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Spin labeling monitors weak host–guest interactions

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A resorcinarene bearing four TEMPO units recognizes small molecules in solutions.

Stable, sterically hindered free radicals are widely used in biochemistry and biophysics as spin labels for monitoring various processes by electron paramagnetic resonance (EPR).[1] Molecular recognition by synthetic receptors can also be followed through spin labeling. For example, two tetramethylpiperidine oxide (TEMPO) radical residues attached to calix[4]- and calix[6]arene platforms were shown to sense complexation of metal cations.[2] Different distances between spins in free hosts and their complexes had a pronounced effect on the multiplicity of the EPR signals. Interactions between free radicals attached to the wider rim of calix[4]arene were used to monitor conformational changes of the calixarene skeleton.[3]

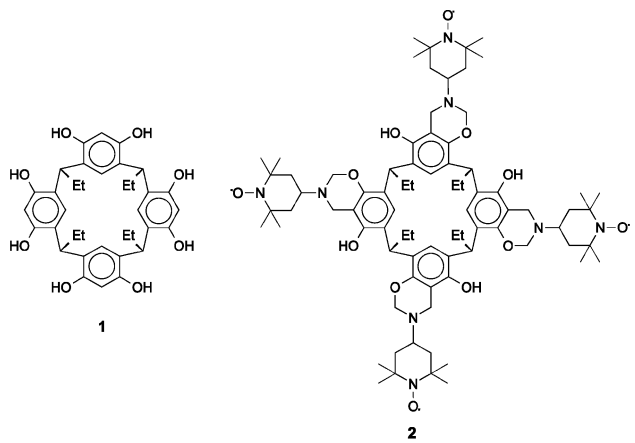
C_4-symmetrical tetrabenzoxazine derivatives of resorcinarenes were shown to include various small guests in the crystalline state.[4] However, in solution no complexation can be detected by NMR spectroscopy. The flexibility of the benzoxazine rings results in very fast uptake and release of the guest.[5,6] Herein we report on the synthesis and structure of paramagnetic resorcinarene **2**,[7] and show that EPR studies reveal its binding properties in solution.

Aminomethylation of resorcinarene **1**[8] (Scheme 1) with four equivalents of commercially available 4-aminoTEMPO and eight equivalents of formaldehyde (MeOH, rt) afforded chiral C_4-symmetrical benzoxazine **2** bearing four TEMPO residues at its wider rim in 38% yield.† Compound **2** precipitated from the reaction mixture and could be purified by simple crystallization. The ^{1}H NMR signals of **2** in $CDCl_3$, although broadened due to paramagnetic effects of the TEMPO residues, are similar to those spectra of known resorcinarene tetrabenzoxazines. The absence of signals for the protons in 2-positions of the resorcinol rings indicates complete aminomethylation, whereas the presence of the resonance at 5.0 ppm reveals the formation of the benzoxazine rings.

Slow crystallization of **2** from the mixture of CH_2Cl_2 and acetonitrile afforded diffraction quality single crystals.‡ As expected, in the crystalline state **2** adopts the cone conformation, stabilized by four intramolecular hydrogen bonds between the hydroxy groups and the oxygens of the benzoxazine rings (Fig. 1). The conformation is slightly distorted since the dihedral angles between pairs of opposite resorcinol rings are 52.6 and 76.5°. The benzoxazine rings are arranged in a distorted C_4-symmetry similar to known resorcinarene tetrabenzoxazines.[8] Although the molecule is chiral, the crystal is a racemate ($P2_1/n$ space group). Three TEMPO residues extend the concavity of the resorcinarene while the remaining residue is directed away from the cavity. This arrangement is most probably caused by packing effects. One acetonitrile molecule disordered over two positions is included in the cavity of **2**. The guest forms C–H···π interactions with the resorcinol rings of the host. In addition, the nitrogen of the acetonitrile molecule is in close contact with the methyl groups of the TEMPO residue (N···C distance 3.5 Å) and the methylene group of the benzoxazine ring (N···C distance 3.5 Å), perhaps due to weak C–H···N interactions. The oxygen atoms of three close TEMPO fragments form a triangle with sides of 5.06, 8.12 and 11.33 Å.

The oxygen atom of the TEMPO fragment directed away from the cavity forms a short contact with the methylene group of a benzoxazine fragment of another molecule (Fig. 2 left). The C···O distance of 3.22 Å indicates intermolecular C–H···O hydrogen bonds. In addition, two TEMPO residues form three centered C–H···O hydrogen bonds; the oxygen is in close contact with the methyl (C–O distance 3.3 Å) and methine groups (C–O distance 3.2 Å). In this way the infinite chains of molecules **2** are formed in the crystal.

X-band EPR spectra of **2** were collected at 295 K§ in non-viscous solvents, conditions under which rapid molecular tumbling can be expected to average anisotropic dipolar interactions. In $CHCl_3$, a change in the line shape of the EPR signal of **2** compared with monomeric 4-aminoTEMPO (Fig. 3a,b) indicates the presence of a weak exchange interaction between spins similar to that seen in nitroxides at high concentration.[9] The effects of exchange are further increased in 1 : 1 $CHCl_3$–CH_3CN (Fig. 3c). The average distances between the radicals are decreased by the conformational change induced by binding of the guest in the cavity. A similar effect was also observed in a 1 : 1 mixture of THF and CH_3CN. No



Scheme 1

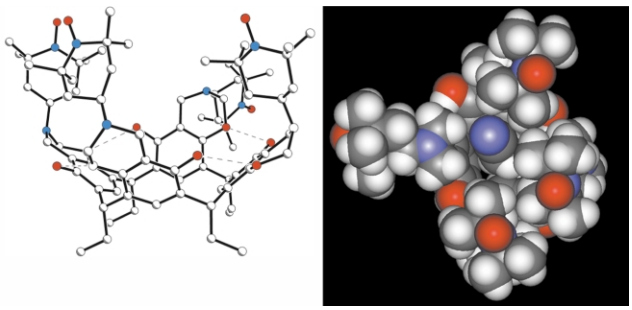


Fig. 1 Molecular structure of **2**·MeCN. Left – side view in ball and stick presentation. Hydrogen atoms and acetonitrile guest are omitted for clarity. Hydrogen bonds are shown in dotted lines. Right – top view in space filling presentation. Only one position of the included acetonitrile molecule is shown.

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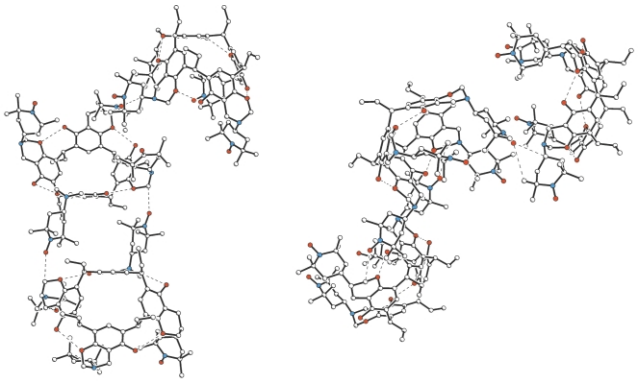

Fig. 2 Crystal packing and intermolecular contacts in **2**·MeCN. Hydrogen atoms and acetonitrile molecules are omitted for clarity. Hydrogen bonds are shown by dotted lines.

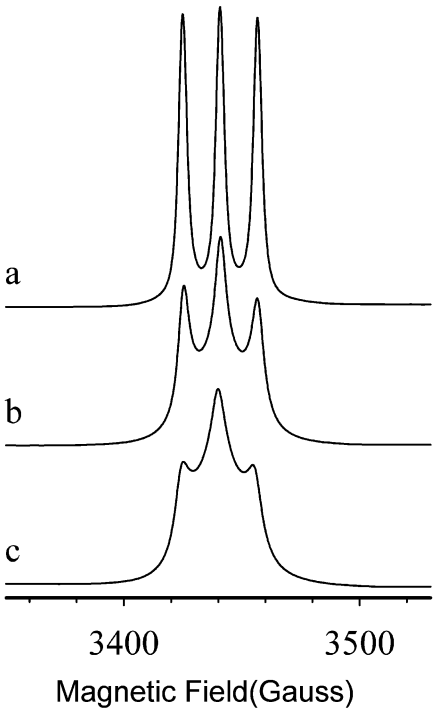

Fig. 3 X-band EPR spectra at 295 K: a) 4-aminoTEMPO (0.4 mM) in $CHCl_3$; b) **2** (0.1 mM) in $CHCl_3$; c) **2** (0.1 mM) in 1 : 1 $CHCl_3$–MeCN. Normalized spectra are displayed as absorption line shapes, obtained as the integral of the measured 1st derivative field modulated signal.

line shape change was observed for 4-aminoTEMPO in 1 : 1 $CHCl_3$–CH_3CN. Spectra of **2** were also recorded in the presence of other potential guest molecules such as CH_3NO_2, CH_2Cl_2 and EtOH, but CH_3CN remained unique in its ability to significantly modulate the EPR line shape. Thus, the effects of exchange interaction between the closely spaced radicals can be used to monitor guest occupation in host–guest complexes.

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Notes and references

† Resorcinarene **1**[7] (0.6 g, 1 mmol) was dissolved in MeOH (3 mL) upon heating to 50 °C and the solution was cooled to room temperature. Formaline (37%, 2 mL, 26 mmol) and acetic acid (2 drops) were added to the solution followed by the solution of 4-aminoTEMPO (1.03 g, 6 mmol) in MeOH (3 mL). The mixture was stirred for 22 h, MeOH (5 mL) was added and the solution was stirred for an additional 20 h. The precipitate formed was filtered off and washed with cold MeOH to give 0.52 g (38%) of the product. The product was recrystallized from CH_2Cl_2–acetonitrile.

‡ *X-ray crystal structure analysis*. Measurements on Bruker Smart diffractometer with CCD-detector, graphite monochromatised MoK_α radiation ($\lambda(MoK_\alpha)$ = 0.71073 Å) at 293 K in a sealed capillary containing mother liquor. The structure was solved by direct methods (R. M. Sheldrick, *Acta Crystallogr., Sect. A*, 1990, **46**, 467) and refinement, based on F^2, was made by full-matrix least-squares techniques (G. M. Sheldrick, *SHELXL-97—A program for crystal structure refinement*, 1997, University of Göttingen, Germany).

CCDC 225623. See http://www.rsc.org/suppdata/cc/b3/b311351e/ for crystallographic data in .cif or other electronic format.

2·MeCN: measurements at 173.0(2) K crystal size 0.3 × 0.2 × 0.1 mm^3, monoclinic, $P2_1/n$, a = 20.200(5) Å, b = 17.983(5) Å, c = 22.885(6) Å, β = 105.023(6)°, V = 8029(4) $Å^3$, Z = 4, ρ_{calcd} = 1.177 g cm^{-3}, $2\theta_{max}$ = 56.26°, μ = 0.079 mm^{-1}, R_{int} = 0.1157, $F(000)$ = 3080, 956 parameters, $R1$ = 0.0852, $wR2$ = 0.2274 (for 5938 refl. $I > 2\sigma(I)$), $R1$ = 0.1668, $wR2$ = 0.2656 (for 19277 unique reflections), S = 1.003, $\Delta\rho$ (min/max) = −0.31/0.61 e $Å^{-3}$.

§ *EPR measurements*. EPR spectra were recorded on an X-band Bruker ESP-300 spectrometer using 10 mW power, 100 KHz field modulation of 2 Gauss. Solvents were stored under N_2 before use. Samples were loaded into a 4 mm diameter cylindrical quartz sample cell containing 19 microbore channels to avoid dielectric loss.

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