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Role of the Methoxy Groups in Cryptophanes for Complexation of Xenon. Conformational Selection Evidenced by $^{129}$Xe-$^1$H NMR SPINOE Experiments.

Patrick Berthault,[b] Céline Boutin,[b] Estelle Léonce,[b] Erwann Jeanneau,[c] and Thierry Brotin[a]

Abstract: We report the laser-polarized $^{129}$Xe and $^1$H NMR spectra of a series of cryptophane derivatives that differ only by the number of methoxy groups attached on their benzene rings and the arrangement syn or anti of the linkers (compounds 6a-s, 9a-s, 12a-s). All these compounds bind xenon even though the characteristic signal of the gas encapsulated in the cavity of the cage-molecule cannot be always detected. Interestingly, the exchange dynamics of xenon strongly depends on the degree of substitution and is different from that of the cryptophane derivatives studied previously. In solution, the $^1$H NMR spectra of these derivatives show the presence of different conformations in a slow exchange regime that can be explained by a decrease of the flexibility of their skeleton. Thanks to $^{129}$Xe-$^1$H dipolar cross-relaxation (SPINOE) spectra we demonstrate that a single conformation present in solution can bind xenon.

Introduction

Xenon is known to show good affinity for organic structures such as the hydrophobic pockets of proteins or hollow organic compounds capable of accommodating substrates in their cavities.[1-4] For instance, it has been reported that xenon presents a good affinity for cryptophane-A (1), a synthetic organic compound with a roughly spherical cavity.[5] Since this discovery, numerous xenon-cryptophane complexes have been thoroughly studied in organic or aqueous solution.[6] These supramolecular complexes also bring attention of the scientific community as they can be used for $^{129}$Xe NMR-based biosensing applications.[7] The remarkable properties of these complexes mainly arise from the high receptiveness of the xenon nuclear spin, which is able to detect subtle changes in its magnetic properties. Thus, when xenon is bound to a host molecule, the magnetic properties of its nucleus are modified resulting in a specific $^{129}$Xe NMR signature for this complex. The possibility to modify the host structure in order to graft a biological site that can specifically recognize a biological target represents another advantage for these systems.[8]

From a more fundamental point of view, $^{129}$Xe NMR spectroscopy appears as the tool of choice for studying cryptophane derivatives with small cavities. For instance, the xenon atom present within the cavity of cryptophane-A congeners shows a chemical shift significantly modified with respect to that of xenon present in the bulk. As an example, in 1,1,2,2-tetrachloroethane-d$_2$ the $^{129}$Xe NMR spectrum of the Xe@cryptophane-A complex shows a chemical shift difference of 160 ppm between xenon in solution and xenon trapped into the cryptophane cavity.[9] The highly polarizable electron cloud of the xenon atom and the shielding of the six aromatic rings are responsible for this shift. Small changes in the structure of these derivatives can also have an impact on the physical properties of the complexes with xenon. For instance, a reduction of the volume of the cavity can modify both the association constant of the complex and the in-out exchange dynamics of xenon. As an example, the $^{129}$Xe NMR spectrum of xenon with cryptophane-111, one of the smallest cryptophanes, gives rise to a very sharp signal at 30 ppm in 1,1,2,2-tetrachloroethane-d$_2$. This chemical shift differs significantly from the one obtained for the Xe@cryptophane-A complex. This complex is also characterized by a very high affinity constant, $K = 10000$ M$^{-1}$ at 293 K.[10] A modified water-soluble cryptophane-111 also exhibits a remarkable xenon binding constant and an unusual downfield-shifted signal.[11] Even though numerous xenon@cryptophane complexes have been studied in the past, a prediction of the physical properties of the complexes (xenon chemical shift, in-out exchange dynamics, binding constant) remains difficult with these systems. Thus, to date it is difficult to establish a clear relationship between the chemical structure of the host molecule and the physical properties of the complexes. This is probably because these molecules show some flexibility. Thus, they can modify the conformation of their linkers to maximize their interaction with xenon. For instance, the ability of the cryptophane skeleton to adapt its conformation upon binding with xenon has been demonstrated by Pines and co-workers using SPINOE experiments.[12] They showed that cryptophane-A changes the conformation of its three O-CH$_2$-CH$_2$O linkers upon encapsulation of xenon.

The study of new xenon@cryptophane derivatives is highly desired for a better understanding of the physical properties of these systems and specially to understand how structural modifications of the cryptophane scaffold can modify the physical properties of the complex. In this article, we report the study of xenon in interaction with several cryptophane-A congeners, whose structures are reported in Scheme 1. These molecules differ from cryptophane-A (1) by the number of methoxy substituents attached on the benzene rings or by the syn or anti arrangement of the three linkers connecting the two CTB units. For sake of simplicity the nomenclature of the new molecules used in this article refers to the number of methoxy substituents attached on the aromatic rings and the anti or syn arrangement of the linkers. For instance, cryptophanes 3a (2) and 3s (3) are molecules that possess only three methoxy substituents attached on the cryptophane scaffold. The subscripts a and s refer to the anti and syn arrangement of the three linkers, respectively. In other Articles these molecules have been called cryptophane-C and D (Collet’s nomenclature), respectively. Similarly, compounds 4 and 5 that contain six methoxy on the same CTB unit, are noted in the text compounds 6a and 6s, respectively. Using the same nomenclature, compounds 6 and 7 are noted 9a and 9s, respectively, whereas compounds 8 and 9 that possess the highest degree of substitution with twelve methoxy substituents attached on the six aromatic rings are noted 12a and 12s, respectively.

With the exception of compound 12s, these cryptophanes are chiral molecules. The chiroptical properties of these derivatives have been thoroughly described in a recent Article.[13] Cryptophane 12a has D$_2$-symmetry and the chirality of this compound originates from the helical arrangement of the three linkers. Compound 12s has a plane of symmetry and is therefore achiral (C$_{5v}$ symmetry). Interestingly, it is noteworthy that the syn-stereoisomer of cryptophane-A has never been isolated. Thus, compound 12s is of high interest in this study. Molecules 6a - 6s and 9a - 9s possess a lower degree of substitution than compounds 12a and 12s, with six and nine methoxy groups, respectively (see Scheme 1). The synthesis of new
derivatives 6a and 6s is described for the first time in this Article. The introduction of additional methoxy groups on the cryptophane backbone and the syn or anti arrangement of the three O-CH₂-CH₂-O linkers are expected to modify the physical properties of the xenon@cryptophane complexes. For instance, the introduction of additional electron-donating substituents on the cryptophane scaffold should have an impact on the caged xenon chemical shift. In addition, the anti or syn arrangement of the linkers should modify the in-out exchange dynamics of xenon.

In this Article, we report both their ¹H NMR and their hyperpolarized ¹²⁹Xe NMR spectra. The spin-hyperpolarization technique is a powerful tool that allows a gain in sensitivity by a factor 10⁻⁶ thus enabling to record ¹²⁹Xe NMR spectra in few seconds with excellent signal to noise ratio. We also report the ¹²⁹Xe-H dipolar cross-relaxation (SPINOE) spectra of these complexes. The results obtained with compounds 6a-s, 9a-s and 12a-s are then compared with those previously obtained with the Xe@1, Xe@3a and Xe@3s complexes. Our results shed light on the role of the substituents in the variation of the physical properties of these complexes. Thanks to ¹²⁹Xe-H SPINOE spectroscopy, we show that a conformational selection occurs upon encapsulation with xenon.

Results and Discussion

Synthesis and characterization of compounds 6-12: compounds 9a-s and 12a-s (Scheme 1) have been prepared according to a known procedure. New derivatives 6a and 6s have been prepared using a similar strategy (see Scheme 2). The known 10,15-dihydro-5H-Trabenzo[a,d,g]cydononone-2,7,12-triol, 10 is allowed to react with 2H-Pyran, tetrahydro-2-[4-(2-iodoethoxy)-3,5-dimethoxyphenyl] methoxy; 11 in the presence of cesium carbonate in DMF to give rise to the cryptophane precursor 12 in good yield (72%). The second ring closing reaction is then performed at 55°C in a mixture of formic acid and CHCl₃. This gives rise to a crude product that contains compounds 6a and 6s in similar proportion. It is noteworthy that both compounds show very similar retention time on silica gel. Compound 6s is eluted first on silica gel and is isolated in 29% yield with high purity. The purification of the second diastereomer 6a is more difficult and requires additional purification steps as described in the experimental section. Compound 4 (6a) is obtained with the same yield (29%). Compounds 6a and 6s have been fully characterized by H, ¹³C NMR spectroscopy and HRMS. ¹H NMR and ¹³C NMR spectra of compounds 6a and 6s are given in Supporting Information (Figures S1-S4 in the Supporting Information).

X-Ray structures of compounds 6a and 6s: Single X-ray quality crystals have been obtained for both diastereomers 6a and 6s in a mixture of chloroform and ethanol. Their crystallographic data of are summarized in Supporting Information (Table S5 in the Supporting Information). Both compounds crystallize in a P2₁₂ space group. For the two structures four molecules are present in the lattice. One CHCl₃ molecule is present in the X-ray structure of 6a and another CHCl₃ molecule is present outside the cavity (Figure S6 in the Supporting Information). The refined structure of 6a also reveals residual electronic density within the cavity that could correspond to a statistical occupation of half the cryptophane molecules by a CHCl₃ molecule. The refined structure of 6s shows two CHCl₃ molecules located outside the cavity and another CHCl₃ molecule present within the cavity of 6s (Figure S7 in the Supporting Information). The refined X-ray structure of compound 6a shows that some disorder is present. Indeed, as often observed in X-ray structures of the cryptophane derivatives, the three linkers can adopt different conformations. While the trans-trans-trans (ttt) and the trans-trans-gauche conformations are present in the X-ray structure of 6a, the X-ray structure of 6s does not show any disorder and the three linkers have an all-trans (ttt) conformation. The non-helical structure of 6s (C₂ symmetry) and the ttt conformation give rise to a large inner cavity, which has been estimated to be 147 Å³. By contrast the mean value calculated for the inner cavity of 6a is 91 Å³. For both compounds, the six methoxy groups show different orientations. Three methoxy groups are located in the plane of the bridges, whereas the three others are located out of the plane of the three benzene rings (cif files). This orientation is consistent with the X-ray structures of cryptophane-A (1) and compounds 9a, 9s, 12a, and 12s previously reported by our group. For instance, the X-ray structure of the cryptophane-A derivative shows that the methoxy groups are in the plane of the benzene rings. In contrast, the introduction of additional methoxy substituents results in a change of orientation of these groups since they are located out of the plane of the benzene rings. A steric hindrance can explain this difference of orientation.

¹²⁹Xe NMR Spectroscopy: For this study, 1,1,2,2-tetrachloroethane-d₈ has been chosen as a solvent because it is not supposed to enter the cavity of these cryptophanes and therefore is not a competitor for...
xenon. In these experiments, xenon gas is added after degassing the organic solutions that contain the different cryptophane derivatives. From the one-scan hyperpolarized $^{129}$Xe NMR spectra of the compounds 6a, 6s, 9a, 9s, 12a and 12s recorded in the same conditions (Figure 2), it is worth noting that the xenon in-out exchange rate exhibits large variation depending on the nature of the cryptophane skeleton. A large peak at 225 ppm that corresponds to xenon free in 1,1,2,2-tetrachloroethane-d$_2$ can be clearly identified in all cases. A second up-field shifted signal of weaker intensity is sometimes located, thus revealing directly the caged xenon environment. For the anti cryptophane 6a at sub-millimolar concentration, in our experimental conditions, a fast exchange situation is observed (or no xenon encapsulation). In contrast, for the compounds 9a and 12a a slow exchange is encountered at sub-millimolar concentration and under the same experimental conditions, and the caged xenon signal is shifted towards higher field when the number of OCH$_3$ groups increases. Somewhat counter intuitively a broader line is observed for Xe@12a than for Xe@9a, indicating a faster xenon in-out exchange. On the other hand, compounds 6s, 9s and 12s do not show any signal corresponding to the complexes. However, it can be mentioned that the $^{129}$Xe NMR spectrum of compound 6s shows a broadening of the free xenon signal suggesting that encapsulation of xenon occurs under not so fast exchange conditions. The xenon in-out exchange is likely to be slowed down by the six methoxy groups on one CTB unit. For compounds 9s and 12s, the free xenon exhibits sharp signals. Consequently, the ability of the xenon atom to enter the cavity of compounds 9s and 12s is questionable. If one considers that the xenon in-out exchange is strongly dependent upon the anti or syn arrangement of the three linkers (slow exchange at the NMR scale for the anti-cryptophanes, fast exchange for the syn-cryptophanes), the two exceptions are cryptophanes 6a and 6s. Cryptophane 6a reveals a behavior that is different from what is observed with cryptophane-A (1) and cryptophane 3a (figure S10 in the Supporting Information). For instance, it has been reported that complexation of xenon with cryptophane shows a broadening of the free xenon signal suggesting that encapsulation of xenon occurs under not so fast exchange conditions. The xenon in-out exchange is likely to be slowed down by the six methoxy groups on one CTB unit. For compounds 9s and 12s, the free xenon exhibits sharp signals. Consequently, the ability of the xenon atom to enter the cavity of compounds 9s and 12s is questionable. If one considers that the xenon in-out exchange is strongly dependent upon the anti or syn arrangement of the three linkers (slow exchange at the NMR scale for the anti-cryptophanes, fast exchange for the syn-cryptophanes), the two exceptions are cryptophanes 6a and 6s. Cryptophane 6a reveals a behavior that is different from what is observed with cryptophane-A (1) and cryptophane 3a (figure S10 in the Supporting Information). For instance, it has been reported that complexation of xenon with cryptophane-A (1) and cryptophane 3a (figure S10 in the Supporting Information). For instance, it has been reported that complexation of xenon with cryptophane shows a broadening of the free xenon signal suggesting that encapsulation of xenon occurs under not so fast exchange conditions. The xenon in-out exchange is likely to be slowed down by the six methoxy groups on one CTB unit. For compounds 9s and 12s, the free xenon exhibits sharp signals. Consequently, the ability of the xenon atom to enter the cavity of compounds 9s and 12s is questionable.

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$^{1}$H NMR spectra of the cryptophane derivatives: the difference observed with compounds 6a-s, 9a-s and 12a-s in the presence of xenon prompted us to examine more carefully their $^{1}$H NMR spectra. We thus report in this Article the $^{1}$H NMR spectra of compounds 6a-s, 9a-s and 12a-s under degassed solution and in the presence of xenon. We also report the $^{129}$Xe-$^1$H cross-relaxation (SPINOE) experiments. These experiments are very useful to establish whether xenon enters or not the cavities of compounds 6a-s, 9a-s and 12a-s since the $^{129}$Xe-$^1$H cross-relaxation only occurs at very short distance. The lack of solvent peak (6.0 ppm) in the SPINOE spectra of compounds 6a-s, 9a-s and 12a-s is a good indicator that these experiments have been successfully performed.

As an example, Figure 3 displays the $^{1}$H NMR spectra of cryptophanes 12a and 12s in degassed solution (Fig. 3a and 3b) and after introduction of xenon into the solution (Fig 3c and 3d). Fig. 3e and 3f display the $^{129}$Xe NMR SPINOE spectra of compound 12a and 12s, respectively. Degassing the solution before introducing xenon is a mandatory step to avoid fast $^{129}$Xe relaxation by paramagnetic molecular oxygen and to avoid possible competing binding with other dissolved gases. Interestingly, we notice that introduction of xenon into the solutions deeply modifies the $^1$H NMR spectra: important changes occur for both the anti and syn cryptophanes. For instance, for the degassed solution of 12a, the aromatic part of the proton spectrum shows two signals with very different intensities. For the sample in the presence of xenon this region now reveals a unique peak that corresponds to the minor NMR signal observed in the case of the degassed solution. The axial protons H$_6$ of the CTB units (4.2 ppm) are also strongly affected by the presence of xenon in solution. For instance, we can notice on Figure 3a that the H$_6$ signals originally observed for the degassed solution have been replaced by another up-field shifted doublet. It is noteworthy that signals characteristic of the three linkers (dashed line) are also strongly affected by the presence or not of xenon inside the solution. Indeed, the two signals initially observed at 4.14 ppm and 3.94 ppm are replaced by an intense multiplet located at 3.99 ppm. The $^{129}$Xe NMR SPINOE sub-spectrum of 12a reveals a simplified spectrum where only the protons that are in close proximity to xenon appear. Dealing with the aromatic protons, for 12a, no change is observable compared to the $^1$H NMR spectrum of the solution with xenon. In contrast, the aliphatic part is strongly simplified (in particular as expected the axial proton signals of the CTB are strongly attenuated).

Figure 2. One-scan $^{129}$Xe NMR spectra of the samples of 6 (a and s), 9 (a and s), 12 (a and s). The subscripts a and s refer to anti and syn arrangement of the linkers. Spectra have been recorded at 11.74 T and 293 K. Each sample contained 0.3 ± 0.03 mg of cryptophane 6a (0.4 - 0.5 mM) in 600 µL of 1,1,2,2-tetrachloroethane-d$_2$, and ca. 10 mg of xenon (representing a concentration of ca. 100 mM).

Figure 3. Comparison of the $^1$H NMR spectra for compounds 12a (left) and 12s (right). The samples were constituted by 0.3 mg of cryptophane 12a in 600 µL of 1,1,2,2-tetrachloroethane-d$_2$, 0.3 mg of cryptophane 12s in 600 µL of 1,1,2,2-tetrachloroethane-d$_2$. From top to bottom, the green spectra were recorded with the degassed solution, the blue ones after introduction of xenon into solution, and the red ones correspond to the results of the SPINOE experiments obtained with the sequence of ref. [20].

The changes observed between the $^1$H spectra of the degassed solution, of the solution in the presence of xenon and of the SPINOE (Fig. 3a, 3b and 3c) suggest that the introduction of xenon into the solution has a strong impact on the conformation of the three linkers. A comparison between these spectra indicates that compound 12a has to change the conformation of the three linkers to accommodate the xenon atom. This effect can clearly be evidenced in the aliphatic region of the spectrum.

For compound 12s, the spectral changes between the three spectra are even more important. The degassed solution of 12s exhibits a $^1$H spectrum with numerous signals present in the aromatic and aliphatic regions. It reveals that compound 12s possesses at least three different conformations of the bridges in slow exchange regime. This is supported by the presence of three well-resolved signals in the aromatic part of the spectrum. The addition of xenon into the solution results in a simplification of the $^1$H NMR spectrum and two NMR signals with close intensities are observed in the aromatic part. The aliphatic part of the $^1$H NMR spectrum is also affected by the presence of xenon. However, this spectral region is not very informative due to the strong overlapping of the different signals present in that region.

From the single $^{129}$Xe NMR spectrum, there was no evidence of xenon encapsulation by cryptophane 12s. But the presence of xenon
inside the cavity of 12s could be ascertained from its $^{129}$Xe-H SPINOE spectrum, revealing some well-defined $^1$H signals. For instance, the aromatic region reveals a single NMR peak, and a strong simplification in the aliphatic part of the spectrum is also clearly observed. Combined together these results show that compounds 9a-12s, the two CTB units, adopt different conformations depending on the experimental conditions. The presence of these conformations seems to be favored in the absence of guest inside the cavity of 12s. Upon addition of xenon, several conformations are still present in solution but only one conformation seems to be able to bind efficiently xenon.

The $^1$H NMR spectra of derivatives 9a-s and 6a-s (Figures. S8 and S9 in the Supporting Information) that possess two CTB units with a different degree of substitution, also result in important spectral changes upon addition of xenon. These compounds possess a lower symmetry ($C_3$) than cryptophanes 12a (D$_3$) or 12s ($C_{12}$). Their corresponding $^1$H NMR spectra are therefore more complicated to interpret due to the presence of additional signals in the aromatic region of the $^1$H NMR spectrum. The $^1$H NMR spectrum of a degassed solution of 9a shows several signals of with different intensities in the aromatic region (Figure S9a in the Supporting Information). Considering the molecular symmetry of 9a and the relative intensities of the signals, we expect three signals in the aromatic region. However five distinct peaks appear in this region, suggesting the presence of several conformations in a slow exchange regime. This is also supported by the presence of two doublets in the aliphatic region of the spectrum for protons $H_2$ and $H_3$. As observed for compounds 12a and 12s, for 9a a simplification of the $^1$H NMR spectrum is observed after introduction of xenon into the solution. For instance, the aromatic region of the Xe@9a complex is dominated by two intense signals (two protons of 9a resonate at the same frequency). Three other signals of much lower intensity are also present in this region and indicate the presence of another conformation in minor proportion. A simplification of the $^1$H NMR spectrum of 9a in the aliphatic region is also observed (Figure S8b in the Supporting Information). The differences observed between these two spectra suggest that a strong rearrangement of the linkers takes place upon addition of xenon. The $^{129}$Xe-H SPINOE of 9a shows a simplified spectrum with respect to that of the Xe@9a complex. Once again, this simplification is clearly visible in the aromatic part of the spectrum since only two intense signals are now visible. Spectral changes are also observed in the aliphatic part. As observed for its diastereomer, compound 9s also shows important spectral modifications upon addition of xenon into the solution. For instance, the $^1$H NMR spectrum of the degassed solution of 9s (Figure S8b in the Supporting Information) shows the presence of at least two major forms in ca. 1:2 ratio and an additional minor form. This situation where two forms dominate, is different from that obtained for 9a, and can be interpreted considering the syn arrangement of the linker. Upon addition of xenon, this ratio is reversed, revealing a clear induction effect of xenon. Interestingly, the $^{129}$Xe-H SPINOE spectrum of 9s shows a simplified spectrum suggesting that a cryptophane with a unique conformation of the linkers can effectively accommodate xenon. Once again, as mentioned for compound 12s, the $^{129}$Xe-H SPINOE technique is a valuable tool to discriminate between the different conformations that bind or not xenon. In the case of compound 9s it can be clearly established that a single conformation of the bridges can accommodate xenon in solution, even though several conformations in a slow exchange regime can be detected on the $^1$H NMR spectrum of 9s.

Among all xenon complexes studied in this Article, compound 6a and its diastereomer 6s have the lowest degree of substitution. The $^1$H NMR spectrum of a degassed solution of 6a (figure S9a in the Supporting Information) is very simple; the presence of 4 aromatic signals is indicative of a single conformation. This is supported by the presence of only one set of signals even when, at first glance, this result may appear surprising considering that compounds with a higher degree of substitution showed several conformations in slow exchange. However, it can be interpreted considering the higher flexibility of compound 6a in which one of the two CTB unit is devoid of substituents. Upon addition of xenon, only slight modifications of the spectrum occurred in the aromatic part of the spectrum. The $^{129}$Xe-H SPINOE sub-spectrum of 6a gives the same signals except the axial and equatorial methylene protons. Consequently, compound 6a exhibits in solution a single conformation of the linkers whatever the xenon concentration (from 10 mM to 100 mM, data not shown). This conformation is able to accommodate xenon, even though no specific $^{129}$Xe NMR signal for the complex is detected at 239K. The $^1$H NMR spectrum of the complex is dominated by $^1$H signals of the cavity. The signal at 7 ppm indicate that the cavity size is too large to be modified upon xenon encapsulation. With compound 6s a different situation occurs: the aromatic region of the $^1$H spectrum for the degassed sample exhibits two sets of signals (Figure. S9d in the Supporting Information). These two conformations are present in a similar proportion but the addition of xenon (Figure S9e in the Supporting Information) modifies their relative proportion, and the SPINOE sub-spectrum indicates a unique conformation able to accommodate xenon (Figure. S9f in the Supporting Information). The helical arrangement of the linkers of the anti-cryptophanes is expected to give them more flexibility upon binding of guest molecules than syn-cryptophanes. It is surprising to observe that compound 6s can modify its conformation to interact with xenon, whereas the $^1$H NMR spectrum of its diastereomer 6a remains unchanged. The $^{129}$Xe-H SPINOE spectrum of 6s (figure S9f in the Supporting Information) shows a simplification of the spectrum indicating that a single conformation can accommodate xenon.

The NMR experiments performed with compounds 3a-s, 6a-s, 9a-s and 12a-s lead to conclusions that may seem counter-intuitive. Indeed, the study of these compounds demonstrates that a correlation between the degree of substitution of the CTB units and the in-out exchange dynamics of xenon cannot be clearly established as initially expected. Similarly, a correlation between the arrangement syn or anti of the linkers and the exchange dynamics of xenon is not direct. Consequently, the in-out exchange dynamics of xenon with these derivatives cannot be explained solely on considerations based on the cavity size of the host molecules and the steric hindrance induced by the presence of the additional methoxy groups. Other factors must be involved to interpret these results. The presence of numerous conformational forms observed for the majority of these derivatives and the ability of these compounds to change the conformation of their linkers give us a clue. The $^1$H NMR spectra of these compounds reveal that the linkers can adopt several conformations (trans or gauche) in solution. Importantly, these conformations are in a slow exchange regime. This is attested by the presence of distinct signals in the aromatic part of these compounds. On the other hand, the $^{129}$Xe-H SPINOE spectra of compounds 6a-6s, 9a-9s and 12a-12s show a clear simplification with respect to their $^1$H NMR spectra. Thus, these experiments demonstrate without exception that all the derivatives studied in this work (with $^{129}$Xe NMR investigation) have a single conformation characteristic of the complexes cannot be always detected. The $^{129}$Xe-H SPINOE spectra also indicate that a single conformation, among those detected by $^1$H NMR spectroscopy, can effectively accommodate xenon. The presence of different conformations of the linkers, even after adding xenon into the solution, seems to be a consequence of the presence of the additional methoxy groups grafted on the CTB units. These substituents rigidly the cryptophane scaffold and constrain the linkers to adopt different conformations. This assumption is supported by the study of the $^1$H NMR spectra of 3a and 3s (Figure S11 in the Supporting Information) that reveal a single conformation for the degassed and gassed samples. In the case of compounds 6s, 9a-9s and 12a-12s these different conformations are in a slow exchange regime. The anti derivatives 9a and 12a, thanks to the helical arrangement of their linkers are more flexible and they can more easily modify the conformation of xenon. In contrast, the syn arrangement (compounds 6s, 9s and 12s) of the linkers seems detrimental and several conformations of the linkers are still present even after introducing xenon. Interestingly, these results show that cryptophane derivatives with opposite arrangement of the linkers can eventually take place between xenon, solvent molecules and the host molecules. Interactions are probably more important in the case of compounds 6a and 6s considering the
larger apertures observed for these two compounds. Molecular dynamics calculations of the complexes using an explicit solvation model would be a valuable tool to interpret these data. Such theoretical approach appears very challenging considering the large size of these molecules and the number of solvent molecules needed for the calculations.

Conclusions

We report a study of the Xe@6a-s, 9a-s and 12a-s complexes by 1H NMR and 125Xe NMR spectroscopy. These compounds differ from their congeners 1-3 by the number of methoxy substituents attached on the cryptophane-222 scaffold and the arrangement syn or anti of the linkers. We show that introduction of additional methoxy groups on the cryptophane backbone has a strong impact on the in-out exchange dynamics of xenon, whereas the lengths of the linkers are the same. Among this series, two cryptophanes have an unexpected behavior. Compound 6a is the only anti-cryptophane in which xenon is in fast exchange on the 125Xe NMR spectrum. 6a is the only syn-cryptophane in which xenon is present in a slow exchange regime, as evidenced by the broadening of the low-field signal. However for these two compounds we cannot exclude a contribution of the solvent molecules to explain these results.

The introduction of methoxy substituents positioned from each side of the linker rigidifies the cryptophane-222 scaffold. Thus, in solution these compounds show several conformations of their bridges in slow exchange dynamics. This effect seems more pronounced with the syn derivatives, which are more rigid. Addition of xenon into the solution modifies the relative distribution of these conformations. The anti derivatives have a higher flexibility show a major conformation for the linkers after introduction of xenon. In contrast, the 1H NMR spectra of the syn derivatives show several conformations in slow exchange even after introduction of xenon. The 125Xe - 1H SPINOE spectra provide additional information and are valuable to distinguish the different conformations that effectively accommodate xenon that the other ones that do not. In our case, the 125Xe - 1H SPINOE spectra reveal that only a single conformational present in solution can bind xenon.

Combined together these results demonstrate that cryptophane derivatives possessing identical linkers can possess very different properties towards xenon encapsulation. This effect can possibly be exploited for 125Xe NMR-based biosensing application. It would be a way to tune the in-out exchange dynamics that play a key role for sensitive NMR detection, helped in that by recent solutions from the literature based on quantitative analysis of saturation-transfer experiments.[1]

Experimental Section

Mass spectra (HRMS LSIMS) were performed by the Centre de Spectrométrie de Masse, University of Lyon, on a ThermoFinnigan MAT 95XL spectrometer. Mass spectra (HRMS LSIMS) were performed by the Centre de Mass Spectrométrie, University of Lyon, on a Thermo Finnigan MAT 95XL spectrometer.

Synthesis of cryptophanes 4 (6a) and 5 (6s).

In a 1000 mL round bottom flask, formic acid (300 mL) was added in one portion to a solution of cryptophane precursor 12 (0.55 g, 0.46 mmol) dissolved in chloroform (300 mL). The solution was stirred for 5 hours at 55°C. The solvents were removed under reduced pressure. Then CHCl3 (100 mL) was added and the solvent was removed under reduced pressure. This procedure was repeated three times in order to remove traces of formic acid via azeotropic distillation. The crude product was then purified on silica gel (eluent: CH2Cl2/Acetone – 90/10) to give rise to a white glassy product (0.58 g). This product contains the two cryptophanes anti-4 (6a) and syn-5 (6s) in the same amount. A second column chromatography (eluent: CH2Cl2/Acetone – 99/01) allows the separation give rise to the pure syn-5 (6s) derivative (0.12 g; 29 %). This compound was then recrystallized in a mixture of CHCl3 and EtOH. Compound 4: 1H NMR (300 MHz, CDCl3) δ 7.11 (3H, s), 7.07 (3H, d, J = 8.7 Hz), 6.67 (3H, d, J = 2.4 Hz), 6.55 (3H, dd, J = 2.4 Hz, J = 8.7 Hz), 4.58 (3H, d, J = 13.5 Hz), 4.28 (3H, d, J = 13.5 Hz), 4.35 – 4.25 (3H, m), 4.25 – 4.10 (3H, m), 4.10 – 3.95 (3H, m), 3.91 (9H, s), 3.87 (18H, m), 3.73 (9H, s), 3.65 – 3.50 (3H, m), 3.46 (3H, d, J = 13.5 Hz). 13C NMR (75.475 MHz, CDCl3, 25°C) δ 157.14 (3C), 152.03 (3C), 151.31 (3C), 140.7 (3C), 139.1 (3C), 136.5 (3C), 132.1 (3C), 130.6 (3C), 125.3 (3C), 118.4 (3C), 113.1 (3C), 110.5 (3C), 69.3 (3C), 65.8 (3C), 60.2 (3C), 55.7 (3C), 36.0 (3C). HRMS (ESI) calcd for C62H45O33N3 [M+Na]+. 1223.5504 found 1223.5503.

This product was then followed by compound 4 (6a), still contaminated by the presence of a small amount of compound 5 (6s). Another column chromatography on silica gel (eluent: CH2Cl2/Acetone – 99/01) gives rise to the pure diasteromer 4 (0.12 g; 29 %). This product was then recrystallized in a mixture of CHCl3 and EtOH. 1H NMR (300 MHz, CDCl3) δ 7.08 (3H, d, J = 8.3 Hz), 7.05 (3H, s), 6.74 (3H, d, J = 2.4 Hz), 6.39 (3H, dd, J = 2.4 Hz, J = 8.3 Hz), 4.62 (3H, d, J = 13.5 Hz), 4.28 (3H, d, J = 13.5 Hz), 4.2 – 4.0 (12H, m), 3.85 (9H, s), 3.83 (3H, d, J = 13.5 Hz), 3.72 (3H, s), 3.49 (3H, d, J = 13.5 Hz). 13C NMR (75.475 MHz, CDCl3, 25°C) δ 157.14 (3C), 152.03 (3C), 151.31 (3C), 140.7 (3C), 139.1 (3C), 136.5 (3C), 132.1 (3C), 130.6 (3C), 125.3 (3C), 118.4 (3C), 113.1 (3C), 110.5 (3C), 69.3 (3C), 65.8 (3C), 60.2 (3C), 55.7 (3C), 36.0 (3C). HRMS (ESI) calcd for C62H45O33N3 [M]+. 895.3688 found 895.3674.

X-ray crystallography: Suitable crystals for all four compounds were selected and mounted on a Gemini kappa-geometry diffractometer (Agilent Technologies UK Ltd) equipped with an Atlas CCD detector and using Cu radiation (λ = 1.5418 Å). Intensities were collected at 150 K for anti-5 and syn-6 by means of the CrystalsPro software Reflection indexing, unit-cell parameters refinement, Lorentz-polarization correction, peak integration and background determination were carried out with the CrystalsPro software.[2] An analytical absorption correction was applied using the modeled faces of the crystal.[3] The resulting set of hkl was used for the structure solution and refinement. The refinement was carried out by direct methods using SIR97 and the least-square refinement on F2 was achieved with the CrystalsPro software.[4] All non-hydrogen atoms were refined anisotropically. The hydrogen atoms were all located in a difference map, but those attached to carbon atoms were repositioned geometrically. The H atoms were initially refined with soft restraints on the bond lengths and to regularize the geometry (C–H in the range 0.93–0.98 Å) and Uiso(H) (in the range 1.2–1.5 times Ueq of the parent atom), after which the positions were...
refined with riding constraints. For compound anti-5, a chlorof orm solvent molecule could be located outside the cryptophane cage. Nevertheless this structure contains additional solvent molecules inside the cryptophane cages that could not be localized. The contribution of the disordered solvent molecules was removed using the SOUUE2 algorithm.[21]

CCDC 1525399, 1525400, contains the supplementary crystallographic data for anti-4 and syn-5, respectively. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

**Laser-Polarized 129Xe NMR spectroscopy:** Hyperpolarized xenon was produced through the spin-exchange method in the batch mode using a home built apparatus based on two coupled 30 W laser diodes.[22] Frozen xenon was stored and transported in a glass reservoir immersed in liquid nitrogen. For the transfer to the NMR tube this reservoir was heated and installed in a vacuum line in the fringe field of the NMR magnet. A hollow NMR spinner reservoir enabled us to condensate hyperpolarized xenon on top of the solution without freezing the solution.

**Acknowledgements**

Support from the French Research Ministry (project ANR-12-BSV5-0003 MAX4US) is greatly acknowledged.

**Keywords:** Cryptophanes • hyperpolarized xenon • molecular recognition • SPINOE • NMR
Supporting Information

Role of the methoxy groups in cryptophanes for complexation of xenon. Conformational Selection Evidenced by $^{129}$Xe-$^1$H NMR SPINOE Experiments.


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[c] Centre de Diffractométrie Henri Longchambon, Université Lyon 1, 5 rue de La Doua, 69100 Villeurbanne, France.

S1: $^1$H NMR (300 MHz) spectrum of compound (4)-6a in CDCl$_3$ solution at 298 K.
S2: $^{13}$C NMR (75.5 MHz) spectrum of compound (4)-6a in CDCl$_3$ solution at 298 K.
S3: $^1$H NMR (300 MHz) spectrum of compound (5)-6s in CDCl$_3$ solution at 298 K.
S4: $^{13}$C NMR (75.5 MHz) spectrum of compound (5)-6s in CDCl$_3$ solution at 298 K.
S5: crystallographic data for diastereomers (4)-6a and (5)-6s.
S6: (top) View of cryptophone (4)-6a (hydrogen atoms and disordered parts have been omitted for clarity). (bottom) Projection of the packing of molecule 6 along the a unit-cell axis.
S7: (top) View of cryptophone (5)-6s (hydrogen atoms and disordered parts have been omitted for clarity). (bottom) Projection of the packing of molecule (5)-6s along the a unit-cell axis.
S8: $^1$H NMR spectra of compounds 9a (left) and 9s (right) for the degassed solutions (spectra a and d); in the presence of xenon (spectra b and e). $^{129}$Xe-$^1$H SPINOE spectra of compounds 9a (spectrum c) and 9s (spectrum f).

S9: $^1$H NMR spectra of compounds 6a (left) and 6s (right) for the degassed solutions (spectra a and d); in the presence of xenon (spectra b and e). $^{129}$Xe-$^1$H SPINOE spectra of compounds 6a (spectrum c) and 6s (spectrum f).

S10: $^{129}$Xe NMR spectra of compounds 3a (left) and 3s (right) at 293K

S11: $^1$H NMR spectra of compounds 3a (left) and 3s (right) for the degassed solutions (spectra a and d); in the presence of xenon (spectra b and e). $^{129}$Xe-$^1$H SPINOE spectra of compounds 3a (spectrum c) and 3s (spectrum f).
S1: $^1$H NMR (300 MHz) spectrum of compound (4)-6s in CDCl$_3$ solution at 298 K.
S2: $^{13}$C NMR (75.5 MHz) spectrum of compound (4)-6a in CDCl$_3$ solution at 298 K.
S3: $^1$H NMR (300 MHz) spectrum of compound (5)-6s in CDCl$_3$ solution at 298 K.
$^{13}$C NMR (75.5 MHz) spectrum of compound (5)-6s in CDCl$_3$ solution at 298 K.
**Table 1:** crystallographic data for diastereomers *anti*-4 and *syn*-5

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<th><em>anti</em>-4</th>
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<td><em>P</em>₂₁/<em>n</em></td>
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<tr>
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<td>12.3095 (1)</td>
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<tr>
<td>b (Å)</td>
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<td>20.8271 (2)</td>
</tr>
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<td>c (Å)</td>
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<tr>
<td>β (°)</td>
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<td>92.3869 (8)</td>
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<td>γ (°)</td>
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<td></td>
<td>1.17 e Å⁻³</td>
<td>0.69 e Å⁻³</td>
</tr>
</tbody>
</table>

S5: crystallographic data for diastereomers (4)-6a and (5)-6s.
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