Formation of specific dipolar microenvironments complementary to dipolar betaine dye by nonionic peptide lipids in nonpolar medium†

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Received 15th October 2008, Accepted 9th February 2009
First published as an Advance Article on the web 18th March 2009
DOI: 10.1039/b818218c

This paper describes the host–guest interaction between nonionic peptide lipids and solvatochromic dipolar betaine dyes in nonpolar aprotic organic solvent. We have serendipitously found that the colour of Reichardt's Dye (referred to as Et(30) hereafter, although the term Et(30) has been used as a polarity parameter) in chlorobenzene unusually blue-shifted in the presence of L-glutamic acid-derived peptide lipid **1** with a benzyloxycarbonylated Gly headgroup. Since it is widely accepted that Et(30) shows negative solvatochromism, *i.e.*, the visible absorption band of this dye blue-shifts as the solvent polarity increases, the blue-shift indicates that Et(30) was in contact with the more polar microenvironment produced by the peptide lipid **1** rather than chlorobenzene under aggregate-free conditions. The binding site was assumed to be N-H$^{\delta+}$ and C=O$^{\delta-}$ attached to both sides of the Gly residue, respectively, *i.e.*, the O$^-$ and N$^+$ of Et(30) complementarily bound to N-H$^{\delta+}$ and C=O$^{\delta-}$ through hydrogen bonding and ion-dipole interaction, respectively. Since Et(30) is practically non-fluorescent, it was not feasible to use fluorescence spectrometry, which is a powerful method for the study of host–guest interactions, in order to specify the binding mode of Et(30). Therefore, a synthetic approach, although very laborious but reliable, has been used in conjunction with solvatochromic probing using visible absorption spectroscopy to specify the binding site on peptide lipid **1**. The binding site has been found to be located on two dipoles, *i.e.*, N-H$^{\delta+}$ and C=O$^{\delta-}$ attached to both sides of the Gly residue, respectively, because introducing steric hindrance into the Gly moiety using several L-α-amino acids with bulky α-substituents interfered with the binding of Et(30). Similar specific binding behaviour of Et(30) was observed by replacing the Gly residue of the lipid **1** with sarcosine (Sar). It was found that self-assembly of the peptide lipid was necessary for effective capture of Et(30). The molecular structural requirements of the peptide lipids that form such specific polar microenvironments complementary to dipolar betaine dyes have also been investigated.

Introduction

Host–guest chemistry through a bottom-up approach both in water and in organic solvents using self-assembling molecules is indispensable to fundamental study and applications in the field of nanoscience and nanotechnology. In the accompanying paper,[1] we have demonstrated that a highly hydrophobic local microenvironment could be produced in water using newly designed self-assembling peptide amphiphiles. On the other hand, it is also of great significance to produce specific polar local microenvironments in nonaqueous and nonpolar media using nonionic and lipophilic self-assembing molecules. Polar guest molecules would prefer the local polar microenvironment rather than the nonpolar organic media. This would enable the construction of host–guest systems using polar molecules in the nonpolar solvent. In general, however, it is difficult to dissolve highly polar molecules in nonpolar organic media without using *e.g.*, lipophilic macrocyclic compounds. Therefore, studying host–guest chemistry using appropriate self-assembling acyclic molecules in nonpolar organic systems has been highly restricted. Since double-chained lipophilic peptide lipids possessing, as dipolar functional groups, at least three amide groups per molecule are soluble in nonpolar aromatic media such as benzene and self-assemble into fibrillar aggregates responsible for gelation,[2] appropriately designed nonionic peptide lipids are regarded as promising candidates for host compounds for specific polar guests soluble in nonpolar organic media. It is widely accepted that polar solutes are hardly solvated in nonpolar solvent, and therefore, dipolar functional groups such as amide groups exist almost bare in such a solvent. This results in much stronger electrostatic interactions between oppositely charged dipolar moieties in nonpolar solvent than in water, because both positive and negative solutes in water are surrounded by the oppositely polarized moiety of water molecules, respectively, and therefore, net charges are considerably reduced. In this respect, appropriately positioned plural amide groups in a peptide lipid molecule would cooperatively act not only as self-assembly driving forces for the lipid but also as receptors towards *e.g.*, lipophilic dipolar betaine molecules through complementary intermolecular interactions such as hydrogen bonding and dipole–dipole interactions in noncompetitive nonpolar media. Fortunately, Et(30), one of the representative solvatochromic dipolar betaine dyes, was found to be soluble in chlorobenzene which did not interfere

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† Electronic supplementary information (ESI) available: (1) Experimental details; (2) Supplementary figures. See DOI: 10.1039/b818218c

with the self-assembly of the lipophilic peptide lipids. Since ET(30) was sparingly soluble in nonpolar organic solvents such as benzene and toluene that have been found suitable for the self-assembly of peptide lipids,[2] chlorobenzene was employed in this study. We herein report on novel supramolecular hosts formed from self-assembled L-glutamic acid-derived nonionic peptide lipids that contain Gly and Sar residues. These lipids were found to capture ET(30) especially when they self-assembled in chlorobenzene as evidenced using the solvatochromic nature of ET(30) under its aggregate-free condition. Furthermore, the methodology of the receptor design has been potentially extended to an ethylenediamine derivative.

Results and discussion

Visible absorption spectral behaviour of ET(30) in chlorobenzene

It is widely accepted that ET(30) exhibits negative solvatochromism,[3] *i.e.*, the visible absorption maximum wavelength (λ_{max}) blue-shifts as the solvent polarity is increased. In fact, its λ_{max} in chlorobenzene, 1-butanol, and methanol (0.15 mM, 20 °C) were 752 nm (Fig. 1), 575 nm, and 515 nm, respectively. Also, ET(30) exhibits slight thermochromism, *i.e.*, the molar extinction coefficient gradually increases as the temperature is raised (Fig. S2†). When 3.0 mM of **1** was added to the chlorobenzene solution of ET(30) at 20 °C, the λ_{max} remarkably blue-shifted to 672 nm (Fig. 1). Since ET(30) is virtually aggregate-free at 0.15 mM in chlorobenzene (Fig. S2),† this blue-shift (hypsochromic shift) of the λ_{max} indicates that ET(30) was in contact with a more polar microenvironment than chlorobenzene. Therefore, the visually observed colour change from green (λ_{max}; 752 nm) to blue (λ_{max}; 672 nm) in chlorobenzene could be ascribed to the solvatochromism of monomeric ET(30) species bound to the polar moieties of lipid **1**. Molecular modelling suggested that the compound **1** (see Scheme 1) and ET(30) formed a host–guest complex as shown in Figs. 2 (a) and S3 (a).†

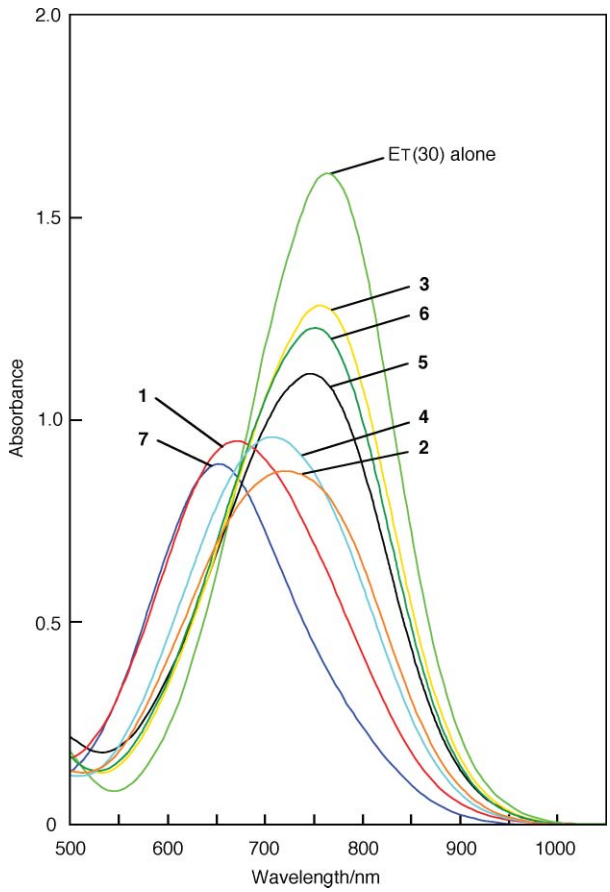


Fig. 1 Visible absorption spectra of ET(30) in the presence of lipids **1–7** in chlorobenzene. Temp., 20 °C, path length of quartz cell: 1.0 cm, [lipid] = 3.0 mM, [ET(30)] = 0.15 mM, [lipid]/[ET(30)] = 20. Receptor ability is in the order of Sar > Gly > Leu > Ala > Ile > Phe > Val under these conditions as evaluated from the extent of the blue-shift.

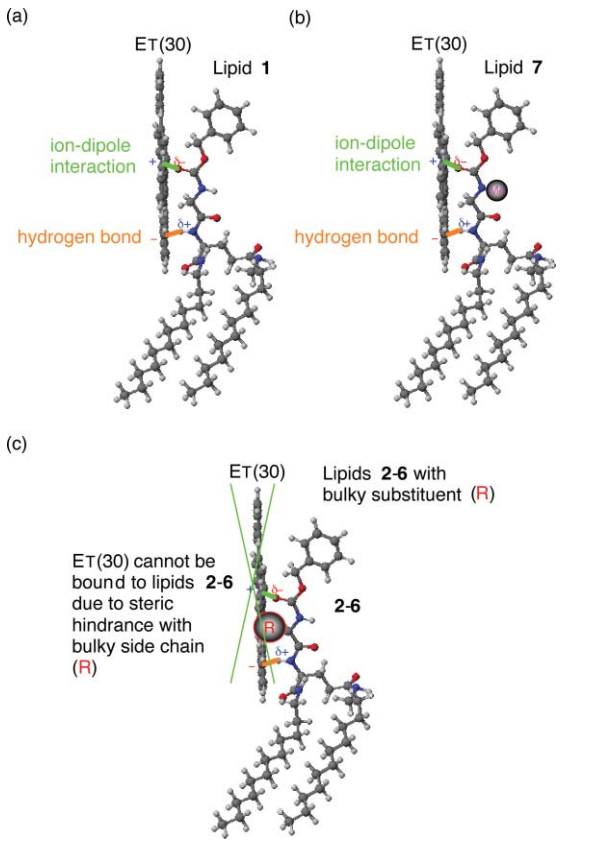
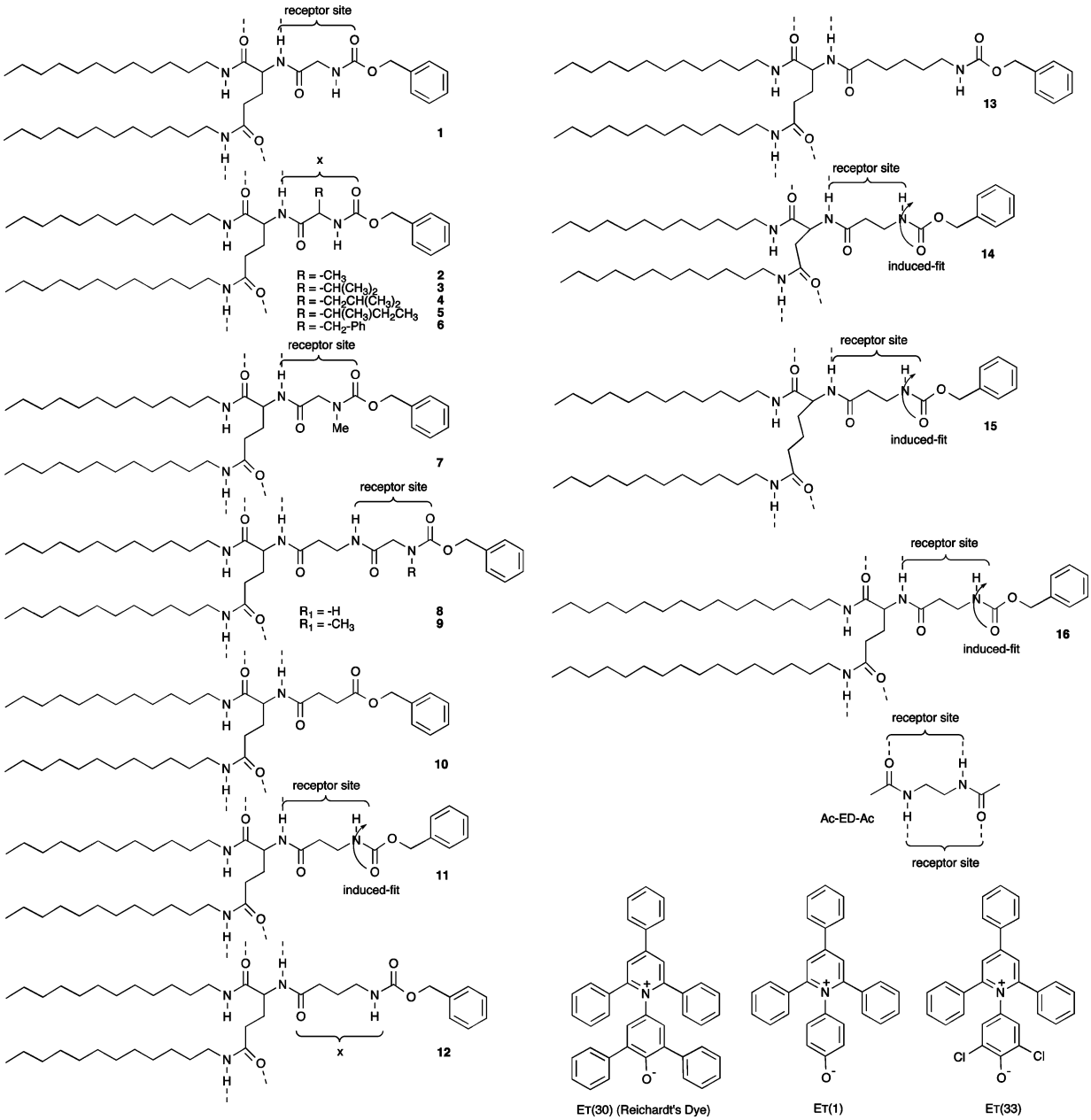


Fig. 2 Schematic representation of complementary intermolecular interactions between ET(30) and the spacer moiety of the dipeptide lipids: (a) dipeptide lipid **1**, (b) dipeptide lipid **7** with an *N*-methylated carbamate group, (c) dipeptide lipids **2–6** with bulky side chains (R) on the spacer moiety.

Specifying binding site, effect of self-assembly, and dye specificity

It was assumed that the receptor moiety on lipid **1** was N^{α}-H of the Gln residue and C=O of the amino-protecting group (Z-group or carbamate) attached to the Gly residue as shown in Figs. 2 (a) and S3 (a). Although, in general, fluorescence spectrophotometry is a powerful tool for the study of host–guest chemistry, ET(30) was found to be practically non-fluorescent. Therefore, it was not feasible to use fluorescence measurement to specify the binding site of ET(30). Thus, a synthetic approach was used, *i.e.*, replacing the original Gly residue of **1** at the receptor moiety with chiral α-amino acid residues. If our assumption were right, various chiral α-amino acid residues with bulky α-substituents would



Scheme 1

interfere with the complementary binding of ET(30) to the N^{α}-H and C=O connected with these α-amino acid residues. Since this approach is very laborious but reliable, we have prepared peptide lipids **2–6** (see Scheme 1) with L-Ala-Z, L-Val-Z, L-Leu-Z, L-Ile-Z, and L-Phe-Z headgroups, respectively. Fig. 1 shows the visible absorption spectra of ET(30) in the presence of lipids **2–6** in chlorobenzene. As expected, bulky substituents interfered with the blue-shift of absorption λ_{max} of ET(30) at 20 °C, *i.e.*, the binding of ET(30).‡ Therefore, our assumption was proved to be right as schematically shown in Fig. 2 (c). It may safely be concluded that the $N–H^{\delta+}$ and $C=O^{\delta-}$ are complementarily bound to the O^- and N^+ of ET(30) through hydrogen bonding and ion–dipole interaction, respectively. This conclusion was further confirmed by using peptide lipid **7** (see Scheme 1) containing a Sar residue as shown in Fig. 2 (b). The *N*-methyl group of the Sar residue did not interfere with the binding of ET(30) at all. Similarly, lipid **10** with a benzyl succinamate-headgroup exhibited the most blue-shifted λ_{max} as shown in Fig. 3. This indicates that the N-H of the Gly residue of **1** has nothing to do with the binding of ET(30). This is consistent with the conclusion described above. It is noted that lipid **12** with a GABA residue as the spacer moiety did not act as a receptor as evidenced by the result in Fig. 3, although, judging from its molecular structure, the GABA residue can complementarily bind to ET(30) as shown in Scheme 1. This

‡ As shown in Table 1, lipids **2**, **4**, and **6** with relatively lower T_c exhibited induced-fit at elevated temperatures below their T_c. This indicates that even some lipids with bulky substituents responsible for steric hindrance at the receptor moiety can undergo induced-fit to some extent when their self-assemblies are loosened at temperatures near their T_c.

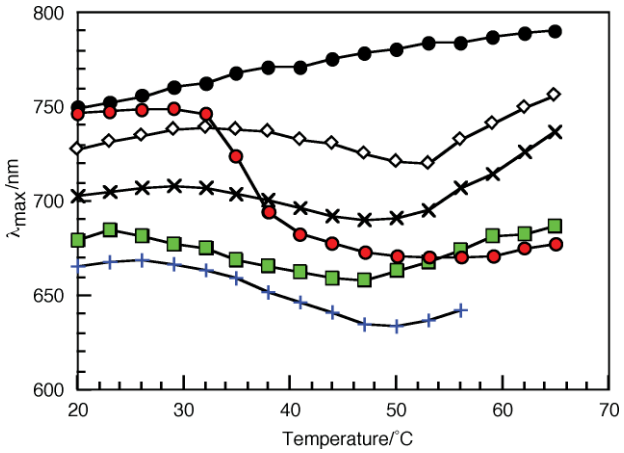


Fig. 3 Temperature dependence of the λ_{max} of ET(30) in the presence of lipids L-**1**, L-**10**, L-**11**, L-**12** and L-**13** in chlorobenzene: ■, L-**1**;**+**, L-**10**; ●, L-**11**; ×, L-**12**; ◇, L-**13**; ●, dye alone; [lipid] = 3.0 mM, [ET(30)] = 0.15 mM, [lipid]/[ET(30)] = 20.

indicates that ET(30) is not incorporated into the lipid assembly from the opposite side of the phenolate anion due to considerable steric hindrance by the side chain. Furthermore, lipids **1**, **7**, **8** and **9** captured ET(30) with an increasing molar ratio of lipid to dye at the constant dye concentration (0.15mM). The representative visible absorption spectra of **8** and **9** are shown in Fig. 4. The temperature dependences of the visible absorption spectra at the respective molar ratios in the heating process (data not shown) are very similar to the spectral changes with decreasing molar ratio at 20 °C in Fig. 4. These results indicate that aggregation of the lipid promotes the binding of ET(30), probably due to multiple interactions cooperating in the supramolecular cavity formed between self-assembled lipids **1**, **7**, **8** and **9**. With respect to dye specificity, similar host–guest interactions were observed when structurally related betaine dyes ET(1) and ET(33) were used instead of ET(30) as listed in Table 1. These results suggest that the host functions of the peptide lipids are not peculiar to ET(30) and are a general phenomena towards dipolar betaine dyes.

Binding constants

The binding constant (K_s) was tentatively determined as reported previously[4b] based on the equation suggested by Sepülveda *et al.*[5] Calculations were based on the following equation: $f/(1-f) = K_s\{[D_t] - [S_t]f\} - K_s[cac]$, in which f denotes the fraction [aggregate-incorporated dye]/[total dye],§ D_t is the total lipid concentration, S_t is the total ET(30) 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. The K_s values of ET(30) for **1**, **7**, **8**, and **9** at 20 °C were 5×10^4 M^{-1}, 3×10^4 M^{-1}, 1×10^4 M^{-1}, and 1×10^4 M^{-1}, respectively. Although these values may contain considerable errors, the K_s values could be used as comparisons for systematic interpretation. It is concluded that separation of the Gly or Sar residue from the L-Gln residue by insertion of a β-Ala residue as a spacer lowered the K_s values. This indicates that

§ Fraction f was determined according to the following equation $f = (\lambda - \lambda_a)/(\lambda_m - \lambda_a)$, where λ, λ_a and λ_m denote the λ_{max} value of ET(30) in the presence of varying concentrations of lipid in chlorobenzene, the λ_{max} value of ET(30) alone in chlorobenzene, and the λ_{max} value of ET(30) completely bound to the lipid assembly in chlorobenzene, respectively. The slope (K_s) could be obtained by plotting $f/(1-f)$ against $\{[D_t] - [S_t]f\}$.

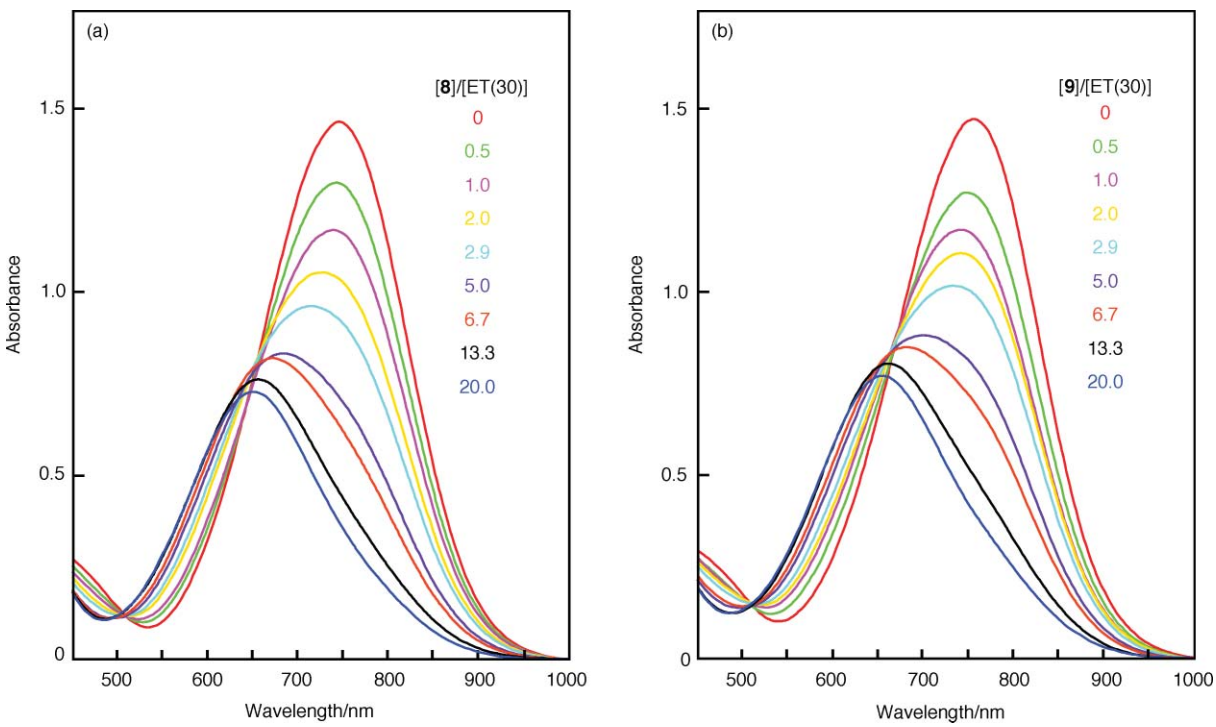


Fig. 4 Visible absorption spectra of ET(30) in the presence of lipids **8** (a) and **9** (b) in chlorobenzene at 20 °C. [ET(30)] = 0.15 mM = const., [lipid]/[ET(30)] = 0, 0.5, 1.0, 2.0, 2.9, 5.0, 6.7, 13.3, 20.0. Path length, 1.0 cm.

Table 1 The λ_{max} of ET(30) in the presence of various peptide lipids in chlorobenzene; [ET(30)] = 0.15 mM, [lipid] = 3.0 mM, [lipid]/[ET(30)] = 20

Peptide lipid	Dye	λ_{max} at 20 °C/nm	Minimum λ_{max}/nm	$\Delta\lambda_{max}$/nm[b]
1	ET(30)	672	659 (44 °C)[c]	13
1	ET(30)	652[a]	652 (20 °C)[a]	0
1	ET(1)	572	–	–
1	ET(33)	569	–	–
2	ET(30)	720	688 (44 °C)[c]	32‡
3	ET(30)	757	757 (20 °C)	0
4	ET(30)	708	697 (41 °C)[c]	11‡
5	ET(30)	753	753 (20 °C)	0
6	ET(30)	746	716 (59 °C)[c]	30‡
7	ET(30)	652	652 (20 °C)	0
8	ET(30)	649	649 (20 °C)	0
8	ET(1)	546	–	–
8	ET(33)	552	–	–
9	ET(30)	655	655 (20 °C)	0
9	ET(1)	549	–	–
9	ET(33)	553	–	–
10	ET(30)	666	635 (50 °C)[c]	31
L-**11**	ET(30)	748[d]	671 (53 °C)[f]	77[g]
L-**11**	ET(30)	718[e]	674 (56 °C)[f]	44[g]
DL-**11**	ET(30)	678[e]	668 (41 °C)[f]	10
12	ET(30)	704	691 (50 °C)[c]	13
13	ET(30)	728	721 (53 °C)[c]	7
14	ET(30)	752	701 (59 °C)[f]	51[g]
15	ET(30)	736[e]	696 (59 °C)[f]	40[g]
16	ET(30)	718[e]	675 (50 °C)[f]	43[g]
None	ET(30)	752	–	–
None	ET(1)	647	–	–
None	ET(33)	633	–	–
None (in MeOH)	ET(30)	513	–	–

[a] [lipid]/[ET(30)] = 6.7, [ET(30)] = 0.15 mM. [b] $\Delta\lambda_{max}$ is defined as subtraction of the minimum λ_{max} in the heating process from λ_{max} at 20 °C. [c] This is ascribed to slight induced-fit. [d] The lipid–dye mixture was prepared without heat treatment. It was found that the λ_{max} value of lipid **11** depended on the heat treatment prior to UV–visible absorption spectral measurement. [e] The blue-shifted λ_{max} from 752 nm is ascribed to initial induced-fit caused by conformational change of the β-Ala residue. [f] The remarkably blue-shifted λ_{max} is ascribed to induced-fit caused by drastic conformational change of the β-Ala residue. [g] The large $\Delta\lambda_{max}$ value could be regarded as a measure of the drastic conformational change of the β-Ala residue

self-assembled L-Gln residue and its neighbours contribute to the enhancement of K_s values cooperatively. This is consistent with the result that binding is promoted as the lipid concentration is increased, *i.e.*, self-assembly of lipid is promoted.

Induced-fit and gel-to-sol phase transition in the heating process

Next, the host–guest interaction between **11** and ET(30) was investigated. ET(30) alone in chlorobenzene (0.15 mM) exhibited a green colour and λ_{max} of 752 nm at 20 °C. When 3.0 mM of **11** was dissolved into the solution of ET(30) by sonication, the λ_{max} slightly blue-shifted to 748 nm at 20 °C as shown in Fig. 3 and Table 1.¶ The λ_{max} blue-shifted to a minimum value of 671 nm with increasing temperature as long as self-assembly of the lipid is maintained. These results indicate that ET(30) interacts with **11** to give a change in colour corresponding to that in more polar solvents in the heating process below T_c. Molecular modelling suggested that ET(30) complementarily bound to N^{α}-H of the Gln residue and C=O of the Z-group attached to the β-Ala residue of **11** due to a drastic conformational change as schematically shown in Fig. 5. Lipids **12** and **13** with an increased number (m = 3 for **12** and m = 5 for **13**) of methylene spacers than in **11** (m = 2) exhibited less effective binding of ET(30) as shown in Fig. 3. Furthermore, the minimum λ_{max} values in the heating processes are closely related to the spacer lengths as listed in Table 1. In other words, the shorter the spacer length is, the more remarkably the λ_{max} blue-shifted as evidenced by the minimum λ_{max} values of lipids **1** (m = 1), **11** (m = 2), **12** (m = 3), and **13** (m = 5). From these results, it is concluded that the induced-fit of the β-Ala residue of lipid **11** is responsible for the blue-shift of λ_{max} with a drastic conformational change from anti to non-anti conformation. Similar behaviours were observed for the structurally related lipids **14** and **15** (see Scheme 1 and Fig. S4†), although detailed behaviours in terms of the temperature dependence of λ_{max} were different depending on the side-chain length that affected T_c values. The gel-to-sol phase transition of self-assembled **11** by heating above T_c led to destruction of the self-assembly of **11** and the host–guest complex of **11** and ET(30), accompanied by restoratoion of the original λ_{max} of ET(30) alone in chlorobenzene. A schematic illustration of the behaviour is shown in Fig. S5 (b).† Similar disassembly behaviours of host–guest complexes at elevated temperatures above their T_c were observed for all the lipids that captured ET(30) in their self-assembled states.

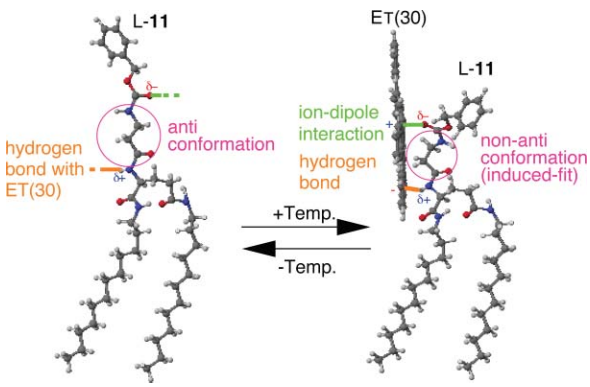


Fig. 5 Schematic representation of complementary intermolecular interactions between ET(30) and the spacer moiety of peptide lipid L-**11**. Adjacent lipids and bilayer structure are omitted for clarity.

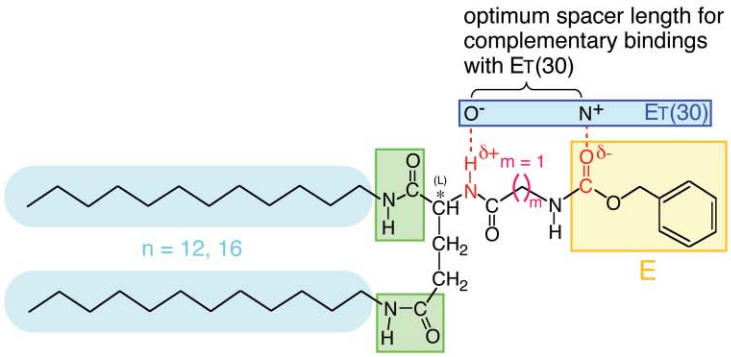
Molecular structural requirements of peptide lipids capable of capturing dipolar dyes

Molecular structural requirements of the peptide lipid for specific incorporation of ET(30) are summarized in Table 2. It is noted that *N′*,*N″*-didodecyl-N^{α}-[(3-carboxy)propanoyl]-L-glutamide ($2C_{12}$-L-Gln-suc)[4b,4c] with a carboxylic acid headgroup, capable of complementary binding with ET(30) judging by its molecular structure, did not induce a blue-shift in ET(30), indicating that ET(30) was blocked at the surface of the lipid assembly probably due to hydrogen bonding between –COOH and ET(30). This suggests that a dipolar headgroup acting as a hydrogen bonding

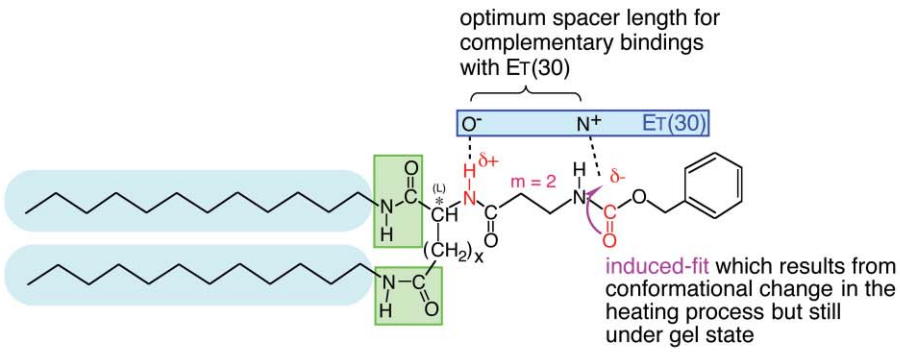
¶ The initial values of λ_{max} of ET(30) at 20 °C were found to be remarkably affected by the heat treatment accompanied by aging at 20 °C prior to visible absorption spectral measurements. This is due to the difference in the extent of the initial induced-fit arising from the difference in the heat treatment procedure. Table 1 shows two kinds of λ_{max} data for L-**11**.

Table 2 Structural requirements of acidic α-amino acid-derived peptide lipids and conditions for effective host–guest interaction with ET(30)

Structural requirements for specific capture of ET(30)



Lipids **11**, **14**, and **15** that show induced-fit due to drastic conformational change



molecular structure, solvent, etc.	effect/condition/result
spacer methylene number (m)	1 or 2
odd-even effect	odd (m = 1) > even (m = 2, induced fit by moderate heating)
carbonyl-containing headgroup (E)	carbamate (urethane), amide, ester
	carboxylic acid-headgroup blocked incorporation of ET(30) at the surface of lipid assembly
side-chain methylene number (x)	longer side-chain is preferred
optical isomer of Gln residue	racemate was preferred for blue-shift of λ_{max}: DL-**11** > L-**11**
connector moiety of double-chain	amide (ester-type lipid does not self-assemble)
alkyl chain of lipid (C_nH_{2n+1})	n = 12, 16
self-assembly of lipid	self-assembly of lipids promotes the incorporation of ET(30)
molar ratio of lipid to ET(30)	more than ca. 5 and not too excess of lipid (because ET(30) tends to be released at higher concentration of lipid)
solvents	nonpolar aprotic solvents such as chlorobenzene as long as ET(30) is soluble

donor is not suitable as a headgroup. Racemic lipid (DL-**11**) corresponding to L-**11** exhibited a more hypsochromic shift of the visible absorption band of ET(30) than L-**11** under the same conditions. It was reported that the molecular packing of glutamic acid-derived enantiomeric lipids is tighter than the corresponding racemates as long as they do not completely phase-separate into respective enantiomeric components.[4b] DSC measurements revealed that the DSC thermograms of L-**11** and DL-**11** are not identical (data are given in the Experimental section: Characterization of lipid aggregates), indicating that DL-**11** is not phase-separated into respective pure enantiomeric components. In general, enantiomeric amphiphiles in aqueous systems are densely packed and favorable to the specific incorporation of organic guests due to the major driving force of hydrophobic interactions between the hydrophobic moiety of the amphiphiles and the incorporated guest. It was found that ET(30) was more effectively captured by DL-**11** than the corresponding enantiomeric L-**11** as evidenced by the remarkable hypsochromic shift of the initial λ_{max} at 20 °C and the minimum λ_{max} at 44 °C of ET(30) (Fig. 6). Since the molecular packing of DL-**11** is assumed to be looser than that of L-**11** because of the lower T_c and smaller ΔH (T_c, 66 °C and 76 °C (ΔH; 10.1 kcal mol^{-1}) for L-**11**; T_c, 54 °C and 67 °C (ΔH; 6.4 kcal mol^{-1}) for DL-**11**), this opposite behaviour compared to conventional aqueous systems may be ascribed to the

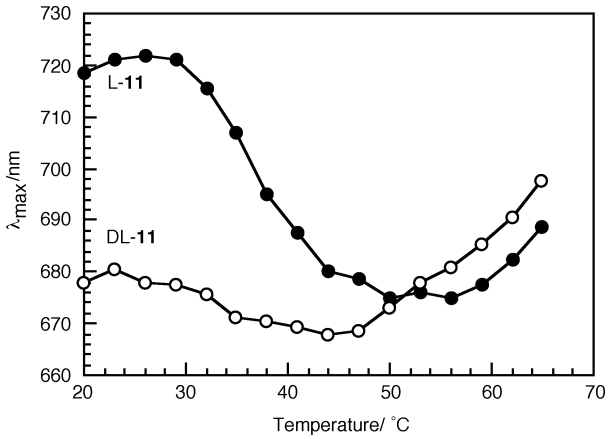


Fig. 6 Temperature dependence of the visible absorption maximum wavelength (λ_{max}) of ET(30) in the presence of lipids L-**11** and DL-**11** in chlorobenzene: [lipid] = 3.0 mM, [ET(30)] = 0.15 mM, [lipid]/[ET(30)] = 20.

absence of hydrophobic interactions in the present non-aqueous non-polar system. The same is true for the molecular structure of the guest dyes, *i.e.*, ET(30) is incorporated into the lipid assembly from the bulky anionic side as schematically shown in Figs. 2, 5, S3, and S5,† and Table 2. This direction in terms of incorporation of ET(30) is supported by the fact that lipid **12** with a GABA residue as the spacer moiety did not capture ET(30) effectively as demonstrated by the data in Fig. 3. This is probably due to steric hindrance between ET(30) and the side chain of lipid **12** as inferred from its chemical structure in Scheme 1. These results appear to be peculiar to non-aqueous systems. Elongation of the double-chain alkyl groups from didodecyl to dihexadecyl was found to effect less influence on the blue-shift of λ_{max} of ET(30) (Fig. S6).† The present findings could be applied to related dipolar molecules other than ET(30), ET(1), and ET(33) as long as they are soluble in the nonpolar organic media in which the peptide lipid can self-assemble. The present findings were extended to a nonionic receptor, *N*,*N*′-diacetylethylenediamine (Ac-ED-Ac). Although Ac-ED-Ac formed precipitates in chlorobenzene at room temperature due to aggregation *via* intermolecular hydrogen bonding, this compound induced a λ_{max} of 669 nm (absorbance; 0.93) in ET(30) under similar conditions. This indicates that diacylated ethylenediamine would also behave as an effective receptor towards ET(30) and its analogues in chlorobenzene, provided that it is attached to an appropriate self-assembling lipophilic lipid capable of complementary intermolecular hydrogen bonding, such as those derived from L-lysine,[1] to serve not only as a lipophilic moiety but also as a support for arrangement of the diacylated ethylenediamine residues in a nonpolar solvent.

Conclusions

It has been found that ET(30) binds to L-glutamic acid-derived double-chain lipids with benzyloxycarbonylated Gly headgroups and changes colour in chlorobenzene as if ET(30) were dissolved in more a polar organic solvent than chlorobenzene. This colour change was ascribed to the formation of a complementary host–guest complex between the peptide lipid and the dipolar ET(30) in nonpolar aprotic chlorobenzene. From the molecular modelling, the binding site was assumed to be polarized N-H$^{\delta+}$ and C=O$^{\delta-}$ attached to the Gly residue as a spacer of the lipid. The binding site was specified by means of introducing steric hindrance arising from bulky α-substituents into the spacer moiety using several L-α-amino acids instead of Gly. As expected, bulky substituents interfered with the binding of ET(30). Therefore, it may safely be concluded that the O$^-$ and N$^+$ of ET(30) complementarily bind to N-H$^{\delta+}$ and the C=O$^{\delta-}$ attached to the Gly residue of the lipid through hydrogen bonding and ion–dipole interaction, respectively. This conclusion was further confirmed by using Sar residue instead of Gly because the *N*-methyl group of Sar residue did not interfere with the binding of ET(30). It is noted that aggregation of the lipid promoted the binding of ET(30), indicating that ET(30) was cooperatively incorporated into the supramolecular cavity of the self-assembled lipids through multiple interactions operating in the supramolecular cavity. Similar host–guest interactions were observed when the structurally related dipolar betaine dyes, ET(1) and ET(33), were used instead of ET(30). Induced-fit was observed when a β-Ala residue was used instead of the Gly residue. This is presumably caused by both optimum spacer length and odd-even effect of spacer methylenes. It was found that the present findings, in terms of the design of a receptor moiety using Gly or Sar, were potentially applicable to diacylated ethylenediamine derivatives.

In conclusion, we have demonstrated that self-assembled nonionic peptide lipids could act as receptors for dipolar dyes in nonpolar aprotic solvent. The dipolar N-H and C=O attached to both sides of the Gly or Sar could complementarily bind to not only dipolar ET(30) but also its analogues (ET(1) and ET(33)) in chlorobenzene. Molecular structural requirements for effective capture of ET(30) have been clarified. Some results different from aqueous systems were found: (*i*) The self-assembled racemic mixture of **11** was rather superior at the capture of ET(30). This is probably because ET(30) was incorporated more easily into the lipid assembly due to less steric hindrance arising from looser molecular packing of the racemic lipid. (*ii*) The phenolate anion side of ET(30) molecule was preferentially incorporated rather than the pyridinium moiety in the middle of ET(30), although the phenolate anion side of ET(30) is rather bulky. These results may be caused by the absence of hydrophobic interactions between adjacent lipids. An ethylenediamine derivative was also found to be potentially applicable to the receptor moiety. Induced-fit behaviour typical of aqueous natural systems was found even in the artificial chlorobenzene system. In view of these results, it is suggested that the positioning of the N-H and C=O groups by a combination of amide and amide, amide and carbamate, amide and ester, or carbamate and ester, respectively, *via* an appropriate spacer would be a promising methodology for the design of a binding site for dipolar molecules in nonpolar aprotic organic media, provided that the molecules containing these dipolar functional groups are soluble in such media. The present findings could be applied to, *e.g.*, normal phase liquid chromatography for the separation of dipolar molecules when appropriate peptide microparticles composed of not only α-amino acids but also various ω-amino acids could be prepared as the stationary phase. It may also be possible to detect glycine residue in peptides and proteins using solvatochromic dipolar dyes if appropriate microenvironments for preferential binding could be produced.

Experimental

Materials and methods

N-Benzyloxycarbonylated α-amino acids (Z-α-amino acids) and β-amino acid used in this study were as follows: *N*-benzyloxycarbonylglysine (Gly-Z), *N*-benzyloxycarbonyl-L-alanine (L-Ala-Z), *N*-benzyloxycarbonyl-L-valine (L-Val-Z), *N*-benzyloxycarbonyl-L-leucine (L-Leu-Z), *N*-benzyloxycarbonyl-L-isoleucine (L-Ile-Z), *N*-benzyloxycarbonyl-L-phenylalanine (L-Phe-Z), *N*-benzyloxycarbonyl-sarcosine (Sar-Z), and *N*-benzyloxycarbonyl-β-alanine (β-Ala-Z). These Z-amino acids were purchased from Kokusan Chemicals. Co., Japan and used as received. *N*,*N*′-Diacetylethylenediamine (Ac-ED-Ac) was purchased from Tokyo Kasei Kogyo Co., Ltd, Japan and used as received. All the peptide compounds containing the *N*-benzyloxycarbonylated α-amino acids were prepared according to ordinary peptide synthesis procedures as described in the preceding paper.[1] All the α-amino acid derivatives denote L-isomers unless otherwise specified. Reichardt's Dye (2,6-diphenyl-4-(2,4,6-triphenyl-1-pyridinio)phenolate) (referred to as E$_T$(30) in this paper), 1-(4-hydroxyphenyl)-2,4,6-triphenylpyridinium hydroxide, inner salt hydrate (referred to as E$_T$(1) hereafter), and 2,6-dichloro-4-(2,4,6-triphenyl-1-pyridinio)phenolate (referred to as E$_T$(33) hereafter) were commercially available and used as received.

Characterization of peptide 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-270, and by elemental analysis with a Yanaco CHN Corder MT-3. It is noted that most of the peptide lipids and their precursors with more than three amide bonds per molecule formed organogels in $CDCl_3$, and therefore, ^{1}H NMR signals were considerably broadened. Therefore, ^{1}H NMR assignment was restricted to main signals, and no appreciable existence of impurities was confirmed to ensure the reliability of elemental analysis.

Preparation of chlorobenzene solutions of lipid–dye mixtures

Peptide lipid was dissolved in chlorobenzene solution of 0.15 mM E$_T$(30) by sonication and heating in 85 °C water unless otherwise noted.‖ After being allowed to stand at 20 °C for several hours in the dark, the solutions were subjected to visible absorption spectral measurements.

Visible absorption spectra measurements

The samples in a 1.0 cm quartz cell were incubated in a sample holder for 10 min at 20 °C. The visible absorption spectra were measured with a JASCO Ubest V-530 spectrophotometer.

Preparation of peptide lipids

All the peptide lipids **1–16** including intermediates **17–20** were prepared according to previously reported methods[4] (see ESI†).

‖ In the case of L-**11**, two kinds of samples with and without heating, respectively, were prepared and the results are shown in Table 1.

Characterization of peptide lipid aggregates

Formation of highly ordered peptide lipid aggregates in chlorobenzene was confirmed by using transmission electron microscopy (TEM) with a JEOL 2000FX transmission electron microscope or Phillips TECNAI F20 S-TWIN. The viscous chlorobenzene solutions (fragmentary gels) of 1.0 mmol dm^{-3} peptide lipids were spotted onto carbon-coated copper grids. The samples were air-dried at room temperature, after which they were post-stained with 2 wt% aqueous ammonium molybdate. Aggregate morphologies of **1–16** in chlorobenzene were observed using TEM. It has been found that all the peptide lipids (**1–16**) formed fibrillar aggregates based on bilayer structures after dissolution in chlorobenzene at 85 °C in water bath and subsequent aging at room temperature. The representative aggregate morphologies of L-**1** and L-**11** are shown in Fig. S1.† Differential scanning calorimetry (DSC) measurements of 20 mM chlorobenzene solutions also supported the existence of self-assembled peptide lipids. The phase transition temperature was measured by DSC with a SEIKO I & E DSC 120. The sample solution (20 mmol dm^{-3}) was sealed in an Ag capsule and scanned using a heating rate of 2.0 °C min^{-1}. Gel-to-sol phase transition temperature (T_c) and transition enthalpy (ΔH) of bilayer-based aggregates estimated by DSC measurements were as follows: T_c, 57 °C (ΔH; 9.3 kcal mol^{-1}) for **1**; T_c, 66 °C (ΔH; 4.8 kcal mol^{-1}) for **2**; T_c, 88 °C (ΔH; 18 kcal mol^{-1}) for **3**; T_c, 51 °C (ΔH; 14 kcal mol^{-1}) for **4**; T_c, 81 °C (ΔH; 6.9 kcal mol^{-1}) for **5**; T_c, 63 °C (ΔH; 14 kcal mol^{-1}) for **6**; T_c, 52 °C (ΔH; 10 kcal mol^{-1}) for **7**; T_c, 33 °C (ΔH; 4.2 kcal mol^{-1}) for **8**; T_c, 25 °C (ΔH; 2.4 kcal mol^{-1}) for **9**; T_c, 91 °C (ΔH; 1.8 kcal mol^{-1}) for **10**; T_c, 66 °C and 76 °C (ΔH; 10.1 kcal mol^{-1}) for L-**11**; T_c, 54 °C and 67 °C (ΔH; 6.4 kcal mol^{-1}) for DL-**11**; T_c, 51 °C and 71 °C (ΔH; 13 kcal mol^{-1}) for **12**; T_c, 65 °C (ΔH; 5.7 kcal mol^{-1}) for **13**; T_c, 77 °C and 83 °C (ΔH; 4.0 kcal mol^{-1}) for **14**; T_c, 42 °C and 80 °C, (ΔH; 11 kcal mol^{-1}) for **15**, and T_c, 71 °C (ΔH; 6.7 kcal mol^{-1}) for **16**.

Molecular modelling

Molecular modelling was performed using CAChe WorkSystem Ver. 4.1.1 for Power Macintosh (1999 Oxford Molecular Ltd.) installed on Macintosh G3 OS 8.6. To begin with, molecular structure was produced using Editor, then transferred to Mechanics (settings: Parameter File: MM2 Parameters; Force Field Type: MM2) to optimize conformation, and finally the results were transferred to Visualizer. The graphics finally obtained were further transferred to software for drawing and then appropriately processed. It is noted that the modelling results were not reproducible, and therefore, the figures produced may be regarded as schematic to some extent.

Acknowledgements

This work was supported in part by the Ministry of Education, Culture, Sports, Science and Technology, Japan (S0801085). We are grateful to Dr. H. Ihara and Dr. M. Takafuji of Kumamoto University for elemental analyses, and Dr. M. Nishida of Kyushu University for taking transmission electron micrographs using JEOL 2000FX and Dr. R. Tomoshige of the Research Centre for Advances in Impact Engineering, Faculty of Engineering, Sojo University for taking transmission electron micrographs

using Phillips TECNAI F20 S-TWIN. We are also grateful to N. Saeki, Y. Dan, S. Yamaura, S. Hirai, T. Sakaguchi, H. Momoi, N. Kanekura, and Y. Umenaga for peptide lipid preparations and technical assistance.

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