Characterisation of Host-Guest Complexes of Supramolecular Self-Assembled Cages using EPR Spectroscopy

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**ABSTRACT**

Host-guest interactions between nitroxide stable radicals and supramolecular coordination cages were investigated using EPR spectroscopy in water and acetonitrile. TEMPO showed negligible association with the cages in water while 4-oxo-TEMPO bound with a strength comparable to that previously reported for related ketones. Carboxylic acid-functionalised nitroxides bound strongly to the acetonitrile-soluble coordination cages. In all cases, host-guest complex formation resulted in significant decreases in the molecular tumbling rate of the guests, with tumbling becoming strongly anisotropic. The polarity of the cage environment in both solvents was found to be intermediate between water and acetonitrile.

**INTRODUCTION**

Self-assembled supramolecular coordination cages and the host-guest complexes they can form are an area of interest due to the variety of potential applications these structures can afford, ranging from the storage and transport of specific molecules (dangerous1,2 or drug-like3,4 for example) to using cages to enable efficient catalysis of chemical reactions5–7 or provide control over reaction dynamics.8,9 Therefore, investigating the binding environments of these structures, and the nature of the interactions between host and guest molecule, is vital to enable further development and understanding of these potential applications.

Although not previously applied to characterisation of coordination cages, EPR spectroscopy is well suited for studying supramolecular interactions by using guest molecules labelled with a stable free radical (e.g., nitroxide). Molecular motion of the guest probe on the ns timescale may be investigated by observing the linewidth changes upon binding. The hyperfine value of the EPR signal of stable radicals is sensitive to changes in polarity, and hence may be used as a reporter on the polarity of the microenvironment around the guest molecule. Deconvoluting complex spectra into contributions from different components can be used to determine equilibrium constants. Finally, interactions between adjacent radical guests can provide information about intermolecular distances. For instance, Fujita et al. observed encapsulation of two radicals in a coordination cage resulting in a radical pair in triplet state.10,11 EPR spectroscopy has been successfully used to characterise a range of supramolecular complexes including cyclodextrins,12–16 cucurbiturils17–22 and resorcinarenes.23,24

In this work, several nitroxide radicals were used to probe the host-guest complexes for cage structures which had not previously been studied in this way. Analysis of EPR spectra allowed us to measure equilibrium constants for host-guest complex formation and characterise the polarity and rotational diffusion parameters of the encapsulated guests.

**EXPERIMENTAL SECTION**

**General Details**

Cages **C1w** and **C2org** and the precursors required were prepared by adapting literature procedures. Full experimental procedures are given in the Supporting Information. All guests used in this work were obtained from commercial sources and used as received.

**EPR Spectroscopy**

EPR spectra were recorded on a Bruker EMXmicro spectrometer. Samples in MeCN were transferred to sealed Pasteur pipettes and were degassed with N2 prior to recording, whilst samples in H2O were recorded in sealed Marienfeld melting point tubes. Samples in H2O were not degassed due to the lower dissolved oxygen content, and to avoid potential issues arising due to difficulty in obtaining a uniform level of degassing in these samples. All samples were run at room temperature except for frozen spectra which were recorded at 125 K. Typical EPR parameters were: Power = 5.024 mW; Frequency = 9.32 GHz, Field Modulation Width = 1.0 G.

Experimental EPR spectra were presented without normalisation, and absolute integrals were reported. To ensure comparison between spectra was possible, measurements of samples in the same solvent were recorded in identical EPR cells, and controls showed that absolute integrals were reproducible to within 2.5 %.

**EPR Simulations**

EWVoigt software developed by Alex I. Smirnov was used for fast motion nitroxide EPR simulations,25 and EasySpin version 5.2.15 was used in MATLAB R2017b for slow-motion simulations and determination of molecular motion parameters.26 EWVoigt was chosen for fast-motion spectra as it allowed for more efficient correction of phase errors in the spectrum than the corresponding function in EasySpin.

**RESULTS AND DISCUSSION**

**Synthesis and characterisation of the cage structures**

The cage structures used in this work were based on those reported by Ward and co-workers,27 and have the general formula [M8L12][X]16, where M = Co2+ or Cd2+, L = C28H22N6, and X = ClO4- or Cl-, as depicted in **Figure 1**.



**Figure 1.** Schematic representation of the cage structures **C1w** ([Co8L12][Cl]16) and **C2org** ([Cd8L12][ClO4]16), highlighting the approximately cubic shape. Only two of the 12 ligands are depicted, illustrating the bis-bidentate binding of the ligand to the metal atoms. The "w" and "org" endings represent solvents (water and acetonitrile) which were used for experiments with these cages.

These structures were chosen due to their reported stability in solid form and in solution,27 and the ability to fine-tune the properties of the cage by substituting metal and counterions. By using paramagnetic Co2+ and diamagnetic Cd2+ ions in cages **C1w** and **C2org** respectively, the interaction between the EPR-active spin probes and an EPR-active or inactive host system could be investigated and compared. Additionally, cage **C1w** (chloride counterions) is water-soluble while cage **C2org** (perchlorate counterions) is soluble in MeCN. Attempts to form a water-soluble diamagnetic Cd2+-based cage with chloride anions were unsuccessful, most likely because Cd2+ is more kinetically labile than Co2+, and so exchange between the linker ligands and aqua ligands in water is faster, leading to destruction of the cage when dissolved in water.28

The approximately 400 Å3 cavity size made the cage a suitable host for the nitroxide guests studied in this work (**Chart 1**), as they occupied around 44 % of the internal cavity volume. Based on the optimal occupancy value suggested by Rebek’s Rule of 55 %,29,30 it was expected that binding of the guests should be reasonably favourable.

**Chart 1.** Structures of the guests used in this work: (**R3**) TEMPO, (**R4**) 4-oxo-TEMPO, (**R5**) 4-carboxy-TEMPO, (**R6**) 3-carboxy-PROXYL, (**R7**) 3-carbamoyl-PROXYL, (**CG8**) hexamethylacetone, (**CG9**) benzoic acid.



The cages were synthesised by adapting literature procedures and characterised by a range of analytical techniques (see Supporting Information).7,27,31,32

**Host-guest interactions in radical+cage (R+C) systems**

In this section, we describe qualitative studies aimed at identifying the types of radical guest molecules that could bind to the cage structures, comparing the behaviour of two cages, one in water, the other in MeCN. The EPR spectra of radical+cage systems are explained qualitatively in terms of a combination of host-guest interactions and additional effects in both solvents. In the second part of the results and discussion section, we present quantitative analysis of selected examples. We use spectrum simulations to extract information about the microenvironment of encapsulated guests, including polarity and rotational motion of the guests, and estimate the binding constants.

Guests **R3** and **R4** were investigated with cage **C1w** in water and guests **R5** and **R6** with cage **C2org** in MeCN. In previous studies with cages of this type hydrophobic factors were found to dominate binding of guests in H2O-soluble cages, whilst H-bonding interactions dominate in the MeCN-soluble analogues.33–35 Inside the cage cavity, two H-bond donor “pockets” are located on opposite corners, at the two *fac* tris-chelate binding sites. Multiple CH bonds from the cage ligands point inwards towards the cage cavity at these positions, and have previously be found to effectively form CH···X hydrogen bonding interactions to bind guest molecules inside cages in organic solvents.36–38 Hence it was expected the more hydrophobic molecules **R3** and **R4** would best suit cage **C1w**, whilst the carboxylic acid functionality of **R5** and **R6** would provide a better H-bonding acceptor group for cage **C2org**.

Stock solutions of the radical guests at 4×10-5 M concentration were prepared and combined with an equal volume of either neat solvent or 1×10-3 M cage solution, yielding radical (R) and radical+cage (R+C) solutions with the same radical concentration: 2×10‑5 M. This ensured that accurate comparisons could be made between the EPR spectra for these solutions.

The initial spectra revealed decreases in signal intensity for the **R+C** samples compared to the cage-free controls (**R**), with the smallest decrease observed for the **R3**+**C1w** sample (Figure 2a), and the largest observed for the **R6**+**C2org** sample (Figure 4b). This is indicative of host-guest interactions, as line broadening which results from slower tumbling of encapsulated guest manifests itself visually as drop in signal intensity.



**Figure 2.** EPR spectra of radical (top, black), radical+cage (middle, blue), radical+cage+competing guest (bottom, red). a) **R3**, **C1w**, **CG8**; b) **R4**, **C1w**, **CG8**. Radical concentration was 2×10-5 M, cage concentration 5×10-4 M and competing guest concentration ~2×10-3 M.

To confirm that these decreases were indeed due to guest-cage binding, a competing guest was added at ~2×10-3 M. For cage **C1w**, hexamethylacetone (**CG8**) was chosen as the competing guest, as previous studies with these cage structures had shown it to bind strongly in an aqueous environment.39 For cage **C2org**, benzoic acid (**CG9**) was chosen as the competing guest as **CG8** was found to bind less strongly to the cage structures in MeCN. It was predicted that binding of radicals **R5** and **R6** to cage **C2org** would occur through the carboxy group, and so using **CG9** as a competing guest also provided this functionality. The additional aromatic ring was expected to enhance the H-bonding acceptor nature of the guest, improving the binding ability as discussed by Turega et al. and thus making **CG9** a suitable competing guest.37

**EPR spectra of R+C system in water**

Upon addition of **CG8** to the radical+**C1w** samples it was observed that for **R3**, the signal intensity did not return to that of radical in neat solvent, and instead remained at a level similar to that of the **R3**+**C1w** spectrum (**Figure 2a**). For **R4** however, the signal intensity did increase above that of the **R4**+**C1w** signal, but did not reach that of the radical in neat solvent (**Figure 2b**). This suggested that an additional broadening effect was present in these samples, and that for **R3**+**C1w**, there was no binding occurring, with the decrease in intensity observed solely due to this additional effect, whilst for **R4**+**C1w** the larger decrease in signal intensity could be ascribed to a combination of binding and the other effect.

To explain these observations, Heisenberg exchange between the radical guest and the paramagnetic cobalt atoms of the cage structure was considered.40 This effect would significantly broaden the EPR signal of the encapsulated radical due to collisions of the radical with the cobalt atoms in close proximity. The broadening would be reduced when the radicals are displaced from the cage by large excess of competing guest, as the radical-cobalt collisions would be less frequent.

To confirm this hypothesis further, a control experiment was run where Co(ClO4)2·6H2O solution was combined with **R4** and **R4**+**CG8** solutions, and EPR spectra recorded (**Figure 3**). Co2+ concentration was the same as in the **R4**+**C1w** solution. However, no binding of **R4** to Co(ClO4)2 was expected in the absence of the cage, so if broadening was observed this could be attributed to Heisenberg exchange.

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**Figure 3.**EPR spectra of solutions of: 4-oxo-TEMPO (**R4**) (top, black); **R4** + Co2+ (middle, green); **R4**+Co2++**CG8** (bottom, red). Concentration of radical is 2×10-5 M in all cases, concentration of Co(ClO4)2• 6H2O was 4×10-3 M and concentration of **CG8** was 2×10-3 M.

The EPR spectra showed that the intensity of **R4**+Co2+ was similar to that of **R4**+Co2++**CG8**, indicating that Heisenberg exchange was broadening the signal. Simulation of the exchange-broadened **R4**+Co2+ spectrum as the convolution of the **R4** spectrum and a Lorentzian line revealed a peak-to-peak width of 0.21 G for the Lorentzian component. This value is in perfect agreement with the literature value of 0.24 G reported for Co(H2O)62+ at the same concentration, also in the presence of **R4** in water, confirming that the broadening was due to Heisenberg exchange between the radical and Co2+.41

Thus, addition of cage **C1w** to both **R3** and **R4** leads to interactions between unbound radical in solution and the Co atoms of the cage, causing broadening of the EPR signal as a result of Heisenberg exchange. However, for **R4**+**C1w**, greater broadening is observed for two reasons: (i) binding of the radical to the cage enhances Heisenberg exchange due to increased proximity of the radical to the Co atoms, and (ii) binding restricts the tumbling motion of the radical. Upon addition of **CG8** to **R4+C1w**, the bound radical is released, and these effects disappear, leaving only interactions between unbound radical and Co atoms of the cage. This confirms that unlike **R3**, **R4** forms a host-guest complex with cage **C1w**, indicating the carbonyl group in **R4** is necessary to facilitate binding, supporting previous literature reports of ketones binding to the cage and suggesting that the hydrophobicity of the cavity is not the sole driving force for binding. On the other hand, **R3** has been reported to bind with moderate affinity to a similar water-soluble cage (with a hydroxylated ligand and ClO4- counterions), K = 1.3×104 M‑1).42 This discrepancy with our observation of no binding of **R3** with the cage **C1w** suggests that the strength of intermolecular interactions is strongly dependent on subtle changes in the host structure, and although good quantitative structure-activity relationships have been reported,35,42 small changes in the host structure (including counterion) can significantly affect intermolecular interactions.

**EPR spectra of R+C system in MeCN.**

Although the EPR signal intensity of **R5** and **R6** was reduced in the presence of cage **C2org** (which indicates host-guest interactions), addition of **CG9** led to a surprisingly large increase in signal intensity, well above that of the radical in neat solvent (**Figure 4).**



**Figure 4.** EPR spectra of radical (top, black), radical+cage (middle, blue), radical+cage+competing guest (bottom, red). a) **R5**, **C2org**, **CG9**; b) **R6**, **C2org**, **CG9**. Radical concentration was 2×10-5 M, cage concentration 5×10-4 M and competing guest concentration ~2×10-3 M.

The effect was attributed to the fact that in solution the radical-containing carboxylic acid may form H-bonded dimers.43,44 Radical dimerisation leads to spin exchange which manifests itself in the EPR spectra as reduction in intensity of the three nitroxide hyperfine lines. Addition of benzoic acid to the radical+cage solution hence resulted not only in displacement of the bound radical, but also disrupted radical-radical dimers, increasing signal intensity.

Addition of benzoic acid to a solution containing only radical **R5**, with no cage present, also led to an apparent increase in intensity, thus confirming the presence and subsequent disruption of dimerisation between the radicals.

To confirm that binding of these radicals was dependent on the presence of the carboxylic acid group, 3-carbamoyl-PROXYL (**R7**), with an amide group in the place of the carboxylic acid was investigated. The spectra of radical **R7** and radical+cage (**R7**+**C2org**) were nearly identical, suggesting that no binding was occurring. This indicates that the carboxylic acid group plays a critical role in forming the host-guest interaction for this system. This big difference between binding properties of **R6** and **R7** is surprising taking into account the similarity between the size/shape and H-bonding acceptor properties (which correlate strongly with the association constant for related systems due to the H-bond donor “pockets” in the cage, *vide supra*42) of the COOH and CONH2 groups. We note that carboxylic acids (e.g., adamantane and aromatic derivatives) were previously reported to show strong binding to such coordination cages in a neutral, undissociated state.45,46

**Quantitative analysis of EPR spectra**

Once the formation of host-guest complexes of **R4**+**C1w, R5**+**C2org** and **R6**+**C2org,** had been established qualitatively, we explored the host-guest interactions of these complexes quantitatively to obtain information about binding constants, the local microenvironment of the cage cavity, and how the radical guests behaved inside. Experiments were performed with radical concentrations 1×10‑5 M, 2×10‑5 M, 4×10‑5 M and 5×10‑5 M, and the resulting spectra simulated to extract the necessary parameters for analysis.

The EPR spectra were simulated as the combination of two components, corresponding to a narrower, unbound component, and a broader, bound radical component. The simulations showed the unbound component with three sharp signals, as expected for a nitroxide radical in the fast-motion regime. However, in the bound component, these signals appear to be far broader, providing evidence of restricted motion (and Heisenberg exchange in the case of cage **C1w**) (**Figure 5a-c**).

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**Figure 5.** a) EPR spectrum of 2×10-5 M **R4** + 5×10-4 M **C1w**. b) EPR spectrum of 2×10-5 M **R5** + 5×10-4 M **C2org** c) EPR spectrum of 4×10-5 M **R6** + 5×10-4 M **C2org.** Experimental (dotted, black), simulated (green), simulated unbound (red), and bound (blue) components.

For **R4+C1w** and **R5+C2org** the bound components were still considered to be in the fast-motion regime, whilst for **R6+C2org** the change in lineshape for the bound component appeared to be significant enough that the motion was considered to be in the slow-motion regime.

For all radical+cage combinations, the spectra for each concentration were simulated with the same set of optimized EPR parameters (**Tables 1-2**), which made it possible to determine the contribution from free and encapsulated guests and hence calculate the association constants for host-guest interaction (**Table 3**, see details in supporting information). This approach assumes that encapsulation is slow on the EPR time scale, which is commonly observed for supramolecular interactions at room temperature.

**Table 1.** Averaged parameters for the simulations of **R**+**C** spectra

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Component | Lorentzian / G[a] | | | Gaussian / G[b] | Nitroxide Hyperfine / G |
| (1) | (0) | (-1) |  |
| **R4**+**C1w** | Unbound | 0.18 | 0.19 | 0.22 | 0.64 | 16.03 |
| **R4**+**C1w** | Bound | 3.73 | 3.68 | 4.50 | 0.14 | 15.74 |
| **R5**+**C2org** | Unbound | 0.18 | 0.22 | 0.40 | 1.56 | 15.79 |
| **R5**+**C2org** | Bound | 4.10 | 3.94 | 7.70 | 0.32 | 16.36 |

[a] Contribution of the Lorentzian function to the linewidth, for ml = 1, 0 and -1.

[b] Contribution of the Gaussian envelope function to the linewidth.

**Table 2.** Averaged parameters for the simulations of **R6**+**C2org** spectra

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Component | Lorentzian / G | Gaussian / G | log[Diffusion Rate / s-1] | | | Rotational Diffusion Rate / s‑1 | | |
| Isotropic | Dxy | Dz | Isotropic | Dxy | Dz |
| Unbound | 0.61 | 1.31 | 9.31 | - | - | 2.04×109 | - | - |
| Bound | 1.40 | 2.57 | - | 7.15 | 7.58 | - | 1.41×106 | 3.80×107 |

**Table 3.** Calculated association constants for each **R**+**C** complex

|  |  |  |  |
| --- | --- | --- | --- |
| Radical | Cage | Solvent | K / 103 M-1 |
| **R4** | **C1w** | H2O | 7.9 ± 0.3 |
| **R5** | **C2org** | MeCN | 9.2 ± 0.6 |
| **R6** | **C2org** | MeCN | 11.9 ± 0.3 |

**Effect of solvent and the nature of functional groups on binding**

Comparison to previous literature investigations with similar H2O- and MeCN-soluble cages revealed comparable association constants to those obtained for coumarin (7.6×103 M-1) and 2-quinolone (6.7×103 M-1) in the water-soluble cage.39 This suggests that **R4** has a similar binding affinity for **C1w**, potentially due to hydrophobic interactions and H-bonding between the cage structure and the carbonyl and nitroxide groups in **R4** being analogous to the interactions between the cage and the carbonyl group and fused aromatic ring in the previously investigated guests. The strength of binding in a similar water-soluble cage has been shown to correlate with several parameters including shape complementarity, hydrophobic interactions, guest flexibility etc.42

In the previously studied MeCN cage,39 these molecules had association constants of 7.8×101M-1 (coumarin) and 6.0×102 M-1 (2-quinolone), showing far weaker binding than the radicals **R5** and **R6** studied in this work in MeCN. The carboxylic acid functional group of the radicals may be the main reason for this, providing greater H-bonding interaction strength than that of the carbonyl of the previous guests.

**Microenvironment of bound guests**

***Polarity***

The EPR simulations revealed a small change in hyperfine value observed for the **R4**+**C1w** (15.74 G) compared to that of the unbound **R4** (16.03 G). The decrease in the hyperfine value suggests that the cavity environment of the cage is less polar than the bulk H2O solution,47–49 supporting observations in previous work.33

In contrast, the increase in hyperfine value for radical **R5**+**C2org** (16.36 G) compared to unbound **R5** (15.79 G) in solution, indicates the binding environment of the radical is more polar than that of the unbound radical for this radical+cage complex. This suggests that the binding environment of the cage structures is more polar than MeCN but less polar than H2O.

It is important to note the change in hyperfine value is only small (~0.3 G), and so strong conclusions may not be drawn from this data alone, as the change in hyperfine value could arise as a simulation artefact due to using the fast-motion approximation to fit the bound components. However, the observations appear to support the previously observed characteristics of the host-guest complexes of cages of this type.

***Rotational Diffusion***

Diffusion parameters were obtained from spectral simulations, allowing characterisation of the molecular motion of the radical guests in their bound and unbound states. For unbound radicals the isotropic diffusion parameter Diso was calculated, whilst for the bound radicals the axial diffusion parameters Dxy and Dz were determined. To assign these values to the rotational diffusion axes D⊥ and D∥ respectively, the nitroxide was approximated as a rigid rod, with one end fixed to the cage and the other terminating in the N-O bond, defining the D∥ axis as parallel to the N-O bond. The angle between D∥ and the magnetic z-axis, βD, in this work was 90º (**Figure 6**).



**Figure 6.** Diagrams showing the orientation of the magnetic axes for nitroxides in this work: Top view (left), side view (centre), and diagram showing rotation about the diffusion axes, where angle between the magnetic z-axis and D∥, given by βD, is 90º (right). D⊥ is perpendicular to D∥.

Using this basis, the Diso and D⊥ and D∥ parameters were calculated for each radical, as shown in **Table 4**.

**Table 4.** Calculated diffusion parameters for each **R**+**C** complex

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Radical | Cage | Solvent | Unbound | Bound | |
| Diso / s-1 | (D⊥) / s-1 | (D∥) / s-1 |
| **R4** | **C1w[a]** | H2O | 9.52×109 | 2.44×108 | 3.98×105 |
| **R5** | **C2org** | MeCN | 2.34×109 | 3.39×105 | 1.63×108 |
| **R6** | **C2org** | MeCN | 2.41×109 | 1.33×106 | 3.93×106 |

[a] Inclusion of 7.54 MHz Heisenberg exchange in the R4+C1w simulation gave better fit to the experimental spectrum than simulation that did not include this parameter. (See Supplementary Figure S2)

The diffusion parameters of unbound guests are similar to the values reported in the literature (2.64×109 - 6.67×109 s-1 for **R3**,19,20 7.58×109 s-1 for **R4** in water,20 3.96×109 s-1 and 1.39×1010 s-1 for TEMPO-Me3N+ and 4-amino-TEMPO in water-saturated dichloromethane, respectively).23

Highly anisotropic diffusion parameters (D⊥ ≠ D∥) suggest that EPR-detected motion is dominated by the tumbling of the guest and the movement of the whole complex (radical+cage) is slow on the EPR timescale (as rotational diffusion of the cubic cage would have been nearly isotropic). The absolute values of the faster diffusion component (108 to 109 s-1) are similar to those for many encapsulated nitroxides, e.g., in disulfide-crosslinked cyclodextrins16 or in larger cryptophanes.50

The D∥ value for **R4**+**C1w** (3.98×105 s-1) and D⊥ for **R5**+**C2org** (3.39×105 s-1) indicate that these motions are almost frozen on the EPR timescale. For **R4**, this is surprising, as H-bonding between the cage and the carbonyl group of the radical should not affect D∥ tumbling. The motion is probably slowed down by a specific steric interaction with the host molecule in this case. Additionally, the relatively large D⊥ for **R4** (2.44×108 s-1), suggests that the motion of the radical within the cavity about this axis is almost unrestricted, potentially indicating a good match between the radical and the cage cavity, such that such a specific interaction and motion can be achieved.

In **R5** and **R6**, the motion about the D⊥ is strongly restricted, presumably due to strong H-bonding of the carboxylic acid. The rod-like, nearly axially-symmetric **R5** shows fast motion about the D∥ axis, whereas tumbling of bent **R6** about this axis is significantly slower.

**CONCLUSIONS**

Using a range of nitroxide radicals as spin probes, EPR spectroscopy has been used to characterise host-guest interactions in supramolecular coordination cages and determine equilibrium constants for binding. In water, 4-oxo-TEMPO showed comparable binding strength to those previously reported for similar ketones. In an organic solvent (acetonitrile), carboxylic acid-functionalised nitroxides showed strong binding to the cages. The environment around the encapsulated guests showed intermediate polarity between water and acetonitrile, and anisotropic rotational diffusion.

ASSOCIATED CONTENT

**Supporting Information**. Synthetic procedures for cages and precursors; EPR sample preparation details; EPR simulation procedures and information

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

ABBREVIATIONS

EPR, electron paramagnetic resonance.

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**Table of Contents**

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