Supramolecular receptors from α-amino acid-derived lipids

Hiroshi Hachisako,*[a] Yutaka Murata[a] and Hirotaka Ihara[b]

[a] *Department of Applied Chemistry, Kumamoto Institute of Technology, 4-22-1 Ikeda, Kumamoto 860-0082, Japan*
[b] *Department of Applied Chemistry & Biochemistry, Faculty of Engineering, Kumamoto University, 2-39-1 Kurokami, Kumamoto 860-8555, Japan*

Received (in Cambridge, UK) 8th May 1999, Accepted 2nd September 1999

Anionic lipids derived from L-, D-, DL-glutamic acids, L-aspartic acid, L-lysine, L-ornithine, and L-2,4-diaminobutyric acid were prepared and the way in which structurally related, solvatochromic cationic styryl dyes were incorporated into these lipid aggregates were investigated. The L- or D-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 bondings 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 L-lysine- and L-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 L-amino acid-derived lipids. A difference in the manner of assembly of L-glutamic acid residues between L-, D-isomers, and DL-mixtures in the lipid aggregates is suggested not only by the λ_{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 α-amino acid residues in appropriately designed lipids can produce specific hydrophobic cavities unless the DL-mixture phase-separates into two enantiomeric components.

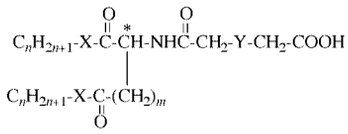
Introduction

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,[1–4] we have reported that certain L-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.[3,4] However, the specific behaviors seemed to be peculiar to the ester-type glutamate lipids such as L-**9**. 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 L-glutamic acid-derived lipids to other kinds of α-amino acid-derived lipids. It is also necessary to gain insight into appropriate molecular designs of component lipids for the supramolecular receptors.

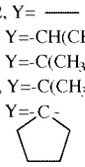
On the other hand, it is known that the side-chains of poly(amino acid)s are crucial for formation of their secondary structures, *e.g.* α-helix and β-structure in water.[5–11] For example, poly(L-lysine) (PLL) can adopt the α-helix and β-structure in water, whereas the helix content of poly(L-ornithine) (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(L-glutamic acid) (PLGA) and poly(L-aspartic acid) (PLAA) which has a shorter side-chain by one methylene than PLGA, and PLO and poly(2,4-diaminobutyric acid) (PLDBA), 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 α-amino acid residues play important roles when assembled in specific ways such as head-to-head orientation of the α-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 α-amino acid residues. The side-chain effect of poly(amino acid)s, by which their secondary structures are remarkably affected, would become apparent in some way by two-dimensionally assembled single α-amino acid residues in supramolecular lipid aggregates.

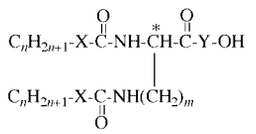
In this paper, we prepared various kinds of α-amino acid-derived anionic lipids with plural amide groups per molecule (L-glutamic acid-derived lipids **1**–**7**, L-aspartic acid-derived lipid **8**, L-lysine-derived lipids **11**–**13**, L-ornithine-derived lipids **14** and **15**, and L-2,4-diaminobutyric acid-derived lipid **16**) in order to investigate the effect of α-amino acid residues and chemical structure requirements on formation of the specific hydrophobic cavities in water, because it had previously been confirmed that the L-glutamic acid-derived lipids (such as L-**1** and L-**2**) with three amide groups per molecule can form complementary hydrogen bonds between neigbouring lipids when assembled with head-to-head orientation.[12,13,17–21] 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_1 hereafter), 2-[4-(dimethylamino)styryl]-*N*-methylpyridinium iodide (abbreviated to St-2C_1 hereafter), and 2-[4-(dimethylamino)styryl]-*N*-ethylpyridinium iodide (abbre-



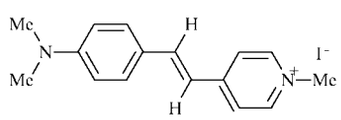
L-**1**; n=12, X=-NH-, m=2, Y=-CH_2-
D-**1**; n=12, X=-NH-, m=2, Y=-CH_2-
DL-**1**; n=12, X=-NH-, m=2, Y=-CH_2-
L-**2**[2]; n=12, X=-NH-, m=2, Y= ——
L-**3**; n=12, X=-NH-, m=2, Y=-$CH(CH_3)$-
L-**4**; n=12, X=-NH-, m=2, Y=-$C(CH_3)_2$-
D-**5**; n=12, X=-NH-, m=2, Y=-$C(CH_3)(CH_2CH_3)$-
L-**6**; n=12, X=-NH-, m=2, Y=-C-



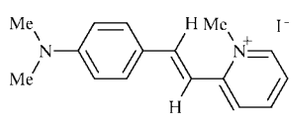
L-**7**; n=4, X=-NH-, m=2, Y=-$CH(CH_3)$-
L-**8**; n=12, X=-NH-, m=1, Y=-CH_2-
L-**9**[1,3,4]; n=18, X=-O-, m=2, Y=-CH_2-
DL-**9**[3,4]; n=18, X=-O-, m=2, Y=-CH_2-
L-**10**[1,3]; n=12, X=-O-, m=2, Y=-CH_2-



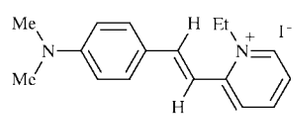
L-**11**; n=16, X=-O-, m=4, Y= ——
L-**12**; n=16, X=-O-, m=4, Y=-$NHCH_2CH_2CO$-
L-**13**; n=15, X= —— , m=4, Y=-$NHCH_2CH_2CO$-
L-**14**; n=16, X=-O-, m=3, Y= ——
L-**15**; n=16, X=-O-, m=3, Y=-$NHCH_2CH_2CO$-
L-**16**; n=16, X=-O-, m=2, Y= ——



St-4C_1



St-2C_1



St-2C_2

viated to St-2C_2 hereafter) not only as a guest molecule but also as microenvironmental probes due to their solvatochromic properties.

Results and discussion

Spectral behaviour of St-4C_1 in the presence of anionic lipid assemblies

As reported previously,[1,4] the wavelengths at the ultraviolet–visible absorption maximum (λ_{max}) of St-4C_1 in water and in methanol are 450 and 475 nm, respectively, regardless of the temperatures. This indicates that the St-4C_1 has no thermochromic properties but exhibits solvatochromic behaviour. When lipid L-**1** is added to the aqueous solution of St-4C_1, λ_{max} shifted to 478 nm, comparable to λ_{max} in methanol at 20 °C. This indicates that St-4C_1 is incorporated into the hydrophobic microenvironment, that is, near the glutamate residue in the lipid aggregates in cooperation with hydrophobic and electrostatic interactions.[1] This conclusion is based on previous results.[1–4] When the temperature is raised, aggregates of L-**1** undergo a gel-to-liquid crystalline phase transition (peak-top temperature, 43 °C by DSC) accompanied by a hypsochromic shift of λ_{max} of St-4C_1. Further increases in temperature up to 65 °C, at which the L-**1** aggregates are in a liquid crystalline state, lead to a λ_{max} of 450 nm (Tables 1 and 2). This wavelength is comparable to the λ_{max} of St-4C_1 alone in water. This indicates that the microenvironment around the electrostatically bound and incorporated St-4C_1 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 L-**3** induced the largest bathochromic shift in λ_{max} to 510 nm at 20 °C. This indicates that the most hydrophobic microenvironment was produced around St-4C_1 by self-assembly of L-**3**. However, no similar bathochromic shifts were induced by lipids **4**–**8**. These results indicate that not only ester-type L-**9**[1,3,4] but also certain kinds of amide-type lipids such as L-**1**, D-**1**, and L-**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 L-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 L-**10** with two dodecyl chains leads to relatively looser packing of component lipids than that of L-**9** with two octadecyl chains.[1,4] In fact, as shown in Table 1, this resulted in no bathochromic shift of the λ_{max} of St-4C_1 in L-**10** aggregates at 20 °C in contrast with a remarkable bathochromic shift in the L-**9** aggregates system. This indicates that the St-4C_1 is located in the polar microenvironment in L-**10** aggregates. However, it is noted that the substitution of amide groups (L-**1**) for the corresponding ester groups (L-**10**) resulted in the bathochromic shift of St-4C_1. This indicates that the St-4C_1 is located in the less polar microenvironment in L-**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 program[12,13,19,20] (abbreviated to CAChe-MM2 hereafter). Three amide groups in L-glutamic acid-derived lipid L-**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 L-**10** is incapable of forming such complementary hydrogen bonds. Such complementary hydrogen bonding by L-**1** occurs even in organic media, *e.g.*, benzene.[12,13,20,21] Owing to the remarkable difference in the visible absorption spectral behaviour between L-**1** and L-**10**, the L-**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.

Effect of cooperation of complementary hydrogen bonding and hydrophobic interactions between lipids on planarity recognition

It is known that increasing the molar ratio of lipid to dye promotes the incorporation of the dye into the hydrophobic region and the λ_{max} value reaches a constant value close to those in

Table 1 Dispersion state of St-4C$_1$ in the presence of various lipids in water ([St-4C$_1$] = 1.5 × 10^{-4} mol dm^{-3} = const., pH 10.0)

Lipid	Aggregate morphologies	[Lipid]/[St-4C$_1$]	λ_{max}/nm of St-4C$_1$ 20 °C	65 °C	Dispersion state of St-4C$_1$ at 20 °C
None (in water)	—	0	450	450	M[a]
None (in methanol)	—	0	475	475[b]	M[a]
L-**1**	Helices and tubules	20	478	450	M[c]
D-**1**	Helices and tubules	20	480	445	M[c]
DL-**1**	Tubules	20	448	448	M[d]
L-**2**[2]	Helices and tubules	20	462	445	M[c]
L-**3**	Tubules	20	510[e]	450	M[c]
L-**4**	Tubules	20	458	455	M[d]
D-**5**	Tubules	20	450	450	M[d]
L-**6**	Vesicular aggregates	20	454	454	M[d]
L-**7**	No structure	20	450	450	M[f]
L-**8**	Tubules	20	452	452	M[d]
L-**9**[1,3,4]	Vesicular aggregates	20	475	444	M[c]
DL-**9**[3,4]	Vesicular aggregates	20	458	444	M[c,d]
L-**10**[1,3]	Tape-like aggregates	20	443	443	M[d]
L-**11**	Vesicular aggregates	20	469	448	M[c]
L-**12**	Tubules and vesicles	20	481	462	M[c]
L-**13**	Tubules and vesicles	20	(525)[g]	(525)[g]	J[g]
L-**14**	Vesicular aggregates	20	456	447	M[c,d]
L-**15**	Tubules and vesicles	20	471	443	M[c]
L-**16**	Vesicular aggregates	20	448	448	M[d]

[a] M; molecularly dispersed monomer of St-4C$_1$. [b] Measured at 60 °C because the boiling point of methanol is 65 °C. [c] M; incorporated monomer as evidenced by the bathochromic shift of λ_{max} 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. [d] M; monomer of St-4C$_1$ bound to the carboxylate and is not incorporated. [e] The anomalous bathochromic shift is not due to the J-aggregates but due to the most hydrophobic microenvironment in which St-4C$_1$ is incorporated. [f] M; molecularly dispersed monomer of St-4C$_1$ because L-**7** shows no aggregation behaviour. [g] J; J-aggregates of St-4C$_1$ 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.

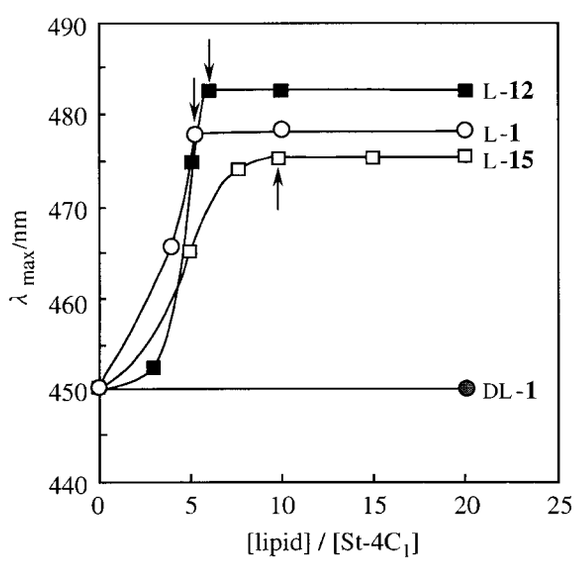


Fig. 1 Relationships between λ_{max} of St-4C$_1$ and molar ratios in the presence of L-**1**, L-**12**, and L-**15**. [St-4C$_1$] = 1.5 × 10^{-4} mol dm^{-3} = const., pH 10, 20 °C. The arrows indicate break points of the complete incorporation with increase in the molar ratio of lipid to St-4C$_1$.

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 is a measure of preferential incorporation (inclusion). Table 2 shows the molar ratio variations of L-**1** to St-4C$_1$, St-2C$_1$, and St-2C$_2$ 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$_1$. The critical molar ratios of complete incorporation of these three dyes indicate that the order of preferential inclusion is St-4C$_1$ > St-2C$_2$ > St-2C$_1$ 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$_1$, 10 for St-2C$_2$, *ca.* 20 for St-2C$_1$. The order of molecular planarity, based on their molecular structures, is considered to be St-4C$_1$ > St-2C$_1$ > St-2C$_2$.[1,3,4] 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$_1$, *ca.* 20° for St-2C$_1$, *ca.* 40° for St-2C$_2$. It is also noted that the order of preferential incorporation of the dyes in the L-**9** aggregate system was St-4C$_1$ > St-2C$_1$ > St-2C$_2$ as reported previously,[1,3,4] indicating that the incorporation is mainly based on planarity recognition rather than the order of hydrophobicity (St-4C$_1$ ≅ St-2C$_1$ < St-2C$_2$). However, the order of incorporation of St-2C$_1$ and St-2C$_2$ 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 the dye. In this respect St-2C$_2$ is more hydrophobic than St-2C$_1$ because of the higher hydrophobicity of the *N*-ethyl group than the *N*-methyl group of St-2C$_1$. Therefore these contradictory results between L-**1** and L-**9** strongly suggest that hydrophobic interactions between longer alkyl chains (2C$_{18}$H$_{37}$-) of lipid L-**9** are preferred for the planarity recognition of the dyes than the collaboration of hydrophobic interactions between relatively shorter alkyl chains (2C$_{12}$H$_{25}$-) 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 λ_{max} 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$_1$ considerably. For example, lipid L-**12** with urethane (carbamate) groups incorporated St-4C$_1$, whereas

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)

Lipid	Dye	[Lipid]/[Dye]	λ_{max} of dye/nm		Dispersion state of dye at 20 °C
			20 °C	65 °C	
None	St-4C$_1$	0	450	450	M[a]
		0 (in MeOH)	475	475[b]	M[a]
None	St-2C$_1$	0	435	435	M[a]
		0 (in MeOH)	460	460[b]	M[a]
None	St-2C$_2$	0	434	434	M[a]
		0 (in MeOH)	460	460[b]	M[a]
L-**1**	St-4C$_1$	20	478	450	M[c]
		10	478	452	M[c]
		5	477	452	M[c]
		4	465	448	M[c,d]
		2	466 (550)[e]	450	M[c] & J[e]
L-**1**	St-2C$_1$	20	463	446	M[c]
		10	457	445	M[c,d]
		4	445	444	M[d]
		2	443	440	M[d]
L-**1**	St-2C$_2$	20	465	443	M[c]
		10	463	445	M[c]
		5	452	445	M[c,d]
L-**2**	St-4C$_1$	20	462	445	M[c]
		10	473 (545)[e]	447	M[c] & J[e]
		4	474 (547)[e]	445	M[c] & J[e]
		2	471 (563)[e]	447	M[c] & J[e]
L-**12**	St-4C$_1$	20	483	468	M[c]
		10	483	469	M[c]
		6	483	465	M[c]
		5	475	458	M[c,d]
		3	453	450	M[c,d]
L-**12**	St-2C$_1$	20	468	456	M[c]
		17.5	468	454	M[c]
		15	460	446	M[c]
		10	455	444	M[c,d]
		5	443	438	M[c,d]
L-**12**	St-2C$_2$	20	463	450	M[c]
		17.5	461	448	M[c]
		15	460	445	M[c]
		10	455	445	M[c,d]
		5	445	438	M[c,d]
L-**15**	St-4C$_1$	20	475	445	M[c]
		15	475	447	M[c]
		10	475	445	M[c]
		7.5	474	445	M[c]
		5	465	443	M[c,d]

[a] M; molecularly dispersed monomer. [b] Measured at 60 °C because the boiling point of methanol is 65 °C. [c] M; incorporated monomer as evidenced by the bathochromic shift of λ_{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.[1–4,22] [d] M; monomer bound to the carboxylate and is not incorporated due to the low molar ratio of lipid to dye. [e] J; 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 L-**13** with amide groups could not incorporate St-4C$_1$ but induced J-aggregates.[23] According to the CPK model and the CAChe-MM2 calculation, no appreciable difference was observed between aggregation modes of L-**12** and L-**13**, shown schematically in Fig. 2. However, the fact that L-**13** induced J-aggregation[23] of St-4C$_1$ strongly suggests that L-**13** is more densely packed than L-**12** and enough to lead to the non-incorporation of St-4C$_1$.† This may be related to the fact that a carbamate group is more polar and/or flexible than a carboxamide group.

Next, incorporation preference of St-4C$_1$, St-2C$_1$, and St-2C$_2$ by aggregates of L-**12** was investigated. Table 2 shows the molar ratio variations of L-**12** to St-4C$_1$, St-2C$_1$, and St-2C$_2$ at fixed dye concentrations (1.5×10^{-4} mol dm^{-3}), respectively. Fig. 1 also shows the molar ratio variations of L-**12** to St-4C$_1$. The critical molar ratios of complete incorporation of these three dyes indicate that the order of preferential inclusion is St-4C$_1$ > St-2C$_1$ > St-2C$_2$ in the L-**12** aggregate systems, because the critical molar ratios estimated from the data in Table 2 are as follows: 6 for St-4C$_1$, 17.5 for St-2C$_2$, more than 20 for St-2C$_1$. This order of incorporation preference is in good agreement with that for L-**9** aggregate systems,[3,4] indicating that the aggregates of L-**12** can recognize the molecular planarity of these dyes more preferentially than their hydrophobicity as well as in the systems of L-**9**.[3,4] Therefore, it is concluded that the recognition of molecular planarity is not restricted to the L-glutamic acid-derived lipids (**1**–**3**, **9**) and may be a general phenomena for appropriately designed α-amino acid-derived lipids such as L-**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-4C$_1$ 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-4C$_1$ 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.[23]

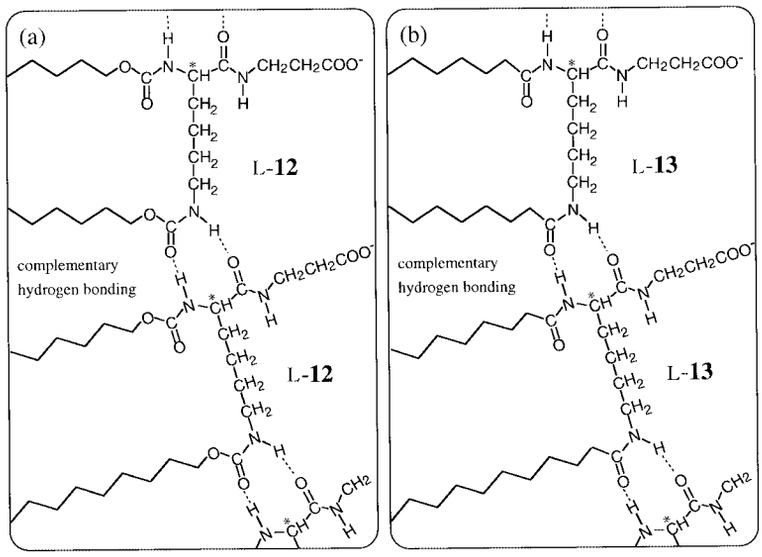


Fig. 2 Schematic representation of complementary hydrogen bonding between amide groups in the main chain and side-chain of lipids of carbamate-type L-**12** (a) and amide-type L-**13** (b).

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

Lipids	Dyes	K_S/dm^3 mol^{-1}
L-**1**	St-4C$_1$	3×10^5
	St-2C$_1$	2×10^3
	St-2C$_2$	2×10^4
L-**9**	St-4C$_1$	1×10^5
	St-2C$_1$	1×10^3
	St-2C$_2$	2×10^3
L-**12**	St-4C$_1$	2×10^4
	St-2C$_1$	2×10^3
	St-2C$_2$	2×10^3
L-**15**	St-4C$_1$	1×10^5

preferentially incorporated into aggregates of L-**12** ($m = 4$) than L-**15** ($m = 3$) which has one less side-chain methylene number than L-**12**, as indicated by the arrows at the critical molar ratios of complete incorporation in Fig. 1. It is also noted that the λ_{max} of St-4C$_1$ in the presence of lipids L-**12** and L-**15** with β-alanine head groups are more bathochromic than those for the corresponding lipids L-**11** and L-**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[1,3] 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 acid)s.

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.*[24,25] are listed in Table 3. Calculations were based on the values listed in Table 2, using the following equation: $f/(1 - f) = K_s\{[D_t] - [S_t]f\} - K_s[\mathrm{cac}]$, in which f denotes the fraction [aggregate-incorporated dye]/[total dye], D_t is the total lipid concentration, S_t is the total dye concentration, and cac is the critical aggregation concentration of the lipid, respectively. For the calculation of f, variation of λ_{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 λ_{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 D-**1** (a) and DL-**1** (b). Aggregate morphologies for D-**1** in water are observed as a mixture of helical and tubular aggregates. Similar aggregates were observed for L-**1** with opposite helical sense (right-handed for D-**1**, left-handed for L-**1**). However, only tubular aggregates were observed and no helical aggregates were observed for the DL-**1**. This suggests that D-**1** and L-**1** are completely miscible, resulting in no helical aggregates, only tubular aggregates. Remarkable bathochromic shift in the λ_{max} of St-4C$_1$ incorporated in aggregates of enantiomeric L-**1**/D-**1** is also consistent with the difference in the polarity of microenvironments in which the St-4C$_1$ is incorporated since no λ_{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

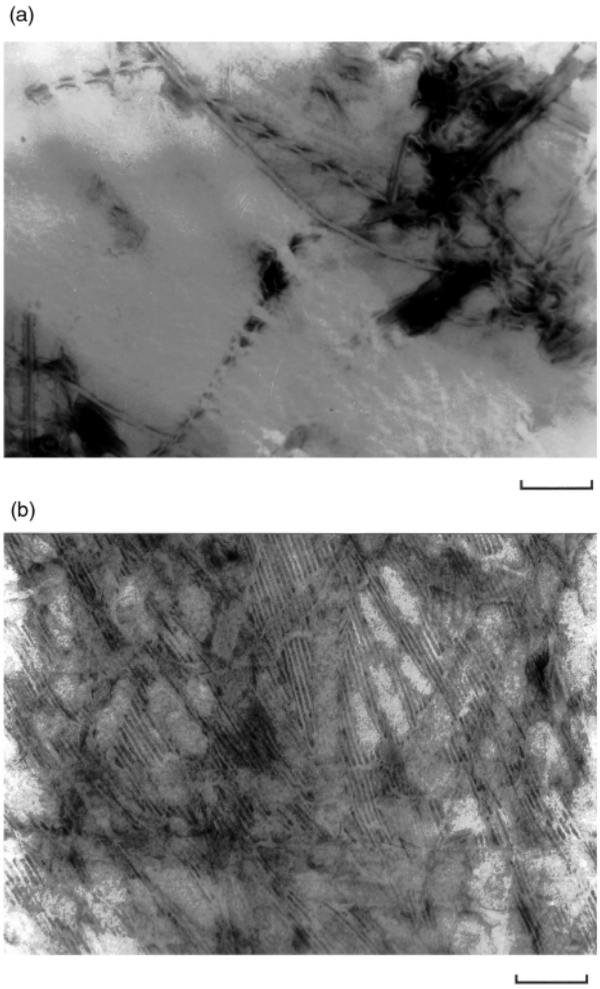


Fig. 3 TEM images of aqueous dispersions of D-**1** (a) and DL-**1** (b) negatively stained by ammonium molybdate. pH 10.0. Scale bars represent 2000 Å, respectively.

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 L-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 L-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 α-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 L-**12** and L-**13** (Fig. 2 and Table 1).

In conclusion, the two-dimensional arrangement of enantiomeric single α-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 L-**1**/D-**1**, possessing such specific hydrophobic cavities are considered to have potential applications to many fields.

Experimental

Materials

L-Glutamic acid, L-lysine monohydrochloride, L-2,4-diaminobutyric acid dihydrochloride, palmitoyl chloride, and Pd black were purchased from Wako Pure Chemicals Co. Ltd. (Japan) and used as obtained. L-Ornithine monohydrochloride and diethyl cyanophosphonate (DECP) were purchased from Aldrich Co. Ltd. and used as obtained. *n*-Hexadecyl chloroformate and *n*-butylamine were purchased from Tokyo Kasei Co. Ltd. (Japan) and used as obtained. St-4C_1, St-2C_1, St-2C_2 were purchased from Aldrich and used as obtained. Other reagents were also used as obtained unless otherwise noted.

Lipid preparation

All the lipids except for L-**2**,[2] L-**9**,[1,3,4] DL-**9**,[3,4] and L-**10**[1,3] were newly synthesized and identified by Fourier transform infrared spectroscopy (FTIR) measurement, ^{1}H-NMR measurement, and elemental analyses.

***N′*,*N″*-Didodecyl-*N*$^{\alpha}$-[4-carboxybutanoyl]-L-glutamide (L-1).** *N′*,*N′*-Didodecyl-L-glutamide (L-**20**[14]) (2.0 g, 4.2×10^{-3} mol) and triethylamine (0.51 g, 5.1×10^{-3} mol) were dissolved in tetrahydrofuran (THF) and stirred with cooling. Glutaric anhydride (0.54 g, 4.7×10^{-3} mol) was added to the solution. After being stirred for 1 day at room temperature, the solution was concentrated *in vacuo*. The residue was recrystallized from methanol and dried *in vacuo* to give a white solid (L-**1**): yield 2.0 g (80%); mp 127–133 °C; ν_{max}(KBr)/cm^{-1} 3298, 2926, 2856, 1702, 1638 and 1560; ^{1}H-NMR ($CDCl_3$) δ 0.78–0.97 (m, 6H, $CH_3 \times 2$), 1.03–1.50 (m, 44H, $(CH_2)_{10} \times 2$, $CH_2 \times 2$), 1.77–2.45 (m, 6H, $CH_2C(=O)NH \times 2$, $CH_2C(=O)$), 2.90–3.40 (m, 4H, $CH_2NHC(=O) \times 2$), 3.60–4.25 (m, 1H, C*H*) (Anal. Found: C, 68.73; H, 12.14; N, 7.50. Calc. for $C_{34}H_{65}N_3O_5$: C, 68.53; H, 11.00; N, 7.05%).

D-**1** and DL-**1** were prepared as described above: D-**1**, yield 75%; mp 127–133 °C; spectral data were identical to those of L-**1**: DL-**1**, yield 80%; mp 97–100 °C; ν_{max}(KBr)/cm^{-1} 3294, 2922, 2854, 1702, 1638 and 1562; ^{1}H-NMR ($CDCl_3$) δ 0.68–0.99 (m, 6H, $CH_3 \times 2$), 1.00–1.76 (m, 44H, $(CH_2)_{10} \times 2$, $CH_2 \times 2$), 1.80–2.42 (m, 6H, $CH_2C(=O)NH \times 2$, $CH_2C(=O)$), 2.90–3.35 (m, 4H, $CH_2NHC(=O) \times 2$), 3.92–4.20 (m, 1H, C*H*) (Anal. Found: C, 66.49; H, 11.60; N, 6.90. Calc. for $C_{34}H_{65}N_3O_5 \cdot 1.0H_2O$: C, 66.49; H, 11.00; N, 6.84%).

The related compounds were prepared as described above, using the corresponding substituted glutaric anhydrides instead of glutaric anhydride: 3-methylglutaric anhydride for L-**3**, 3,3-dimethylglutaric anhydride for L-**4**, 3-ethyl-3-methylglutaric anhydride for D-**5**, 3,3-tetramethyleneglutaric anhydride for L-**6**, and 3-methylglutaric anhydride for L-**7**. L-**3**, yield 78%; mp 138–141 °C; ν_{max}(KBr)/cm^{-1} 3298, 2922, 2854, 1702, 1636 and 1562; ^{1}H-NMR ($CDCl_3$) δ 0.60–1.00 (m, 9H, $CH_3 \times 3$), 1.00–1.84 (m, 43H, $(CH_2)_{10} \times 2$, $C(=O)CH_2CH(CH_3)$, CH_2), 1.85–2.60 (m, 6H, $CH_2C(=O)NH \times 2$, $CH_2C(=O)$), 2.80–3.40 (m, 4H, $CH_2NHC(=O) \times 2$), 4.42–4.56 (m, 1H, CH) (Anal. Found: C, 67.88; H, 11.92; N, 6.80. Calc. for $C_{35}H_{67}N_3O_5 \cdot 0.5H_2O$: C, 67.88; H, 11.07; N, 6.79%). L-**4**, yield 79.9%; mp 100–102 °C; ν_{max}(KBr)/cm^{-1} 3298, 2920, 2854, 1638 and 1560; ^{1}H-NMR ($CDCl_3$) δ 0.73–1.00 (m, 12H, $CH_3 \times 4$), 1.00–1.67 (m, 42H, $(CH_2)_{10} \times 2$, CH_2), 1.90–2.45 (m, 6H, $CH_2C(=O)NH \times 2$, $CH_2C(=O)$), 2.90–3.43 (m, 4H, $CH_2NHC(=O) \times 2$), 4.30–4.60 (m, 1H, C*H*) (Anal. Found: C, 68.55; H, 11.32; N, 6.61. Calc. for $C_{36}H_{69}N_3O_5 \cdot 0.4H_2O$: C, 68.55; H, 11.15; N, 6.66%). D-**5**, yield 76%; mp 103–105 °C; ν_{max}(KBr)/cm^{-1} 3452, 1649 and 1562; ^{1}H-NMR ($CDCl_3$) δ 0.78–1.00 (m, 12H, $CH_3 \times 4$), 1.12–1.49 (m, 44H, $(CH_2)_{10} \times 2$, *$CHCH_2$, CH_2), 1.90–2.50 (m, 6H, $CH_2C(=O)NH \times 2$, $CH_2C(=O)$), 2.97–3.39 (m, 4H, $CH_2NHC(=O) \times 2$), 4.43–4.56 (m, 1H, C*H*) (Anal. Found: C, 68.37; H, 12.54; N, 7.14. Calc. for $C_{37}H_{71}N_3O_5 \cdot 0.7H_2O$: C, 68.37; H,

11.22; N, 6.47%). L-**6**, yield 96%; mp 45–50 °C; ν_{max}(KBr)/cm^{-1} 3294, 2926, 2856, 1711, 1638 and 1560; ^{1}H-NMR ($CDCl_3$) δ 0.72–1.00 (m, 6H, $CH_3 \times 2$), 1.01–1.80 (m, 50H, $(CH_2)_{10} \times 2$, $(CH_2)_4$, *$CHCH_2$), 1.86–2.61 (m, 6H, $CH_2C(=O)$-NH × 2, $CH_2C(=O)$), 3.00–3.43 (m, 4H, $CH_2NHC(=O) \times 2$), 3.92–4.20 (m, 1H, *CH) (Anal. Found: C, 69.47; H, 11.18; N, 6.34. Calc. for $C_{38}H_{71}N_3O_5 \cdot 0.7H_2O$: C, 69.47; H, 11.01; N, 6.40%).

***N*′,*N*″-Dibutyl-*N*$^{\alpha}$-benzyloxycarbonyl-L-glutamide (L-18).** *N*-Benzyloxycarbonyl-L-glutamic acid (L-Glu(Z))[15] (4.0 g, 1.4×10^{-2} mol), *n*-butylamine (2.3 g, 3.1×10^{-2} mol), and triethylamine (4.4 g, 4.3×10^{-2} mol) were dissolved in THF (50 cm^3). The solution was cooled to 0 °C, and DECP (5.8 g, 3.3×10^{-2} mol) was added to the solution. After being stirred for 1 day at room temperature, 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 solution was dried over Na_2SO_4 and concentrated *in vacuo* to give a waxy solid (L-**18**): yield 5.5 g (98%); mp 172–174 °C; ν_{max}(KBr)/cm^{-1} 3296, 3094, 1690, 1644 and 1539; ^{1}H-NMR ($CDCl_3$) δ 0.68–1.08 (t, 6H, $CH_3 \times 2$), 1.08–1.83 (m, 8H, $CH_3(CH_2)_2 \times 2$), 1.83–2.29 (m, 2H, *$CHCH_2$), 2.29–2.51 (m, 2H, *$CHCH_2CH_2C(=O)$), 3.01–3.60 (m, 4H, $CH_2NHC(=O) \times 2$), 4.00–4.40 (m, 1H, *CH), 4.88–5.27 (s, 2H, $CH_2C_6H_5$), 7.20–7.64 (s, 5H, C_6H_5).

***N*′,*N*″-Dibutyl-L-glutamide (L-21).** L-**18** (3.5 g, 8.9×10^{-3} mol) was dissolved in ethanol (300 cm^3) with heating and Pd black (1 g) was added to the solution. H_2 gas was bubbled slowly into the solution for 6 hours. After removal of the benzyl group, Pd black was removed by filtration. The solution was concentrated and dried *in vacuo* to give a waxy solid (L-**21**): yield 1.9 g (85%); ν_{max}(KBr)/cm^{-1} 3318, 2962, 2934, 2874, 1647 and 1553; ^{1}H-NMR ($CDCl_3$) δ 0.68–1.10 (t, 6H, $CH_3 \times 2$), 1.10–1.78 (m, 8H, $CH_3(CH_2)_2 \times 2$), 1.85–2.20 (m, 2H, *$CHCH_2CH_2C(=O)$), 2.20–2.71 (m, 2H, $CH_2CH_2C(=O)NH$), 2.93–3.50 (m, 4H, $CH_2NHC(=O) \times 2$), 4.00–4.40 (m, 1H, *CH).

***N*′,*N*″-Dibutyl-*N*$^{\alpha}$-[3-(methyl)-4-carboxybutanoyl]-L-glutamide (L-7).** L-**21** (1.0 g, 3.89×10^{-3} mol) was dissolved in 50 cm^3 of THF. 3-Methylglutaric anhydride (0.60 g, 4.66×10^{-3} mol) and triethylamine (0.47 g, 4.66×10^{-3} mol) were added to the solution with cooling. After being stirrerd for 1 day, the solution was concentrated *in vacuo*. The residue was recrystallized from methanol and dried *in vacuo* to give a waxy solid (L-**7**): yield 29%; mp 139–146 °C; ν_{max}(KBr)/cm^{-1} 1702, 1647 and 1553; ^{1}H-NMR ($CDCl_3$) δ 0.75–1.10 (t, 9H, $CH_3 \times 3$), 1.10–1.63 (m, 11H, $CH_3(CH_2)_2 \times 2$, *$CHCH_2CH_2C(=O)$, $CH_2CH(CH_3)CH_2$), 1.70–2.62 (m, 6H, $CH_2C(=O)NH \times 2$, $CH_2C(=O)$), 2.70–3.43 (m, 4H, $CH_2NHC(=O) \times 2$), 3.80–4.40 (m, 1H, *CH) (Anal. Found: C, 57.39; H, 9.39; N, 10.83. Calc. for $C_{19}H_{35}N_3O_5 \cdot 1.6H_2O$: C, 57.39; H, 9.66; N, 10.57%).

***N*-(Benzyloxycarbonyl)-L-aspartic acid (L-Asp(Z)).** L-Asp(Z) was prepared similarly according to the method reported previously:[14] Yield 5.6 g (65%); mp 115–116 °C (lit.[15] 116 °C).

***N*′,*N*″-Didodecyl-*N*$^{\alpha}$-(benzyloxycarbonyl)-L-aspartamide (L-19).** L-Asp(Z) (2.0 g, 7.5 × 10^{-3} mol), 1-aminododecane (3.0 g, 1.6×10^{-2} mol), and triethylamine (2.3 g, 2.3 × 10^{-2} mol) were dissolved in THF (150 cm^3). The solution was cooled to 0 °C, and DECP (3.3 g, 1.9×10^{-2} mol) was added to the solution. After being stirred for 1 day at room temperature, 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 solution was dried over Na_2SO_4 and concentrated *in vacuo* to give a white powder (L-**19**): yield 3.8 g (85%); mp 150–162 °C; ν_{max}(KBr)/cm^{-1} 3300, 2922, 1688, 1657 and 1543; ^{1}H-NMR ($CDCl_3$) δ 0.67–1.01 (t, 6H, CH_3), 1.01–1.81 (m, 40H, $(CH_2)_{10} \times 2$), 2.26–2.87 (m, 2H, *$CHCH_2$), 2.87–3.52 (m, 4H, $CH_2NHC(=O) \times 2$), 4.26–4.72 (m, 1H, CH), 4.99–5.24 (s, 2H, $CH_2C_6H_5$), 7.20–7.68 (s, 5H, C_6H_5).

***N*′,*N*″-Didodecyl-L-aspartamide (L-22).** L-**19** (3.0 g, 5.0 × 10^{-3} 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 (L-**22**): yield 2.0 g (85%); mp 102 °C; ν_{max}(KBr)/cm^{-1} 3304, 2922, 2854, 1632 and 1545; ^{1}H-NMR ($CDCl_3$) δ 0.76–1.00 (t, 6H, CH_3), 1.13–1.80 (m, 40H, $(CH_2)_{10} \times 2$), 2.45–2.71 (m, 2H, *$CHCH_2$), 3.02–3.40 (m, 4H, $CH_2NHC(=O) \times 2$), 3.51–3.82 (m, 1H, *CH).

***N*′,*N*″-Didodecyl-*N*$^{\alpha}$-(4-carboxybutanoyl)-L-aspartamide (L-8).** L-**8** was prepared similarly as L-**1** using L-**22** instead of L-**20**[14] to give a white powder (L-**8**): yield 59%; mp 112–118 °C; ν_{max}(KBr)/cm^{-1} 3292, 2922, 2854, 1700, 1647 and 1545; ^{1}H-NMR ($CDCl_3$) δ 0.75–1.00 (m, 6H, $CH_3 \times 2$), 1.04–2.12 (m, 40H, $(CH_2)_{10} \times 2$), 2.06–2.70 (m, 6H, $CH_2C(=O)NH \times 2$, $CH_2C(=O)$), 2.84–3.35 (m, 4H, $CH_2NHC(=O) \times 2$), 3.67–4.08 (m, 1H, *CH) (Anal. Found: C, 65.43; H, 11.29; N, 7.81. Calc. for $C_{33}H_{63}N_3O_5 \cdot 1.3H_2O$: C, 65.43; H, 10.93; N, 6.94%).

***N*$^{\alpha}$,*N*$^{\varepsilon}$-Bis(hexadecyloxycarbonyl)-L-lysine (L-11).** L-Lysine monohydrochloride (5.0 g, 2.7×10^{-2} mol) was dissolved in water. The solution was cooled to 0 °C, and a 20 cm^3 portion of water containing 4.3 g (1.1×10^{-1} mol) of NaOH was added to the solution at 0 °C. Then a 100 cm^3 of acetone containing 12.5 g (4.10×10^{-2} mol) of *n*-hexadecyl chloroformate 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 (L-**11**): yield 6.9 g (62%); mp 71–75 °C; ν_{max}(KBr)/cm^{-1} 1694 and 1557; ^{1}H-NMR ($CDCl_3$) δ 0.70–1.01 (m, 6H, $CH_3 \times 2$), 1.01–1.95 (m, 62H, $(CH_2)_{14} \times 2$, *$CH(CH_2)_3$), 3.04–3.41 (m, 2H, $NHCH_2$), 3.90–4.50 (m, 5H, $CH_2O \times 2$, CH) (Anal. Found: C, 69.51; H, 11.44; N, 4.09. Calc. for $C_{40}H_{78}N_2O_6 \cdot 0.2H_2O$: C, 69.51; H, 11.50; N, 4.05%).

***N*$^{\alpha}$,*N*$^{\delta}$-Bis(hexadecyloxycarbonyl)-L-ornithine (L-14).** L-**14** was prepared as described above using L-ornithine monohydrochloride instead of L-lysine hydrochloride: yield 11.8 g (93.4%); mp 59.5–63.0 °C; ν_{max}(KBr)/cm^{-1} 3318, 2962, 2934, 1690 and 1545; ^{1}H-NMR ($CDCl_3$) δ 0.70–1.05 (m, 6H, $CH_3 \times 2$), 1.05–1.95 (m, 60H, $(CH_2)_{14} \times 2$, *$CH(CH_2)_2$), 3.05–3.40 (m, 2H, $NHCH_2$), 3.90–4.20 (m, 5H, $CH_2O \times 2$, *CH) (Anal. Found: C, 68.94; H, 11.25; N, 4.05. Calc. for $C_{39}H_{76}N_2O_6 \cdot 0.6H_2O$: C, 68.94; H, 11.45; N, 4.12%).

β-Alanine benzyl ester toluene-*p*-sulfonate (23). Benzyl alcohol (17.0 g, 0.157 mol), β-alanine (10.0 g, 0.135 mol), and toluene-*p*-sulfonic acid monohydrate (25.6 g, 0.135 mol) were suspended in 400 cm^3 of toluene and refluxed for 5 h, removing water azeotropically. The reaction mixture was concentrated *in vacuo* and an excess amount of diethyl ether was added. The precipitates formed were collected and dried *in vacuo* to give white plates (**23**): yield 38.0 g (96.3%); mp 138–139 °C (lit.[16] 138–139 °C); ν_{max}(KBr)/ cm^{-1} 3066, 1736, 1232 and 1156.

***N*$^{\alpha}$,*N*$^{\varepsilon}$-Bis(hexadecyloxycarbonyl)-*N*-[2-(benzyloxycarbonyl)-ethyl]-L-lysinamide (L-24).** L-**11** (3.0 g, 4.39×10^{-3} mol) was dissolved in 100 cm^{-3} of chloroform. Then 30 cm^{-3} portion of THF containing β-alanine benzyl ester hydrotoluene-*p*-

sulfonate (**23**) (1.66 g, 4.89×10^{-3} mol) and triethylamine (1.33 g, 1.32×10^{-2} 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% $NaHCO_3$, 0.2 M HCl, and water. The solution was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was recrystallized from methanol and dried *in vacuo* to give a white powder (L-**24**): yield 2.5 g (77%); mp 72–76 °C; ν_{max}(KBr)/cm^{-1} 3296, 2962, 2934, 1738, 1698, 1651 and 1557; ^{1}H-NMR ($CDCl_3$) δ 0.70–1.00 (m, 6H, CH_3 × 2), 1.00–1.90 (m, 62H, $(CH_2)_{14}$ × 2, *CH(CH_2)$_3$), 2.45–2.70 (m, 2H, CH_2C(=O)), 3.03–3.72 (m, 4H, NHCH_2 × 2), 3.90–4.51 (m, 5H, CH_2O × 2, *CH), 5.05–5.20 (m, 2H, C$H_2C_6H_5$), 7.20–7.50 (m, 5H, C_6H_5) (Anal. Found: C, 71.77; H, 10.76; N, 5.57. Calc. for $C_{50}H_{89}N_3O_7$: C, 71.77; H, 10.63; N, 5.02%).

***N*$^{\alpha}$,*N*$^{\delta}$-Bis(hexadecyloxycarbonyl)-*N*-[2-(benzyloxycarbonyl)-ethyl]-L-ornithinamide (L-25).** L-**25** was prepared as described above using L-**14** instead of L-**11**: yield 2.0 g (54%); mp 53.0–57.9 °C; ν_{max}(KBr)/cm^{-1} 3308, 2962, 2934, 1738, 1692, 1651 and 1545; δ 0.70–1.05 (m, 6H, CH_3 × 2), 1.05–1.95 (m, 60H, $(CH_2)_{14}$ × 2, *CH(CH_2)$_2$), 2.45–2.71 (m, 2H, CH_2C(=O)O), 3.05–3.85 (m, 4H, NHCH_2 × 2), 3.90–4.20 (m, 4H, CH_2O × 2), 4.60–5.01 (m, 1H, *CH), 5.05–5.20 (m, 2H, C$H_2C_6H_5$), 5.25–5.50, 7.28–7.40 (m, 5H, C_6H_5) (Anal. Found: C, 69.97; H, 10.72; N, 4.73. Calc. for $C_{49}H_{87}N_3O_7 \cdot 0.6H_2O$: C, 69.97; H, 10.57; N, 5.00%).

***N*$^{\alpha}$,*N*$^{\varepsilon}$-Bis(hexadecyloxycarbonyl)-*N*-[2-carboxyethyl]-L-lysinamide (L-12).** L-**24** (1.47 g, 1.73×10^{-3} mol) was dissolved in ethanol (300 cm^3) with heating and Pd black (1 g) was added to the solution. H_2 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 (L-**12**): yield 0.95 g (73%); mp 126–129 °C; ν_{max}(KBr)/cm^{-1} 3326, 2962, 2934, 1698, 1655 and 1555; ^{1}H-NMR ($CDCl_3$) δ 0.70–1.00 (m, 6H, CH_3 × 2), 1.00–1.40 (m, 62H, $(CH_2)_{14}$ × 2, *CH(CH_2)$_3$), 2.45–2.70 (m, 2H, CH_2C(=O)), 3.00–3.70 (m, 4H, NHCH_2 × 2), 3.90–4.15 (m, 5H, CH_2O × 2, *CH) (Anal. Found: C, 67.78; H, 10.95; N, 5.51. Calc. for $C_{43}H_{83}N_3O_7 \cdot 0.4H_2O$: C, 67.78; H, 11.10; N, 5.52%).

***N*$^{\alpha}$,*N*$^{\delta}$-Bis(hexadecyloxycarbonyl)-*N*-[2-carboxyethyl]-L-ornithinamide (L-15).** L-**15** was prepared as described above using L-**25** instead of L-**24**: yield 1.1 g (84%); mp 45.0–52.0 °C; ν_{max}(KBr)/cm^{-1} 3310, 2962, 2934, 1692, 1653 and 1547; δ 0.70–1.05 (m, 6H, CH_3 × 2), 1.05–1.95 (m, 60H, $(CH_2)_{14}$ × 2, *CH(CH_2)$_2$), 2.45–2.71 (m, 2H, CH_2C(=O)OH), 2.95–3.75 (m, 4H, NHCH_2 × 2), 3.80–4.50 (m, 5H, CH_2O × 2, *CH) (Anal. Found: C,67.39; H, 11.09; N, 5.33. Calc. for $C_{41}H_{79}N_3O_7 \cdot 0.3H_2O$: C, 67.39; H, 10.97; N, 5.75%).

L-Lysine benzyl ester bis(toluene-*p*-sulfonate) (L-26). L-**26** was prepared according to the literature:[16] white powder, yield 2.0 g (13%); mp 148–151 °C; ν_{max}(KBr)/cm^{-1} 3436, 3034, 1752, 1526, 1218 and 1176.

***N*$^{\alpha}$,*N*$^{\varepsilon}$-Bis(hexadecanoyl)-L-lysine benzyl ester (L-28).** L-**26** (1.90 g, 3.27×10^{-3} mol) was dissolved in 80 cm^3 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 cm^3 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 1 day at room temperature, the solution was washed with 5% $NaHCO_3$, 0.2 M HCl, and water. The solution was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was recrystallized from methanol and dried *in vacuo* to give a white powder (L-**28**): yield 1.7 g (70%); mp 92.0–94.5 °C; ν_{max}(KBr)/cm^{-1} 3318, 2962, 2934, 1750, 1642 and 1555; ^{1}H-NMR ($CDCl_3$) δ 0.70–1.00 (m, 6H, CH_3 × 2), 1.00–1.40 (m, 58H, $(CH_2)_{13}$ × 2, *CH(CH_2)$_3$), 2.05–2.30 (m, 4H, CH_2C(=O)), 3.05–3.40 (m, 4H, NHCH_2), 4.40–4.82 (m, 1H, *CH), 5.10–5.20 (s, 2H, C$H_2C_6H_5$), 6.20–6.45 (m, 2H, N*H*CH$_2$), 7.20–7.50 (m, 5H, C_6H_5).

***N*$^{\alpha}$,*N*$^{\varepsilon}$-Bis(hexadecanoyl)-L-lysine (L-29).** L-**28** (1.68 g, 2.58×10^{-3} mol) was dissolved in 400 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 h. After removal of 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 (L-**29**): yield 1.3 g (88%); mp 120–125 °C; ν_{max}(KBr)/cm^{-1} 3318, 2962, 2934, 1717, 1644 and 1560; ^{1}H-NMR ($CDCl_3$) δ 0.70–1.00 (m, 6H, CH_3 × 2), 1.00–1.40 (m, 58H, $(CH_2)_{13}$ × 2, *CH(CH_2)$_3$), 2.05–2.30 (m, 4H, CH_2C(=O)), 3.01–3.42 (m, 4H, NHCH_2), 4.42–4.83 (m, 1H, *CH) (Anal. Found: C, 72.74; H, 11.87; N, 4.39. Calc. for $C_{38}H_{74}N_2O_4 \cdot 0.2H_2O$: C, 72.74; H, 11.98; N, 4.47%).

***N*$^{\alpha}$,*N*$^{\varepsilon}$-Bis(hexadecanoyl)-*N*-[2-(benzyloxycarbonyl)ethyl]-L-lysinamide (L-30).** L-**29** (1.10 g, 1.83×10^{-3} mol) was dissolved in 100 cm^3 of chloroform. A 30 cm^3 portion of THF containing 0.71 g (2.0×10^{-3} mol) of **23** and 0.56 g (5.5×10^{-3} mol) of triethylamine. DECP (0.45 g, 2.6×10^{-3} mol) was added to the mixture and stirred for 30 min with cooling and subsequently for 1 day at room temperature. The solution was washed with 5% $NaHCO_3$, 0.2 M HCl, and water. The solution was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was recrystallized from methanol and dried *in vacuo* to give a white powder (L-**30**): yield 1.2 g (88%); mp 96–105 °C; ν_{max}(KBr)/cm^{-1} 3308, 2962, 2934, 1651 and 1551 (Anal. Found: C, 70.40; H, 10.71; N, 4.83. Calc. for $C_{48}H_{85}N_3O_5 \cdot 2.2H_2O$: C, 70.40; H, 10.94; N, 5.11%).

***N*$^{\alpha}$,*N*$^{\varepsilon}$-Bis(hexadecanoyl)-*N*-(2-carboxyethyl)-L-lysinamide (L-13).** L-**30** (0.90 g, 1.19×10^{-3} mol) was dissolved in 400 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 7 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 to give white powder (L-**13**): yield 0.58 g (73%); mp 126–129 °C; ν_{max}(KBr)/cm^{-1} 3310, 2962, 2934, 1717, 1644 and 1560 (Anal. Found: C, 70.55; H, 11.48; N, 5.91. Calc. for $C_{41}H_{79}N_3O_5 \cdot 0.2H_2O$: C, 70.55; H, 11.50; N, 6.02%).

L-2,4-Diamonobutyric acid benzyl ester bis(toluene-*p*-sulfonate) (L-27). L-2,4-Diaminobutyric acid dihydrochloride (0.90 g, 4.71×10^{-3} mol) and toluene-*p*-sulfonic acid monohydrate (2.15 g, 1.13×10^{-2} mol) were suspended in 60 cm^3 of benzyl alcohol and refluxed for 12 h. Excess amount of diethyl ether was added to the solution. The precipitates formed were collected and dried *in vacuo* to give a white powder (L-**27**): yield 2.1 g (79%); mp 101–125 °C; ν_{max}(KBr)/cm^{-1} 3066, 1758, 1731, 1226 and 1178.

***N*$^{\alpha}$,*N*$^{\gamma}$-Bis(hexadecyloxycarbonyl)-L-diaminobutyric acid benzyl ester (L-31).** L-**27** (1.00 g, 1.82×10^{-3} mol) was dissolved in 80 cm^3 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 cm^3 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% $NaHCO_3$, 0.2 M HCl, and water. The solution was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was recrystallized from methanol and dried *in vacuo* to give a white powder (L-**31**): yield 0.77 g (56%); mp 38.0–42.6 °C; ν_{max}(KBr)/cm^{-1} 3310, 2962, 2934, 1738, 1690 and 1545; ^{1}H-NMR ($CDCl_3$)

δ 0.70–1.00 (m, 6H, $CH_3 \times 2$), 1.00–1.80 (m, 58H, $(CH_2)_{14} \times 2$, *$CHCH_2$), 2.80–3.30 (m, 2H, $NHCH_2$), 3.50–3.80 (m, 1H, *CH), 3.85–4.20 (m, 4H, $CH_2O \times 2$), 5.15–5.20 (m, 2H, $CH_2C_6H_5$), 7.20–7.45 (s, 5H, C_6H_5).

L-N^{α},N^{γ}-Bis(hexadecyloxycarbonyl)-2,4-diaminobutyric acid (L-16). L-**31** (0.77 g, 1.0×10^{-3} mol) was dissolved in 250 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 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 (L-**16**): yield 0.60 g (87%); mp 57.7–58.8 °C; ν_{max}(KBr)/cm^{-1} 3310, 2962, 2934, 1698 and 1562; ^{1}H-NMR ($CDCl_3$) δ 0.70–1.05 (m, 6H, $CH_3 \times 2$), 1.00–1.80 (m, 58H, $(CH_2)_{14} \times 2$, *$CHCH_2$), 2.80–3.30 (m, 2H, $NHCH_2$), 3.50–3.80 (m, 1H, *CH), 3.85–4.20 (m, 4H, $CH_2O \times 2$).

Characterization of lipids

The chemical structures of all the compounds synthesized were confirmed by Fourier transform infrared spectroscopy (FTIR) measurement with a JASCO FT/IR-7000, by ^{1}H 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 Ultrasonic Generator with a 4280S type vibrator produced by Kaijyo Denki Co. Ltd. Then the pH was adjusted with hydrochloric acid and sodium hydroxide.

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 molybdenate. 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: 43 °C for L-**1**; 35, 42, and 45 °C for D-**1**; 36, 44, and 46 °C for DL-**1**; 38 °C for L-**2**; 40 °C for L-**3**; no detection for L-**4**; 47, 57, 54, 39, and 27 °C for D-**5**; 23 °C for L-**6**; no detection for L-**7**; 88 °C for L-**8**; 46 °C for L-**11**; 68 °C for L-**12**; 79 °C for L-**13**; 52 °C for L-**14**; 52 and 76 °C for L-**15**; 62 °C for L-**16**. It was also observed using TEM that most of the lipids (except for L-**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 dyes.

‡ Most of the L-glutamic acid-derived lipids with three amides per molecule formed an organic gel in $CDCl_3$.[12,13,17,18] Therefore, ^{1}H-NMR signals were considerably broadened.

Acknowledgements

We are grateful to Professor Hideto Shosenji of Kumamoto University for elemental analyses, and Dr M. Nishida and J. Sugise for taking transmission electron micrographs. We are also grateful to T. Ishikawa, T. Mishiro, N. Saeki, S. Uchiyama, N. Kanekura, K. Urashi and D. Goto for lipid preparations and technical assistance.

References

1 H. Hachisako, T. Yamazaki, H. Ihara, C. Hirayama and K. Yamada, *J. Chem. Soc., Perkin Trans. 2*, 1994, 1671.
2 H. Hachisako, Y. Motozato, R. Murakami and K. Yamada, *Chem. Lett.*, 1992, 219.
3 H. Hachisako, T. Yamazaki, H. Ihara, C. Hirayama and K. Yamada, *J. Chem. Soc., Perkin Trans. 2*, 1994, 1681.
4 H. Hachisako, T. Yamazaki, R. Murakami and K. Yamada, *Liq. Cryst.*, 1993, **15**, 723.
5 M. J. Grourke and J. H. Gibbs, *Biopolymers*, 1971, **10**, 795.
6 G. Blauer and Z. B. Alfassi, *Biochim. Biophys. Acta*, 1967, **133**, 206.
7 M. J. Grourke and J. H. Gibbs, *Biopolymers*, 1967, **6**, 586.
8 S. R. Chadhuri and J. T. Yang, *Biochemistry*, 1965, **4**, 1249.
9 M. Hatano, M. Yoneyama, I. Ito, T. Nozawa and M. Nakai, *J. Am. Chem. Soc.*, 1969, **91**, 2165.
10 I. Satake and J. T. Yang, *Biochem. Biophys. Res. Commun.*, 1973, **54**, 930.
11 H. Yamamoto and J. T. Yang, *Biopolymers*, 1974, **13**, 1093.
12 H. Ihara, K. Shudo, C. Hirayama, H. Hachisako and K. Yamada, *Liq. Cryst.*, 1996, **20**, 807.
13 H. Ihara, K. Shudo, M. Takafuji, C. Hirayama, H. Hachisako and K. Yamada, *Jpn. J. Polym. Sci. Tech.*, 1995, **52**, 606.
14 H. Ihara, H. Hachisako, C. Hirayama and K. Yamada, *Liq. Cryst.*, 1987, **2**, 215.
15 M. Bergmann and L. Zervas, *Ber.*, 1932, **65**, 1192.
16 N. Izumiya and S. Makisumi, *Nippon Kagaku Zasshi*, 1957, **78**, 662.
17 H. Ihara, H. Hachisako, C. Hirayama and K. Yamada, *J. Chem. Soc., Chem. Commun.*, 1992, 1244.
18 H. Hachisako, H. Ihara, T. Kamiya, C. Hirayama and K. Yamada, *J. Chem. Soc., Chem. Commun.*, 1997, 19.
19 H. Hachisako, H. Ihara and K. Yamada, *Recent Res. Dev. Pure Applied Chem.*, 1998, **2**, 59.
20 H. Ihara, M. Yoshitake, M. Takafuji, T. Yamada, T. Sagawa, C. Hirayama and H. Hachisako, *Liq. Cryst.*, 1999, **26**, 1021.
21 M. Takafuji, H. Ihara, C. Hirayama, H. Hachisako and K. Yamada, *Liq. Cryst.*, 1995, **18**, 97.
22 N. Nakashima, H. Fukushima and T. Kunitake, *Chem. Lett.*, 1981, 1555.
23 A. H. Herz, *Adv. Colloid Interface Sci.*, 1977, **8**, 237.
24 C. Hirose and L. Sepúlveda, *J. Phys. Chem.*, 1981, **85**, 3689.
25 C. A. Bunton, F. Rivera and L. Sepúlveda, *J. Org. Chem.*, 1978, **43**, 1166.

Paper 9/03956B