Supporting Information Section

Molecular recognition of methamphetamine by carboxylatopillar[5]arene: drug-dependent complexation stoichiometry and insights into medical applications

Eduardo V. Silveira,*[a] Eduardo H. Wanderlind,[a] Andrieli K. Masson,[a] Vanessa Nascimento,[b] Pâmella S. Cordeiro,[b] Ricardo F. Affeldt[a] and Gustavo A. Micke[a]

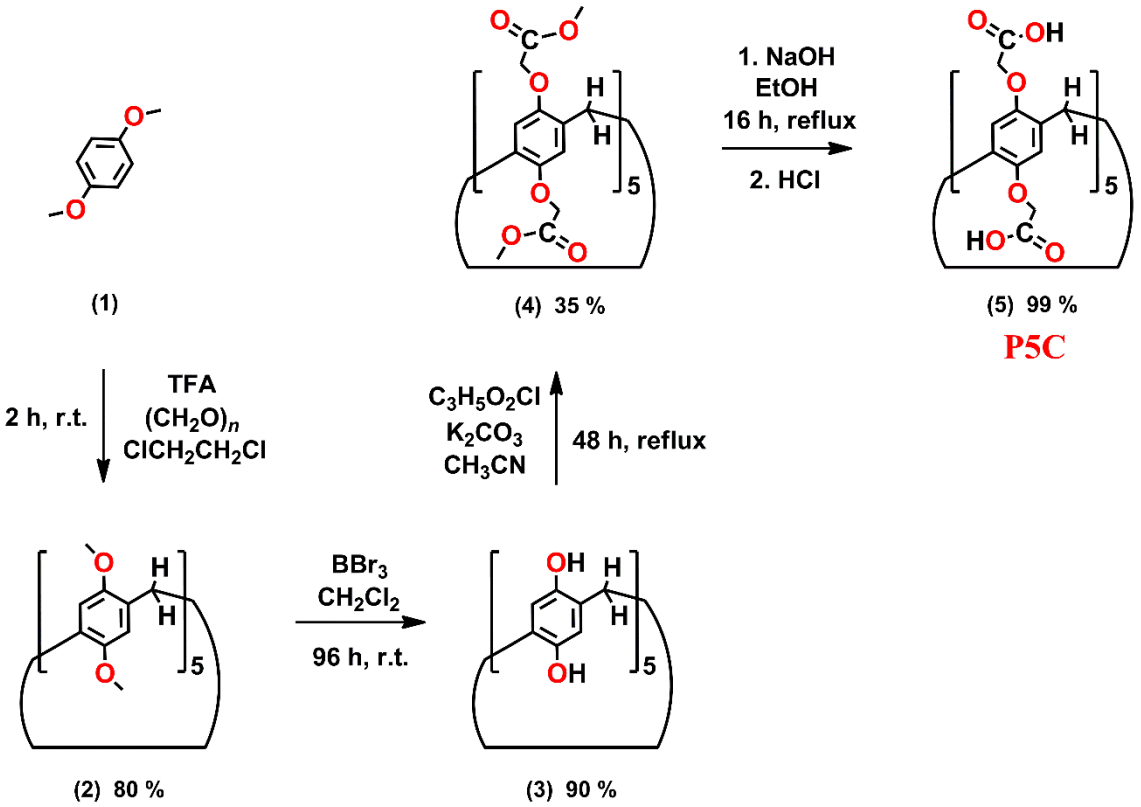
[a] *Department of Chemistry, Federal University of Santa Catarina, Florianópolis-SC, 88040-900, Brazil. *E-mail: eduardovieira.chem@gmail.com*

[b] *Department of Organic Chemistry, Fluminense Federal University, Niterói-RJ, 24020-150, Brazil.*

Section

1. Synthesis of Carboxylatopillar[5]arene (P5C)

The synthetic route used to obtain the carboxylatopillar[5]arene (P5C) was based on the literature,[1,2] with modifications. Scheme S1 shows the four reaction steps for P5C synthesis: (step 1) cyclization promoted by Lewis acid, (step 2) demethylation with BBr_3, (step 3) alkylation with methyl chloroacetate and (step 4) saponification followed by acidification.



Scheme S1. Synthetic route used to obtain P5C.

(Step 1): Under stirring paraformaldehyde (21.70 mmol) was added to a solution of 1,4-dimethoxybenzene **(1)** (21.70 mmol) in dry 1,2-dichloroethane (195 ml). The reaction mixture was stirred for some minutes and trifluoroacetic acid was added. After 2 h, the solution was poured in MeOH and the solid formed collected by filtration, yielding 80% of compound **(2)** as a solid after recrystallization. ^{1}H NMR (300 MHz, $CDCl_3$, TMS), δ (ppm): 6.88 (s, 1H); 3.76 (s, 1H); 3.73 (s, 3H). ^{13}C APT NMR (75 MHz, $CDCl_3$, residual solvent as reference), δ (ppm): 150.46 (C); 128.33 (C); 113.39 (CH); 55.51 (CH_3); 29.37 (CH_2).

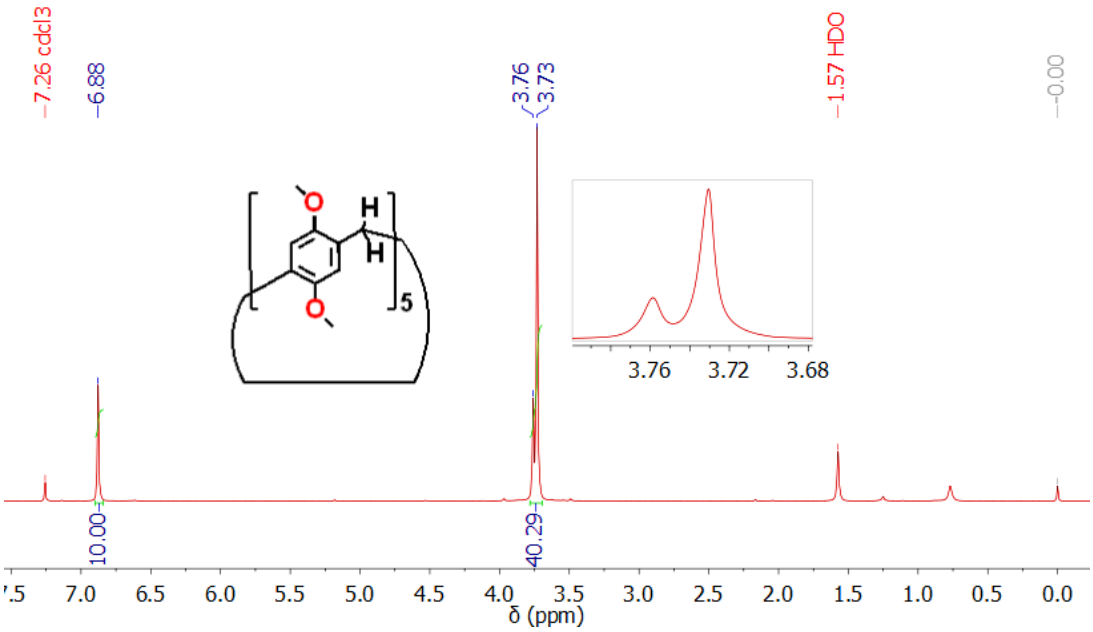


Fig. S1A ^{1}H NMR spectrum of **(2)** ($CDCl_3$; 300 MHz; 25.0 ºC; TMS).

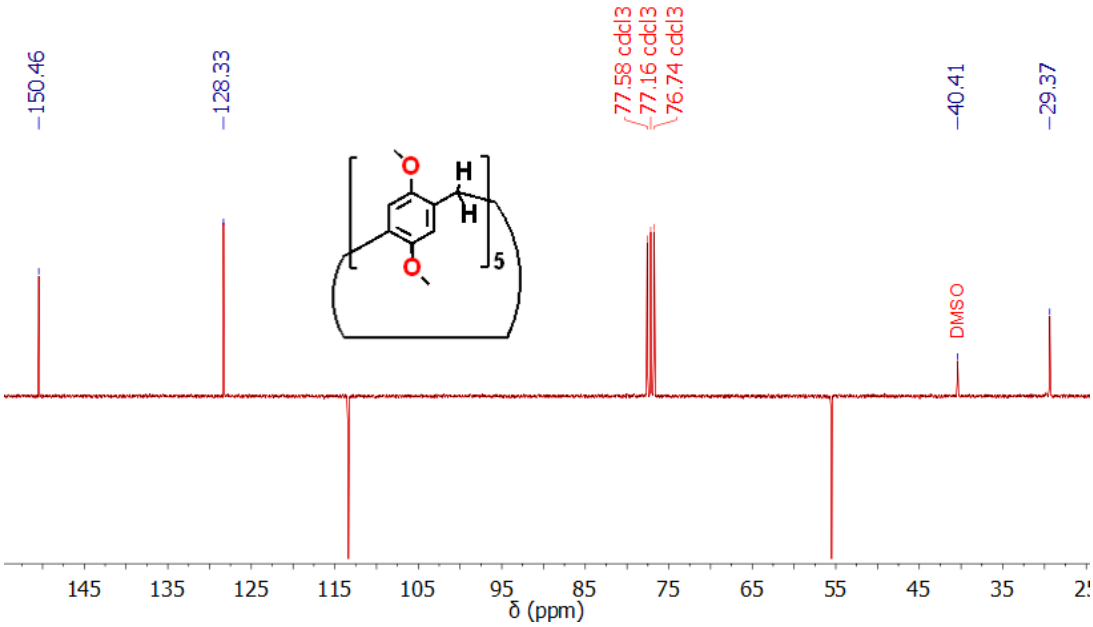


Fig. S1B ^{13}C APT NMR spectrum of **(2)** ($CDCl_3$; 75 MHz; 25.0 ºC; residual solvent as reference).

(Step 2): To a solution of **(2)** (0.800 mmol) in CH_2Cl_2 (24 ml), boron tribromide (38.9 mmol) was added at 0 °C. The reaction mixture was stirred under N_2 atmosphere for 96 h at room temperature. H_2O was added and the system was stirred for further 72 h. The solid formed was collected by filtration, washed with H_2O and dried under vacuum to yield 90% of compound **(3)**. ^{1}H NMR (300 MHz, Acetone-D_6, TMS), δ (ppm): 8.09 (s,

1H); 6.68 (s, 1H); 3.58 (s, 1H). ^{13}C APT NMR (75 MHz, Acetone-D_6, residual solvent as reference), δ (ppm): 147.44 (C); 127.99 (C); 118.27 (CH); 30.65 (CH_2).

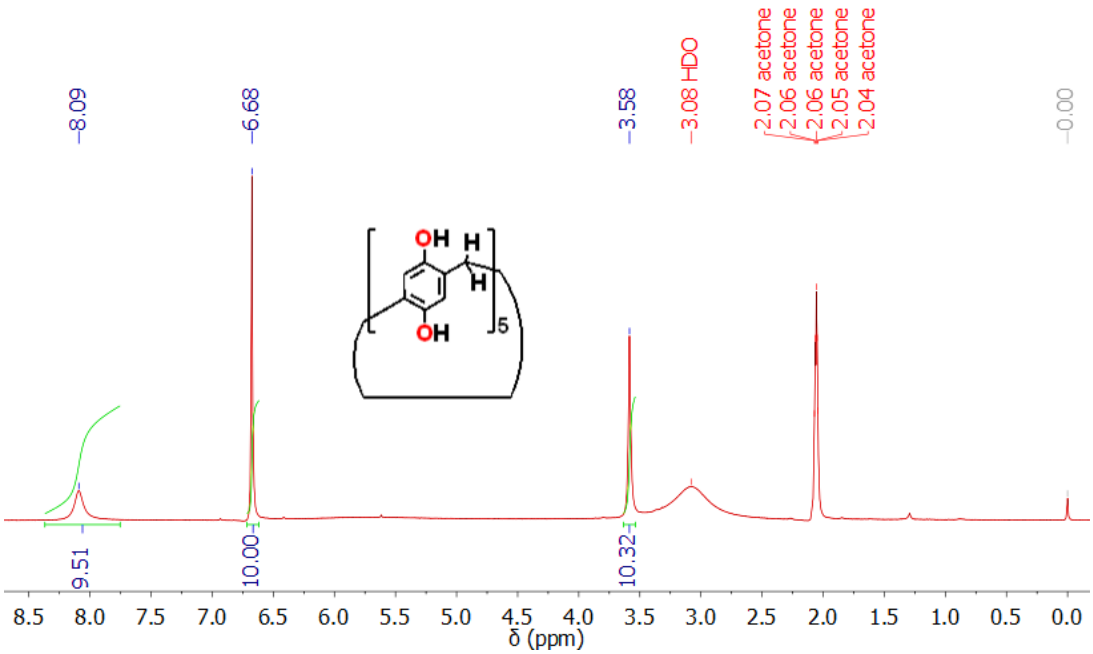


Fig. S2A ^{1}H NMR spectrum of **(3)** (Acetone-D_6; 300 MHz; 25.0 ºC; TMS).

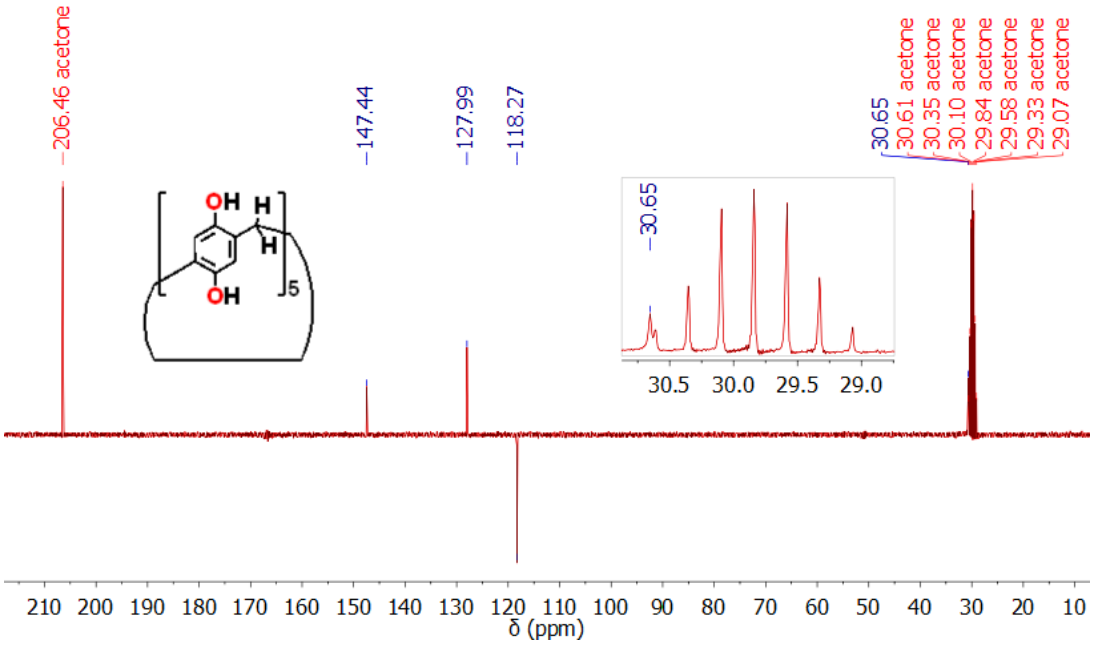


Fig. S2B ^{13}C APT NMR spectrum of **(3)** (Acetone-D_6; 75 MHz; 25.0 ºC; residual solvent as reference).

(Step 3): Methyl chloroacetate (1.64 mmol) and K_2CO_3 (3.28 mmol) were added to a solution of **(3)** (0.082 mmol) in dry CH_3CN (3.5 ml). The mixture was kept under reflux

for 48 h and then filtered and washed with $CHCl_3$. The solvent was removed giving the product **(4)** with 35% of yield after purification. ^1H NMR (500 MHz, $CDCl_3$, TMS), δ (ppm): 6.99 (s, 1H); 4.55 (s, 2H); 3.85 (s, 1H); 3.55 (s, 3H). ^{13}C APT NMR (75 MHz, $CDCl_3$, residual solvent as reference), δ (ppm): 169.93 (C); 149.07 (C); 128.63 (C); 114.62 (CH); 65.63 (CH_2); 51.85 (CH_3); 29.43 (CH_2).

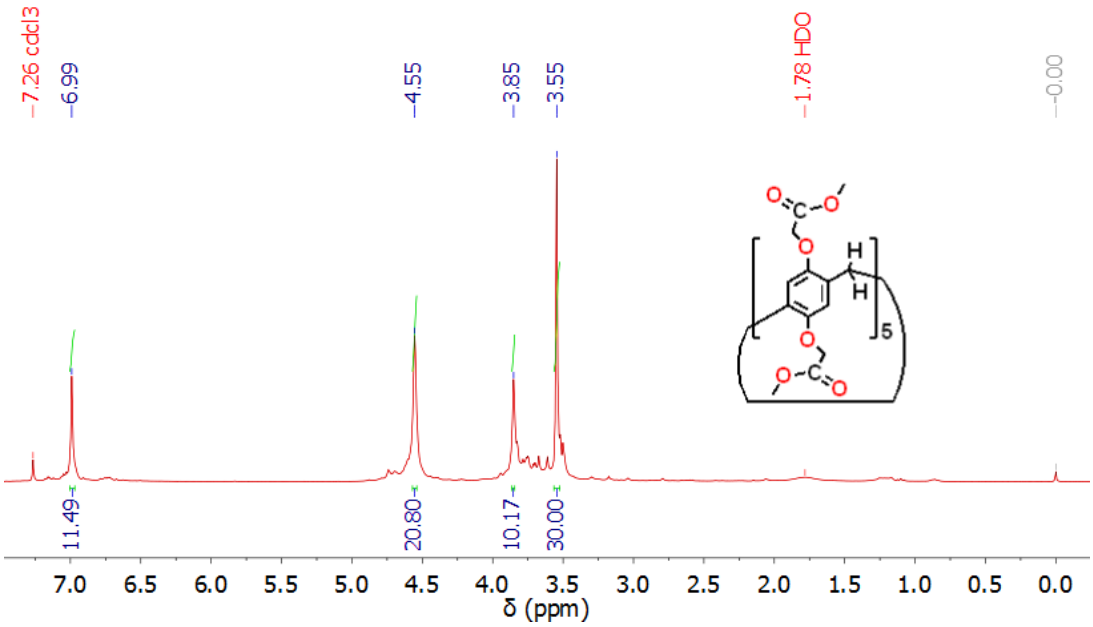


Fig. S3A ^1H NMR spectrum of **(4)** ($CDCl_3$; 500 MHz; 25.0 ºC; TMS).

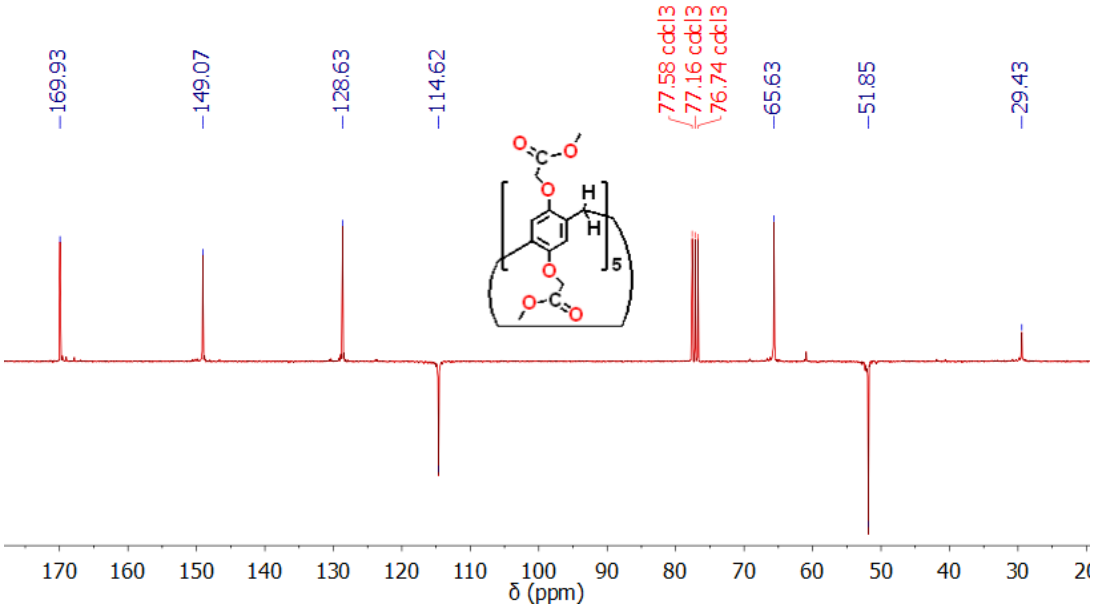


Fig. S3B ^{13}C APT NMR spectrum of **(4)** ($CDCl_3$; 75 MHz; 25.0 ºC; residual solvent as reference).

(Step 4): A solution of **(4)** (0.046 mmol) in EtOH (4 mL) was treated with 40% aqueous sodium hydroxide (4 mL) in reflux for 16 h. The reaction mixture was concentrated under reduced pressure and acidified with HCl. The precipitated product was collected by filtration, washed with H_2O and dried under vacuum to yield 99% of compound **(5)** (P5C). ^{1}H NMR (200 MHz, DMSO-D_6, TMS), δ (ppm): 7.06 (s, 1H); 4.66 (d, J = 15.9 Hz, 1H); 4.41 (d, J = 15.8 Hz, 1H); 3.73 (s, 1H). ^{13}C{^{1}H} NMR (50 MHz, DMSO-D_6, residual solvent as reference), δ (ppm): 170.29; 148.62; 127.58; 114.29; 65.39; 28.48.

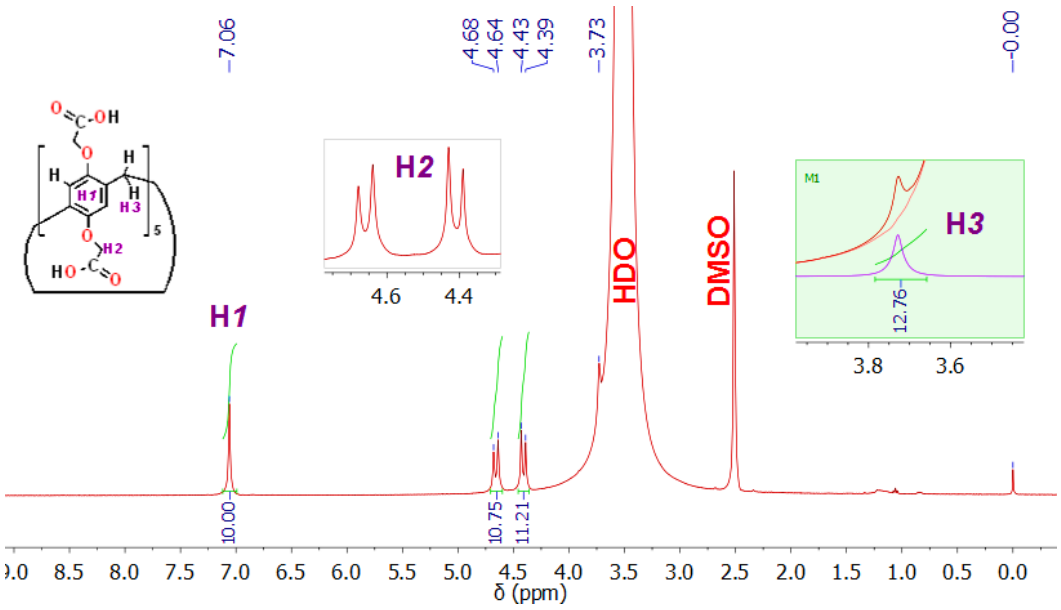


Fig. S4A ^{1}H NMR spectrum of P5C (DMSO-D_6; 200 MHz; 25.0 ºC; TMS). The total area of the H*3* (superposed by HDO) was obtained by fitting of its line.

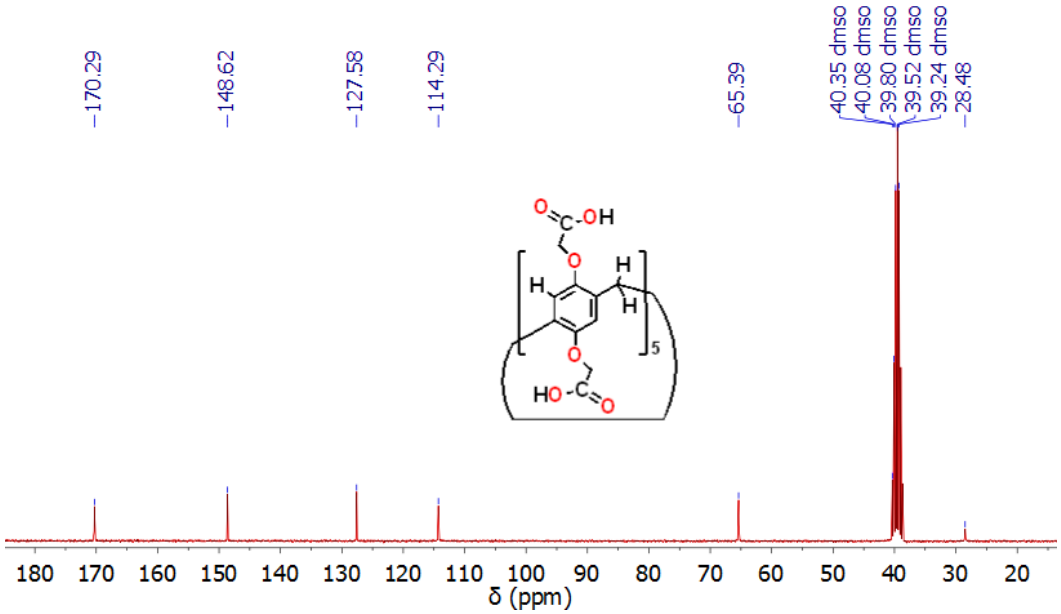


Fig. S4B ^{13}C{^{1}H} NMR spectrum of P5C (DMSO-D_6; 50 MHz; 25.0 ºC; residual solvent as reference).

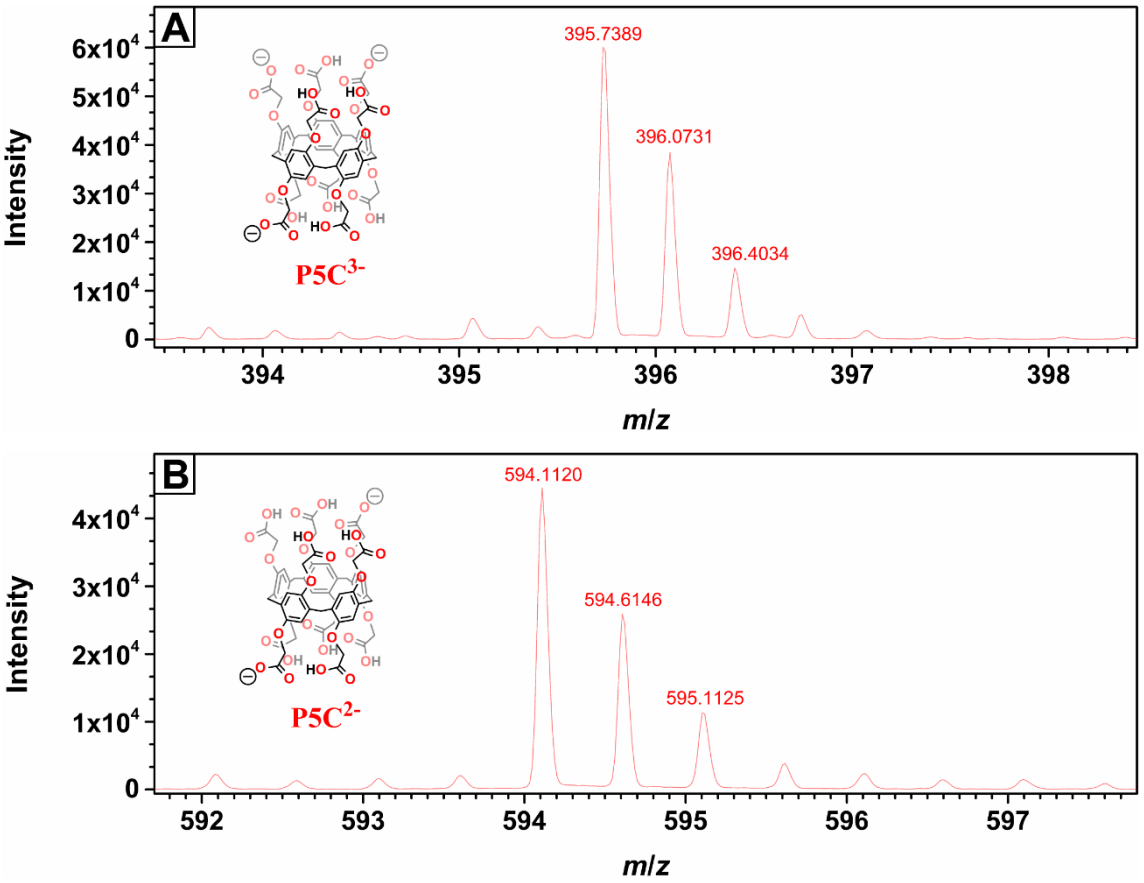


Fig. S5 HRMS/ESI-TOF spectra of aqueous solution of P5C in negative ion mode. For $[P5C–3H^+]^{3-}$ ($C_{55}H_{47}O_{30}$): calculated 395.7383, found 395.7389 (**A**). For $[P5C–2H^+]^{2-}$ ($C_{55}H_{48}O_{30}$), calculated 594.1124, found 594.1120 (**B**).

2. Synthesis of Methamphetamine (Meth)

The methamphetamine was obtained by reduction of the pseudoephedrine by the Moscow method (I_2 + P) followed by purification (acid-base extractions and Kugelrohr distillation). Yield of 53% as a colorless oil. ^{1}H NMR (200 MHz, MeOD-D_4, TMS), δ (ppm): 7.32–7.16 (m, 5H); 2.77 (m, 2H); 2.51 (m, 1H); 2.35 (s, 3H); 1.00 (d, J = 6.2 Hz, 3H). ^{13}C{^{1}H} NMR (50 MHz, MeOD-D_4, residual solvent as reference), δ (ppm): 140.47; 130.29; 129.49; 127.32; 57.69; 43.78; 33.64; 19.05.

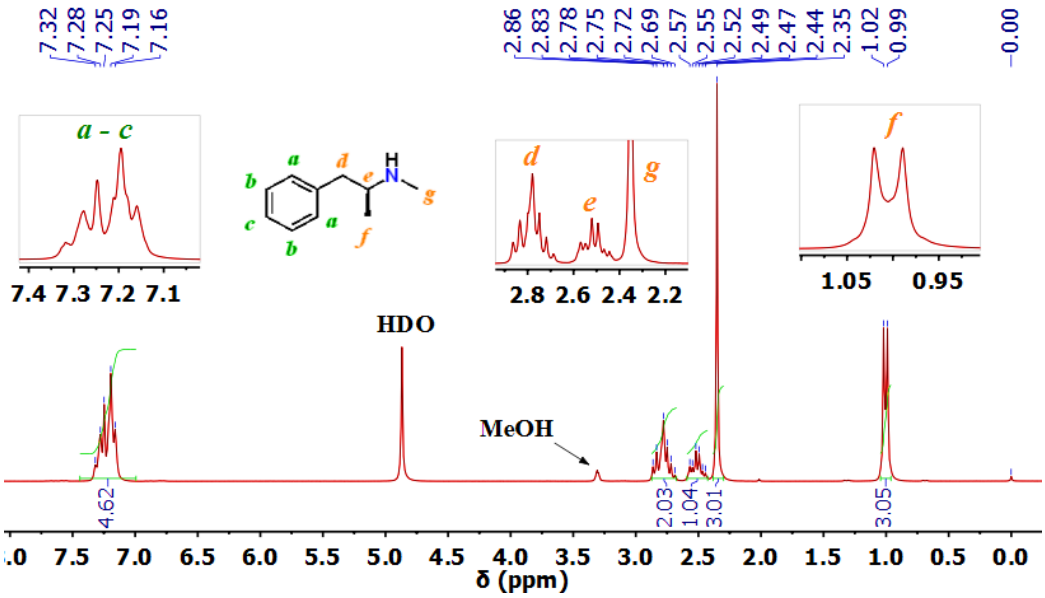


Fig. S6A ^{1}H NMR spectrum of methamphetamine (MeOD-D_4; 200 MHz; 25.0 ºC; TMS).

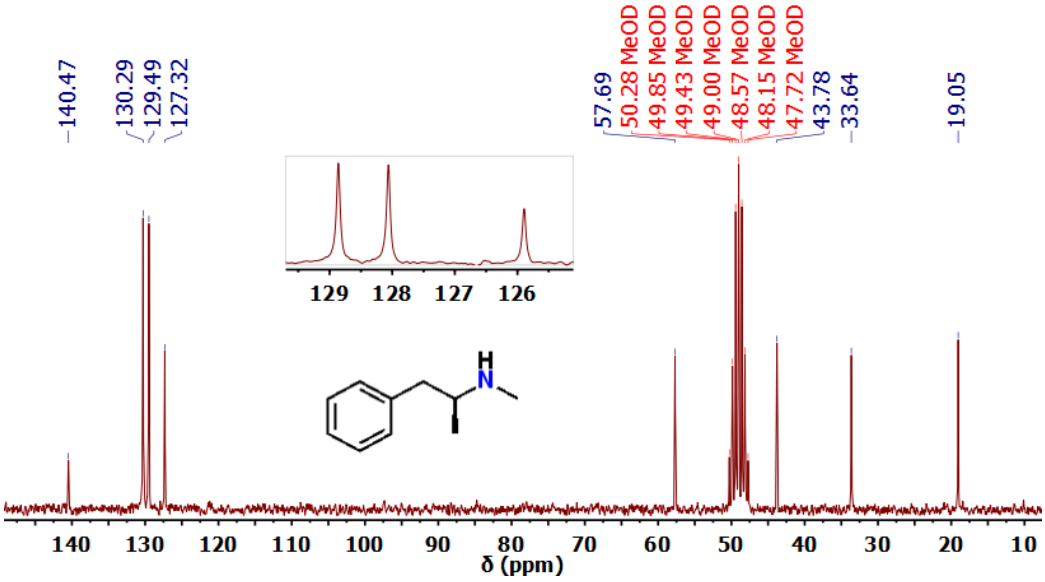


Fig. S6B ^{13}C{^{1}H} NMR spectrum of methamphetamine (MeOD-D_4; 50 MHz; 25.0 ºC; residual solvent as reference).

3. Potentiometric Titration

A potentiometric titration of P5C was performed in H_2O:CH_3CN 8:2 (v/v), employing a Hanna HI1330 combined glass electrode coupled to a Hanna HI 113 pH meter. A sample containing P5C (4.675×10^{-6} mol) at an initial pH of 9.685 was titrated at 22.0 ºC under N_2 with a standard HCl solution (8.8305×10^{-3} M) containing the same H_2O:CH_3CN ratio. All solutions, including those for the HCl solution standardization, were prepared with previously boiled deionized water. The titration profile was analyzed employing the CurtiPot software.[3]

The data fitting of the potentiometric titration profile of P5C (Fig. 1, see manuscript) was performed considering the independence of its carboxyl groups and equivalence between its two portals, resulting in 5 different pK_as for the macrocycle, as shown below. From this, the relative distribution of species was calculated as a function of pH (Fig. S7), with the electrostatic destabilization effect between the carboxyl groups increasing with pH.

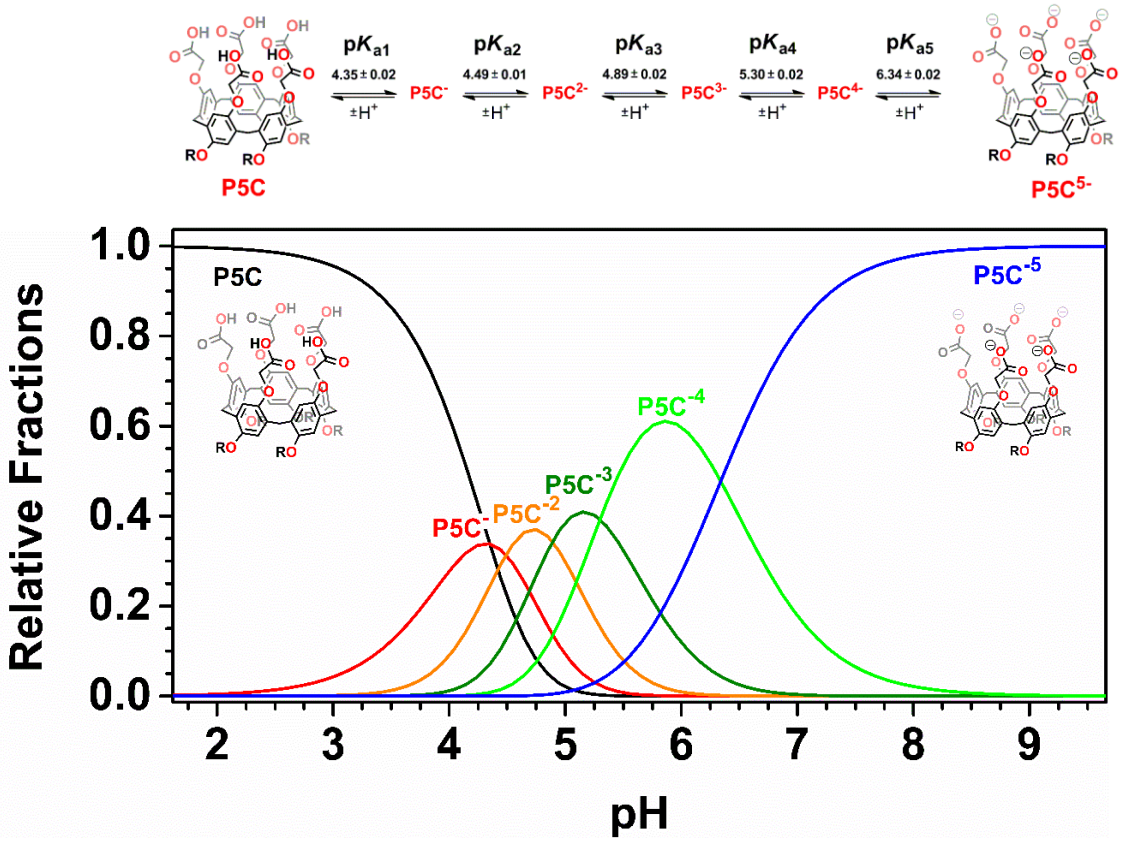


Fig. S7 Relative fractions of the P5C species as a function of pH.

4. NMR Experiments

The NMR experiments were performed using Bruker AC 200 and 400 MHz spectrometers. For the ^{1}H NMR titration, to the NMR tube containing the P5C solution ([P5C] = 11.51 mM in D_2O; pD 7.00; [Bis-Tris methane] = 0.01 M; TMSP), aliquots of concentrated solutions of Meth were added (D_2O; pD 7.00; [Bis-Tris methane] = 0.01 M; TMSP), and after its total homogenization the spectra were collected. Mixing time of 225 ms was used in the ^{1}H ROESY experiment.

4.1 ^{1}H NMR titration

The ^{1}H NMR titration was performed to provide structural elucidation of the complexes and the affinity between P5C and Meth. Fig. 2 (see manuscript) show the binding isotherms for all hydrogens of the Meth and P5C, these data being obtained from the successive spectra shown below.

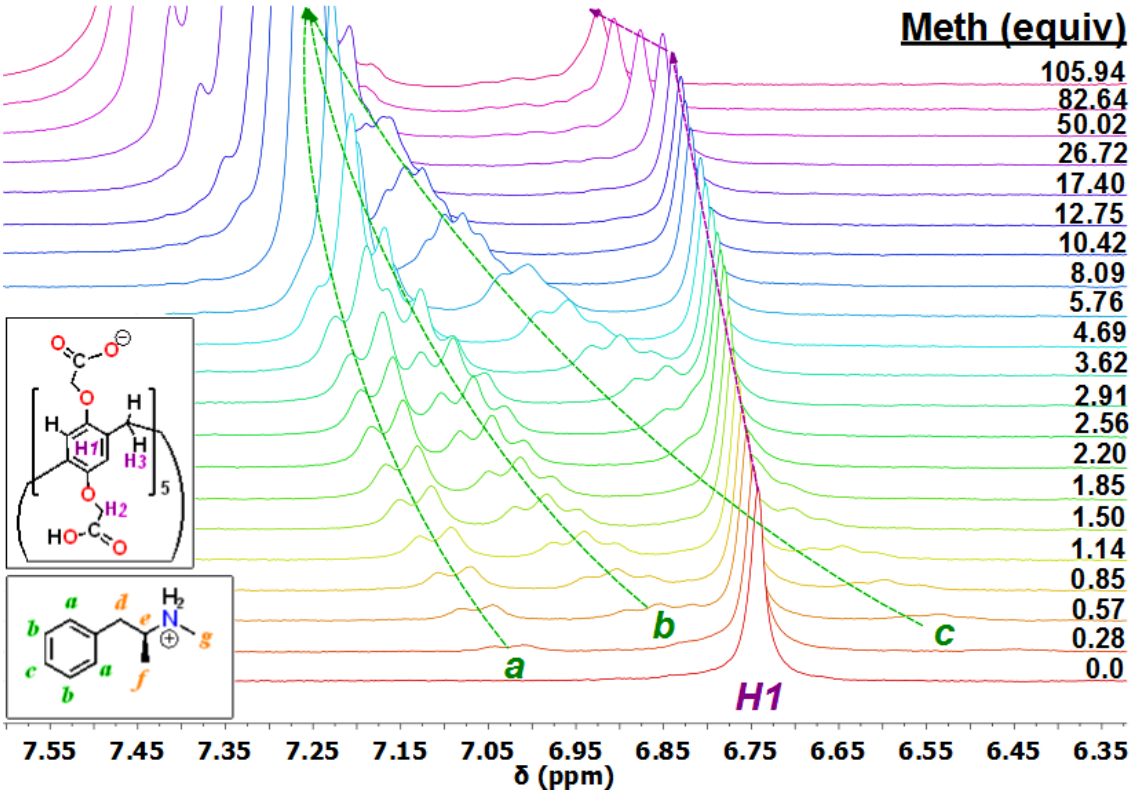


Fig. S8 Successive spectra of the ^{1}H NMR titration at different conditions of $[Meth]_0/[P5C]_0$ showing the chemical shifts observed for the aromatic hydrogens of Meth (H*a*, H*b* and H*c*) and P5C (H*1*).

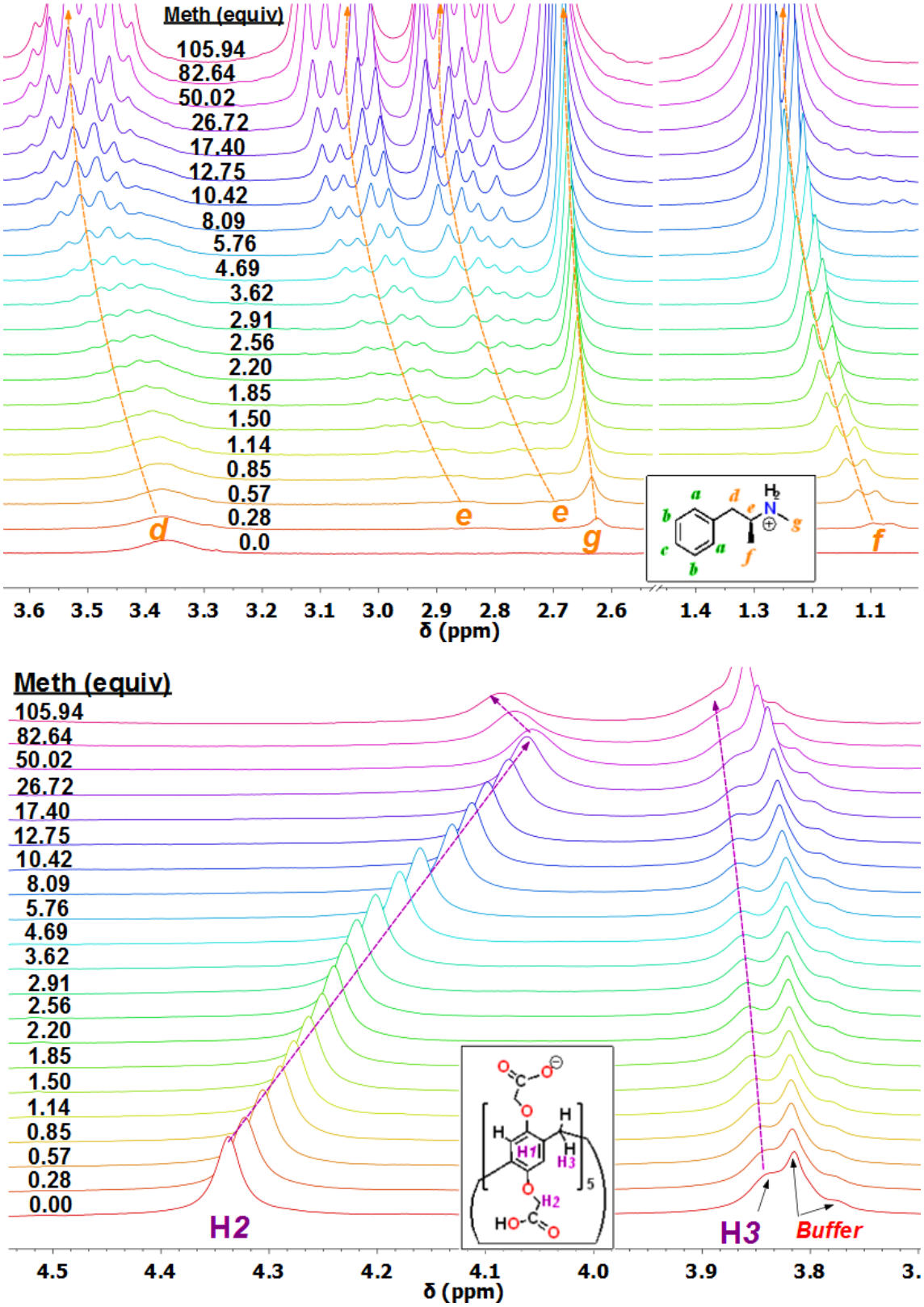


Fig. S9 Successive spectra of the ^{1}H NMR titration at different conditions of $[Meth]_0/[P5C]_0$ showing the chemical shifts observed for the aliphatic hydrogens of Meth (H*d*, H*e*, H*g* and H*f*, **Above**) and P5C (H*2* and H*3*, **Below**).

4.2 Treatment of the ^{1}H NMR titration data

The graphic of Δδ *vs*. $[Meth]_0/[P5C]_0$ for H*f* hydrogen was fitted to H:G, H:G_2 and H_2:G to investigate the stoichiometry and magnitude of the binding constants. For this, different models were used,[4] all presented below:

H:G stoichiometry

Assuming only the existence of the H:G complex (H + G ⟛ H:G), the binding constant is defined by:

$$K_{1:1} = \frac{[\mathrm{H{:}G}]}{[\mathrm{H}]\cdot[\mathrm{G}]}$$

Since the free H and G concentrations can not be measured directly, an alternative approach to use $[P5C]_0$ and $[Meth]_0$ can be solved, reaching:

$$[\mathrm{H{:}\,G}] = \frac{1}{2}\left\{[\mathrm{P5C}]_0 + [\mathrm{Meth}]_0 + \frac{1}{K_{1:1}} - \sqrt{\left([\mathrm{P5C}]_0 + [\mathrm{Meth}]_0 + \frac{1}{K_{1:1}}\right)^2 - 4[\mathrm{P5C}]_0[\mathrm{Meth}]_0}\right\}$$

Lastly, the experimental data was fitted to the Equation S1:

$$\Delta\delta = \delta_{1:1}\left(\frac{[\mathrm{H{:}G}]}{[\mathrm{H}]_0}\right) \qquad \text{(S1)}$$

H:G_2 stoichiometry

Assuming the existence of high order complexes in relation to Meth (H + G ⟛ H:G + G ⟛ H:G_2), the macroscopic binding constants are defined by:

$$K_{1:1} = \frac{[\mathrm{H{:}G}]}{[\mathrm{H}]\cdot[\mathrm{G}]} \qquad K_{1:2} = \frac{[\mathrm{H{:}G_2}]}{[\mathrm{H{:}G}]\cdot[\mathrm{G}]}$$

Thus, the free Meth concentration can be determined by the cubic equation below:

$$a[\mathrm{Meth}]^3 + b[\mathrm{Meth}]^2 + c[\mathrm{Meth}] - [\mathrm{Meth}]_0 = 0$$

Where:

$$a = K_{1:1}K_{1:2}$$

$$b = K_{1:1}(2K_{1:2}[\mathrm{P5C}]_0 - K_{1:2}[\mathrm{Meth}]_0 + 1)$$

$$c = K_{1:1}([\mathrm{P5C}]_0 - [\mathrm{Meth}]_0) + 1$$

Lastly, the experimental data was fitted to the Equation S2:

$$\Delta\delta = \frac{\delta_{1:1}K_{1:1}[\mathrm{Meth}] + \delta_{1:2}K_{1:1}K_{1:2}[\mathrm{Meth}]^2}{1 + K_{1:1}[\mathrm{Meth}] + K_{1:1}K_{1:2}[\mathrm{Meth}]^2} \qquad \text{(S2)}$$

H_2:G stoichiometry

Assuming the existence of high order complexes in relation to P5C (H + G $\rightleftharpoons$ H:G + H $\rightleftharpoons$ H_2:G), the macroscopic binding constants are defined by:

$$K_{1:1} = \frac{[\mathrm{H{:}G}]}{[\mathrm{H}]\cdot[\mathrm{G}]} \qquad K_{2:1} = \frac{[\mathrm{H_2{:}G}]}{[\mathrm{H{:}G}]\cdot[\mathrm{H}]}$$

Thus, the free P5C concentration can be determined by the cubic equation below:

$$a[\mathrm{P5C}]^3 + b[\mathrm{P5C}]^2 + c[\mathrm{P5C}] - [\mathrm{P5C}]_0 = 0$$

Where:

$$a = K_{1:1}K_{2:1}$$

$$b = K_{1:1}(2K_{2:1}[\mathrm{Meth}]_0 - K_{2:1}[\mathrm{P5C}]_0 + 1)$$

$$c = K_{1:1}([\mathrm{Meth}]_0 - [\mathrm{P5C}]_0) + 1$$

Lastly, the experimental data was fitted to the Equation S3:

$$\Delta\delta = \frac{\delta_{1:1}[\mathrm{Meth}]_0K_{1:1}[\mathrm{P5C}] + 2\delta_{2:1}[\mathrm{Meth}]_0K_{1:1}K_{2:1}[\mathrm{P5C}]^2}{[\mathrm{H}]_0(1 + K_{1:1}[\mathrm{P5C}] + K_{1:1}K_{2:1}[\mathrm{P5C}]^2)} \qquad \text{(S3)}$$

For fitting data Bindfit online tool was used. Table S1 shows the values of $K_{1:1}$ and $K_{1:2}$ or $K_{2:1}$ for H*f*. To access each data file click at Bindfit (blue color) or use the corresponding link. Alternatively, these data are shown in the Fig. S10.

Table S1. Binding constants obtained from fitting experimental data of H*f* to Equations S1-S3 (using Bindfit online tool, supramolecular.org).

Stoichiometry	Link	$K_{1:1}$ (M^{-1})	$K_{1:2}$ or $K_{2:1}$ (M^{-1})
H:G	Bindfit[(a)]	83.42 ± 2.9%	---
H:G_2	Bindfit[(b)]	$(4.42 \times 10^{14}) \pm 10^{8}$%	20.96 ± 2.3%
H_2:G	Bindfit[(c)]	23.42 ± 5.3%	28.72 ± 5.3%

[(a)] http://app.supramolecular.org/bindfit/view/ac04073e-05eb-42ed-adb8-e18583988a79
[(b)] http://app.supramolecular.org/bindfit/view/16ff20f6-bc9e-4d69-847a-a47dc737628b
[(c)] http://app.supramolecular.org/bindfit/view/733e249f-89b5-426a-958d-b856bb38a306

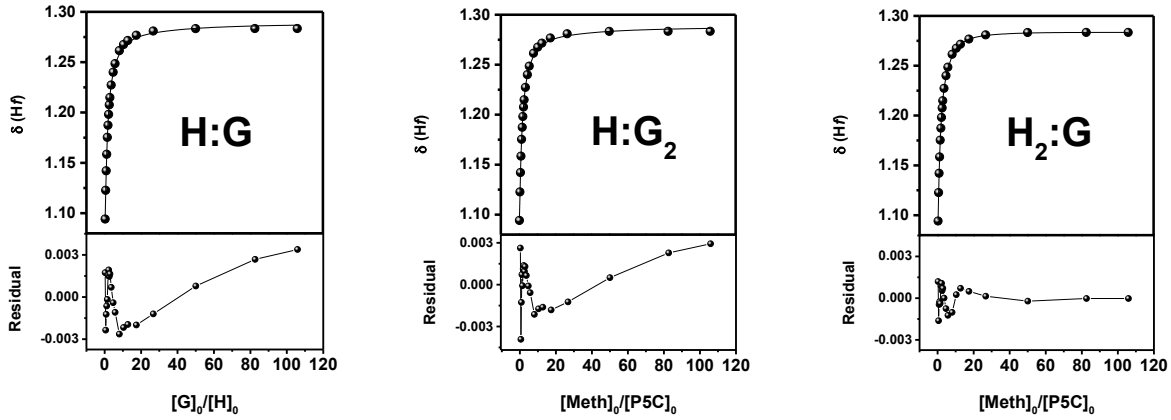


Fig. S10 ^{1}H NMR chemical shifts for H*f* in response to the increased $[Meth]_0/[P5C]_0$ ratio, with data fitting and residuals to H:G (**left**), H:G_2 (**middle**) and H_2:G (**right**) stoichiometries (pD 7.00; [Bis- Tris methane] = 10.0 mM; 25.0 ºC; 200 MHz).

5. DLS Experiment

A dynamic light scattering (DLS) titration under the same conditions of ^{1}H NMR titration was performed on a ZetaPlus Brookhaven equipment to evaluate the hydrodynamic diameter (D_H) as a function of the Meth addition. Solutions were prepared and manipulated in a clean chamber (TROX Chapel class 100) using nitrile gloves (free of talc) in a certified white laboratory. All samples were filtered twice through PVDF 0.20 μm filters (ChromafilXtraCA-20/25) before titration.

The first titration point (P5C only) revealed a D_H value close to 1 nm (Fig. S11A), being consistent with sizes already determined for structurally similar pillararenes.[5] The increase in $[Meth]_0/[P5C]_0$ ratio (Fig. S11B-D) resulted in the appearance of species close to 2 nm, which corroborates the formation of dimers of P5C, that is, the observed H_2:G complex with Meth. We emphasize that the size range measurable by the equipment (2 nm to 3 μm) can generate some inaccuracy in the distribution of the D_H values, which lead us to consider this data only to exclude the possibility of formation of larger aggregates or tube-type self-assemblies (**P5C--Meth--P5C--Meth--P5C**…).

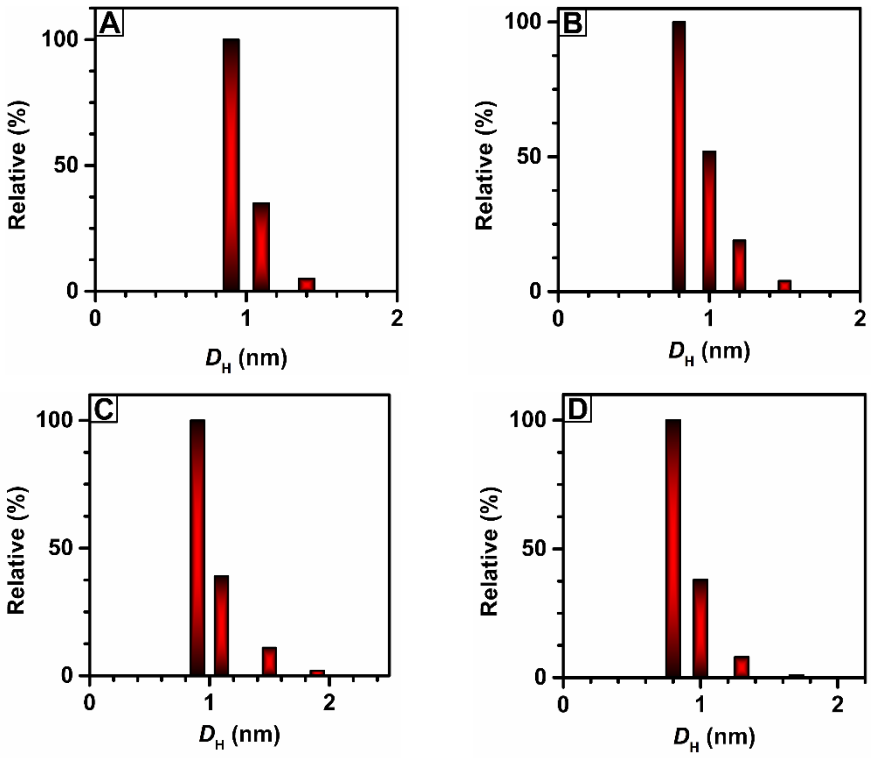


Fig. S11 D_H distribution of aqueous solutions of P5C (**A**) and $[Meth]_0/[P5C]_0$ ratios of 5 (**B**), 15 (**C**) and 30 (**D**), (pH 7.00; [P5C] = 10.0 mM; [Bis-Tris methane] = 10.0 mM; 25.0 ºC).

6. References

(1) G. Yu, M. Xue, Z. Zhang, J. Li, C. Han and F. Huang, *J. Am. Chem. Soc.* 2012, **134**, 13248–13251.

(2) J. Yang, G. Yu, D. Xia and F. Huang, *Chem. Commun*., 2014, **50**, 3993–3995.

(3) I. G. R. Gutz. Available at: http://www.iq.usp.br/gutz/Curtipot.html. Accessed: 2 May 2019.

(4) P. Thordarson, *Chem. Soc. Rev.*, 2011, **40**, 1305–1323.

(5) M. Panneesrselvam, M. D. Kumar, M. Jaccob and R. V. Solomon, *ChemistrySelect*. 2018, **3**, 1321–1334.