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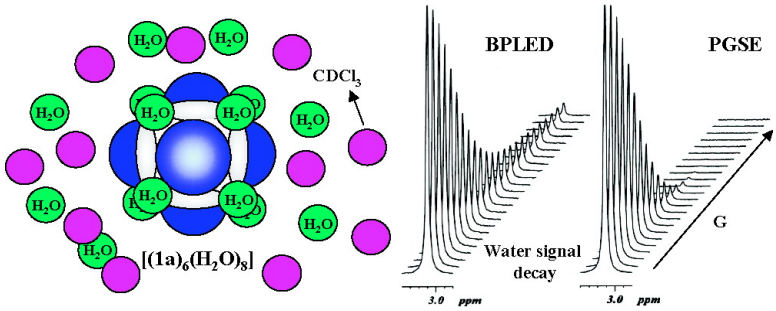

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Diffusion Measurements for Molecular Capsules: Pulse Sequences Effect on Water Signal Decay

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Abstract: Diffusion NMR and, more recently, diffusion ordered spectroscopy (DOSY) are gaining popularity as efficient tools for the characterization of supramolecular systems in solution. Here, using diffusion NMR of hydrogen-bond molecular capsules, we demonstrate that the use of different diffusion sequences may have a dramatic effect on exchanging peaks. In fact, we found that the signal decay of the water peak in $[(\mathbf{1a})_6(H_2O)_8]$ is monoexponential in the pulsed gradient spin−echo (PGSE) and stimulated echo (PGSTE) sequences and biexponential in the longitudinal eddy current delay (LED) and the bipolar longitudinal eddy current delay (BPLED) sequences, routinely used in modern DOSY experiments. By performing these diffusion measurements on molecular capsules, in which water is not part of the molecular capsules, we demonstrate that this phenomenon is observed only for water molecules that exchange between two sites that differ considerably in their diffusion coefficients. Degeneration of the LED or the BPLED sequences into PGSTE-type sequences by shortening the te period resulted in the disappearance of the extra slow diffusing component. The origin, as well as the implications of the different results obtained from conventional diffusion sequences, such as the PGSE and PGSTE as compared with the LED and BPLED sequences generally used in DOSY experiments, are briefly discussed.

Introduction

Modern fields of chemistry, such as supramolecular, pharmaceutical, and combinatorial chemistry, deal with complex structures and mixtures in solution. As a consequence, there is a constant need for additional analytical methods for the characterization of such systems. Indeed, in recent years, diffusion NMR[1] is gaining more popularity in supramolecular, pharmaceutical, and combinatorial chemistry.[1,2] Diffusion NMR was used, inter alia, to evaluate association constants,[2c,d,3] probe encapsulation,[4] study the structure and aggregation mode of organometallic complexes,[5] determine dendrimer's generation and structure,[6] and elucidate the structure of the self-assembled metallosupramolecular systems[7a−e] and other supramolecular systems.[7f−g] In combinatorial chemistry, diffusion NMR was suggested as an alternative method for screening lead compounds without the need for mixture separation.[2a,b,8] The applications of diffusion NMR to supramolecular and combinatorial chemistry were recently reviewed.[1d]

There are several basic pulse sequences for measuring diffusion with NMR (Figure 1).[1,9] These are the pulsed gradient

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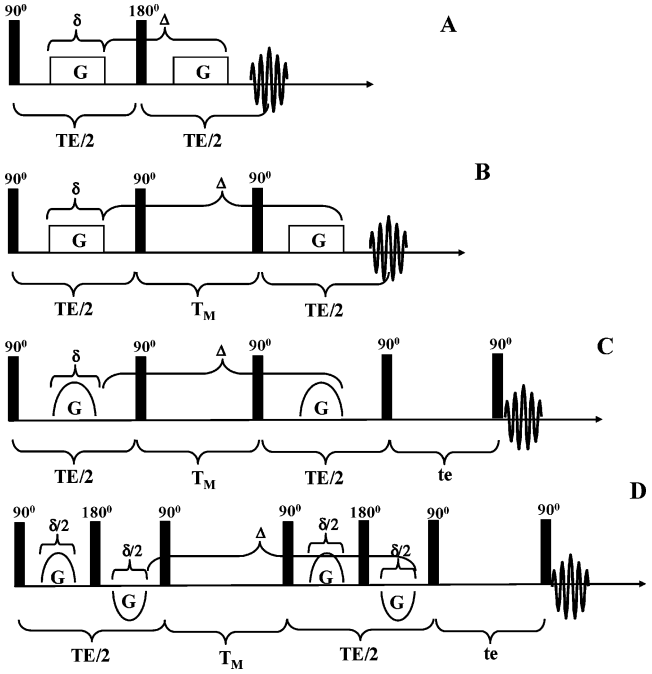
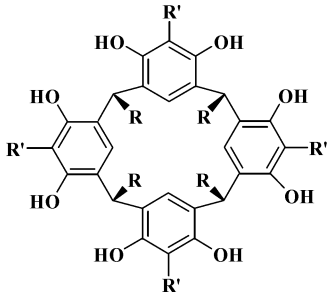


Figure 1. Four basic pulse sequences used for measuring diffusion by NMR: (A) PGSE;[9a] (B) PGSTE;[9b] (C) LED;[9c] (D) BPLED.[9d]

spin echo (PGSE),[9a] the pulsed gradient stimulated echo (PGSTE),[9b] the longitudinal eddy current delay (LED),[9c] and the bipolar longitudinal eddy current delay (BPLED)[9d] sequences. In recent years, LED and BPLED, the sequences used in diffusion ordered spectroscopy (DOSY),[1c,9c,d] are gaining importance in diffusion measurements of organic systems using conventional high-resolution spectrometers. However, in systems with exchange, both the experimental parameters and the pulse sequence used may have a pronounced effect on the obtained results, as will be presented in this article.

In the past decade, noncovalent molecular capsules attracted much interest.[10] Recently, we used diffusion NMR to study the structure and the role of water molecules in the molecular capsules of resorcin[4]arene (**1a**) and pyrogallol[4]arene (**1b**) (Chart 1).[11] It was found that **1a** self-assembles into a hexameric capsule of the $[(\mathbf{1a})_6(H_2O)_8]$-type, where the eight water molecules are in fast exchange with bulk water, while **1b** self-assembles into a hexameric capsule of the $(\mathbf{1b})_6$-type.[11b,d] These results are in agreement with the findings in the solid state.[12] Subsequently, it was found that the encapsulation of an ammonium salt by the hexamer of **1a** releases the water molecules from the supramolecular structure of the capsule.[11c]

Chart 1



1a R= $C_{11}H_{23}$ R'=H

1b R= $C_{11}H_{23}$ R'=OH

In the course of these studies we found that different diffusion pulse sequences used had a dramatic effect on the signal decay and, hence, the extracted diffusion coefficients of some of the signals. In fact, in some of these sequences, the resulting signal decay may lead to misinterpretation, emphasizing the special care that should be paid to the selection of the appropriate diffusion experiment in some systems. This paper describes the origin for this phenomenon and discusses the implications of these observations with regards to the diffusion NMR characterization of systems with exchange.

Result and Discussion

Figure 2 shows the signal decay of the water peak (A and B) and one of the peaks of **1a** (C and D) in the $CDCl_3$ solution of $[(\mathbf{1a})_6(H_2O)_8]$ as a function of the gradient strength (G) in the BPLED (A and C) and PGSE (B and D) sequences.

This figure demonstrates that although the same signal decay is observed for the signal of **1a** in the two sequences (Figure 2C,D), the signal decay of the water peak, in the two diffusion

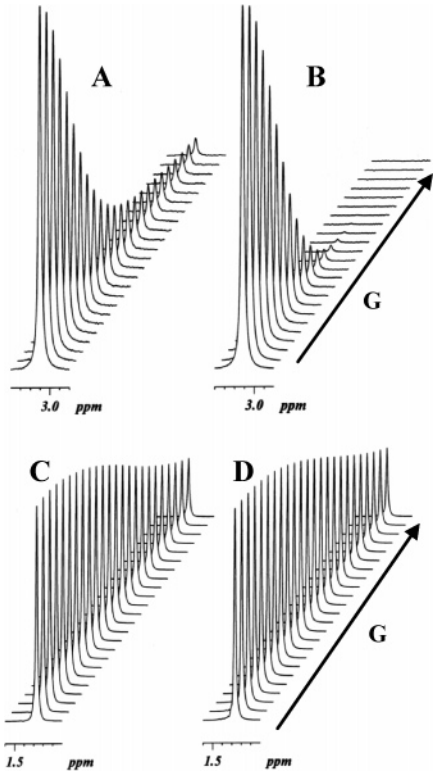


Figure 2. ^{1}H NMR signal decay as a function of the gradient strength (G) (400 MHz, 298 K) of water (A and B) and one of the peaks of **1a** (C and D) in the $CDCl_3$ solution of $[(\mathbf{1a})_6(H_2O)_8]$ obtained with the BPLED (A and C) and PGSE (B and D) sequences.

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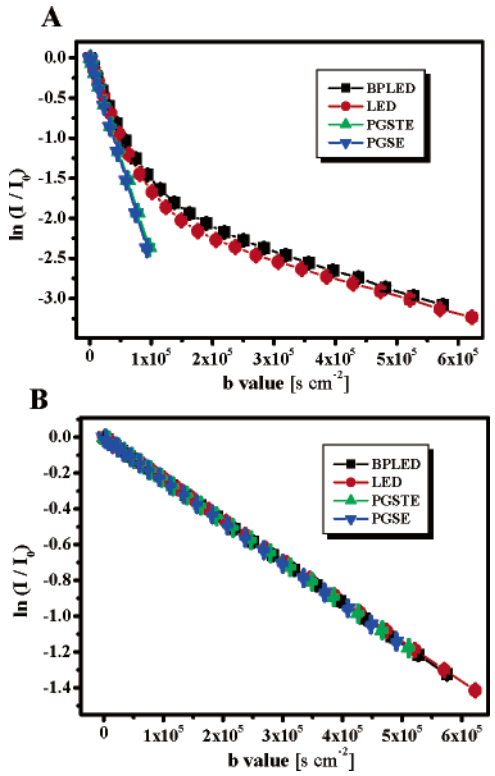

Figure 3. Natural log of the normalized signal decay ($\ln(I/I_0)$) as a function of *b* value (400 MHz, 298 K) of water (A) and one of the peaks of **1a** (B) in a $CDCl_3$ solution of $[(\mathbf{1a})_6(H_2O)_8]$ obtained with the BPLED, LED, PGSTE, and PGSE sequences.

sequences, differs dramatically (Figure 2A,B). Clearly in the BPLED sequence an extra slow diffusion component is observed only for the water peak. This point is further illuminated in Figure 3 that shows the normalized signal decay, in a logarithmic scale, as a function of the diffusion weighting in the four pulse sequences used in this study.

Figure 3A shows that the water signal decay in the PGSE and PGSTE experiments is identical but differs from that obtained from the LED and BPLED experiments, which in turn is also very similar. While the water signal decay is monoexponential in the first two sequences (i.e. PGSE and PGSTE), it is clearly not monoexponential in the LED and BPLED experiments. However, it is important to note that, in these four experiments, the signal decay of the peaks of **1a** is exactly the same (Figure 3B). The diffusion coefficient extracted for **1a**, in these four diffusion experiments, was the same and was found to be $(0.23 \pm 0.01) \times 10^{-5}$ cm^2 s^{-1}. These results rule out the possibility that the effect is due to experimental errors in the acquisition of any of these diffusion experiments. The diffusion coefficient extracted from the PGSE and PGSTE for the water peak in this molecular capsule is the weighted average of the eight water molecules in the molecular capsule of **1a** and the remaining bulk water in the solution. However, as it has been found that the different water pools in the $CDCl_3$ solution of the molecular capsule of **1a** are in fast exchange, one should expect monoexponential signal decay for that water peak under these experimental conditions. Therefore, the extra slow diffusing component of the water peak observed only in the LED and BPLED experiments is surprising at first glance. It was found that the diffusion coefficient extracted from the slow diffusing component of the water signal in the LED and BPLED

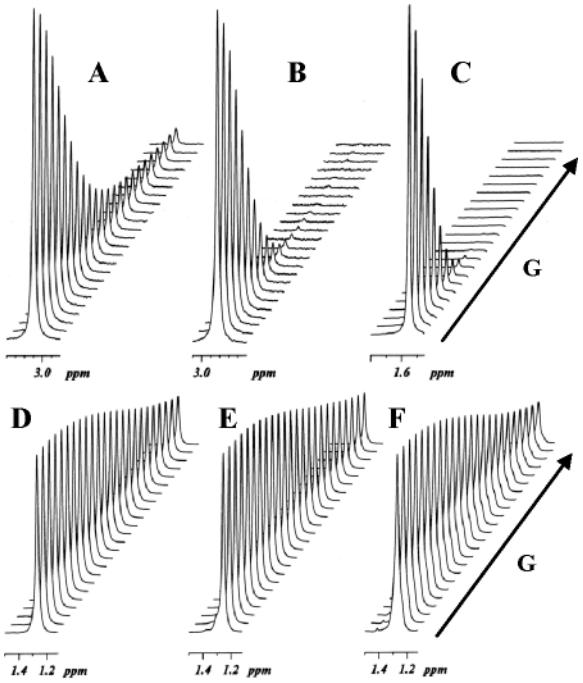

Figure 4. ^{1}H NMR signal decay as a function of the gradient strength (G) (400 MHz, 298 K) of the water peak (A−C) and one of the peaks of the macrocycle (D−F) in different $CDCl_3$ solutions as extracted from the BPLED sequence. (A) and (D) show a 20 mM solution of **1a**, (B) and (E) a 20 mM solution of **1a** in the presence of THABr, and (C) and (F) a 7 mM solution of **1b**. In all solutions the macrocycle:water ratio was ~6:20.

experiments is very similar to the diffusion coefficient of **1a**. The diffusion coefficient of the fast component of the water in these sequences, however, approaches the diffusion coefficients of the water molecules obtained from the PGSE and the PGSTE sequences.

This peculiar behavior of the water peak is observed only for water molecules that are part of the supramolecular structure of the capsules as demonstrated in Figure 4. This figure shows the signal decay for the water and one representative peak of the macrocycle in the capsule in the BPLED experiment performed with exactly the same experimental parameters for three different systems. Figures 4A,D, 4B,E, and 4C,F are for the $CDCl_3$ solutions of **1a**, **1a** in the presence of tetrahexylammonium bromide ((THA)Br), and **1b**, respectively. Although very similar signal decays are observed for the macrocycle peak (Figure 4D−F), the water signal decay is clearly different for the three systems (Figure 4A−C).

These results, presented graphically in Figure 5, for solutions where the macrocycle:water ratio was about 6:20, show that the extra slow diffusing component is observed only for the water signal in the system where the water molecules are known to be part of the supramolecular structure.[11] In this system, the water peak represents two pools of water which differ considerably in their diffusion coefficients.

In principle, in isotropic systems exhibiting free diffusion, the deviation of the signal decay from linearity may be due to chemical exchange and/or the effect of the intermolecular nuclear overhauser effect (NOE),[13,14] sometime referred to as transferred NOE.

(13) The effect of exchange on diffusion was studied extensively, mostly for cases in which fast exchange prevails on the chemical shift scale but also for cases of slow exchange on the chemical shift scale. For a few selected examples, see: (a) Andrasko, J. *Biochim. Biophys. Acta* **1976**, *428*, 304−311. (b) Kärger, J.; Pfeifer, H.; Heink, W. *Adv. Magn. Reson.* **1988**, *12*,

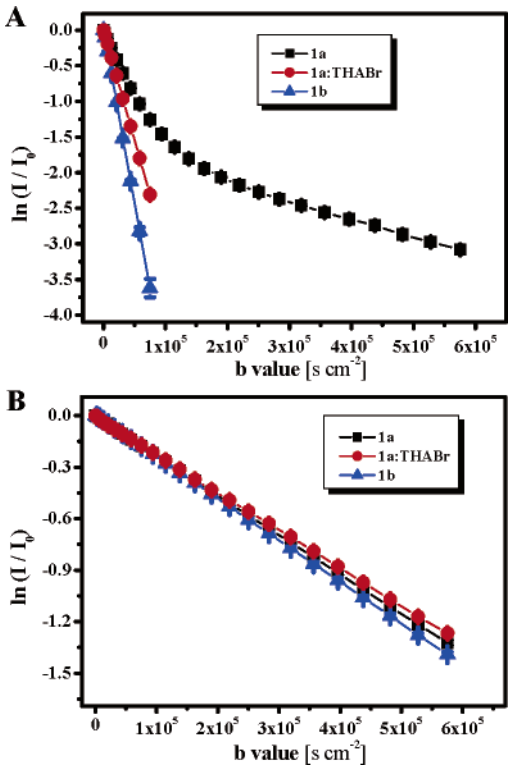

Figure 5. Natural log of the normalized signal decay ($\ln(I/I_0)$) as a function of *b* value (400 MHz, 298 K) of water (A) and one of the peaks of the macrocycle (B) as extracted from the BPLED sequence, for the $CDCl_3$ solutions of **1a**, **1a**:(THA)Br, and **1b**.

Since the diffusion experiments shown in Figures 2 and 3 were performed on the same sample with the same diffusion time, and since the deviation from linearity in the signal decay of the water peak was observed only in two of the four sequences used, we suspected that the different signal decays, between the two types of sequences, originate from the NOE, which develops during the eddy current delay (the te period) of the LED and BPLED sequences (Figure 1C,D). To further verify this issue, we performed a series of BPLED (and LED; data not shown) experiments in which the te parameter was varied while all other parameters in the sequences were kept constant. Figure 6 shows the signal decay for the water peak (Figure 6A−D) and one representative peak of **1a** in the capsule (Figure 6E−H) when the te was varied from 150 to 4 ms. The data clearly show that while this parameter had no effect on the signal decay of the peak of **1a**, the signal decay of the water peak changes considerably. Shortening the te caused a gradual disappearance of the slow diffusing component affording for the BPLED experiment with a te of 4 ms, a signal decay which is very similar to that observed in the PGSTE experiment under the same experimental conditions.

Shortening the te degenerates the LED or BPLED sequences into a PGSTE-like sequence. The fact that no deviation from linearity is observed in the PGSTE experiments or the degenerated LED and BPLED sequences implies that the transferred NOE, which may develop during the mixing time of the diffusion time of these sequences, is not sufficient to detect this effect. These results suggest that the additional extra diffusion component of the water signal originates from the NOE effect that develop during the te period following the diffusion tagging which occurs during the first part of the sequences. Monoexponential water signal decay was also observed in the PGSTE experiments acquired with long diffusion and mixing times, suggesting that these parameters are not crucial for the observation of this phenomenon. These conclusions are further corroborated by the fact that a slow diffusing component is also observed for the water peak in a modified PGSE sequence to which a 90−delay−90 sequence was added before the acquisition.

Figure 7 summarizes the diffusion coefficients extracted for "free" water in a $CDCl_3$ solution and for the water peak in the $CDCl_3$ solution of **1a** obtained with the different sequences. This figure shows, inter alia, that the diffusion coefficient extracted from the BPLED experiment acquired with a te of 4 ms is in fact very similar to the diffusion coefficients obtained from the PGSE and the PGSTE experiments for the water peak.

In addition, as expected, these diffusion coefficients are different from the diffusion coefficient of "free" water in chloroform as they represent a weighted average between bulk water in the $CDCl_3$ solution and water in the molecular capsule. The asymptotic value of the fast diffusing component in the BPLED experiment collected with a te of 50 ms is much smaller than that of free water. In fact, it is even somewhat smaller than the average values obtained from the PGSE, PGSTE, and the BPLED (with a te of 4 ms) experiments. The results mean that the slow and fast diffusing components observed in the LED and BPLED experiments when the te is in the order of 50 ms do not represent free and bound water as one could erroneously conclude from such data having done these experiments without comparing the results with the results obtained from the more conventional PGSE and PGSTE experiments. It should be noted that since the observed effect can only be detected when the water molecules are part of the supramolecular structure, additional information could be extracted from the LED and BPLED experiments. The deviation from linearity can serve as an indication for large NOE interactions in multicomponent systems that occupy different sites which differ considerably in their diffusion coefficients.

In conclusion, we have shown that in systems where exchange and NOE may prevail, special care should be paid to select the most appropriate pulse sequence for measuring diffusion. In systems where there are chances for strong NOE interactions between nuclei that differ considerably in their diffusion coefficients, a more complete description of these systems can be achieved by acquiring and comparing the diffusion results of the two conventional diffusion sequences, i.e., PGSE[9a] and PGSTE,[9b] and the two more modern LED[9c] and BPLED[9d] sequences generally used in DOSY packages.[1c] One should be aware that, in such cases, the length of the te has an effect on extracted diffusion coefficients which may result in erroneous interpretation of the diffusion data and hence system characterization.

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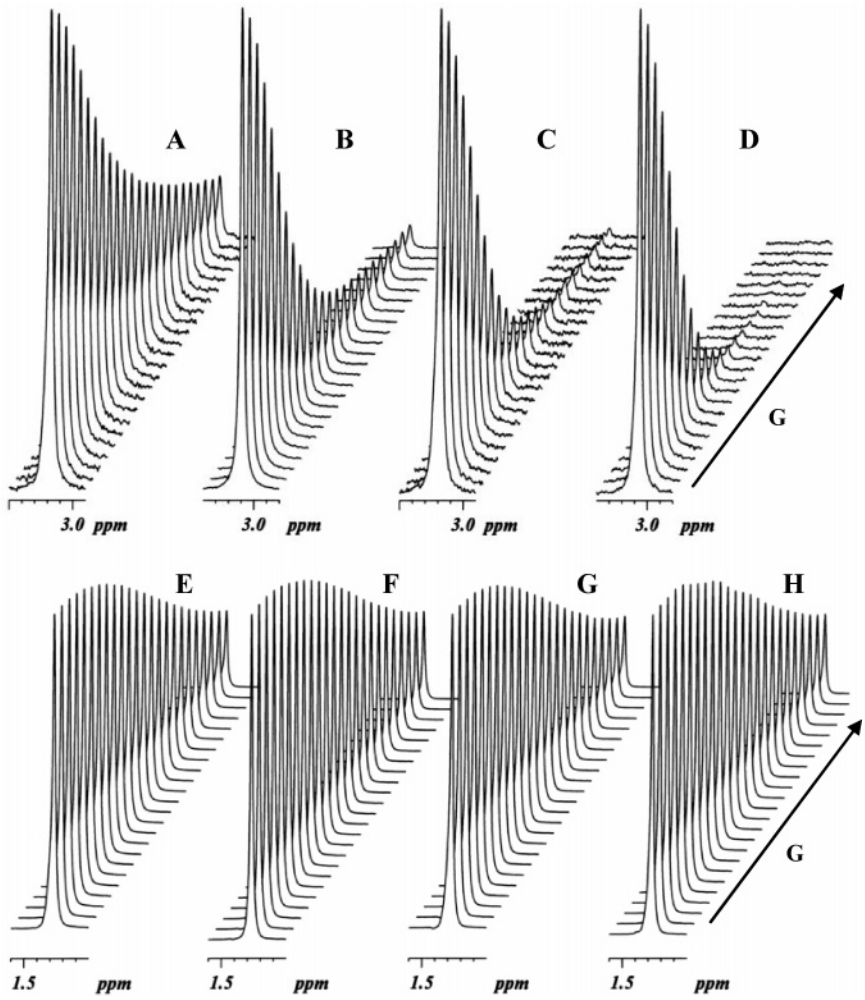

Figure 6. ^{1}H NMR signal decay as a function of the gradient strength (G) (400 MHz, 298 K) of water (A−D) and one of the peaks of **1a** (E−H) in a $CDCl_3$ solution as extracted from the BPLED sequence with different te's: (A, E) 150 ms; (B, F) 50 ms; (C, G) 14 ms; (D, H) 4 ms.

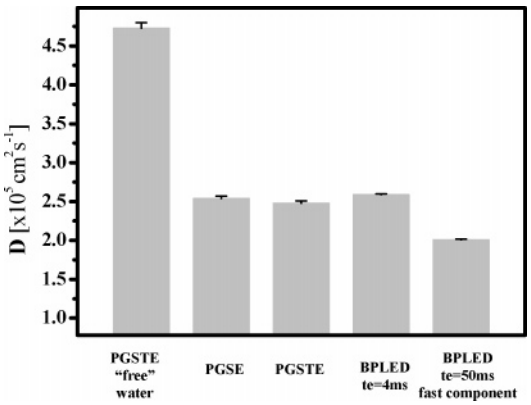

Figure 7. Diffusion coefficients of "free" water in $CDCl_3$ and water in a 20 mM $CDCl_3$ solution of **1a** as extracted from different NMR diffusion measurements. The type of diffusion experiment used is indicated.

Experimental Section

General Methods. NMR diffusion measurements were performed on a 400 MHz Avance Bruker NMR spectrometer equipped with a Great1 gradient system capable of producing magnetic field pulse gradients in the *z*-direction of about 50 G cm^{-1}. All experiments were carried out using a 5 mm inverse probe. Measurements were performed at least three times, and the reported diffusion coefficients are the mean ± standard deviation of at least three experiments. Only data where the correlation coefficients of $\ln(I/I_0)$ versus $\gamma^2\delta^2g^2(\Delta - \delta/3)$ for the nonwater peaks were higher than 0.999 are reported. The measurements were all preformed at 298 K. All diffusion measurements were performed in a 4 mm NMR tube inserted in a 5 mm NMR tube, which acts as a thermal insulating system and increase the accuracy and reproducibility of the diffusion measurements by reducing the chance for convections in the sample. This precaution is more important when diffusion NMR experiments are performed on nonviscous solvents with low boiling point and heat capacity.

The diffusion experiments were performed using the four different pulse sequences, PGSE, PGSTE, LED, and BPLED (Figure 1), with the following parameters:

PGSE. Rectangular pulsed gradients, of 3 ms duration, were incremented from 0 to 40.2 G cm^{-1} in 24 steps, the pulse gradient separation was 48 ms, and the echo time (TE) was 60 ms. In addition, a PGSE sequence, to which a 90−τ_m−90 sequence was added before acquisition, was performed with the same parameters when τ_m was set to 50 ms.

PGSTE. The same parameters as the PGSE sequence were used, the only difference being that the pulse gradient separation was 50 ms (instead of 48 ms) and the echo time was set to 56 ms (instead of 60 ms). The mixing time (T_M) in the PGSTE experiment was 22 ms.

LED. Sine-shape pulsed gradients, of 4 ms of duration, were incremented from 0.7 to 32.2 G cm^{-1} in 24 steps, and the pulse gradient separation was 54 ms. The echo time, the mixing time, and eddy current delay (te) were 56, 26, and 50 ms, respectively.

BPLED. The same parameters as those in the LED sequence were used except for the pulse gradient separation and mixing time that were 56 and 22 ms, respectively. The te effect was measured by performing the BPLED or the LED sequences with the above parameters four different times with te's of 150, 50, 14, and 4 ms.

The diffusion measurements were performed on three different $CDCl_3$ solutions: a 20 mM solution of **1a**; a 20 mM solution of **1a** and (THA)Br; a 7 mM solution of **1b**. In all these solutions, the ratio between the macrocycle and water was ~6:20.

Materials. All starting materials, guest molecules, reagents, and the deuterated solvent ($CDCl_3$) were purchased from Aldrich (Milwaukee, WI) and used as supplied. Compounds **1a**,**b** were prepared according to modifications of the previously published procedure.[11,15]

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