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Solid phase lipid synthesis (SPLS) for construction of an artificial glycolipid library†

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A simple solid phase lipid synthesis (SPLS) is proposed for construction of an artificial glycolipid library; this method is so convenient and flexible that we have efficiently constructed a glycolipid library; some of these glycolipids formed into stable bilayer aggregates in aqueous solution, suggesting that the glycolipids obtained by SPLS are useful as a suitable model of naturally occurring glycolipids.

Recent development in the field of glycobiology has clearly established that naturally occurring glycolipids have various key functions in biological systems.[1] Despite such important roles, fundamental questions on the relationship of the functions to the molecular structures are poorly understood. This is partially due to the complicated and diverse structures of natural glycolipids. An appropriate model and synthetic scheme are now required.[2] We describe herein a simple and convenient strategy for synthesis of artificial glycolipids based on a solid phase method (SPLS). Using the present method we efficiently constructed a small glycolipid library.

Typical examples of the designed artificial glycolipids are shown in Fig 1. Since the artificial glycolipids can be divided into several structural modules to provide flexibility to SPLS,[3] we planned to combine these modules on a resin in sequence (Scheme 1). The Merrifield resin modified with a 3-hydoxymethyl-4-nitrophenoxy linker was employed as a solid support.[4] Initially, phenylisopropyl Fmoc-aspartate as a connector part is attached to the resin using a condensation reagent (DIC). After deprotection of the Fmoc group, succinic anhydride was reacted with the amine site to introduce succinic amide as a spacer, followed by condensation of dialkylamine with the remaining carboxylic acid site to form a hydrophobic tail.[5] Then, the phenylisopropyl group was cleaved by 2% TFA to afford a free β-carboxylic acid of the aspartic acid connector,[6] which was next connected with aminoethyl glycoside,[7, 8] a polar head group. Finally, the artificial glycolipid thus synthesized, was released from the solid support. Two distinct methods were used for the cleavage from the resin, (i) hydrazinolysis,[9] or (ii) photolysis followed by methanolysis.[4] In the hydrazinolysis, cationic lipids bearing a hydrazide were afforded,† whereas anionic lipids bearing a carboxylate were obtained in the photolysis sequence.† All glycolipids were purified by gel chromatography [Sephadex LH20, eluent : $CHCl_3$–MeOH (1/1)], and identified by MALDI-TOF mass and NMR spectroscopies.† The overall yield for the six or seven steps ranges from 33 to 91%. Using this SPLS method, a small library was prepared as summarized in Table 1.

In order to confirm the fundamental capability to form a lipid membrane, we next investigated aggregation properties of the artificial glycolipids using conventional physicochemical measurements. Glc-asp(NH_2)-$2C_2$ and Glc-asp(NH_2)-$2C_6$ were solu-

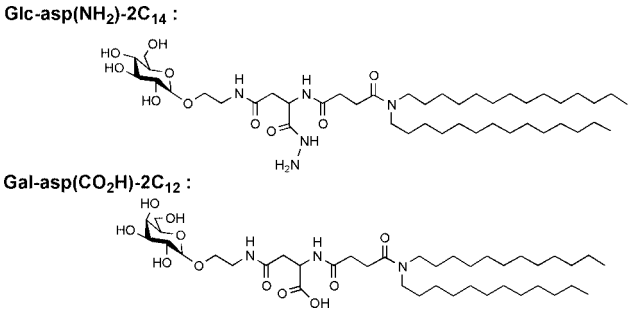

Fig. 1 Typical examples of artificial glycolipids. The glycolipids are abbreviated as 'sugar–connector(charge)–tail'. Sugar denotes the sugar head structure (Glc, Gal, Man), connector denotes the connection part of the lipid (asp), charge denotes the surface charge [NH_2 (cationic) or CO_2H (anionic)], and tail denotes the hydrophobic tail group ($2C_{12}$, $2C_{14}$, $2C_{16}$ *etc.*).

† Details of the release of Glc-asp(NH_2)-$2C_{12}$ from resin **5** and the synthesis of Glc-asp(CO_2H)-$2C_{12}$ are available as electronic supplementary information (ESI). See http://www.rsc.org/suppdata/cc/b0/b002545n/

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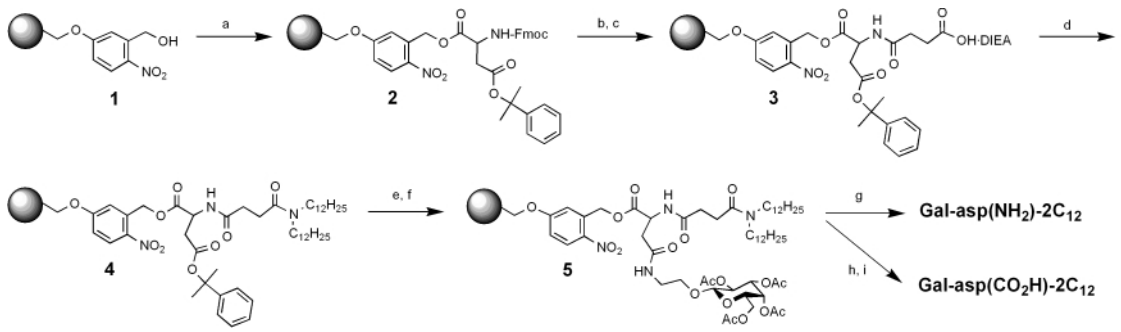

Scheme 1 Typical synthetic route towards artificial glycolipids (Gal-asp(NH_2)–$2C_{12}$, and Gal-asp(CO_2H)–$2C_{12}$). *Reagents and conditions*: (a) Fmoc-Asp(Pip)-OH (3.0 equiv.), DIC (3.0 equiv.), DMAP (3.0 equiv.), HOBT (4.0 equiv.), DMF, 25 °C, 10 h, 83%; (b) piperidine (20%), DMF, 25 °C, 30 min.; (c) succinic anhydride (4.0 equiv.), DIEA (4.0 equiv.), DMF, 25 °C, 14 h; (d) didodecylamine (3.0 equiv.), pyBOP (3.0 equiv.), DIEA (3.0 equiv.), DMF, 25 °C, 10 h; (e) TFA (2%), CH_2Cl_2, 25 °C, 3 h; (f) 2-aminoethyl-*O*-tetraacetyl glucopyranoside (3.0 equiv.), pyBOP (3.0 equiv.), DIEA (3.0 equiv.), DMF, 25 °C, 10 h; (g) $NH_2NH_2·H_2O$ (50 equiv.), THF–MeOH (4/1, v/v), 25 °C, 5 h, 67% over six steps; (h) *hν*, THF, 25 °C, 24 h; (i) NaOMe (1.0 equiv./unit), CH_2Cl_2–MeOH (3/1, v/v), 25 °C, 3 h, 39% over seven steps. Pip = phenylisopropyl, DIC = *N*,*N*′-diisopropylcarbodiimide, DMAP = 4-dimethylaminopyridine, HOBT = 1-hydroxybenzotriazole, DIEA; = *N*,*N*′-diisopropylethylamine, pyBOP = benzotriazol-1-yl-oxytrispyrrolidinophosphonium hexafluorophosphate.

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Table 1 Yields and aggregation properties of artificial glycolipids synthesized by SPLS

Compound	Total yield (%)	Aggregation properties in water		
		Diameter[a]/nm	Morphology[b]	T_c[c]/°C
Gal-asp(CO_2H)-2C_{12}	39	40–350	Vesicles	< 0
Gal-asp(NH_2)-2C_{12}	67	120–690	Vesicles	< 0
Man-asp(NH_2)-2C_{12}	91	660–1230	Vesicles	< 0
Glc-asp(NH_2)-2C_2	49	< 5	No aggregates	Not measured
Glc-asp(NH_2)-2C_6	56	< 5	No aggregates	Not measured
Glc-asp(NH_2)-2C_{10}	68	170–810	Vesicles	< 0
Glc-asp(NH_2)-2C_{12}	81	50–90, 200–810	Vesicles	< 0
Glc-asp(NH_2)-2C_{14}	59	90–370	Vesicles, helical rods	4–5
Glc-asp(NH_2)-2C_{16}	60	140–430	Vesicles, helical rods, tubes	23–25
Glc-asp(NH_2)-2C_{18}	33	Not dispersed		

[a] The aggregates were assumed to be spherical in calculation of diameters. [b] All samples were negatively-stained with uranyl acetate. [c] Phase transition behaviors of the bilayer membrane were estimated by DSC.

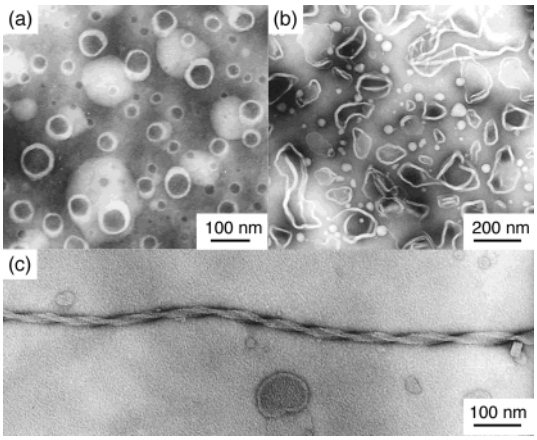

Fig. 2 TEM images of aggregates formed in 1.0 mM aqueous dispersions of artificial glycolipids. All samples were incubated at 35 °C for 12 h, followed by staining with uranyl acetate. The pH of the dispersed solution was *ca.* 6.2. (a) Gal-asp(NH_2)–2C_{12}, (b) Glc-asp(NH_2)–2C_{12}, (c) Glc-asp(NH_2)–2C_{14}.

ble in water without sonication whereas Glc-asp(NH_2)-2C_{18} was not dispersed even by prolonged sonication. Other glycolipids swelled in water, and then yielded a homogeneous dispersion displaying light-scattering properties upon sonication. The size distribution of the glycolipid aggregates in aqueous solution was studied by dynamic light scattering measurement (DLS, Otsuka DLS 7000). For Glc-asp(NH_2)-2C_2 and Glc-asp(NH_2)-2C_6, no or less than a few nm diameter of aggregates was determined, suggesting that these were dissolved as monomers or small micelles. DLS for the other lipids dispersed in aqueous solutions showed aggregate sizes is in the range 40–1230 nm.

Direct observation of these aggregates was conducted by transmission electron microscopy (TEM). No aggregates were observed for Glc-asp(NH_2)-2C_2 or Glc-asp(NH_2)-2C_6, consistent with data of their solubilities and DLS measurements. Fig. 2 shows typical TEM images of the artificial glycolipids. Both Glc-asp(NH_2)-2C_{12} and Glc-asp(CO_2H)-2C_{12} predominantly form into unilamellar vesicles. The layer width of these vesicles was roughly estimated to be 10 nm, which corresponds to the bimolecular length of Glc-asp(NH_2)-2C_{12}. Similarly, spherical vesicles were observed for Gal-asp(NH_2)-2C_{12} and Man-asp(NH_2)-2C_{12}, indicating that the morphology of these aggregates is not dependent on the head group structure of the monosaccharide. When the tail part becomes longer as for Glc-asp(NH_2)-2C_{14} and Glc-asp(NH_2)-2C_{16}, more developed structures such as helical rods and/or long tubes were observed, as well as vesicles.

Membrane fluidity of these aggregates was estimated by differential scanning calorimetry (DSC). The glycolipids bearing didodecylamine [Glc-asp(CO_2H)-2C_{12} and Glc-, Gal- or Man-asp(NH_2)-2C_{12}], do not show any endothermic peaks, suggesting that the phase transition temperature (T_c) is lower than 0 °C. Glc-asp(NH_2)-2C_{14} shows a rather broad peak at *ca.* 4–5 °C and the T_c of Glc-asp(NH_2)-2C_{16} was determined as 23–25 °C. The corresponding phase transition enthalpy (ΔH) was also determined to be 4–5 kcal mol^{-1} for Glc-asp(NH_2)-2C_{14} and 8–9 kcal mol^{-1} for Glc-asp(NH_2)-2C_{16}, comparable to that of typical natural phospholipids. It is clear that T_c gradually rises with an increase in the hydrophobic chain length, but these artificial glycolipids are still in the fluidic liquid crystal phase at room temperature.

In conclusion, we have developed a convenient SPLS method and successfully applied it to the construction of a small library of artificial glycolipids. All physicochemical data clearly demonstrated that some glycolipids form into stable aggregates consisting of bilayer membranes in aqueous solution. The proposed SPLS scheme is so flexible that we may envision a large library of glycolipids which confers valuable insights for the design of novel saccharide-based biomaterials, as well as elucidation of biological functions of natural glycolipids.

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10 ^{1}H NMR spectra of the final products obtained by hydrazinolysis suggest that the glycolipids were partially racemized at the connector site (8/2 = L-asp/D-asp).