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Preparation of Artificial Glycolipids and Their Aggregation Behavior in Aqueous Media

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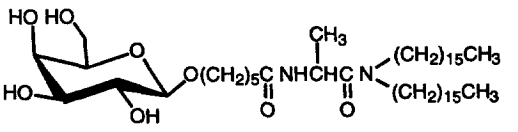
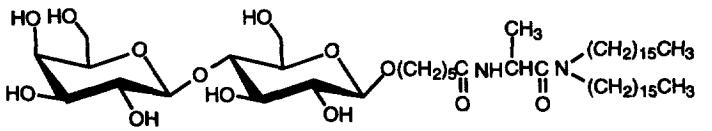
Abstract: *N*,*N*-Dihexadecyl-N^{α}-[6-(β-D-galactopyranosyl)hexanoyl]-L-alaninamide and *N*,*N*-dihexadecyl-N^{α}-[6-{(β-D-galactopyranosyl)-(1→4)-*O*-(D-glucopyranosyl)}hexanoyl]-L-alaninamide, which have an amino acid residue interposed between a polar sugar moiety and a hydrophobic double-chain segment individually, were prepared and characterized as novel artificial glycolipids. Morphological studies by means of differential scanning calorimetry and dynamic light scattering as well as electron microscopy clarified that these lipids tend to form stable bilayer membranes by themselves in aqueous media. © 1997 Elsevier Science Ltd.

Specific proteins and lipids are known to be major components of cell membranes. Although morphological structures of phospholipids have been extensively investigated in connection with their biological functions, their complexity and chemical instability have necessitated development of more stable membrane-forming amphiphiles. Meanwhile, glycolipids behave as biological receptors or carriers capable of transmitting physiological information in biomembranes, and their pure species are obtainable in limited amounts from natural resources. Therefore, it is quite important from the viewpoint of biomimetic chemistry to prepare artificial glycolipids. Natural glycolipids, especially monoglycolipids, can not form stable bilayer membranes by themselves;[1,2] therefore, a vesicle-forming lipid is usually added to the glycolipids in order to get stable bilayer vesicles. Artificial glycolipids, which can form stable bilayer membranes by themselves, are



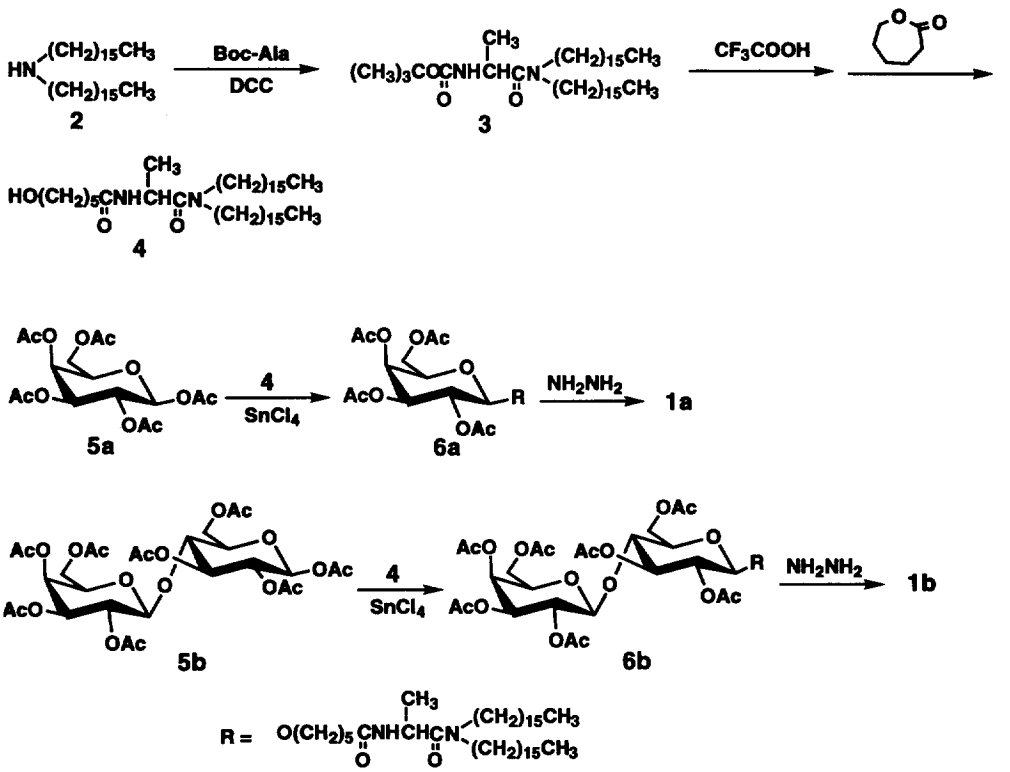
1a



1b

expected to be used as a material for drug delivery or DNA transfection with targeting ability.[3,4] We have reported previously that peptide lipids, having an amino acid residue and two long alkyl chains, form quite stable bilayer assemblies in aqueous media due to formation of the hydrogen-belt region in the intramembrane domain.[5] In this work, we prepared novel glycolipids, **1a** having a galactose unit and **1b** having a lactose unit, involving an amino acid residue interposed between a sugar moiety and an aliphatic double-chain and investigated their morphological behavior.

Artificial glycolipids (**1a** and **1b**) were prepared according to reaction steps as shown in Scheme 1. Compound **3**, which was prepared by the reaction between *N*-(*t*-butoxycarbonyl)-L-alanine (Boc-Ala) and dihexadecylamine (**2**) in the presence of *N,N*-dicyclohexylcarbodiimide (DCC) according to the previous method,[5] was dissolved in dry dichloromethane including trifluoroacetic acid to eliminate the Boc group. After evaporated to dryness, the residue was dissolved in diethyl ether. ε-Caprolactone was then added to it and the mixture was stirred for 88 h at room temperature. The resulting product was purified by column chromatography (Wakogel C-300 / ethyl acetate) to give compound **4** in a yield of 29%. Compound **6a** was obtained by the reaction between β-D-galactose pentaacetate (**5a**) and the compound **4** in the presence of tin tetrachloride in dry dichloromethane,[6] and purified by gel-filtration chromatography with Sephadex LH-20 in a yield of 38% (only the β-anomer).[7] Compound **1a** was obtained by the reaction with **6a** and hydrazine, and purified by gel-filtration chromatography with Sephadex LH-20 in a yield of 52% (β-anomer).[8] The glycolipid with lactose (**1b**) was also prepared by the same procedure (a molar ratio of α/β, 1/9).[9]



Scheme 1

The aggregation behavior of glycolipids **1a** and **1b** was examined by differential scanning calorimetry (DSC) and dynamic light scattering (DLS) as well as electron microscopy. Firstly, the phase transition from gel to the liquid crystalline state for the lipids was monitored by DSC. As for the aqueous dispersion of **1a** at pH 7.0, the phase transition parameters, maximum temperature (T_m), enthalpy change (ΔH),and entropy change (ΔS) are as follows: T_m, 23.0 °C; ΔH, 27.1 kJ mol^{-1}; ΔS, 90.3 J mol^{-1}. As for the dispersion solution of **1b**, the phase transition parameters are as follows: T_m, 23.6 °C; ΔH, 29.6 kJ mol^{-1}; ΔS, 98.7 J mol^{-1}. These values are comparable to those for ionic peptide lipids capable of forming stable bilayer aggregates.[10] Secondly, the aggregate size for **1a** in the dispersion state was measured by DLS and found to be in a range of 200–400 nm. Furthermore, electron microscopy was applied to samples negatively stained with uranyl acetate to clarify the aggregate morphology, showing formation of spherical particles in a diameter range of 200–300 nm. These results indicate that the present novel glycolipids definitely form bilayer vesicles in aqueous media. After the dispersion sample was sonicated with a probe-type sonicator at 30 W for 5 min, the particle size became smaller (a diameter range of 90–100 nm) as evidenced by DLS. Electron micrographs for **1a** and **1b** obviously show the formation of bilayer vesicles as shown in Fig. 1.

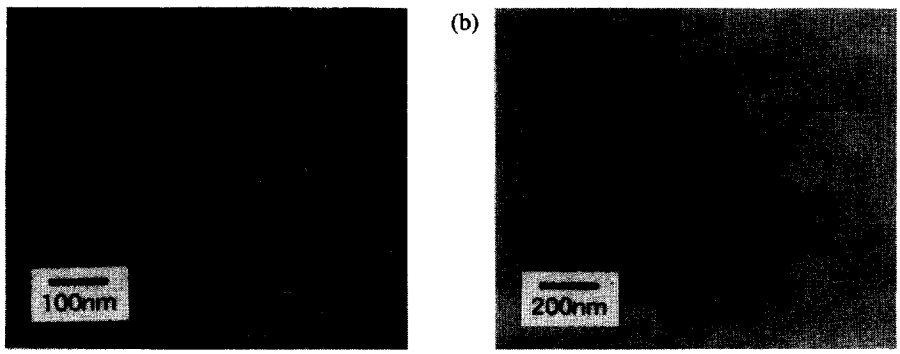


Fig. 1. Electron micrographs negatively stained with uranyl acetate: (a) 1 mmol dm^{-3} aqueous solution of **1a** sonicated for 5 min with a probe-type sonicator at 30W; (b) 1 mmol dm^{-3} aqueous solution of **1b** sonicated for 5 min with a probe-type sonicator; taken on a JEOL JEM-200CX electron microscope installed at the Research Laboratory for High Voltage Electron Microscopy of Kyushu University.

In conclusion, we succeeded in preparation of artificial glycolipids involving a galactosyl or a lactosyl unit, which form stable bilayer aggregates in aqueous media. Natural glycolipids are usually difficult to form stable bilayer vesicles by themselves. As for the present artificial glycolipids, a hydrogen-belt domain may be constructed through intermolecular hydrogen-bonding interaction between amino acid residues in each vesicle. Such intravesicular interaction may act to tighten the aggregate structure.

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7. **6a**: ^{1}H-NMR ($CDCl_3$, 500MHz): δ= 0.88 [t, 6H, *J* =7 Hz, $(CH_2)_{15}C\underline{H}_3$], 1.25-1.70 [m, 65H, $CH_2(C\underline{H}_2)_{14}CH_3$, $CH(C\underline{H}_3)$, and $OCH_2(C\underline{H}_2)_3CH_2CO$], 1.98-2.15 [4s, 3H each, OAc], 2.17 [t, 2H, *J* =8 Hz, $O(CH_2)_4C\underline{H}_2CO$], 3.05-3.55 [m, 5H, $C\underline{H}_2(CH_2)_{14}CH_3$ and $OC\underline{H}_2(CH_2)_4CO$], 3.87-3.91 [m, 2H, $OC\underline{H}_2(CH_2)_4CO$ and H-5], 4.04-4.17 [m, 2H, H-6], 4.44 [d, 1H, *J* =8 Hz, H-1], 4.82 [m, 1H, $C\underline{H}(CH_3)$], 5.01 [dd, 1H, *J* =3, 10 Hz, H-3], 5.20 [dd, 1H, *J* =10, 10 Hz H-2], 5.45 [d, 1H, *J* =3 Hz, H-4], 6.53 [d, 1H, *J* =8 Hz, $CON\underline{H}CH(CH_3)$]; Found: C, 67.13; H, 10.17; N,2.64%; Calcd for $C_{75}H_{116}O_{20}N_2$: C, 67.31; H, 10.27; N, 2.85%.
8. **1a**: ^{1}H NMR (CD_3OD, 500MHz): δ=0.88 [t, 6H, *J* =7 $(CH_2)_{15}C\underline{H}_3$], 1.27-1.72 [m, 65H, $CH_2(C\underline{H}_2)_{14}CH_3$, $CH(C\underline{H}_3)$ and $OCH_2(C\underline{H}_2)_3CH_2CO$], 2.22 [t, 2H, *J* =7 Hz, $O(CH_2)_4C\underline{H}_2CO$], 3.15-3.52 [m, 7H, $C\underline{H}_2(CH_2)_{14}CH_3$, H-2, H-3 and H-5], 3.72 [m, 3H, H-6 and $OC\underline{H}_2$ $(CH_2)_4CO$], 3.82 [d, 1H, *J* =2 Hz, H-4], 3.89 [m, 1H, $OC\underline{H}_2$ $(CH_2)_4CO$], 4.19 [d, 1H, *J* =8 Hz,H-1], 4.76 [m, 1H, $CON\underline{H}CH(CH_3)$]; Found: C, 68.89; H, 11.21; N, 3.22%; Calcd for $C_{47}H_{92}O_8N_2 \cdot 0.5H_2O$: C, 68.65; H, 11.40; N, 3.46%.
9. **6b**: The ratio of α- and β-anomers was determined by ^{1}H-NMR spectroscopy. ^{1}H-NMR ($CDCl_3$, 500MHz): β-anomer: δ= 0.88 [t, 6H, *J* =7 Hz, $(CH_2)_{15}C\underline{H}_3$], 1.25-1.64 [m, 65H, $CH_2(C\underline{H}_2)_{14}CH_3$, $CH(C\underline{H}_3)$ and $OCH_2(C\underline{H}_2)_3CH_2CO$], 1.96-2.19 [m, 23H, OAc and $O(CH_2)_4C\underline{H}_2CO$], 3.05-3.80 [m, 9H, $C\underline{H}_2(CH_2)_{14}CH_3$, $OC\underline{H}_2(CH_2)_4CO$, H-5 Gal, H-4, H-5 Glc], 4.05-4.16 [m, 3H, H-6 Gal and H-6a Glc], 4.40-4.50 [m, 3H, H-1 Gal, H-6b and H-1 Glc], 4.80 [m, 1H, $NHC\underline{H}(CH_3)$], 4.87 [dd, 1H, *J* =8, 9 Hz, H-2 Glc], 4.94 [dd, 1H, *J* =3, 10 Hz, H-3 Gal], 5.11 [dd, 1H, *J* =8, 10 Hz, H-2 Gal], 5.19 [t, 1H, *J* =9 Hz H-3 Glc], 5.35 [d, 1H, *J* =3 Hz, H-4, Gal], 6.53 [d, 1H, *J* =8 Hz, $CON\underline{H}CH(CH_3)$]; α-anomer: 3.73 [t, 1H, *J* =9 Hz, H-4, Glc], 5.46 [t, 1H, *J* =9 Hz, H-3, Glc]; Found: C, 63.31; H, 9.19; N,2.25%; Calcd for $C_{67}H_{116}O_{20}N_2$: C, 63.38; H, 9.20; N, 2.20%. **1b**: ^{1}H NMR (CD_3OD, 500MHz): δ=0.90 [t, 6H, *J* =7, $(CH_2)_{15}C\underline{H}_3$], 1.23-1.80 [m, 65H, $CH_2(C\underline{H}_2)_{14}CH_3$, $CH(C\underline{H}_3)$ and $OCH_2(C\underline{H}_2)_3CH_2CO$], 2.20 [t, 2H, *J* =7, $O(CH_2)_4C\underline{H}_2CO$], 4.28 [d, 1H, *J* =8Hz, H-1 β-Glc], 4.73-4.78 [m, 2H, $CON\underline{H}CH(CH_3)$ and H-1 α-Glc]; Found: C, 65.59; H, 10.54; N, 2.80%; Calcd for $C_{53}H_{102}O_{13}N_2$: C, 65.26; H, 10.54; N, 2.87%.
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