Electronic supplementary information

Molecular Selective Binding of Basic Amino Acids by a Water-soluble Pillar[5]arene

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Contents

Materials and methods.

CP5A host[1] were prepared according to our previously reported method. Native L-α-Aminoacid guests were commercially available and used as received. ^{1}H NMR, ^{13}C NMR and 2D NOESY spectra were recorded on a Bruker AV500 instrument. D_2O was adjusted to pD 7.2 with 1 M NaOD or 1 M DCl. The value was verified on a pH meter calibrated with two standard buffer solutions. pH readings were converted to pD by adding 0.4 units.[2]

Copies of ^{1}H NMR and ^{13}C NMR spectra of CP5A.

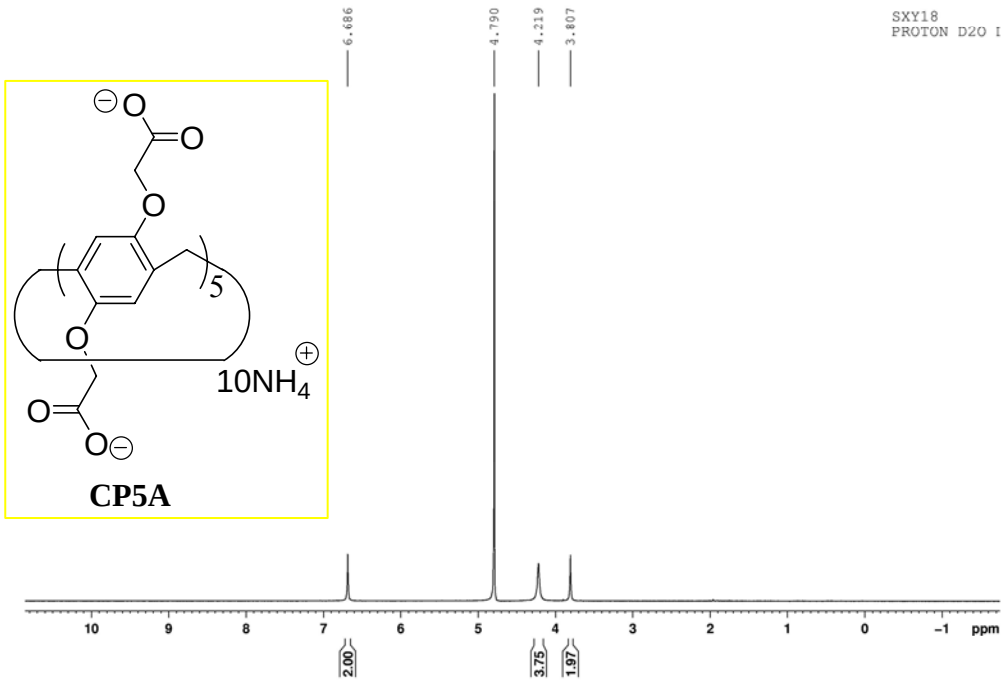

Figure S1. ^{1}H NMR spectrum (500 MHz) of CP5A in D_2O.

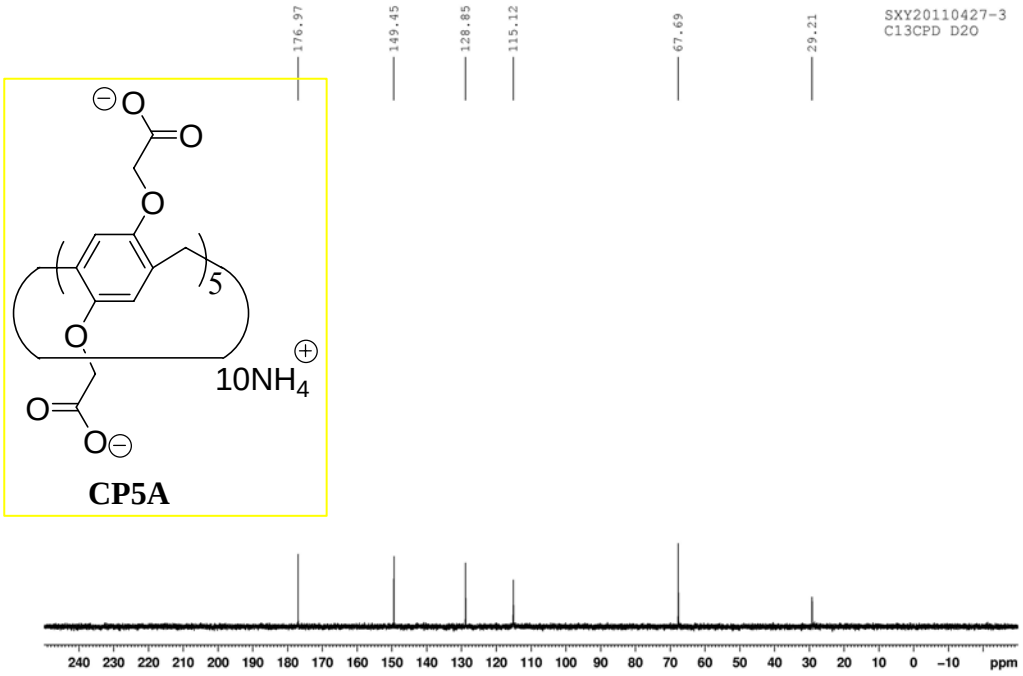

Figure S2. ^{13}C NMR spectrum (125 MHz) of CP5A in D_2O.

^{1}H NMR spectra of guests in the absence and presence of CP5A.

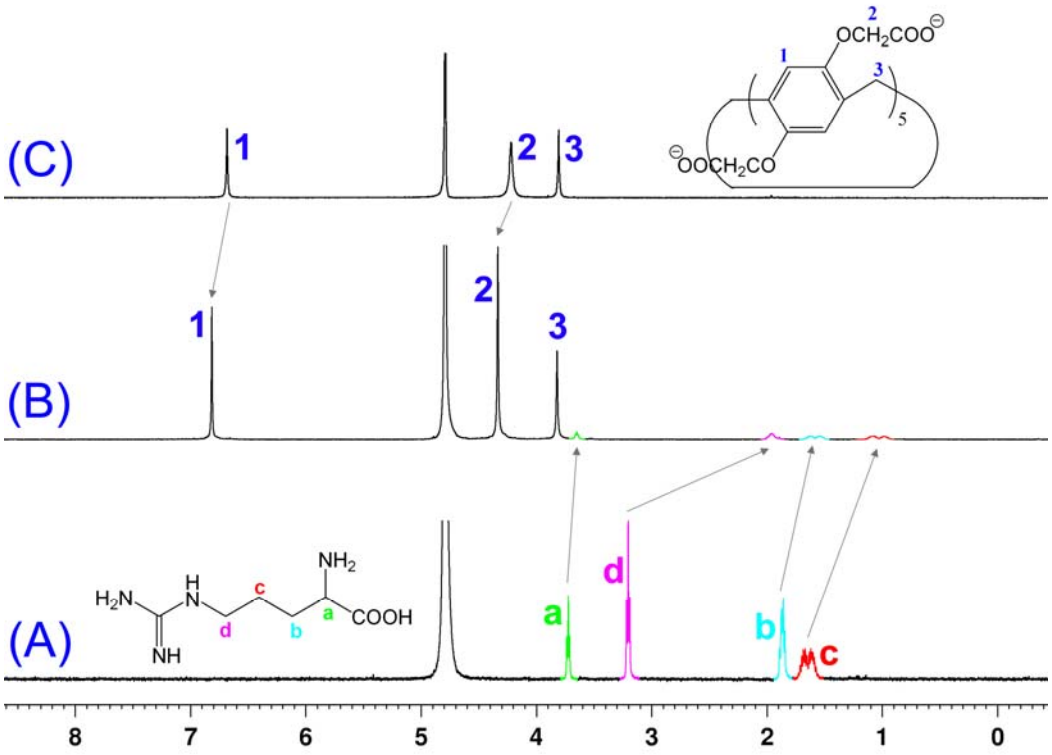

Figure S3. ^{1}H NMR spectra (500 MHz) of (A) Arg, (B) Arg + CP5A, and (C) CP5A in D_2O (pD 7.2) at 2.6–3.0 mM.

Figure S3 shows the ^{1}H NMR spectra of Arg recorded in the absence and in the presence of approximately 1.0 equiv of CP5A host. From Figure S3b, in the presence of CP5A, the peaks for $H_{a\sim d}$ protons of Lys display substantial upfield shifts and broadening effects compared to the free guest ($\Delta\delta = -0.07 \sim -1.25$ ppm) as a consequence of inclusion-induced shielding effects,[3] indicating that the host is fully threaded by Arg guest.

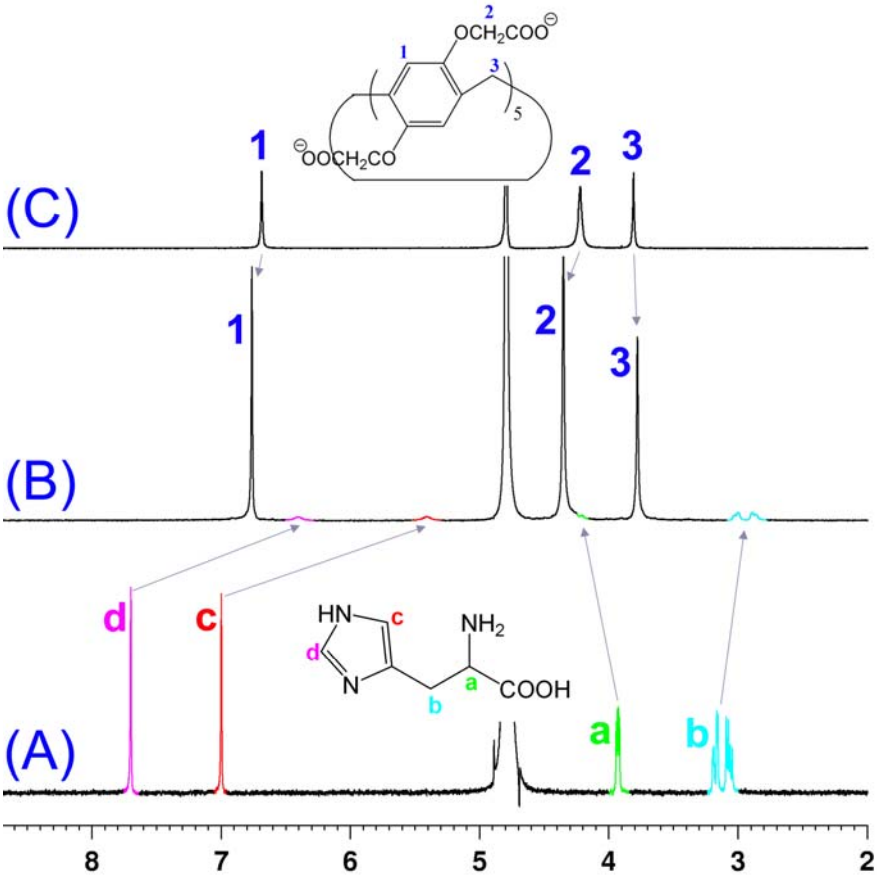

Figure S4. ^{1}H NMR spectra (500 MHz) of (A) His, (B) His + CP5A, and (C) CP5A in D_2O (pD 7.2) at 2.7–3.0 mM.

Figure S4 shows the ^{1}H NMR spectra of His recorded in the absence and in the presence of approximately 1.0 equiv of CP5A host. From Figure S4b, in the presence of CP5A, the peaks for imidazole protons H_c and H_d and methylene protons H_b display substantial upfield shifts and broadening effects compared to the free guest ($\Delta\delta$ = −0.19 ~ −1.59 ppm) as a consequence of inclusion-induced shielding effects,[3] indicating that the guest's imidazole unit is deeply included in the cavity of CP5A. At the same time, the signal corresponding to the α-proton of amino acid (H_a) shifts slightly downfield ($\Delta\delta$ = 0.28 ppm), which is characteristic of the protons being located at just outside the host's cavity portal.[4]

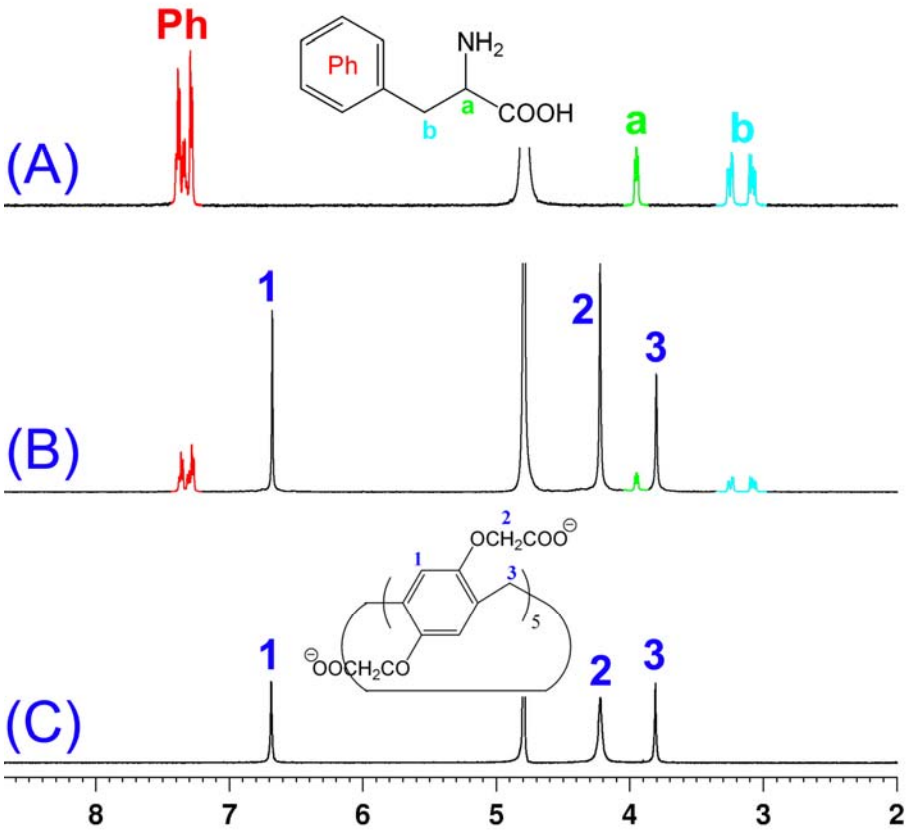

Figure S5. ^{1}H NMR spectra (500 MHz) of (A) Phenylalanine (Phe), (B) Phe + CP5A, and (C) CP5A in D_2O (pD 7.2) at 2.6–3.0 mM.

Very different with three basic amino acids (Arg, Lys and His), Phenylalanine (Phe) did not show obvious NMR changes when mixing with CP5A host. Similar with Phe, no obvious NMR changes were found for Alanine, Isoleucine, Methionine, Tyrosine, Serine, Asparagine, Glycine, Threonine, Valine, Glutamine, Glutamic acid, Leucine, Cysteine, Aspartic acid, Proline and Tryptophane either, indicating that CP5A did not form inclusion complexes with these amino acids or at least had very weak interactions.

Table S1 shows the chemical shift change [Δδ (ppm)] values of CP5A host upon complexation with 20 native L-α-Aminoacids.

Table S1. The chemical shift change[a] [Δδ (ppm)] values of CP5A host upon complexation with 20 native L-α-Aminoacids. See Scheme 1 and Figure 1 for position labels 1−3 of CP5A.

Guest		Δδ (ppm)		
		H_1	H_2	H_3
Lysine	Lys	0.09	0.11	[b]
Arginine	Arg	0.13	0.11	0.01
Histidine	His	0.08	0.13	−0.03
Alanine	Ala	[b]	0.01	[b]
Isoleucine	Ile	[b]	0.01	[b]
Methionine	Met	[b]	0.01	[b]
Tyrosine	Tyr	[b]	0.01	[b]
Serine	Ser	[b]	[b]	[b]
Asparagine	Asn	[b]	[b]	[b]
Glycine	Gly	[b]	[b]	[b]
Threonine	Thr	[b]	0.01	[b]
Valine	Val	[b]	[b]	[b]
Glutamine	Gln	[b]	[b]	[b]
Glutamic acid	Glu	[b]	−0.02	[b]
Leucine	Leu	[b]	[b]	[b]
Cysteine	Cys	[b]	[b]	[b]
Aspartic acid	Asp	[b]	−0.02	[b]
Phenylalanine	Phe	[b]	[b]	[b]
Proline	Pro	[b]	[b]	[b]
Tryptophane	Trp	−0.01	[b]	[b]

[a] Δδ = δ(complexed host) − δ(free host); Negative values indicate upfield shift. The concentrations of CP5A host and amino acid guests are 2.4–3.0 mM. [b] The chemical shift change is very small ($|\Delta\delta| < 0.01$).

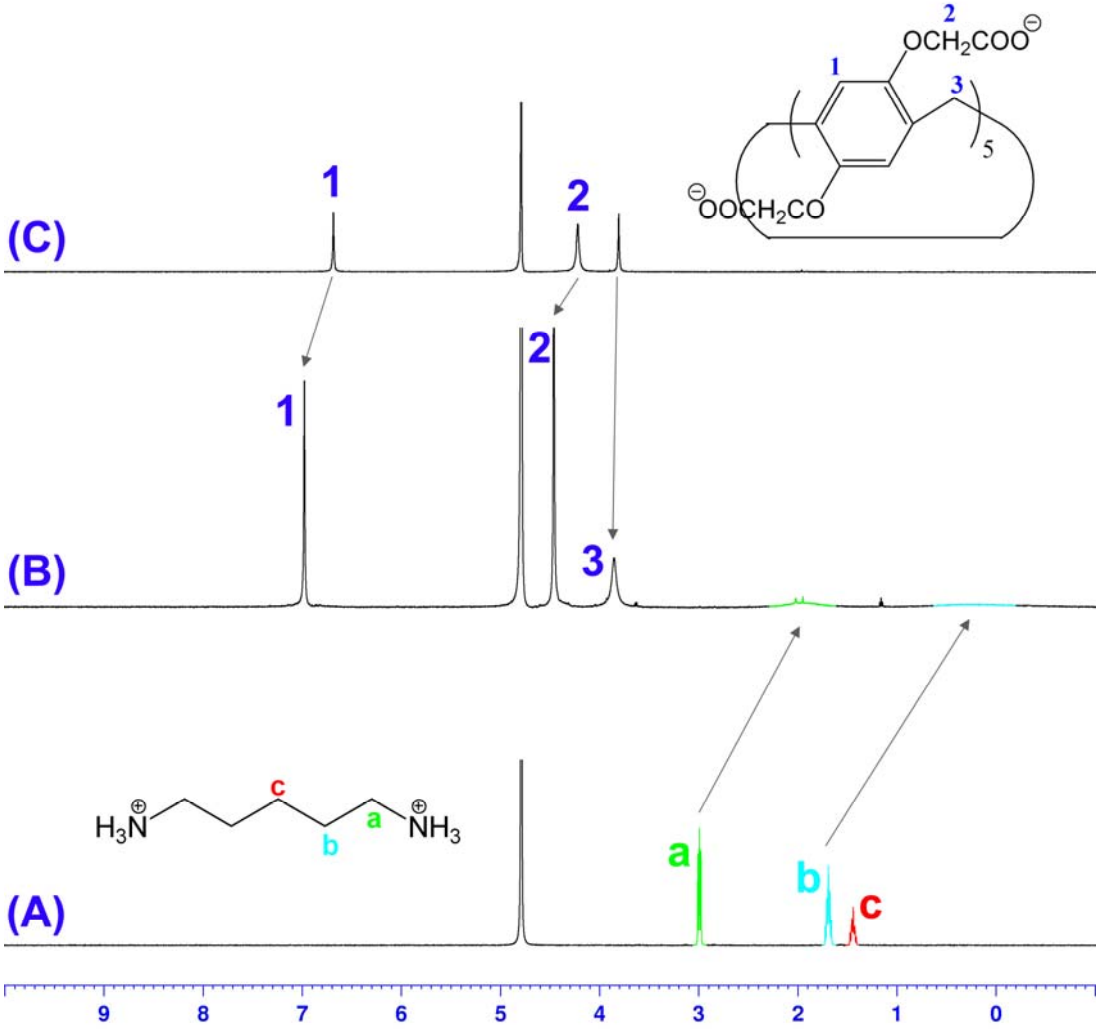

Figure S6. ^{1}H NMR spectra (500 MHz) of (A) Cad, (B) Cad + CP5A, and (C) CP5A in D_2O (pD 7.2) at 2.7–3.0 mM.

Figure S6 shows the ^{1}H NMR spectra of Cad recorded in the absence and in the presence of approximately 1.0 equiv of CP5A host. From Figure S6b, in the presence of CP5A, the signals of Cad's methylene protons (H_a, H_b and H_c) exhibit a very substantial upfield shift and broadening effects compared to the free guest. Typically, the broadening effects were so remarkable that the signals of H_c could not be observed in the ^{1}H NMR spectrum. These results reveal that the host engulfs the guest, which thus leads to an efficient shield[3] toward guest protons.

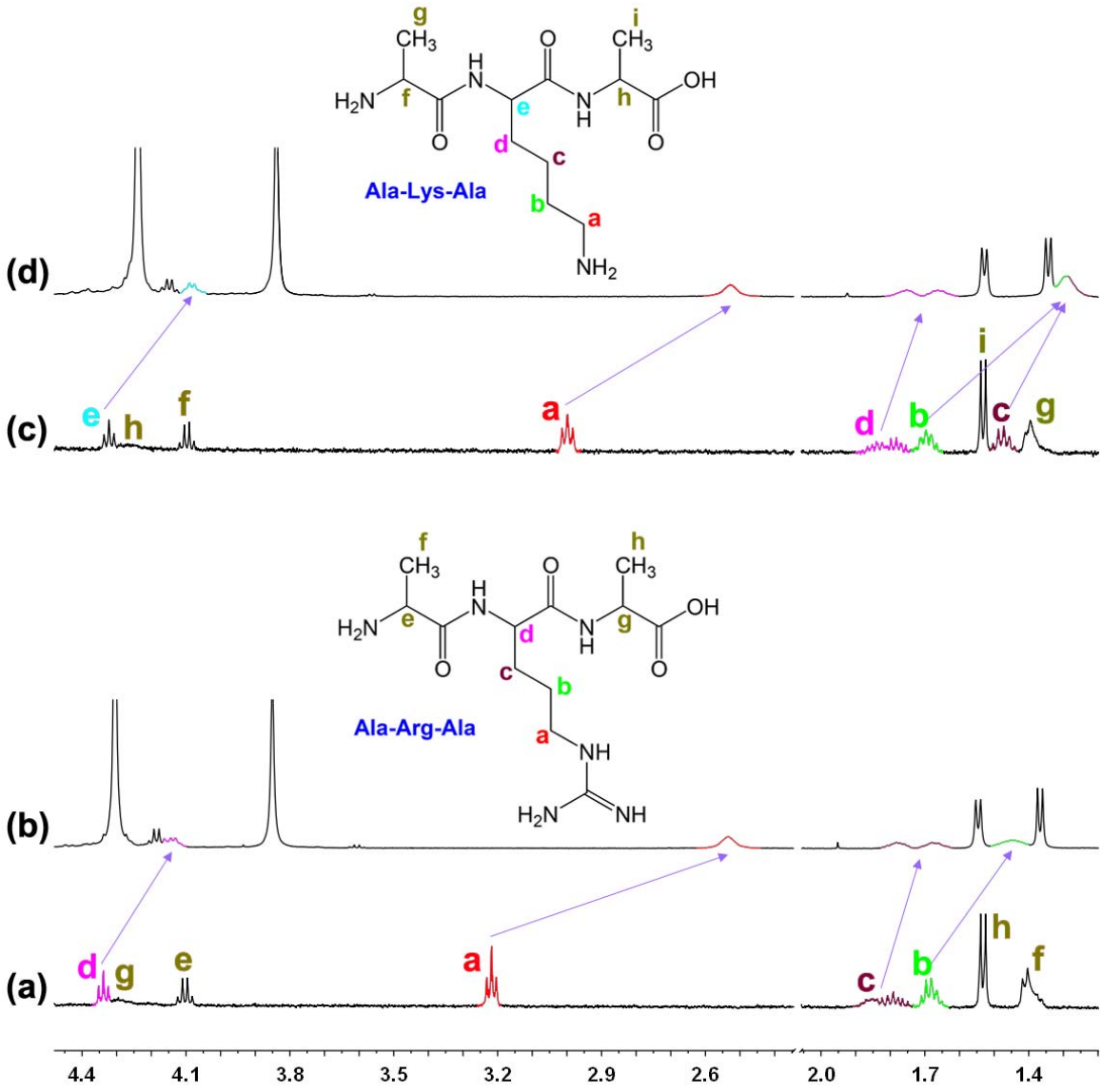

Figure S7. ^{1}H NMR spectra (500 MHz) of (A) Ala-Arg-Ala, (B) Ala-Arg-Ala + CP5A, (C) Ala-Lys-Ala and (D) Ala-Lys-Ala + CP5A, in D_2O (pD 7.2) at 2.7–2.8 mM.

2D NOESY spectra of Lys/Arg⊂CP5A complexes.

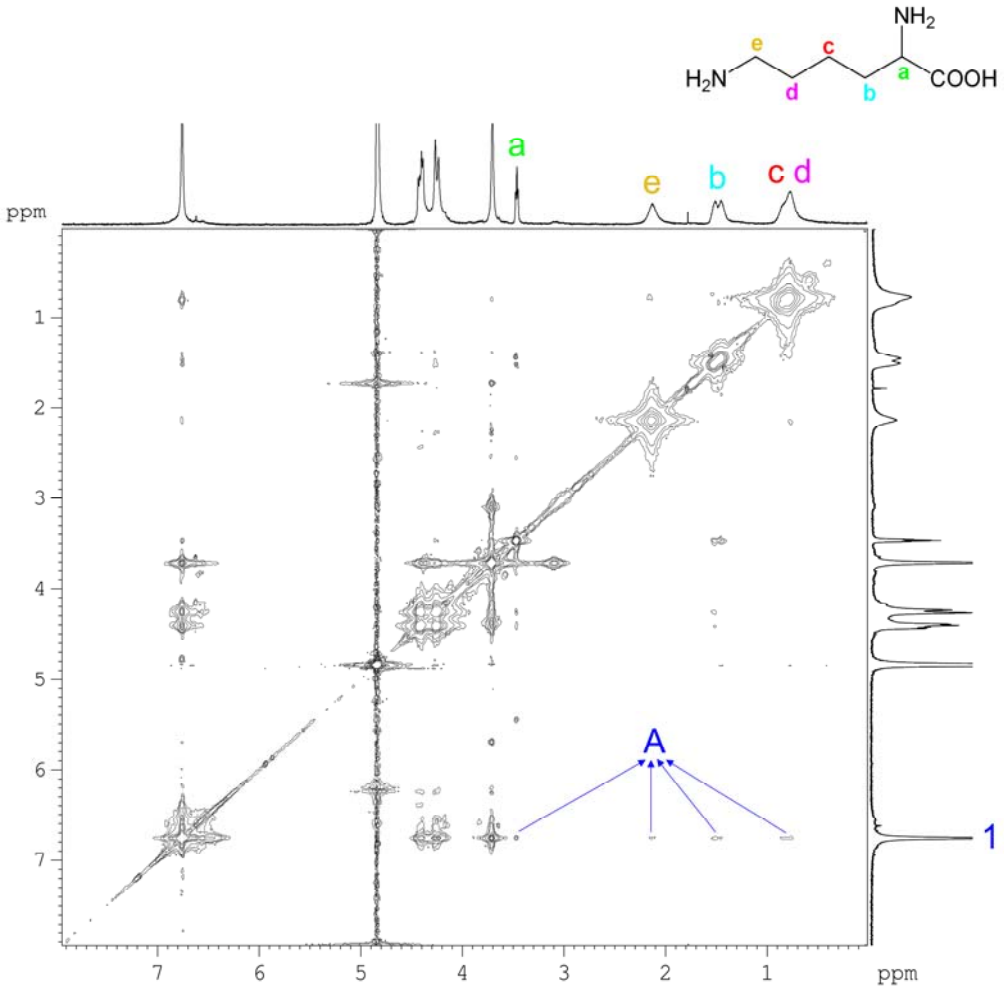

Figure S8. 2D NOESY analysis of Lys with CP5A in D_2O with a mixing time of 300 ms. (500 MHz, 283 K. The concentrations of host and guest are 15 and 20 mM, respectively)

As shown in Figure S8, NOE correlations were observed between methylene protons of Lys and aromatic protons (H_1) of CP5A (see the NOE cross-peaks A), which also confirms the inclusion geometry.[5]

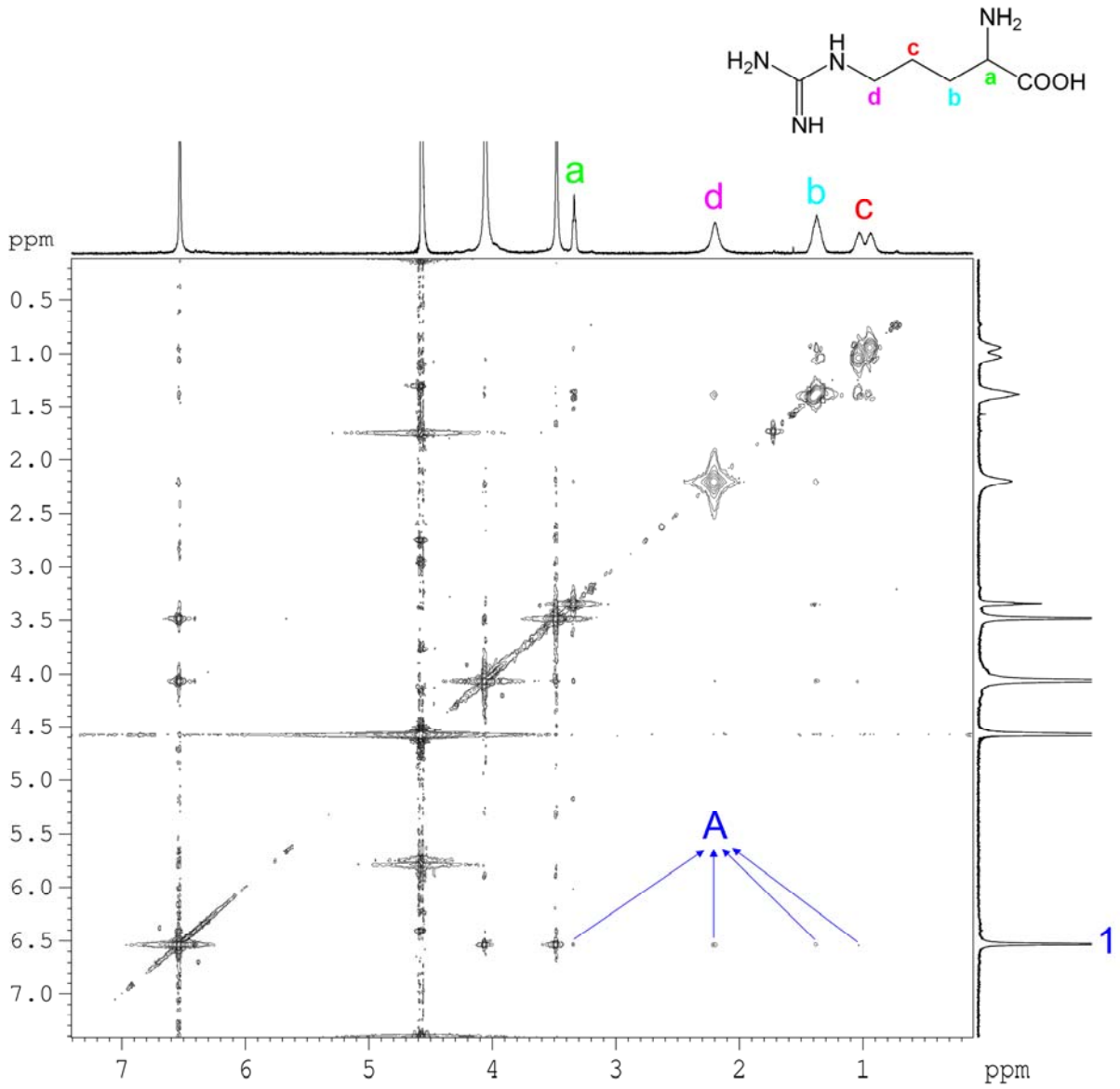

Figure S9. 2D NOESY analysis of Arg with CP5A in D_2O with a mixing time of 300 ms. (500 MHz, 283 K. The concentrations of host and guest are 15 and 20 mM, respectively)

As shown in Figure S9, NOE correlations were observed between methylene protons of Arg and aromatic protons (H_1) of CP5A (see the NOE cross-peaks A), which also confirms the inclusion geometry.[5]

Determination of the association constants.

In the present host-guest systems, chemical exchange is fast on the NMR time scale. To determine the association constant, NMR titrations were done with solutions which had a constant concentration of CP5A and varying concentrations of guest. Assuming 1:1 inclusion complexation stoichiometry between CP5A and the guests, the association constants (K_a) could be calculated by analyzing the sequential changes in chemical shift changes of CP5A host that occurred with changes in guest concentration. Using the nonlinear curve-fitting method, the association constant was obtained for each host-guest combination from the following equation[6]:

$$A = (A_\infty/[\text{P5A}]_0)\ (0.5[\text{G}]_0 + 0.5([\text{P5A}]_0+1/K_a)-(0.5\ ([\text{G}]_0^2+(2[\text{G}]_0(1/K_a - [\text{P5A}]_0)) + (1/K_a + [\text{P5A}]_0)^2)^{0.5}))$$

Where A is the chemical shift change of H_1 on CP5A host at $[G]_0$, A_∞ is the chemical shift change of H_1 when the host is completely complexed, $[P5A]_0$ is the fixed initial concentration of the CP5A host, and $[G]_0$ is the initial concentration of guest.

For each guest examined, the plot of A as a function of $[G]_0$ gave an excellent fit ($R > 0.98$), verifying the validity of the 1:1 inclusion complexation stoichiometry assumed. Additionally, the 1:1 inclusion complexation stoichiometry has also been proved by job's experiments (Figure S12).

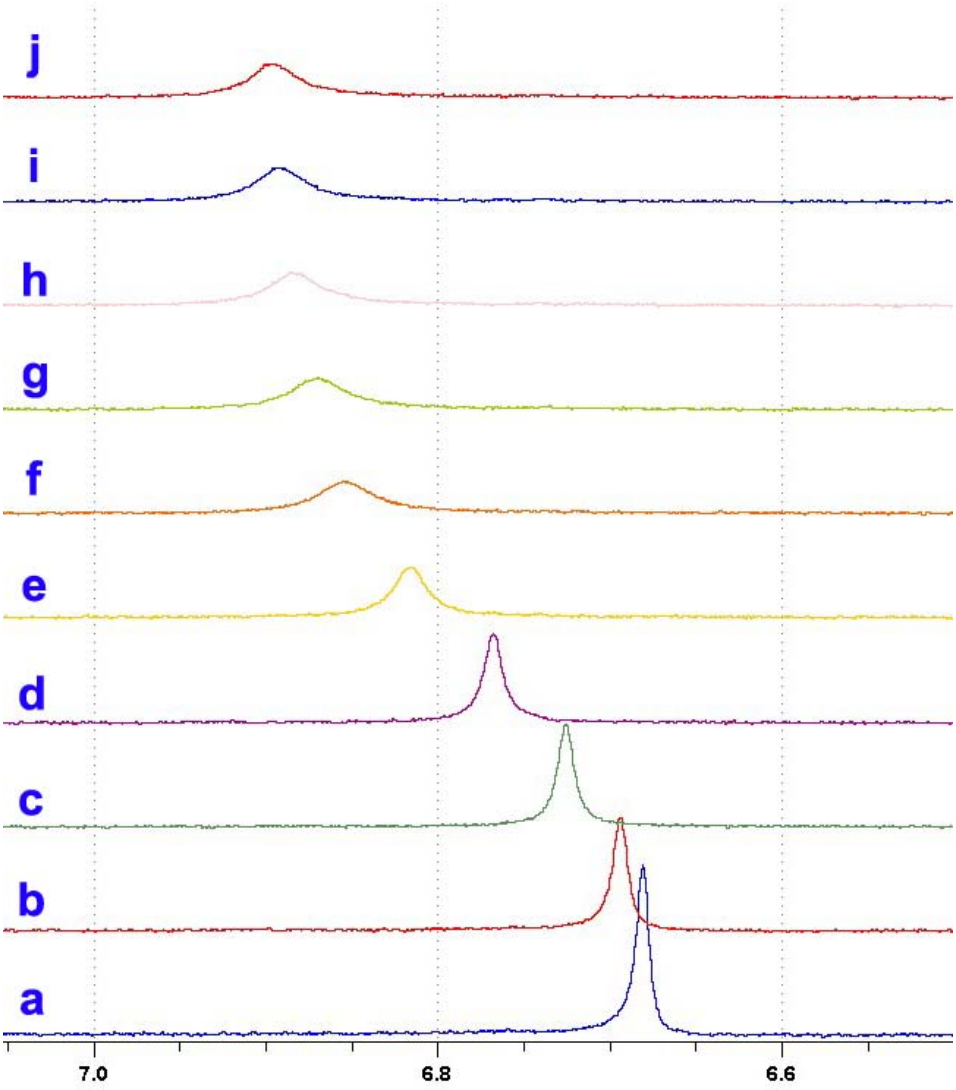

Figure S10. Partial ^{1}H NMR spectra (500 MHz, D_2O, 298 K) of CP5A at a concentration of 1.0 mM upon addition of Lys: (a) 0 mM, (b) 0.079 mM, (c) 0.35 mM, (d) 0.74 mM, (e) 1.4 mM, (f) 2.4 mM, (g) 3.2 mM, (h) 4.4 mM, (i) 5.6 mM, and (j) 6.6 mM.

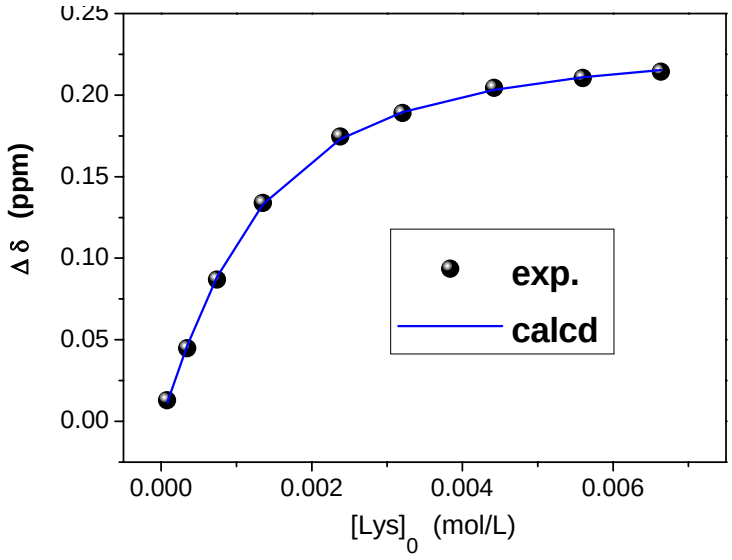

Figure S11. The non-linear curve-fitting (NMR titrations) for the complexation of CP5A host (1.0 mM) with Lys in D_2O at 298 K. The concentration of Lys was 0, 0.079, 0.35, 0.74, 1.4, 2.4, 3.2, 4.4, 5.6, 6.6 mM.

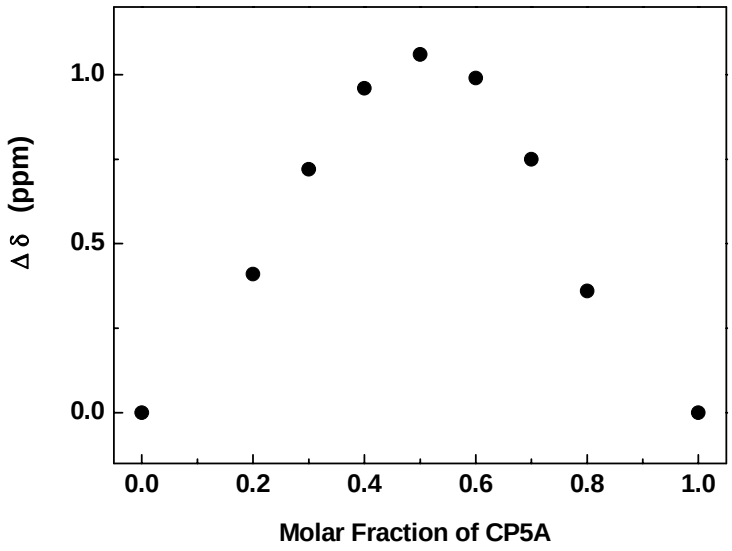

Figure S12. Job plot showing the 1 : 1 stoichiometry of the complex between CP5A and Lys in D_2O (pD 7.2) by plotting the Δδ in chemical shift of the guest's methylene proton H_e (for proton designations, see Figure 1) observed by ^1H NMR spectroscopy against the mole fraction of CP5A ([host] + [guest] = 6.0 mM).

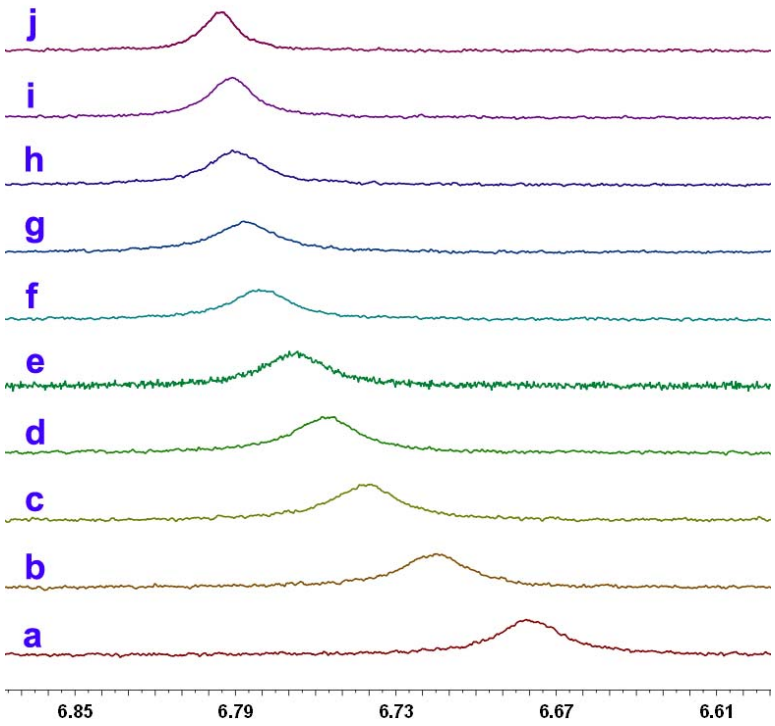

Figure S13. Partial ^{1}H NMR spectra (500 MHz, D_2O, pD 7.2, 298 K) of CP5A at a concentration of 1.0 mM upon addition of Ala-Arg-Ala: (a) 0 mM, (b) 0.30 mM, (c) 0.70 mM, (d) 1.2 mM, (e) 1.7 mM, (f) 2.2 mM, (g) 2.9 mM, (h) 4.0 mM, (i) 5.4 mM, and (j) 7.6 mM.

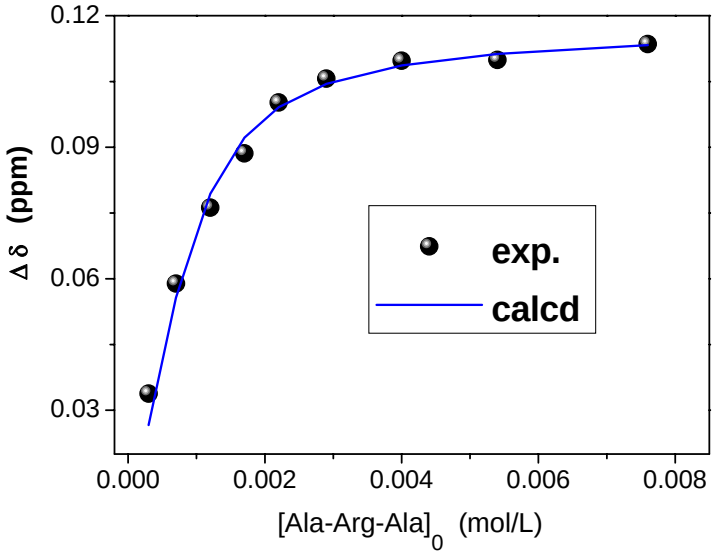

Figure S14. The non-linear curve-fitting (NMR titrations) for the complexation of CP5A host (1.0 mM) with Ala-Arg-Ala in D_2O (pD 7.2) at 298 K. The concentration of Ala-Arg-Ala was 0, 0.30, 0.70, 1.2, 1.7, 2.2, 2.9, 4.0, 5.4, 7.6 mM.

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