 65 °C with stirring under N_2 and then hydroxybenzoic acid or methyl gallate was added. After heating for several hours, aq 2 M HCl and CH_2Cl_2 were added to the solution with stirring at room temperature. The aqueous phase was separated from the CH_2Cl_2 phase and successively extracted with CH_2Cl_2. The combined CH_2Cl_2 phases were dried over Na_2SO_4 and concentrated in vacuo. FCC (Merck silica gel 60, 230–400 mesh) with CH_2Cl_2–hexane (ca. 1:1) and recrystallization from ethanol gave alkyl alkoxybenzoate as a white powder for **1**, **3**–**6** or colorless oil for **2**.

Alcoholysis (long alkyl ester to methyl ester) for 7–10. The above long alkyl ester and 0.1 M sodium methoxide in methanol (10 mL) were dissolved in CH_2Cl_2, followed by stirring at room temperature for 14 h under N_2; if the solid of the starting ester was observed after vigorous stirring, a small portion of CH_2Cl_2 was added until complete dissolution. The solution was neutralized with Amberlite IR-120 (plus) resin, filtered, and concentrated in vacuo. Recrystallization of the residue from ethanol gave pure methyl ester as a white needle-like powder.

Aminolysis (ester to amide) for 11–16. Alkyl alkoxybenzoate was added to a mixture of 2-aminoethanol (45 mL) and freshly distilled DMF (58 mL) under N_2, and the mixture was heated to 110 °C. After the ester was completely consumed, aq 2 M HCl was added to the ice-cooled solution. The aqueous solution was extracted with $CHCl_3$ and the combined extracts were dried over Na_2SO_4 and concentrated in vacuo. Recrystallization from methanol for **11**–**14**, methanol–ethanol for **15** or ethyl acetate for **16** afforded *N*-(2-hydroxyethyl)amide, lipid analogue, as a white powder.

Glycosylation for 22–29. Lipid analogue and 2,3,4,6-tetra - *O* - benzoyl - α - D - mannopyranosyl trichloroacetimidate[9] (Bz-Man-imidate) were dissolved in distilled CH_2Cl_2 under Ar. The solution was cooled to 0 °C with stirring and then a diluted CH_2Cl_2 solution of TMSOTf was added dropwise for a few min. After stirring for several hours at room temperature, the reaction mixture was quenched with a small amount of Et_3N and concentrated in vacuo. Purification by silica gel column chromatography (Wakogel FC-40FM) with 50% ethyl acetate–hexane yielded benzoyl-protected glycolipid as a white solid. Unchanged lipid analogue was recovered by collecting some fractions after eluting the glycolipid on the column chromatography. The total amount of the

glycoside was used in the following deprotection reaction without recrystallization.

Deprotection for 30–37. The above benzoyl-protected glycolipid and 0.1 M sodium methoxide in methanol were dissolved in CH_2Cl_2, and the solution was stirred at room temperature (**30**–**34**, **36**–**37**) or under moderate heating (**35**; ca. 40 °C) for several hours under N_2. The solution was neutralized with Amberlite IR-120 (plus) resin, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (Wakogel FC-40FM) with 10% methanol–$CHCl_3$ and recrystallized from methanol to give glycolipid analogue as a white solid. The melting points of **30**–**37** are not presented here, because the solids were amorphous to provide broad and indefinite melting points. Yields in parenthesis (see below) are overall yields for glycosylation and deprotection based on consumed lipid analogues.

Octadecyl 4-octadecyloxybenzoate (1). 1-Bromooctadecane (10.0 g, 30 mmol), K_2CO_3 (20.7 g, 0.15 mol) and 4-hydroxybenzoic acid (2.07 g, 15 mmol) in DMF (200 mL) for 30 h gave **1** (8.44 g, 88%); mp 76–77 °C; ^{1}H NMR ($CDCl_3$) δ = 7.98 (2H, d, J = 9 Hz, 2,6-H), 6.90 (2H, d, J = 9 Hz, 3,5-H), 4.27 (2H, t, J = 7 Hz, COO–CH_2), 4.00 (2H, t, J = 7 Hz, O–CH_2), 1.77 (4H, m, O–C–CH_2), 1.43 (4H, br-m, O–C_2–CH_2), 1.26 (56H, br-m, O–C_3–$C_{14}H_{28}$), 0.88 (6H, t, J = 7 Hz, O–C_{17}–CH_3). MS (FAB) found: m/z 644. Calcd for $C_{43}H_{79}O_3$: $[M+H]^+$, 644.

Dodecyl 3,5-bis(dodecyloxy)benzoate (2). 1-Bromododecane (6.02 g, 24 mmol), K_2CO_3 (5.53 g, 40 mmol) and 3,5-dihydroxybenzoic acid (1.23 g, 8.0 mmol) in DMF (60 mL) for 23 h gave **2** (3.92 g, 74%); mp 32 °C; ^{1}H NMR ($CDCl_3$) δ = 7.15 (2H, d, J = 2 Hz, 2,6-H), 6.63 (1H, t, J = 2 Hz, 4-H), 4.28 (2H, t, J = 7 Hz, COO–CH_2), 3.96 (4H, t, J = 6 Hz, O–CH_2), 1.76 (6H, m, O–C–CH_2), 1.43 (6H, br-m, O–C_2–CH_2), 1.26 (48H, br-m, O–C_3–C_8H_{16}), 0.88 (9H, t, J = 6 Hz, O–C_{11}–CH_3). MS (FAB) found: m/z 660. Calcd for $C_{43}H_{79}O_4$: $[M+H]^+$, 660.

Tetradecyl 3,5-bis(tetradecyloxy)benzoate (3). 1-Bromotetradecane (7.01 g, 24 mmol), K_2CO_3 (3.32 g, 24 mmol) and 3,5-dihydroxybenzoic acid (1.23 g, 8.0 mmol) in DMF (50 mL) for 23 h gave **3** (4.86 g, 82%); mp 42–43 °C; ^{1}H NMR ($CDCl_3$) δ = 7.15 (2H, d, J = 2 Hz, 2,6-H), 6.63 (1H, t, J = 2 Hz, 4-H), 4.28 (2H, t, J = 7 Hz, COO–CH_2), 3.96 (4H, t, J = 6 Hz, O–CH_2), 1.76 (6H, m, O–C–CH_2), 1.43 (6H, br-m, O–C_2–CH_2), 1.26 (60H, br-m, O–C_3–$C_{10}H_{20}$), 0.88 (9H, t, J = 7 Hz, O–C_{13}–CH_3). MS (FAB) found: m/z 744. Calcd for $C_{49}H_{91}O_4$: $[M+H]^+$, 744.

Hexadecyl 3,5-bis(hexadecyloxy)benzoate (4). 1-Bromohexadecane (7.37 g, 24 mmol), K_2CO_3 (3.33 g, 24 mmol) and 3,5-dihydroxybenzoic acid (1.24 g, 8.0 mmol) in DMF (50 mL) for 27 h gave **4** (4.89 g, 74%); mp 51 °C; ^{1}H NMR ($CDCl_3$) δ = 7.15 (2H, d, J = 2 Hz, 2,6-H), 6.63 (1H, t, J = 2 Hz, 4-H), 4.29 (2H, t, J = 7 Hz, COO–CH_2), 3.96 (4H, t, J = 7 Hz, O–CH_2), 1.76 (6H, m, O–C–CH_2), 1.43 (6H, br-m, O–C_2–CH_2), 1.26 (72H, br-m, O–C_3–$C_{12}H_{24}$), 0.88 (9H, t, J = 7 Hz, O–C_{15}–CH_3). MS (FAB) found: m/z 828. Calcd for $C_{55}H_{103}O_4$: $[M+H]^+$, 828.

Octadecyl 3,5-bis(octadecyloxy)benzoate (5). 1-Bromooctadecane (10.0 g, 30 mmol), K_2CO_3 (21.1 g, 0.15 mol) and 3,5-dihydroxybenzoic acid (1.54 g, 10 mmol) in DMF (150 mL) for 27 h gave **5** (7.63 g, 84%); mp 56 °C; IR (KBr) 1713 (C=O), 1597 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$) δ = 7.15 (2H, d, J = 2 Hz, 2,6-H), 6.63 (1H, t, J = 2 Hz, 4-H), 4.28 (2H, t, J = 7 Hz, COO–CH_2), 3.96 (4H, t, J = 6 Hz, O–CH_2), 1.76 (6H, m, O–C–CH_2), 1.43 (6H, br-m, O–C_2–CH_2), 1.25 (84H, br-m, O–C_3–$C_{14}H_{28}$), 0.88 (9H, t, J = 7 Hz, O–C_{17}–CH_3). MS (FAB) found: m/z 912. Calcd for $C_{61}H_{115}O_4$: $[M+H]^+$, 912.

Methyl 3,4,5-tris(octadecyloxy)benzoate (6). Triether **6** was prepared by previously reported procedures;[8] see ref 8 for data of **6**.

Methyl 3,5-bis(dodecyloxy)benzoate (7). Dodecyl ester **2** (659 mg, 1.0 mmol) yielded **7** (484 mg, 96%); alternative preparation and characterization of benzoate **7** was reported in the literature.[13] See ref 13 for data of **7**.

Methyl 3,5-bis(tetradecyloxy)benzoate (8). Tetradecyl ester **3** (743 mg, 1.0 mmol) yielded **8** (541 mg, 96%); mp 71–72 °C; ^{1}H NMR ($CDCl_3$) δ = 7.15 (2H, d, J = 2 Hz, 2,6-H), 6.63 (1H, t, J = 2 Hz, 4-H), 3.96 (4H, t, J = 7 Hz, O–CH_2), 3.89 (3H, s, $COOCH_3$), 1.77 (4H, m, O–C–CH_2), 1.44 (4H, br-m, O–C_2–CH_2), 1.26 (40H, br-m, O–C_3–$C_{10}H_{20}$), 0.88 (6H, t, J = 7 Hz, O–C_{13}–CH_3). MS (FAB) found: m/z 561. Calcd for $C_{36}H_{65}O_4$: $[M+H]^+$, 561.

Methyl 3,5-bis(hexadecyloxy)benzoate (9). Hexadecyl ester **4** (827 mg, 1.0 mmol) yielded **9** (615 mg, 100%); mp 77 °C; ^{1}H NMR ($CDCl_3$) δ = 7.15 (2H, d, J = 2 Hz, 2,6-H), 6.63 (1H, t, J = 2 Hz, 4-H), 3.96 (4H, t, J = 6 Hz, O–CH_2), 3.89 (3H, s, $COOCH_3$), 1.77 (4H, m, O–C–CH_2), 1.44 (4H, br-m, O–C_2–CH_2), 1.26 (48H, br-m, O–C_3–$C_{12}H_{24}$), 0.88 (6H, t, J = 7 Hz, O–C_{15}–CH_3). MS (FAB) found: m/z 618. Calcd for $C_{40}H_{73}O_4$: $[M+H]^+$, 618.

Methyl 3,5-bis(octadecyloxy)benzoate (10). Octadecyl ester **5** (911 mg, 1.0 mmol) yielded **10** (675 mg, 100%); mp 82–83 °C; IR (KBr) 1722 (C=O), 1601 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$) δ = 7.15 (2H, d, J = 2 Hz, 2,6-H), 6.63 (1H, t, J = 2 Hz, 4-H), 3.96 (4H, t, J = 6 Hz, O-CH_2), 3.89 (3H, s, $COOCH_3$), 1.77 (4H, m, O–C–CH_2), 1.44 (4H, br-m, O–C_2–CH_2), 1.25 (56H, br-m, O–C_3–$C_{14}H_{28}$), 0.88 (6H, t, J = 7 Hz, O–C_{17}–CH_3). MS (FAB) found: m/z 674. Calcd for $C_{44}H_{81}O_4$: $[M+H]^+$, 674.

***N*-(2-Hydroxyethyl)-4-octadecyloxybenzamide (11).** Aminolysis of **1** (322 mg, 0.50 mmol) for 64 h afforded **11** (175 mg, 81%) as a white needle-like powder; mp 109–110 °C; ^{1}H NMR ($CDCl_3$) δ = 7.74 (2H, d, J = 9 Hz, 2,6-H), 6.91 (2H, d, J = 9 Hz, 3,5-H), 6.49 (1H, br-t, NH), 3.99 (2H, t, J = 7 Hz, 4–O–CH_2), 3.84 (2H, q, J = 5 Hz, N–C–CH_2), 3.62 (2H, q, J = 5 Hz, N–CH_2), 2.66 (1H, t, J = 5 Hz, OH), 1.79 (2H, m, O–C–CH_2), 1.45 (2H, br-m, O–C_2–CH_2), 1.25 (28H, br-m, O–C_3–$C_{14}H_{28}$), 0.88 (3H,

t, $J=6$ Hz, O–C_{17}–CH_3). MS (FAB) found: m/z 434. Calcd for $C_{27}H_{48}NO_3$: $[M+H]^+$, 434.

***N*-(2-Hydroxyethyl)-3,5-bis(dodecyloxy)benzamide (12).** Aminolysis of **7** (251 mg, 0.50 mmol) for 21 h afforded **12** (233 mg, 88%); mp 82–83 °C; ^{1}H NMR ($CDCl_3$) $\delta=6.87$ (2H, d, $J=2$ Hz, 2,6-H), 6.57 (1H, br-t, 4-H), 6.52 (1H, br-t, NH), 3.96 (4H, t, $J=6$ Hz, 3,5-O–CH_2), 3.84 (2H, q, $J=5$ Hz, N–C–CH_2), 3.62 (2H, q, $J=5$ Hz, N–CH_2), 2.48 (1H, t, $J=5$ Hz, OH), 1.77 (4H, m, O–C–CH_2), 1.43 (4H, br-m, O–C_2–CH_2), 1.26 (32H, br-m, O–C_3–C_8H_{16}), 0.88 (6H, t, $J=6$ Hz, O–C_{11}–CH_3). MS (FAB) found: m/z 534. Calcd for $C_{33}H_{60}NO_4$: $[M+H]^+$, 534.

***N*-(2-Hydroxyethyl)-3,5-bis(tetradecyloxy)benzamide (13).** Aminolysis of **8** (280 mg, 0.50 mmol) for 21 h afforded **13** (266 mg, 90%); mp 90–91 °C; ^{1}H NMR ($CDCl_3$) $\delta=6.87$ (2H, d, $J=2$ Hz, 2,6-H), 6.57 (1H, br-t, 4-H), 6.53 (1H, br-t, NH), 3.96 (4H, t, $J=7$ Hz, 3,5-O–CH_2), 3.83 (2H, q, $J=5$ Hz, N–C–CH_2), 3.61 (2H, q, $J=5$ Hz, N–CH_2), 2.50 (1H, t, $J=5$ Hz, OH), 1.77 (4H, m, O–C–CH_2), 1.44 (4H, br-m, O–C_2–CH_2), 1.26 (40H, br-m, O–C_3–$C_{10}H_{20}$), 0.87 (6H, t, $J=7$ Hz, O–C_{13}–CH_3). MS (FAB) found: m/z 591. Calcd for $C_{37}H_{68}NO_4$: $[M+H]^+$, 591.

***N*-(2-Hydroxyethyl)-3,5-bis(hexadecyloxy)benzamide (14).** Aminolysis of **9** (307 mg, 0.50 mmol) for 21 h afforded **14** (293 mg, 91%); mp 95 °C; ^{1}H NMR ($CDCl_3$) $\delta=6.87$ (2H, d, $J=2$ Hz, 2,6-H), 6.57 (1H, br-t, 4-H), 6.53 (1H, br-t, NH), 3.96 (4H, t, $J=7$ Hz, 3,5-O–CH_2), 3.83 (2H, q, $J=5$ Hz, N–C–CH_2), 3.61 (2H, q, $J=5$ Hz, N–CH_2), 2.50 (1H, t, $J=5$ Hz, OH), 1.77 (4H, m, O–C–CH_2), 1.44 (4H, br-m, O–C_2–CH_2), 1.25 (48H, br-m, O–C_3–$C_{12}H_{24}$), 0.87 (6H, t, $J=6$ Hz, O–C_{15}–CH_3). MS (FAB) found: m/z 647. Calcd for $C_{41}H_{76}NO_4$: $[M+H]^+$, 647.

***N*-(2-Hydroxyethyl)-3,5-bis(octadecyloxy)benzamide (15).** Aminolysis of **10** (336 mg, 0.50 mmol) for 21 h afforded **15** (330 mg, 94%); mp 95–96 °C; IR (KBr) 3398 (O–H), 3298 (N–H), 1628 (C=O), 1597 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$) $\delta=6.87$ (2H, d, $J=2$ Hz, 2,6-H), 6.57 (1H, br-t, 4-H), 6.53 (1H, br-t, NH), 3.96 (4H, t, $J=7$ Hz, 3,5-O–CH_2), 3.83 (2H, q, $J=5$ Hz, N–C–CH_2), 3.61 (2H, q, $J=5$ Hz, N–CH_2), 2.49 (1H, t, $J=5$ Hz, OH), 1.77 (4H, m, O–C–CH_2), 1.44 (4H, br-m, O–C_2–CH_2), 1.25 (56H, br-m, O–C_3–$C_{14}H_{28}$), 0.87 (6H, t, $J=7$ Hz, O–C_{17}–CH_3). MS (FAB) found: m/z 703. Calcd for $C_{45}H_{84}NO_4$: $[M+H]^+$, 703.

***N*-(2-Hydroxyethyl)-3,4,5-tris(octadecyloxy)benzamide (16).** Aminolysis of **6** (1.13 g, 1.2 mmol) with 2-aminoethanol (109 mL) in DMF (163 mL) for 14 h afforded **16** (894 mg, 77%); mp 83–85 °C; ^{1}H NMR ($CDCl_3$) $\delta=6.96$ (2H, s, 2,6-H), 6.48 (1H, br-t, NH), 4.00 (4H, t, $J=6$ Hz, 3,5-O–CH_2), 3.98 (2H, t, $J=6$ Hz, 4-O–CH_2), 3.84 (2H, t, $J=5$ Hz, N–C–CH_2), 3.62 (2H, q, $J=5$ Hz, N–CH_2), 1.77 (6H, m, O–C–CH_2), 1.46 (6H, br-m, O–C_2–CH_2), 1.25 (84H, br-m, O–C_3–$C_{14}H_{28}$), 0.87 (9H, t, $J=7$ Hz, O–C_{17}–CH_3). MS (FAB) found: m/z 971. Calcd for $C_{63}H_{120}NO_5$: $[M+H]^+$, 971.

***N*-(2-Hydroxyethyl)-*N*-methyl-3,5-bis(octadecyloxy) benzamide (17).** Ester **5** (1.74 g, 1.9 mmol) was added to neat 2-(methylamino)ethanol (76 mL) under N_2, and the mixture was heated to 110 °C for 16 h. Allowing the reaction mixture to stand at room temperature for 4 days gave a crude product as a solid. The resulting precipitates were filtered and washed with methanol. Recrystallization from methanol afforded pure **17** (1.23 g, 90%) as a white powder; mp 83–85 °C; IR (KBr) 3341 (O–H), 1616 (C=O), 1587 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$ at 323 K[14]) $\delta=6.53$ (2H, d, $J=2$ Hz, 2,6-H), 6.49 (1H, br-t, 4-H), 3.94 (4H, t, $J=7$ Hz, 3,5-O–CH_2), 3.87 (2H, br-s, N–C–CH_2), 3.67 (2H, br-s, N–CH_2), 3.06 (3H, s, N–CH_3), 1.76 (4H, m, O–C–CH_2), 1.44 (4H, br-m, O–C_2–CH_2), 1.27 (56H, br-m, O–C_3–$C_{14}H_{28}$), 0.89 (6H, t, $J=7$ Hz, O–C_{17}–CH_3). MS (FAB) found: m/z 717. Calcd for $C_{46}H_{86}NO_4$: $[M+H]^+$, 717.

2-Hydroxyethyl 3,5-bis(octadecyloxy)benzoate (18). Methyl ester **10** (67.4 mg, 100 μmol) was added to a mixture of 1,2-dihdroxyethane (135 mL), 1,2-dimethoxyethane (134 mL) and concd H_2SO_4 (17 mL). After refluxing for 31 h, the reaction mixture was cooled to room temperature and extracted with hexane. The combined extracts were washed with aq satd $NaHCO_3$ and aq satd NaCl, dried over Na_2SO_4 and concentrated in vacuo. Purification by FCC with 0–10% ether-CH_2Cl_2 and recrystallization from methanol–ethanol gave **18** (62.2 mg, 88%) as a white powder; mp 71 °C; IR (KBr) 3537 (O–H), 1701 (C=O), 1605 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$) $\delta=7.16$ (2H, d, $J=2$ Hz, 2,6-H), 6.64 (1H, t, $J=2$ Hz, 4-H), 4.45 (2H, t, $J=5$ Hz, COO-CH_2), 3.96 (6H, m, 3,5-O–CH_2, COO–C–CH_2), 2.01 (1H, t, $J=6$ Hz, OH), 1.77 (4H, m, O–C–CH_2), 1.44 (4H, br-m, O–C_2–CH_2), 1.25 (56H, br-m, O–C_3–$C_{14}H_{28}$), 0.87 (6H, t, $J=7$ Hz, O–C_{17}–CH_3). MS (FAB) found: m/z 704. Calcd for $C_{45}H_{83}O_5$: $[M+H]^+$, 704.

3,5-Bis(octadecyloxy)benzyl alcohol (19). To a dry THF solution (22 mL) of the above pure **10** (448 mg, 665 μmol) was added $LiAlH_4$ (403 mg, 10.6 mmol) under N_2 and the mixture was then heated to 40 °C for 12 h. After water (100 mL) was added dropwise, the solution was extracted with $CHCl_3$. The combined extracts were washed with aq 2 M HCl and aq 4% $NaHCO_3$, dried over Na_2SO_4 and concentrated in vacuo. Purification by recrystallization from methanol gave **19** (414 mg, 97%) as a white powder; mp 68–70 °C; IR (KBr) 3518 (O–H), 1589 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$) $\delta=6.50$ (2H, d, $J=2$ Hz, 2,6-H), 6.37 (1H, br-t, 4-H), 4.62 (2H, d, $J=6$ Hz, 1-CH_2), 3.93 (4H, t, $J=7$ Hz, O–CH_2), 1.76 (4H, m, O–C–CH_2), 1.43 (4H, br-m, O–C_2–CH_2), 1.25 (56H, br-m, O–C_3–$C_{14}H_{28}$), 0.88 (6H, t, $J=7$ Hz, O–C_{17}–CH_3). MS (FAB) found: m/z 645. Calcd for $C_{43}H_{80}O_3$: M^+, 645.

3,5-Bis(octadecyloxy)benzyl bromide (20). A solution of alcohol **19** (322 mg, 499 μmol), triphenylphosphine (160 mg, 609 μmol), and carbon tetrabromide (415 mg, 1.25 mmol) in dry CH_2Cl_2 (16 mL) was stirred at room temperature for 4 h under Ar. The solution was washed with aq satd $NaHCO_3$ and aq satd NaCl, dried over Na_2SO_4 and concentrated in vacuo. After purification

by FCC with CH_2Cl_2, the eluate was evaporated to give **20** (318 mg, 90%) as a white solid; mp 70–73 °C; IR (KBr) 1595 (C=C), 550 cm^{-1} (C–Br); ^1H NMR ($CDCl_3$) δ = 6.51 (2H, d, J = 2 Hz, 2,6-H), 6.37 (1H, br-t, 4-H), 4.40 (2H, s, 1-CH_2), 3.92 (4H, t, J = 6 Hz, O–CH_2), 1.76 (4H, m, O–C–CH_2), 1.43 (4H, br-m, O–C_2–CH_2), 1.25 (56H, br-m, O–C_3–$C_{14}H_{28}$), 0.88 (6H, t, J = 7 Hz, O–C_{17}–CH_3). MS (FAB) found: m/z 708. Calcd for $C_{43}H_{80}BrO_2$: $[M+H]^+$, 708.

2-Hydroxyethyl 3,5-bis(octadecyloxy)benzyl ether (21). Sodium hydride (60% in mineral oil; 6.0 mg, 0.15 mmol) was added to a solution of **20** (31.2 mg, 44 μmol) and ethylene glycol (16.1 mg, 0.26 mmol) in THF (4 mL) under Ar. The mixture was refluxed for 3 days, and aq 2% HCl was added to the ice-chilled mixture. The resulting mixture was extracted with CH_2Cl_2, washed aq satd NaCl, dried over Na_2SO_4, and evaporated to dryness. FCC with 1% methanol–CH_2Cl_2 afforded **21** (22.1 mg, 73%); mp 51–54 °C; IR (KBr) 3504 (O–H), 1601 (C=C), 1177 cm^{-1} (C–O–C); ^1H NMR ($CDCl_3$) δ = 6.47 (2H, d, J = 2 Hz, 2,6-H), 6.38 (1H, br-t, 4-H), 4.48 (2H, s, 1-CH_2), 3.92 (4H, t, J = 7 Hz, 3,5-O–CH_2), 3.75 (2H, br-q, 1-C–O–C–CH_2), 3.59 (2H, t, J = 5 Hz, 1-C–O–CH_2), 1.98 (1H, br-t, OH), 1.76 (4H, m, O–C–CH_2), 1.43 (4H, br-m, O–C_2–CH_2), 1.25 (56H, br-m, O–C_3–$C_{14}H_{28}$), 0.87 (6H, t, J = 7 Hz, O–C_{17}–CH_3). MS (FAB) found: m/z 689. Calcd for $C_{45}H_{84}O_4$: M^+, 689.

***N*-[*O*-(2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl)oxyethyl]-4-octadecyloxybenzamide (22).** Glycosylation of **11** (69.0 mg, 0.16 mmol) with Bz-Man-imidate (ca. 0.16 mmol) in CH_2Cl_2 (21 mL) and 50 mM TMSOTf in CH_2Cl_2 (0.32 mL) for 3 h gave **22**, and all of **11** was consumed; ^1H NMR ($CDCl_3$) δ = 8.1–7.3 (20H, m, C_6H_5), 7.82 (2H, d, J = 12 Hz, 2,6-H), 6.92 (2H, d, J = 12 Hz, 3,5-H), 6.67 (1H, br-t, NH), 6.10 (1H, t, J = 10 Hz, Man-4-H), 5.92 (1H, dd, J = 3, 10 Hz, Man-3-H), 5.75 (1H, br-t, Man-2-H), 5.15 (1H, br-d, Man-1-H), 4.66 (1H, dd, J = 2, 10 Hz, Man-6-H), 4.46 (1H, m, Man-6-H), 4.43 (1H, m, Man-5-H), 4.07 (1H, m, N–C–CH), 3.95 (2H, t, J = 7 Hz, O–CH_2), 3.79 (3H, m, N–C–CH, N–CH_2), 1.76 (2H, m, O–C–CH_2), 1.45 (2H, br-m, O–C_2–CH_2), 1.25 (28H, br-m, O–C_3–$C_{14}H_{28}$), 0.87 (3H, t, J = 7 Hz, O–C_{17}–CH_3).

***N*-[*O*-(2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl)-oxyethyl]-3,5-bis(dodecyloxy)benzamide (23).** Glycosylation of **12** (126 mg, 0.24 mmol) with Bz-Man-imidate (ca. 0.16 mmol) in CH_2Cl_2 (6.4 mL) and 11 mM TMSOTf in CH_2Cl_2 (0.72 mL) for 5 h gave **23**, and 63.9 mg of **12** was recovered; ^1H NMR ($CDCl_3$) δ = 8.1–7.2 (20H, m, C_6H_5), 6.95 (2H, d, J = 2 Hz, 2,6-H), 6.71 (1H, br-t, NH), 6.58 (1H, br-t, 4-H), 6.12 (1H, t, J = 10 Hz, Man-4-H), 5.91 (1H, dd, J = 3, 10 Hz, Man-3-H), 5.74 (1H, br-t, Man-2-H), 5.15 (1H, br-d, Man-1-H), 4.68 (1H, d, J = 10 Hz, Man-6-H), 4.48 (1H, m, Man-6-H), 4.43 (1H, m, Man-5-H), 4.07 (1H, m, N–C–CH), 3.95 (4H, t, J = 6 Hz, O–CH_2), 3.78 (3H, m, N–C–CH, N–CH_2), 1.72 (4H, m, O–C–CH_2), 1.39 (4H, br-m, O–C_2–CH_2), 1.25 (32H, br-m, O–C_3–C_8H_{16}), 0.88 (6H, t, J = 6 Hz, O–C_{11}–CH_3).

***N*-[*O*-(2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl)oxyethyl] - 3,5 - bis(tetradecyloxy)benzamide (24).** Glycosylation of **13** (107 mg, 0.18 mmol) with Bz-Man-imidate (ca. 0.12 mmol) in CH_2Cl_2 (11 mL) and 11 mM TMSOTf in CH_2Cl_2 (0.54 mL) for 5 h gave **24**, and 57.0 mg of **13** was recovered; ^1H NMR ($CDCl_3$) δ = 8.1–7.2 (20H, m, C_6H_5), 6.95 (2H, d, J = 2 Hz, 2,6-H), 6.71 (1H, br-t, NH), 6.59 (1H, br-t, 4-H), 6.12 (1H, t, J = 10 Hz, Man-4-H), 5.91 (1H, dd, J = 3, 10 Hz, Man-3-H), 5.74 (1H, br-t, Man-2-H), 5.16 (1H, br-d, Man-1-H), 4.68 (1H, d, J = 11 Hz, Man-6-H), 4.48 (1H, m, Man-6-H), 4.44 (1H, m, Man-5-H), 4.07 (1H, m, N–C–CH), 3.95 (4H, t, J = 6 Hz, O–CH_2), 3.78 (3H, m, N–C–CH, N–CH_2), 1.72 (4H, m, O–C–CH_2), 1.39 (4H, br-m, O–C_2–CH_2), 1.25 (40H, br-m, O–C_3–$C_{10}H_{20}$), 0.88 (6H, t, J = 6 Hz, O–C_{13}–CH_3).

***N*-[*O*-(2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl)oxyethyl] - 3,5 - bis(hexadecyloxy)benzamide (25).** Glycosylation of **14** (77.1 mg, 0.12 mmol) with Bz-Man-imidate (ca. 80 μmol) in CH_2Cl_2 (16 mL) and 11 mM TMSOTf in CH_2Cl_2 (0.36 mL) for 5 h gave **25**, and 44.0 mg of **14** was recovered; ^1H NMR ($CDCl_3$) δ = 8.1–7.2 (20H, m, C_6H_5), 6.95 (2H, d, J = 2 Hz, 2,6-H), 6.68 (1H, t, J = 5 Hz, NH), 6.58 (1H, br-t, 4-H), 6.11 (1H, t, J = 10 Hz, Man-4-H), 5.91 (1H, dd, J = 3, 10 Hz, Man-3-H), 5.74 (1H, dd, J = 1, 2 Hz, Man-2-H), 5.16 (1H, d, J = 1 Hz, Man-1-H), 4.68 (1H, d, J = 10 Hz, Man-6-H), 4.48 (1H, m, Man-6-H), 4.44 (1H, m, Man-5-H), 4.07 (1H, m, N–C–CH), 3.96 (4H, t, J = 6 Hz, O–CH_2), 3.78 (3H, m, N–C–CH, N–CH_2), 1.73 (4H, m, O–C–CH_2), 1.39 (4H, br-m, O–C_2–CH_2), 1.25 (48H, br-m, O–C_3–$C_{12}H_{24}$), 0.88 (6H, t, J = 7 Hz, O–C_{15}–CH_3).

***N*-[*O*-(2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl)oxyethyl] - 3,5 - bis(octadecyloxy)benzamide (26).** Glycosylation of **15** (56.3 mg, 80 μmol) with Bz-Man-imidate (ca. 80 μmol) in CH_2Cl_2 (5.6 mL) and 5.5 mM TMSOTf in CH_2Cl_2 (0.36 mL) for 17 h gave **26**, and 14.7 mg of **15** was recovered; ^1H NMR ($CDCl_3$) δ = 8.1–7.2 (20H, m, C_6H_5), 6.95 (2H, d, J = 2 Hz, 2,6-H), 6.71 (1H, t, J = 5 Hz, NH), 6.58 (1H, br-t, 4-H), 6.11 (1H, t, J = 10 Hz, Man-4-H), 5.91 (1H, dd, J = 3, 10 Hz, Man-3-H), 5.74 (1H, dd, J = 1, 2 Hz, Man-2-H), 5.15 (1H, d, J = 1 Hz, Man-1-H), 4.67 (1H, d, J = 10 Hz, Man-6-H), 4.47 (1H, m, Man-6-H), 4.44 (1H, m, Man-5-H), 4.06 (1H, m, N–C–CH), 3.95 (4H, t, J = 6 Hz, O–CH_2), 3.78 (3H, m, N–C–CH, N–CH_2), 1.72 (4H, m, O–C–CH_2), 1.39 (4H, br-m, O–C_2–CH_2), 1.25 (56H, br-m, O–C_3–$C_{14}H_{28}$), 0.87 (6H, t, J = 6 Hz, O–C_{17}–CH_3).

***N*-[*O*-(2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl)oxyethyl]-3,4,5-tris(octadecyloxy)benzamide (27).** Glycosylation of **16** (156 mg, 0.16 mmol) with Bz-Man-imidate (ca. 0.16 mmol) in CH_2Cl_2 (16 mL) and 50 mM TMSOTf in CH_2Cl_2 (0.80 mL) for 1.5 h gave **27**, and 5.9 mg of **16** was recovered; ^1H NMR ($CDCl_3$) δ = 8.1–7.3 (20H, m, C_6H_5), 7.04 (2H, s, 2,6-H), 6.66 (1H, br-t, NH), 6.11 (1H, t, J = 10 Hz, Man-4-H), 5.90 (1H, dd, J = 3, 10 Hz, Man-3-H), 5.73 (1H, br-dd, Man-2-H), 5.15 (1H, br-d, Man-1-H), 4.68 (1H, d, J = 10 Hz, Man-6-H), 4.47 (1H, m, Man-6-H), 4.43 (1H, m, Man-5-H), 4.02 (7H, m, N–C–CH, O–CH_2), 3.78 (3H, m, N–C–CH, N–CH_2), 1.76 (6H, m, O–C–CH_2), 1.41 (6H, br-m,

O–C_2–CH_2), 1.25 (84H, br-m, O–C_3–$C_{14}H_{28}$), 0.87 (9H, t, $J=6$ Hz, O–C_{17}–CH_3).

***N*-Methyl-*N*-[*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)oxyethyl]-3,5-bis(octadecyloxy) benzamide (28).** Glycosylation of **17** (57.5 mg, 80 μmol) with Bz-Man-imidate (59.2 mg, 80 μmol) in CH_2Cl_2 (5.7 mL) and 50 mM TMSOTf in CH_2Cl_2 (0.40 mL) for 4 h gave **28**, and 10.5 mg of **17** was recovered; ^{1}H NMR ($CDCl_3$ at 323 K[14]) δ = 8.1–7.2 (20H, m, C_6H_5), 6.56 (2H, d, $J=2$ Hz, 2,6-H), 6.49 (1H, t, $J=2$ Hz, 4-H), 6.10 (1H, t, $J=10$ Hz, Man-4-H), 5.88 (1H, dd, $J=3$, 10 Hz, Man-3-H), 5.70 (1H, br-t, Man-2-H), 5.14 (1H, br-s, Man-1-H), 4.69 (1H, dd, $J=2$, 12 Hz, Man-6-H), 4.53 (1H, dd, $J=5$, 12 Hz, Man-6-H), 4.44 (1H, br-d, Man-5-H), 4.08 (1H, br-m, N–C–CH), 3.94 (4H, t, $J=7$ Hz, O–CH_2), 3.82 (3H, br-m, N–C–CH, N–CH_2), 3.18 (3H, s, N–CH_3), 1.72 (4H, m, O–C–CH_2), 1.45 (4H, br-m, O–C_2–CH_2), 1.28 (56H, br-m, O–C_3–$C_{14}H_{28}$), 0.89 (6H, t, $J=6$ Hz, O–C_{17}–CH_3).

***O*-(2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl)oxyethyl 3,5-bis(octadecyloxy)benzyl ether (29).** Glycosylation of **21** (13.8 mg, 20 μmol) with Bz-Man-imidate (ca. 30 μmol) in CH_2Cl_2 (2 mL) and 5.5 mM TMSOTf in CH_2Cl_2 (0.14 mL) for 10 h gave **29**, and 1.5 mg of **21** was recovered; ^{1}H NMR ($CDCl_3$) δ = 8.1–7.2 (20H, m, C_6H_5), 6.52 (2H, d, $J=2$ Hz, 2,6-H), 6.37 (1H, br-t, 4-H), 6.14 (1H, t, $J=10$ Hz, Man-4-H), 5.94 (1H, dd, $J=3$, 10 Hz, Man-3-H), 5.75 (1H, br-dd, Man-2-H), 5.17 (1H, br-d, Man-1-H), 4.69 (1H, dd, $J=2$, 12 Hz, Man-6-H), 4.56 (2H, s, 1-CH_2), 4.52 (1H, m, Man-5-H), 4.46 (1H, dd, $J=4$, 12 Hz, Man-6-H), 3.99 (1H, m, 1-C–O–C–CH), 3.91 (4H, t, $J=7$ Hz, 3,5-O–CH_2), 3.84(1H, m, 1-C–O–C–CH), 3.75 (2H, t, $J=4$ Hz,1-C–O–CH_2), 1.71 (4H, m, O–C–CH_2), 1.39 (4H, br-m, O–C_2–CH_2), 1.26 (56H, br-m, O–C_3–$C_{14}H_{28}$), 0.88 (6H, t, $J=6$ Hz, O–C_{17}–CH_3).

***N*-[*O*-(α-D-Mannopyranosyl)oxyethyl]-4-octadecyloxybenzamide (30).** Deprotection of **22** with 0.1 M MeONa/MeOH (8 mL) in CH_2Cl_2 (2 mL) for 17 h gave **30** (39.8 mg, 42%); ^{1}H NMR ($(CD_3)_2CO$) δ = 7.85 (2H, d, $J=9$ Hz, 2,6-H), 7.77 (1H, br-t, NH), 6.95 (2H, d, $J=9$ Hz, 3,5-H), 4.78 (1H, br-d, Man-1-H), 4.03 (2H, t, $J=6$ Hz, 4-O–CH_2), 3.98 (1H, m, Man-2-H), 3.78 (5H, m, N–C–CH_2, Man-3,4,6-H), 3.60 (8H, m, N–CH_2, Man-5,6-H, Man-2,3,4,6-OH), 1.77 (2H, m, O–C–CH_2), 1.46 (2H, br-m, O–C_2–CH_2), 1.27 (28H, br-m, O–C_3–$C_{14}H_{28}$), 0.86 (3H, br-t, O–C_{17}–CH_3). MS (FAB) found: *m*/*z* 596. Calcd for $C_{33}H_{58}NO_8$: $[M+H]^+$, 596.

***N*-[*O*-(α-D-Mannopyranosyl)oxyethyl]-3,5-bis(dodecyloxy)benzamide (31).** Deprotection of **23** with 0.1 M MeONa/MeOH (8 mL) in CH_2Cl_2 (2 mL) for 8 h gave **31** (45.3 mg, 56%); ^{1}H NMR ($CDCl_3$ at 323 K) δ = 6.86 (2H, d, $J=2$ Hz, 2,6-H), 6.56 (2H, br-m, 4-H, NH), 4.87 (1H, br-d, Man-1-H), 3.95, 3.84, 3.8–3.5 (18H, m, 3,5-O–CH_2, N–C–CH_2, N–CH_2, Man-2,3,4,5,6,6-H, Man-2,3,4,6-OH), 1.76 (4H, m, O–C–CH_2), 1.44 (4H, br-m, O–C_2–CH_2), 1.28 (32H, br-m, O–C_3–C_8H_{16}), 0.89 (6H, t, $J=7$ Hz, O–C_{11}–CH_3). MS (FAB) found: *m*/*z* 696. Calcd for $C_{39}H_{69}NO_9$: M^+, 696.

***N*-[*O*-(α-D-Mannopyranosyl)oxyethyl]-3,5-bis(tetradecyloxy)benzamide (32).** Deprotection of **24** with 0.1 M MeONa/MeOH (6 mL) in CH_2Cl_2 (2 mL) for 8 h gave **32** (34.7 mg, 55%); ^{1}H NMR ($CDCl_3$ at 323 K) δ = 6.87 (2H, d, $J=1$ Hz, 2,6-H), 6.70 (1H, br-t, NH), 6.55 (1H, br-t, 4-H), 4.87 (1H, br-d, Man-1-H), 3.93, 3.86, 3.64, 3.57 (18H, m, 3,5-O–CH_2, N–C–CH_2, N–CH_2, Man-2,3,4,5,6,6-H, Man-2,3,4,6-OH), 1.74 (4H, m, O–C–CH_2), 1.43 (4H, br-m, O–C_2–CH_2), 1.28 (40H, br-m, O–C_3–$C_{10}H_{20}$), 0.89 (6H, t, $J=7$ Hz, O–C_{13}–CH_3). MS (FAB) found: *m*/*z* 775. Calcd for $C_{43}H_{77}NNaO_9$: $[M+Na]^+$, 775.

***N*-[*O*-(α-D-Mannopyranosyl)oxyethyl]-3,5-bis(hexadecyloxy)benzamide (33).** Deprotection of **25** with 0.1 M MeONa/MeOH (4 mL) in CH_2Cl_2 (2 mL) for 8 h gave **33** (26.5 mg, 64%); ^{1}H NMR ($CDCl_3$ at 323 K) δ = 6.87 (2H, d, $J=2$ Hz, 2,6-H), 6.58 (1H, br-t, 4-H), 6.38 (1H, br-t, NH), 4.90 (1H, br-d, Man-1-H), 3.98 (5H, t, $J=7$ Hz, 3,5-O–CH_2, Man-2-H), 3.83 (5H, m, N–C–CH_2, Man-3,4,6-H), 3.68 (4H, m, N–CH_2, Man-5,6-H), 2.41 (1H, d, $J=5$ Hz, Man-3-OH), 2.36 (1H, br-d, Man-4-OH), 2.25 (1H, d, $J=4$ Hz, Man-2-OH), 1.99 (1H, br-t, Man-6-OH), 1.78 (4H, m, O–C–CH_2), 1.47 (4H, br-m, O–C_2–CH_2), 1.28 (48H, br-m, O–C_3–$C_{12}H_{24}$), 0.89 (6H, t, $J=7$ Hz, O-C_{15}-CH_3). MS (FAB) found: *m*/*z* 831. Calcd for $C_{47}H_{85}NNaO_9$: $[M+Na]^+$, 831.

***N*-[*O*-(α-D-Mannopyranosyl)oxyethyl]-3,5-bis(octadecyloxy)benzamide (34).** Deprotection of **26** with 0.1 M MeONa/MeOH (5 mL) in CH_2Cl_2 (3 mL) for 26 h gave **34** (39.0 mg, 76%); ^{1}H NMR ($CDCl_3$ at 323 K) δ = 6.87 (2H, d, $J=2$ Hz, 2,6-H), 6.58 (1H, br-t, 4-H), 6.37 (1H, br-t, NH), 4.90 (1H, br-d, Man-1-H), 3.98 (5H, t, $J=7$ Hz, 3,5-O–CH_2, Man-2-H), 3.85 (5H, m, N–C–CH_2, Man-3,4,6-H), 3.68 (4H, m, N–CH_2, Man-5,6-H), 2.37 (1H, d, $J=6$ Hz, Man-3-OH), 2.30 (1H, d, $J=3$ Hz, Man-4-OH), 2.19 (1H, d, $J=4$ Hz, Man-2-OH), 1.94 (1H, t, $J=6$ Hz, Man-6-OH), 1.78 (4H, m, O–C–CH_2), 1.46 (4H, br-m, O–C_2–CH_2), 1.28 (56H, br-m, O–C_3–$C_{14}H_{28}$), 0.89 (6H, t, $J=7$ Hz, O–C_{17}–CH_3). MS (FAB) found: *m*/*z* 887. Calcd for $C_{51}H_{93}NNaO_9$: $[M+Na]^+$, 887.

***N*-[*O*-(α-D-Mannopyranosyl)oxyethyl]-3,4,5-tris(octadecyloxy)benzamide (35).** Deprotection of **27** with 0.1 M MeONa/MeOH (8 mL) in CH_2Cl_2 (8 mL) for 8 h gave **35** (107 mg, 61%); ^{1}H NMR ($CDCl_3$) δ = 6.98 (2H, s, 2,6-H), 6.34 (1H, br-t, NH), 4.90 (1H, br-d, Man-1-H), 4.02 (7H, m, 3,4,5-O–CH_2, Man-2-H), 3.83 (5H, m, N–C–CH_2, Man-3,4,6-H), 3.66 (4H, m, N–CH_2, Man-5,6-H), 2.37 (1H, d, $J=6$ Hz, Man-3-OH), 2.30 (1H, br-d, Man-4-OH), 2.19 (1H, d, $J=4$ Hz, Man-2-OH), 1.93 (1H, t, $J=6$ Hz, Man-6-OH), 1.81 (6H, m, O–C–CH_2), 1.53 (6H, br-m, O–C_2–CH_2), 1.28 (84H, br-m, O–C_3–$C_{14}H_{28}$), 0.89 (9H, t, $J=7$ Hz, O–C_{17}–CH_3). MS (FAB) found: *m*/*z* 1132. Calcd for $C_{69}H_{129}NO_{10}$: M^+, 1132.

***N*-[*O*-(α-D-Mannopyranosyl)oxyethyl]-*N*-methyl-3,5-bis(octadecyloxy)benzamide (36).** Deprotection of **28** with 0.05 M MeONa/MeOH (1 mL) in CH_2Cl_2 (1 mL) for 20 h gave **36** (15.2 mg, 26%); ^{1}H NMR ($CDCl_3$ at 323 K[14]) δ = 6.49 (3H, br-s, 2,4,6-H), 4.85 (1H, br-s, Man-1-H), 3.95 (5H, t, $J=6$ Hz, 3,5-O–CH_2, Man-2-H), 3.84 (5H, br-m, N–C–CH_2, Man-3,4,6-H), 3.64 (4H, br-

m, N–CH_2, Man-5,6-H), 3.06 (3H, br-s, N–CH_3), 1.77 (4H, br-m, O–C–CH_2), 1.45 (4H, br-m, O–C_2–CH_2), 1.28 (56H, br-m, O–C_3–$C_{14}H_{28}$), 0.92 (6H, t, $J=7$ Hz, O–C_{17}–CH_3). MS (FAB) found: m/z 901. Calcd for $C_{52}H_{95}NNaO_9$: $[M+Na]^+$, 901.

***O*-(α-D-Mannopyranosyl)oxyethyl 3,5-bis(octadecyloxy)-benzyl ether (37).** Deprotection of **29** with 0.1 M MeONa/MeOH (0.5 mL) in CH_2Cl_2 (3.5 mL) for 15 h gave **37** (11.9 mg, 78%); ^1H NMR ($CDCl_3$ at 323 K) $\delta=6.46$ (2H, d, $J=2$ Hz, 2,6-H), 6.38 (1H, br-t, 4-H), 4.89 (1H, br-d, Man-1-H), 4.47 (2H, s, 1-CH_2), 3.94 (5H, t, $J=7$ Hz, 3,5-O–CH_2, Man-2-H), 3.87 (2H, br-s, 1-C–O–C–CH_2), 3.82 (3H, m, Man-3,4,6-H), 3.63 (4H, m, 1-C–O–CH_2, Man-5,6-H), 2.56 (4H, br-s, Man-2,3,4,6-OH), 1.76 (4H, m, O–C–CH_2), 1.45 (4H, br-m, O–C_2–CH_2), 1.28 (56H, br-m, O–C_3–$C_{14}H_{28}$), 0.89 (6H, t, $J=7$ Hz, O–C_{17}–CH_3). MS (FAB) found: m/z 874. Calcd for $C_{51}H_{94}NaO_9$: $[M+Na]^+$, 874.

Preparation of liposomes

An aqueous suspension of liposome was prepared by either of the following two procedures: (a) a 5 mM ethanol solution (500 μL) of glycolipid was mixed with a 20 mM ethanol solution (500 μL) of EPC (SIGMA, L-α-phosphatidylcholine from frozen egg yolk). The mixed ethanol solution (400 μL) was injected into a magnetically stirred HBS-N buffer (4.6 mL; Biacore AB) through a Hamilton syringe (Hamilton, Reno) with 20 μL/min at 313 K. The resulting suspension was partially evaporated to afford an almost ethanol-free liposomal suspension; or (b) a 2 mM chloroform solution (100 μL) of glycolipid, an 8 mM chloroform solution (100 μL) of EPC, chloroform (600 μL) and methanol (200 μL) was mixed in a vial. The mixture was dried with a rotary evaporator and kept under high vacuum for at least 2 h. The lipid mixture was hydrated in a HBS-N buffer (1 mL) and shaken with a tube mixer. One-half of the suspension of multilamellar vesicles was extruded 11 times to give small unilamellar vesicles through two polycarbonate membrane filters (AVESTIN, pore diameter 100 nm) mounted in the mini-extruder (AVESTIN, LiposoFast) fitted with two 500 μL Hamilton syringes.[15]

SPR experiments

The hydrophobic surface of an HPA chip (Biacore AB) was washed with 20 mM CHAPS solution (25 μL, 5 μL/min; PIERCE). The above suspension of liposomes was injected (60 μL, 1 μL/min) to generate an artificial lipid monolayer. An aq 10 mM NaOH solution (5 μL, 5 μL/min) washed away the weakly attached lipid vesicles. After changing the solvents to a running buffer (HBS-N buffer), binding assays were carried out by injection of the aqueous solution of a protein (10 μL, 10 μL/min). An aq 10 mM NaOH solution (10 μL, 10 μL/min) removed the binding protein to regenerate the lipid surface. Aq 20 μM BSA (SIGMA, Albumin, Bovine), ricin tetramer (WAKO, Ricin 120 solution) and Con A (Nacalai Tesque, Concanavalin A from Jack Beans) buffer solutions were used for the present measurement. The used HPA chip was finally recycled by washing with an aqueous 40 mM solution of octyl glucoside (25 μL, 10 μL/min).

Acknowledgements

We thank Dr. Tomohiro Miyatake of Ryukoku University for measurement of FAB-MS spectra and Professor Dr. Masakazu Kikuchi of Ritsumeikan University for measurement of SPR sensorgrams.

References and Notes

1. Drickamer, K.; Taylor, M. E. *Annu. Rev. Cell Biol.* **1993**, *9*, 237.
2. Varki, A. *Glycobiology* **1993**, *3*, 97.
3. (a) Mortell, K. H.; Weatherman, R. V.; Kiessling, L. L. *J. Am. Chem. Soc.* **1996**, *118*, 2297. (b) Mortell, K. H.; Gingras, M.; Kiessling, L. L. *J. Am. Chem. Soc.* **1994**, *116*, 12053.
4. Lundquist, J. J.; Toone, E. J. *Chem. Rev.* **2002**, *102*, 555.
5. A review of syntheses of sphingosine: Koskinen, P. M.; Koskinen, A. M. P. *Synthesis* **1998**, 1075.
6. (a) Kunitake, T. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 709. (b) Blackburn, C. C.; Schnaar, R. L. *J. Biol. Chem.* **1983**, *258*, 1180. (c) Auzély-Velty, R.; Benvegnu, T.; Mackenzie, G.; Haley, J. A.; Goodby, J. W.; Plusquellec, D. *Carbohydr. Res.* **1998**, *314*, 65. (d) Pozsgay, V. *Org. Lett.* **1999**, *1*, 477.
7. (a) Lockhoff, O.; Stadler, P. *Carbohydr. Res.* **1998**, *314*, 13. (b) Conboy, J. C.; McReynolds, K. D.; Gervay-Hague, J.; Saavedra, S. S. *J. Am. Chem. Soc.* **2002**, *124*, 968.
8. Tamiaki, H.; Obata, T.; Azefu, Y.; Toma, K. *Bull. Chem. Soc. Jpn.* **2001**, *74*, 733.
9. Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21.
10. (a) Toma, K.; Sato, R.; Tamiaki, H. JP Patent 2001–122889, 2001. (b) Tamiaki, H.; Azefu, Y.; Toma, K.; Sato, R. JP Patent 2001–253896, 2001. (c) Sato, R.; Toma, K.; Tamiaki, H. JP Patent 2002–30091, 2002.
11. (a) Kalb, E.; Frey, S.; Tamm, L. K. *Biochim. Biophys. Acta* **1992**, *1103*, 307. (b) Kuziemko, G. M.; Stroh, M.; Stevens, R. C. *Biochemistry* **1996**, *35*, 6375.
12. Sharon, N.; Lis, H. *LECTINS*; Chapman and Hall: London, 1989.
13. Metzger, R. M.; Wiser, D. C.; Laidlaw, R. K.; Takassi, M. A. *Langmuir* **1990**, *6*, 350.
14. At 293 K, the ^1H NMR spectrum was complex due to the *cis*/*trans* mixture (see text). At 323 K, the *cis* and *trans* conformers exchanged so rapidly in the NMR measurement time scale that the spectrum was simple.
15. MacDonald, R. C.; MacDonald, R. I.; Menco, B.P.M.; Takeshita, K.; Subbarao, N. K.; Hu, L. R. *Biochim. Biophys. Acta* **1991**, *1061*, 297.