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Supporting Information

Advanced ^{19}F-NMR Studies Shed New Light on Encapsulation of Isosteric Guests in the Hexameric Capsules of Resorcin[4]arenes and Pyrogallol[4]arenes

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Materials. All starting materials, reagents, guest molecules and the deuterated solvents were purchased from Sigma-Aldrich and were used as received. Compounds **1** and **2** were prepared according to modifications of the previously published procedures.[1,2]

General. ^{1}H NMR and ^{19}F NMR spectra, diffusion NMR measurements and ^{19}F-GEST NMR measurements were performed on 11.7T Avance III Bruker NMR spectrometer.

Diffusion NMR. For the diffusion measurements, the samples were placed in 4mm NMR tubes that were then placed coaxially in 5mm NMR tubes, which act as a thermal insulating system and increase the accuracy and reproducibility of the diffusion measurements by reducing the chance of convections in the sample. This precaution is more important when diffusion NMR experiments are performed on non-viscous solvents with low boiling points and heat capacities. Diffusion NMR were performed with a z-gradient system capable of producing maximal gradient pulses of about 50 gauss cm^{-1}. ^{1}H- and ^{19}F-diffusion NMR experiments were performed using the longitudinal eddy currents delays (LED) pulse sequence.[3] Sine-shaped pulse gradients, of 4ms duration, were incremented from 0.7 to 32.2 gauss cm^{-1} in 10 steps, the pulse gradient separation was 50ms, and the t_e was set to 5ms. The diffusion coefficients were extracted from:

$$ln\ I/I_0=-\gamma^2\delta^2G^2(2/\pi)^2(\Delta-\delta/4)D=-bD$$

where I and I_0 are the echo intensity, in the presence and absence of the gradient pulse, respectively, γ is the gyromagnetic ratio, G is the pulse gradient strength, $2/\pi$ is a geometrical correction factor due to the sine shape of the pulse gradients used, δ is the duration of the pulse gradient, Δ is the time interval between the leading edges of the pulse gradient used, and D is the diffusion coefficient. The diffusion coefficients were extracted from the slope of the plot of ln I/I_0 versus the b value. All diffusion NMR data were acquired at 298K and were obtained in triplicate. The given values represent means ± the standard deviation.

^{19}F-GEST. ^{19}F-GEST experiments were performed on 11.7T NMR instrument at 470MHz. A pre-saturation pulse with varying intensity and of 2sec of duration was applied prior to the 90° pulse. The frequency of the pre-saturation pulse was swept from $\Delta\omega$ = +3.2ppm to $\Delta\omega$ = -3.2ppm in 128 steps relative to the frequency of the free guest

that was set to 0ppm. In addition, a reference ^{19}F-NMR spectrum (S_0) was collected with a pre-saturation pulse applied at $\Delta\omega$ of +15ppm. For each frequency offset ($S_{\Delta\omega}$), four scans were collected using a repetition time of 8sec, resulting in a collection time of 31sec per ^{19}F-NMR spectrum. The total collection time of the entire GEST experiment was 70minutes. For k_{out} values estimation the z-spectra of the multi-B1 GEST experiments were fitted using the Bloch–McConnell equations, as recently described. [4-8] Simulations were performed on the z-spectra using custom-written scripts in MATLAB version 8.2.0.701 (The MathWorks, Natick, MA). The code for data fitting can be found at http://www.cest-sources.org/doku.php?id=start and can be found in the publication by Zaiss and Bachert.[4-8]

Table S1: Integration ratios of the signals of **1** and **2** (4.3-5ppm) vs. the signals of encapsulated **3** and **4**, in C_6D_6 and $CDCl_3$ solutions of 30mM of **1** and **2** and 10mM of **3** and **4.**

Sample	**Normalized integration ratios of host signals at 4-5ppm vs signals of encapsulated guests** [a]			
	#1	#2	#3	Average
1/3 in C_6D_6	1:0.42	1:0.44	1:0.49	0.45±0.04
1/3 in $CDCl_3$	1:0.63	1:0.64	1:0.64	0.63±0.01
1/4 in C_6D_6	1:0.28	1:0.27	1:0.30	0.28±0.02
1/4 in $CDCl_3$	NA	NA	NA	NA
2/3 in C_6D_6	1:0.17	1:0.18	1:0.19	0.18±0.01
2/3 in $CDCl_3$	1:0.12	1:0.12	1:0.13	0.12±0.01
2/4 in C_6D_6	1:0.40	1:0.41	1:0.40	0.40±0.01
2/4 in $CDCl_3$	NA	NA	NA	NA

[a] Taking into account that **4** has 3 hydrogens less than **3**.

For **1** in the C_6D_6 solutions, the encapsulation preference of **4** over **3** is 0.28/0.45 = 0.62, while for **2**, in the same solvent, it is 0.40/0.18 = 2.22 resulting in a preference ratio of more than 3 for the two capsules.

Table S2. ^{1}H Diffusion coefficients for representative peaks of **1**, **2**, **3** and **4** (500MHz, 298K) in the $CDCl_3$ solutions of 30mM of **1** or **2** and 10mM of **3** and **4**.

Sample	peak (ppm)	^{1}H Diffusion coefficients [x10^{-5} cm^2 s^{-1}]	
		No CD_3OD	With CD_3OD
1/**3** in $CDCl_3$	-0.06 (encapsulated **3**)	0.24 ± 0.01	NA
	1.9 (free **3**)	1.00 ± 0.01	1.10 ± 0.02[a]
	4.3 (**1**)	0.25 ± 0.01	0.41 ± 0.01[a]
	7.28 ($CHCl_3$)	1.96 ± 0.05	2.30 ± 0.02[a]
1/**4** in $CDCl_3$	2.0 (free **4**)	0.95 ± 0.01	
	4.3 (**1**)	0.25 ± 0.01	
	7.28 ($CHCl_3$)	1.90 ± 0.02	
2/**3** in $CDCl_3$	0.37 (encapsulated **3**)	0.23 ± 0.01	NA
	1.9 (free **3**)	1.06 ± 0.01	0.94 ± 0.01[b]
	4.3 (**2**)	0.23 ± 0.01	0.37 ± 0.01[b]
	7.28 ($CHCl_3$)	2.34 ± 0.01	1.91 ± 0.17[b]
2/**4** in $CDCl_3$	2.0 (free **4**)	1.01 ± 0.01	
	4.4 (**2**)	0.23 ± 0.01	
	7.28 ($CHCl_3$)	2.29 ± 0.02	

[a] For disruption of hexameric capsules of **1**/**3** in $CDCl_3$ 45µl of CD_3OD were added. [b] For disruption of hexameric capsule of **2**/**3** in $CDCl_3$ 150µl of CD_3OD were added.

Table S3. ^{19}F Diffusion coefficients of **4** (470MHz, 298K) in the C_6D_6 and $CDCl_3$ solutions of 30mM of **1** or **2** and 10mM of **4**.

Sample	Peak (ppm)	^{19}F Diffusion coefficients [x10^{-5} cm^2 s^{-1}]
1/**4** in C_6D_6	-143 (free **4**)	0.96 ± 0.01
2/**4** in C_6D_6	-145.7 (encapsulated **4**)	0.21 ± 0.01
	-143 (free **4**)	0.98 ± 0.01
1/**4** in $CDCl_3$	-143 (free **4**)	0.93 ± 0.01[a]
2/**4** in $CDCl_3$	-143 (free **4**)	1.03 ± 0.01

[a] Partial overlap with the small signal of the encapsulated guest4.

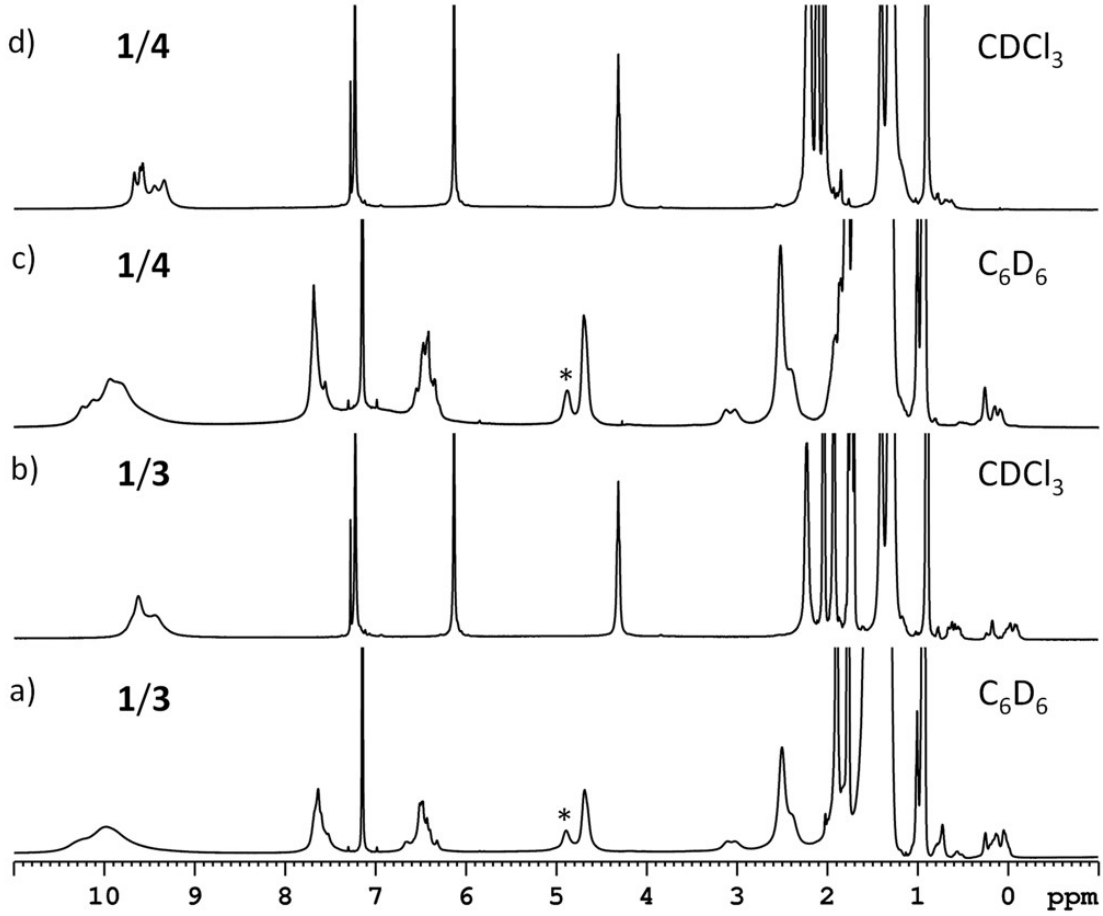

Figure S1. ^{1}H-NMR spectra (500MHz, 298K) of the solutions of 30mM of **1** and 100mM of **3** (a ,b) and **4** (c, d) in C_6D_6 (a, c) and $CDCl_3$ (b, d). The * symbols represent signals of the higher aggregates of **1** in C_6D_6.

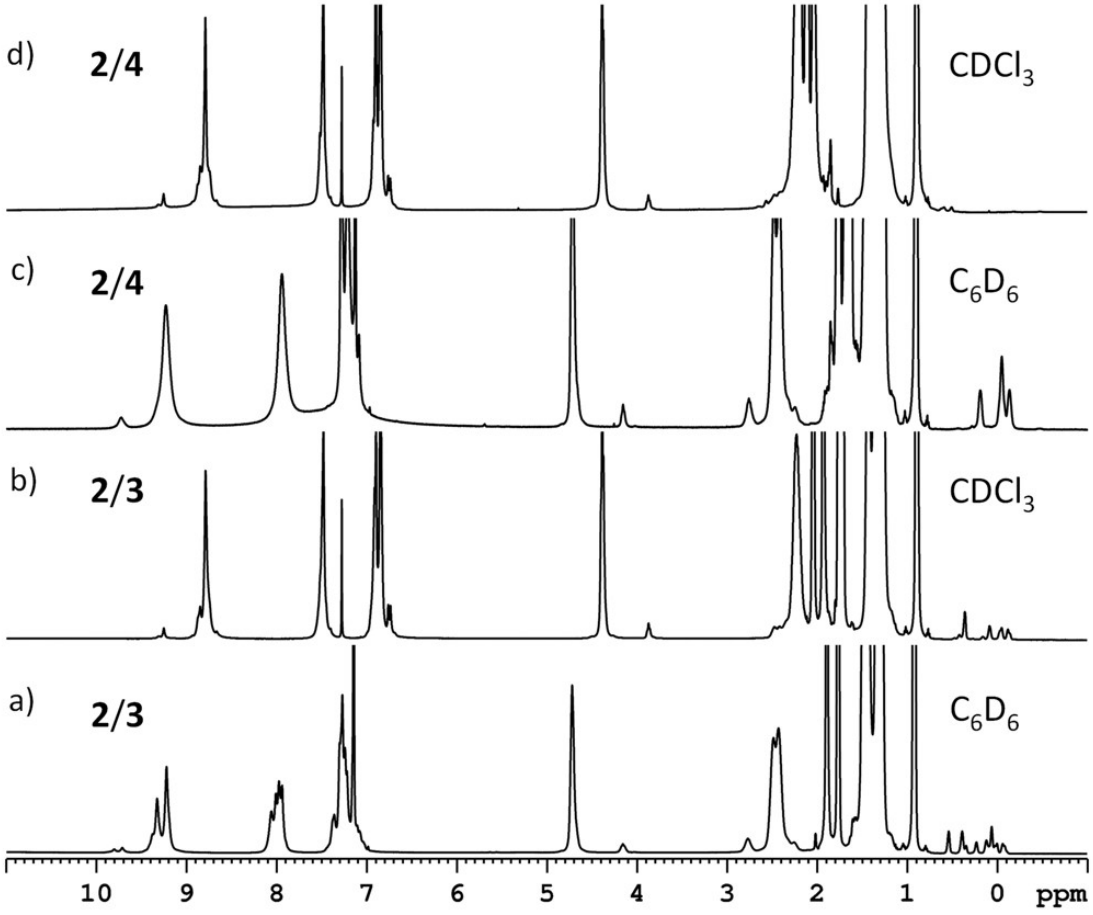

Figure S2. ^{1}H-NMR spectra (500MHz, 298K) of the solutions of 30mM of **2** and 100mM of **3** (a ,b) and **4** (c, d) in C_6D_6 (a, c) and $CDCl_3$ (b, d).

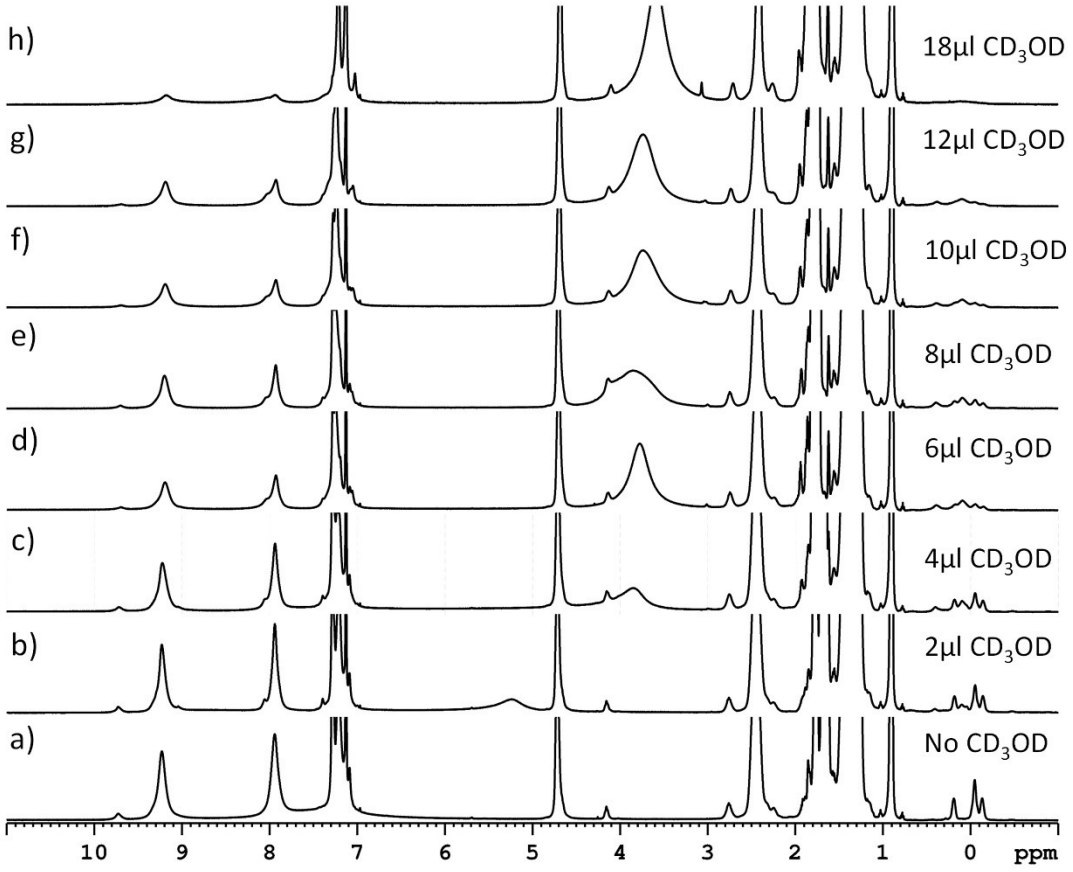

Figure S3. ^{1}H-NMR spectra (500MHz, 298K) of the C_6D_6 solution of 30mM of **2** and 100mM of **4** a) before and after addition of b) 2µl, c) 4µl, d) 6µl, e) 8µl, f) 10µl, g) 12µl and h) 18µl CD_3OD.

Table S4. ^{1}H Diffusion coefficients for representative peaks of **2**, and **4** (500MHz, 298K) in the C_6D_6 solution of 30mM of **2** and 100mM of **4**.

Sample	Peak (ppm)	^{1}H Diffusion coefficients [x10^{-5} cm^2 s^{-1}]			
		No CD_3OD	With 8µl CD_3OD	With 12µl CD_3OD	With 18µl CD_3OD
2/**4** in C_6D_6	-0.13 (encapsulated **4**)	0.20 ± 0.01	NA	NA	NA
	1.7 (free **4**)	0.80 ± 0.01	0.91 ± 0.01	0.91 ± 0.01	0.96 ± 0.01
	4.7 (**2**)	0.19 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.22 ± 0.01
	7.15 (C_6H_6)	1.77 ± 0.01	1.97 ± 0.01	1.94 ± 0.01	2.13 ± 0.02

Table S5. ^{19}F Diffusion coefficients of **4** (470MHz, 298K) in the C_6D_6 solution of 30mM of **2** and 100mM of **4** before and after addition of methanol.

Sample	Peak (ppm)	^{19}F Diffusion coefficients [x10^{-5} cm^2 s^{-1}]			
		No CD_3OD	With 8µl CD_3OD	With 12µl CD_3OD	With 18µl CD_3OD
2/**4** in C_6D_6	-145.7 (encapsulated **4**)	0.18 ± 0.01	NA	NA	NA
	-143.0 (free **4**)	0.81 ± 0.01	0.92 ± 0.01	0.91 ± 0.01	0.96 ± 0.01

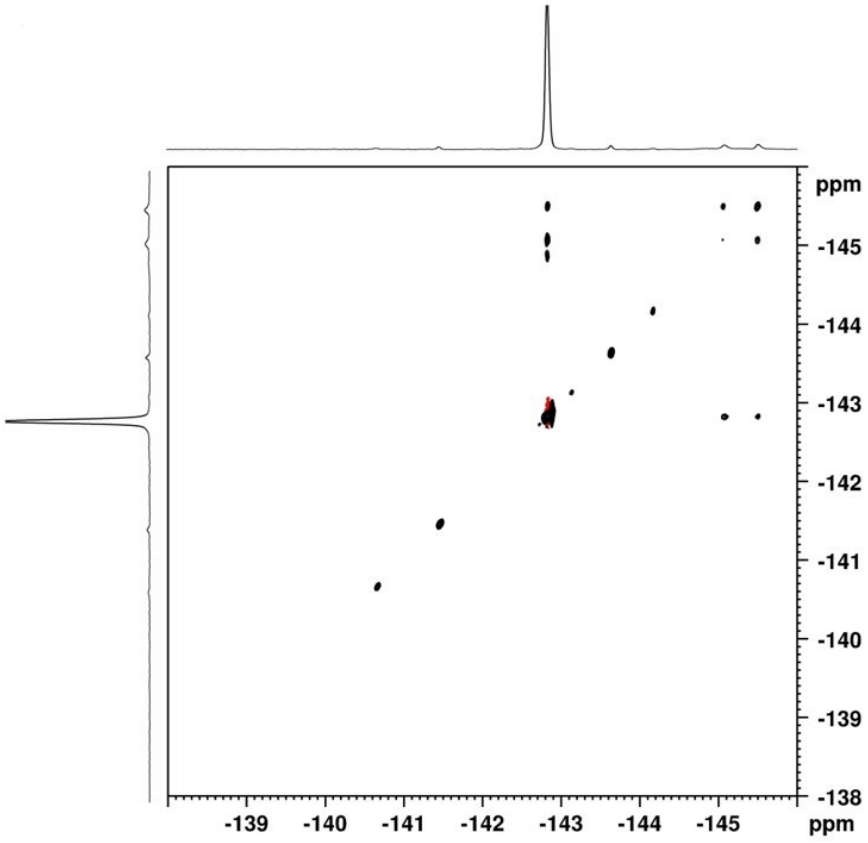

Figure S4. ^{19}F 2D NOESY experiment performed on the C_6D_6 solution of 30mM of **2** and 10mM of **4** (470MHz, 298K) after addition of 8µl of CD_3OD. The spectra was collected 18hours after sample preparation.

4. References

(1) Avram, L.; Cohen, Y. *J. Am. Chem. Soc.* **2002**, *124*, 15148-15149.

(2) Avram, L.; Cohen, Y. *J. Am. Chem. Soc.,* **200**4, *126*, 11556-11563.

(3) Gibbs, S. J.; Johnson, C. S. Jr., *J. Magn. Reson*., **1991**, 93, 395-402.

(4) M. Zaiss, P. Bachert, *NMR Biomed.,* **2013**, 26, 507-518.

(5) M. Zaiss, Z. Zu, J. Xu, P. Schuenke, D. F. Gochberg, J. C. Gore, M. E. Ladd, P. Bachert, *NMR Biomed*., **2015**, 28, 217-230.

(6) M. Zaiss, G. Angelovski, E. Demetriou, M. T. McMahon, X. Golay, K. Scheffler, *Magn. Reson. Med*., **2018**, 79, 1708-1721.

(7) L. Avram, V. Havel, R. Shusterman-Krush, M. A. Iron, M. Zaiss, V. Sindelar, A. Bar-Shir, *Chem. Eur. J*., **2019**, 25, 1687-1690.

(8) R. Shusterman-Krush, L. Grimm, L. Avram, F. Biedermann, A. Bar-Shir, *Chem. Sci.,* **2021***,* 12, 865-871.