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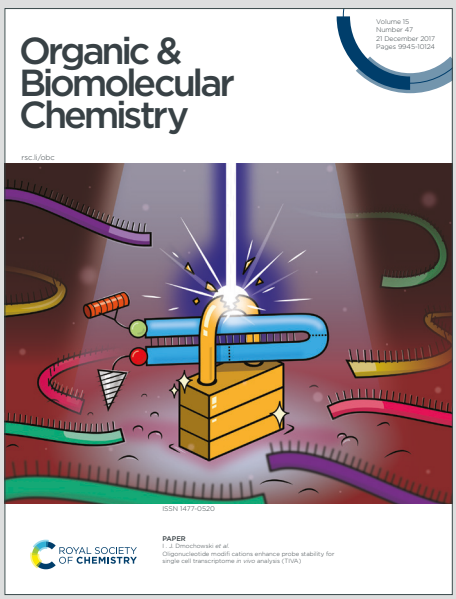

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Homo- and heterotypic pentameric cyclophanes exhibiting fascinating host-guest binding properties

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Osamu Hayashida,*[a] Hayato Saruwatari,[a] Syota Minami [a] and Takaaki Miyazaki [a]

Tetraaza[2.2.1.2.2.1]paracyclophane (**L**) and tetraaza[6.1.6.1]paracyclophane (**S**), which have larger and smaller cavities, respectively, were utilized to develop cationic cyclophane (CP) pentamers as water-soluble hosts. Homotypic CP pentamer **L5**, in which five **L**s are linked in a divergent fashion, as well as heterotypic CP pentamer **hetero5** consisting of one **L** and four **S**s were synthesized. **L5** and **hetero5** exhibited fascinating molecular recognition in guest-binding toward stocky guests such as 1-pyrenebutyric acid (PBA) and perylene-3,9-dicarboxylic acid (PeDA), and elongated guests such as 6-*p*-toluidinonaphthalene-2-sulfonate (TNS) and 4-*N*,*N*-dimethylamino-azobenzene-4'-sulfonyl derivative (CDab). That is, **L5** potently captured PBA and PeDA, with binding constants in the order 10^6 M^{-1}, whereas **hetero5** potently captured not only PBA but also TNS and CDab. The guest-binding ability of **L5** and **hetero5** to preferred guests was significantly improved compared to the corresponding monocyclic CPs **L1** and **S1**, which reflects an increase in the local concentration of the macrocycles. Furthermore, both PBA and CDab molecules were simultaneously and efficiently incorporated into the macrocycles of **hetero5**, which was confirmed by fluorescence spectroscopy.

Introduction

Cyclophanes (CPs) and related macrocyclic compounds that possess internal hydrophobic cavities for guest-binding are quintessential molecular scaffolds in host-guest chemistry.[1] In particular, azacyclophanes such as tetraaza[6.1.6.1]paracyclophane[2] (**S**, Fig. 1) can be easily modified by introducing various hydrophilic functional groups onto the nitrogen atoms.[3] Water-soluble CPs can be very powerful tools in supramolecular chemistry[4] and biology.[5] Applications in areas such as molecular recognition[6], drug delivery[7] and in vivo use[8] are progressing, and it is expected that they will contribute to even more innovative technologies in the future.[9] We have previously developed various water-soluble CPs[10] consisting of a single macrocyclic skeleton. For example, non-ionic water-soluble CP,[11] which was prepared by introducing four hydrophilic side chains with a terminal galactose residue into **S**, exhibited moderate guest-binding affinities toward hydrophobic guest molecules such as 6-*p*-toluidinonaphthalene-2-sulfonate (TNS).[12] Although hydrophobic interactions are the primary driving force for host-guest complex formation in aqueous media, other recognition forces, such as electrostatic interactions, also become effective in well-desolvated and hydrophobic microenvironment.[13] Indeed, cationic water-soluble CP bearing terminal ammonium side chains (**S1**, Fig. 1) can bind more strongly to anionic and hydrophobic TNS with opposite charges.[14] Furthermore, we recently developed cationic CP pentamers based on molecular design in which five **S** macrocycles are covalently linked as a water-soluble homotypic pentameric CP host[15]. The CP pentamers were found to capture the identical guest molecule with much higher guest-binding affinity than the monomeric CP, **S1**.[15, 16]

On the other hands, the size and shape of the intramolecular cavity of the azacyclophane can be freely designed, enabling guest selectivity based on size and shape. For these reasons, we focused on tetraaza[2.2.1.2.2.1]paracyclophane[17] (**L**, Fig. 1) as a larger macrocycle than **S** and recently developed a pseudo-rotaxane-type cyclophane-chromophore conjugate and particularly investigated its optical properties.[17] Such large macrocycles are expected to be able to bind stocky guest molecules more potently than elongated guests such as TNS. However, the molecular recognition behaviour of water-soluble CPs based on **L** have not been widely studied to date. In this regard, we became interested in the design and molecular recognition capabilities of water-soluble CPs based on **L**. On these grounds, we designed a cationic water-soluble CP with a larger internal cavity and terminal ammonium side chains (**L1**, Fig. 1). Additionally, to further improve the binding affinity to stocky guests, we also designed a cationic and homotypic CP pentamer **L5** based on **L**, in which polar side chains bearing terminal ammonium groups were incorporated into the outer four macrocycles, conferring water-solubility to the resulting host (Fig. 1). Such a CP pentamer containing five large macrocycles is expected to be able to bind stocky guest molecules even more strongly than **L1**. In addition, it would be interesting to develop hosts that can strongly bind both stocky and elongated guests. We also designed a cationic and heterotypic CP pentamer **hetero5**, which is composed of one **L** as a larger cavity and four **S**s as a smaller one (Fig. 1). Similarly, polar side chains with

[a] Department of Chemistry, Faculty of Science, Fukuoka University, Nanakuma 8-19-1, Fukuoka 814-0180, Japan

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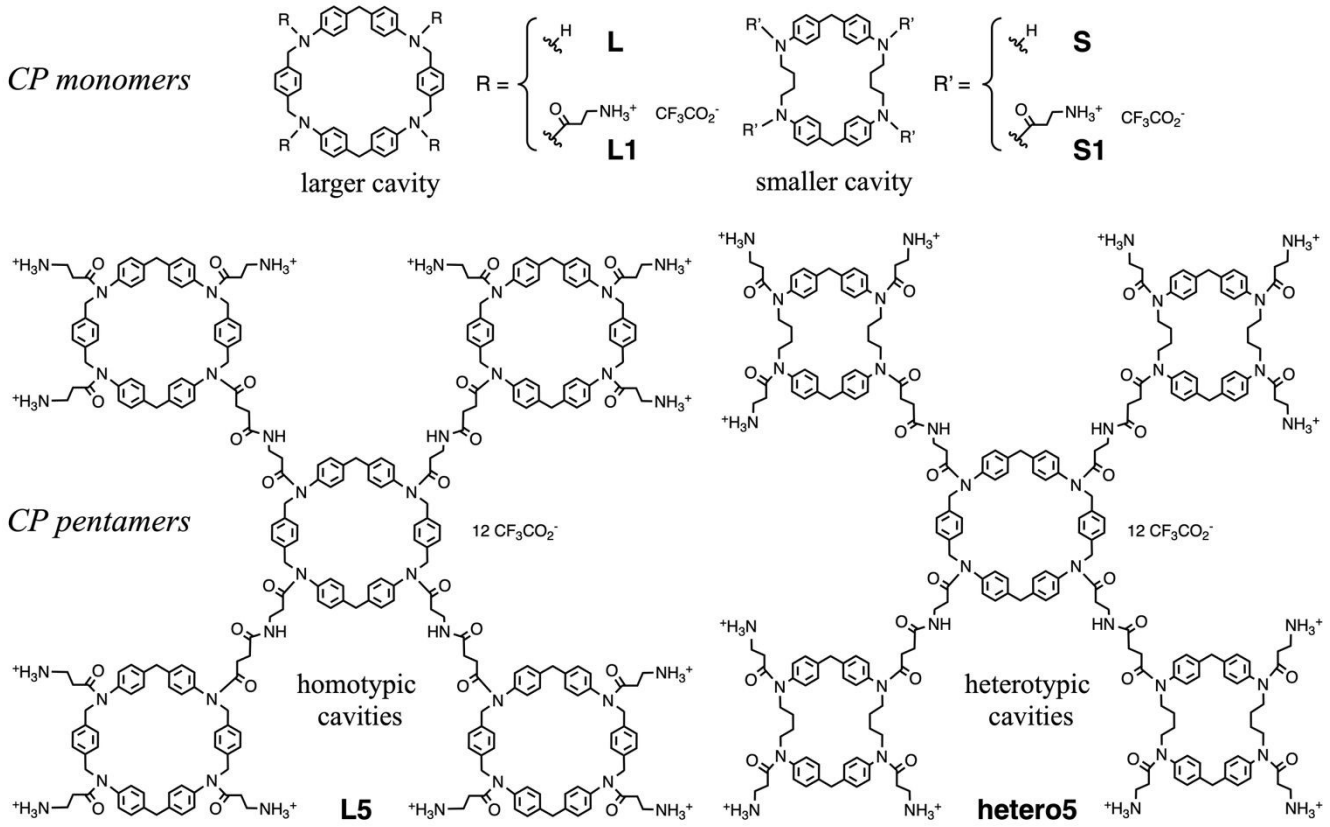

Fig. 1 CP monomers based on **L** and **S** (**L1** and **S1**, respectively) as well as homo- and heterotypic CP pentamers (**L5** and **hetero5**, respectively).

terminal ammonium groups were incorporated into the external macrocycles to confer water-solubility on the resulting host. This paper report on the molecular recognition based-on the cavity size of cationic CP pentamers **L5** and **hetero5**, focusing on their preparation and the behaviour of improved guest-binding affinity due to increased local concentration of the macrocycles. In addition, we also report the simultaneous binding of two different guest molecules to these cationic CP pentamers.

Results and discussion

Syntheses of cationic CP pentamers based on large macrocycle

Both CPs, **L** and **S**, have two *N*, *N*'-diaminodiphenylmenthane (DAPM) units. In the molecular structure of compound **S**, DAPM units are bridged by a flexible and short tetramethylene group (Fig. 1). On the other hand, compound **L** is composed of the two DAPM units and two more rigid and longer *p*-xylene groups that form a bridge between the two nitrogen atoms (Fig. 1). A computational study of **L** and **S** performed using the Avogadro software (v. 1.2.0) using a force field (MMFF94),[18] suggested that the internal cavities of **L** and **S** are different in size and shape. The cavity size of **L** is about 1.25 and 1.18 nm in width (distances between the diagonal nitrogen atoms), which are longer than those of **S** (1.09 and 1.07 nm) (see SI, Fig. S1). We hypothesized that the difference in the internal cavities might be reflected in the guest-binding ability and decided to develop water-soluble CP monomers and pentamers using these two types of CPs. First, we designed **L1** as a cationic CP monomer with the larger internal cavity by introducing the same terminal ammonium side chains as **S1** into **L** (Fig. 1). Aiming to further improve guest-binding affinity through increasing local concentration of the macrocycles, we also designed a cationic and homotypic CP pentamer **L5**, in which five **L** macrocycles are linked in a divergent fashion via a spacer (Fig. 1). Similarly, we also designed a cationic and heterotypic CP pentamer **hetero5** consisting of two different sized CPs, one **L** and four **S**s, as a binding site for both large and small-sized guest molecules, respectively (Fig. 1).

Cationic CP monomer **L1** as well as cationic CP pentamers **L5**

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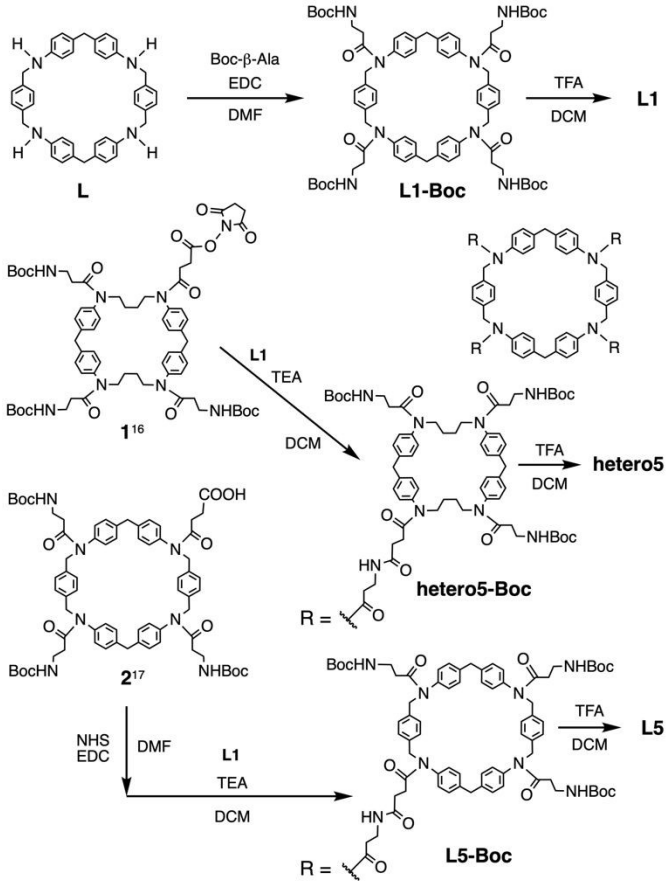
and **hetero5** were synthesized according to the reaction sequence shown in Scheme 1. A precursor (**L1-Boc**) of **L1** was prepared by condensation of **L** with excess Boc-β-alanine in the presence of 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide (EDC) in dry *N*,*N*-dimethylformamide (DMF) in a yield (63 %). Compound **L1** was obtained from **L1-Boc** by removal of the Boc-protecting groups with trifluoroacetic acid (TFA) in dry dichloromethane (DCM) in a good yield (97 %). In the previous paper, we reported the synthesis of key compound **1**, a derivative of **S** bearing a carboxylic acid succinimidyl ester and three Boc-β-alanine residues.[16] A precursor (**hetero5-Boc**) of heterotypic CP pentamer **hetero5** was obtained by a reaction of **L1** with excessive amount of **1** in the presence of triethylamine (TEA) in a good yield (84 %). Similarly, a derivative of **L** with a carboxylic acid succinimidyl ester and three Boc-β-alanine residues was prepared from compound **2**,[17] a derivative of **L** with a carboxylic acid and three Boc-β-alanine residues, in the presence of *N*-hydroxysuccinimide (NHS) and EDC. A precursor (**L5-Boc**) of homotypic CP pentamer **L5** was obtained by a reaction of **L1** with excessive amount of the succinimidyl ester derivative of CP in a yield (57 %). Finally, removal of the protecting groups from **hetero5-Boc** and **L5-Boc** using TFA afforded cationic heterotypic and homotypic CP pentamers, **hetero5** and **L5**, respectively, in good yields (87 and 86%, respectively). All new compounds, which were purified by size exclusion chromatography, were identified by ^{1}H and ^{13}C{^{1}H} NMR spectroscopy, matrix-assisted laser desorption time of flight mass spectrometry (MALDI-TOF MS), and elemental analysis (see SI).



Scheme 1 Preparation of cationic CP monomer **L1** as well as homotypic and heterotypic CP pentamers (**L5** and **hetero5**, respectively).

Molecular recognition of stocky guests by water-soluble CPs

Despite the presence of a hydrophobic macrocyclic skeleton, compounds **L1**, **S1**, **L5**, and **hetero5** were soluble in water at concentrations of at least μM in the neutral pH ranges, owing to the peripheral polar side chains. These compounds having hydrophobic internal cavities were expected to act as water-soluble hosts. To evaluate the molecular recognition behaviour of these water-soluble hosts, we first used 1-pyrenebutyric acid (PBA) and perylene-3,9-dicarboxylic acid (PeDA) as fluorescent and hydrophobic guests (Fig. 2). These fluorescent guest molecules are expected to behave as anionic species in neutral aqueous solutions. Of these, the molecular widths of PBA and PeDA are relatively wide, while that of TNS is slim (see SI, Fig. S1). These relatively larger and stocky compounds, PBA and PeDA, contain pyrene[19] and perylene[20] moieties, respectively, and emit characteristic fluorescence. Host-guest complexes formation of **L1**, **S1**, **L5**, and **hetero5** with PBA and PeDA was investigated in 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethane-sulfonic acid (HEPES) buffer (0.01 M, pH 7.4, 0.15 M with NaCl) by fluorescence titration experiments, under conditions where the concentration of hosts was in large excess over the

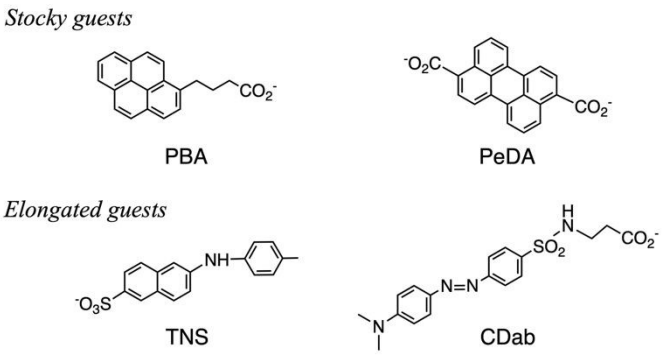


Fig. 2 Stocky and elongated guests.

concentration of guests. To suppress the formation of 1:2 host-guest complexes, an excess of hosts was added compared to the concentration of guests. When large excess **L1** (2 - 22.5 μM) was added to an aqueous solution containing PBA (0.2 μM), a fluorescence intensity originated from PBA was subjected to decrease with a saturation behaviour (Fig. 3a). Such a decrease in the fluorescence intensity of pyrene derivatives is also observed in the formation of host-guest complexes with hosts having amino groups such aminomethylated calixarene.[21] As shown in Fig. 3b, when large excess **S1** was used as a host instead of **L1**, a similar change in fluorescence spectrum was observed, but the spectral change was relatively small. On the other hand, when excess **L5** and **hetero5** (0.5 - 4.5 μM) were added to an aqueous solution containing PBA (0.2 μM), the fluorescence intensities originated from PBA were efficiently reduced with a saturation behaviour (Fig. 3c, d). The binding constants (*K*) for 1:1 host-guest complexes were evaluated by using online BindFit v0.5 program[22] (see SI, Fig. S20) and were summarized in Table 1.

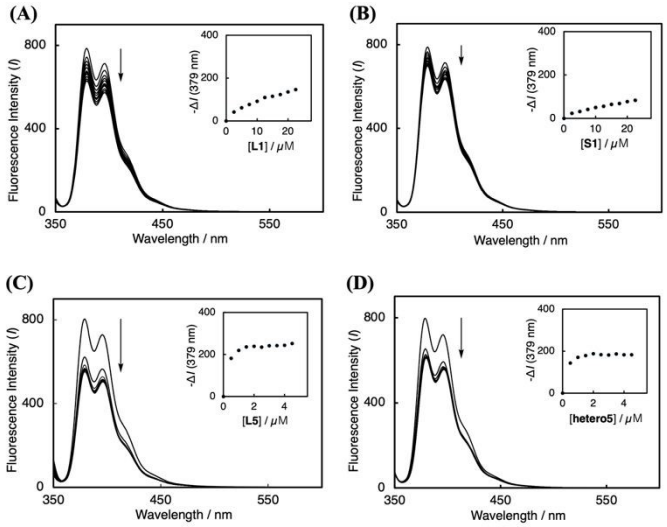


Fig. 3 Fluorescence spectra of PBA (0.2 μM) upon the addition of (A) **L1**, (B) **S1**, (C) **L5** and (D) **hetero5** in aqueous HEPES buffer at 298K. Inset: the corresponding titration curves. Ex 341 nm.

Table 1 The binding constants (*K*) for 1:1 host-guest complexes of the hosts with PBA and PeDA at 298K.

host	K / M^{-1}	
	PBA	PeDA
S1	$5.3 \times 10^4 \pm 6 \times 10^3$	$1.9 \times 10^4 \pm 3 \times 10^3$
L1	$5.9 \times 10^4 \pm 1 \times 10^4$	$7.1 \times 10^4 \pm 1 \times 10^4$
L5	$5.1 \times 10^6 \pm 2 \times 10^6$	$1.9 \times 10^6 \pm 2 \times 10^5$
hetero5	$9.6 \times 10^6 \pm 1 \times 10^6$	$9.8 \times 10^5 \pm 1 \times 10^5$

As regards molecular recognition by CP monomers toward PBA, the *K* values for complexation of **S1** and **L1** with PBA were approximately the same (*K*, 5.3 x 10^4 and 5.9 x 10^4 M^{-1} for **S1** and **L1**, respectively), indicating that PBA molecules may fit well into the internal cavities of **S1** and **L1**. Enhanced guest-binding affinity was also observed for **L5** and **hetero5** with PBA. Thus, the *K* values of **L5** and **hetero5** toward PBA were *ca*. 87- and 160-fold larger than that of **L1**, respectively (Table 1). The enhanced guest-binding ability is likely due to the local concentration effect of the macrocycles, as previously reported.[16] We reported that as the number of macrocycles increases, the rate constant of the binding remains almost unchanged, but the rate constant of dissociation decreases.[16] Furthermore, when PeDA was used as even stocky guest instead of PBA in the fluorescence titration experiments, similar changes in the fluorescence spectra were observed, as shown in Fig. 4, although the spectral change were much smaller in the case of **S1**. The evaluated *K* value for complexation of **L1** with PeDA was 7.1 x 10^4 M^{-1}, whereas the *K* value of **S1** was 1.9 x 10^4 M^{-1} (Table 1, see SI, Fig. S21). These results indicate that PeDA molecules also prefer **L1** over **S1**. In other words, the combination of **S1** and PeDA showed a size mismatch between host and guest. In addition, the evaluated *K* values of **L5** and **hetero5** with PeDA were *ca*. 26- and 13-fold larger than that of **L1**, respectively, which reflects an increase in the local concentration of the macrocycles (Table 1, see SI, Fig. S21). Electrostatic interactions may also play an important role in the formation of suitable host-guest complexes.[23] For instance, cationic host **L1** bound dianionic PeDA slightly more potently than monoanionic PBA, reflecting favourable electrostatic interactions between host and guest molecules (Table 1). However, because monoanionic PBA bound more potently to **L5** and **hetero5** than did dianionic PeDA, hydrophobic interactions appear to be more important for the formation of host-guest complexes between the cationic and hydrophobic CP pentamers and these anionic guests. **L1** has a total charge of +4 per CP, while **L5** and **hetero5** only have a total charge of +2.4 per CP. Therefore, hydrophobic interactions are likely to dominate over

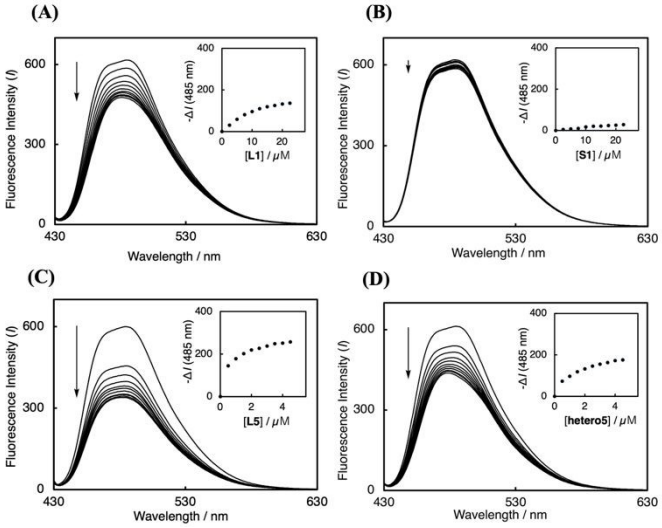


Fig. 4 Fluorescence spectra of PeDA (0.2 μM) upon the addition of (A) **L1**, (B) **S1**, (C) **L5** and (D) **hetero5** in aqueous HEPES buffer at 298K. Inset: the corresponding titration curves. Ex 418 nm.

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electrostatic interaction in guest-binding behaviour of **L5** and **hetero5**. **L5** and **hetero5** are characterized as five covalently bonded macrocyclic molecules, providing effective hydrophobic binding sites for these guest molecules. Among the host-guest combinations investigated here, the combination of **hetero5** and PBA gave the strongest *K* value (9.6 x 10^6 M^{-1}). Unfortunately, the solubilities of **L1**, **S1**, **L5**, and **hetero5** at mM concentrations in D_2O were low, making it impossible to perform NMR measurements to obtain information about the host-guest complex configuration.

Molecular recognition of elongated guests by water-soluble CPs

We investigated the molecular recognition of **L1**, **S1**, **L5** and **hetero5** toward elongated guests. As mentioned previously, TNS, environment-sensitive fluorescent probe, was frequently employed as a hydrophobic and anionic guest in host-guest chemistry, because its emission is very sensitive, both in intensity and wavelength, to the microenvironmental polarity of the surrounding medium.[12] Herein, we used TNS as an elongated and small guest because the molecular size of TNS is smaller than that of PeDA and PBA. The molecular recognition behaviour of **L1**, **S1**, **L5** and **hetero5** was evaluated in HEPES buffer (0.01 M, pH 7.4, 0.15 M with NaCl) by fluorescence titration experiments. These titration experiments were also performed under conditions where the concentration of hosts was in large excess over the concentration of TNS. Upon the addition of large excess **S1** (2.5 - 25 μM) to an aqueous solution containing TNS (1.0 μM), a fluorescence intensity originated from TNS was subjected to increase with a saturation behaviour, although the spectral changes are relatively small (Fig. 5b). The *K* value was 2.1 x 10^4 M^{-1} which was evaluated by using online BindFit v0.5 program[22] (Table 2, see SI, Fig. S22). In the case of **L1**, similar spectral changes are observed as shown in Fig. 5a and evaluated *K* value was 5.3 x 10^3 M^{-1}. The result that TNS

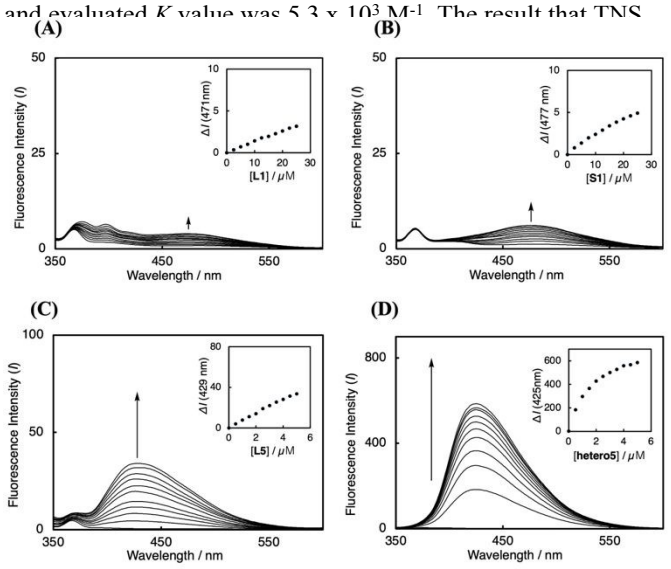

Fig. 5 Fluorescence spectra of TNS (1.0 μM) upon the addition of (A) **L1**, (B) **S1**, (C) **L5** and (D) **hetero5** in aqueous HEPES buffer at 298K. Inset: the corresponding titration curves. Ex 326 nm.

bound to **S1** more than 3 times stronger than **L1** reflects the fact that the TNS molecule fits into the internal cavity of **S1**. In the case of **hetero5**, when **hetero5** was added to an aqueous solution containing TNS (1.0 μM), significant changes in the fluorescence spectra of TNS were observed, showing saturation behaviour (Fig. 5d). The *K* value (1.0 x 10^6 M^{-1}) for complexation of **hetero5** for TNS was *ca*. 58-fold larger than that of **S1** (1.8 x 10^4 M^{-1}), which reflects an increase in the local concentration of the macrocycles (Table 2). This is because **hetero5** has four **S** macrocycles suitable for capturing a TNS molecule. In the case of **L5**, when **L5** was added to an aqueous solution containing TNS (1.0 μM), moderate increases in fluorescence intensity were observed with saturation behaviour (Fig. 5c). However, no significant enhancement in *K* was observed for **L5** (5.7 x 10^4 M^{-1}), although similar CP pentamer (Table 2). This is likely because **L5** has five L macrocycles, but no **S** macrocycle suitable for capturing TNS molecules. The microenvironmental polarity experienced by the bound TNS molecule was evaluated from the fluorescence maximum (λ_{max}).[12] The λ_{max} values for TNS in **S1**, **L1**, **L5** and **hetero5** were 477, 471, 429 and 425 nm, respectively. In general, the fluorescence intensity of TNS increases with a short wavelength shift as the polarity of the microenvironment of the surrounding medium decreases. Therefore, the microenvironment of binding site of each host sensed by the bound TNS became increasingly hydrophobic in the following order: **S1** < **L1** << **L5** < **hetero5**. The guest binding sites of the CP pentamers were much more hydrophobic than those of the CP monomers. Because these cyclophane pentamers have five hydrophobic macrocycles.

Then, we investigated the guest-binding behaviour of **L1**, **S1**, **L5** and **hetero5** toward another elongated guest, CDab[10c] (see SI, Fig. S1) by electronic absorption spectroscopy. CDab is a 4-*N*,*N*-dimethylamino-azobenzene-4'-sulfonyl (dabsyl) derivatives, which are a non-fluorescent chromophore frequently used as a dark quencher.[24] The binding affinity of the CP pentamers toward CDab was too strong to be evaluated in water, so the titration experiments were carried out in a mixed solvent of HEPES buffer (0.01 M, pH 7.4, 0.15 M with NaCl) and methanol (6:4 v/v) to reduce hydrophobic interactions. An absorption intensity originated from CDab, decreased along with a concomitant blue shift of its absorption maximum upon the addition of large excess **L1**, **S1**, **L5** and **hetero5**, reflecting the formation of host-guest complexes, as shown in Fig. 6. The binding constants (*K*) for 1:1 host-guest complexes were calculated on the basis of spectroscopic data obtained at various concentrations of the hosts by using online BindFit v0.5 program[22] (see SI, Fig. S23) and were summarized in Table 2. The *K* value of **S1** toward CDab was slightly larger than that of **L1**, indicating that CDab molecules prefer **S1** over **L1**. In addition, the values of **L5** and **hetero5** were much enhanced than those of **L1** and **S1**, which reflects an increase in the local concentration of the macrocycles.

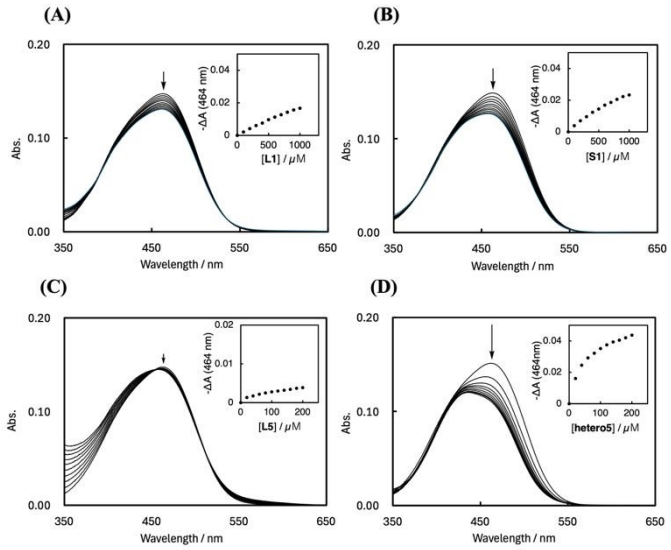

Fig. 6 Absorption spectra of CDab (10 μM) upon the addition of (A) **L1**, (B) **S1**, (C) **L5** and (D) **hetero5** in aqueous HEPES buffer/methanol (6:4 v/v) at 298K. Inset: the corresponding titration curves.

host	K / M^{-1}	
	TNS[a]	CDab[b]
S1	$1.8 \times 10^4 \pm 3 \times 10^3$	$7.3 \times 10^2 \pm 2 \times 10^2$
L1	$5.3 \times 10^3 \pm 7 \times 10^2$	$4.7 \times 10^2 \pm 2 \times 10^2$
L5	$5.7 \times 10^4 \pm 2 \times 10^4$	$1.2 \times 10^4 \pm 2 \times 10^3$
hetero5	$1.0 \times 10^6 \pm 1 \times 10^5$	$2.4 \times 10^4 \pm 9 \times 10^2$

a) in HEPES buffer
b) in HEPES buffer / methanol (6:4 v/v)

Simultaneous binding of different guests to CP pentamers

Heterotypic CP pentamer **hetero5** exhibited good guest-binding affinity for both the stocky guest PBA and the elongated guest TNS, as mentioned above. Furthermore, even in HEPES buffer/methanol (6:4 v/v), **hetero5** bound CDab with K value of 2.4 x 10^4 M^{-1}. As a demonstration experiment for simultaneous binding of two types of guest molecules into **hetero5**, we used PBA as a good fluorescent guest molecule as well as CDab as a dark quencher. Upon the addition of excess CDab to an aqueous solution of PBA (0.5 μM) in the presence of **hetero5** (10 μM), a fluorescence intensity originated from PBA decreased with a saturation behavior, as shown in Fig. 7a. On the other hand, in the control experiment in which **hetero5** was absent, the spectral change of PBA was relatively small (Fig. 7b). These results indicated that both PBA and CDab molecules were simultaneously incorporated into the macrocycles of **hetero5**, where the distance between the two guest molecules was reduced, making quenching more effective. This can be attributed to fluorescence resonance energy transfer[25] between the fluorescence donor PBA and quencher CDab incorporated into the **hetero5**. In the case of monomeric CPs, upon the addition of CDab to aqueous solutions of PBA (0.5 μM) in the presence of **S1** (50 μM) and **L1** (50 μM), the quenching effects were almost the same regardless of the presence or absence of **L1** and **S1** (see SI, Fig. S24). These results suggested that **L1** and **S1** bound only one guest molecule and could not simultaneously bind to two types of guest molecules. In addition, in the case of **L5**, quenching behavior similar to that observed with **hetero5** was also observed, but less effective than that observed with **hetero5** (see SI, Fig. S24). That is, as CDab was added to the aqueous solution containing of PBA and **L5**, the spectral changes decreased more slowly. These differences in the quenching efficiency between **L5** and **hetero5** may be due to the weaker binding affinity of **L5** with

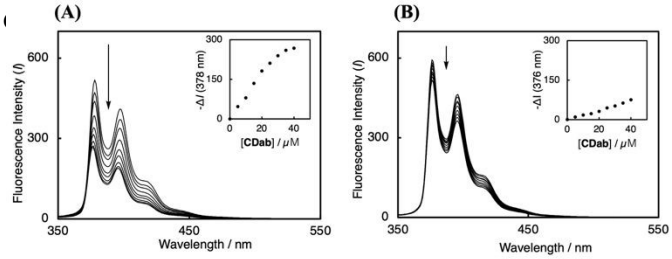

Fig. 7 Fluorescence spectral changes of PBA (0.5 μM) upon the addition of CDab in the (A) presence and (B) absence of **hetero5** (10 μM) in aqueous HEPES buffer at 298K. The corresponding titration curves. Ex 341 nm.

Conclusions

To expand the functionality of the water-soluble CPs, we utilized tetraaza[2.2.1.2.2.1]paracyclophane **L** and tetraaza[6.1.6.1]-paracyclophane **S** to provide larger and smaller cavities, respectively. Aiming to further improve guest-binding affinity, we developed cationic CP pentamers, such as homotypic **L5**, in which five **L** macrocycles are linked in a divergent fashion via spacers. Furthermore, a heterotypic CP pentamer, **hetero5**, featuring internal cavities of different sizes, was designed to simultaneously capture two different guest molecules. **Hetero5** was composed of one **L** with a larger cavity and four **S**s with smaller cavities. Anionic and hydrophobic guests such as PBA and PeDA were used as stocky guests, while TNS and CDab were used as elongated guests. **L5** and **hetero5** exhibited fascinating molecular recognition behaviour in guest-binding. Specifically, **L5** potently captured the stocky guests PBA and PeDA, with K in the order 10^6, whereas **hetero5** potently captured not only PBA but also the elongated guests TNS and CDab. The guest-binding ability of **L5** and **hetero5** was significantly enhanced compared to the monocyclic CPs **L1** and **S1**, reflecting an increase in the local concentration of the macrocycles. Furthermore, both PBA and CDab molecules were simultaneously incorporated into the macrocycles of **hetero5**, which was confirmed by fluorescence spectroscopy. Although the current study focused on quenching of fluorescence, future

work may involve developing supramolecular systems based on fluorescent donor and acceptor guest molecules.

Experimental

General experimental methods

Elemental analyses were determined using an elemental analyzer J-Science Lab JM11. UV-Vis spectra were taken on a JASCO V730 spectrometer. NMR spectra were taken on a Bruker Avance III 400 spectrometer, while Bruker autoflex speed was used for MALDI-TOF MS measurements.

Materials

Tetraaza[2.2.1.2.2.1]paracyclophane L and CP derivative having a carboxylic acid residue and three Boc-β-alanine residues (**2**) were prepared according to the literature reported previously[16]. Succinimidyl ester derivative of tetraaza[6.1.6.1]paracyclophane having Boc-protected-β-alanine residues **1** was prepared according to the literature reported previously.[16] Compound **2**, a derivative of **L** with a carboxylic acid and three Boc-β-alanine residues, was prepared according to the literature reported previously.[17] Anionic dabsyl guest (CDab) was prepared according to the literature[10c].

Synthesis of CP having four Boc-β-Ala residues (L1-Boc)

A solution of L (260 mg, 0.43 mmol) in dry DMF (2 mL) was added dropwise to a solution of Boc-β-alanine (541 mg, 2.86 mmol) and EDC (548 mg, 2.86 mmol) in dry DMF (2 mL), and the mixture was stirred for 12 h at room temperature. The solvent was eliminated under reduced pressure, and the residue was dissolved in ethyl acetate (EtOAc, 200 mL) and washed with saturated aqueous sodium chloride (50 mL). After being dried ($MgSO_4$), the solution was evaporated to dryness under reduced pressure. The residue was chromatographed on a column of silica gel (SiO_2) with ethyl acetate as eluent. The product fraction was dried in vacuo to give a white solid. 0.35g (63%): ^{1}H NMR (400 MHz, $CDCl_3$, 298K) δ 1.42 (s, 36H), 2.22 (m, 8H), 3.33 (m, 8H), 3.94 (s, 4H), 4.82 (s, 8H), 5.33 (m, 4H), 6.81 (d, J = 8.0 Hz, 8H), 7.05 (s, 8H), and 7.08 (d, J = 8.0 Hz, 8H). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$, 298K) δ 25.6, 28.4, 34.8, 36.4, 41.0, 52.4, 78.9, 128.6, 128.9, 129.9, 136.5, 139.9, 140.3, 155.9, and 171.5. Found: C, 67.22; H, 7.04; N, 8.34, Calcd for $C_{74}H_{92}N_8O_{12}\cdot 2H_2O$: C, 67.25; H, 7.32; N, 8.48. MALDI-TOF MS m/z 1309.5 $[M + Na]^+$, where M shows $C_{74}H_{92}N_8O_{12}$.

Synthesis of Cationic CP monomer (L1)

To a solution of **L1-Boc** (102 mg, 0.079 mmol) in dry DCM (4 mL) was added TFA (1 mL). The mixture was stirred for overnight at ambient temperature. After the solvent was distilled off on a rotatory evaporator, methanol (10 mL) was added to the residue, and this procedure was repeated 3 times to remove remaining TFA. Evaporation of the solvent on a rotatory evaporator gave a white solid. The solid was chromatographed on a column of Sephadex LH-20 with methanol as an eluent to purify. Evaporation of the main fraction on a rotatory evaporator gave a white solid (cyclophane-tetraamine TFA salt, 103 mg, 97%): ^{1}H NMR (400 MHz, CD_3OD, 298K) δ 2.48 (t, J = 12 Hz, 8H), 3.16 (t, J = 12 Hz, 8H), 4.01 (s, 4H), 4.87 (s, 8H), 6.94 (d, J = 8.0 Hz, 8H), 7.09 (s, 8H), and 7.20 (d, J = 8.0 Hz, 8H). ^{13}C{^{1}H} NMR (100 MHz, CD_3OD, 298K) δ 31.1, 35.6, 40.4, 51.8, 112.5, 115.4, 118.3, 121.2, 128.5, 128.8, 129.8, 136.4, 138.9, 141.2, and 170.2. Found: C, 54.36; H, 4.75; N, 7.91, Calcd for $C_{62}H_{64}F_{12}N_8O_{12}\cdot 2H_2O$: C, 54.07; H, 4.98; N, 8.14. MALDI-TOF MS m/z 886.1 $[M + H]^+$, 909.0 $[M + Na]^+$, where M shows $C_{54}H_{60}N_8O_4$ (as a free amine of cyclophane derivative).

Synthesis of a precursor of hetero5 (hetero5-Boc)

A solution of succinimidyl ester derivative of tetraaza[6.1.6.1]-paracyclophane having Boc-protected-β-alanine residues 1 (220 mg, 0.18 mmol) in dry DCM (5 mL) was added dropwise to a solution of L1 (20 mg, 0.015 mmol) and TEA (1 mL) in dry DCM (2 mL), and the mixture was stirred for 9 days at room temperature while monitoring the progress of the reaction using MALDI-TOF MS. The solvent was evaporated under reduced pressure, and the crude product was purified by gel filtration chromatography on column of Sephadex LH-20 with methanol as eluant. Evaporation of the product fraction under reduced pressure gave a white solid (66 mg, 84%): ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ 1.42 (s, 140H), 2.10 (m, 24H), 2.23 (m, 16H), 2.39 (m, 8H), 3.26 (m, 24H), 3.42 (m, 8H), 3.62 (m, 32H), 3.95 (s, 20H), 4.82 (s, 8H), 5.33 (m, 12H), 6.62 (m, 4H), and 7.02 (m, 88H). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$, 293K) δ 24.8, 28.4, 29.9, 31.3, 34.3, 34.7, 35.2, 36.4, 41.0, 48.6, 52.4, 78.9, 128.2, 128.4, 128.5, 128.8, 130.0, 130.2, 136.5, 139.8, 140.1, 140.4, 140.5, 155.8, 171.4, 171.5, and 172.0. Found: C, 67.29; H, 7.35; N, 9.18. Calcd for $C_{302}H_{384}N_{36}O_{48}\bullet 6H_2O$: C, 67.24; H, 7.40; N, 9.35. MALDI-TOF MS (positive mode): m/z 5310 $[M + Na]^+$, where M shows $C_{302}H_{384}N_{36}O_{48}$.

Synthesis of Cationic heterotypic CP pentamer (hetero5)

To a solution of **hetero5-Boc** (59 mg, 0.011 mmol) in dry DCM (1 mL) was added TFA (0.3 mL). The mixture was stirred for 2 h at room temperature. After the solvent was distilled off on a rotatory evaporator, methanol (10 mL) was added to the residue, and this procedure was repeated 3 times to remove remaining TFA. Evaporation of the solvent on a rotatory evaporator gave a white solid. The crude product was purified by gel filtration chromatography on column of Sephadex LH-20 with methanol as eluant. Evaporation of the product fraction under reduced pressure gave a white solid (53 mg, 87%): ^{1}H NMR (400 MHz, CD_3OD, 298K) δ 1.31 (m, 32H), 1.64 (m, 8H), 1.99 (m, 16H), 2.17 (m, 24H), 2.92 (m, 24 H), 3.25 (m, 8H), 3.57 (m, 32 H), 3.90 (s, 20H), 4.82 (m, 8H), and 7.02 (m, 88H). ^{13}C{^{1}H} NMR (100 MHz, CD_3OD, 293K) δ 24.0, 24.2, 29.5, 30.4, 33.1, 33.7, 35.4, 36.2, 40.4, 51.8, 112.5, 115.4, 118.3, 121.2, 128.0, 128.1, 128.5, 128.7, 129.7, 130.0, 130.1, 136.5, 139.5, 140.0, 141.1, 141.5, 161.4, 161.7, 170.7, 171.5, 172.0, and 173.0. Found: C, 54.71; H, 6.07; N, 8.75, Calcd for $C_{266}H_{300}F_{36}N_{36}O_{48}\bullet 20H_2O$: C, 54.95; H, 5.89; N, 8.67. MALDI-TOF MS m/z 4109 $[M + Na]^+$, where M shows $C_{242}H_{288}N_{36}O_{24}$ (as a free amine of cyclophane derivative).

Synthesis of A precursor of L5 (L5-Boc)

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EDC (68 mg, 0.35 mmol) and NHS (45 mg, 0.39 mmol) were added to a solution of carboxylic acid of cyclophane (**2**,[16] 211 mg, 0.17 mmol) in dry DMF (2 mL), and the resulting mixture was stirred for 12 h at room temperature. The solution was evaporated to dryness under reduced pressure. Ethyl acetate (EtOAc, 200 mL) was added to the residue, and the mixture was then washed with saturated aqueous sodium chloride (50 mL). After being dried ($MgSO_4$), the solution was evaporated to dryness under reduced pressure. The residue was chromatographed on a column of silica gel (SiO_2) with EtOAc as eluent. Evaporation of the product (succinimidyl ester derivative of cyclophane) fraction under reduced pressure gave a white solid (157 mg, 69%). The resulting solid was used in the next reaction. A solution of the succinimidyl ester derivative of cyclophane (143 mg, 0.11 mmol) in dry DCM (5 mL) was added dropwise to a solution of **L1** (18 mg, 0.014 mmol) and TEA (1 mL) in dry DCM (2 mL), and the mixture was stirred for 14 days at room temperature while monitoring the progress of the reaction using MALDI-TOF MS. The solvent was evaporated under reduced pressure, and the crude product was purified by gel filtration chromatography on column of Sephadex LH-20 with methanol as eluant. Evaporation of the product fraction under reduced pressure gave a white solid (64 mg, 82%): ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ 1.41 (s, 108H), 2.22 (m, 32H), 2.35 (m, 8H), 2.45 (m, 8H), 3.32 (m, 24H), 3.43 (m, 8H), 3.93 (m, 20H), 4.81 (s, 40H), 5.35 (m, 12H), 6.63 (m, 4H), and 6.97 (m, 120H). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$, 293K) δ 28.4, 29.9, 31.4, 34.4, 34.9, 35.2, 36.5, 41.0, 52.5, 52.6, 79.1, 128.6, 128.8, 129.0, 130.0, 136.4, 136.6, 136.8, 140.0, 140.2, 140.3, 156.0, 171.7, 171.8, and 172.0. Found: C, 69.67; H, 6.72; N, 8.53. Calcd for $C_{334}H_{384}N_{36}O_{48}$•$5H_2O$: C, 69.63; H, 6.89; N, 8.75. MALDI-TOF MS (positive mode): m/z 5694 $[M + Na]^+$, where M shows $C_{334}H_{384}N_{36}O_{48}$.

Synthesis of Cationic homotypic CP pentamer (L5)

To a solution of **L5-Boc** (37 mg, 0.0065 mmol) in dry DCM (1 mL) was added TFA (0.3 mL). The mixture was stirred for 2 h at room temperature. After the solvent was distilled off on a rotatory evaporator, methanol (10 mL) was added to the residue, and this procedure was repeated 3 times to remove remaining TFA. Evaporation of the solvent on a rotatory evaporator gave a white solid. The crude product was purified by gel filtration chromatography on column of Sephadex LH-20 with methanol as eluant. Evaporation of the product fraction under reduced pressure gave a white solid (33 mg, 86%): ^{1}H NMR (400 MHz, CD_3OD, 298K) δ 2.20 (m, 16H), 2.30 (m, 8H), 2.38 (m, 24H), 3.06 (m, 24H), 3.29 (m, 8H), 3.81 (m, 20H), 4.70 (m, 40H), and 6.90 (m, 120H). ^{13}C{^{1}H} NMR (100 MHz, CD_3OD, 293K) δ 23.7, 24.8, 25.4, 28.1, 29.6, 30.0, 34.8, 46.1, 109.8, 112.7, 122.9, 123.2, 124.0, 124.2, 130.4, 130.7, 131.1, 133.2, 133.7, 134.1, 135.3, 135.4, 135.6, 135.7, 155.7, 156.1, 164.5, 166.0, 166.7, and 167.6. Found: C, 59.11; H, 5.69; N, 8.26. Calcd for $C_{298}H_{300}F_{36}N_{36}O_{48}$•$13H_2O$: C, 58.95; H, 5.41; N, 8.30. MALDI-TOF MS (positive mode): m/z 4495 $[M + Na]^+$, where M shows $C_{274}H_{288}N_{36}O_{24}$ (as a free amine of cyclophane derivative).

Binding constants of the hosts with fluorescence guests

To each solution of PBA (0.2 μM), PeDA (0.2 μM), and TNS (1.0 μM) in HEPES buffer (0.01 M, pH 7.4, 0.15 M with NaCl) were added increasing amounts of the hosts, and the fluorescence spectra were recorded after each addition at 298K. The binding constants (K) for 1:1 host-guest complexes were calculated from the obtained titration dada by using online binding program (BindFit v0.5).[22] For host-guest complexes such as 1:2, reasonable calculation results could not be obtained. We performed the experiment three or more times and calculated the K values with good reproducibility.

Binding constants of the hosts with CDab

To each solution of CDab (10 μM) in HEPES buffer (0.01 M, pH 7.4, 0.15 M with NaCl) were added increasing amounts of the hosts, and the absorption spectra were recorded after each addition at 298K. Similarly, the K values were also calculated from the obtained titration dada by using binding program (BindFit v0.5).[22]

Simultaneous binding of PBA and CDab to the hosts

To each solution of PBA (0.5 μM) in the absence or presence of the hosts in HEPES buffer (0.01 M, pH 7.4, 0.15 M with NaCl) were added increasing amounts of CDab, and the absorption spectra were recorded after each addition at 298K.

Conflicts of interest

There are no conflicts of interest to declare.

Data availability

The data that support the findings of this study are available in the published article and its supplementary information (SI). Supplementary information: NMR and MALDI-TOF MS spectra for compounds **L1-Boc**, **L1**, **hetero5-Boc**, **hetero5**, **L5-Boc**, and **L5**, additional fluorescence spectra, and fitting of fluorescence titration data.

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The data supporting this article have been included as part of the Supplementary Information (SI).

The following sentences are included in the text of manuscript.

Data availability

The data that support the findings of this study are available in the published article and its supplementary information (SI). Supplementary information: NMR and MALDI-TOF MS spectra for compounds **L1-Boc**, **L1**, **hetero5-Boc**, **hetero5**, **L5-Boc**, and **L5**, additional fluorescence spectra, and fitting of fluorescence titration data.