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MOLECULAR RECOGNITION BY MACROCYCLIC RECEPTORS HAVING MULTIPLE HYDROPHOBIC BRANCHES IN A SYNTHETIC BILAYER MEMBRANE

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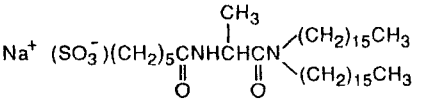
Hybrid molecular assemblies were prepared in combinations of a synthetic peptide lipid, involving an L-alanine residue interposed between an anionic head group and a hydrophobic double-chain segment, with cationic macrocyclic hosts, a steroid cyclophane bearing four rigid steroid moieties and octopus cyclophanes having eight flexible hydrocarbon branches. On addition of the cyclophanes to multi-walled bilayer membranes composed of the anionic lipid, thermodynamic parameters (ΔH and ΔS) associated with the phase transition between the gel and liquid-crystalline states were subjected to changes that are consistent with the formation of the hybrid assemblies. Anionic fluorescent guests, 8-anilinonaphthalene-1-sulphonate and 6-*p*-toluidinonaphthalene-2-sulphonate, were effectively incorporated into the hydrophobic cavities provided by the cationic cyclophanes embedded in the bilayer membrane through hydrophobic and electrostatic interactions. The guest-binding modes of the hybrid assemblies are classified into two types; a guest is included in the proximity of the hydrogen-belt domain of the bilayer membrane in one mode, and a guest is incorporated into the hydrophobic bilayer domain composed of double hydrocarbon chains of the lipid in another.

INTRODUCTION

In recent years, much attention has been focused on molecular recognition by artificial macrocycles in order to mimic specific functions of naturally occurring supramolecular hosts, such as enzymes and receptors.[1–8] While molecular recognition abilities of such artificial hosts have been generally examined in solution and solid states, biological receptors exhibit characteristic molecular recognition in combination with biomembranes. In this regard, guest recognition by supramolecular assemblies composed of an artificial macrocycle and a bilayer membrane has been studied only to a limited extent.[8,9]

We have been employing functionalized cyclophanes as artificial enzymes and receptors,[5,10–12] while adopting bilayer aggregates formed with synthetic peptide lipids, which have α-amino acid residue(s) interposed between a polar head moiety and a hydrophobic double-chain segment through amide linkages, as structural and functional models of biomembranes.[13] On the above-mentioned ground, we report here on molecular recognition by hydrophobic cyclophane derivatives which are embedded in the bilayer membrane formed with an anionic peptide lipid (**1**), two types of hydrophobic macrocycles being used as receptor models, a steroid cyclophane bearing four rigid steroid moieties (**2**) and octopus cyclophanes having eight flexible hydrocarbon branches (**3** and **4**). These cationic macro-



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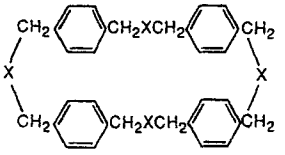
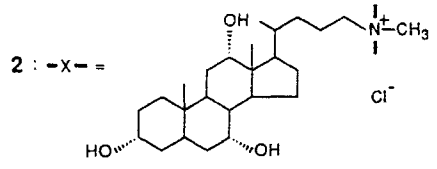
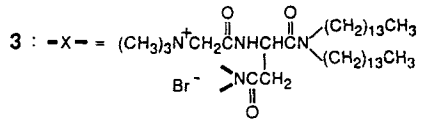
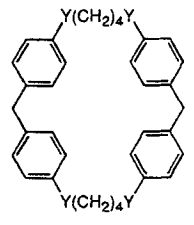
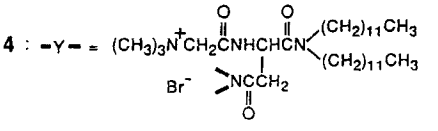
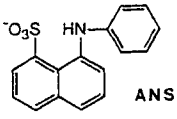




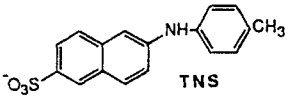








ANS



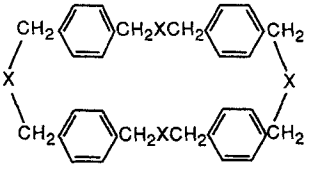
TNS

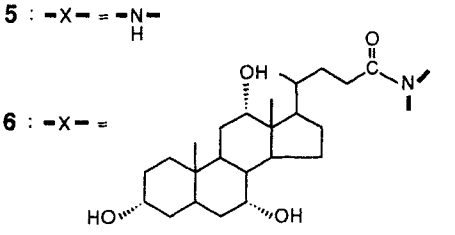
cycles afforded hybrid assemblies with the anionic peptide lipid and acted as effective artificial receptors toward anionic organic guests, such as 8-anilinonaphthalene-1-sulphonate (ANS) and 6-*p*-toluidinonaphthalene-2-sulphonate (TNS). Characteristic features of molecular recognition by the receptor models embedded in the bilayer membrane are clarified in this work.[14]

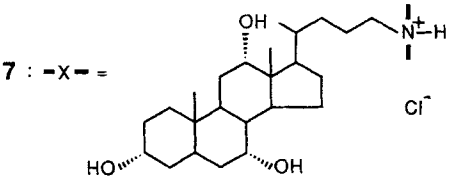
EXPERIMENTAL

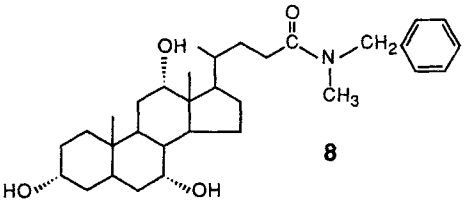
Materials. Magnesium bis(8-anilinonaphthalene-1-sulphonate) [$Mg(ANS)_2$] and potassium 6-*p*-toluidinonaphthalene-2-sulphonate [K(TNS)] of guaranteed reagent grade were obtained as fluorescent probes from Nacalai Tesque (Kyoto, Japan) and used without further purification. An anionic peptide lipid, sodium *N,N*-dihexadecyl-N^{α}-(6-sulphohexanoyl)-L-alaninamide (**1**),[15] and an octopus cyclophane, *N,N',N'',N'''*-tetrakis{3-(*N,N*-ditetradecylcarbamoyl)-3-[(trimethylammonio)acetamido]propanoyl}-2, 11, 20, 29-tetraaza[3.3.3.3]paracyclophane tetrabromide (**3**),[16] were prepared according to the methods reported previously.

N,N',N'',N'''-Tetrakis(3α,7α,12α-trihydroxy-5β-cholan-24-oyl)-2,11,20,29-tetraaza[3.3.3.3]paracyclophane (**6**). Ethyl chloroformate (260 mg, 2·4 mmol)









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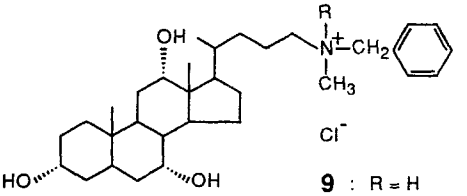
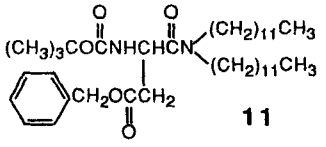


9 : R = H

10 : R = CH_3



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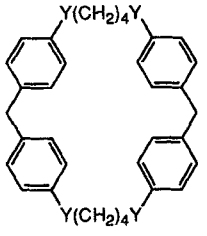
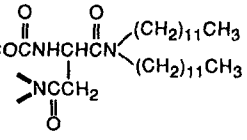


12: -Y- = -N-
H

13: -Y- =



was added to a mixture of cholic acid (3α,7α,12α-trihydroxy-5β-cholan-24-oic acid; 970 mg, 2·4 mmol) and dry triethylamine (240 mg, 2·4 mmol) in dry tetrahydrofuran (30 ml) at room temperature, and the reaction vessel was placed immediately in an ice-bath for 20 min while the mixture was gently stirred. A filtrate of the mixture was added to a dry tetrahydrofuran (60 ml) solution of 2,11,20,29-tetraaza[3.3.3.3]paracyclophane[17] (**5**; 190 mg, 0·40 mmol), and the resulting mixture was stirred for 20 h at room temperature. The solvent was removed under reduced pressure and the residue was purified by liquid chromatography on a column of silica gel (Wakogel C-100) with methanol as eluent. Evaporation of the solvent under reduced pressure gave a white solid (670 mg, 82%): m.p. 216–223 °C; IR (KBr disc), 3412 (OH), 2938 and 2867 (CH) and 1629 (C=O) cm^{-1}; ^1H NMR (CD_3SOCD_3, 373 K), δ = 0·60 [12H, s, 18-H (steroid)], 0·82 [12H, s, 19-H (steroid)], 0·90 [12H, d, J = 5·9 Hz, 21-H (steroid)], 2·3 [8H, m, 23-H (steroid)], 3·21 [4H, m, 3-H (steroid)], 3·52 (4H, d, J = 3·4 Hz, OH), 3·63 [8H, m, 12-H (steroid) and OH], 3·77 [4H, m, 7-H (steroid)], 3·85 (4H, d, J = 4·4 Hz, OH), 4·39 (16H, br s, CH_2Ph) and 6·86 (16H, s, ArH); mass spectrum (FAB-MS), m/z 2039 ($M^+ + H$); calculated MW for $C_{128}H_{188}N_4O_{16}$, 2039 ($M^+ + H$). Analysis: calculated for $C_{128}H_{188}N_4O_{16}\cdot 3H_2O$, C 73·46, H 9·34, N 2·68; found, C 73·54, H 9·20, N 2·71%.

N,N′,N″,N‴-Tetrakis(3α,7α,12α-trihydroxy-5β-cholan-24-yl)-2,11,20,29-tetraaza[3.3.3.3]paracyclophane tetrahydrochloride (**7**). A solution of **6** (100 mg, 0·050 mmol) in dry tetrahydrofuran (20 ml) was stirred at room temperature under nitrogen atmosphere for 1 h. Borane–dimethyl sulphide (borane content 10 mol dm^{-3}; 3 ml, 30 mmol) was added to the solution, and the mixture was stirred for 1 h under nitrogen atmosphere. The dissociated dimethyl sulphide was evaporated off and the mixture was refluxed for 10 h under nitrogen atmosphere. The solvent was removed under reduced pressure, aqueous hydrochloric acid (1 mol dm^{-3}; 10 ml) was added to the residue and the mixture was refluxed for 10 h. The resulting mixture was cooled to room temperature and insoluble materials were recovered. This crude product was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol as eluent. Evaporation of the solvent under reduced pressure gave a white solid (87 mg, 83%): m.p. 199–202 °C; IR (KBr disc), 3381 (OH), and 2931 and 2864 (CH) cm^{-1}; ^1H NMR (CD_3SOCD_3, 293 K), δ = 0·60 [12H, s, 18-H (steroid)], 0·82 [12H, s, 19-H (steroid)], 0·98 [12H, br s, 21-H (steroid)], 3·62 [4H, m, 12-H (steroid)], 3·80 [4H, m, 7-H (steroid)], 4·33 (16H, m, CH_2Ph) and 7·39 (16H, m, ArH). Analysis: calculated for $C_{128}H_{200}Cl_4N_4O_{12}$, C 72·22, H 9·47, N 2·63; found, C 72·25, H 9·47, N 2·89%.

N,N′,N″,N‴-Tetrakis(3α,7α,12α-trihydroxy-5β-cholan-24-yl)-N,N′,N″,N‴-tetramethyl-2,11,20,29-tetraazonia[3.3.3.3]paracyclophane tetrachloride (**2**). Methyl iodide (5·0 g, 35 mmol) and potassium carbonate (830 mg, 5·4 mmol) were added to **7** (174 mg, 0·082 mmol) dissolved in dry *N,N*-dimethylformamide (25 ml) and the mixture was stirred for 69 h at room temperature. After precipitates had been removed from the mixture by filtration, the filtrate was evaporated to dryness under reduced pressure. Water (10 ml) was added to the residue and insoluble materials were recovered. The resulting iodide salt was converted into the chloride salt by ion-exchange chromatography on a column of Dowex 1-X8 (Cl^-) with methanol as eluent. The solvent was removed under reduced pressure and the crude product was purified by gel filtration chromatography on a column of Toyopearl HW-40F with methanol as eluent. Evaporation of the product fraction *in vacuo* gave a pale yellow solid (149 mg, 84%): m.p. 207–210 °C; IR (KBr disc), 3380 (OH), and 2935 and 2860 (CH) cm^{-1}; ^1H NMR (CD_3SOCD_3, 293 K), δ = 0·59 [12H, s, 18-H (steroid)], 0·81 [12H, s, 19-H (steroid)], 0·95 [12H, br s, 21-H (steroid)], 3·61 [4H, m, 12-H (steroid)], 3·66 (13H, s, CH_3N^+), 3·79 [4H, m, 7-H (steroid)], 4·60 (16H, m, CH_2Ph) and 7·55 (16H, m, ArH). Analysis: calculated for $C_{132}H_{208}Cl_4N_4O_{12}\cdot 3H_2O$, C 70·81, H 9·63, N 2·50; found, C 70·85, H 9·26, N 2·85%.

N-Benzyl-N-methyl-3α,7α,12α-trihydroxy-5β-cholan-24-amide (**8**). This compound was prepared by conden-

sation of cholic acid (3·3 g, 8·0 mmol) with *N*-methylbenzylamine (0·97 g, 8·0 mmol) in a manner similar to that applied to the synthesis of **6**. The crude product was purified by liquid chromatography on a column of silica gel (Wakogel C-100) with acetone as eluent to give a white solid (3·0 g, 74%): m.p. 99–101 °C; IR (KBr disc), 3400 (OH), 2933 and 2867 (CH), and 1629 (C=O) cm^{-1}; ^1H NMR (CD_3SOCD_3, 373 K), $\delta = 0·61$ [3H, s, 18-H (steroid)], 0·94 [3H, d, $J = 6·5$ Hz, 21-H (steroid)], 2·3 [2H, m, 23-H (steroid)], 3·23 [1H, m, 3-H (steroid)], 3·52 (1H, d, $J = 3·9$ Hz, OH), 3·65 [2H, m, 12-H (steroid) and OH], 3·78 [1H, m, 7-H (steroid)], 3·85 (4H, d, $J = 4·4$ Hz, OH), 4·51 (2H, s, CH_2Ph) and 7·3 (5H, m, ArH). Analysis: calculated for $C_{32}H_{49}NO_4$, C 75·11, H 9·65, N 2·74; found, C 74·72, H 9·70, N 2·53%.

N-Methyl-N-(3α,7α,12α-trihydroxy-5β-cholan-24-yl)-benzylamine hydrochloride (**9**). This compound was prepared by reduction of **8** (100 mg, 0·20 mmol) with borane–dimethyl sulphide (borane content 10 mol dm^{-3}; 0·5 ml, 5 mmol) in a manner similar to that applied to the synthesis of **7**. The crude product was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol as eluent to give a pale brown, glassy solid (85 mg, 81%): m.p. 118–120 °C; IR (KBr disc), 3387 (OH) and 2926 and 2862 (CH) cm^{-1}; ^1H NMR ($CDCl_3$, 293 K), $\delta = 0·8–1·0$ [9H, m, 18-H, 19-H, and 21-H (steroid)], 2·68 (3H, s, CH_3N^+), 2·90 [2H, m, 24-H (steroid)], 4·17 (2H, br s, CH_2Ph) and 7·45 (5H, m, ArH). Analysis: calculated for $C_{32}H_{52}ClNO_3$, C 71·95, H 9·81, N 2·62; found, C 72·00, H 9·39, N 2·55%.

N,N-Dimethyl-N-(3α,7α,12α-trihydroxy-5β-cholan-24-yl)benzylammonium chloride (**10**). This compound was prepared by quaternization of **9** (200 mg, 0·40 mmol) with methyl iodide (1·7 g, 12 mmol) followed by ion exchange in a manner similar to that applied to the synthesis of **2**. The crude product was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol as eluent to give a pale yellow, glassy solid (144 mg, 72%): m.p. 143–146 °C; IR (KBr disc), 3390 (OH) and 2924 and 2860 (CH) cm^{-1}; ^1H NMR ($CDCl_3$, 293 K), $\delta = 0·8–1·0$ [9H, m, 18-H, 19-H, and 21-H (steroid)], 3·29 (6H, br s, CH_3N^+), 5·00 (2H, br s, CH_2Ph) and 7·49 (5H, m, ArH). Analysis: calculated for $C_{33}H_{54}ClNO_3$, C 72·30, H 9·93, N 2·55; found, C 71·98, H 9·73, N 2·65%.

N,N-Didodecyl-N$^{\alpha}$-(tert-butoxycarbonyl)-L-isoasparagine β-benzyl ester (**11**). Dicyclohexylcarbodiimide (2·37 g, 11·6 mmol) was added to a dry dichloromethane (20 ml) solution of *N*$^{\alpha}$-(*tert*-butoxycarbonyl)-L-aspartic acid β-benzyl ester (3·36 g, 10·4 mmol) at 0 °C and the mixture was allowed to stand at the same temperature while being stirred for 20 min. Didodecylamine (3·54 g, 10·0 mmol) in dry dichloromethane (25 ml) was added to the mixture and the resulting mixture was stirred for 4 h at 0 °C. An insoluble material (*N,N'*-dicyclohexylurea) was removed by filtration, the solvent was eliminated under reduced pressure and the residue was dissolved in ethyl acetate (60 ml). After the solution had been allowed to stand overnight at 0 °C, the precipitates that had formed were removed by filtration. The filtrate was washed with 10% aqueous citric acid (4 × 60 ml), 4% aqueous sodium hydrogencarbonate (3 × 60 ml) and saturated aqueous sodium chloride (60 ml) in this sequence. Water was removed from the ethyl acetate solution by treatment with silicone-treated filter-paper (Whatman 1PS) and the solution was evaporated to dryness under reduced pressure. The residue was purified by liquid chromatography on a column of silica gel (Wakogel C-100) with ethyl acetate as eluent, followed by gel filtration chromatography on a column of Toyopearl HW-40F with methanol as eluent. Evaporation of the solvent under reduced pressure gave a pale yellow oil (2·52 g, 38%): TLC (Wako silica gel 70FM; methanol), $R_F = 0·41$; IR (neat), 3300 (NH), 2926 and 2854 (CH) and 1736, 1711, and 1636 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 293 K), $\delta = 0·88$ [6H, t, $J = 6·8$ Hz, $(CH_2)_{11}CH_3$], 1·26 [40H, m, $CH_2(CH_2)_{10}CH_3$], 1·42 [9H, s, $(CH_3)_3CO$], 2·63 [1H, dd, $J_{vic} = 6·5$ Hz and $J_{gem} = 15·6$ Hz, CH_2COO (non-equivalent)], 2·82 [1H, dd, $J_{vic} = 6·5$ Hz and $J_{gem} = 15·6$ Hz, CH_2COO (non-equivalent)], 3·2–3·5 [4H, m, $CH_2(CH_2)_{10}CH_3$], 4·96 (1H, m, NHC*H*CO), 5·11 (2H, s, benzyl), 5·28 (1H, d, $J = 8·8$ Hz, CON*H*CH) and 7·34 (5H, m, ArH).

N,N',N'',N'''-Tetrakis[3-(N,N-didodecylcarbamoyl)-3-[(tert-butoxycarbonyl)amino]propanoyl]-1,6,20,25-tetraaza[6.1.6.1]paracyclophane (**13**). Palladium black (10% Pd, 1·0 g) was added to **11** (2·36 g, 3·78 mmol) in tetrahydrofuran (40 ml) and hydrogen was introduced into the mixture at room temperature for 22 h with stirring. The catalyst was removed by filtration and the solvent was eliminated under reduced pressure. The residue was chromatographed on a column of silica gel (Wakogel C-100) with ethyl acetate as eluent, and the product fraction was dried *in vacuo* to give a pale yellow oil (1·40 g, 67%). Removal of the benzyl group was confirmed by IR and ^1H NMR spectroscopy. Dicyclohexylcarbodiimide (0·48 g, 2·33 mmol) was added to a solution of the deprotected derivative of **11** (1·30 g, 2·29 mmol) in dry dichloromethane (20 ml) at 0 °C and the mixture was allowed to stand for 20 min at the same temperature while being stirred. 1,6,20,-25-Tetraaza[6.1.6.1]paracyclophane[18] (**12**; 0·26 g, 0·51 mmol) in dry dichloromethane (20 ml) was added to the mixture and the resulting mixture was stirred for

4 h at 0 °C and for an additional 23 h at room temperature. An insoluble material (*N,N'*-dicyclohexylurea) was removed by filtration and the filtrate was evaporated to dryness under reduced pressure. The residue was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol–chloroform (1 : 1, v/v) as eluent. Evaporation of the product fraction under reduced pressure gave a pale yellow solid (1.00 g, 73%): m.p. 98–100 °C; TLC [Wako silica gel 70FM; methanol–ethyl acetate (1 : 1, v/v)], $R_F = 0{\cdot}62$; IR (KBr disc), 3281 (NH), 2925 and 2854 (CH) and 1707 and 1647 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 293 K), $\delta = 0{\cdot}88$ [24H, t, $J = 6{\cdot}8$ Hz, $(CH_2)_{11}CH_3$], 1·25 [160H, m, $CH_2(CH_2)_{10}CH_3$], 1·40 [36H, s, $(CH_3)_3CO$], 1·5 [8H, m, $NCH_2(CH_2)_2CH_2N$], 2·0–2·6 [8H, m, $COCH_2CH$], 3·0–3·7 [24H, m, $NCH_2(CH_2)_2CH_2N$ and $CH_2(CH_2)_{10}CH_3$], 3·92 (4H, s, $PhCH_2Ph$), 4·93 (4H, m, NHCHCO), 5·07 [2H, br, CONHCH (non-equivalent)], 5·35 [2H, br, CONHCH (non-equivalent)] and 6·9–7·3 (16H, m, ArH).

N,N',N'',N'''-Tetrakis{3-(N,N-didodecylcarbamoly)-3-[(trimethylammonio)acetamido]propanoyl}-1,6,20,-25-tetraaza[6.1.6.1]paracyclophane tetrabromide (**4**). Trifluoroacetic acid (7 ml) was added to a dry dichloromethane (25 ml) solution of **13** (0·89 g, 0·33 mmol) and the mixture was stirred for 3 h at room temperature. After the solution had been evaporated to dryness under reduced pressure, the residue was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol as eluent. Evaporation of the solvent under reduced pressure gave a pale yellow glass (650 mg, 84%). Removal of four *tert*-butoxycarbonyl groups was confirmed by IR and ^1H NMR spectroscopy. The deprotected derivative of **13** (500 mg, 0·22 mmol) and triethylamine (0·7 ml, 5 mmol) were dissolved in dry dichloromethane (20 ml) and the solution was cooled to 0 °C. Bromoacetyl chloride (0·47 g, 5·5 mmol) in dry dichloromethane (20 ml) was added dropwise to the solution at 0 °C with stirring and the mixture was stirred for 2 h at 0 °C and for an additional 18 h at room temperature. The resulting mixture was evaporated to dryness under reduced pressure. The residue was dissolved in dichloromethane (100 ml) and washed with 4% aqueous sodium hydrogencarbonate (50 ml), 4% aqueous citric acid (50 ml) and saturated aqueous sodium chloride (2 × 50 ml) in this sequence. Water was removed from the ethyl acetate solution by treatment with silicone-treated filter-paper (Whatman 1PS), the solution was evaporated to dryness under reduced pressure and the residue was dissolved in tetrahydrofuran (30 ml). Dry trimethylamine gas was introduced into the solution for 2·5 h at room temperature and the resulting solution was stirred at the same temperature for 18 h. The solution was evaporated to dryness under reduced pressure and the residue was purified by gel filtration chromatography on a column of Toyopearl HW-40F with methanol as eluent. Evaporation of the product fraction under reduced pressure gave a pale brown solid (397 mg, 82%): m.p. 152–153 °C; IR (KBr disc), 2925 and 2853 (CH) and 1680 and 1649 (C=O) cm^{-1}; ^1H NMR (CD_3SOCD_3, 373 K), $\delta = 0{\cdot}84$ [24H, t, $J = 6{\cdot}8$ Hz, $(CH_2)_{11}CH_3$], 1·25 [160H, m, $CH_2(CH_2)_{10}CH_3$], 1·46 [8H, m, $NCH_2(CH_2)_2CH_2N$], 2·23 [4H, m, $COCH_2CH$ (non-equivalent)], 2·48 [4H, m, $COCH_2CH$ (non-equivalent)], 3·0–3·6 [24H, m, $NCH_2(CH_2)_2CH_2N$ and $CH_2(CH_2)_{10}CH_3$], 3·20 [36H, s, $N^+(CH_3)_3$], 3·93 (4H, s, $PhCH_2Ph$), 4·04 [8H, s, $COCH_2N^+(CH_3)_3$], 5·12 (4H, m, NHCHCO), 7·08 [8H, d, $J = 8{\cdot}3$ Hz, NArH(*ortho*)], 7·29 [8H, d, $J = 8{\cdot}3$ Hz, NArH(*meta*)] and 8·59 (2H, d, $J = 8{\cdot}0$ Hz, CONHCH). Analysis: calculated for $C_{166}H_{296}Br_4N_{16}O_{12}$, C 65·85, H 9·85, N 7·40; found, C 66·02, H 9·76, N 7·19%.

Measurements. Melting points were measured with a Yanagimoto MP-S1 apparatus (hot-plate type). Elemental analyses were performed at the Microanalysis Center of Kyushu University. IR spectra were recorded on a JEOL JIR-AQS20M FT-IR spectrophotometer. NMR spectra were taken on a JEOL JNM-GX270 spectrometer. A JEOL JMS HX-100 spectrometer was used for fast atom bombardment mass spectrometry (FAB-MS). A MicroCal MC-2 ultrasensitive scanning calorimeter was used for differential scanning calorimetry (DSC). Surface tension measurements were performed at room temperature with a Shimadzu ST-1 surface tensometer assembled by the Wilhelmy principle, and dynamic light-scattering measurements were carried out with a Photal (Otsuka Electronics) DLS-700 dynamic light-scattering spectrophotometer (He–Ne laser, 632·8 nm) equipped with an NEC PC-9801 RA personal computer. Fluorescence spectra were taken on a Hitachi 650-40 spectrofluorimeter. Steady-state fluorescence polarization data were obtained with a Union Giken FS-501A spectrophotometer equipped with a Sord M200 Mark II microcomputer, and fluorescence lifetimes were recorded on a Horiba NAES-1100 time-resolved spectrofluorimeter.

Preparation of hybrid assemblies. Preparation of hybrid assemblies was performed by mixing an aqueous solution of a cyclophane derivative (**2**, **3** or **4**) with an aqueous dispersion of the peptide lipid (**1**) in a 1 : 40 molar ratio. Each mixture was allowed to stand for 10 h at 30 °C before measurements. For all measurements with the hybrid assemblies, final concentrations were maintained at $1{\cdot}0 \times 10^{-5}$ and $4{\cdot}0 \times 10^{-4}$ mol dm^{-3} for the macrocyclic host and the lipid, respectively. A hybrid assembly composed of steroid derivative **10** ($4{\cdot}0 \times 10^{-5}$ mol dm^{-3}) and of **1** ($4{\cdot}0 \times 10^{-4}$ mol dm^{-3}) was also prepared for reference measurements.

RESULTS AND DISCUSSION

Molecular recognition behaviour of macrocyclic receptors in aqueous solution

Octopus cyclophanes are unique host molecules capable of performing molecular recognition toward various hydrophobic guests through the induced-fit mechanism.[16,19–22] This interaction mode originates from the flexible character of multiple alkyl branches introduced into a rigid macrocyclic skeleton. In aqueous media, each of those octopus cyclophanes can provide a relatively large and hydrophobic binding site constructed with a macrocyclic ring and hydrocarbon chains. In addition, electrostatic and charge-transfer interactions come into play in the course of molecular recognition by octopus cyclophanes when appropriate structural components are incorporated into the host.

Recently, we have clarified aggregation behaviour and induced-fit* molecular recognition performed in aqueous media by a cationic octopus cyclophane (**3**), having L-aspartate residues as connector units interposed between a rigid 2,11,20,29-tetraaza[3.3.3.3]paracyclophane skeleton and four double-chain hydrocarbon segments.[16] Guest molecules such as ANS and TNS are incorporated into the three-dimensional cavity provided intramolecularly by the macrocyclic ring and the eight hydrocarbon chains even when the octopus cyclophane undergoes aggregation.

We now designed and prepared another octopus cyclophane (**4**) having a 1,6,20,25-tetraaza[6.1.6.1]paracyclophane skeleton in order to evaluate the induced-fit effect provided by multiple hydrocarbon segments in guest recognition behaviour, in view of previous studies on molecular recognition by the internal cavity of **12** toward hydrophobic guests.[18,24] The chain length of the hydrocarbon segments for host **4** is slightly shorter than that for host **3**. Consequently, well resolved ^{1}H NMR spectra were observed for the host–guest complexes with the former host in aqueous media relative to those with the latter, by suppressing mutual aggregation interactions of the hydrophobic branches. Formation constants (K) for the 1:1 host–guest complexes of cyclophanes with ANS and TNS and the corresponding free energy changes (ΔG) were evaluated in aqueous media by fluorescence spectroscopy in a manner similar to that reported previously,[22] as indicated in Table 1. When aqueous stock solutions of the hosts were employed, the guest-binding behaviours of **2**–**4** are almost identical.

Table 1. Formation constants (K) and free energies of formation (ΔG) for complexes of cyclophane hosts in aqueous phosphate buffer (10 mmol dm^{-3}, pH 8·0) at 30·0 °C[a]

	K/dm^3 mol^{-1} ($-\Delta G$/kJ mol^{-1})[b]	
	Guest	
Host	ANS	TNS
Cyclophane **2**	$3·3 \times 10^5$ (32·0)	$2·0 \times 10^6$ (36·5)
Cyclophane **3**[c]	$5·3 \times 10^5$ (33·2)	$1·4 \times 10^6$ (35·6)
Cyclophane **4**	$1·5 \times 10^6$ (35·8)	$4·0 \times 10^6$ (38·3)

[a] Concentrations in mol dm^{-3}: guests, $1·0 \times 10^{-6}$; cyclophanes, $3·3 \times 10^{-7}$–$1·0 \times 10^{-5}$.
[b] $-\Delta G$ is given in parentheses.
[c] Taken from Ref. 16.

The cationic steroid cyclophane **2**, which is constructed with four steroid moieties and a 2,11,20,29-tetraaza[3.3.3.3]paracyclophane skeleton, exhibited different guest recognition behaviour to **3** and **4**. The critical aggregate concentration (CAC) value, as evaluated by means of surface tension measurements based on the Wilhelmy principle, is $3·5 \times 10^{-5}$ mol dm^{-3} at room temperature (30 °C). Formation of relatively large aggregates with hydrodynamics diameters (d_{hy}) of 130–140 nm was observed in a concentration range above the CAC. The formation constants for the 1:1 host–guest complexes of **2** with ANS and TNS were evaluated in a concentration range below its CAC (Table 1). As for complexation with ANS, the K value is much greater than that for the corresponding simple macrocycle, *N*, *N'*, *N''*, *N'''*-tetramethyl-2,11,20,29-tetraaza[3.3.3.3]paracyclophane (550 dm^3 mol^{-1} at pH 2),[25] and comparable to those for the octopus cyclophanes.[16] The microscopic polarity experienced by ANS at the guest-binding site provided by **2** was evaluated from a fluorescence maximum (λ_{em}) observed for the guest; E_T^N[16,26,*] 0·41, λ_{em} 463 nm. The result, along with additional rationalization by the CPK molecular model study, suggests strongly that the steroid cyclophane is able to incorporate one ANS molecule

* Although the induced-fit concept was originally given by Koshland[23] to explain the specific interaction of substrates with enzyme molecules, the term has now become a more general one for explanation of host–guest interactions. In this work the term means that a host molecule such as octopus cyclophane **3** undergoes conformational and configurational changes that are induced by complexation with a guest molecule, so as to attain a tight binding interaction between them.

* The microenvironmental polarity parameter E_T^N is defined as

$$E_T^N = [E_T(\text{solvent}) - E_T(\text{TMS})]/[E_T(\text{water}) - E_T(\text{TMS})] = [E_T(\text{solvent}) - 30·7]/32·4$$

E_T(solvent), E_T(TMS), and E_T(water) are based on transition energies in kcal mol^{-1} (1 kcal = 4·184 kJ) for the longest wavelength solvatochromic absorption band of a specific pyridinium-*N*-phenoxide betaine dye in a solvent of choice, tetramethylsilane (TMS) and water, respectively.[26] The E_T^N values assigned to water and TMS are 1·000 and 0·000 as the most polar and apolar solvents, respectively.

into its three-dimensionally extended hydrophobic cavity created by the four steroid moieties and the macrocyclic skeleton in a similar manner as performed by the octopus cyclophanes. It is noteworthy, however, that the specific structural rigidity of the steroid moieties is reflected in the rotational correlation time (θ) of the guest incorporated into the host. The θ value was evaluated from the observed values of steady-state fluorescence polarization (P) and fluorescence lifetime (τ) on the basis of Perrin's equation:[27]

$$1/P - 1/3 = (1/P_0 - 1/3)(1 + \tau/\theta) \quad (1)$$

where P_0 refers to the maximum value of P for a probe without any rotational motion; P, τ, P_0 and θ are 0·140, 13·6 ns, 0·427[16] and 5·7 ns, respectively, for ANS at 30·0 °C. The θ value thus obtained is much smaller than that for the identical guest included in octopus cyclophane **3** (θ = 23·0 ns).[16] This means that the flexible hydrocarbon chains of the octopus cyclophane are capable of grasping the guest more tightly than the rigid hydrophobic moieties of the steroid cyclophane.

Formation of hybrid assemblies

A biomembrane is an excellent example of supramolecular assemblies, in which various functional molecules are structurally organized for molecular recognition. In order to develop artificial supramolecular systems capable of mimicking biomembrane functions, we became interested in molecular recognition by macrocyclic hosts embedded in synthetic bilayer membranes.

We have previously clarified that anionic peptide lipid **1** affords multi-walled bilayer aggregates when dispersed in aqueous media, as confirmed by negative staining electron microscopy.[15] Under such circumstances, the two octopus cyclophanes **3** and **4** and the steroid cyclophane **2** were individually incorporated into the bilayer membrane. Phase transition parameters (temperature at peak maximum of DSC, T_m; enthalpy change, ΔH; entropy change, ΔS) and hydrodynamic diameters (d_{hy}) for the bilayer aggregates in the presence and absence of the macrocycles were evaluated by means of ultrasensitive differential scanning calorimetry and dynamic light-scattering measurements, respectively, as summarized in Table 2. On addition of each host to the aqueous dispersion of **1**, there was little change in the peak temperature for phase transition from the gel to the liquid-crystalline state (T_m). However, there are a 3–23% decrease in ΔH, a 4–23% decrease in ΔS, an 18–33% increase in the half-width of the endothermic peak ($\Delta T_{1/2}$) and a 21–26% decrease in d_{hy}, reflecting the formation of the hybrid assemblies. When steroid derivative **10**, a monomeric analogue of **2** with respect to the steroid fragment, was added to the aqueous dispersion of **1** in a 1 : 10 molar ratio, the phase transition parameters and the d_{hy} value were affected to much lesser extents (Table 2), reflecting its weaker perturbation effect on the bilayer membrane structure.

Molecular recognition by hybrid assemblies

ANS and TNS are well known fluorescent probes whose emission is extremely sensitive to changes in microenvironmental properties around the molecules.[28,29] These fluorescent probes were employed in this work to evaluate molecular recognition behaviour of the hybrid assemblies formed with the macrocyclic hosts and the peptide lipid. Figure 1(A) shows fluorescence spectra of ANS in an aqueous solution of **1** with and without cyclophane **2**. The solution was sonicated with a probe-type sonicator at 30 W power for 1 min to obtain the single-walled vesicle. While the anionic ANS alone interacts weakly with the anionic lipid aggregate, the hybrid assembly formed with **1** and **2** strongly incorporates ANS into its hydrophobic domain. Such a drastic change in the microenvironment experienced by the guest is evident from an increase in the fluorescence intensity and a blue shift of the fluorescence maximum. An analogous spectral change was observed for an aqueous dispersion of the hybrid assembly prepared without sonication, although the background intensity was raised owing to light scattering from the aggregates.

Another host TNS is also effectively bound to the

Table 2. Phase transition parameters and hydrodynamic diameters for aggregates in the dispersion state[a]

Aggregate[b]	T_m/°C	ΔH/kJ mol^{-1}	ΔS/J K^{-1} mol^{-1}	$\Delta T_{1/2}$/°C	d_{hy}[c]/nm
Lipid **1**	24·3	32·6	110	0·39	380
Lipid **1** + Cyclophane **2**	24·4	31·5	106	0·47	300
Lipid **1** + Cyclophane **3**	24·2	25·2	85	0·52	300
Lipid **1** + Cyclophane **4**	24·4	29·7	99	0·46	280
Lipid **1** + Steroid **10**	24·7	32·0	107	0·39	310

[a] Measured in aqueous phosphate buffer (10 mmol dm^{-3}, pH 8·0).
[b] Concentrations in mol dm^{-3}: lipid, 4·0 × 10^{-4}; cyclophanes, 1·0 × 10^{-5}; steroid **10**, 4·0 × 10^{-5}.
[c] Measured at 30·0 °C.

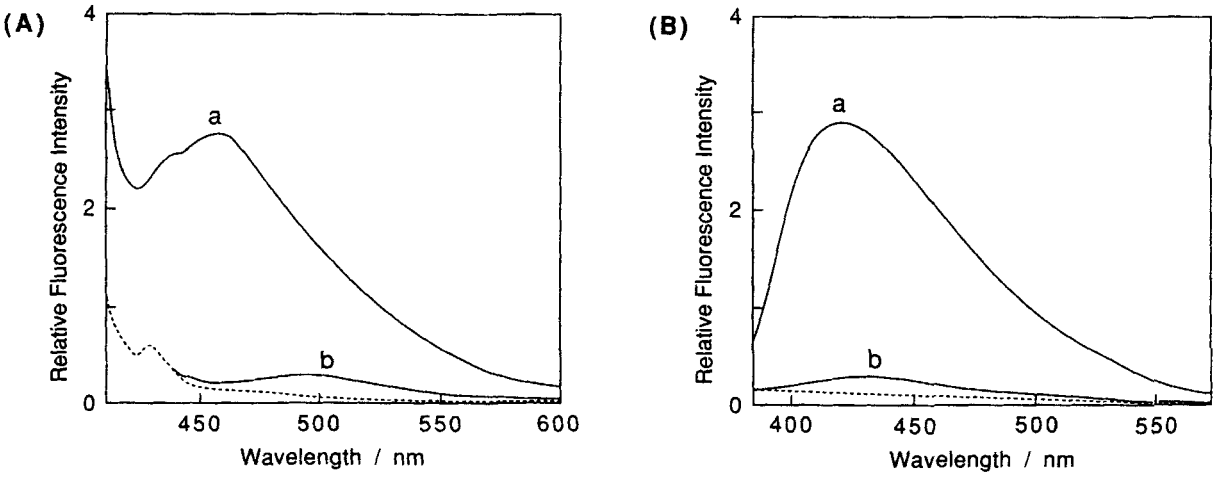


Figure 1. Fluorescence spectra of (A) ANS in a sonicated solution of **1** and (B) TNS in an aqueous dispersion of **1**, (a) with and (b) without **2** in aqueous phosphate buffer (10 mmol dm^{-3}, pH 8·0) at 20·0 °C. Concentrations in mol dm^{-3}: guests, $1{\cdot}0 \times 10^{-6}$; **1**, $4{\cdot}0 \times 10^{-4}$; **2**, $1{\cdot}0 \times 10^{-5}$. Excitation wavelengths: 375 and 324 nm for ANS and TNS, respectively. The dotted lines refer to background spectra originating from **1** alone

identical hybrid assembly, as shown in Figure 1(B). Each of the hybrid systems composed of the octopus cyclophane (**3** or **4**) and peptide lipid **1**, also exhibited effective guest-binding behaviour in comparison with the cyclophane-free system, as reflected in the fluorescence features of ANS and TNS, which are similar to those shown in Figure 1. On the other hand, the hybrid assembly formed with steroid derivative **10** and lipid **1** in a molar ratio of 1 : 10 did not enhance the fluorescence intensities of the guests to any significant extent.

The results may provide a guidepost for designing hybrid assemblies which are capable of performing effective binding of anionic and hydrophobic guests that are not incorporated into the anionic bilayer membrane itself; macrocyclic hosts with polycationic charges provide effective binding sites in the anionic bilayer membrane. Unfortunately, the guest-binding constants were not obtained because the light-scattering phenomena caused by these aggregates interfered with the exact evaluation of the fluorescence intensities of the guests.

The microenvironmental polarity parameters for ANS and TNS bound to various hosts are given in Table 3. These values were found to be independent of temperature in the range 10–40 °C. In the absence of any macrocyclic hosts, ANS is bound to the membrane in its surface domain while TNS to the hydrogen-belt domain[30,31]* interposed between the polar surface region and the hydrophobic domain composed of double-chain segments in the light of the E_T^N values; the microenvironments for the former and the latter are close to that provided by water ($E_T^N = 1{\cdot}000$) and equivalent to that in ethanol ($E_T^N = 0{\cdot}654$), respectively. Such a difference in the microenvironmental polarity presumably comes from the difference in molecular shape; TNS is more slender than ANS.

The steroid cyclophane provides less polar microenvironments for ANS and TNS by forming a hybrid assembly with the peptide lipid. To our surprise, the microenvironment around the ANS molecule incorporated into the hybrid assembly is equivalent to that provided by hexane ($E_T^N = 0{\cdot}009$). In contrast, the microscopic polarity experienced by TNS in the identical hybrid assembly is as polar as pentan-1-ol ($E_T^N = 0{\cdot}568$). On the other hand, both of the octopus cyclophanes and their hybrid assemblies with the peptide lipid incorporate both guest molecules into the comparable binding sites with respect to the microenvironmental polarity.

In order to evaluate microscopic viscosity around the guest incorporated into the hybrid assemblies, the steady-state fluorescence polarization measurements were performed for ANS and TNS in a temperature range above and below the T_m. As shown in Figure 2(A), the P value for ANS incorporated into steroid cyclophane **2** in aqueous solution remains nearly

* The biomembrane was proposed to have a tripartite structure: a hydrophobic interior domain composed of hydrocarbon chains, a polar surface domain composed of hydrophilic head groups and a hydrogen-belt domain interposed between these two, where intermolecular hydrogen-bonding interactions among constituent molecular components tend to enhance the morphological stability of the biomembrane.[32]

constant at 0·14 in the range 10–40 °C. In the case of the corresponding hybrid assembly formed with the peptide lipid, however, the P value decreases significantly as temperature is raised, along with a slight inflection in the phase transition temperature range. Hence the ANS molecule is obviously incorporated into the hydrophobic domain of the aggregate, in which molecular motion of the guest is subjected to change by the phase transition. The P value for ANS bound to octopus cyclophane **4** in aqueous solution decreases monotonously as temperature is raised. Although the P vs temperature correlation line for the hybrid system composed of cyclophane **4** and lipid **1** is not much separated from that for **4** alone in aqueous solution, the P value for the former is subjected to change by temperature in a biphasic manner, exhibiting a slight inflection in the T_m range. Intrinsically, similar correlations between temperature and P were observed for TNS, as shown in Figure 2(B).

Table 3. Microenvironmental polarity parameters (E_T^N) for guests bound to hosts in aqueous phosphate buffer (10 mmol dm^{-3}, pH 8·0) at 30·0 °C[a]

Host	E_T^N (λ_{ex}, nm; λ_{em}, nm)[b]	
	ANS	TNS
None	1·000 (350, 515)	1·000 (366, 500)
Lipid **1**	0·90 (375, 500)	0·66 (324, 430)
Cyclophane **2**	0·41 (375, 463)	0·66 (324, 430)
Lipid **1** + Cyclophane **2**	0·01 (375, 458)	0·58 (324, 421)
Cyclophane **3**[c]	0·57 (375, 467)	0·63 (322, 426)
Lipid **1** + Cyclophane **3**	0·57 (375, 467)	0·63 (322, 426)
Cyclophane **4**	0·58 (375, 468)	0·55 (324, 418)
Lipid **1** + Cyclophane **4**	0·57 (375, 467)	0·58 (324, 421)

[a] Concentrations in mol dm^{-3}: lipid, $4·0 \times 10^{-4}$; cyclophanes, $1·0 \times 10^{-5}$; guests, $1·0 \times 10^{-6}$.
[b] Excitation and emission maxima are given in parentheses, in this sequence.
[c] Taken from Ref. 16.

Since the P value is subject to change by the fluorescence lifetime (τ) and the rotational correlation time (θ), as mentioned above, these values for ANS bound to the hosts were evaluated at 20 and 30 °C in the presence and absence of the bilayer membrane (Table 4). All the τ values for ANS bound to the hosts are large relative to that in water ($\tau = 0·55$ ns).[33] This means that the guest molecules are placed in hydrophobic microenvironments well separated from the bulk aqueous phase. It is noteworthy that the θ values for ANS in the hybrid assembly formed with the steroid cyclophane are much larger than those in the hybrid assemblies formed with the octopus cyclophanes. It has been reported that the θ values for ANS bound to liposomal membranes formed with lecithin and those bound to various proteins are in ranges 3–6 and 9–63 ns, respectively.[28] Hence the θ value for ANS incorporated into the gel state of the hybrid assembly formed with the steroid cyclophane and the peptide lipid seems to be the largest one for ANS so far observed. It must be noted that such remarkable restriction of the molecular motion can be achieved by a combination of the steroid cyclophane, which is incapable of performing tight guest binding by

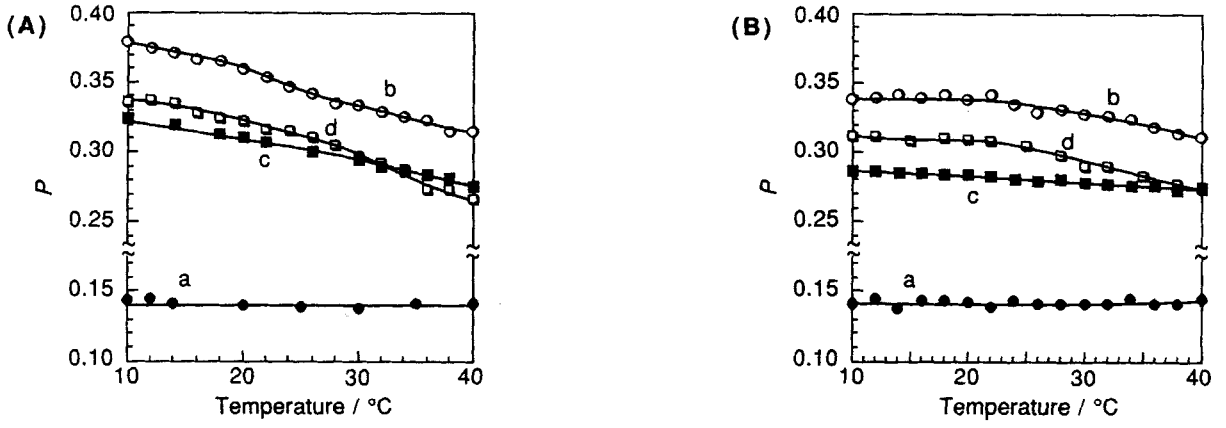


Figure 2. Correlations of temperature with steady-state fluorescence polarization of (A) ANS and (B) TNS bound to the following hosts in aqueous phosphate buffer (10 mmol dm^{-3}, pH 8·0): (a) **2**; (b) **1** + **2**; (c) **4**; (d) **1** + **4**; lipid **1** being used in the dispersion state. Concentrations in mol dm^{-3}: guests, $1·0 \times 10^{-6}$; **1** $4·0 \times 10^{-4}$; cyclophanes, $1·0 \times 10^{-5}$

Table 4. Steady-state fluorescence polarization values (P), fluorescence lifetimes (τ), and rotational correlation times (θ) for ANS bound to hosts in aqueous phosphate buffer (10 mmol dm^{-3}, pH 8·0)[a]

Host	Temp/°C	P	τ/ns	θ/ns
Cyclophane **2**	20·0	0·139	15·4	6·4
	30·0	0·140	13·6	5·7
Lipid **1** + Cyclophane **2**	20·0	0·358	15·0	66·7
	30·0	0·332	13·5	40·5
Cyclophane **3**	20·0	0·333	11·9	36·2
	30·0	0·302	11·1	23·0
Lipid **1** + Cyclophane **3**	20·0	0·326	11·4	31·6
	30·0	0·280	10·9	17·8
Cyclophane **4**	20·0	0·309	13·6	30·5
	30·0	0·293	13·0	24·4
Lipid **1** + Cyclophane **4**	20·0	0·321	12·6	32·7
	30·0	0·295	12·0	23·0

[a] Concentrations in mol dm^{-3}: lipid, $4{\cdot}0\times10^{-4}$; cyclophanes, $1{\cdot}0\times10^{-5}$; ANS, $1{\cdot}0\times10^{-6}$.

itself, and the anionic peptide lipid, which cannot bind an anionic guest effectively in its aggregated state. In contrast, the molecular motion of ANS bound to the octopus cyclophanes is not subjected to significant change in the hybrid assemblies formed with the peptide lipid.

The guest-binding modes of the present hybrid assemblies are classified into two types as shown schematically in Figure 3; a guest molecule is included in the proximity of the hydrogen-belt domain of the bilayer membrane on the one hand, and is bound to the hydrophobic region composed of the hydrocarbon double chains on the other. The hybrid assemblies formed with the octopus cycloplane and the peptide lipid exercise the former binding mode toward both ANS and TNS. A structural difference in the macrocyclic skeleton between the octopus cyclophanes (**3** and **4**) scarcely affects the guest-binding behaviour. On the other hand, the hybrid assembly formed with the steroid cyclophane and the peptide lipid exercises the former and latter binding modes toward TNS and ANS, respectively.

In conclusion, this study has demonstrated characteristic features of molecular recognition by hybrid assemblies formed with an anionic peptide lipid and cationic macrocyclic hosts toward hydrophobic anion guests. The octopus cyclophanes are suitable hosts for performing the induced-fit molecular recognition by their eight flexible arms not only in aqueous solution but also in bilayer membranes. On the other hand, the steroid cyclophane tends to bind guest molecules much less tightly than the octopus cyclophanes in aqueous solution owing rigid structural nature of the steroid moieties. When the hybrid assembly is formed with the peptide lipid, however, the steroid cyclophane behaves as an artificial receptor molecule much superior to the octopus cyclophanes, exhibiting a marked guest discrimination.

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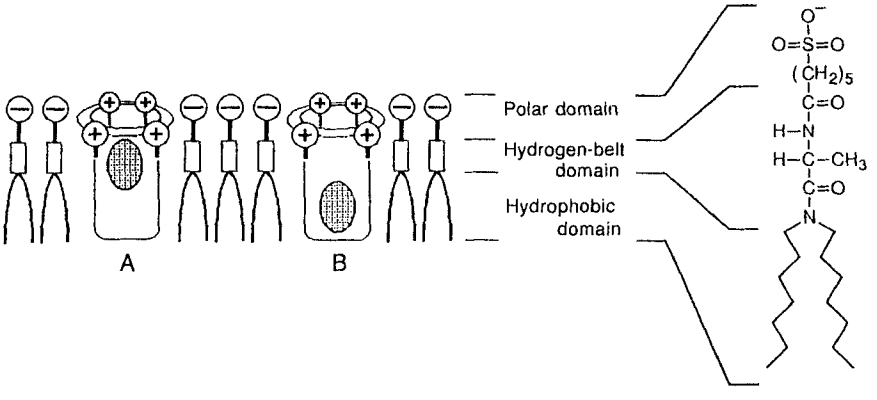

Figure 3. Schematic representation of guest-binding modes of the hybrid assemblies; a guest molecule is located (A) in the hydrogen-belt domain and (B) in the hydrophobic domain

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