Anionic lipids derived from \( \alpha \), \( \beta \), and \( \gamma \) glutamic acids, \( \alpha \)-aspartic acid, \( \alpha \)-lysine, \( \alpha \)-ornithine, and \( 1,2,4 \)-diaminobutyric acid were prepared and the way in which structurally related, solvatocromatic cationic styryl dyes were incorporated into these lipid aggregates were investigated. The \( \alpha \)- or \( \beta \)-glutamic acid-derived lipids with glutaric acid headgroups aggregated to form specific hydrophobic cavities which exhibited inclusion ability for the styryl dyes mainly based on the planarity recognition. The formation of such specific hydrophobic cavities can be achieved not only by introducing amide groups capable of complementary hydrogen bonds between neighbouring lipids in the aggregates but also by introducing appropriate spacer methylenes into, for example, glutarate headgroups. Side-chain methylenes of the amino acid residue were also found to play a significant role in the formation of specific hydrophobic cavities as well as the spacer methylenes. Such cavities were also formed for appropriately designed \( \alpha \)-lysine- and \( \alpha \)-ornithine-derived lipids. These results indicate that the specific incorporation is not peculiar to the glutamic acid-derived lipids but a general phenomenon for the aggregates from appropriately designed \( \alpha \)-amino acid-derived lipids. A difference in the manner of assembly of \( \alpha \)-glutamic acid residues between \( \alpha \)-, \( \beta \)-, and \( \gamma \)-isomers, and \( \alpha \beta \)-mixtures in the lipid aggregates is suggested not only by the \( \Delta_{\text{max}} \) shift of incorporated styryl dyes but also by aggregate morphologies as evidenced by the TEM observations. These results suggest that the two-dimensional assembly of pure enantiomeric \( \alpha \)-amino acid residues in appropriately designed lipids can produce specific hydrophobic cavities unless the \( \alpha \beta \)-mixture phase-separates into two enantiomeric components.

Introduction

Considerable attention has been focused on inclusion compounds and their molecular designs. Since the inclusion abilities of the conventional inclusion compounds such as cyclodextrins, calixarenes, cyclophanes, are restricted mostly by their primary structures (covalently linked macrocyclic structure), the size and the kind of guest molecule may be restricted to a considerable extent. Our interests have been focused on the construction of inclusion compounds by using self-assembly of component amphiphiles. In our previous papers, we have reported that certain \( \alpha \)-glutamic acid-derived anionic lipids formed specific hydrophobic cavities for cationic dyes. The cavities were able to incorporate the cationic dyes due mainly to molecular planarity of the dyes.” However, the specific behaviors seemed to be peculiar to the ester-type glutamate lipids such as \( \alpha \)-lysine. In order to generalize the inclusion behaviour by a wide variety of self-assembling compounds, it is necessary to extend the molecular structure from the \( \alpha \)-glutamic acid-derived lipids to other kinds of \( \alpha \)-amino acid-derived lipids. It is also necessary to gain insight into appropriate molecular designs of component lipids for the supramolecular receptors.

On the other hand, it is known that the side-chains of poly(amino acids) are crucial for formation of their secondary structures, e.g. \( \alpha \)-helix and \( \beta \)-structure in water. For example, poly(\( \alpha \)-lysine) (PLL) can adopt the \( \alpha \)-helix and \( \beta \)-structure in water, whereas the helix content of poly(\( \alpha \)-ornithine) (PLO), which has a shorter side-chain by one methylene than PLL, is considerably lower under the same conditions. Similar tendencies were observed for the following pairs, e.g. poly(\( \alpha \)-glutamic acid) (PLGA) and poly(\( \alpha \)-aspartic acid) (PLAA) which has a shorter side-chain by one methylene than PLGA, and PLO and poly(2,4-diaminobutyric acid) (PDLBA), which has a shorter side-chain by one methylene than PLO. In these cases, the difference in only one methylene group is crucial for the formation of secondary structures.

We are interested in how \( \alpha \)-amino acid residues play important roles when assembled in specific ways such as head-to-head orientation of the \( \alpha \)-amino acid-containing lipids. The effect of the side-chain on the intermolecular interaction between assembled amino acid residues would be very significant. Therefore, we have been investigating the construction of specific hydrophobic cavities which exhibit inclusion behaviour by means of self-assembly of appropriately designed lipids containing single \( \alpha \)-amino acid residues. The side-chain effect of poly(\( \alpha \)-amino acids), by which their secondary structures are remarkably affected, would become apparent in some way by two-dimensionally assembled single \( \alpha \)-amino acid residues in supramolecular lipid aggregates.

In this paper, we prepared various kinds of \( \alpha \)-amino acid-derived anionic lipids with plural amide groups per molecule (\( \alpha \)-glutamic acid-derived lipids \( 1-7 \), \( \alpha \)-aspartic acid-derived lipid \( 8 \), \( \alpha \)-lysine-derived lipid \( 10-13 \), \( \alpha \)-ornithine-derived lipids \( 14 \) and \( 15 \), and \( \alpha \)-2,4-diaminobutyric acid-derived lipid \( 16 \)) in order to investigate the effect of \( \alpha \)-amino acid residues and chemical structure requirements on formation of the specific hydrophobic cavities in water, because it had previously been confirmed that the \( \alpha \)-glutamic acid-derived lipids (such as \( 1-1 \) and \( 1-2 \)) with three amide groups per molecule can form complementary hydrogen bonds between neighbouring lipids when assembled with head-to-head orientation. The effect of the amino acid residues on the inclusion and the molecular recognition were investigated using the structurally related cationic styryl dyes (stilbazolium derivatives), 4-[4-(dimethylamino)styryl]-N-methylpyridinium iodide (abbreviated to St-4C, hereafter), 2-[4-(dimethylamino)styryl]-N-methylpyridinium iodide (abbreviated to St-2C, hereafter), and 2-[4-(dimethylamino)styryl]-N-ethylpyridinium iodide (abbreviated to St-2E, hereafter), and 2-[4-(dimethylamino)styryl]-N-ethylpyridinium iodide (abbreviated to St-2E, hereafter).
When lipid 1-1 is added to the aqueous solution of St-4Cl, $\lambda_{\text{max}}$ shifted to 478 nm, comparable to $\lambda_{\text{max}}$ in methanol at 20°C. This indicates that St-4Cl is incorporated into the hydrophobic microenvironment, that is, near the glutamate residue in the lipid aggregate in cooperation with hydrophobic and electrostatic interactions.² This conclusion is based on previous results.¹,⁴ When the temperature is raised, aggregates of 1-1 undergo a gel-to-liquid crystalline phase transition (peak-top temperature, 43°C by DSC) accompanied by a hypsochromic shift of $\lambda_{\text{max}}$ of St-4Cl. Further increases in temperature up to 65°C, at which the 1-1 aggregates are in a liquid crystalline state, lead to a $\lambda_{\text{max}}$ of 450 nm (Tables 1 and 2). This wavelength is comparable to the $\lambda_{\text{max}}$ of St-4Cl alone in water. This indicates that the microenvironment around the electrostatically bound and incorporated St-4Cl becomes more hydrophilic. A similar mechanism accounts for the hypsochromic shifts observed for other lipids in Table 1 with an increase in temperature (see footnotes of Tables 1 and 2). Note that, among the lipids in Table 1, aggregates of 1-3 induced the largest bathochromic shift in $\lambda_{\text{max}}$ to 510 nm at 20°C. This indicates that the most hydrophobic microenvironment was produced around St-4Cl by self-assembly of 1-3. However, no similar bathochromic shifts were induced by lipids 4-8. These results indicate that not only ester-type 1-9,1,4 but also certain kinds of amide-type lipids such as 1-1, 1-10, and 1-3 can produce specific hydrophobic cavities by self-assembly. Also note that slight changes in chemical structures considerably affect the polarity of hydrophobic cavities produced by self-assembly of these 1-glutamic acid-derived lipids.

### Effect of complementary hydrogen bonds between neighbouring lipids on formation of hydrophobic cavities

It is believed to be reasonable that the lipid 1-10 with two dodecyl chains leads to relatively looser packing of component lipids than that of 1-9 with two octadecyl chains.¹,⁴ In fact, as shown in Table 1, this resulted in no bathochromic shift of the $\lambda_{\text{max}}$ of St-4Cl in 1-10 aggregates at 20°C in contrast with a remarkable bathochromic shift in the 1-9 aggregates system. This indicates that the St-4Cl is located in the polar microenvironment in 1-10 aggregates. However, it is noted that the substitution of amide groups (1-1) for the corresponding ester groups (1-10) resulted in the bathochromic shift of St-4Cl. This indicates that the St-4Cl is located in the less polar microenvironment in 1-1 aggregates and that the amide groups can stabilize the molecular packing by complementary intermolecular hydrogen bonding. The complementary hydrogen bonds were previously examined by using the Corey-Pauling-Koltun (CPK) model and molecular mechanics (MM2) calculation in the CAChe molecular modeling program¹²,¹³,¹⁸,²⁰ (abbreviated to CAChe-MM2 hereafter). Three amide groups in 1-glutamic acid-derived lipid 1-1 can collaborate between neighbouring lipids in a similar manner to the related lipids with different headgroups.¹²,¹³,¹⁹,²⁰ assuming such conformations are possible only under head-to-head orientation. On the other hand, ester-type lipid 1-10 is incapable of forming such complementary hydrogen bonds. Such complementary hydrogen bonding by 1-1 occurs even in organic media, e.g., benzene.¹²,¹³,¹⁸,²¹ Owing to the remarkable difference in the visible absorption spectral behaviour between 1-1 and 1-10, the 1-1 aggregates are regarded as a model system showing cooperation of plural weak intermolecular interactions (hydrogen bonding and hydrophobic interactions in this case) essential in supramolecular chemistry.

### Results and discussion

#### Spectral behaviour of St-4Cl in the presence of anionic lipid assemblies

As reported previously,¹,⁴ the wavelengths at the ultraviolet-visible absorption maximum ($\lambda_{\text{max}}$) of St-4Cl in water and in methanol are 450 and 475 nm, respectively, regardless of the temperatures. This indicates that the St-4Cl has no thermochromic properties but exhibits solvatochromic behaviour.

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**References**

¹,²,³,⁴,⁵,⁶,⁷,⁸,⁹,¹⁰,¹¹,¹²,¹³,¹⁴,¹⁵,¹⁶,¹⁷,¹⁸,¹⁹,²⁰,²¹

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**Equations**

- $\lambda_{\text{max}}$ (maximum wavelength)
- CAChe-MM2 (molecular modeling program)
- CPK model (Corey-Pauling-Koltun model)
- MM2 (molecular mechanics)
- St-4Cl (stearic acid-4-chloro-1,8-naphthalimide)
- 1-glutamic acid-derived lipid
- 1-glutamic acid-derived lipid assemblies

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**Tables**

- Table 1: Spectral behaviour of St-4Cl in the presence of anionic lipid assemblies
- Table 2: Effects of complementary hydrogen bonds on formation of hydrophobic cavities
dispersed dyebound to the carboxylate and is not organic solvents.22 In general, it is believed that the lower the critical molar ratio at which the dyes are incorporated completely, the more easily the dyes are incorporated into the lipid aggregates. Therefore, the critical molar ratio a measure of the incorporation is mainly based on planarity recognition (inclusion). Table 2 shows the molar ratio variations of l-1 to St-4C, St-2C, and St-2C at fixed dye concentrations (1.5 × 10−4 mol dm−3), respectively. Fig. 1 also shows the molar ratio variations of l-1 to St-4C. The critical molar ratios of complete incorporation of these three dyes indicate that the order of preferential inclusion is St-4C > St-2C2 > St-2C in the l-1 aggregate systems because the critical molar ratios estimated from the data in Table 2 are as follows: 5 for St-4C, 10 for St-2C, ca. 20 for St-2C, i. The order of molecular planarity, based on their molecular structures, is considered to be St-4C > St-2C > 13,46 A semiempirical quantum mechanical calculation using MOPAC in the CAChe molecular modeling program also supported the order of molecular planarity in terms of the rotation angle of two aromatic rings in a molecule: 0° for St-4C, ca. 20° for St-2C, ca. 40° for St-2C. It is also noted that the order of preferential incorporation of the dyes in the l-9 aggregate system was St-4C > St-2C > St-2C as reported previously,4,13,4 indicating that the incorporation is mainly based on planarity recognition rather than the order of hydrophobicity (St-4C > St-2C > St-2C). However, the order of incorporation of St-2C and St-2C in the l-9 system is inverted in the l-1 system. In general, the higher the hydrophobicity of the dye, the more easily the dyes are incorporated at lower molar ratios of lipid to dye. In this respect St-2C is more hydrophobic than St-2C, because of the higher hydrophobicity of the N-ethyl group than the N-methyl group of St-2C. Therefore these contradictory results between l-1 and l-9 strongly suggest that hydrophobic interactions between longer alkyl chains (2C16H33) of lipid l-9 are preferred for the planarity recognition of the dyes than the collaboration of hydrophobic interactions between relatively shorter alkyl chains (2C6H13) and complementary hydrogen bonding between amide groups of lipid l-1.

Effect of amino acid residues on inclusion and planarity recognition

It is noted that the specific hydrophobic cavities were also formed from L-lysine-derived lipids (l-11 and l-12) and the l-ornithine-derived lipid (l-14 and l-15) as shown in Tables 1 and 2, as evidenced by the bathochromic shift of λmax at 20°C. This indicates that the formation of such specific hydrophobic cavities is not restricted to the l- or D-glutamic acid-derived lipids (1-3, 9). However, a subtle difference in the chemical structures between lipids l-12 and l-13 affected the incorporation of St-4C considerably. For example, lipid l-12 with urethane (carbamate) groups incorporated St-4C, whereas

Table 1 Dispersion state of St-4C in the presence of various lipids in water ([St-4C] = 1.5 × 10−4 mol dm−3 = const., pH 10.0)

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Aggregate morphologies</th>
<th>[Lipid]/[St-4C]</th>
<th>λmax/nm of St-4C</th>
<th>Dispersion state of St-4C at 20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (in water)</td>
<td>—</td>
<td>0</td>
<td>450</td>
<td>450</td>
</tr>
<tr>
<td>None (in methanol)</td>
<td>—</td>
<td>0</td>
<td>475</td>
<td>475</td>
</tr>
<tr>
<td>l-1</td>
<td>Helices and tubules</td>
<td>20</td>
<td>478</td>
<td>450</td>
</tr>
<tr>
<td>d-1</td>
<td>Helices and tubules</td>
<td>20</td>
<td>480</td>
<td>445</td>
</tr>
<tr>
<td>DL-1</td>
<td>Tubes</td>
<td>20</td>
<td>448</td>
<td>448</td>
</tr>
<tr>
<td>l-2</td>
<td>Helices and tubules</td>
<td>20</td>
<td>462</td>
<td>445</td>
</tr>
<tr>
<td>l-3</td>
<td>Tubes</td>
<td>20</td>
<td>510</td>
<td>450</td>
</tr>
<tr>
<td>l-4</td>
<td>Tubes</td>
<td>20</td>
<td>458</td>
<td>455</td>
</tr>
<tr>
<td>d-5</td>
<td>Tubes</td>
<td>20</td>
<td>450</td>
<td>450</td>
</tr>
<tr>
<td>l-6</td>
<td>Vesicular aggregates</td>
<td>20</td>
<td>454</td>
<td>454</td>
</tr>
<tr>
<td>l-7</td>
<td>No structure</td>
<td>20</td>
<td>450</td>
<td>450</td>
</tr>
<tr>
<td>l-8</td>
<td>Tubes</td>
<td>20</td>
<td>452</td>
<td>452</td>
</tr>
<tr>
<td>l-9-1,3,4</td>
<td>Vesicular aggregates</td>
<td>20</td>
<td>475</td>
<td>444</td>
</tr>
<tr>
<td>DL-9-1,4</td>
<td>Vesicular aggregates</td>
<td>20</td>
<td>458</td>
<td>444</td>
</tr>
<tr>
<td>l-10</td>
<td>Tape-like aggregates</td>
<td>20</td>
<td>443</td>
<td>443</td>
</tr>
<tr>
<td>l-11</td>
<td>Vesicular aggregates</td>
<td>20</td>
<td>469</td>
<td>448</td>
</tr>
<tr>
<td>l-12</td>
<td>Tubes and vesicles</td>
<td>20</td>
<td>481</td>
<td>462</td>
</tr>
<tr>
<td>l-13</td>
<td>Tubes and vesicles</td>
<td>20</td>
<td>(525)</td>
<td>(525)</td>
</tr>
<tr>
<td>l-14</td>
<td>Vesicular aggregates</td>
<td>20</td>
<td>456</td>
<td>447</td>
</tr>
<tr>
<td>l-15</td>
<td>Tubes and vesicles</td>
<td>20</td>
<td>471</td>
<td>443</td>
</tr>
<tr>
<td>l-16</td>
<td>Vesicular aggregates</td>
<td>20</td>
<td>448</td>
<td>448</td>
</tr>
</tbody>
</table>

* M: molecularly dispersed monomer of St-4C. Measured at 60°C because the boiling point of methanol is 65°C. M: incorporated monomer as evidenced by the bathochromic shift of λmax, comparable to that in methanol with an increase in lipid concentration. In general, increasing the molar ratio of lipid to dye leads to an aggregate-to-monomer transition of the dye. M: monomer of St-4C bound to the carboxylate and is not incorporated. The anomalous bathochromic shift is not due to the J-aggregates but due to the most hydrophobic microenvironment in which St-4C is incorporated. M: molecularly dispersed monomer of St-4C, because l-7 shows no aggregation behaviour. J, J-aggregates of St-4C bound to the surface of lipid aggregates: this is supported by the fact that J-aggregates are converted to monomeric dye species when the molar ratio of lipid to dye is increased as shown in Table 2.
the CAChe-MM2 calculation, no appreciable difference was observed between aggregation modes of J-aggregation schematically in Fig. 2. However, the fact that three dyes indicate that the order of preferential inclusion is also shows the molar ratio variations of molar ratio of lipid to dye.

oxamide group.
carbamate group is more polar and/or

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Table 2 Dispersion state of various dyes in the presence of various lipids in water ([Dye] = 1.5 × 10⁻⁴ mol dm⁻³ = const., pH 10.0)

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Dye</th>
<th>[Lipid]/[Dye]</th>
<th>(\lambda_{\text{max}}) of dye/nm</th>
<th>Dispersion state of dye at 20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>St-4C₁</td>
<td>0 (in MeOH)</td>
<td>450 at 20°C; 450 at 65°C</td>
<td>M⁺</td>
</tr>
<tr>
<td>None</td>
<td>St-2C₁</td>
<td>0 (in MeOH)</td>
<td>435 at 20°C; 435 at 65°C</td>
<td>M⁺</td>
</tr>
<tr>
<td>None</td>
<td>St-2C₂</td>
<td>0 (in MeOH)</td>
<td>434 at 20°C; 434 at 65°C</td>
<td>M⁺</td>
</tr>
<tr>
<td>1-1</td>
<td>St-4C₁</td>
<td>17.5</td>
<td>461 at 20°C; 461 at 65°C</td>
<td>M⁺</td>
</tr>
<tr>
<td>1-1</td>
<td>St-2C₁</td>
<td>10</td>
<td>455 at 20°C; 455 at 65°C</td>
<td>M⁺</td>
</tr>
<tr>
<td>1-1</td>
<td>St-2C₂</td>
<td>5</td>
<td>443 at 20°C; 443 at 65°C</td>
<td>M⁺</td>
</tr>
<tr>
<td>1-2</td>
<td>St-4C₁</td>
<td>10</td>
<td>473 at 20°C; 473 at 65°C</td>
<td>M⁺</td>
</tr>
<tr>
<td>1-12</td>
<td>St-4C₁</td>
<td>10</td>
<td>483 at 20°C; 483 at 65°C</td>
<td>M⁺</td>
</tr>
<tr>
<td>1-12</td>
<td>St-2C₁</td>
<td>10</td>
<td>468 at 20°C; 468 at 65°C</td>
<td>M⁺</td>
</tr>
<tr>
<td>1-12</td>
<td>St-2C₂</td>
<td>10</td>
<td>461 at 20°C; 461 at 65°C</td>
<td>M⁺</td>
</tr>
<tr>
<td>1-15</td>
<td>St-4C₁</td>
<td>10</td>
<td>475 at 20°C; 475 at 65°C</td>
<td>M⁺</td>
</tr>
</tbody>
</table>

\(\ast\) M⁺; molecularly dispersed monomer. \(\ast\) Measured at 60°C because the boiling point of methanol is 65°C. \(\ast\) M⁺ incorporated monomer as evidenced by the bathochromic shift of \(\lambda_{\text{max}}\) comparable to that in methanol with increase in lipid concentrations. In general, increasing the molar ratio of lipid to dye leads to aggregate-to-monomer transition of the dye. Therefore, it is concluded that J aggregates bound to the surface of lipid aggregates: this is supported by the fact that, in general, aggregated dye species are converted to monomeric dye species when the molar ratio of lipid to dye is increased. The anomalous bathochromic shifts shown in parentheses do not result from dye incorporation.

lipid 1-13 with amide groups could not incorporate St-4C₁ but induced J aggregates.⁵ According to the CPK model and the CAChe-MM2 calculation, no appreciable difference was observed between aggregation modes of 1-12 and 1-13, shown schematically in Fig. 2. However, the fact that 1-13 induced J-aggregation⁴ of St-4C₁ strongly suggests that 1-13 is more densely packed than 1-12 and enough to lead to the non-incorporation of St-4C₁.† This may be related to the fact that a carbamate group is more polar and/or flexible than a carbamoyl group.

Next, incorporation preference of St-4C₁, St-2C₁, and St-2C₂ by aggregates of 1-12 was investigated. Table 2 shows the molar ratio variations of 1-12 to St-4C₁, St-2C₁, and St-2C₂ at fixed dye concentrations (1.5 × 10⁻⁴ mol dm⁻³), respectively. Fig. 1 also shows the molar ratio variations of 1-12 to St-4C₁. The critical molar ratios of complete incorporation of these three dyes indicate that the order of preferential inclusion is Sn-4C₁ > St-2C₁ > St-2C₂ in the 1-12 aggregate systems, because the critical molar ratios estimated from the data in Table 2 are as follows: 6 for St-4C₁, 17.5 for St-2C₂, and 20 for St-2C₁. This order of incorporation preference is in good agreement with that for 1-9 aggregate systems,⁵ indicating that the aggregates of 1-12 can recognize the molecular planarity of these dyes more preferentially than their hydrophobicity as well as in the systems of 1-9.⁵ Therefore, it is concluded that the recognition of molecular planarity is not restricted to the \(\alpha\)-glutamic acid-derived lipids (1-3, 9) and may be a general phenomena for appropriately designed \(\alpha\)-amino acid-derived lipids such as 1-12.

Effect of side-chain methylene number of amino acid residue on inclusion and planarity recognition

It is noted that the bathochromic shifts of St-4C₁ are more significant for the corresponding lipids with longer side-chains (11 > 14 > 16, and 12 > 15) as shown in Table 1. These results suggest that a longer side-chain is preferred for the formation of more hydrophobic cavities. It is noted that St-4C₁ are more

† It is considered that the J aggregates are not incorporated into the lipid aggregates because they are believed to be composed of 4-7 dye molecules per dye aggregate.⁵
aggregates of the speciﬁc polar headgroups also play an important role for formation of β-ene numbers. These results support the previous conclusion

1,3-cyclohexane dicarboxamide-type dyes. Binding constant (Table 3)

Fig. 2  Schematic representation of complementary hydrogen bonding

between amide groups in the main chain and side-chain of lipids of carboxamate-type 1-12 (a) and amide-type 1-13 (b).

Table 3  Binding constant ($K_s$) of the cationic dyes to the anionic lipid aggregates of 1-1, 1-9, and 1-12

<table>
<thead>
<tr>
<th>Lipids</th>
<th>Dyes</th>
<th>$K_s$/(dm$^3$/mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-1</td>
<td>St-4C$_1$</td>
<td>$3 \times 10^4$</td>
</tr>
<tr>
<td></td>
<td>St-2C$_1$</td>
<td>$2 \times 10^4$</td>
</tr>
<tr>
<td>1-9</td>
<td>St-4C$_1$</td>
<td>$1 \times 10^4$</td>
</tr>
<tr>
<td></td>
<td>St-2C$_1$</td>
<td>$1 \times 10^4$</td>
</tr>
<tr>
<td>1-12</td>
<td>St-4C$_1$</td>
<td>$2 \times 10^4$</td>
</tr>
<tr>
<td></td>
<td>St-2C$_1$</td>
<td>$2 \times 10^4$</td>
</tr>
<tr>
<td></td>
<td>St-2C$_2$</td>
<td>$1 \times 10^4$</td>
</tr>
<tr>
<td></td>
<td>St-4C$_2$</td>
<td>$1 \times 10^4$</td>
</tr>
<tr>
<td>1-15</td>
<td>St-4C$_1$</td>
<td>$1 \times 10^4$</td>
</tr>
</tbody>
</table>

preferentially incorporated into aggregates of 1-12 ($m = 4$) than 1-15 ($m = 3$) which has one less side-chain methylene number than 1-12, as indicated by the arrows at the critical molar ratios of complete incorporation in Fig. 1. It is also noted that the $\lambda_{\max}$ of St-4C$_1$ in the presence of lipids 1-12 and 1-15 with β-alanine head groups are more bathochromic than those for the corresponding lipids 1-11 and 1-14 without β-alanine residues as shown in Table 1. This indicates that the spacer methylene numbers between α-amino acid residues and polar headgroups also play an important role for formation of the specific hydrophobic cavities as well as the side-chain methylene numbers. These results support the previous conclusion

that the St-4C$_1$ is incorporated in between the polar headgroups (including hydrophobic spacer methylenes) and α-amino acid residues (including side-chain) with the cooperation of electrostatic interaction and hydrophobic interaction under aggregation of the lipids with head-to-head orientation. It is, therefore, concluded that the specific hydrophobic cavities can be produced by single α-amino acid residue-containing lipids, based on the cooperation of hydrophobic interactions (by long chain alkyl groups, side-chain methylenes, and spacer moieties) and complementary hydrogen bonding between amide or carbamate groups. The balance of the cooperation can be regulated by appropriate molecular designs. Hydrophobicity of side-chain methylenes of amino acids has a large influence on the formation of the specific hydrophobic cavities as well as on the secondary structures of the corresponding poly(α-amino acids).

Binding constants of cationic dyes to the anionic lipid aggregates

The binding constant ($K_s$) determined from the slope of the equation suggested by Sepúlveda et al.\textsuperscript{24,25} are listed in Table 3. Calculations were based on the values listed in Table 2, using the following equation: $f(1 - f) = K_s[D_s - [S]}] - K_{cac}$, in which $f$ denotes the fraction [aggregate-incorporated dye]/[total dye], $D_s$ is the total lipid concentration, $S$ is the total dye concentration, and cac is the critical aggregation concentration of the lipid, respectively. For the calculation of $f$, variation of $\lambda_{\max}$ was used instead of the variation of absorbance, because absorbance was less reproducible for the dyes used in this study. It is noted that the $K_s$ values for the St-4C$_1$ were remarkably enhanced as compared with those for St-2C$_1$ and St-2C$_2$. Although $K_s$ values for St-4C$_1$ are considered to contain considerable experimental errors because only two data points were used for the calculation of the slope ($K_s$) due to the drastic change of $\lambda_{\max}$ for St-4C$_1$ at the narrow molar ratio range. However, these $K_s$ values may be used as comparisons for systematic interpretation.

Formation of uniform tubular aggregates from racemic glutamic acid-derived lipids

Fig. 3 shows the TEM images for lipids 1-1 (a) and DL-1 (b). Aggregate morphologies for 1-1 in water are observed as a mixture of helical and tubular aggregates. Similar aggregates were observed for 1-1 with opposite helical sense (right-handed for 1-1), left-handed for 1-1). However, only tubular aggregates were observed and no helical aggregates were observed for the DL-1. This suggests that 1-1 and 1-1 are completely miscible, resulting in no helical aggregates, only tubular aggregates. Remarkable bathochromic shift in the $\lambda_{\max}$ of St-4C$_1$ incorporated in aggregates of enantiomeric L-1/DL-1 is also consistent with the difference in the polarity of microenvironments in which the St-4C$_1$ is incorporated since no $\lambda_{\max}$ shift was observed in the aggregates of DL-1 even at a high molar ratio as shown in Fig. 1. These results indicate that the specific hydrophobic cavities are formed not from racemic but from enantiomeric L-glutamic acid-derived lipids.

Conclusions

It has been clarified that the formation of supramolecular receptors from α-amino acid-derived lipids is possible as long as the component lipids are appropriately designed. The molecular structure requirements clarified/suggested in this study are as follows. i) Enantiomeric α-amino acids should be used because the Dl-glutamic acid-derived lipids were incapable of inclusion. However, if the racemic lipids phase-separate into

two enantiomeric components in water, the racemic lipids would show the same results as enantiomeric ones. ii) A relatively longer spacer is preferred for the design of the lipid. iii) Acidic amino acid (glutamic acid) and basic amino acids (lysine, ornithine) with relatively longer side-chains can be used. iv) For the \( \alpha \)-glutamic acid-derived lipids, it is possible to use ester bondings instead of amide bondings when much longer alkyl chains are used.\(^{1,4}\) In other words, when alkyl chains are relatively shorter, such as dodecyl groups, amide groups should be used instead of ester groups. v) An enantiomeric \( \alpha \)-glutamic acid-derived lipid with at least three amide groups is a necessary condition for the formation of supramolecular helical aggregates. The corresponding racemic lipids led to only the tubular aggregates but no helical aggregates. vi) Although the assembling manner of \( \alpha \)-amino acid-derived lipids in water can be inferred to some extent using CPK molecular model, CAChe-MM2, and/or CAChe-MOPAC, it cannot necessarily be precisely predicted whether the lipid aggregates are capable of specific inclusion of dyes, as is schematically seen for t-12 and t-13 (Fig. 2 and Table 1).

In conclusion, the two-dimensional arrangement of enantiomeric single \( \alpha \)-amino acid residues in anionic lipid assemblies can produce specific hydrophobic cavities. Although the present system is not necessarily superior to those of conventional macrocyclic receptors, it may be meaningful that one methodology has been demonstrated without using the closed structure. Supramolecular receptors, e.g., helical aggregates from lipid t-1/0-1, possessing such specific hydrophobic cavities are considered to have potential applications to many fields.
N',N'-Dibutyl-N'-benzoylcarbonyl-L-γ-lactamidene (t18).  
N-Benzoylcarbonyl-L-glutamic acid (L-5)\(\text{H} \cdot \text{Na}_{\text{OH}}\) (3.0 g, 5.0 \times 10^{-3} \text{ mol}) was dissolved in 300 cm\(^3\) of ethanol with heating and Pd black (1 g) was added to the solution. H\(_2\) gas was bubbled slowly into the solution for 5 hours. After removal of the benzyl group, Pd black was removed by filtration. The solution was concentrated in vacuo. The residue was recrystallized from methanol to give a white powder (t19): yield 1.2 g (68%); mp 138–139 °C; \(\nu \text{CHC} = 3304, 2922, 2854, 1632, 1545 \text{ \text{cm}^{-1}}\).

N',N'-Dibutyl-N'-benzoylcarbonyl-L-γ-lactamidene (t17).  
3-Methylglutaric anhydride (0.59 g, 4.66 \times 10^{-3} \text{ mol}) was dissolved in 200 cm\(^3\) of THF. The solution was concentrated in vacuo, and the residue was dissolved in 200 cm\(^3\) of chloroform. The solution was washed with 1 M NaOH, 0.2 M HCl, and water. The residue was dried over Na\(_2\text{SO}_4\) and concentrated in vacuo to give a waxy solid (t18): yield 232 mg (96%); mp 172–174 °C; \(\nu \text{CHC} = 3304, 2922, 2854, 1647 \text{ \text{cm}^{-1}}\).

N',N'-Dibutyl-N'-benzoylcarbonyl-L-γ-lactamidene (t16).  
\(\text{CH}_{10} \text{COCl}\) (5.8 g, 3.3 \times 10^{-3} \text{ mol}) was dissolved in 50 cm\(^3\) of acetone containing 12.5 g (4.10 \times 10^{-3} \text{ mol}) of hexadecyl chlorofluoroam was added dropwise to the solution and vigorously stirred for 2 days to give a slurry-like suspension. The pH of the mixture was adjusted to 2 and stirred for 3 h. After filtration, the residue was recrystallized from ethanol and dried in vacuo to give a white solid (t17): yield 6.9 g (62%); mp 71–75 °C; \(\nu \text{CHC} = 3304, 2922, 2854, 1700, 1647 \text{ \text{cm}^{-1}}\).

N',N'-Dibutyl-N'-benzoylcarbonyl-L-γ-lactamidene (t15).  
\(\text{CH}_{10} \text{COCl}\) (3.0 g, 5.0 \times 10^{-3} \text{ mol}) was dissolved in 200 cm\(^3\) of THF. The solution was concentrated in vacuo, and the residue was recrystallized from methanol and dried in vacuo to give a waxy solid (t16): yield 232 mg (96%); mp 172–174 °C; \(\nu \text{CHC} = 3304, 2922, 2854, 1647 \text{ \text{cm}^{-1}}\).

N',N'-Dibutyl-N'-benzoylcarbonyl-L-γ-lactamidene (t14).  
\(\text{CH}_{10} \text{COCl}\) (3.0 g, 5.0 \times 10^{-3} \text{ mol}) was dissolved in 200 cm\(^3\) of THF. The solution was concentrated in vacuo, and the residue was recrystallized from methanol and dried in vacuo to give a waxy solid (t15): yield 232 mg (96%); mp 172–174 °C; \(\nu \text{CHC} = 3304, 2922, 2854, 1647 \text{ \text{cm}^{-1}}\).

N',N'-Dibutyl-N'-benzoylcarbonyl-L-γ-lactamidene (t13).  
\(\text{CH}_{10} \text{COCl}\) (3.0 g, 5.0 \times 10^{-3} \text{ mol}) was dissolved in 200 cm\(^3\) of THF. The solution was concentrated in vacuo, and the residue was recrystallized from methanol and dried in vacuo to give a waxy solid (t14): yield 232 mg (96%); mp 172–174 °C; \(\nu \text{CHC} = 3304, 2922, 2854, 1647 \text{ \text{cm}^{-1}}\).
sulfonate (23) (1.66 g, 4.89 × 10⁻³ mol) and triethylamine (1.33 g, 1.32 × 10⁻³ mol) was added to the solution and stirred with cooling. Then DECP (1.07 g, 6.10 × 10⁻³ mol) was added to the solution with cooling. After being stirred for 1 day at room temperature, the solution was washed with 5% NaHCO₃, 0.2 M HCl, and water. The solution was dried over Na₂SO₄ and concentrated in vacuo. The residue was recrystallized from methanol and dried in vacuo to give a white powder (t-24): yield 2.5 g (77%); mp 72–76°C; νmax(KBr)/cm⁻¹ 3296, 2962, 2934, 1738, 1698, 1651 and 1557; δ-H-NMR (CDCl₃) δ 0.70–1.00 (m, 6H, CH₃), 1.05–1.95 (m, 60H, (CH₂)₄₂ × 2, *CH(CH₃)₂), 2.45–2.70 (m, 2H, CH₂C(O)), 3.03–3.72 (m, 4H, NHCH₂ × 2), 3.90–4.51 (m, 5H, CH₂O × 2, *CH), 5.05–5.20 (m, 2H, CH₂C(Ο)H₂), 7.20–7.50 (m, 5H, C₆H₅) (Anal. Found: C, 71.77; H, 10.63; N, 5.02%).

\( N^+\beta-N^-\text{Bis(hexadecyloxyphenyl)-N-[2-(benzyloxybenzyl)-}
\text{ethyl]-l-ornithinamide (t-25).} \) t-25 was prepared as described above using 1-t-14 instead of t-11: yield 2.0 g (54%); mp 53.0–
57.9°C; νmax(KBr)/cm⁻¹ 3308, 2962, 2934, 1738, 1692, 1651 and 1555; δ-H-NMR (CDCl₃) δ 0.70–1.00 (m, 6H, CH₃), 1.05–1.95 (m, 60H, (CH₂)₄₂ × 2, *CH(CH₃)₂), 2.45–2.71 (m, 2H, CH₂C(O)), 3.05–3.85 (m, 5H, NHCH₂ × 2), 3.90–4.20 (m, 4H, CH₂O × 2, 4.60–6.00 (m, 2H, CH₂C(Ο)H₂), 5.05–5.20 (m, 2H, CH₂C(Ο)H₂), 5.25–
5.50, 7.28–7.40 (m, 5H, C₆H₅) (Anal. Found: C, 69.97; H, 10.72; N, 4.73). Calc. for C₃₄H₅₇NO₂·0.6H₂O: C, 69.97; H, 10.57; N, 5.00%.

\( N^+\beta-N^-\text{Bis(hexadecyloxyphenyl)-N-[2-carboxyethyl]-}
\text{l-lysinate (t-12).} \) t-24 (1.47 g, 1.73 × 10⁻³ mol) was dissolved in ethanol (300 cm³) with heating and Pd black (1 g) was added to the solution. H₂ gas was bubbled slowly into the solution for 6 h. After removal of the benzyl group, Pd black was removed by filtration. The solution was concentrated and dried in vacuo to give a white powder (t-12): yield 0.95 g (73%); mp 126–129°C; νmax(KBr)/cm⁻¹ 3326, 2962, 2934, 1698, 1655 and 1555; δ-H-NMR (CDCl₃) δ 0.70–1.00 (m, 6H, CH₃), 1.00–1.40 (m, 62H, (CH₂)₄₂ × 2, *CH(CH₃)₂), 2.45–2.70 (m, 2H, CH₂C(Ο)H₂), 3.00–3.70 (m, 5H, NHCH₂ × 2), 3.90–4.15 (m, 5H, CH₂O × 2, *CH) (Anal. Found: C, 76.78; H, 10.95; N, 5.51. Calc. for C₃₄H₅₇NO₂·0.4H₂O: C, 76.78; H, 11.10; N, 5.52%).

\( N^+\beta-N^-\text{Bis(hexadecyloxyphenyl)-N-[2-carboxyethyl]-}
\text{l-ornithinamide (t-15).} \) t-15 was prepared as described above using t-14 instead of t-11: yield 1.1 g (84%); mp 45.0–52.0°C; νmax(KBr)/cm⁻¹ 3310, 2962, 2934, 1692, 1653 and 1547; δ-H-NMR (CDCl₃) δ 0.70–1.05 (m, 6H, CH₃), 1.05–1.95 (m, 60H, (CH₂)₄₂ × 2, *CH(CH₃)₂), 2.45–2.71 (m, 2H, CH₂C(O)), 2.95–3.75 (m, 4H, NHCH₂ × 2), 3.80–4.50 (m, 5H, CH₂O × 2, *CH) (Anal. Found: C, 67.39; H, 11.09; N, 5.33. Calc. for C₃₄H₅₇NO₂·0.3H₂O: C, 67.39; H, 10.97; N, 5.75%).

\( 1\text{-Lysine benzyl ester bis(toluene-p-sulfonate) (t-26).} \) t-26 was prepared according to the literature.16 white powder, yield 2.0 g (13%); mp 148–151°C; νmax(KBr)/cm⁻¹ 3436, 3034, 1752, 1526, 1218 and 1176.

\( N^+\beta-N^-\text{Bis(hexadecanoyl)-l-lysine benzyl ester (t-29).} \) t-28 (1.90 g, 3.27 × 10⁻³ mol) was dissolved in 80 cm³ of chloroform. The solution was cooled to 0°C, and triethylamine (1.65 g, 1.64 × 10⁻² mol) was added to the solution. A 20 cm³ portion of chloroform containing 1.98 g (7.19 × 10⁻² mol) of palmitoyl chloride was added dropwise to the solution with cooling. After being stirred for 2 days at room temperature, the solution was washed with 5% NaHCO₃, 0.2 M HCl, and water. The solution was dried over Na₂SO₄ and concentrated in vacuo. The residue was recrystallized from methanol and dried in vacuo to give a white powder (t-28): yield 1.7 g (70%); mp 92.0–94.5°C; νmax(KBr)/cm⁻¹ 3318, 2962, 2934, 1750, 1642 and 1555; δ-H-NMR (CDCl₃) δ 0.70–1.00 (m, 6H, CH₃ × 2), 1.00–1.40 (m, 58H, (CH₂)₄₂ × 2, *CH(Η₂)₂), 2.05–2.30 (m, 4H, CH₂C(Ο)), 3.05–3.40 (m, 4H, NHCH₂), 4.40–4.82 (m, 1H, *CH), 5.10–5.20 (m, 2H, CH₂C(Ο)H₂), 6.20–6.45 (m, 4H, 2H, NHCH₂), 7.20–7.50 (m, 5H, C₆H₅).

\( N^+\beta-N^-\text{Bis(hexadecanoyl)-l-diaminoobutyric acid benzyl ester (t-31).} \) t-27 (1.00 g, 1.82 × 10⁻³ mol) was dissolved in 80 cm³ of chloroform. The solution was cooled to 0°C, and triethylamine (0.92 g, 9.1 × 10⁻³ mol) was added to the solution. A 20 cm³ portion of chloroform containing 1.2 g (4.0 × 10⁻³ mol) of n-hexadecyl chloroformate was added dropwise to the solution with cooling. After being stirred for 1 day at room temperature, the solution was washed with 5% NaHCO₃, 0.2 M HCl, and water. The solution was dried over Na₂SO₄ and concentrated in vacuo. The residue was recrystallized from methanol and dried in vacuo to give a white powder (t-31): yield 0.77 g (56%); mp 38.0–42.6°C; νmax(KBr)/cm⁻¹ 3310, 2962, 2934, 1738, 1690 and 1545; δ-H-NMR (CDCl₃)
δ 0.70–1.00 (m, 6H, CH2 × 2), 1.00–1.80 (m, 58H, (CH2)4 × 2, *CH(CH3)2) 2.80–3.30 (m, 2H, NHCH2), 3.50–3.80 (m, 1H, *CH), 3.85–4.20 (m, 4H, CH2O × 2), 5.15–5.20 (m, 2H, CH2CH2CH2), 7.20–7.45 (s, 5H, C6H5).

1-L,N,N,N’-Bis(hexadecyloxycarbonyl)-2,4-diaminobutyric acid (t-16), t-31 (0.77 g, 1.0 × 10⁻³ mol) was dissolved in 250 cm³ of ethanol with heating and Pd black (1 g) was added to the solution. H₂ gas was bubbled slowly into the solution for 3 h. After removal of the benzyl group, Pd black was removed by filtration. The solution was concentrated in vacuo. The residue was recrystallized from methanol and dried in vacuo to give white powder (t-16): yield: 0.60 g (87%); mp 57.7–58.8 °C; δ max(KBr)/\cm⁻¹ 3310, 2962, 2934, 1698 and 1562; 1H-NMR (CDCl3) δ 0.70–1.05 (m, 6H, CH₂ × 2), 1.00–1.80 (m, 58H, (CH₂)₄ × 2, *CH(CH₃)₂) 2.80–3.30 (m, 2H, NHCH₂), 3.50–3.80 (m, 1H, *CH), 3.85–4.20 (m, 4H, CH₂O × 2).

Characterization of the chemical structures of all the compounds synthesized were confirmed by Fourier transform infrared spectroscopy (FTIR) measurement with a JASCO FT/IR-7000, by 1H NMR measurement with a JEOL JNM-EX-90, and by elemental analysis with a Yanaco CHN Corder MT-3.

Preparation of aqueous solutions of lipids

The lipids were suspended in water (pH 10) and quickly heated in hot water prior to sonication. The suspension was then sonicated using a Yanoako CHN Corder MT-3.

Preparation of an aqueous lipid–dye mixture

All solutions of the lipid and dye mixtures were prepared by addition of the stock solution of the dyes to aqueous dispersions of the lipids and subsequent sonication. After adjustment of the pH to 10.0 with sodium hydroxide and hydrochloric acid, the solutions were used for visible absorption spectra measurements.

Visible absorption spectra measurements

The samples in a 1 mm quartz cell were incubated for 15 min at selected temperatures. The visible absorption spectra were measured with a JASCO Ubest 35 spectrophotometer.

Characterization of lipid aggregates

Formation of highly ordered lipid aggregates in water was confirmed by using transmission electron microscopy (TEM) with a JEOL 2000FX transmission electron microscope. The aqueous samples (6 × 10⁻⁴ mol dm⁻³, pH 10) were spotted onto carbon-coated copper grids. The samples were air-dried at room temperature, after which they were stained with 2 wt% aqueous ammonium molybdate. The phase transition temperature was measured by differential scanning calorimetry (DSC) with a SEIKO I&E DSC 120. The sample solution (20 mmol dm⁻³, pH 10) was sealed in an Ag capsule and scanned using a heating rate of 2 °C min⁻¹. Gel-to-liquid crystalline phase transition temperatures are as follows: 45 °C for t-1; 35, 42, and 45 °C for t-15; 36, 44, and 46 °C for t-16; 38 °C for t-1, 40 °C for t-3; no detection for t-4; 47, 57, 54, 39, and 27 °C for t-5; 23 °C for t-6; no detection for t-7; 88 °C for t-8; 46 °C for t-11; 68 °C for t-12; 79 °C for t-13; 52 °C for t-14; 52 and 76 °C for t-15; 62 °C for t-16. It was also observed using TEM that most of the lipids (except for t-7 with short alkyl chains) formed highly-oriented lipid aggregates based on lipid bilayer structures in water. Aggregate morphologies are summarized in Table 1. It is noted that there is no particular relationship between the different aggregate morphologies and the dispersion states of the dyestatic.

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