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Cholesterol-Armed Cyclens for Helical Metal Complexes Offering Chiral Self-Aggregation and Sensing of Amino Acid Anions in Aqueous Solutions

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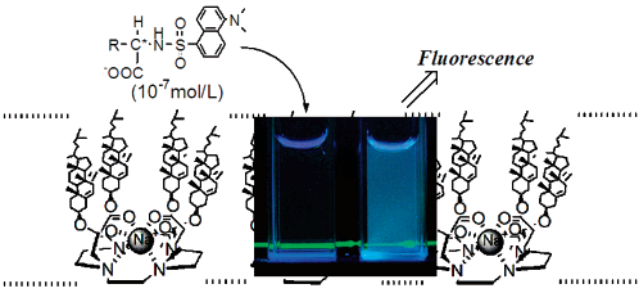

Cholesterol-armed cyclens worked as octadentate receptors for Na^+, Ca^{2+}, and Y^{3+} complexes in which four chiral cholesterol-functionalized sidearms were bundled and asymmetrically twisted above cyclen−metal complex platforms. Since the resulting helical metal complexes included chiral, hydrophobic cholesterol residues and charged, hydrophilic metal sites as well as asymmetric coordination geometries, they exhibited unique amphiphilic properties and provided chiral self-aggregates in aqueous solutions. Light scattering, fluorescence, and TEM characterizations demonstrated that Na^+ complex with cholesterol-armed cyclen gave a particularly stable self-aggregate in aqueous solution and offered supramolecular environments effective for sensing and detection of amino acid anions. Various dansylamino acid derivatives (dansyl = 5-(dimethylamino)-1-naphthalenesulfonyl) were nicely accommodated in the helicate aggregates to give highly enhanced fluorescence signals, which could be detected by the naked eye at 10^{-7} mol/L level. Their inclusion behaviors were analyzed by a Langmuir-type equation, indicating that enantiomer-selective inclusion occurred. MM/MD calculations and circular dichroism (CD) studies further suggested that cholesterol-armed cyclen helicates have chiral and hydrophobic cavities upon self-aggregation, in which the dansylamino acid anions were specifically accommodated. Since these helicates exhibited nonselective binding abilities in solvent extraction experiments of dansylamino acid anions, uncommon chiral recognition and sensing functions were generated by supramolecular alignments of the chiral metal helicates in the aqueous solutions.

Introduction

Various types of receptor molecules were recently designed and their metal complexes had highly organized structures and sophisticated functions.[1] Representatives of these are the helical metal complexes, so-called "helicates", with one or more coordinating receptors, which have well-defined coordination topology and high stability even in solution states. In addition to various oligopyridine ligand−metal complexes,[2] a series of hexacoordinate transition metal complexes and octa-/nonacoordinate lanthanide complexes have received considerable attention as functional helicates.[3] Armed cyclens having four

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cation-ligating sidearms were typically reported to form helical complexes with alkali, alkaline earth and lanthanide metal cations, which often had twisted square anti-prismatic structures.[4] When chiral substituents are attached to the cyclen system, the four sidearms stand up in the same direction above the macrocyclic ring and are asymmetrically arranged in a quadruply helical fashion. Wainwright et al. reported that Cd^{2+} complex with chiral alcohol-armed cyclen further formed ternary complexes with D- and L-histidinate anions in DMSO solution.[5a,b] Parker et al. recently employed Yb^{3+} complex with chiral, heptadentate amide-armed cyclen in recognition of lactate and alaninate anions in aqueous solutions.[5c,d] Since these chiral helicates have potential as a new class of metallo-receptors, synthetic approaches are required at not only the molecular level but also the supramolecular level, so that they can have defined cavities effective for precise recognition.

As frequently found in protein-based supramolecular systems,[6] the highly structured helicates are expected to exhibit their sophisticated functionalities via supramolecular aggregation through noncovalent interactions. Since there are many structural variations, synthetic metal helicates can be versatile building blocks for new supramolecular receptors, catalysts, devices, and related systems along this line. In earlier communications,[7] we demonstrated that cholesterol-armed cyclen **1**−Na^+ complex exhibited chiral and amphiphilic natures and spontaneously formed a stable self-aggregate in aqueous solution. Although several kinds of metal complex-based amphiphiles have been developed,[8] this helicate provided specific environments for guest accommodation based on the following chiroptical features: (1) assembling of chiral, hydrophobic cholesterol residues; (2) helicity of octadentate cyclen-metal complex; and (3) supramolecular alignment of chiral helicates. The aggregate of cyclen **1**−Na^+ complex nicely accommodated dansylglycine anion and asymmetrically fixed its conformation to induce the circular dichroism (CD) signals.[7] Here, we combine armed cyclen chemistry with self-aggregation technology for supramolecular architectures, in which a series of cholesterol-armed cyclen complexes with Na^+, Ca^{2+} and Y^{3+} cations are integrated at the supramolecular level. Some of them are applicable in sensing and detection of dansylamino acid anions of 1×10^{-7} mol/L by the naked eye (see picture in Abstract). Enantiomer determination of the employed dansyl amino acids was reported using capillary electrophoresis and fluorescence methods, in which glycopeptide antibiotics and an α-acid glycoprotein were typically used as chiral selectors.[9] Although the cholesterol-armed cyclen metal complexes rarely exhibited such anion recognition and sensing behaviors in homogeneous solution systems, they offered the sophisticated functions upon self-aggregation in aqueous media.

Results and Discussion

1. Cholesterol-Armed Cyclens for Chiral Helicates. Several armed cyclens were reported to bind Na^+, Ca^{2+}, and trivalent lanthanide cations quite strongly via octa-coordination from parent cyclen nitrogen atoms and donor sidearms.[4] NMR, CD, X-ray, and CPL characterizations have shown that only single C_4 orientation of the armed cyclen around the metal center predominated when chiral substituents were introduced on the four sidearms. Although cholesterol-armed cyclens **1** and **2** (Figure 1) similarly formed chiral helicates, their metal complexes were further furnished with hydrophobic cholesterol groups and the trapped metal cation as chiral amphiphiles for self-aggregation. NaCl complex with cyclen **1** is large (10 Å × 10 Å × 25 Å) with an inner void and exhibited high stability in solution (log $K = 11.0$ in ethanol).[4f] Figure 2 indicates ^{13}C NMR spectra of cyclen **1**−NaCl complex measured in $CDCl_3$ at various temperatures. The signals for two cyclen ring carbons separately resonated at 53.0 and 48.4 ppm at <308 K, while the singlet signal was observed at 75.5 ppm for N-CH_2-CO− carbons of four sidearms. Since cyclen **1** itself gave no diastereomeric signal for cyclen ring carbons even at 233 K, its Na^+ complex was confirmed to have quadruply helical structure with C_4 symmetry. Increasing the

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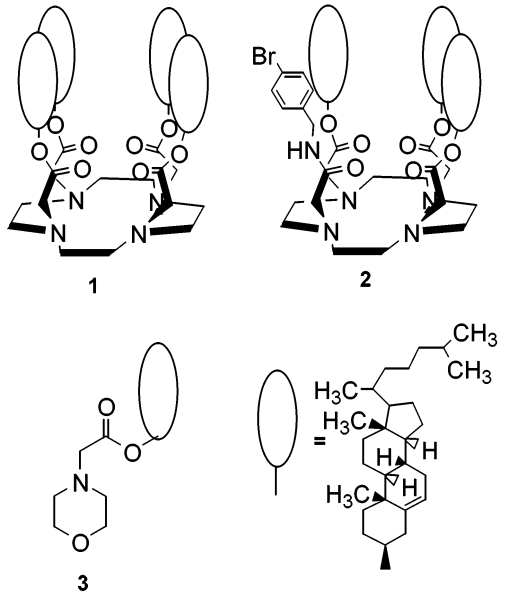

FIGURE 1. Cholesterol-armed cyclen **1** and its derivatives **2** and **3**.

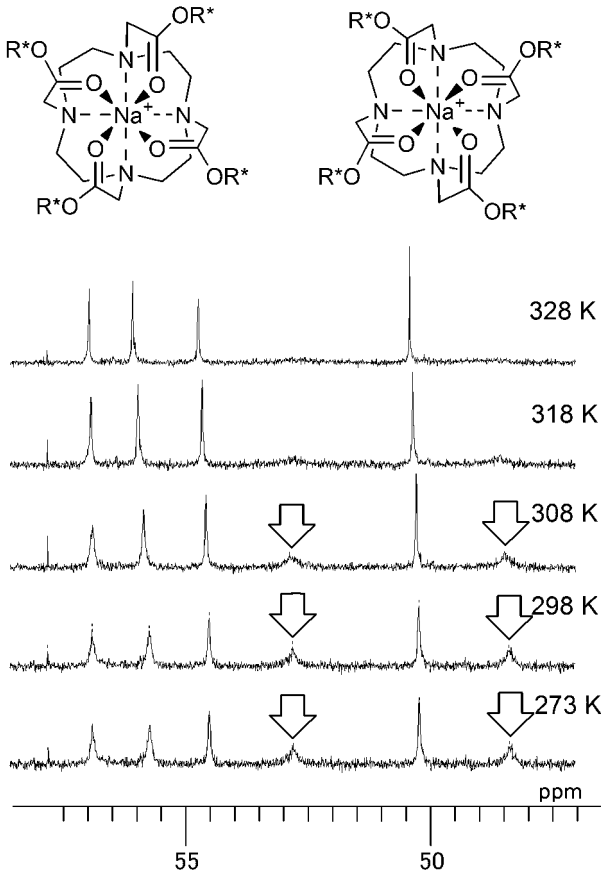

FIGURE 2. Two possible diastereomers and ^{13}C NMR spectra of cyclen **1**−NaCl complex. Conditions: cyclen **1**−NaCl, 4×10^{-2} mol/L in $CDCl_3$.

sample temperature to 318 K caused disappearance of the cyclen ring signals, suggesting that intramolecular ligand exchange occurred at higher temperature. Ca^{2+} and Y^{3+} complexes were prepared by mixing cyclen **1** and corresponding metal triflates in $CH_3CN/CHCl_3$. Its Ca^{2+} complex exhibited similar NMR spectral profiles to those with Na^+ complex, though Y^{3+} complex gave broad and unidentified ^1H NMR signals.

When four planar and hydrophobic cholesterol moieties stand up above the Na^+ - cyclen complex platform, a substantial box-like cavity can arise from juxtaposition of the four cholesterol planes. Although the suitable crystal for X-ray structural determination was not obtained, modeling experiments combined with molecular mechanics and molecular dynamics calculations were carried out. These support that cyclen **1**−Na^+ complex can have a chiral, hydrophobic cavity suitable for guest accommodation and self-aggregation. As schematically illustrated in Figure 3, two types of three-dimensional structures were postulated for cholesterol-armed cyclen−Na^+ complex: a saucer-shaped open-form and a stewpan-shaped closed-form. We have reported that some ester and amide-armed cyclen−Na^+ complexes had twisted anti-prismatic structures in solid states and preferred saucer-shaped structures due to the steric factors involving four sidearm-substituents.[4c,f] Therefore, cyclen **1**−Na^+ complex was first optimized as a saucer-shaped structure using the CAChe MM2 force field. Two diastereomeric structures with clockwise and anticlockwise helicities (Figure 2) were optimized to have similar stabilities. When these saucer-shaped structures were used for MD calculations, the stewpan-shaped structures were newly generated from both clockwise and anticlockwise precursors. Effective interactions between the cholesterol arms are believed to be the driving force for structural changes from the saucer-shaped to the stewpan-shaped forms. Five initial geometries of the stewpan-shaped close-form were extracted from trajectories of MD simulations. An optimized structure of this close-form is shown in Figure 3. In this complex, the four cholesterol moieties stand up in the same direction to provide a chiral, hydrophobic cavity. Since both interior and exterior surfaces of the complex are composed of hydrophobic planes, this can be effective not only for guest accommodation but for self-aggregation. Similar stewpan-shaped structures had been established in other types of armed cyclen complexes.[5] Although various cholesterol derivatives are known to provide chiral, hydrophobic environments for molecular recognition and self-aggregation,[10,11] the present type of armed cyclen helicates is suggested to offer effective arrangements of the chiral, four cholesterol sidearms for supramolecular recognition and aggregation.

2. Self-Aggregation of Cholesterol-Armed Cyclen−Metal Complexes. When cyclen **1**−NaCl complex was dispersed in the aqueous ethanolic solution (H_2O/EtOH = 80/20, v/v), this spontaneously formed very stable self-aggregate and gave no precipitate from the aqueous solution after 10 days. Its aggregation behaviors were characterized by fluorescence titrations, light-

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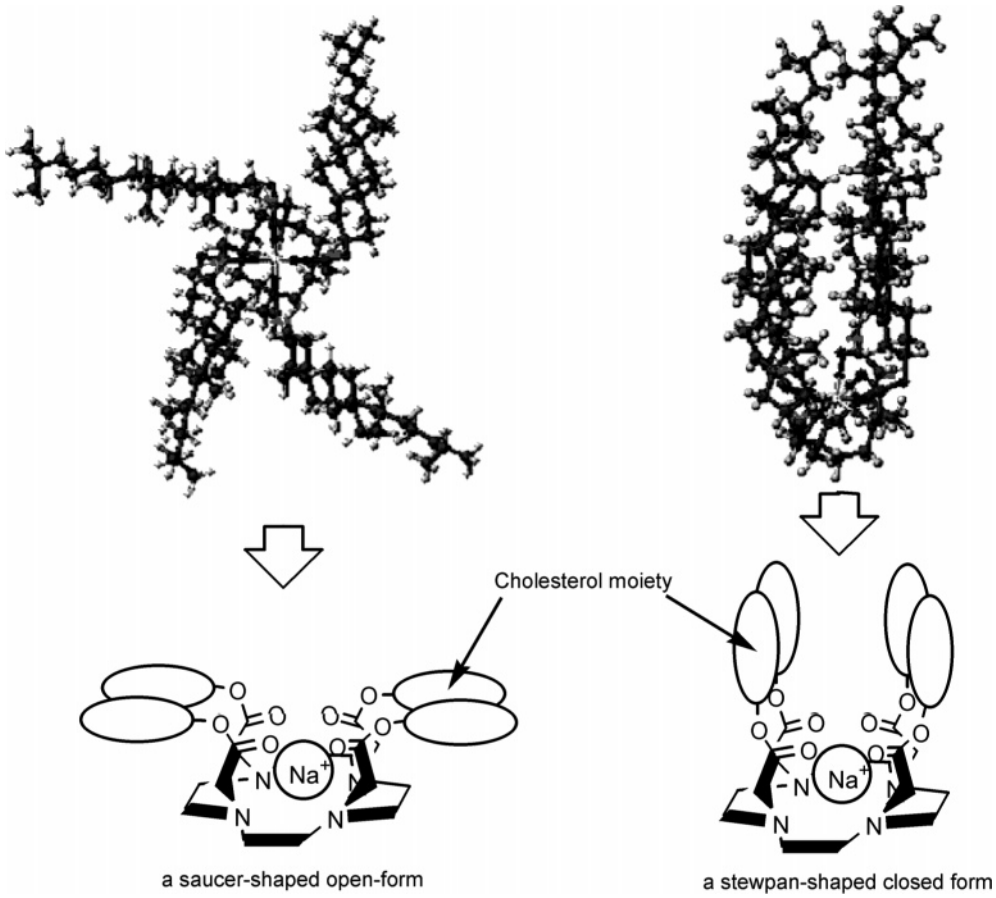

FIGURE 3. Schematic illustration of two preferred conformations of cyclen **1**−Na^+ complex and their optimized structures (top views).

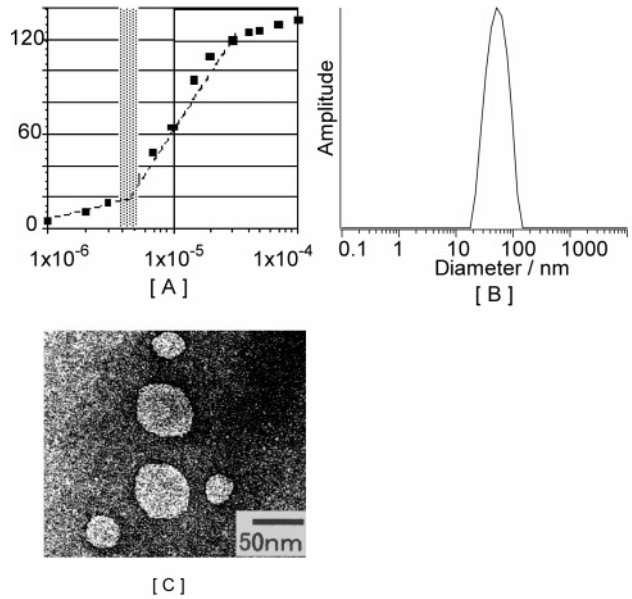

FIGURE 4. Self-aggregate of armed cyclen **1**−NaCl complex in H_2O/EtOH (80/20 v/v, pH = 7.2). (A) Fluorescence titration with dansyl-L-phenylalanine. (B) Size distribution by dynamic light scattering. (C) TEM picture.

scattering experiments, and TEM observations (Figure 4). Its critical aggregation concentration (cac) was estimated as 4.0×10^{-6} mol/L by titration with fluorescent dansyl-L-phenylalanine anion. When cyclen **1**−Na^+ complex was gradually added to the aqueous solution of dansyl-L-phenylalanine anion (1×10^{-5} mol/L), its fluorescence maximum continuously shifted from 538 to 498 nm and the intensity recorded at 498 nm was gradually increased. Pronounced changes in the fluorescence intensity occurred around 4.0×10^{-6} mol/L of cyclen **1**−Na^+ complex (Figure 4A). Dynamic light scattering experiments showed that the resulting self-aggregate had a mean hydrodynamic radius of 60 nm, when 1×10^{-4} mol/L of the Na^+ complex was dispersed (Figure 4B). A TEM picture taken after treatment of uranyl acetate indicates that dispersed self-aggregates of 60 nm or larger were observed (Figure 4C). When the size distributions were measured at 20−60 °C, the average distribution was confirmed as almost constant, revealing that the formed self-aggregate was stable even at a wide range of temperature. Ca^{2+} and Y^{3+} complexes with armed cyclen **1** similarly formed self-aggregates in the aqueous ethanolic solutions, but their particle sizes were somewhat different from that with Na^+ complex: 112 nm for Ca^{2+} complex and 29 nm for Y^{3+} complex. Since these three cations have almost the same ion radii,[12] the charge state of the metal center played an important role in the self-aggregation processes.

Armed cyclen **2**−Na^+ complex also formed self-aggregate in the aqueous ethanol solutions, though up to 30% ethanol content is required to give a spectroscopi-

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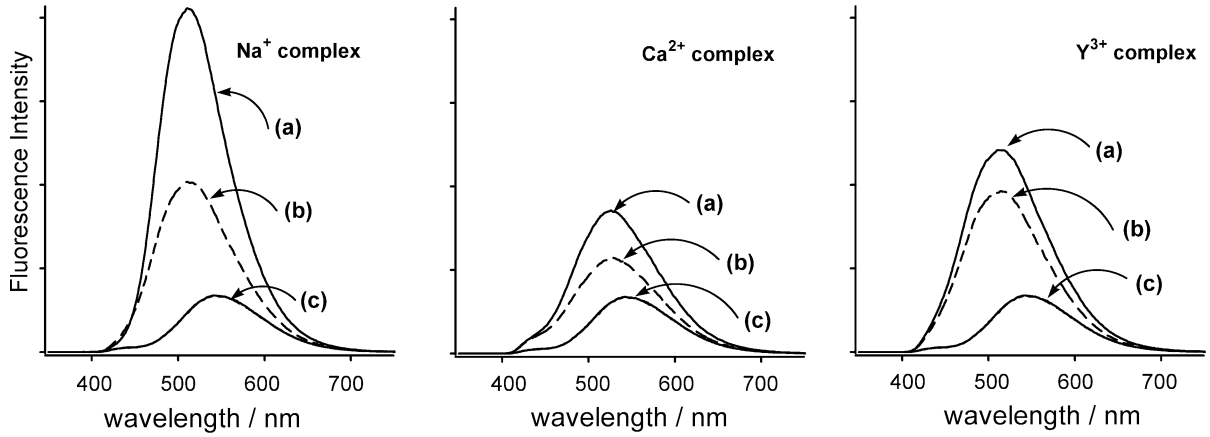

FIGURE 5. Fluorescence sensing of dansyl-L- and -D-leucine anions with cyclen **1**−metal complex aggregates: (a) dansyl-L-leucine (bound), (b) dansyl-D-leucine (bound), (c) dansyl-L- and -D-leucine (free). Conditions: cyclen metal complex, 3.3×10^{-5} mol/L; dansylleucine, 1.0×10^{-6} mol/L; in H_2O/EtOH (80/20 v/v); pH = 7.2.

TABLE 1. Fluorescence and Binding Behaviors of Dansylamino Acid Anions in Armed Cyclen 1−NaCl Self-Aggregate

	fluorescence[a]			binding[c]		
	intensity (λ_{max}/nm)					
dansylamino acid	no aggregate	aggregate	enhancement[b]	K/M^{-1}	n/m	log D
L-valine	27 (536)	213 (504)	7.9	$(2.3 \pm 0.8) \times 10^5$	0.59	−1.35
D-valine	26 (537)	185 (505)	7.1	$(1.7 \pm 0.3) \times 10^5$	0.38	
L-*t*-leucine	37 (534)	258 (504)	7.0	$(2.8 \pm 0.4) \times 10^5$	0.55	−1.05
D-*t*-leucine	36 (537)	226 (506)	6.3	$(1.9 \pm 0.3) \times 10^5$	0.30	
L-leucine	32 (533)	207 (507)	6.5	$(3.1 \pm 0.4) \times 10^5$	0.35	−0.88
D-leucine	32 (532)	158 (505)	4.9	$(1.4 \pm 0.2) \times 10^5$	0.30	
L-phenylglycine	32 (539)	200 (514)	6.3	$(4.5 \pm 0.7) \times 10^5$	0.67	−1.03
D-phenylglycine	32 (538)	250 (504)	7.8	$(8.8 \pm 1.7) \times 10^5$	0.65	
L-phenylalnine	25 (538)	229 (498)	9.2	$(4.2 \pm 0.6) \times 10^5$	0.53	−0.60
D-phenylalnine	25 (539)	203 (505)	8.1	$(6.0 \pm 1.4) \times 10^5$	0.55	

[a] Conditions: cyclen **1**−NaCl, 1.0×10^{-4} mol/L; dansylamino acid, 1.0×10^{-5} mol/L; in H_2O/EtOH (80/20, pH = 7.2). [b] Fluorescence intensity at λ_{max} in the presence of aggregate/fluorescence intensity at λ_{max} in the absence of aggregate. [c] Conditions: see the Experimental Section.

cally clear solution. Its cac value (1.6×10^{-5} mol/L) and averaged particle-size (30 nm) were different from those of cyclen **1**−Na^+ complex in H_2O/EtOH (70/30, w/w), indicating that the sidearm substitution altered the detailed profile of the self-aggregate. When CD spectrum of cyclen **2**−Na^+ complex in ethanol was compared with that in aqueous ethanol solution, the sign of CD signal around 230 nm changed drastically (see the Supporting Information, Figure S1): a negative sense in EtOH → a positive sense in H_2O/EtOH. These CD spectral changes clearly indicate that the self-aggregation greatly altered chiral environments around the phenyl chromophore of the cyclen complex. Probably, the three cholesterol-functionalized sidearms of cyclen **2** were compressed upon self-aggregation to stabilize the stewpan-shaped conformation (see Figure 3). Similar cavity shape conversion of the receptor was reported in the monolayer system composed of steroid cyclophane,[11c] in which four steroid moieties were rearranged upon monolayer formation at the air/water interface to offer the hydrophobic cavity effective for guest accommodation.

3. Fluorescence Sensing of Amino Acid Anions in Aqueous Media. Dansylamino acid anions were nicely accommodated in armed cyclen **1**−metal complex aggregates and exhibited characteristic fluorescence enhancements. The fluorescence profiles of the bound dansylamino acid anions were largely dependent on the nature of the metal center as well as mole ratio of guest to complex amphiphile. Figure 5 compares the enhanced fluorescence profiles of dansyl-L- and D-leucine anions upon inclusion into the self-aggregates of cyclen **1**−Na^+, −Ca^{2+}, and −Y^{3+} complexes. Among them, cyclen **1**−Na^+ complex exhibited the largest fluorescence enhancement: 6.1 times for Na^+ complex; 2.5 times for Ca^{2+} complex; and 3.6 times for Y^{3+} complex. The intensity difference between the guest enantiomers was observed in each helicate aggregate, and Na^+ complex also offered a greater difference than Ca^{2+} and Y^{3+} complexes. Several dansylamino acids of 10^{-7} mol/L were detected by the naked eye[13] in the presence of cyclen **1**−Na^+ complex aggregate (see picture in Abstract).

Cyclen **1**−Na^+ complex gave the most stable self-aggregate in the aqueous media, and its anion recognition and sensing behaviors were characterized by fluorescence

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method (Table 1). When an excess of the self-aggregate (1.0×10^{-4} mol/L) was added to an aqueous ethanol solution of dansyl-L- or D-phenylalanine anion (1.0×10^{-5} mol/L), the fluorescence maxima of the bound anions shifted from 538 to 498 nm for L-isomer and to 505 nm for D-isomer. Under the employed conditions (see Table 1), the fluorescence intensities were enhanced 9.2-fold for dansyl-L-phenylalanine anion and 8.1-fold for its D-isomer upon inclusion, indicating that the two enantiomers were located under somewhat different circumstances. In contrast, the enantiomers of aliphatic amino acid anions exhibited the enhanced fluorescence signals upon inclusion, but their maxima appeared at almost the same wavelengths: 507 and 505 nm for dansylleucine; 504 and 506 nm for dansyl-*tert*-leucine; and 504 and 505 nm for dansylvaline. These observations suggest that the phenyl residues of aromatic amino acids occupy unique positions in the self-aggregate. The fluorescence maxima of the dansyl derivatives generally relate to the polar natures of the employed solvents. The fluorescence maxima of dansyl-L-leucine were recorded in the absence of self-aggregate at 533 nm in H_2O (pH = 7.2), 503 nm in ethanol, and 490 nm in $CHCl_3$. Therefore, cyclen **1**−Na^+ complex aggregate provided an ethanol-like environment around the dansyl chromophores. With an increase of ethanol content in the aqueous aggregate solution, the intensity of the observed fluorescence signal decreased, and there was no enhancement in 60% of ethanol aqueous solution. Since the particle sizes could not be determined under such ethanol-rich conditions by light scattering method, cyclen **1**−Na^+ complex in a nonaggregated form did not have an effective cavity to accommodate dansylamino acid anions. Although several chiral disk-shaped molecules were reported to exhibit chirality-amplification by stacking in highly ordered chiral columns in the solutions,[14] the present type of helicates generated unique anion-sensing functions upon self-aggregation in aqueous media. Cationic dansylethylenediamine hydrochloride was also employed as a fluorescence probe. Since its fluorescence intensity was rarely enhanced in the presence of cyclen **1**−Na^+ complex aggregate, the electrostatic attraction between the metal center of the cyclen complex and $-CO_2^-$ group of the dansylamino acid anion was essentially involved as well as hydrophobic interaction between cholesterol moieties and dansyl group in the aggregate inclusion processes.

4. Quantitative Studies of Anion Inclusion. The inclusion behaviors of dansylamino acid anions with cyclen **1**−Na^+ complex aggregate were quantitatively analyzed using the following Langmuir-type equation

$$m[A]_b/[1 - Na^+]_o = n\,K\,[A]_u/(1 + K\,[A]_u)$$

where $[A]_b$ and $[A]_u$ are concentrations of bound and unbound guest anions, and m and n are numbers of cyclen **1**−Na^+ complex and saturated guests in the single self-aggregate. According to this equation, K values were estimated for enantiomers of dansyl-valine, *tert*-leucine, leucine, phenylglycine, and phenylalanine by curve-fitting method (see the Supporting Information). As summarized in Table 1, the self-aggregate of cyclen **1**−Na^+ complex preferred L-isomers of aliphatic amino acids to their D-isomers but favored D-isomers of aromatic amino acids. The K values (L/mol) were estimated as 2.3×10^5 and 1.7×10^5 for dansyl-L- and -D-valine anions, 2.8×10^5 and 1.9×10^5 for dansyl-L- and -D-*t*-leucine anions, 3.1×10^5 and 1.4×10^5 for dansyl-L- and -D-leucine anions, 4.5×10^5 and 8.8×10^5 for dansyl-L- and -D-phenylglycine anions; and 4.2×10^5 and 6.0×10^5 for dansyl-L- and -D-phenylalanine anions. The mole ratio of n/m ranged from 0.30 to 0.67, and two possible guest accommodation modes of intra- and inter-complex bindings were postulated. The log D values were calculated at pH = 7.2 using the PALLAS program (version 3.0, CompuDrug Chemistry Ltd.)[15] as measures of hydrophobicity for dansylamino acid anions (Table 1). No clear relationship between K values and log D values of the guests was found, but aromatic amino acid anions with large log D values were more strongly bound than aliphatic amino acids with small log D values. The highest L/D selectivity was recorded as 2.2 in the case of leucine derivative, while the highest D/L one was estimated as 2.0 with phenylglycine anion. As described above, dansylamino acid anions were bound by cyclen **1**−Na^+ complex via electrostatic attraction and hydrophobic interaction. Since aromatic and aliphatic amino acid derivatives exhibited different trends in K values and enantiomer selectivity, aromatic residues of amino acid anions provided further interactions with cyclen metal complex.

Circular dichroism (CD) analysis provided structural information of the dansylamino acid anions incorporated in the chiral helicate aggregates. The employed dansylamino acid anions changed CD signs upon incorporation. Typically, dansyl-L-leucine anion exhibited an intense CD signal around 260 nm with a negative sign in the aqueous solution but a positive sign in the self-aggregate of cyclen **1**−Na^+ complex (Figure 6). In contrast, dansyl-D-leucine anion gave a positive sign in the aqueous solution and a negative sign in the self-aggregate. Polonski et al. had reported that the Cotton effect observed with chiral dansylamino acid was originated from asymmetrical twisting of the sulfonamide group in relation to the naphthalene plane under the influence of hydrogen atom in the *peri*-position.[16] Based on this model, the observed inversion of the CD signs suggests the conformational change between A and B in Figure 6. Since the most bulky substituent R of the leucine anion predominantly occupies the sterically least hindered position, dansyl-L-leucine anion favors an "anti-clockwise" form to offer the negative CD signal in the bulk aqueous solution (A in Figure 6). When this anion is included in the self-aggregate, the CO_2^- group becomes more bulky than the substituent R, because of electrostatic interaction with large cyclen **1**−Na^+ cation. This means that dansyl-L-leucine anion has a "clockwise" form and gives the positive CD signal in the self-aggregate (B in Figure 6). Since the D-isomer has apparently reversed profiles, the

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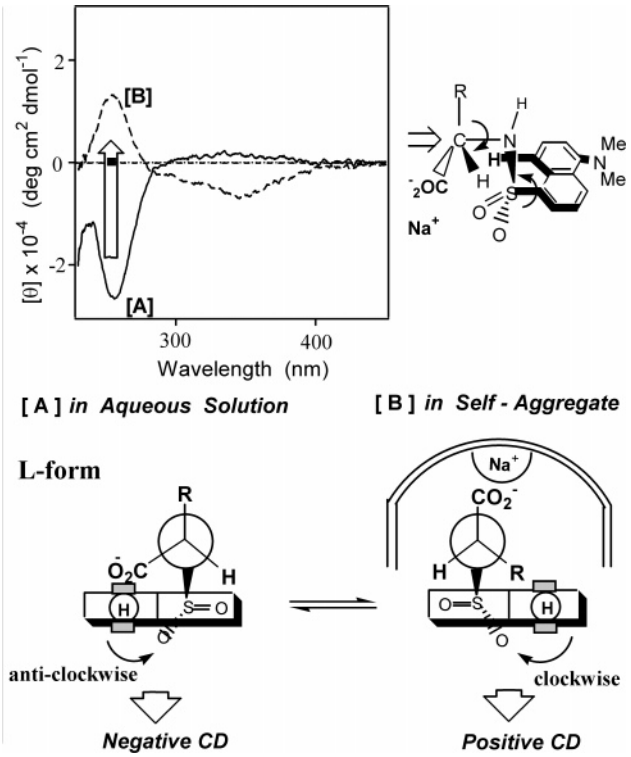

FIGURE 6. CD spectral changes of dansyl-L-leucine and its preferred conformations (A) in aqueous solution and (B) in self-aggregate of cyclen **1**−NaCl aggregate.[17]

bound amino acid guest was thought to have a "frozen" conformation in the self-aggregate.

The cholesterol-armed cyclen metal complexes also interacted with dansylamino acid anions in organic media but exhibited no enantiomer selectivity. When an aqueous solution of racemic dansyl-L- and D-leucine anion (2.0×10^{-4} mol/L, each, 2.0 mL) was shaken with a $CHCl_3$ solution of cyclen **1**−NaCl complex (2.0×10^{-4} mol/L, 2.0 mL), the guest anion was quantitatively but not enantiomer-selectively extracted. Although cyclen **1**−Na^+ complex has an asymmetric helical structure even in the organic media, its nonaggregated form simply behaved like a cationic surfactant and rarely responded to the guest chirality.[5] Thus, supramolecular alignments of the helical complexes generated characteristic anion recognition and sensing functions. When cholesteroxycarbonyl-4-methylmorpholine **3** (2.0×10^{-5} mol/L) was dispersed with NaCl (5.0×10^{-6} mol/L) in aqueous ethanol solution (20/80, v/v, pH = 7.2), TEM experiments confirmed its self-aggregation. Since the addition of this aggregate rarely influenced the fluorescence spectra of the dansylamino acid anions, it could not provide an effective environment for anion inclusion. Albumin and γ-cyclodextrin were also employed as receptors for dansylamino acids.[18] Although they are well-known to accommodate aromatic guest anions, only albumin exhibited the enhanced fluorescence signals of the dansylamino acid anions in the neutral aqueous solutions. This exhibited larger fluorescence signal for L-leucine than D-leucine, but smaller signal for L-*t*-leucine than D-*t*-leucine. Since this protein has several different binding sites for substrate accommodation, dansylamino acids were confirmed to locate in the different cavities. Therefore, the present type of cyclen metal complexes was demonstrated to be unique building blocks for supramolecular aggregates, which offered enantiomer recognition and visual sensing of amino acid anions in aqueous solutions. The proper organization of cholesterol-functionalized sidearms, octadentate chiral armed cyclen platform, and charged metal center offered water-soluble aggregates with notable anion recognition and sensing functions. Since similar phenomena were not observed at the molecular level, self-aggregation of the helicates can be considered an effective method of generating sophisticated functionalities.

Experimental Section

Materials. Dansyl-L-valine, -L-leucine, and -L-phenylalanine were commercially available and used after recrystallization from CH_3OH/H_2O. Other dansylamino acids were synthesized by the reported methods.[19] Their optical purities were determined as >99% by chiral HPLC analysis (SUMICHIRAL OA-3200). The $[\alpha]_D$ values of the synthesized derivatives were as follows: dansyl-D-valine, −22.1 (c = 0.5, CH_3OH); dansyl-D-leucine, +29.2 (c = 0.5, CH_3OH); dansyl-L-*tert*-leucine, +56.2 (c = 0.5, CH_3OH); dansyl-D-*tert*-leucine, −55.5 (c = 0.5, CH_3OH); dansyl-D-phenylalanine, +55.5 (c = 0.5, CH_3OH); dansyl-L-phenylglycine, +50.6 (c = 0.5, CH_3OH); dansyl-D-phenylglycine, −50.7 (c = 0.5, CH_3OH).

1,4,7,10-Tetrakis(cholesteryloxycarbonylmethyl)-1,4,7,-10-tetraazacyclododecane (1). After a solution of cyclen tetrahydrochloride (644 mg, 2.02 mmol) and Cs_2CO_3 (9.80 g, 30.1 mmol) in CH_3CN (15 mL) was refluxed for 1 h, a solution of cholesterol choloroacetate (5.58 g, 12.0 mmol) in $CHCl_3$ (20 mL) was added dropwise. The resulting mixture was refluxed for 5 h and then filtered. After solvent was evaporated, the residue was washed with ether and recrystallized from $CHCl_3$/ether, $CHCl_3$/acetone and then $CHCl_3/CH_3CN$ (14%): mp 196−202 °C decomp; $[\alpha]_D = -29.3$ (c = 0.5, $CHCl_3$); IR (KBr) ν 1738 cm^{-1} (br); ^1H NMR ($CDCl_3$) δ 0.68 (s, 12H), 0.80−2.20 (m, 104H), 0.85 (d, J = 1.2 Hz, 12H), 0.87 (d, J = 1.5 Hz, 12H), 0.91 (d, J = 6.1 Hz, 12H), 1.01 (s, 12H), 2.31 (d, J = 8.1 Hz, 8H), 2.82 (s, 16H), 3.36 (s, 8H), 4.66 (br m, 4H), and 5.36 (br, 4H); ^{13}C NMR ($CDCl_3$) δ 11.85, 18.72, 19.32, 21.04, 22.55, 22,81, 23.85, 24.28, 27.93, 28.00, 28.33, 31.86, 31.91, 35.80, 36.19, 36.58, 37.02, 38.26, 39.51, 39.74, 42.31, 50.03, 52.07, 56.11, 56.16, 56.70, 73.96, 122.66, 139.59, and 171.14. Anal. Calcd for $C_{124}H_2O_4N_4O_8$: C, 79.26; H, 10.94; N, 2.98. Found: C, 79.09; H, 10.97; N, 2.97.

1,4,7,10-Tetrakis(cholesteryloxycarbonylmethyl)-1,4,7,-10-tetraazacyclododecane (1)−NaCl Complex. After a solution of cyclen tetrahydrochloride (0.65 g, 2.05 mmol) and Na_2CO_3 (3.22 g, 30.4 mmol) in CH_3CN (15 mL) was refluxed for 1.5 h, a solution of cholesterol chloroacetate (5.56 g, 12.0 mmol) in $CHCl_3$ (40 mL) was added dropwise. The resulting mixture was refluxed for 7 h and then filtered. After the solvent was evaporated, the residue was washed with ether and recrystallization from $CHCl_3/CH_3CN$ gave white needle crystals of **1**−NaCl·5H_2O complex (71%): mp 151−152 °C dec; $[\alpha]_D = -83.4$ (c = 0.5, $CHCl_3$); IR (neat) ν 1725 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.70 (s, 12H), 0.80−2.40 (m, 112H), 0.86 (d, 12H, J

(17) In the presence of aggregate, the concentrations of bound and unbound dansylleucine were estimated to be 0.8×10^{-4} and 1.0×10^{-4} M, respectively. The [θ] value used in the graph was obtained by dividing the θ value by total concentration of dansylleucine, 1.8×10^{-4} M.

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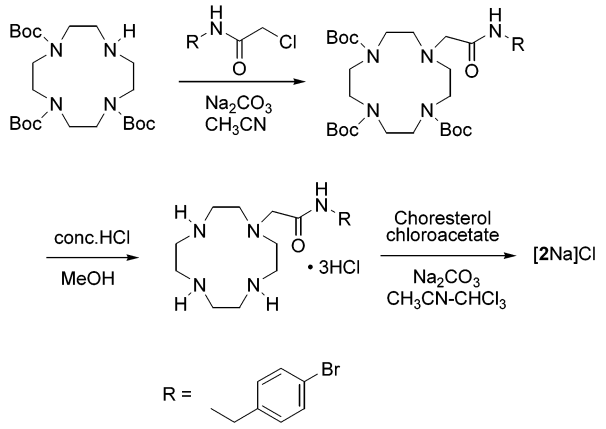

FIGURE 7. Synthesis of armed cyclen **2**.

= 1.5 Hz), 0.88 (d, 12H, J = 1.7 Hz), 0.92 (d, 12H, J = 6.3 Hz), 0.99 (s, 12H), 2.15−2.35 (br m, 4H), 2.40−2.70 (br m, 8H), 2.98 (br d, 4H, J = 17.3 Hz), 3.25−3.45 (br m, 4H), 3.45 (br d, 4H, J = 17.1 Hz), 4.53 (br m, 4H), and 5.26 (br 4H); ^{13}C NMR ($CDCl_3$) δ 11.93, 18.69, 19.08, 21.03, 22.54, 22.77, 23.57, 24.26, 27.64, 27.97, 28.13, 31.63, 32.49, 35.69, 36.20, 36.36, 36.90, 38.15, 39.57, 39.90, 42.32, 48.46, 50.34, 53.01, 54.80, 56.07, 57.23, 75.48, 122.49, 139.71, and 173.49. Anal. Calcd for $C_{124}H_{204}N_4O_8$·NaCl·5H_2O: C, 73.46; H, 10.64; N, 2.76. Found: C, 73.76; H, 10.49; N, 2.76.

1-(4-Bromophenyl)methylcarbamoylmethyl-4,7,10-tris-(cholesteryloxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (2)−NaCl Complex (See Figure 7). *N*-(4-Bromophenyl)methyl-2-chloroethanamide[20] was synthesized by mixing a CH_2Cl_2 (100 mL) solution of 4-(bromophenyl)methylamine (1.96 g, 8.81 mmol) and triethylamine (2.24 g, 22.1 mmol) with a CH_2Cl_2 solution (50 mL) of 2-chloroacetyl chloride (1.51 g, 13.4 mmol) at 0 °C. After 1.5 h stirring at 0 °C, the organic phase was washed with water (100 mL), 1 M hydrochloric acid (100 mL × 2) and sat. NaCl solution (100 mL), evaporated, and dried over $MgSO_4$. Recrystallization from CH_2Cl_2/hexane gave needle crystals (1.33 g, 5.07 mmol): 58% yield; mp 124−125 °C; IR (KBr) ν 3270, 1661, 1558, 1245, and 796 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.11 (s, 2H), 4.45 (d, 2H, J = 6.1 Hz), 6.88 (br s, 1H), 7.18 (m, 2H), and 7.48 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 42.6, 43.2, 121.7, 129.5, 131.9, 136.3, and 165.9; MS (FAB, pos) m/z 262 (M + H$^+$).

1-(4-Bromophenyl)methylcarbamoylmethyl-4,7,10-tris-(*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecane was prepared by the reaction of 1,4,7-tris(*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecane (993 mg, 2.10 mmol)[21] and *N*-(4-bromophenyl)methyl-2-chloroethanamide (822 mg, 3.14 mmol) in the presence of K_2CO_3 (647 mg, 4.68 mmol) and KI (156 mg, 0.940 mmol) in dry CH_3CN (20 mL). After refluxing for 15 h under N_2, the solvent was evaporated and the residue was dissolved in CH_2Cl_2 and filtered. The filtrate was chromatographed on silica gel with CH_2Cl_2/AcOEt (1/5, v/v) to give 1.26 g (85%) of amorphous product: IR (KBr) ν 1689, 1540, 1250, and 1167 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.38 (br s, 18H), 1.48 (s, 9H), 3.19 (s, 2H), 3.37 + 3.47 (br s, 12H), 4.37 (d, 2H, J = 6.1 Hz), 7.17 (m, 2H), 7.28 (br s, 1H), and 7.43 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 28.3, 28.5, 42.6, 47.6, 50.0, 60.3, 80.0, 121.0, 129.5, 131.6, 137.6, 155.6, and 171.2; MS (FAB, pos) m/z 698 (M + H$^+$).

1-(4-Bromophenyl)methylcarbamoylmethyl-1,4,7,10-tetraazacyclododecane·3HCl was synthesized by deprotection of 1-(4-bromophenyl)methylcarbamoylmethyl-4,7,10-tris-(*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecane (1.22 g, 1.74 mmol) with concd HCl (1.5 mL) in MeOH (10 mL). After stirring for 2 h, the solvent was removed. White hygroscopic precipitate was obtained (571 mg, 65%) by adding Et_2O to the MeOH solution: IR (KBr) ν 1653, 1551, and 1071 cm^{-1}; ^{1}H NMR (D_2O) δ 2.8 (br s, 8H), 2.9−3.1 (8H), 3.36 (s, 2H), 4.22 (s, 2H), 7.08 (d, 2H, J = 8.1 Hz) and 7.41 (d, 2H, J = 8.2 Hz); ^{13}C NMR (D_2O) δ 42.9, 43.3, 45.1, 50.4, 56.0, 129.7, 132.3, 137.4, and 173.8; MS (FAB, pos) m/z 398 (M + H$^+$). Anal. Calcd for $C_{17}H_{28}N_5OBr$·3HCl·1.5H_2O: C, 38.18; H, 6.41; N, 13.10. Found: C, 38.33; H, 6.41; N, 13.08.

1-(4-Bromophenyl)methylcarbamoylmethyl-4,7,10-tris-(cholesteryloxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (2)−NaCl complex was prepared as described below. 1-(4-Bromophenyl)methylcarbamoylmethyl-1,4,7,10-tetraazacyclododecane·3HCl (151 mg, 0.298 mmol) and Na_2CO_3 (328 mg, 3.09 mmol) were refluxed in CH_3CN (7 mL) for 1 h under N_2. Cholesterol chloroacetate (631 mg, 1.36 mmol) dissolved in $CHCl_3$ (10 mL) was added dropwise and refluxed for 50 h. After solvent was removed, CH_2Cl_2 was added, and the insoluble inorganic materials were filtered using cerite. After evaporation, the residue was repeatedly reprecipitated from CH_2Cl_2/hexane followed by centrifugation to remove excess cholesterol chloroacetate. Colored impurities were removed using charcoal (160 mg × 2) in CH_2Cl_2 solution which finally gave colorless crystals from CH_2Cl_2/Et_2O (230 mg, 42%): mp 198−200 °C; $[\alpha]^{27}_D$ = −63 (c = 0.50, $CHCl_3$); IR (KBr) ν 1732, 1668, and 1213 cm^{-1}; ^{1}H NMR ($CDCl_3$, 50 °C) δ 0.68 (s, 3H), 0.70 (s, 3H × 2), 0.87 (d, 18H, J = 6.0 Hz), 0.92 (d, 6H, J = 6.7 Hz), 0.94 (d, 3H, J = 6.4 Hz), 0.96 (s, 3H), 0.98 (s, 6H), 0.9−1.2 (33H), 1.2−1.7 (33H), 1.7−2.1 (15H), 2.1−3.4 (25H), 3.62 (s, 2H), 4.28 (br s, 1H), 4.48 (br s, 1H), 4.62 (m, 3H), 5.10 (br s, 1H), 5.31 (m, 2H), 7.24 (m, 2H), 7.31 (m, 2H), and 9.99 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 °C) δ 11.9, 12.1, 18.8, 18.9, 19.0, 19.2, 19.3, 21.2, 21.3, 22.6, 22.8, 22.8, 23.9, 24.0, 24.1, 24.1, 24.4, 24.5, 27.5, 27.9, 28.0, 28.1, 28.1, 28.2, 28.3, 31.9, 31.9, 32.0, 32.1, 32.3, 32.5, 35.8, 35.9, 35.9, 36.0, 36.3, 36.4, 36.6, 36.6, 36.7, 37.0, 37.1, 37.3, 38.0, 38.2, 38.3, 39.7, 39.9, 40.0, 40.1, 42.4, 42.5, 42.5, 50.1, 50.3, 50.4, 55.2, 55.2, 56.2, 56.4, 56.5, 56.9, 56.9, 57.1, 57.2, 75.5, 75.2, 75.6, 120.2, 123.0, 123.1, 129.6, 131.2, 139.1, 139.4, 139.9, 172.6, and 172.9; MS (FAB, pos) m/z 1699 (2 + Na$^+$). Anal. Calcd for $C_{104}H_{166}BrN_5O_7$·NaCl·(H_2O)$_{3.5}$: C; 69.40, H; 9.69, N; 3.89. Found: C; 69.19, H; 9.38, N; 3.82.

Synthesis of Cholesteryl Morpholinoacetate (3). This was similarly prepared by reaction of morpholine and cholesterol chloroacetate (65%): mp 123−124 °C; $[\alpha]^{25}_D$ = −30.4 (c = 2.0, $CHCl_3$); IR (KBr) ν 1729 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.68 (s, 3H), 0.85 (d, 3H, J = 1.3 Hz), 0.87 (d, 3H, J = 1.3 Hz), 0.91 (d, 3H, J = 6.4 Hz), 1.02 (s, 3H), 0.8−2.1 (m, 26H), 2.33 (d, 2H, J = 7.7 Hz), 2.59, 3.75 (t x 2, 8H, J = 4.7 Hz), 3.18 (s, 2H), 4.66 (br m, 1H), and 5.38 (d, 1H, J = 4.4 Hz); ^{13}C NMR ($CDCl_3$) δ 11.84, 18.70, 19.29, 21.01, 22.54, 22.80, 23.81, 24.26, 27.78, 28.00, 28.20, 31.84, 31.89, 35.78, 36.16, 36.57, 36.93, 38.11, 39.50, 39.71, 42.30, 50.00, 53.28, 56.12, 56.67, 59.90, 66.80, 74.73, 122.82, 139.44, and 169.49. Anal. Calcd for $C_{33}H_{55}NO_3$: C, 77.14; H, 10.79; N, 2.73. Found: C, 77.01; H, 10.77; N, 2.73.

Preparation of Self-Aggregates. An ethanol solution of cholesterol-armed cyclen **1**−NaCl complex (0.50 μmol/1 mL) was diluted with Bis-Tris-HCl buffer solution (6.3 × 10^{-3} M, pH = 7.2). The resulting aqueous solution (H_2O/EtOH = 80/20, v/v) was spectroscopically clear and gave no precipitate for several days. Light scattering experiments indicated that the particle sizes of the self-aggregates were slightly changed (50−70 nm) after aging, centrifugation or other procedures. The self-aggregate of morpholine derivative **3** was similarly prepared, though it did not complex with Na$^+$ cation. Ca^{2+} and Y^{3+} complexes with cyclen **1** were *in situ* prepared. When 1.5 equiv of Ca^{2+} triflate was typically mixed with free cyclen **1**, the cation complexation process was monitored by ESI-MS and NMR measurements. After evaporation, the ethanol solution of the complex was diluted with aqueous buffer solution. Both

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complexes formed stable aggregates in Bis-Tris-HCl buffer solutions (pH = 7.2, H_2O/EtOH = 80/20, v/v). Since the Na^+ complex with cyclen **2** required high ethanol contents to disperse in the solutions, we usually employed aqueous ethanol solution (H_2O/EtOH = 70/30, w/w) in this case.

Molecular Modeling of Cyclen 1−Na^+ Complex. CAChe (ver.5.04, Fujitsu Ltd. Japan) was used for molecular modeling. To obtain initial structures of the large cyclens, Δ- and Λ-types of Na^+ complexes with cyclen having four CH_2COOH arms were optimized using the CAChe MM2 force field. The four H atoms on the sidearms were replaced by cholesterol arms to obtain initial structures which were used for optimizing complexes with the saucer-shaped open-form geometry as shown in Figure 3. Five structures with different stewpan-shaped closed-form geometry were extracted from trajectories of MD simulations of 40 ps at 298 K. MM2 calculations were performed to optimize the cyclen **1**−Na^+ complex.

Fluorescence and CD Measurements. Fluorescence experiments were carried out in a 20% ethanol aqueous solution (Bis-Tris-HCl buffer solution: 6.3×10^{-3} M; pH = 7.2). The initial concentrations of self-aggregate and guest dansylamino acid anions are shown in each Table and Figure. The fluorescence spectra of the dansylamino acid anions were recorded upon excitation at 327 nm. CD experiments were similarly performed.

Langmuir-Type Binding Studies. Dansylamino acid (1.0×10^{-6}−4.0×10^{-5} mol/L) was added to an aqueous solution of cyclen **1** − NaCl complex aggregate (3.0×10^{-5} mol/L). The fluorescence spectral changes of each dansylamino acid were observed by excitation at 327 nm in H_2O/EtOH (80/20, pH = 7.2) and analyzed based on the equation described above. The obtained Langmuir-type plots were employed to estimate the *K* values listed in Table 1 (see Figure S2 in the Supporting Information).

Liquid−Liquid Extraction. Determination of distribution percentage for each dansylamino acid was determined by adding a $CHCl_3$ solution of the cyclen **1**−NaCl complex (4.0×10^{-4} mmol in 2.0 mL) to an aqueous solution of racemic dansylamino acid (8.0×10^{-4} mmol in 2.0 mL, pH was adjusted to be 12.2 with NaOH). After the mixture had been stirred for 2 h, the concentration of the dansylamino acid in the aqueous phase was determined by UV spectroscopic method (monitored at 320 nm) and its enantiomeric excess % was determined by chiral HPLC method (SUMICHIRAL OA-3200, eluted with 0.01 mol/L $CH_3CO_2NH_4$/CH_3OH). We confirmed that negligible amounts of dansylamino acids were distributed into the $CHCl_3$ phase in the absence of cyclen **1**−NaCl complex (<1%).

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Supporting Information Available: CD spectral changes of cyclen **2**−NaCl complex and Langmuir-type analysis data for inclusion of dansylamino acids. This material is available free of charge via the Internet at http://pubs.acs.org.

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