Chromatographic Separation of Aromatic Guest Compounds by a Gel-Immobilized Water-Soluble Cyclophane

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An agarose gel upon which a water-soluble cyclophane having three quaternary nitrogens was immobilized, Gel-QCP (3), was synthesized and its properties as a chromatographic stationary phase were studied. The affinity of Gel-QCP for several neutral and anionic aromatic guests was broadly consistent with that of the parent cyclophane (2). This suggests that the guest separation is attributable to the formation of inclusion complexes between the cyclophane moiety of Gel-QCP and the guests. Non-specific electrostatic interaction also influences anionic guests, but this effect is reduced by using an aqueous solution of NaCl as an eluent.

Keywords macrocycle; water-soluble cyclophane; hydrophobic cavity; immobilization; agarose gel; inclusion chromatography; separation; aromatic guest

Water-soluble cyclophanes constitute a promising class of totally synthetic hosts having hydrophobic cavities to capture organic guests in water.[2] We have previously reported that a series of paracyclophanes having diphenylmethane skeletons, represented by CP44 (**1**), form inclusion complexes with neutral and anionic aromatic guest molecules in acidic water (pH < 2).[3a] The guest inclusion by these hosts was found to occur with particular geometry and remarkable selectivity, based on host–guest recognition of steric structure and charge.[3b,c] It has also been shown that the N-quaternized derivative (QCP44, **2**) of **1** retains binding ability and selectivity comparable to those of **1**.[3d,e] Host **2** is a versatile modification of **1**, since it is highly soluble in water over the whole pH range.

Application of host molecules to chromatography provides an efficient means for separation based on inclusion of the guest molecule in the well-defined cavity of the host. By host–guest inclusion chromatography based on such a principle, separation of guest molecules having similar structures that are difficult to separate by ordinary chromatographic methods would be possible, as exemplified by inclusion chromatography using cyclodextrin-immobilized silica gel columns.[4] Water-soluble cyclophanes are another class of host compounds having well-defined hydrophobic cavities, and are totally synthetic hosts which may be modifiable as required to include a wide range of organic guests. Accordingly, the chromatographic application of this class of host compounds is expected to afford a versatile system for separation of organic guests with similar structures.[5] In this paper we describe the synthesis of a new type of gel upon which water-soluble cyclophane having diphenylmethane units is immobilized (**3**, Gel-QCP), and its application to the separation of aromatic guest compounds.

Host **4** having one amide nitrogen and three quaternary ammonium nitrogens was found essentially to retain the ability of cyclophane **2** having four quaternary ammonium nitrogens to form inclusion complexes with aromatic guests.[6] The tendencies of guest selectivity were also retained. Hence, Gel-QCP having three quaternary ammonium nitrogens, like **4**, was also expected to have the ability to form inclusion complexes with selectivity. As a solid support we chose ECH Sepharose 4B, which is an agarose gel bearing carboxyl group through a spacer, and which in itself scarcely shows non-specific adsorption of solutes.

Results and Discussion

Synthesis Gel-QCP (**3**) was synthesized from compound **5**[6b] as shown in Chart 2. Compound **6**, having one deprotected nitrogen, was prepared by partial hydrolysis of **5**, using a limited amount of base to avoid hydrolysis of more than one trifluoroacetyl group. Thus, treatment of **5** with 0.5 eq of KOH in tetrahydrofuran (THF)–MeOH gave a mixture containing mostly **5** and **6**.[6b] Since these were difficult to separate from each other, the mixture was reacted directly with di-*tert*-butyl dicarbonate (Boc_2O). The resulting mixture of **5** and **7** was treated with benzene, and undissolved **5** was filtered off. Retreatment with benzene, followed by silica gel column chromatography, gave pure **7** in 28% yield from **5**. Hydrolysis of the trifluoroacetyl groups of **7** afforded the triamine **8**. The exhaustive

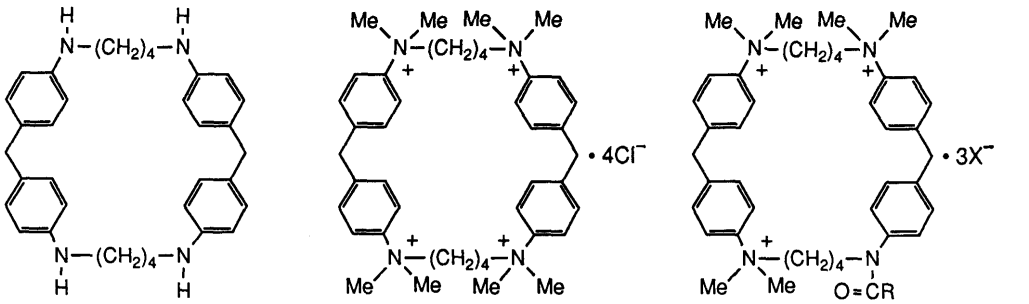
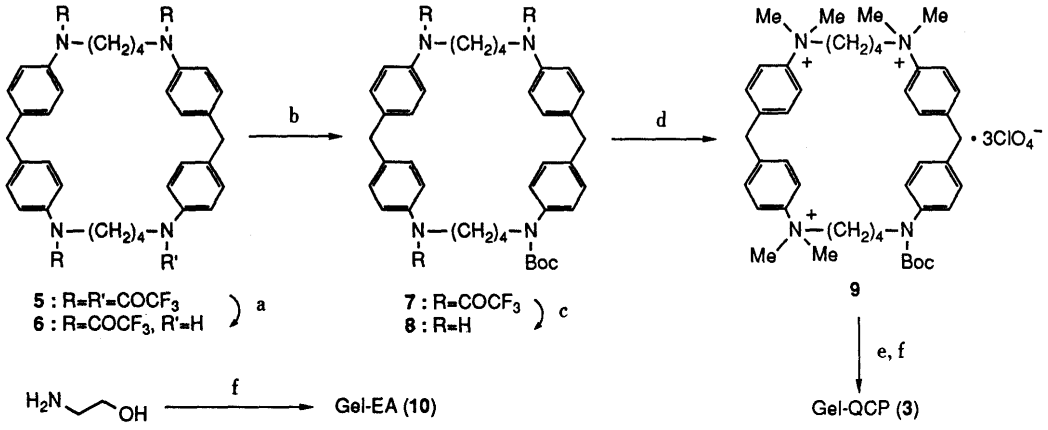


Chart 1



a) KOH, MeOH-THF b) Boc_2O, CH_2Cl_2 (28%, 2 steps) c) KOH, MeOH-THF, (94%)
d) CH_3I, n-Bu_3N, DMF-H_2O, (34%) e) HCl/H_2O-MeOH f) ECH Sepharose 4B, EDC, pH4.4

Chart 2

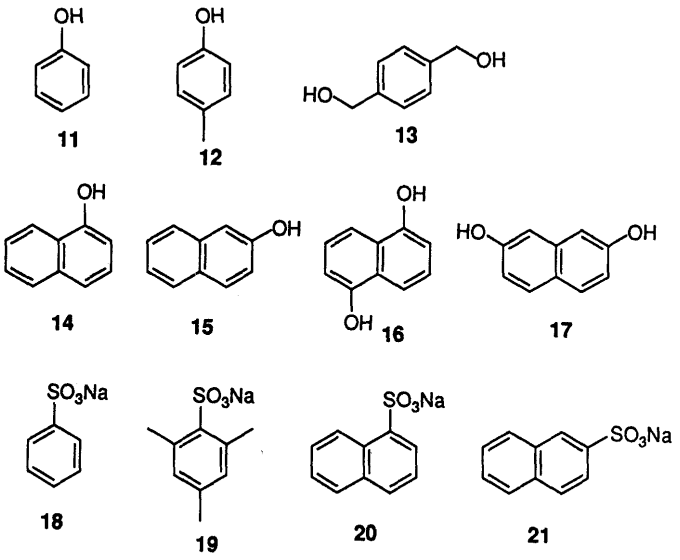


Chart 3

N-quaternization of **8** was carried out by the procedure of Sommer *et al.* (methyl iodide, tri-*n*-butylamine).[7] The quaternized compound (iodide) was converted to the corresponding perchlorate by ion exchange column chromatography using Dowex 1 (ClO_4 form) and purified by recrystallization from H_2O. This quaternized compound (**9**) was deprotected with HCl and then coupled with ECH Sepharose 4B in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC; water-soluble carbodiimide). Based on the microanalysis of nitrogen of the resulting gel (Gel-QCP) and ECH Sepharose 4B, about 66% of the carboxyl group attached to ECH Sepharose 4B was found to be coupled with the cyclophane.

For control experiments the gel (**10**, Gel-EA) in which ethanolamine was coupled with ECH Sepharose 4B was prepared.

Separation of Aromatic Guests We investigated the chromatographic behavior of neutral and anionic aromatic guests (**11**—**21**, Chart 3). The chromatographic separation experiments using Gel-QCP, Gel-EA and ECH Sepharose 4B columns were carried out under the following conditions: column size, 10 mm × 68 or 72 mm; flow rate, 0.62 ml/min; temperature, 23 °C; detection, ultraviolet (UV) at 254 nm.

Table I. Retention Times of Aromatic Guests on Gel-QCP, Gel-EA and ECH Sepharose 4B Columns[a]

Guest	Eluent	Retention time (min)		
		Gel-QCP	Gel-EA	ECH Sepharose 4B
11	H_2O	12	11	
12	H_2O	12	10	
13	H_2O	12	10	
14	H_2O	18	11	
15	H_2O	22	11	
16	H_2O	19	12	
17	H_2O	25	12	
11[b]	1 M NaCl in H_2O	12	12	
14[b]	1 M NaCl in H_2O	21	12	
15[b]	1 M NaCl in H_2O	25	12	
18[b]	0.01 M NaCl in H_2O	34	15	15
19[b]	0.01 M NaCl in H_2O	47	15	15
20[b]	0.01 M NaCl in H_2O	59	19	15
21[b]	0.01 M NaCl in H_2O	>70	19	15

a) Column size, 10 mm × 72 mm unless otherwise noted; flow rate, 0.62 ml/min; temperature, 23 °C; detection, UV (254 nm). b) Column size, 10 mm × 68 mm.

Under these conditions the retention time of acetone was 10 min (eluent, H_2O) regardless of the gel used.

Retention times of the guests are shown in Table I. When water was used as an eluent for the neutral aromatic guests **11**—**17**, all of these neutral guests were retained more strongly (showed longer retention times) in Gel-QCP than in Gel-EA column. Furthermore, the naphthyl derivatives (**14**—**17**) were retained in Gel-QCP more strongly than the phenyl derivatives (**11**—**13**), and the β-substituted naphthalenes (**15**, **17**) were retained more strongly than the α-substituted naphthalenes (**14**, **16**). When Gel-EA was used, all of the neutral guests showed almost the same retention times.

The anionic aromatic guests **18**—**21** were not eluted from Gel-QCP within 2 h when water was used as an eluent. Therefore, an aqueous solution of NaCl was used. The retention times of the sulfonates (**18**—**21**) eluted with 0.01 M NaCl are shown in Table I. Of these anionic aromatic guests, the naphthyl derivatives (**20**, **21**) were retained in Gel-QCP more strongly than the phenyl derivatives (**18**, **19**), and the β-substituted naphthalene **21** was retained

TABLE II. Retention Times of **18** and **20** on Gel-QCP Column Using Different Eluents[a]

	Retention time (min)		
	1 M NaCl in H_2O	0.05 M NaCl in H_2O	0.05 M NaCl in MeOH–H_2O 1:1 (v/v)
18	12	16	14
20	14	23	14

a) Column size, 10 mm × 68 mm; flow rate, 0.62 ml/min; temperature, 23 °C; detection, UV (254 nm).

TABLE III. Stability Constants (K_s) of the Host–Guest Complexes between **2** and Aromatic Guests[a]

Guest	Solvent	K_s/M^{-1}	
12	D_2O	1.1×10^2	
17	D_2O	1.5×10^3	*b*)
17	1 M NaCl in D_2O	6.9×10^2	
18	D_2O	1.0×10^3	
19	D_2O	9.0×10^2	*b*)
20	D_2O	2.8×10^3	
21	D_2O	1.6×10^4	
21	1 M NaCl in D_2O	8.8×10^2	

a) K_s of the 1:1 complexes in water (D_2O) determined by ^{1}H-NMR at 28 ± 2 °C. *b*) Taken from reference 3*e*.

more strongly than the α-substituted naphthalene **20**. Gel-EA showed slight selectivity for the naphthalene guests, and ECH Sepharose 4B itself showed no selectivity.

The effect of the eluent used on the anionic guests **18** and **20** was examined as shown in Table II. The higher the concentration of NaCl, the shorter the retention times of the anionic guests were. When an aqueous-methanol solution of NaCl was used in place of the aqueous solution, the retention times were shortened and **18** and **20** were eluted from Gel-QCP with the same retention time.

Stability constants of the 1:1 inclusion complexes between host **2** (QCP44) and the aromatic guests were determined by proton-nuclear magnetic resonance (^{1}H-NMR) (Table III). The following properties were observed as to the guest binding by this host. Host **2** forms more stable complexes (1) with the naphthyl derivatives than with the phenyl derivatives, (2) with the β-substituted naphthalenes than with the α-substituted naphthalenes, and (3) in D_2O than in 1 M NaCl, especially for a sulfonate guest.

The trends of the affinity of the Gel-QCP column to the guests are consistent with the complexation selectivities exhibited by host **2**. It is reasonable, therefore, to assume that the formation of inclusion complexes between the cyclophane moiety of Gel-QCP and the guests plays a major role in the chromatographic separation.

The formation of inclusion complexes by this type of cyclophane is based mainly on hydrophobic interaction as well as electrostatic interaction.[3c] The order of the retention times with the H_2O eluent (**20**, **21** > **18**, **19**; **21** > **20**; Table I) as well as the markedly stronger retention of guest **20** (but not of the less hydrophobic guest **18**) with the H_2O eluent than the H_2O–MeOH eluent of the same NaCl concentration clearly shows that hydrophobic interaction is the major factor in the guest separation by the Gel-QCP column.

However, non-specific electrostatic interaction works especially well for the anionic guests **18**—**21**. In the case of using water as an eluent, the marked increase in the retention time for these anionic guests as compared with the neutral guests can be reasonably attributed to the electrostatic interaction between the ammonium groups of the cyclophane moiety and the sulfonate group of the guests. In turn, the decrease in retention time of the anionic guests with increasing concentration of NaCl in the eluent is probably due to a decreased host–guest interaction as a consequence of competition between the guest anions and chloride ions; that is supported by the much weaker complexation between **2** and **21** in 1 M NaCl/D_2O than in D_2O (Table III).[8] The decreased retention time and the failure of separation when using the H_2O–MeOH eluent in place of H_2O eluent at the same NaCl concentration (Table II) is attributed to the weakened hydrophobic interaction, resulting in an increase in the relative importance of the non-specific electrostatic interaction as the controlling factor.

Some other factors must be taken into account for the interaction between Gel-QCP and the guests. In the case of using 1 M NaCl as an eluent, the retention times of the neutral guests **11**, **14**, and **15** were somewhat longer than in the case of using water (Table I). This result is in contrast with the case of the anionic guests (*vide supra*) and cannot be explained by the decrease in host–guest complexation in the presence of NaCl (Table III). Therefore, this increased retention time may be attributable to an increased adsorption of the neutral guests on the agarose gel.

Although guests **18** and **19** showed similar stability constants for the complexation with **2**, guest **18** eluted out from Gel-QCP faster than **19** (34 min *vs.* 47 min, Table I). This indicates some difference in the guest selectivity between **2** and the cyclophane bound to the gel.

In conclusion, Gel-QCP shows guest selectivity on the basis of recognition by the formation of inclusion complexes with the cyclophane moiety. In addition, non-specific electrostatic interaction operates for anionic guests; it can be reduced by using an aqueous solution of NaCl as an eluent.

Experimental

General *N*,*N*-Dimethylformamide (DMF) was distilled under reduced pressure. CH_2Cl_2 was distilled over P_2O_5 before use. THF was freshly distilled from sodium–benzophenone ketyl under argon. Melting points were measured on a Büchi 510 melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a JASCO DS-701G diffraction grating IR spectrophotometer. ^{1}H-NMR spectra were measured on a JEOL JNM-FX 100 Fourier transform NMR spectrometer (100 MHz). Chemical shifts are reported in δ values in ppm with tetramethylsilane (TMS) as an internal standard. In cases where D_2O was used as a solvent, a TMS capillary was used as an external standard. Mass spectra (MS) were recorded on a JEOL JMS-01 SG-2 or a JEOL JMS-DX 300 mass spectrometer. High performance liquid chromatography (HPLC) was conducted with a Hitachi 635 liquid chromatograph using a Radial Pak 8PSC × 10 μ (Waters Associates), with 0.15 M aqueous $Et_3N \cdot HBr$ in 45% CH_3CN (v/v) (pH adjusted to 7) as an eluent. Detection was done at 254 nm. Gel filtration chromatography was performed on a column packed with Toyopearl HW-40S. Anion exchange chromatography was carried out on a column packed with Dowex 1 (ClO_4 form). ECH Sepharose 4B (3 ml) (Pharmacia) was washed with 0.5 M NaCl (240 ml) on a glass filter before use. Standard buffer solutions (pH 4.01, 10.02) were purchased from Nacalai Tesque, Inc.

1-*tert*-Butoxycarbonyl-6,20,25-tris(trifluoroacetyl)-1,6,20,25-tetraaza-[6.1.6.1]paracyclophane (7)[9] A solution of KOH (111 mg, 1.7 mmol) in

THF–MeOH (1 : 1, 84 ml) was added dropwise to a stirred solution of **5** (3.01 g, 3.4 mmol) in THF (420 ml) over a period of 35 min. After being stirred at room temperature overnight, the reaction mixture was evaporated. CH_2Cl_2 (400 ml) and saturated aqueous $NaHCO_3$ (300 ml) were added to the residue, and the organic layer was washed successively with water (300 ml) and brine (300 ml), dried over anhydrous K_2CO_3, filtered and evaporated to give a white solid (2.73 g). This solid (2.60 g) was dissolved in hot CH_2Cl_2 (13 ml), and a solution of Boc_2O (0.49 g, 2.3 mmol) was added. The reaction mixture was refluxed for 66 h and then evaporated to give a white solid (2.88 g). A part (1.74 g) of this solid was stirred in benzene (100 ml) for 4.5 h, and the residual solid was filtered off. The filtrate was evaporated to give a pale yellow solid (0.83 g). This solid was taken up in benzene (50 ml), and the mixture was stirred for 2 h then filtered. The filtrate was evaporated to give a white amorphous solid (0.79 g). The crude product was purified by column chromatography (silica gel, CH_2Cl_2–Et_2O (40 : 1)) to give **7** as a white amorphous solid (0.48 g, 28% from **5**), which was reprecipitated from CH_2Cl_2–hexane. White powder, mp 210.5—211.5 °C(dec.). IR (KBr) cm^{-1}: 1690 ($CF_3\underline{C}ONR_2$, $RO\underline{C}ONR_2$). ^{1}H-NMR ($CDCl_3$) δ: 1.43 (9H, s, $C\underline{H}_3$), 1.53 (8H, m, $NCH_2C\underline{H}_2$), 3.69 (8H, m, $NC\underline{H}_2CH_2$), 3.95, 4.01 (2H × 2, two s, $ArC\underline{H}_2Ar$), 7.10 (16H, m, Ar$\underline{H}$). FAB-MS *m/z*: 893 ($M^+ + 1$). *Anal.* Calcd for $C_{45}H_{45}F_9N_4O_5$: C, 60.54; H, 5.08; N, 6.27. Found: C, 60.77; H, 5.10; N, 6.56.

1-*tert*-Butoxycarbonyl-1,6,20,25-tetraaza[6.1.6.1]paracyclophane (8) A solution of KOH (295 mg, 4.5 mmol) in MeOH (14 ml) was added to a solution of **7** (331 mg, 0.37 mmol) in THF (14 ml), and the mixture was stirred at room temperature overnight. After evaporation of the solvent, the residue was dissolved in CH_2Cl_2 (100 ml). This solution was successively washed with water (100 ml × 2) and brine (100 ml), dried over K_2CO_3, filtered, and evaporated to give **8** as a white amorphous solid (210 mg, 94%). An analytical sample was obtained by reprecipitation from CH_2Cl_2–hexane. White powder, mp 168—168.5 °C (dec.). IR (KBr) cm^{-1}: 3380 (N$\underline{H}$), 1690 ($RO\underline{C}ONR_2$). ^{1}H-NMR ($CDCl_3$) δ: 1.40 (12H, s, $C\underline{H}_3$, N$\underline{H}$), 1.50—1.70 (8H, m, $NCH_2C\underline{H}_2$), 3.00—3.60 (8H, m, $NC\underline{H}_2CH_2$), 3.70 (2H, s, $ArC\underline{H}_2Ar$), 3.80 (2H, m, $ArC\underline{H}_2Ar$), 6.40—7.20 (16H, m, Ar$\underline{H}$). MS *m/z*: 604 (M^+). *Anal.* Calcd for $C_{39}H_{48}N_4O_2$: C, 77.45; H, 8.00; N, 9.26. Found: C, 77.16; H, 7.97; N, 9.42.

1-*tert*-Butoxycarbonyl-6,6,20,20,25,25-hexamethyl-1-aza-6,20,25-triazonia[6.1.6.1]paracyclophane Triperchlorate (9) *n*-Bu_3N (135 ml, 567 mmol) and CH_3I (35 ml, 562 mmol) were added in several portions to a stirred solution of **8** (0.58 g, 0.96 mmol) in DMF (10 ml) over a period of 7 d. Since stirring was difficult due to the precipitate that separated during the reaction, approximately 50 ml of water was added to dissolve the precipitate. The progress of quaternization was checked by HPLC using 0.15 M $Et_3N\cdot HBr$ in CH_3CN–H_2O as an eluent, as described above (general). The reaction mixture was evaporated, then the resulting yellow solid (2.27 g) was dissolved in water (1000 ml), and washed with CH_2Cl_2 (500 ml × 5). Water was removed *in vacuo*, and the residue was purified by gel filtration chromatography (Toyopearl HW40S, MeOH as an eluent) to give a yellow solid (666 mg). This was applied to an anion exchange column (Dowex 1, ClO_4 form, MeOH–H_2O (1 : 1) as an eluent) to exchange the counter anion from iodide to perchlorate. After evaporation of the eluate, the residue was recrystallized from water to give **9** as colorless fine needles (329 mg, 34%). This product was hygroscopic and hence was air-dried until the weight became constant, mp 176.5—179 °C (dec.). An analytical sample was obtained by additional recrystallization from water and air-drying over a period of 2 d, mp 177.5—180.5 °C (dec.). IR (KBr) cm^{-1}: 3420 (O$\underline{H}$), 1680 ($RO\underline{C}ONR_2$), 1395 ($N^+\underline{C}H_3$, $CC\underline{H}_3$), 1080, 620 (ClO_4^-). ^{1}H-NMR (acetone-d_6) δ: 1.36 (9H, s, $C\underline{C}H_3$), 1.50 (8H, br, $NCH_2C\underline{H}_2$), 3.69, 3.76, 3.82 (6H × 3, three s, $N^+C\underline{H}_3$), 4.00 (8H, br, $NC\underline{H}_2CH_2$), 4.05, 4.19 (2H × 2, two s, $ArC\underline{H}_2Ar$), 7.03 (2H, d, J = 8.4 Hz, BocNAr$\underline{H}$ (*ortho*)), 7.26 (2H, d, J = 8.4 Hz, BocNAr$\underline{H}$ (*meta*)), 7.47—7.96 (14H, m, the rest of the aromatic protons). *Anal.* Calcd for $C_{45}H_{63}Cl_3N_4O_{14}\cdot 1.5H_2O$: C, 53.13; H, 6.54; Cl, 10.45; N, 5.51. Found: C, 53.16; H, 6.43; Cl, 10.47; N, 5.53.

Gel-QCP (3) A solution of **9** (58.5 mg, 58 μmol) in H_2O–MeOH (1 : 1, 5 ml) was treated with 1 N HCl (2.5 ml). The mixture was stirred for 5 min, then the solvent was removed *in vacuo* to give a white solid. Water (10 ml) was added to a solution of this solid in acetone (2 ml), and the pH of the solution was adjusted to 4.2—4.4 with 1 N HCl and 1 N NaOH. To this was added a suspension of ECH Sepharose 4B (3 ml of a swollen gel) in water (15 ml). Then a solution of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (water-soluble carbodiimide, 0.72 g, 3.8 mmol) in water (15 ml) adjusted to pH 4.4—4.6 was added. After shaking of the reaction mixture for 1 h, the pH was adjusted again to 4.4 with 1 N NaOH and 1 N HCl, and shaking was continued at room temperature for 23 h. The gel was collected on a glass filter (3G) and washed successively with water (150 ml), standard buffer solutions of pH 10.02 and 4.01 (6 ml × 3, 6 ml × 3, applied alternately) and water (150 ml). The resulting gel (Gel-QCP) was used for the chromatographic experiments.

Preparation of Analytical Sample of ECH Sepharose 4B An analytical sample of ECH Sepharose 4B was obtained as follows. ECH Sepharose 4B (0.5 ml) was washed successively with water (20 ml), MeOH (2 ml × 6) and Et_2O (2 ml × 6). The resulting white solid was dried *in vacuo* for 24 h and air-dried until the weight became constant. *Anal.* Found: N, 0.64 ± 0.03 (the average of two samples).

Preparation of Analytical Sample of Gel-QCP Gel-QCP (0.5 ml) was washed successively with 1 M NaCl (20 ml), water (20 ml), MeOH (2 ml × 6) and Et_2O (2 ml × 6) on a glass filter (3G). The resulting white solid was dried *in vacuo* for 5 h and air-dried until the weight became constant. *Anal.* Found: N, 1.94. On the basis of this microanalysis, about 66% of the carboxyl group attached to ECH Sepharose 4B was calculated to be derivatized with the cyclophane unit (**9**).

Gel-EA (10) Ethanolamine (8.5 μl, 141 μmol) was dissolved in water (25 ml), and the pH of the solution was adjusted to 4.4—4.6 with 1 N HCl and 1 N NaOH. This solution was added to a suspension of ECH Sepharose 4B (3 ml) in water (20 ml). Then a solution of EDC (1.34 g, 7.0 mmol) in water (25 ml) adjusted to pH 4.4—4.6 was added. After shaking of the reaction mixture for 1 h, the pH was adjusted again to 4.4 with 1 N NaOH and 1 N HCl, and shaking was continued at room temperature for 24 h. The gel was collected on a glass filter (3G) and washed successively with water (400 ml), standard buffer solutions of pH 10.02 and 4.01 (5 ml × 3, 5 ml × 3, applied alternately) and water (10 ml). The resulting gel (Gel-EA) was used for the chromatographic experiments.

Chromatographic Separation Using Gels Columns of 10 mm diameter and 68 or 72 mm length was made with the gels (Gel-QCP, Gel-EA and ECH Sepharose 4B). Separation experiments were carried out at 23 °C and the detection of guests was done at 254 nm. Aqueous guest solutions (5 mM, 3 μl) were injected into the column. To prevent collapse of the gel owing to pressure, the flow rate was adjusted to 0.62 ml/min under the control of a peristaltic pump (Pharmacia). Reproducibility of the retention time was within ±6%.

Determination of Stability Constants of Complexes between 2 and Guests D_2O solutions containing a fixed amount of guest and various amounts of **2** were prepared, and their ^{1}H-NMR spectra were recorded. The stability constants were calculated from the host-induced upfield shifts of the guest proton signals (TMS was used as an external standard) by non-linear least-squares fitting of the saturation plot.[10] In the case of the complexation between **2** and **21** in 1 M NaCl, the guest signals were broad and not assignable. Therefore, 1 M NaCl in D_2O solutions containing a fixed amount of **2** and various amounts of **21** were prepared, and the stability constant was calculated from the guest-induced upfield shifts of the methyl proton signal of **2**. The experiments were carried out below the critical aggregation concentration of **2** ($> 1 \times 10^{-1}$ M) determined by the ring method (surface tension measurement) and the ^{1}H-NMR method.

Acknowledgment The authors are grateful to Daicel Chemical Industries, Ltd., for partial financial support.

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8) The opposite effect, *i.e.*, that the increase in NaCl concentration would cause a stronger hydrophobic interaction, does not seem to play an important role.
9) For convenience, a conventional cyclophane nomenclature is used throughout this paper. See: D. J. Cram and J. Abell, *J. Am. Chem. Soc.*, **77**, 1179 (1955).
10) The non-linear curve fitting procedure with the least-squares method was done with the program (DELTA) devised by Dr. A. Itai, Faculty of Pharmaceutical Sciences, University of Tokyo.