

Measurement of Inclusion Complex Formation between Cyclophane and Biological Relevant Amino Acids Using Electrospray Ionization, Cold-Spray Ionization and Fast Atom Bombardment Mass Spectrometry

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The investigation of the host–guest complex formations between cyclophane (TGDMAP) (**1**) as a host and L-acidic amino acids such as L-glutamic acid (Glu) and L-aspartic acid (Asp) as guests was carried out using fast atom bombardment (FAB), electrospray ionization (ESI) and cold-spray ionization (CSI) mass spectrometry (MS). The stability constant (K_s) values obtained by the three different MS methods almost agreed. However, the complex ion peaks of a novel cyclophane (CPCn) (**2**) with Glu and Asp were not observed in FAB-MS. Then, these host–guest complex formations by use of CSI-MS and ESI-MS was examined, as the results, these complex ion peaks were observed clearly and the measurement values by the two MS methods are mostly in agreement. It was concluded that ESI-MS and CSI-MS are available for the determination of K_s value as well as FAB-MS.

Key words cyclophane; glutamic acid; aspartic acid; electrospray ionization (ESI)-MS; cold-spray ionization (CSI)-MS; FAB-MS

¹H-NMR measurement is widely used for determination of host–guest complex formation, however, the method cannot be used for measuring confirmation of host–guest complex formation in case either the peaks of guest and host overlap each other or these signals show broad. Recently, it reported that mass spectrometry can be used to detect noncovalent association complexes.^{1–4)}

We reported that FAB-MS is useful method as well as ¹H-NMR method not only for confirmation of TGDMAP (**1**) and biological relevant phosphates such as nucleotide complex geometry but also for determination of stability constant (K_s) values of the complexes because the K_s value calculated by FAB-MS (100 M⁻¹ for UMP) was most similar to the K_s value calculated by ¹H-NMR spectrometry (110 M⁻¹ for UMP).^{5–7)} These results show that the binding stability in aqueous solution is preserved in the gas phase.

In this report, investigation on the complex formation of **1** with L-glutamic acid (Glu) and L-aspartic acid (Asp) as guests which play an important role in receptor-signaling in

the brain was made by FAB-MS, cold-spray ionization mass spectrometry (CSI-MS), electrospray ionization mass spectrometry (ESI-MS). The K_s values of **1**·Glu and **1**·Asp were obtained by the three different MS methods almost agreed. On the other hand, a novel water-soluble cyclophane (**2**) as a host bearing 1,4,7,10-tetraazacyclododecane group on the alkyl bridge as branches of cyclophane having diphenylmethane skeleton in order to bind to the carboxyl group and also lead to remedy the solubility of cyclophane was designed. The synthesis of the novel cyclophane (**2**) is shown in Chart 1. Examination on the complex formation of **2** with Glu was performed by ¹H-NMR spectrometry in 0.1 M phosphate buffer solution (pD 6.8). However, the ¹H-NMR spectrum of Glu was complicated and the chemical shift changes of Glu in the presence of **2** were negligible. Therefore, it was impossible to calculate the K_s values between **2** and Glu and also the complex ion peaks of **2** with Glu and Asp were not observed in FAB-MS. For the purpose to solve the problem, we investigated the measurement of the K_s values by use of ESI-MS and CSI-MS. Especially ionization procedure of CSI-MS^{8,9)} which is milder than that of ESI-MS is expected to provide a powerful means to study noncovalent host–guest complexes such as hydrogen bond formation. Our primary purpose in this report is to judge if ESI-MS and CSI-MS as well as FAB-MS are useful for determination of the complex formations of **2**·L-acidic amino acids such as Glu and Asp when FAB-MS cannot be used for measuring confirmation of host–guest complex formation as described above. In fact, the complex ion peaks were observed clearly by using ESI-MS and CSI-MS instead of FAB-MS, and also the K_s values calculated by the two different mass spectrometry methods almost agreed.

Results and Discussion

In order to determine the complex formation and K_s value of **1** with Glu or Asp, three ionizing methods (FAB-MS, ESI-

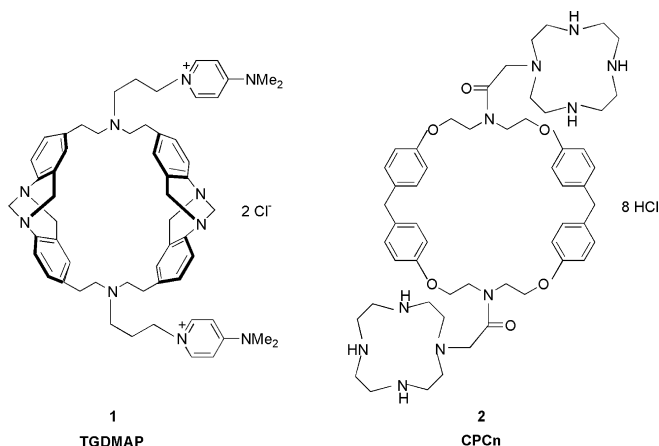


Fig. 1. Structure of Cyclophane Host

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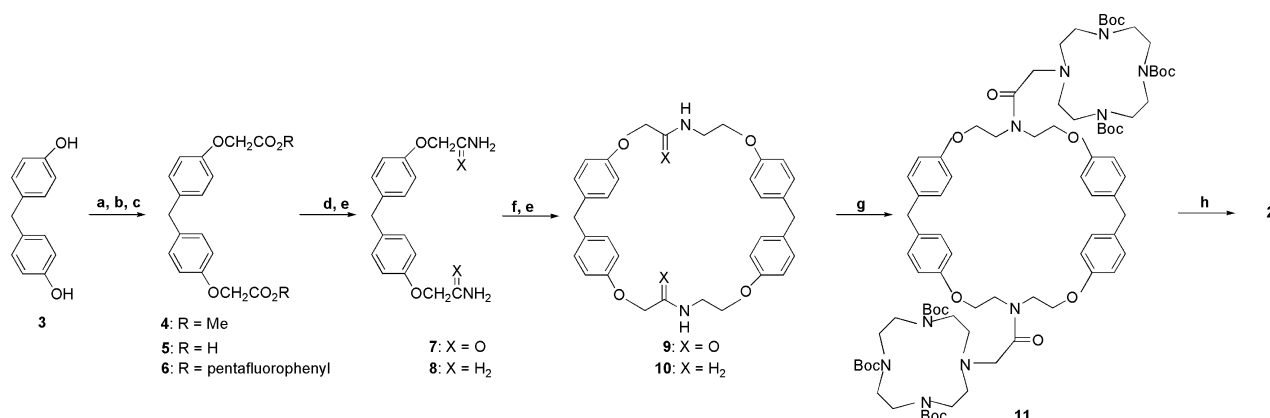


Chart 1

MS and CSI-MS) were investigated. FAB-MS spectrum for a 1 : 1 mixture of an equivalent amount (2.5 mmol) of **1** and Glu in 50% glycerol contained in 20 mmol of triethylammonium acetate (TEAA) solution (pH 7.0) showed the peak of the 1 : 1 complex at m/z 1054.7 $[(1-2\text{Cl})+(\text{Glu})-\text{H}]^+$ and molecular ion peaks of higher mass could not be detected (Fig. 2). Figure 3 shows ESI and CSI mass spectra obtained for an equivalent amount (0.1 mmol) of **1** and Glu in 2 mmol of TEAA solution (pH 7.0). These data suggest that **1** forms the 1 : 1 complex with Glu. The 1 : 1 stoichiometry for the complex between **1** and Glu was also confirmed by using Job's method^{10,11)} of continuous variations using FAB-MS. The Job's method gave a maximum at 0.5, indicative of a 1 : 1 stoichiometry (Fig. 4). The K_s value of the complex was determined using the absolute intensity of the complex on the basis of double reciprocal plots according to the previous report.⁷⁾ The plots gave excellent linearity with a correlation coefficient ($r=0.997$) (Fig. 5). In case of using Asp as a guest, the similar results was obtained and the 1 : 1 complex ion peak $[(1-2\text{Cl})+(\text{Asp})-\text{H}]^+$ of **1** with Asp was shown at m/z 1040.7 in FAB-MS and at m/z 1040.8 in ESI-MS and CSI-MS, respectively (data not shown). The K_s values obtained by FAB-MS (70 M^{-1} for Glu and 60 M^{-1} for Asp) measurement method were mostly identical to the results of CSI-MS (105 M^{-1} for Glu and 80 M^{-1} for Asp) and ESI-MS (120 M^{-1} for Glu and 75 M^{-1} for Asp) measurement methods in spite of using different ionization, temperature and solvent. The binding constants (K_s) are summarized in Table 1.

However, the complex ion peaks of between a novel cyclophane (CPCn) (**2**) and Glu and Asp were not observed in FAB-MS measurement method. Then, the measurement of these host-guest complex formations by use of CSI-MS and ESI-MS was examined. An equivalent amount (0.2 mmol) of **2**, Glu and Asp were dissolved in 2 mmol of TEAA solution (pH 7.0) and then were mixed at a rate of 1 : 1. The ion peaks corresponding to $[\mathbf{2}+\text{Glu}+\text{H}]^+$ was observed at m/z 1110.9 in positive mode ESI-MS and at m/z 1110.7 in CSI-MS (Fig. 6) and $[\mathbf{2}+\text{Asp}+\text{H}]^+$ was observed at m/z 1096.8 (data not shown). The ESI-MS measurement of host-guest complex ion intensity advantaged over the CSI-MS, therefore the examination of the K_s value of the **2**·Glu and **2**·Asp complexes, and the Job's plots were performed using ESI-MS.

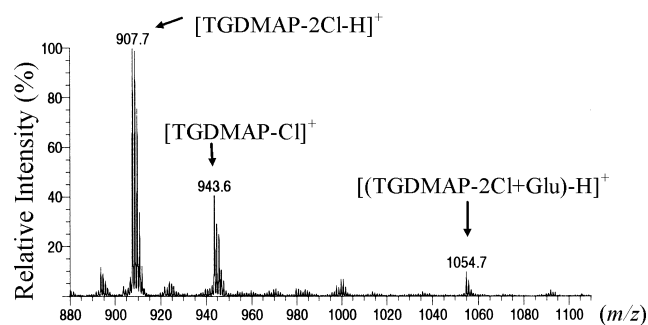


Fig. 2. FAB Mass Spectrum for the Inclusion Complex Formation between TGDMAP and Glu in a Glycerol Matrix

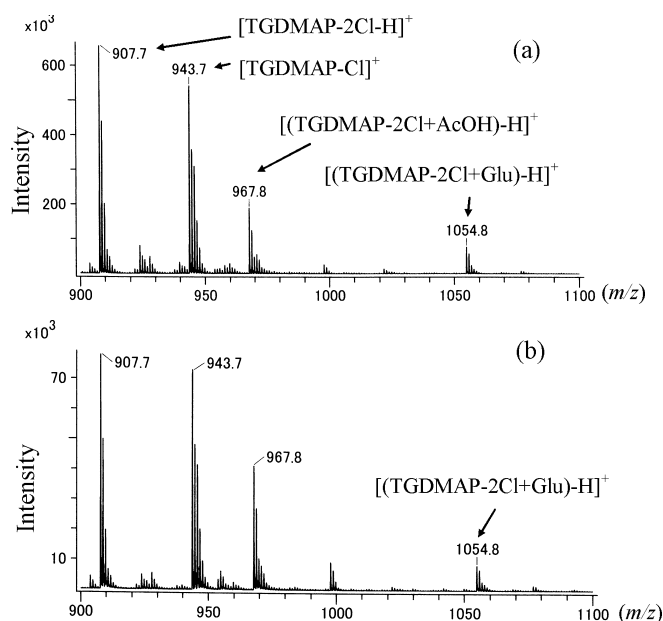


Fig. 3. Comparison of (a) ESI and (b) CSI Mass Spectra for the Inclusion Complex Formation between TGDMAP and Glu in a TEAA Solution

The Job's methods gave a maximum at 0.5, indicative of a 1 : 1 stoichiometry monitored the absolute intensity at m/z 1110.9 for Glu and at m/z 1096.8 for Asp. The good correlation coefficients were obtained in the double reciprocal plots

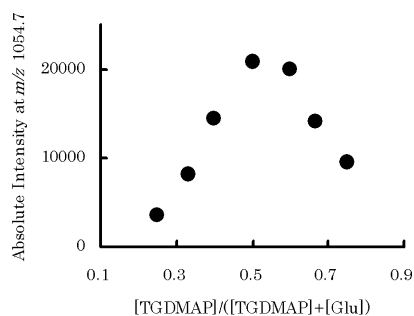


Fig. 4. Job's Plots for the Inclusion Complex Formation between TGDMAP and Glu

The absolute intensity was measured at m/z 1054.7 (host-guest complex ion). The total concentration (TGDMAP+Glu) was 5 mmol.

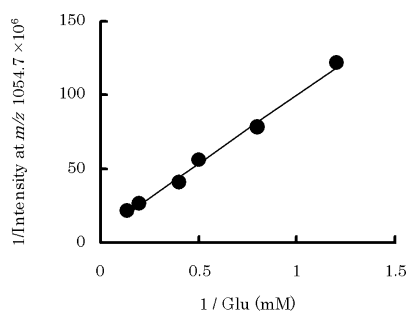


Fig. 5. The Double Reciprocal Plots for the Inclusion Complex Formation between TGDMAP and Glu

The absolute intensity was measured at m/z 1054.7 (host-guest complex ion). The final concentration of TGDMAP was 2.5 mmol and Glu concentration ranges from 0.83 mmol to 7.5 mmol.

Table 1. The K_s (M^{-1}) Values of Guests in the Presence of TGDMAP or CPCn by FAB, ESI and CSI-MS

Method	TGDMAP		CPCn	
	Glu	Asp	Glu	Asp
FAB-MS	70	60	N.D.	N.D.
ESI-MS	120	75	340	115
CSI-MS	105	80	N.T.	N.T.

N.D.: not detected. N.T.: not tested.

for the inclusion complex formation for K_s measurements of **2**·Glu and **2**·Asp monitored the absolute intensity of the host-guest complex ion. As shown in Table 1, the K_s value of Glu and Asp were obtained $340 M^{-1}$ and $115 M^{-1}$, respectively. In the case of using **1**, the K_s values of the **2**·Glu and **2**·Asp in ESI-MS were obtained $120 M^{-1}$ and $75 M^{-1}$, respectively.

In conclusion, a novel cyclophane (**2**) works as host that form complexes with Glu and Asp as guests and the ability of inclusion complex formation of **2** has stronger than **1**. The ESI-MS and CSI-MS methods were found to be a convenient way to evaluate the stability constant of host-guest complexes because the ionizing methods of ESI-MS and CSI-MS, which are milder than that of FAB-MS, are useful for confirmation of host-guest complex which cannot be detected by FAB-MS. In this experiments, although the determined binding constants did not differ significantly between CSI-MS and ESI-MS, CSI-MS is milder ionization than that of ESI-MS and FAB-MS, could apply for determination of a

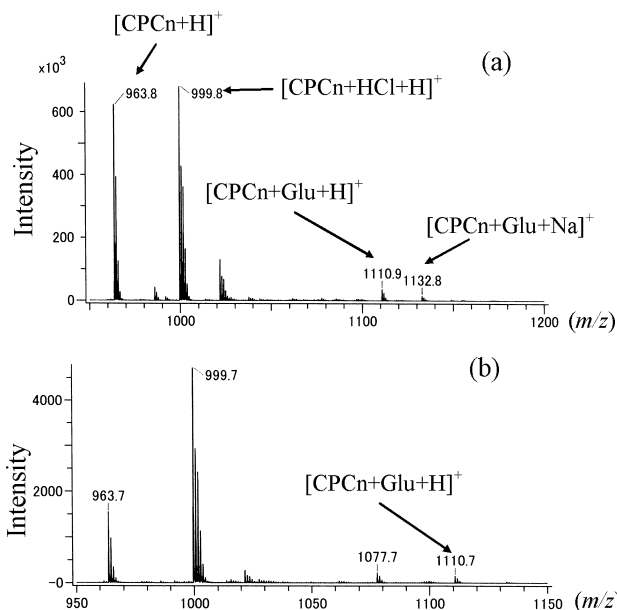


Fig. 6. Comparison of (a) ESI and (b) CSI Mass Spectra for the Inclusion Complex Formation between CPCn and Glu

weak noncovalent inclusion complexes in host-guest chemistry.

Experimental

Apparatus Melting points were determined using a Yanagimoto Melting point Apparatus Yanaco MP and were uncorrected. 1H -NMR was recorded on a JEOL JNM-LA400 spectrometer containing tetramethylsilane as standard. FAB-MS measurement was performed using a JEOL JMS-GC-mate instrument equipped with double-focusing mass analyzer. ESI-MS and CSI-MS measurements were performed using a JEOL JMS-T100CS instrument equipped with time-of-flight mass analyzer. Elemental analyses were performed on a Perkin Elmer 2400 II CHN analyzer.

Reagents The following reagents were commercially available and used without further purification: Borane-methyl sulfide complex ($BH_3 \cdot DMS$), Sigma-Aldrich Chemical; 4,4'-dihydroxydiphenylmethane, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, N,N' -dicyclohexylcarbodiimide, pentafluorophenol, guest compounds (L-glutamic acid and L-aspartic acid potassium salt) triethylammonium acetate (TEAA) solution (pH 7.0); Tokyo Kasei Kogyo. All organic solvents were purchased from Wako Pure Chemical.

4,4'-Bis(methoxycarbonylmethoxy)diphenylmethane (4) A mixture of 4,4'-dihydroxydiphenylmethane (**3**) (1.0 g, 5 mmol), methyl bromoacetate (1.53 g, 10 mmol) and K_2CO_3 (1.38 g, 10 mmol) in DMF (20 ml) was stirred at room temperature. After 24 h, the reaction mixture was filtered. The filtrate was extracted with EtOAc (50 ml $\times$ 3), washed with brine and dried over $MgSO_4$. After removal of the solvent under reduced pressure, the residue was purified by column chromatography on silica gel with EtOAc:CHCl₃ (1:9) as an eluent to give 1.6 g, 93% as a colorless solid. An analytical sample was obtained by recrystallizing this material from EtOAc-hexane, colorless needles. mp 51–52 °C. 1H -NMR ($CDCl_3$) δ =3.80 (6H, s), 3.85 (2H, s), 4.60 (4H, s), 6.82 (4H, d, J =8.8 Hz), 7.08 (4H, d, J =8.8 Hz). EI-MS m/z : 344 [M^+]. HR-EI-MS m/z : 344.1256 (Calcd for $C_{19}H_{20}O_6$: 344.1259). Anal. Calcd for $C_{19}H_{20}O_6$: C, 66.27; H, 5.85. Found: C, 66.54; H, 5.91.

4,4'-Bis(carboxymethoxy)diphenylmethane (5) A mixture of **4** (1.19 g, 3.46 mmol) and 5N KOH/MeOH (4 ml) in MeOH (40 ml) was refluxed 2 h. After removal of the solvent under reduced pressure, the residue was dissolved in 100 ml of H_2O . The solution was extracted with EtOAc (100 ml). The aqueous solution was acidified to pH=1 with 10% HCl and extracted with EtOAc (300 ml). The EtOAc layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to give a colorless powder (1.09 g, 100%). An analytical sample was obtained by recrystallizing this material from EtOAc-hexane, colorless needles. mp 199–200 °C. 1H -NMR ($DMSO-d_6$) δ =3.79 (2H, s), 4.59 (4H, s), 6.80 (4H, d, J =8.8 Hz), 7.10 (4H, d, J =8.8 Hz), 12.90 (2H, s). EI-MS m/z : 316 [M^+]. HR-EI-MS m/z : 316.0944 (Calcd for $C_{17}H_{16}O_6$: 316.0946). Anal. Calcd for

$C_{17}H_{16}O_6$: C, 64.55; H, 5.10. Found: C, 64.60; H, 5.11.

4,4'-Bis(pentafluorophenoxy)carbonylmethoxy)diphenylmethane (6) A mixture of **5** (2.95 g, 9.3 mmol), pentafluorophenol (3.46 g, 18.8 mmol) and *N,N'*-dicyclohexylcarbodiimide (3.88 g, 18.8 mmol) in THF (100 ml) was stirred at room temperature for 24 h. The mixture was filtered, and the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with CH_2Cl_2 as an eluent to give 5.56 g, 92% as a colorless solid. An analytical sample was obtained by recrystallizing this material from EtOAc–hexane, colorless needles. mp 135–136 °C. 1H -NMR ($CDCl_3$) δ =3.90 (2H, s), 4.97 (4H, s), 6.89 (4H, d, J =8.4 Hz), 7.13 (4H, d, J =8.4 Hz). FAB-MS m/z : 648 [M^+]. FAB-HR-MS m/z : 648.0628 (Calcd for $C_{29}H_{14}F_{10}O_6$: 648.0630). Anal. Calcd for $C_{29}H_{14}F_{10}O_6$: C, 53.72; H, 2.18. Found: C, 53.73; H, 2.14.

4,4'-Bis(carbamoylmethoxy)diphenylmethane (7) To a solution of **6** (4.0 g, 6.17 mmol) in THF (30 ml) was added 25% NH_4OH (12 ml) at room temperature. After stirring for 12 h, sat. $NaHCO_3$ (200 ml) was added to the reaction mixture. The precipitate was collected by filtration, washed with H_2O , EtOH and Et_2O and dried under vacuum at 60 °C to give 1.9 g, 98% as a colorless powder which was used in the next step without further purification. mp 233–234 °C. 1H -NMR ($DMSO-d_6$) δ =3.80 (2H, s), 4.36 (4H, s), 6.85 (4H, d, J =8.5 Hz), 7.11 (4H, d, J =8.8 Hz), 7.32 (2H, s), 7.43 (2H, s). FAB-MS m/z : 315 [$M+H$] $^+$. HR-FAB-MS m/z : 315.1346 (Calcd for $C_{17}H_{19}N_2O_4$: 315.1344). Anal. Calcd for $C_{17}H_{18}N_2O_4$: C, 64.96; H, 5.77; N, 8.91. Found: C, 64.74; H, 5.67; N, 8.69.

4,4'-Bis(2-aminoethoxy)diphenylmethane (8) A mixture of **7** (314 mg, 1 mmol) and $BH_3 \cdot DMS$ (1.16 ml, 12 mmol) in THF (12 ml) was stirred for 24 h at 80 °C under N_2 atmosphere, then was cooled to room temperature. Six milliliters of 0.7 M hydrogen chloride–MeOH solution was added, and the mixture was refluxed for 0.5 h, and evaporated under reduced pressure. The residue was basified with 25% NH_4OH . The mixture was extracted with CH_2Cl_2 , washed with brine and dried over Na_2SO_4 . Removal of the solvent under reduced pressure afforded a pale yellow oil, which was purified by column chromatography on silica gel with $CHCl_3$:MeOH:25% NH_4OH (100:40:4) as an eluent to give 243 mg, 85% as a colorless amorphous powder which was used in the next step without further purification. 1H -NMR (CD_3OD) δ =2.99 (4H, t, J =5.6 Hz), 3.82 (2H, s), 3.98 (4H, t, J =5.6 Hz), 6.84 (4H, d, J =8.4 Hz), 7.07 (4H, d, J =8.4 Hz). FAB-MS m/z : 287 [$M+H$] $^+$. FAB-HR-MS m/z : 287.1757 (Calcd for $C_{17}H_{23}N_2O_2$: 287.1759). Anal. Calcd for $C_{17}H_{22}N_2O_2$: C, 71.30; H, 7.74; N, 9.78. Found: C, 71.51; H, 7.78; N, 9.52.

9,27-Dioxo-10,26-diaza-7,13,23,29-tetraoxa[7.1.7.1]paracyclophane (9) A mixture of **6** (973 mg, 1.5 mmol), **8** (430 mg, 1.5 mmol) and TEA (2.1 ml, 15 mmol) in CH_2Cl_2 (300 ml) was stirred at 60 °C under N_2 atmosphere. After 24 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with EtOAc:MeOH (9:1) as an eluent to give 590 mg, 69% as a colorless solid. An analytical sample was obtained by recrystallizing this material from EtOAc–hexane, colorless fine needles. mp 195–196 °C. 1H -NMR ($CDCl_3$) δ =3.53 (2H, s), 3.70–3.734 (4H, m), 3.80 (2H, s), 3.92 (4H, t, J =5.2 Hz), 6.63 (4H, d, J =8.8 Hz), 6.71 (4H, d, J =8.8 Hz), 6.87 (4H, d, J =8.8 Hz), 6.94 (2H, t, J =5.2 Hz), 7.05 (4H, d, J =8.8 Hz). FAB-MS m/z : 567 [$M+H$] $^+$. FAB-HR-MS m/z : 567.2496 (Calcd for $C_{34}H_{35}N_2O_6$: 567.2495). Anal. Calcd for $C_{34}H_{34}N_2O_6 \cdot 1/3 H_2O$: C, 71.30; H, 6.10; N, 4.90. Found: C, 71.36; H, 6.11; N, 4.83.

10,26-Diaza-7,13,23,29-tetraoxa[7.1.7.1]paracyclophane (10) A mixture of **9** (610 mg, 1.07 mmol) in THF (13 ml) was stirred under N_2 atmosphere. $BH_3 \cdot DMS$ (1.3 ml, 1.34 mmol) was added dropwise. The reaction mixture was stirred for 24 h at 80 °C, and then 0.7 ml of 0.7 M hydrogen chloride–MeOH solution was added. After 0.5 h, the reaction mixture was evaporated under reduced pressure. The residue was basified with excess 25% NH_4OH . The mixture was extracted with CH_2Cl_2 (100 ml), washed with brine and dried over Na_2SO_4 . Removal of the solvent, the residue was purified by column chromatography on silica gel with $CHCl_3$:MeOH:25% NH_4OH (100:10:1) as an eluent to give 480 mg, 83% as a colorless powder. An analytical sample was obtained by recrystallizing this material from CH_2Cl_2 –hexane, colorless fine needles. mp 135–136 °C. 1H -NMR ($CDCl_3$) δ =3.00 (8H, t, J =4.8 Hz), 3.80 (4H, s), 4.06 (8H, t, J =4.8 Hz), 6.75 (8H, d, J =8.4 Hz), 7.00 (8H, d, J =8.4 Hz). FAB-MS m/z : 539 [$M+H$] $^+$. FAB-HR-MS m/z : 539.2908 (Calcd for $C_{34}H_{39}N_2O_4$: 539.2909). Anal. Calcd for $C_{34}H_{38}N_2O_4$: C, 75.81; H, 7.11; N, 5.20. Found: C, 75.82; H, 7.39; N, 5.20.

***N,N'*-Bis[tris(4,7,10-*tert*-butoxycarbonyl)-1,4,7,10-tetraazacyclododecane-1-ylacetyl]-10,26-diaza-7,13,23,29-tetraoxa[7.1.7.1]-paracyclophane (11)** A mixture of **10** (54 mg, 0.1 mmol), 1-carboxymethyl-4,7,10-tris(*tert*-butoxycarbonyl)-1,4,7,10-tetraazacyclododecane¹²⁾ (106 mg, 0.2

mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (45 mg, 0.23 mmol) in CH_2Cl_2 (5 ml) was stirred at r.t. for 18 h under N_2 atmosphere. The reaction mixture was diluted with CH_2Cl_2 (10 ml), washed with 2 N NaOH and dried over Na_2SO_4 . The solvent was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with EtOAc:hexane (3:1) and EtOAc as eluent to give a colorless amorphous powder (115 mg, 74%). mp 151–153 °C. 1H -NMR ($CDCl_3$) δ =1.43 (36H, s), 1.46 (18H, s), 3.04 (8H, br), 3.22–3.42 (16H, br), 3.45–3.62 (8H, br), 3.72–3.82 (16H, m), 4.05–4.12 (4H, m), 4.17–4.22 (4H, m), 6.62 (2H, d, J =8.5 Hz), 6.66 (2H, d, J =8.5 Hz), 6.70 (2H, d, J =8.5 Hz), 6.73 (2H, d, J =8.5 Hz), 6.94–7.00 (8H, m). FAB-MS m/z : 1564 [$M+H$] $^+$. FAB-HR-MS m/z : 1563.9335 (Calcd for $C_{84}H_{127}N_{10}O_{18}$: 1563.9329). Anal. Calcd for $C_{84}H_{126}N_{10}O_{18}$: C, 64.51; H, 8.12; N, 8.96. Found: C, 64.40; H, 8.29; N, 8.81.

***N,N'*-Bis(1,4,7,10-tetraazacyclododecane-1-ylacetyl)-10,26-diaza-7,13,23,29-tetraoxa[7.1.7.1]paracyclophane Octahydrochloride (2)** Cyclophane (**11**) (100 mg, 0.064 mmol) was dissolved in THF (1 ml), to which conc. HCl (0.2 ml) was added. After the reaction mixture was stirred at r.t. for 12 h. The reaction mixture was diluted with CH_2Cl_2 (10 ml), washed with 2 N NaOH and dried over Na_2SO_4 . The solvent was diluted with THF (10 ml) and the precipitate was collected by filtration, washed with THF, and dried to give a white powder (80 mg, 100%). mp 280–282 °C (decomp). 1H -NMR (D_2O 0.1 M phosphate buffer (pD 6.8)) δ =2.76–2.89 (32H, m), 3.41–3.53 (16H, m), 3.04 (8H, br), 3.76 (4H, br), 3.84–3.88 (4H, m), 6.18 (2H, d, J =8.3 Hz), 6.31 (2H, d, J =8.3 Hz), 6.36 (2H, d, J =8.3 Hz), 6.40 (2H, d, J =8.3 Hz), 6.63–6.69 (8H, m). FAB-MS 963 [$M-8HCl+H$] $^+$. FAB-HR-MS m/z : 963.6206 (Calcd for $C_{54}H_{79}N_{10}O_6$: 963.6183). Anal. Calcd for $C_{54}H_{86}Cl_8N_{10}O_6$: C, 51.68; H, 6.91; N, 11.16. Found: C, 51.75; H, 7.08; N, 11.35.

FAB-MS Measurements for the Complex Formation Measurement conditions were as follows: equimolar solutions (5 mmol) of host and guest were prepared in 20 mmol of triethylammonium acetate (TEAA) solution containing 50% glycerol (pH 7.0). An equal volume (5 μ l) of the host and guest solution was mixed and then 1 μ l of the mixture was loaded. Xe was employed as a fast atom bombardment gas. Scanning was performed from m/z 100 to 2000 in 10 s and several scans were summed to obtain the final spectrum. All of FAB mass spectra of the mixture were employed positive-ion mode.

ESI-MS Measurements In the case of using of TGDMA (1) as host, ESI-MS measurement conditions and sample preparation procedures were as follows: the probe heater temperature was set at 250 °C and needle, ring and orifice voltages were held at 2000, 20 and 130, in positive-ion mode, respectively. On the other hand, in the case of using of CPCn (2) as host, the probe heater temperature was set at 250 °C and needle, ring and orifice voltages were held at 1800, 22 and 33, in positive-ion mode, respectively. Equimolar solutions (0.2 mmol) of host and guest were prepared in 2 mmol of TEAA solution (pH 7.0). An equal volume of the host and guest solution was mixed and then the sample solution was introduced into the spectrometer at a flow rate of 1.5 ml/h using a syringe pump.

CSI-MS Measurements An orthogonal cold-spray apparatus equipped with a spray temperature control system using liquid N_2 was used and the spray temperature was set at 4 °C. In the case of using of TGDMA (1) as host, needle, ring and orifice voltages were held at 1800, 18 and 140, in positive-ion mode, respectively. In the case of using of CPCn (2) as host, needle, ring and orifice voltages were held at 2000, 18 and 33, in positive-ion mode, respectively. Sample preparation procedures and flow rate followed in ESI-MS measurement.

Determination of K_s Values of the Complexes by FAB-MS, ESI-MS and CSI-MS The K_s values of the host–guest complexes were determined using the absolute intensities of host–guest complexes on the basis of the double reciprocal plots. For FAB-MS measurement, the stock solutions of the host and all of the guests were prepared in 20 mmol of TEAA solution (pH 7.0) containing 50% glycerol. The concentration of the host was 2.5 mmol, while those of the guest ranges from 0.83 mmol to 7.5 mmol (6 points). For ESI-MS and CSI-MS measurements, the stock solutions of the host and all of the guests were prepared in 2 mmol of TEAA solution (pH 7.0). The concentration of the host was 0.1 mmol, while guest ranges from 0.05 mmol to 0.3 mmol (5 points). The double reciprocal plots ($1/\text{absolute intensity (I)}$ vs. $1/[G]$) gave excellent linearity with a correlation coefficient $r \geq 0.997$ (in FAB-MS measurement) and 0.989 (in ESI-MS and CSI-MS measurements).

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