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ARTICLE *in* ORGANIC LETTERS · NOVEMBER 2009

Impact Factor: 6.36 · DOI: 10.1021/ol902391u · Source: PubMed

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ORGANIC LETTERS

2009
Vol. 11, No. 24
5690−5693

EPR and NMR Investigation on the Interactions of Nitroxide Probes with Resorcin[4]arene Molecular Capsules

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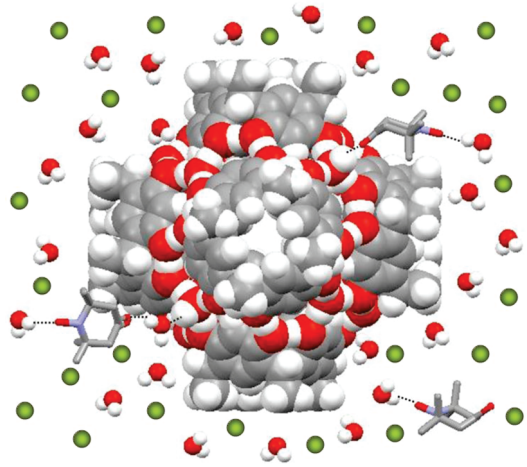
Received October 16, 2009

ABSTRACT



EPR experiments in water-saturated CH_2Cl_2 solution clearly indicate that oxotempo is not included inside hexameric molecular capsules of resorcin[4]arene. In fact, the tumbling rate of oxotempo only experiences minor changes when resorcinarene is present in the solution. However, NMR spectroscopic data suggest that oxotempo engages in labile hydrogen-bonding interactions with water molecules interacting with the resorcinarene molecular capsules.

The self-assembly of resorcin[4]arenes in nonpolar solvents has received considerable attention in the past few years.[1] The initial discovery of the formation by compound **1** (see structures in Figure 1) of hexameric molecular capsules in the solid state[2] was followed by a number of reports[3,4] on the self-assembly of the more soluble analogues **2** and **3** in the solution phase. We have recently reported the use of nitroxide spin probes and electron paramagnetic resonance (EPR) spectroscopy as a powerful method to investigate this self-assembly system in dichloromethane solution.[5] The paramagnetic nature of the nitroxides obviously allows their use as probes in EPR spectroscopic experiments,[6] but their effects on the NMR resonances of neighboring nuclei also constitutes a well established methodology to investigate the system.[7] Here, we contrast the experimental results obtained with these two methods and address an apparent disparity, which provides strong experimental evidence for a unique, labile interaction between oxotempo and water molecules near the external surface of the resorcinarene molecular capsules.

As we have previously reported,[5] our EPR experiments clearly reveal that the nitroxide probes shown in Figure 1 can be classified into two distinct groups in terms of their

(1) (a) MacGillivray, L. R.; Atwood, J. L. *Angew. Chem., Int. Ed.* **1999**, *38*, 1018–1033. (b) Rebek, J., Jr. *Angew. Chem., Int. Ed.* **2005**, *44*, 2068–2078.

(2) MacGillivray, L. R.; Atwood, J. L. *Nature* **1997**, *389*, 469–472.

10.1021/ol902391u
Published on Web 11/17/2009

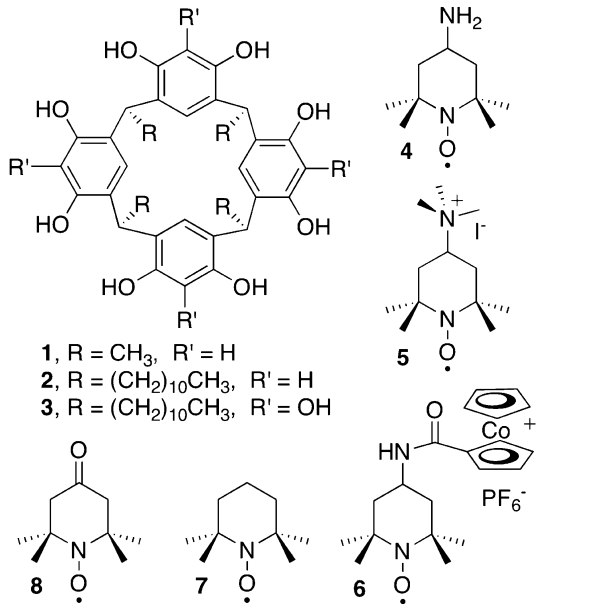


Figure 1. Structures of the compounds used in this work.

interactions with resorcinarene **2**. The first group is composed of nitroxides **4**−**6**, which are incorporated inside hexameric capsules of **2**, as evidenced by the broadening of the high-field peak in the EPR spectrum of the corresponding nitroxide when excess **2** is added to the solution. This reflects the decreasing tumbling rate of the nitroxide probe upon encapsulation inside the hexameric assembly.[8] The hyperfine splitting constant (a_N) also increases slightly from the value observed for the free nitroxide in dichloromethane solution. In striking constrast, the EPR spectrum of the two remaining nitroxides (compounds **7** and **8**) are little affected by the presence of excess resorcinarene **2**. For nitroxide **8**, the a_N value is unchanged and the correlation time (inversely related to the tumbling rate in solution) goes from 1.3×10^{-11} s in the absence of **2** to 2.1×10^{-11} s in the presence of 30 equiv of **2**. Similar data were measured with **7**, unequivocally indicating that neither **7** nor **8** are encapsulated inside hexameric **2**.[9]

The EPR spectra obtained with compound **8** (often referred to as oxotempo) are shown in Figure 2. These spectra are

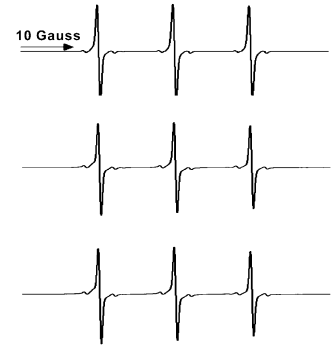


Figure 2. EPR spectra of 0.1 mM **8** in water-saturated CH_2Cl_2 solution (top) in the absence and in the presence of (middle) 6 equiv and (bottom) 12 equiv of host **2**.

not very sensitive, as mentioned above, to the presence of resorcinarene. During the course of this investigation, we ran parallel NMR spectroscopic experiments with the goal of gathering further information on the location of the nitroxide spin probes inside the molecular capsules of **2**. A set of typical results is shown in Figure 3, which depicts the

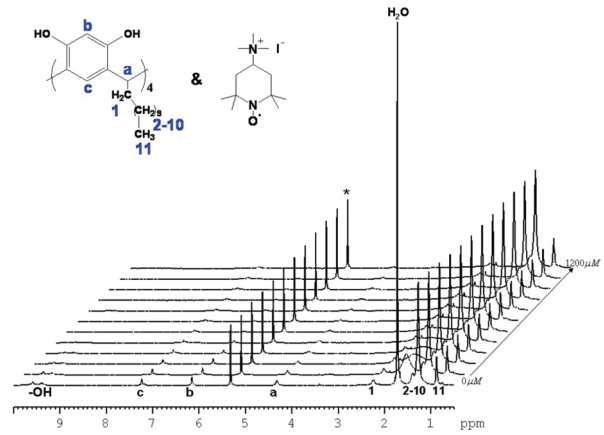


Figure 3. ^{1}H NMR spectra (400 MHz, water-saturated CD_2Cl_2) of resorcinarene **2** as a function of the added concentration of spin probe **5**.

^{1}H NMR spectra recorded for resorcinarene **2** in water-saturated CD_2Cl_2 solution as a function of the added concentration of nitroxide **5**. Clearly, the presence of this nitroxide broadens

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(8) The presence of hexameric molecular capsules was verified by measuring the diffusion coefficient of **2** using NMR PGSE techniques.

(9) We have shown (ref 5) that nitroxide encapsulation by hexameric **2** leads to a substantial increase (50- to 100-fold) in the correlation time.

many of the resonances of the resorcinarene protons. Specifically, the peaks corresponding to the resorcinarene core, that is the hydroxide protons, the aromatic protons (b and c, see Figure 3 for proton labels), the bridge protons (a) and the directly attached methylene protons (1) on the pendant aliphatic chains are all substantially broadened by the spin probe. Notice, however, that the rest of the protons on the pendant undecyl chains (resonating at δ <1.7 ppm) do not undergo any significant broadening. These findings are consistent with the EPR data and unequivocally reveal that the cationic spin probe is encapsulated within the central cavity of the hexameric assembly of **2**, thus affecting and broadening the core protons and leaving the outside protons on the host side chains unaffected.

All EPR and NMR experiments were conducted in water-saturated CH_2Cl_2 (or CD_2Cl_2) as it is well established that water molecules are necessary to complete the network of hydrogen bonds that glue together the six molecules of host **2** into a capsular assembly. The peak for the water protons, clearly visible at 1.7 ppm in Figure 3, also undergoes extensive and effective broadening upon addition of the spin probe. Since there is a large excess of water molecules in the solution, this finding indicates that they exchange quickly between the bulk solution and the hydrogen bound positions on the capsule's surface, leading to the observed broadening of the water peak.

Similar NMR spectroscopic experiments with nitroxides **4** and **6** yield basically the same results (see Figures S3−S6, Supporting Information); that is, the nitroxide causes substantial broadening of the peaks corresponding to the core protons on the host and the water protons, while the outer aliphatic protons on the host side chains remain unaffected. As in the case of nitroxide **5**, this is consistent with the EPR spectroscopic data and the proposed encapsulation of nitroxides **4**−**6** by the hexameric resorcinarene capsules.

Using nitroxide **8** (oxotempo) led to the NMR spectroscopic data shown in Figure 4. The presence of oxotempo

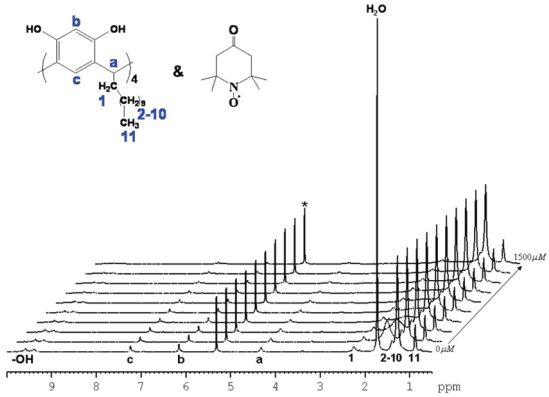

Figure 4. ^{1}H NMR spectra (400 MHz, water-saturated CD_2Cl_2) of resorcinarene **2** as a function of the added concentration of spin probe **8**.

has a much smaller effect on the proton resonances of the resorcinarene core as compared to what we observed with nitroxides **4**−**6**. As always, the aliphatic protons on the side chains are not broadened at all. These findings are consistent with the EPR spectroscopic evidence that provides unequivocal support against encapsulation of **8**. However, the peak corresponding to the water protons undergoes substantial and effective broadening in the presence of **8**. In order to shine some light on this unexpected finding, we also run similar experiments with unfunctionalized tempo (compound **7**). In this case, the NMR spectroscopic data (Figures S9−S10, Supporting Information) show minimal broadening of the signals for the core protons of the resorcinarene and no broadening for the signals of the aliphatic chain protons, while the water peak experiences some broadening, but its magnitude is considerably less pronounced than in the case of **8**.

Obviously intrigued by the pronounced and rather unexpected broadening of the water proton resonance caused by oxotempo, we ran several control experiments. For instance, in the absence of resorcinarene **2**, similar concentrations of oxotempo cause very little broadening of the water peak in water-saturated CD_2Cl_2 (Figure S11, Supporting Information). This finding clearly demonstrates that preferential solvation of **8** by H_2O over CD_2Cl_2 is not a factor in the observed results. We also took advantage of the well-known fact that pyrogallolarene (host **3**) does not require any water molecules to form the corresponding hexameric capsular assembly ($\mathbf{3}_6$); that is, in water-saturated CD_2Cl_2 solutions of host **3**, there are no water molecules hydrogen bound to the capsule. Interestingly, in the presence of host **3**, nitroxide **8** also fails at broadening the water peak in water-saturated CD_2Cl_2 solution (Figure S12, Supporting Information). From the results of these control experiments (see also Figure S14, Supporting Information), we conclude that the broadening of the water resonance by oxotempo requires the presence of water molecules interacting with the hexameric capsule of **2** via hydrogen bonding.

The fact that oxotempo (**8**) is considerably more effective than tempo (**7**) at broadening the NMR resonance of the water molecules in the presence of capsules of **2** led us to hypothesize that the carbonyl oxygen in the former compound may be important. We reasoned that if the carbonyl oxygens engage in hydrogen bonding interactions with the water molecules, large concentrations of molecules with structure similar to **8** may disrupt the molecular interactions and thus decrease or eliminate the observed effect on the NMR water peak. Therefore, we used 1,4-cyclohexanedione (CHD) for this purpose. Figure 5 shows some interesting NMR data in this regard. Addition of 100 mM CHD to a solution containing 3.0 mM host **2** and 0.08 mM nitroxide **5** has very little effect on the NMR spectra (Figures 5A and 5B), beyond the expected appearance of a large resonance for the protons of CHD at 2.7 ppm. Our previous EPR and NMR experiments demonstrate that, in this case, the nitroxide is encapsulated in the central cavity of the hexameric assembly. Therefore, the presence of CHD does not affect capsule formation or the encapsulation of **5**. The degree of broadening of the water resonance remains unchanged, because the broadening is largely due to the proximity

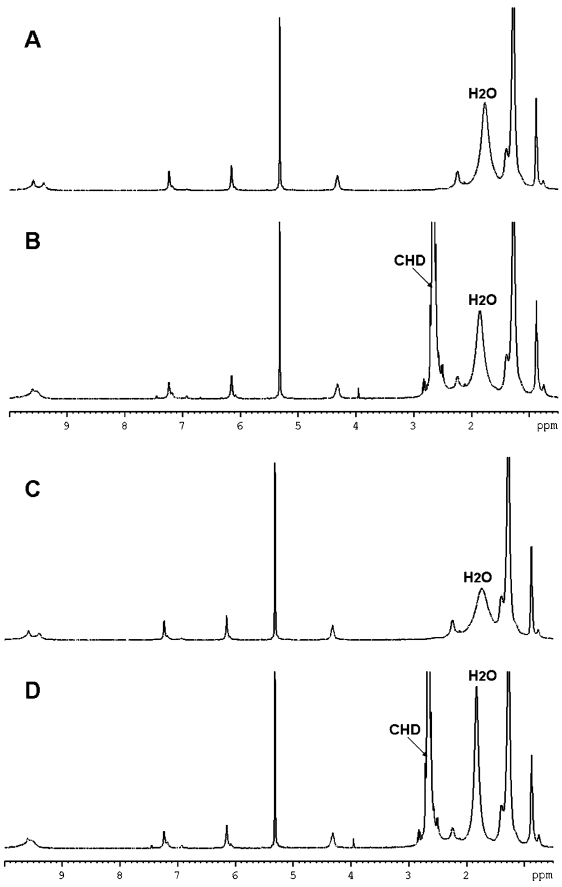

Figure 5. ^{1}H NMR spectra (400 MHz, water-saturated CD_2Cl_2) of 3.0 mM resorcinarene **2** in the presence of (A) 0.08 mM **5**, (B) 0.08 mM **5** + 100 mM CHD, (C) 0.1 mM **8**, and (D) 0.1 mM **8** + 100 mM CHD.

between the capsule-attached, hydrogen bound water molecules (in fast exchange with bulk water molecules) and the capsule-trapped spin probe.

A very different picture develops when oxotempo is used as the spin probe. As shown in Parts C and D of Figure 5, addition of 100 mM CHD to a solution containing 3 mM resorcinarene **2** and 0.1 mM **8** leads to a considerable narrowing of the water peak. This finding indicates that CHD interferes with the interaction between oxotempo and the water molecules. Since this interaction must take place outside the hexameric capsule, as our experimental evidence indicates that oxotempo is not encapsulated, it is reasonable to conclude that CHD competes with oxotempo for hydrogen bonding sites on the water molecules, leading to a sharper water peak.

While the correlation between EPR and NMR spectroscopic data is generally very good, a puzzling aspect of these results is the need to reconcile our observation (NMR spectroscopic data) on the hydrogen bonding of **8** to water molecules, which takes place to a measurable extent only in the presence of capsules of **2**, with our observation on the relatively small effect of the same capsules on the tumbling rate of this nitroxide probe in solution (EPR spectroscopic data). The necessary presence of the capsules clearly suggests that only water molecules hydrogen-bound to the assemblies (or in their immediate vicinity) may interact effectively with nitroxide **8** and these interactions slow down in a detectable way the tumbling of the nitroxide in solution. The tumbling rate of **8** does not decrease nearly as much as the tumbling rates of nitroxides **4**−**6**, as the latter are fully encapsulated. We can only rationalize these results by assuming that oxotempo only hydrogen binds to water molecules which are partially dislodged from the capsular assembly, that is, not reaching their full capacity for hydrogen bonding to the assembly. In this way, the hydrogen bond between water and oxotempo would be extremely labile and would keep the nitroxide free from attachment to the large capsular assembly, preserving a relatively fast tumbling rate. Alternatively, the water molecules may accumulate in the vicinity of the hexameric capsular assembly, where their enhanced local concentration may lead to more effective hydrogen bonding with nitroxide **8**.

This work shows that the combination of EPR and NMR spectroscopies constitutes a powerful tool for the investigation of the complex resorcinarene self-assembling system in solvents of low polarity.

Acknowledgment. The authors are grateful to the National Science Foundation for the support of this work (to A.E.K., CHE-848637). We thank Prof. Jamie D. Walls, University of Miami, for his useful advice.

Supporting Information Available: Additional spectroscopic data as mentioned in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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