# Encapsulated or Not Encapsulated? Mapping Alcohol Sites in Hexameric Capsules of Resorcin[4]arenes in Solution by Diffusion NMR Spectroscopy**

Sarit Slovak, Liat Avram, and Yoram Cohen

Noncovalent molecular capsules are labile and dynamic molecular species. Thus, the probing of their structure in solution is challenging, especially as the number of building units of the supramolecular system increases. Different noncovalent interactions have been used to construct such molecular capsules,[1] among which hydrogen bonds have played a pivotal role.[2–5] Hydrogen-bonded molecular capsules have been studied in the solid state,[2] in solution,[3,4] and recently also in the gas phase.[5] A decade ago, it was demonstrated that diffusion NMR spectroscopy[6] is a useful tool for probing encapsulation in solution.[7] Diffusion NMR spectroscopy revealed for example that the resorcin[4]arenes[8] **1a,b** self-assembled spontaneously in organic solvents

1a R=C₁₁H₂₃
1b R=isobutyl
1c R=CH₃
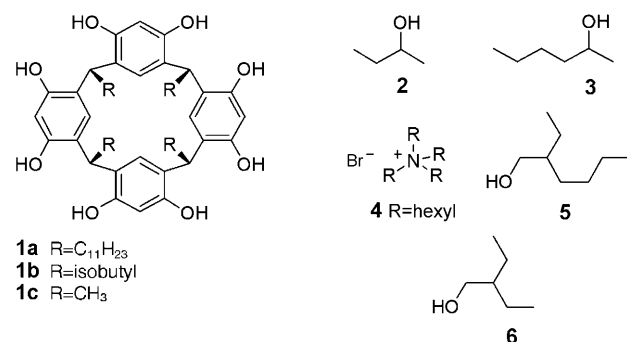
into hexameric capsules composed of six subunits of **1a,b** and eight water molecules.[8a–c] In such capsules, water molecules were found to be part of the capsule network and in fast exchange on the ¹H NMR time scale with the bulk water in the solution.[8a–c,f]

Nearly two decades ago, Aoyama and co-workers probed the interaction of **1a** with different alcohols and proposed the formation of 1:1 host–guest complexes.[9] Recently, Ugono and Holman reported the crystal structure of hexameric capsules of **1c** in which six of the eight water molecules were

replaced with 2-ethylhexanol (**5**).[10] Mattay and co-workers used diffusion NMR spectroscopy to probe the self-assembly of **1a** with various alcohols in water-free solutions and revealed that the alcohol used influences the self-assembly process. Different species were obtained when different alcohols were used.[11] Herein we show on the basis of diffusion NMR spectroscopy that, contrary to common belief, high-field chemical shifts of neutral guests, such as alcohols, in the presence of hexameric capsules of **1a** do not necessarily imply guest encapsulation. We show that despite similar ¹H NMR spectra in the presence of hexameric capsules, only some alcohols are indeed encapsulated, whereas others are part of the hexameric structure of the capsules.

In the ¹H NMR spectrum of a solution prepared from **1a** (25 mM) and 2-butanol (**2**; 1:10) in dry CDCl₃, new peaks were observed in the high-field region for **2** with a ratio to **1a** of 3–4:6 (Figure 1a). In previous studies of **1a**, such high-field peaks, which are in slow exchange with the bulk solution, were considered an indication of guest encapsulation in the hexameric capsule of **1a**.[4a,b] Therefore, one could erroneously conclude that these peaks represent the encapsulation of 2-butanol molecules in the hexameric capsule of **1a**.

However, the results of the diffusion measurements performed on this system were surprising and showed that interpretation of the data is more complex than it appears at first glance. The apparent diffusion coefficient (ADC) for the high-field peaks suspected to represent encapsulated 2-butanol molecules $[(0.63 \pm 0.03 \times 10^{-5})\ cm^2 s^{-1}]$ was much higher than that extracted for **1a** $[(0.24 \pm 0.01 \times 10^{-5})\ cm^2 s^{-1}]$ when diffusion was measured with the longitudinal eddy current delay (LED) sequence.[12] Since the
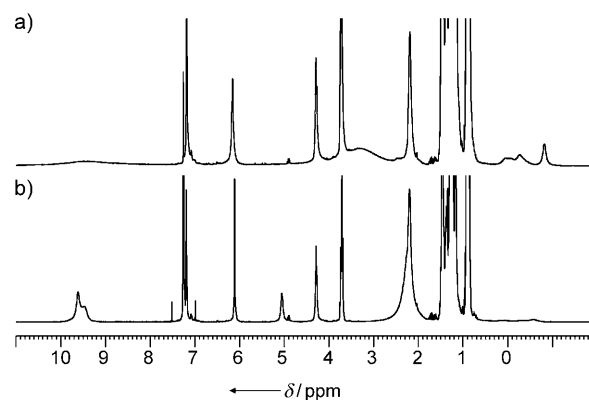
[*] S. Slovak, Dr. L. Avram, Dr. Y. Cohen
School of Chemistry, The Sackler Faculty of Exact Sciences
Tel Aviv University, Ramat Aviv, Tel Aviv 69978 (Israel)
Fax: (+972) 3-6407469
E-mail: ycohen@post.tau.ac.il

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Figure 1. ¹H NMR spectra of **1a** in the presence of **2** in dry CDCl₃ a) before and b) after the addition of water.

encapsulated guest and the host diffuse together as a single entity, their diffusion coefficient should be identical.[6,7] In addition to the difference in the diffusion coefficients, we also observed that the signal decay (measured with the LED sequence) of the high-field peaks was not monoexponential as is usually found for peaks of encapsulated guests in such systems.[8] We therefore began to suspect that **2** plays a different role in the self-assembly of **1a** in chloroform under our experimental conditions. We extracted two different diffusion coefficients from the signal decay of the high-field peaks: $(2.02 \pm 0.03 \times 10^{-5})$ cm^{2}s^{-1} and $(0.32 \pm 0.08 \times 10^{-5})$ cm^{2}s^{-1}. These values resemble the diffusion coefficients of free **2** $[(2.28 \pm 0.02 \times 10^{-5})$ cm^{2}s$^{-1}]$ and hexamers of **1a** $[(0.24 \pm 0.01 \times 10^{-5})$ cm^{2}s$^{-1}]$ in chloroform.

Alcohols in a solution of the hexameric capsule of **1a** can, in principle, occupy three main locations with the four arrangements shown in Figure 2: they can be encapsulated (site a in Figure 2), be part of the hexameric structure (sites b1 or b2 in Figure 2), or be in the bulk solution (site c in Figure 2). Since it is less likely that encapsulated 2-butanol molecules will be exchanged or will exchange magnetization with 2-butanol molecules in the bulk solution on the ^{1}H NMR time scale, we assumed that, under our experimental conditions, some of the water molecules in the structure of the hexameric capsule of **1a** were replaced by 2-butanol. Such molecules of **2** (in sites b1 or b2 in Figure 2) can exchange magnetization with molecules of **2** in the bulk solution.

To corroborate our findings, we studied how the eddy-current delay (t_e) of the LED sequence, which should have no effect on the diffusion results, affected the signal decay of the peaks of **1a** and the high-field peaks of **2** in this system and hence the diffusion results for these peaks (Figure 3). The signal decay of **1a** was monoexponential and was unaffected by the change in t_e (Figure 3a). In contrast, the signal decay of "bound" **2** was biexponential and was affected dramatically by the change in t_e (Figure 3b). As the delay t_e was increased, the slow component gradually disappeared, and the fast component became more predominant. Thus, when the eddy-current delay t_e was relatively long, we actually sampled more of the "free" component of **2**. On the basis of these results, we suspected that the high-field peaks of **2** do not represent encapsulated alcohol molecules but rather molecules of **2** which are part of the formed hexamers, as was recently shown for **5** in the solid state by Ugono and Holman.[10] We concluded that about three or four molecules of **2** are on the shell or the surface of the capsule (sites b1 or b2 in Figure 2); however, because of the high-field shift of the peaks of **2**, site b1 seems to be more consistent with the experimental results. Interestingly, the same trends were observed when 2-hexanol (**3**) was used instead of **2** (see Figure S1 in the Supporting Information).
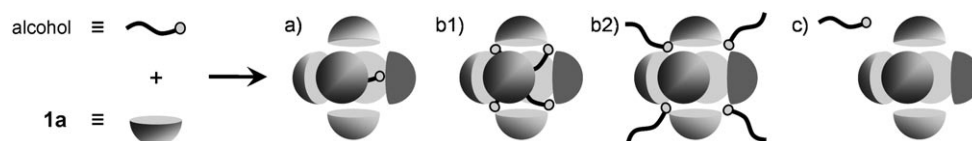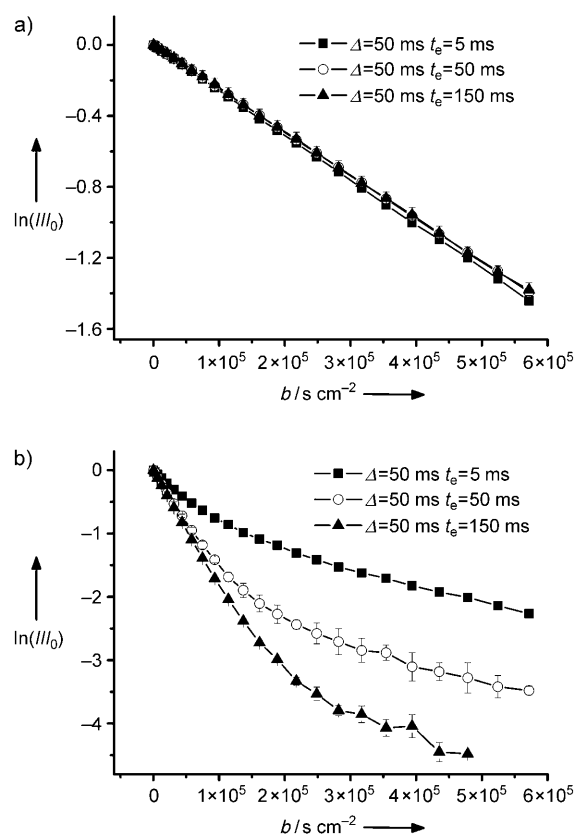

Figure 3. Natural logarithm of the normalized signal decay ($\ln(I/I_0)$) as a function of the b value (400 MHz, 298 K) for one of the peaks of a) **1a** and b) "bound" **2** in CDCl$_3$ as extracted from the LED sequence with different delay periods t_e: 5 ms (■), 50 ms (○), and 150 ms (▲). Δ is the time separation between gradient pulses.

To test our hypothesis that the molecules of **2** replace some of the water molecules in the structure of the hexamer of **1a** and occupy the surface of the hexameric capsule, we added water to the sample. Indeed, the signals at high field in the ^{1}H NMR spectrum disappeared (Figure 1b), probably because of the formation of a $[(\mathbf{1a})_6(H_2O)_8]$-type hexamer.[8] Similar results were observed for the solution of hexameric **1a** with **3**, in which case we found that three molecules of **3** were on the surface of the hexamer prior to the addition of water.

We decided to explore whether these diffusion characteristics apply to guests that are known to be only encapsulated within the capsule of **1a**. We chose tetrahexylammonium bromide (**4**) as a reference, since it is well-known that one molecule of this guest is encapsulated within the hexameric capsule of **1a**.[4a,8a] We prepared a 20 mM solution of **1a** in CDCl$_3$, to which we added **4**. As expected, the ^{1}H NMR spectrum revealed new peaks in the high-field region for **4** with a ratio to **1a** of 1:6; these peaks were attributed to the encapsulated guest (see Figure S2a in the Supporting Information).[4a,8a]

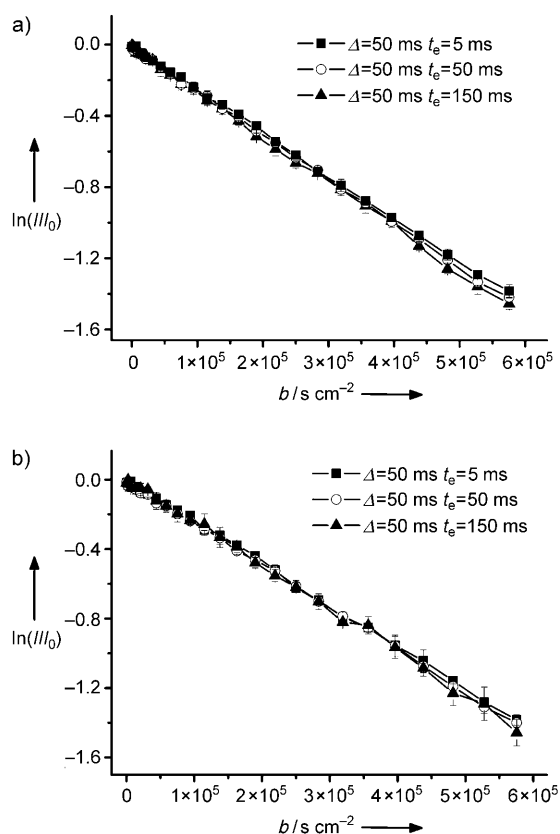Figure 4 shows the natural logarithm of the normalized signal decay ($\ln(I/I_0)$) as

Figure 2. Possible sites a–c occupied by alcohols in a solution of the hexameric capsule of **1a**.

Figure 4. Natural logarithm of the normalized signal decay ($\ln(I/I_0)$) as a function of the b value (400 MHz, 298 K) for one of the peaks of a) **1a** and b) encapsulated **4** in CDCl$_3$, as extracted from the LED sequence with different delay periods t_e: 5 ms (■), 50 ms (○), and 150 ms (▲).

a function of the b values of one of the peaks of **1a** (Figure 4a) and encapsulated **4** (Figure 4b) in CDCl$_3$, as extracted from the LED sequence with different delays t_e. The signal decay for the two species in the sample (**1a** and encapsulated **4**) was found to be monoexponential and the same at all sampled t_e values, and the extracted diffusion coefficient for both **1a** and **4** was $0.24 \pm 0.01 \times 10^{-5}$ cm^{2}s^{-1}. Since **4** is encapsulated inside the hexamer of **1a**, both exchange and magnetization transfer between the "free" (site c in Figure 2) and encapsulated **4** molecules (site a in Figure 2) should be slow with respect to the time scale of the diffusion NMR experiments and thus should not be manifested in the signal decay even at long delay times t_e. Therefore, it is reasonable to assume that in the case of **2**, in which the signal decay was influenced by the change in t_e, the exchange or magnetization transfer between "free" and "bound" **2** is faster. Consequently, we suggest that "bound" **2** molecules, despite the high-field chemical shifts of their hydrogen atoms, are not encapsulated guests but are in fact integrated into the structure of the hexameric capsule (site b, or more precisely b1, in Figure 2). Again, because of the high-field peaks of **2** and **3**, arrangement b1 seems to represent the system better than b2. These results indicate that diffusion measurements with the LED sequence, and particularly the effect of t_e on the diffusion results, can be used to identify the

site occupied by the alcohols in the hexameric capsule on the basis of the magnetization transfer that occurs during the t_e period of the LED sequence. These results were corroborated by 2D NOESY (see Figure S3 in the Supporting Information). As expected, the addition of water to the solution of **1a** and **4** in CDCl$_3$ had no effect on the high-field peaks of the encapsulated molecule **4** (see Figure S2b in the Supporting Information).

Recently, Ugono and Holman solved the crystal structure of hexameric capsules of **1c** with **5** and found that this alcohol replaces water molecules on the surface of the capsule.[10] They also reported that three disordered molecules of **5** existed within the cavity of **1c**.[10] We therefore examined whether **5** behaves in the same way with **1a** in solution as with **1c** in the solid state. The same diffusion experiments were performed on a solution of **1a** (25 mM) with **5** (1:10) in dry CDCl$_3$. The ^{1}H NMR spectrum showed new high-field peaks that could correspond to encapsulated or bound **5**. A 6:2 ratio of **1a** to molecules of **5** associated with the capsules was derived from the peak integrals. Figure S4 in the Supporting Information presents the ^{1}H NMR signal decay as a function of gradient strength (G) for the peaks of guests **2** and **5** (Figure S4a–f) as well as **1a** (Figure S4g–l) in CDCl$_3$. The extracted ADCs are presented in Figure 5.
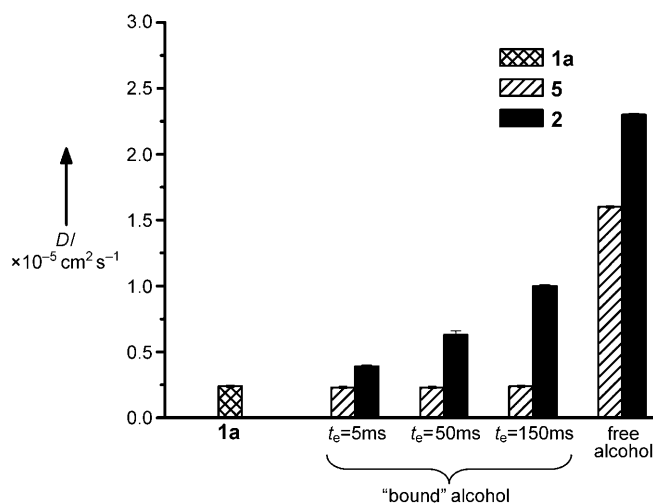

Figure 5. Apparent diffusion coefficients of **1a** in CDCl$_3$, for the high-field peaks of **5** and **2** in solutions of **1a** in CDCl$_3$, and of free **5** and **2** in CDCl$_3$ as a function of t_e, as extracted from the LED sequence. The results reported for the different alcohols were obtained by fitting the signal decay to a monoexponential decay function.

We found that the ADC for "bound" **2** increased with increasing t_e. At very long delays t_e it approached that of free **2** in CDCl$_3$ (Figure 5). However, the ADC for "bound" **5** is exactly the same as that extracted for the peak of **1a** at all t_e values used (Figure 5). These results imply that two molecules of **5** are encapsulated in the hexameric capsule of **1a** under the experimental conditions used, in contrast to the findings for **1c** and **5** in the solid state.[10] The results for 2-ethylbutanol (**6**) in the presence of **1a** were similar to those found for **5** (see Figure S6 in the Supporting Information).

In conclusion, contrary to common belief, the presence of high-field peaks of potential alcohol guests in the ¹H NMR spectrum of the hexameric capsules of resorcin[4]arenes does not necessarily imply guest encapsulation within the capsule. The ¹H NMR spectra of solutions of **1a** with **2** and **3** showed high-field peaks due to bound **2** and **3** that did not correspond to encapsulated guests but rather to molecules of **2** and **3** that replaced some of the water molecules and were part of the structure of the hexameric capsule of **1a**. Alcohols that are part of the capsule network and are not encapsulated can still be exchanged or can exchange magnetization with alcohols in the bulk solution. The alcohols **5** and **6** are truly encapsulated in the capsules and therefore cannot be exchanged or exchange magnetization with the alcohols in the bulk solution on the time scale of the present NMR experiments. Despite the observation of high-field peaks for alcohols in the presence of the hexameric capsule of **1a**, only diffusion measurements, and more specifically the dependency of the LED signal decay on t_e, can quickly indicate which of the alcohol guests are indeed encapsulated in the capsule and which are located on the surface, where they maintain the structure of the capsule. Hence, these diffusion NMR spectroscopy results, which were corroborated by NOE experiments, enable specific mapping of the sites occupied by alcohols in the hexameric capsule and demonstrate how new and unique insight can be gained when diffusion NMR spectroscopy is used to study the structure and dynamics of such labile supramolecular systems in solution.

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