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Cucurbituril derivatives as oxygen carriers

Cucurbit[n]urils are rigid cage-molecules of pumpkin-like shape, made of n-glycoluril units, able to bind mainly neutral molecules and cations. In this work, we investigate the binding of three cucurbit[5]uril derivatives with dioxygen O_2 and show that one of them, namely per-hydroxylated cucurbit[5]uril, $(OH)_{10}CB[5]$, is able to significantly bind dioxygen gas at physiological temperature, even in the presence of sodium chloride at the concentration of injectable solution in blood. As cucurbit[n]urils studied up to now reveal low toxicity, per-hydroxylated cucurbit[5]uril appears as a promising precursor to design a host able to transport O_2 in a haemoglobin substitute solution.

Keywords: cucurbituril; dissolved gas binding; supramolecular chemistry; oxygen carrier

Introduction

Cucurbit[n]urils (*1*) (CBn) are macrocycles based on n-glycoluril units that possess a constricted hydrophobic cavity synthetic pumpkin-like shape molecules with polar carbonyls at the rigid portals (*2*, *3*). These molecules, including CB[5], the smallest member of the family with n=5, compound **1** on Figure 1, are poorly soluble in pure water, about 0.3 mM for CB[5] (*4*) and 0.02 mM for CB[6] (*4–6*) at 293 K, and in most common organic solvents (*4*, *7–9*). The functionalization of their methine moieties, R on Figure 1, which is a challenging task, induces a huge increase of solubility in water (*10–14*). For example, the solubility of the decamethylcucurbit[5]uril **2** is about 8 mM in pure water (*4*, *15*), 27 times higher than that of the native cucurbit[5]uril. No data is available for per-hydroxylated cucurbit[5]uril **3**, but a preliminary analysis shows that the water solubility of $(OH)_{10}CB[5]$ **3** is at least 0.7 mM at 293 K. The functionalization provides the opportunity to synthesize multi-functional molecules, paving the way to an important research area in the field of coordination and supramolecular chemistry (*16*). For example, per-hydroxylated cucurbit[6]uril, similar to $(OH)_{10}CB[5]$ **3** but containing

6 glycoluril units instead of 5, has been grafted on silica gel for applications in chromatography (*17*, *18)* and in the biodegradation of aliphatic hydrocarbons (*19*). Kim et al. have proposed other applications of cucurbituril **3**, including the depollution of water and air, the sensing of organic molecules and metals ions, and the use of **3** as additives, drug carriers, packing materials of chromatographic columns and catalysts for chemical reactions (*20)*. [Figure 1 near here]

Cucurbiturils are able to bind neutral small molecules (*21–23*), including noble gases (*24–27*) and non-noble gases (*27*, *28*). They are located in the cavity, while cations are located at the portals (*29–31*). A chloride anion has been found in the cavity of cucurbit[5]uril in the crystal phase (*32–34*) but this probably arises from the packing forces of the crystal. Nitrate anions also weakly bind cucurbit[5]uril, but only in acidic solutions). However, to the best of our knowledge, no significant binding between anions and cucurbiturils in pure water was reported. As $Me_{10}CB[5]$ **2** has been found to bind O_2 in the gas phase (*29)*, in pure water and in the solid phase (*27)*, the present study aims at investigating the binding of cucurbit[5]uril **1** and two derivatives, decamethylcucurbit[5]uril **2** and $(OH)_{10}CB[5]$ **3**, with O_2 in aqueous solution in the absence or presence of cations.

Material and methods

Chemicals

CB[5] **1** was purchased from Sigma Aldrich and used without purification. $Me_{10}CB[5]$ **2** was synthesized as described in the literature (*35)*. $(OH)_{10}CB[5]$ **3** was prepared following a previously reported procedure (*36)*, slightly modified as follows: a mixture of CB[5] (48.0 mg, 0.058 mmol, 1 eq) and $K_2S_2O_8$ (187.0 mg, 0.59 mmol, 12 eq) in distilled water (2.9 mL) was degassed by bubbling nitrogen, and then heated and stirred

at 358 K for 6 h under nitrogen. After cooling to room temperature, the resulting precipitate was discarded by filtration. The filtrate was half-concentrated under reduced pressure. Dropwise addition of acetone allowed then precipitation of residual impurities that were removed by filtration. Finally the filtrate was concentrated under reduced pressure and purified on an Amberlyst A21 resin (Fluka 20-50 mesh), obtaining a pH 2 solution. After filtration on a sinter filter (P4 porosity), the filtrate was concentrated under reduce pressure to give the desired compound $(OH)_{10}CB[5]$ **3** (28.0 mg, 49 %), in accordance with the reported analysis.

Methods

Cucurbit[5]urils **1**, **2** and **3** were independently dissolved at 0.7-1.1 mM in D_2O or, for **3**, in DMSO-d_6, in NMR tubes equipped with Teflon valves from Young Scientific Glassware Ltd. The solutions were first degassed through several cycles of agitation followed by an expansion of the gas phase in a known volume under vacuum. NMR experiments were performed on a Bruker Avance II spectrometer, operating at a frequency of 500.13 MHz for ^1H and equipped with a 5 mm-inverse broadband probe with z-gradient. Quantitative ^1H NMR spectra were recorded at 293 K with 32768 complex data points and an acquisition time of 2.7 s. The classical inversion-recovery method was used to measure the proton longitudinal relaxation rates R_1, with 16 to 64 delays d between inversion and excitation ranging from 10^{-3} to 18 s. The spectra were recorded with a random order of d values to avoid artefacts from incomplete magnetization recovery between acquisitions. After Fourier transformation, the spectra were phased and baseline corrected in Bruker Topspin 3.2 software. The R_1 values were extracted from the integrals of signals corresponding to cucurbiturils and HDO, and used as inputs for a non-linear least squares fitting (nls function from the R software), by adjusting I_0, A and R_1 in the equation

$$I(d)=I_0*[1-2A \exp(-d\ R_1)] \qquad\qquad (1)$$

^{1}H spectra were recorded again after the addition of a known pressure of O_2. O_2 was introduced in the previously degassed NMR tubes either by expansion of the gas previously contained in a syringe directly connected to the tube, for a pressure of 1 bar, or, for higher pressures, by condensation of O_2 in a tube immersed in a liquid nitrogen bath. In all cases, the pressure value was obtained after the experiment by expanding the gas into a chamber equipped with a manometer. Knowledge of all volumes leads to the estimate of the pressure in the tube for each R_1 value determination. According to the Henry's law, applicable with great accuracy at the pressures used in this work, the dissolved oxygen concentration $[O_2]$ is proportional to the pressure above the solution (*37*). As compound **1** does not significantly bind O_2 (see Results Section), it was discarded for subsequent experiments.

Quantitative ^{1}H NMR spectra were recorded for compounds **2** and **3**, at 0.32 mM, in the presence of 0.15 M NaCl, at 310 K, both degassed and under various O_2 pressures. For $(OH)_{10}CB[5]$ **3** with NaCl, inversion-recovery spectra with presaturation of the HDO signal were recorded, with 64 delays *d* ranging from 5.10^{-4} to 2 s in a random order, and an interscan delay of 2 s.

The O_2-cucurbituril binding constant of $(OH)_{10}CB[5]$ **3** in a 0.15 M NaCl solution was then estimated by fitting the R_1 values against the O_2 concentration. At 310 K, the O_2 solubility in such a solution is 0.18 mM/bar (*38*). Assuming that, for a given NMR signal assigned to a cucurbituril site, R_1 is the sum of a diamagnetic term R_{1d}, measured for the degassed tube, and of a paramagnetic term, R_{1p}, proportional to the average number of O_2 molecules complexed by the cucurbituril, the binding constant K was extracted by fitting R_1 against the O_2 concentration. Two models were tested,

assuming a 1:1 or a 2:1 O_2:cucurbituril complex. Details are given in the Supporting Information.

The presence of ammonium, potassium, sodium and all other metal ions was characterized by mass spectrometry. First, infusion in positive ion electrospray ionization mode on a Waters XEVO™ TQ-S Mass Spectrometry (ESI-MS) was performed on three 0.1 - 1.3 mM samples of each **1**-**3** molecules, diluted to a concentration of 10 µg/mL in a H_2O:CH_3OH, 1:1 v:v solution. The presence of complexes between each cucurbituril and ammonium, sodium and potassium ions was evaluated. Second, the presence of potassium, sodium and other metal ions in aqueous millimolar solutions of compounds **1**, **2** and **3** has been determined by quadrupole ICP-MS (Thermo Electron iCAP Q) in collision cell mode (KED) to eliminate mass interferences. The solutions were scanned between 6 and 260 atomic mass units. The concentration of potassium in the CB[5] **1** and $(OH)_{10}$CB[5] **3** solutions were measured by external calibration after dilution of a certified K standard (SPEX, MultiElement CertiPrep). Measurements on this K standard were repeated 6 times before averaging. Two dilutions of the CB[5] **1** and $(OH)_{10}$CB[5] **3** samples were prepared and analysed. All dilutions were performed by weighting.

Results

The paramagnetic character of dioxygen represents a very powerful probe of the local interaction of the molecule with solutes through electron-nucleus dipolar relaxation in NMR. The effect of the O_2 binding as a function of the nature of the equatorial substituent R of the cucurbit[5]uril appears unambiguously on the quantitative ^{1}H NMR spectra recorded in pure water (Figure 2). At 293 K, under ca. 1 bar of dioxygen, i.e. with 0.25 mM of free O_2 in solution (*38*), the signals of CB[5] **1** broaden only marginally. We conclude that **1** does not significantly bind O_2. On the contrary, a

tremendous broadening of the signals of $Me_{10}CB[5]$ **2** is observed, showing that **2** binds O_2 in pure water. Signals of $(OH)_{10}CB[5]$ **3** are duplicated. Those marked by a star are broaden in the presence of O_2, unlike those marked by a circle. Therefore, only molecules of **3** marked by a star are able to substantially bind O_2. Table S1 contains the full-width half maximum of H_{ax} of CB[5] **1,** $Me_{10}CB[5]$ **2** and $(OH)_{10}CB[5]$ **3** in pure water as a function of O_2 concentration [Figure 2 near here]

The intrinsic properties of dioxygen binding of each cucurbituril should be decorrelated from the presence of cations, known to bind cucurbiturils and therefore potential competitors of O_2. Their presence has been investigated by ICP-MS and positive ion ESI-MS in **1**, **2** and **3** solutions. Na^+ and some other metal ions have been detected by ICP-MS in the CB[5] **1** sample but in non-significant amount. Conversely, K^+ has been titrated to 2 equivalents in a solution of **1** (Figure S1). Taking the reported binding constant of K^+ with CB[5] **1** of 20 M^{-1} (*39*), less than 10% of the cucurbiturils of the 0.35 mM solution bind K^+. This may have a little influence on the binding of O_2 to CB[5]. The NH_4^+ cation has also been detected by positive ion ESI-MS in compound **1** (Figure S2). Its presence at the portals of the cucurbituril is probably weak, as it shows a low binding constant of 10-20 M^{-1} (*39*). NH_4^+ should therefore not impede O_2 to reach the cavity and to stay there. This is in agreement with previous results obtained by X-Ray crystallography, mass spectra and NMR (*27*, *29*, *30*). Considering the absence of influence of these cations on the binding properties and the lack of O_2 binding in these experimental conditions, it confirms that CB[5] **1** is unsuitable as an *in vivo* O_2 carrier. ICP-MS indicates that no Na^+ or other metal ions are present in a solution of $Me_{10}CB[5]$ **2**. ESI-MS of compound **2** show peaks characteristic of cucurbiturils encapsulating Na^+ and K^+. However, these signals are not significant because Na^+ and K^+ ions are present in the water used in the ESI-MS infusion experiments with

concentrations around 1.3 nM and 4.3 nM for K^+ and Na^+, respectively, i.e. a

concentration slightly lower than that of cucurbiturils **1-3**, around 10 nM . In ESI-MS,

these cations facilitate the formation of adducts. We conclude that $Me_{10}CB[5]$ **2** is able

to bind O_2 in the absence of cations.

MS analysis of a solution of $(OH)_{10}CB[5]$ **3** has also been performed.

Unfortunately, ESI-MS of $(OH)_{10}CB[5]$ **3** did not show either the molecular ion peak or

its complex with NH_4^+, Na^+ or K^+. Furthermore, the titration of K^+ by ICP-MS has been

performed and shows that $(OH)_{10}CB[5]$ contains ca. 4 equivalents of K^+ (Figure S1). K^+

may have an influence on the capacity of **3** to bind O_2, especially if the corresponding

binding constant is much higher than that reported when CB[5] **1** is concerned (*39*).

This might explain the unexpected occurrence of two pairs of doublets for the

methylene protons of $(OH)_{10}CB[5]$ **3**. In order to identify other molecules susceptible to

impede O_2 binding and explain the duplication of NMR signals, ^{1}H NMR analysis of **3**

has been performed with DMSO-d_6 as the solvent to observe exchangeable protons.

Firstly, translational diffusion NMR shows that the two pairs of doublets of **3**, marked

by stars and circles, have very close diffusion properties (Figure S3a). This indicates

that the difference between both forms does not come from different intermolecular

interactions between cage-molecules, of molecular mass on the order of 1 kDa, but

rather from the binding of molecules of much lower mass. As $(OH)_{10}CB[5]$ **3** has been

synthesized from CB[5] **1**, whose ^{1}H NMR spectrum discards the presence of CB[6], no

$(OH)_{2n}CB[n]$ derivatives, with n higher than 5, is present. The simple, symmetric [1]H

NMR spectrum discards a partial hydroxylation of CB[5]. A [1]H-NOESY spectrum has

been recorded in DMSO-d_6. It reveals the presence of sharp signals assigned to

ammonium and slightly less sharp signals tentatively assigned to other protonated

ammonium salts (Figure S3b). Indeed, these last signals, but not those of ammonium,

show exchange with the very broad signal assigned to residual water and hydroxyls of $(OH)_{10}CB[5]$. The most intense of those signals tentatively assigned to ammonium salts also show correlations with non-exchangeable protons of $(OH)_{10}CB[5]$ **3**. Moreover, the signals assigned to ammonium salts show smaller diffusion coefficients than those of NH_4^+, though higher than that of methylene protons of **3**, indicating a complexation with a notable influence of exchange with residual water. It has indeed been reported a high binding constant, ca 2.10^4 M^{-1} between 1,6-diaminohexane and $Me_{10}CB[5]$ **2** in acidic conditions (*39*). This tends to indicate that, at least in part of the cage-molecules, ammonium salts are present.

O$_2$, as a paramagnetic molecule, has a huge influence on NMR properties of neighbouring nuclei, particularly on the transversal relaxation rate R_2, acting on the line-width of the signal, and on the longitudinal relaxation rate R_1. The signal corresponding to the methyl protons of $Me_{10}CB[5]$ **2**, at about 1.8 ppm, is only slightly enlarged (Figure 2). This is easily understandable assuming that O$_2$ acts on relaxation mainly through a dipolar process, whose efficiency drops off with a dependence on the sixth power of the distance between the two involved nuclei. Based on the crystallographic structure of $Me_{10}CB[5]$ **2** in the presence of O$_2$ (*27*), a reduced distance was calculated between on the one hand protons of each family (H_{ax}, H_{eq}, Me) of the cucurbituril and on the other hand the centre of the cavity (refer to Fig. 1 for H_{ax} and H_{eq} nomenclature). Reduced distances to H_{ax}, H_{eq} and Me protons of cucurbiturils are 4.8, 5.4 and 6.3 Å, respectively. The higher distance between the centre of the cavity and the methyl protons explains the lower signal broadening. In solution, the location of O$_2$, as other neutral molecules, inside the cavity of the cucurbituril, is in adequacy with previous results obtained by crystallography (*29–31*). These observations have been quantified by fitting the relaxation rate R_1 of each signal in NMR spectra of compounds

1, **2** and **3**, R_1 being a more accessible parameter than R_2. Examples of fits are given in Figure S4, and full data in Figure S5. As expected in the extreme narrowing regime, the R_1 values increase as signals broaden. The difference of relaxation rate with and without O_2 is mainly due to dipolar interaction between unpaired electrons of O_2 and the surrounding nuclei. This relaxation mechanism is efficient only for a long correlation time of the relative position of the nuclear and unpaired electronic spins in the external magnetic field. This correlation time is much shorter when O_2 is not maintained close to the cucurbiturils. As a proof, the longitudinal relaxation rate of the proton of the HDO signal is measured to 0.026 ± 0.006 s^{-1} for the degassed solution of CB[5] **1**, and only slightly increases to 0.056 ± 0.004 s^{-1} under 1 bar pressure of O_2. On the contrary, dipolar relaxation is effective when O_2 has a long residence time, most probably in the core of cucurbituril. For example, all proton signals of Me$_{10}$CB[5] **2** and doublets assigned to H$_{ax}$ and H$_{eq}$, marked by a star in the O_2@**3** complex (Figure 2) show large relaxation rate changes. O_2 acts also slightly on the chemical shift of surrounding nuclei. It was also observed that the signal of H$_{ax}$ responsive to oxygen binding are slightly downfield-shifted within the titration. This shift is of 14 Hz between degassed sample and the sample under 5 bar oxygen pressure. The shift may either come from the paramagnetism of O_2 or a different set of conformations of the cucurbitutil and surrounding solvent molecules. The unicity of the signal is in accordance with a fast exchange regime during the whole titration. This shows that the kinetics of binding and release of O_2 is of 20 Hz or higher in our experimental conditions.

The significant binding of O_2 with Me$_{10}$CB[5] **2** and a part of (OH)$_{10}$CB[5] **3** in pure water and at 293 K lead us to study by ^{1}H NMR the O_2 binding to compounds **2** and **3** in physiological-like conditions, i.e. 310 K and in the presence of 0.15 M of NaCl, the concentration of the classical injectable saline solution. Sodium ions proved to have

a low but measurable affinity to CB[5] **1** in pure water, with a binding constant of 71 ± 2 M^{-1} at 298 K (*39*). They may therefore efficiently compete with O_2 as guest of derivatives of cucurbit[5]uril. [Figure 3 near here]

The ^{1}H NMR spectra of compounds **2** and **3** in a 0.15 M deuterated saline solution are shown in Figure 3. Sharp signals are observed for $Me_{10}CB[5]$ **2** in the presence or the absence of O_2, suggesting that Na^+ impedes O_2 to bind the cage-molecule. This thermodynamic result can be brought closer to a kinetic observation in the gas phase: it has been shown that the release of O_2 from **2** is much slower when ammonium are present, because they form lids at the portals of the cucurbituril (*29*). The occurrence of highly concentrated Na^+ in blood (*40*) makes cucurbituril **2** not suitable to transport O_2. On the contrary, one pair of doublets of **3**, marked by stars on Figure 3 and assigned to H_{ax}, is broadened. The full-width half-maximum values of the signals of **3** are reported in Table S2 as a function of O_2 concentration. Cucurbituril **3** is therefore a good candidate for O_2 binding purposes.

For future applications, it is also important to characterize the kinetics and thermodynamics of binding of dioxygen with $(OH)_{10}CB[5]$ **3**. At 310 K, the pair of doublets of H_{eq} in the same environment is masked by the HDO signal. Therefore, the binding affinity of O_2 with **3** in the saline water solution has been characterized by extracting the R_1 values of the H_{ax} signals marked by a star as a function of O_2 pressure, then by fitting these values over the 1:1 and 1:2 host:guest models given in Supporting Information. For **3**, the presence of a second set of NMR signals of properties unaffected by O_2 does not influence the obtained binding constants. Indeed, concerning the molecules corresponding to the first set of doublets, only the fraction of cucurbiturils that contain O_2 guests is relevant, and not their absolute concentration. The results of the fits are given on Figure 4. Clearly, the model where at most one O_2

molecule can be encapsulated in the cucurbituril cavity is the best. In the 1:1 model for $O_2@\mathbf{3}$, the binding constant K is estimated to 3000 ± 700 M^{-1}. The model fits particularly well the R_1 values at low O_2 concentration: $R_{1d} = 2.1 \pm 0.8$ s^{-1}. The fitted value for R_{1p} is much higher, 51 ± 6 s^{-1}, as expected from the paramagnetic character of O_2. [Figure 4 near here]

Discussion

In the present work, we investigate the binding of dioxygen by three cucurbit[5]uril derivatives. NMR spectroscopy is well-suited to detect the binding of O_2 by cucurbit[5]uril derivatives, as its paramagnetic property strongly influences the parameters of the surrounding nuclear spins, particularly their transversal relaxation rate acting on the line-width of signals, and their longitudinal relaxation rate R_1. We show by ^1H NMR studies that $Me_{10}CB[5]$ **2** and $(OH)_{10}CB[5]$ **3**, but not $CB[5]$ **1**, are able to bind dioxygen in pure water. Subtle differences in gas binding as a function of the functionalization of methine sites of cucurbit[5]uril were reported (*41*). This may partly explain the difference of binding properties of O_2 in the cucurbit[5]uril derivatives **1**-**3**.

The nature and concentration of the cations present in solution has also been reported, in the case of sodium and potassium, to have a substantial influence (*41*). We confirm this result as $(OH)_{10}CB[5]$ **3**, but not $Me_{10}CB[5]$ **2**, significantly binds O_2 at 310 K in a 0.15 M sodium chloride solution, whereas both binds O_2 at 293 K in pure water. The concentration of the most abundant cations in blood, sodium, potassium and calcium, are respectively about 140, 4.5 and 1.2 mM (*40*). The binding constants of these three cations with $CB[5]$ **1** in water at 298 K have been reported to similar values, in the range 20-71 M^{-1} (*39*). When dealing with $Me_{10}CB[5]$ **2** as the host, values of the same order of magnitude were reported, 13 and 41 M^{-1} for potassium and calcium, respectively. Due to their low concentration in blood and low binding constant to

cucurbituril, potassium and calcium are expected to not efficiently influence O_2 complexation with cucurbit[5]uril derivatives.

The occurrence of a fraction, here about half, of **3** unable to bind O_2 has been further investigated. Discarding the hypotheses of assigning this form to stable aggregates, symmetric cucurbiturils formed by 6 glycoluril units or more, and CB[5] moieties bearing less than 10 hydroxyl "equatorial" substituents, these $(OH)_{10}CB[5]$ probably host molecules that act as competitors with O_2. Two candidates have been pointed out. It has been reported that K^+ binding constant is dependent on the equatorial substituents of CB[5] (*41*). K^+ might have a high affinity to **3**, contrary to its affinity with **1** and **2**. Alkyl-ammonium cations are likely to bind CB[6] (*42*) and also CB[5], though with much weaker binding constants (*39*). Ammonium salts might also bind a part of $(OH)_{10}CB[5]$ **3**. They may come from a partial degradation of **3** and may be located at the portal of the cucurbituril. Removing these molecules should involve a higher capacity to bind O_2.

From the experimental $O_2@$**3** binding constant, the solubility of oxygen in saline solution and the fraction of **3** available to bind O_2, a concentration of about 20-30 mM of cucurbituril **3** is required to efficiently transport O_2 at the same concentration as in blood, i.e. about 7.4 mM of O_2, corresponding to 190 ml of O_2 per liter. The toxicity has also to be considered. No data are available on the toxicity of $(OH)_{10}CB[5]$ **3**. However, *in vitro* and *in vivo* studies of another cucurbituril, differing from CB[5] **1** by the occurrence of 7 glycoluril units instead of 5, revealed a very low toxicity (*43*, *44*). For example, a maximum tolerated dosage of 250 mg.kg^{-1} is demonstrated after intravenous delivery in mice (*45*).

Cucurbiturils may find application for oxygen carriers in the conception of viable haemoglobin substitutes, among other approaches (*46*). It is also expected that **3**

can be used as an oxygenator of a culture medium of aerobic microalgae. Molecular design and new synthesis and purification steps will now be performed to optimize cucurbiturils derivatives, particularly with regard to the choice of the equatorial substituents, in the purpose of getting optimized binding properties, high solubility and low toxicity. These optimized cucurbiturils may complement another molecule able to bind bicarbonate, produced from the waste product CO_2. Bambusurils, made of glycoluril units as cucurbiturils, are known to efficiently bind anions in water (*47*), and it has been shown that they can be functionalized (*48, 49*). Together, a cucurbituril and a bambusuril may therefore be the major constituents of a haemoglobin substitute solution.

Declaration of interest statement

After an agreement between the CEA and Mr Benoît Prieur, this discovery has been first patented in Europe and a start-up has been created by Dr Benoît Prieur. All commercial propositions have to be addressed to him by mail: prieur7@gmail.com.

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Figure captions

Figure 1. Cucurbiturils CB[5] **1**, $Me_{10}CB[5]$ **2**, $(OH)_{10}CB[5]$ **3** used in this work.
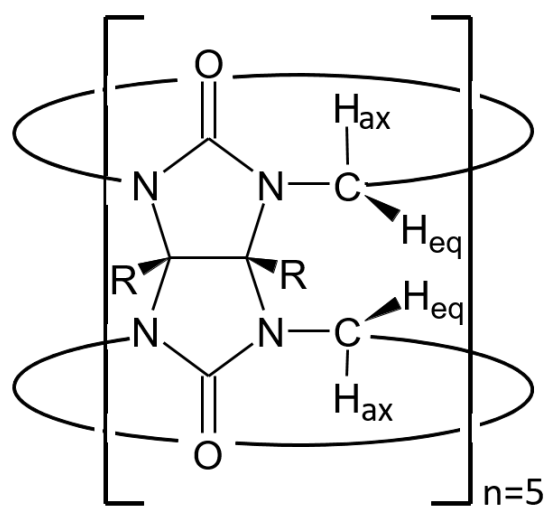
Figure 2. ^1H NMR spectra of CB[5] **1**, $Me_{10}CB[5]$ **2**, $(OH)_{10}CB[5]$ **3** in D_2O, at 293 K, at 1.1, 1.0 and 0.7 mM, respectively, either degassed or under ca. 1 bar of O_2. For **3**, stars are assigned to signals of molecules hosting O_2. Signals marked by circles are not sensitive to O_2, see text.

Figure 3. ^1H NMR spectra of $Me_{10}CB[5]$ **2** and $(OH)_{10}CB[5]$ **3**, at 0.32 mM, in a 0.15 M NaCl solution in D_2O, at 310 K, either degassed or in the presence of ca. 1 bar of O_2. For **3**, stars and circles are assigned as in Figure 2.

Figure 4. Fits of relaxation rates R_1, in s^{-1}, for the doublet of H_{ax} of $(OH)_{10}CB[5]$ **3** marked by a star, in a 0.15 M NaCl water solution, at 310 K, as a function of O_2 concentration, in M, for a 1:1 (solid line) and a 2:1 (dotted line) host:guest model, respectively. Concentration of **3** is 0.32 mM. Experimental errors on fitted R_1 are displayed.

Word count: 5263 words

Figure 1

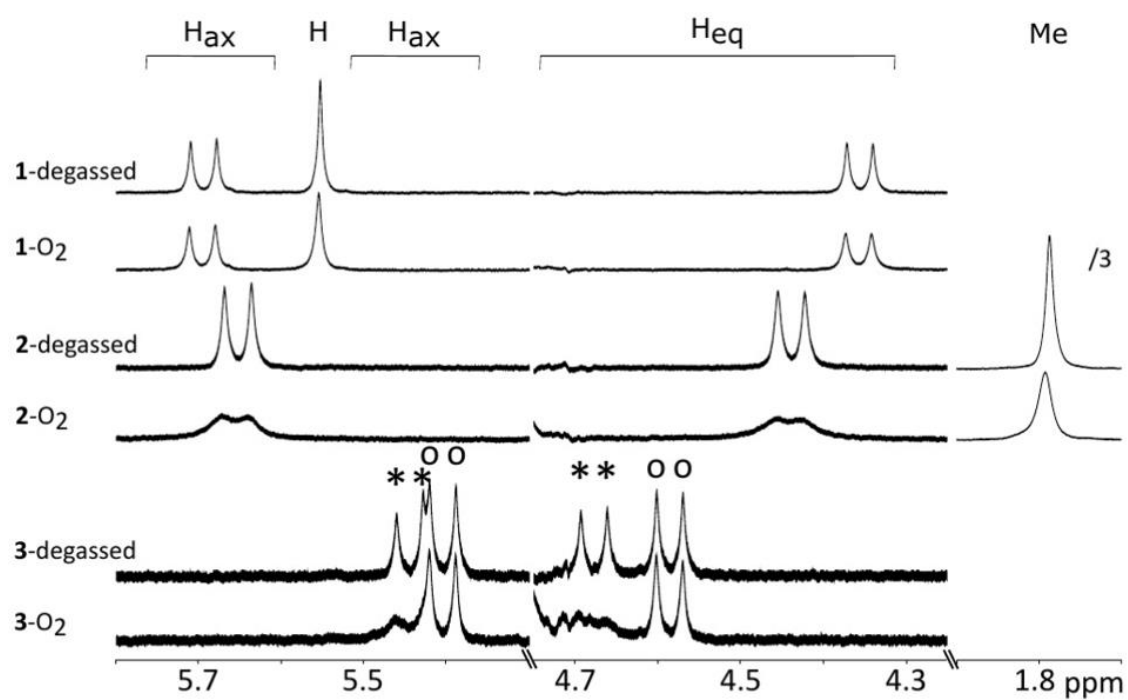**1**: R = H; **2**: R = Me; **3**: R = OH

Figure 2

Figure 3