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EPR sensing of metal and organic cations using a novel spin-labelled dibenzo-24-crown-8-ether

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The synthesis of novel dibenzo-24-crown ether substituted nitroxides and their use as spin probes for the detection of cation guests by EPR is reported. Formation of a host-guest complex between the proposed spin probes and several cations, both organic and inorganic, was evidenced by a significant change in the value of the benzylic and nitrogen EPR hyperfine splittings upon complexation. This favorable feature provided a reliable EPR sensor which is able to selectively distinguish different cationic guests.

Introduction

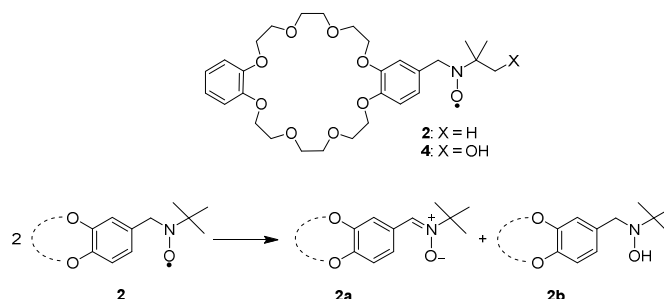
Crown ethers, discovered more than 50 years ago by the Nobel Prize winner Charles Pedersen,¹ are cyclic organic compounds composed of several repeating oligo-ethylene oxide ether units which are known to bind different cationic species.

Owing to their strong binding affinities to various metal ions and chemical species, members of the crown ether family have been widely applied in the design of smart sensor systems.² As an example, fluorophores connected to crown ether moieties have been developed as chemosensors for metal ions.³

The combined use of nitroxide⁴ spin labels and electron paramagnetic resonance (EPR) spectroscopy represents a complementary and useful tool for the characterization of supramolecular assemblies.⁵ Thus, a possible approach to the investigation of crown ethers as cation complexing agents is through EPR spectral studies of nitroxide spin-labeled crown ethers. Actually, examples of spin-labelled crown ethers have been reported in the past. In the case of mono spin labelled however they were found to be rather poor EPR reporting agents for alkali metal cations.⁶ A remarkable exception was represented by the spin labelled monoazacrown reported by Sosnovsky et al.⁷ In this case the participation of the sodium cation in the molecular structure of spin labelled complex was established by observing the coupling of the unpaired electron with Na⁺ nuclei by EPR. However, interaction with other cations was not described.

Di- or tri-spin labelled crown ethers have also been reported in the literature.⁸ Although modulation of spin-spin contact generally reported the binding of K⁺ cation, this interaction did not permit to differentiate the complexation of diverse cationic guests.

Very recently, two examples of [2]rotaxanes incorporating a spin-labelled crown ether have also been studied.^{9,10} In these systems it was demonstrated the shuttling process of the paramagnetic wheel along a dumbbell composed of dialkylammonium and 4,4'-bipyridinium recognition sites by EPR spectroscopy.



Scheme 1

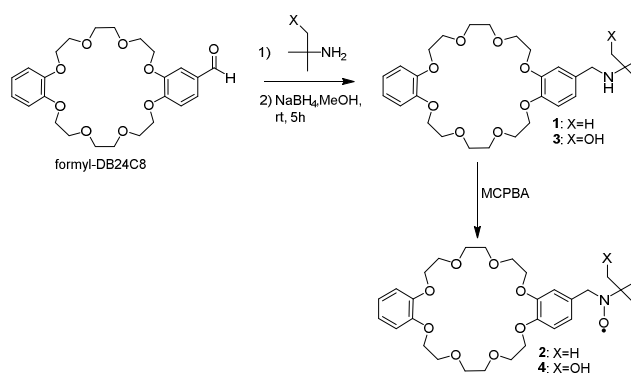
Here, we report the synthesis and the characterization of novel dialkyl nitroxide radicals (**2** and **4**, Scheme 1) bringing a dibenzo-24-crown-8-ether (DB24C8) group which are able to probe complexation of metal and organic cations by EPR spectroscopy. Due to disproportionation into the corresponding hydroxylamine and nitronium (see Scheme 1), the proposed nitroxide framework is characterized by a reduced lifetime if compared to sterically hindered nitroxides like TEMPO.⁴ Nevertheless, it was chosen as the paramagnetic unit because: 1) it can be easily generated by *in situ* oxidation of the

corresponding amine precursor and 2) it provides the favourable EPR features already found in the related benzyl *tert*-butyl nitroxide, which has been largely exploited by our group to investigate supramolecular complexes.¹¹

In particular, the formation of a supramolecular assembly with this class of nitroxide radicals by a complexing agent normally produces significant differences in the resonance fields of $M_1(2H\beta)$ EPR spectral lines in solution compared to the uncomplexed radical. These large spectral changes can arise both/or from the change of the nitrogen hyperfine splitting, a_N , induced by the different polar environment of the complexing host, and the strong variation of the benzylic protons coupling, $a_{2H\beta}$, due to conformational changes occurring upon complexation.¹¹ It will be shown that a sizeable change in the value of the benzylic and nitrogen EPR hyperfine splittings upon complexation was generally observed depending on the nature of the complexed cationic guests.

Results and discussion

Scheme 2 summarizes the synthetic steps to prepare the spin probes **2** and **4**. In particular, the amine **1** obtained by condensation of formyl-DB24C8¹² with *tert*-butyl amine and reduction with NaBH₄, was successively oxidized directly inside an EPR tube to the corresponding nitroxide **2** by addition of 3-chloroperbenzoic acid (MCPBA). Similarly, probe **4** was produced by oxidation of the nitroxide precursor **3** using 2-amino-2-methyl-1-propanol as the starting amine (see experimental section).



Scheme 2

Good EPR spectrum of nitroxide **2** was obtained by oxidation of amine **1** (1.0 mm) with MCPBA (1.0 mm) in CH₂Cl₂ (DCM) at 298 K (see Figure 1a).

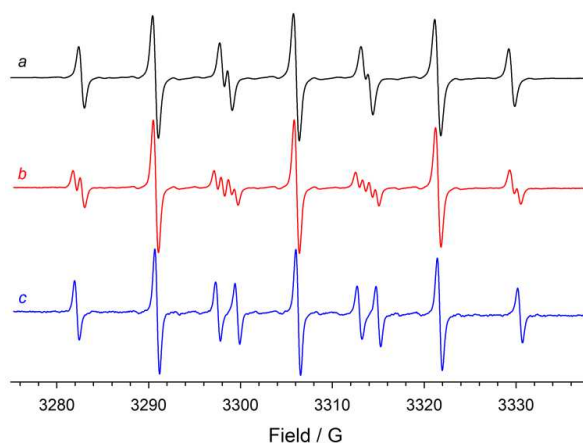


Figure 1. EPR spectra of **2** in CH₂Cl₂ at 298 K, a) in absence of Na⁺; b) in presence of 7 mM Na⁺; c) in Na⁺ saturated solution.

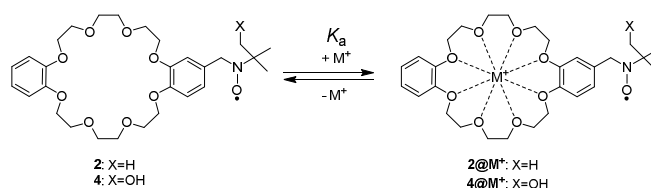
The spectrum was easily interpreted on the basis of the coupling of the unpaired electron with the nitrogen and with the two benzylic protons (see Table 1).

The host properties of **2** towards cations which are known to be selectively complexed by crown ether derivatives, were then investigated by EPR. Alkaline metal M⁺ iodide or picrate salts are practically insoluble in CH₂Cl₂. However complete solubilisation of metal salts was observed after addition of an excess of the starting amine **1**. This clearly suggests that host **1** maintains the complexation ability of crown ethers towards alkaline cations M⁺.

Table 1. EPR parameters of spin-labelled host **2**.

Solvent	Guest	a_N / G	a_{2H} / G
DCM	-	15.32	8.06
ACN	-	15.38	8.35
DCM	Li ⁺	15.32	8.06
DCM	Na ⁺	15.37	8.73
ACN	Na ⁺	15.38	8.72
DCM	K ⁺	15.36	8.56
ACN	K ⁺	15.42	8.69
DCM	Cs ⁺	15.36	8.47
DCM	Bn ₂ NH ₂ ⁺	15.44	8.47
DCM	5H ³⁺	15.43	8.58
DCM	5 ²⁺	15.54	9.94

When the oxidant, MCPBA, is added to these complexes, the corresponding EPR spectrum shows additional signals (Figure 1b, M⁺=Na⁺; Figure 2b, M⁺=K⁺) assigned to the complexed radical **2**@M⁺ in equilibrium with the free nitroxide **2** (Scheme 3).



Scheme 3

When the concentration of metal cations was increased until metal salt precipitation was obtained, the spectrum of the complexed radical **2**@M⁺ became dominant (Figure 1c, M⁺=Na⁺; Figure 2c, M⁺=K⁺), allowing the easy measurement of the spectroscopic parameters of the complexed nitroxide.

This behaviour is shared by Na⁺, K⁺, Cs⁺ cations, while Li⁺, known to be poorly complexed by large crown ethers,¹³ did not lead to any observable change in the EPR spectrum of the free nitroxide (see Table 1).

Inspection of Table 1 indicates that while the value of a_N does not change significantly, that of $a(2H_\beta)$ varies considerably upon complexation, giving rise to significant differences in the resonance fields of $M_1(2H_\beta) = \pm 1$ lines of the complexed and free species.

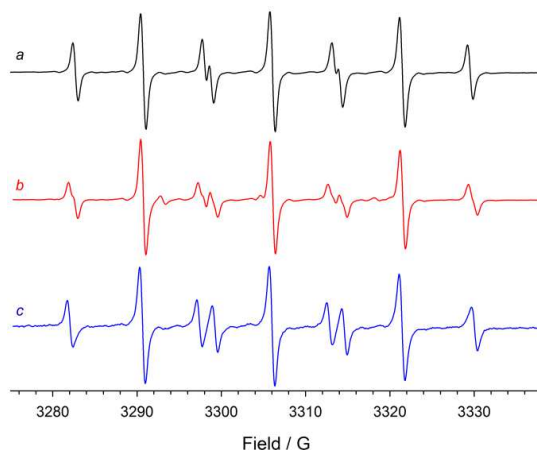


Figure 2. EPR spectra of **2** in CH₂Cl₂ at 298 K, a) in absence of K⁺; b) in presence of 7 mM K⁺; c) in K⁺ saturated solution.

In particular, $a(2H_\beta)$ increases inversely with cation size, being 8.47 G in the presence of Cs⁺ and rising monotonically to 8.73 G in the presence of Na⁺ (8.06 G is the value of the nitroxide in the absence of alkali-metal cation). The observation that nitrogen splitting does not change significantly, strongly suggests that the variation observed in the benzylic coupling value is not due to a general medium effect, that is, a change in the polarity of the environment surrounding the probe, but is caused by a specific interaction with M⁺. According to the Heller–McConnell equation, the value of the hyperfine splitting constant for β -protons in alkyl nitroxides is mainly determined by the dihedral angle between the symmetry axis of the $2p_z$ -orbital of nitrogen and the N-C-H β plane.¹⁴ The change in the geometry of crown ether upon formation of the complex with the metal cation (**2**@M⁺) is also responsible of a variation in the geometry adopted around the benzylic bond giving rise to a substantial change of β -H couplings.

Similar results were also obtained with probe **4** and the corresponding EPR parameters are reported in Table 2.

Table 2. EPR parameters of spin-labelled host **4**.

Solvent	Guest	a_N / G	a_{2H} / G
DCM	-	15.38	8.48
DCM	Na ⁺	15.36	9.07
DCM	K ⁺	15.35	8.98
DCM	Cs ⁺	15.33	8.86
DCM	Bn ₂ NH ₂ ⁺	15.42	8.90
DCM	5H ³⁺	15.39	9.01
DCM	5 ²⁺	15.49	10.0

We checked the possibility to obtain quantitative information on the complexation of metal cations by recording EPR spectra of **2** in ACN, a solvent in which alkaline metal cation iodides are soluble.

In ACN, however, the smaller spectroscopic differences between the free and complexed probes in comparison to those measured in CH₂Cl₂ (see Table 1), do not allow the detection of separate EPR signals for these two species (see Figure 3). In this case, the degree of complexation, p (%), when varying the concentration of M⁺, can be obtained by measuring the apparent value of the benzylic protons coupling constant and by using the following expression

$$p(\%) = \frac{[2@M^+]}{[2]_0} \times 100 = \frac{a_{EPR} - a_{free}}{a_{complex} - a_{free}} \times 100 \quad (1)$$

In equation 1, $[2]_0$ is the total amount of nitroxide, a_{EPR} represents the apparent value of a_{2H} measured from the EPR spectrum while a_{free} and $a_{complex}$ are the benzylic protons coupling constants in the free and complexed nitroxides, respectively. In Figure 3 (circles) is reported the dependence of the degree of complexation on K⁺ concentrations calculated by putting in equation 1 the a_{EPR} values of radical **2** measured in ACN at 298 K.

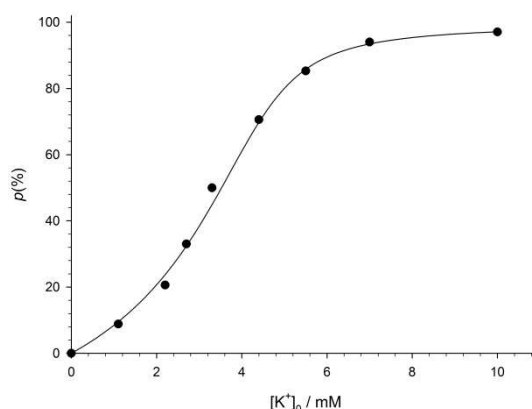


Figure 3. Complexation degree, p , determined by EPR (circles) at 298 K in ACN as function of K⁺ for nitroxide **2** (0.15 mM) produced by oxidation of **1** (5 mM). The line represents the theoretical dependence of p on K⁺ concentration calculated by means of equations 1-3 and by introducing $K_a = 6900 \text{ M}^{-1}$.¹⁵

This sigmoid dependence, however, could not be well-modelled by assuming a value for the association constant, K_a , equal to that reported in the literature in ACN for the unlabelled DB24C8 ($K_a = 6900 \text{ M}^{-1}$)¹⁵ and by admitting a simple standard 1:1 binding model as that reported in Scheme 3.

As mentioned in the introduction, nitroxide probes like **2** and **4**, having hydrogen atoms in β -positions, are characterized by a reduced lifetime if compared to sterically hindered nitroxides.⁴ This behaviour is largely determined by their ability to disproportionate into the corresponding hydroxylamine and nitron (see Scheme 1).

A steady-state concentration of nitroxide is, thus, observed inside the EPR tube when the rates of formation and destruction are approximately equal.[‡] In our case, this condition is reached few minutes after mixing the reagents in the oxidation step. The amount of nitroxide measured from the EPR spectrum does not generally exceed 3% of the starting amine. Under these conditions, the diamagnetic species containing the crown ether unit (**CE**_{dia}), *i.e.* the starting amine **1** and the disproportion products **2a** and **2b**, represent the larger fraction of metal receptor present in solution and cannot be ignored. As a consequence, the concentration of M⁺ available for complexation (M^{+}_{eff}) by the paramagnetic probe is much lower than that initially dissolved in solution (M^{+}_0) and its quantity inversely depends on the amount of diamagnetic crown ethers derivatives actually present in solution. This was definitively proved by recording a series of EPR spectra of **2** in the presence of a constant amount of M⁺ and by varying the initial concentration of the amine precursor and the oxidant.

As an example, Figure 4 (circle symbols) reports the dependence of the degree of complexation of **2** on the amount of host **1** measured in the presence of two different concentrations of Na⁺. For each sample, the quantity of peracid used in the oxidation step was optimized to maintain nearly constant the relative amount of nitroxide and starting amine ($[1]_0/[2]_0 \approx 60$).

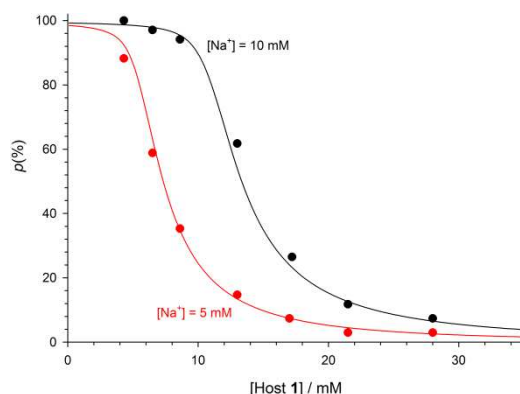


Figure 4. Nitroxide complexation degree, p , determined by EPR (dot symbols) at 298 K in ACN as function of host **1** concentration for different amounts of Na^+ . The lines represent the theoretical dependence of p on host **1** concentration calculated by equations 1-3 introducing $[\mathbf{2}]_0 = [\mathbf{1}]_0/60$ and $K_a = 1.3 \times 10^4 \text{ M}^{-1}$.¹⁵

As it has been supposed, the ratio between complexed and free radical significantly decreases as the starting amine concentration increases. On these bases, the equilibria for metal complexation by the investigated spin probes that must be considered are the following ones:

$$K_a = \frac{[\mathbf{CE}_{dia}@\mathbf{M}^+]}{([\mathbf{CE}_{dia}]_0 - [\mathbf{CE}_{dia}@\mathbf{M}^+])([\mathbf{M}^+]_0 - [\mathbf{CE}_{dia}@\mathbf{M}^+])} \quad (2)$$

$$K_a = \frac{[\mathbf{2}@\mathbf{M}^+]}{([\mathbf{M}^+]_{eff} - [\mathbf{2}@\mathbf{M}^+])([\mathbf{2}]_0 - [\mathbf{2}@\mathbf{M}^+])} \quad (3)$$

where: *i*) $[\mathbf{CE}_{dia}]_0$ is the total amount of EPR silent crown ether derivatives ($[\mathbf{1}] + [\mathbf{2a}] + [\mathbf{2b}] = [\mathbf{1}]_0$) corresponding to the initial amount of starting amine; *ii*) $[\mathbf{M}^+]_{eff} = [\mathbf{M}^+]_0 - [\mathbf{CE}_{dia}@\mathbf{M}^+]$ is the effective concentration of metal cation available in solution to the spin probe; *iii*) $[\mathbf{2}]_0$ is the total amount of nitroxide that can be determined quantitatively by double integration of the EPR spectrum.⁹

As shown in Figures 3 and 4, the dependence of complexation degree of **2** on M^+ (Figure 3) or starting amine (Figure 4) was remarkably modelled by solving simultaneously the reported equilibria and assuming for all labelled crown ether derivatives a K_a value very similar to that reported in the literature for the unsubstituted DB24C8.

In Figure 5a is also reported the EPR spectrum of **1** recorded in CH_2Cl_2 after the addition of dibenzylammonium cation (Bn_2NH_2^+). Once again the EPR spectroscopic parameters (see Table 1) differ from those recorded in CH_2Cl_2 indicating the formation of the complex $\mathbf{2}@\text{Bn}_2\text{NH}_2^+$.

Because the EPR read-out discriminates between different cationic guests, we evaluated the EPR behaviour of radical probes **2** or **4** in a pseudorotaxane containing both dialkylammonium and 4,4'-bipyridinium (viologen) recognition sites in the thread ($\mathbf{5H}^{3+}$, Scheme 4). Again, evidence for the formation of the pseudorotaxane in CH_2Cl_2 was obtained by observing complete solubilisation of $\mathbf{5H}^{3+}$, which is almost completely insoluble in chlorinated hydrocarbons, after the host addition.

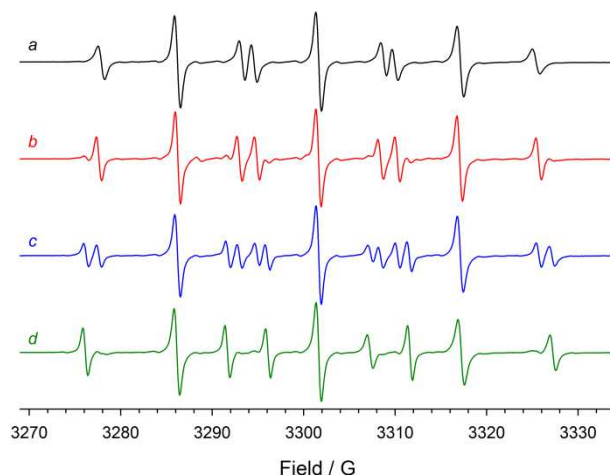
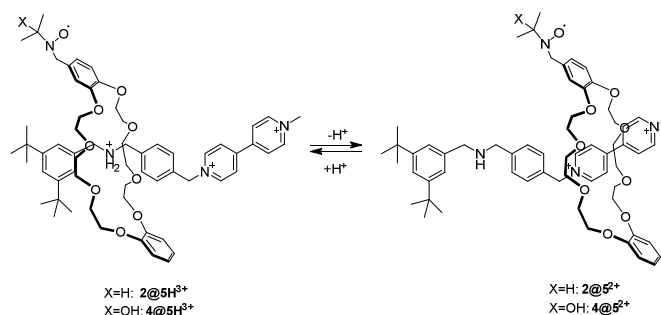


Figure 5. EPR spectra of **2** recorded in CH_2Cl_2 at 298 K in the presence of: a) Bn_2NH_2^+ ; b) $\mathbf{5H}^{3+}$; c) $\mathbf{5H}^{3+}$ and 0.5 eq. DIPEA; d) $\mathbf{5H}^{3+}$ and an excess of DIPEA.

Figure 5b shows the EPR spectrum of the pseudorotaxane $\mathbf{2}@\mathbf{5H}^{3+}$ recorded in CH_2Cl_2 at 298 K, after the oxidation of $\mathbf{1}@\mathbf{5H}^{3+}$ with MCPBA. The values of $a_N = 15.42 \text{ G}$ and $a_{2H} = 8.59 \text{ G}$ (see Table 1) are close to those observed for the $\mathbf{2}@\text{Bn}_2\text{NH}_2^+$

complex. According to literature data,¹⁶ these data suggest that the macrocycle predominantly encircles the ammonium group.



Scheme 4

Treatment of **1@5H³⁺** in CH₂Cl₂ with 0.5 eq. of the non-nucleophilic diisopropylethylamine (*i*Pr₂EtN, DIPEA), which is strong enough to deprotonate the NH₂⁺ centre, led to the appearance of a new radical species (Figure 5c). This new signal is characterized by very different coupling constants ($a_N=15.54$ G and $a_{2H}=9.94$ G) and was attributed to a new complex in which the ring is shifted from the ammonium site to the viologen unit (see Scheme 4).¹⁶ The simultaneous presence of both signals indicates that the exchange between the protonated and the deprotonated forms of the pseudorotaxane must be significantly slower than the EPR timescale.

Addition of increasing amounts of *i*Pr₂EtN to **2@5H³⁺** produced a noticeable increase in the intensity of this new signal which became predominant in the presence of an excess of base (Figure 5d). Finally, the addition of trifluoroacetic acid after addition of *i*Pr₂EtN caused the recovery of the initial EPR spectrum as a consequence of the return of the macrocycle to the ammonium site station.

Conclusions

In summary, we have developed novel radical probes that feature a crown ether moiety. Benzyl protons EPR coupling constants are affected by cation binding, which leads to distinguish between different cationic guests. Cation amounts in the order of mM concentrations can be easily detected by this method. The general good agreement with quantitative data previously determined suggests that EPR can be usefully employed to study complexation by crown ethers even in supramolecular systems of higher complexity when traditional methods based on NMR or fluorescence cannot be applied.

Experimental

Synthetic procedures.

Compound 1. A stirred mixture of formyl-DB24C8 (250 mg, 0.52 mmol) and *tert*-butylamine (82 μ L, 0.788 mmol, 1.5 eq.) in dry methanol (19 mL) was refluxed for 18 h. Formation of the imine intermediate was checked by TLC (CH₂Cl₂/MeOH 9:1). The mixture was then cooled to room temperature and NaBH₄ (78 mg, 2.1 mmol, 4 eq.) was added in small portions. The mixture was stirred at rt for 5 h and quenched with 1 M HCl (3 mL). Methanol was evaporated under vacuum and the residue suspended in 1 M NaOH (10 mL), and stirred for 15 min. The aqueous layer was extracted with CH₂Cl₂ (4x10 mL), washed with water and brine (40 mL each) and dried over MgSO₄. The solvent was removed *in vacuo* to obtain a yellowish solid. The crude product was purified by silica gel chromatography (eluent CH₂Cl₂/MeOH/NH₄OH 25% 90:10:1) to obtain an off white sticky solid (176 mg, 63%) ¹H-NMR (400 MHz, CDCl₃) δ 1.18 (s, 9 H), 3.65 (s, 2 H), 3.82 (s, 8 H), 3.88-3.91 (m, 8 H), 4.09-4.18 (m, 8H), 6.77-6.95 (m, 7 H); ¹³C NMR (101 MHz, CD₂Cl₂) δ 149.64, 149.43, 148.56, 121.94, 115.43, 114.87, 114.61, 71.57, 70.38, 69.83, 54.00, 47.05, 28.88. ESI-MS (m/z): 534.2 [M+H]⁺, 556 [M+Na]⁺.

Compound 3. Formyl-DB24C8 (250 mg, 0.52 mmol, 1 eq.) was reacted with 2-amino-2-methyl-1-propanol (49 mg, 0.54 mmol, 1.05 eq.) in methanol (19 mL) under reflux for 18 h. Formation of the imine intermediate was observed by TLC (CH₂Cl₂/MeOH 9:1). The mixture was then cooled to room temperature and NaBH₄ (78 mg, 2.1 mmol, 4 eq.) was added to the reaction mixture in small portions. The mixture was stirred at rt for 5 h and the reaction quenched with 1 M HCl (3 mL). Methanol was evaporated under vacuum and the residue suspended in 1 M NaOH (10 mL), stirred for 15 min and the aqueous layer extracted with CH₂Cl₂ (4x10 mL), washed with water and brine (40 mL each) and dried with MgSO₄. The solvent was removed *in vacuo* to obtain a yellowish solid. The crude product was purified by silica gel chromatography (eluent CH₂Cl₂/MeOH 9:1 then CH₂Cl₂/MeOH/NH₄OH 90:10:1) to obtain a white sticky solid (160 mg, 55%). ¹H-NMR (400 MHz, CD₂Cl₂) δ 1.11 (s, 6 H), 3.28 (s, 2 H), 3.60 (s, 2 H), 3.76 (s, 8 H), 3.83-3.86 (m, 8 H), 4.09-4.14 (m, 8H), 6.81-6.89 (m, 7

H); ¹³C NMR (101 MHz, CD₂Cl₂) δ 149.64, 148.52, 134.98, 121.93, 121.32, 114.86, 71.59, 70.40, 69.82, 68.81, 54.00, 46.50, 24.42. ESI-MS (*m/z*) 550 [M+H]⁺, 572 [M+Na]⁺.

3,5-Di-*tert*-butylbenzyl-4-[(4,4'-(methylbipyridinium)methyl] benzylammonium Tris(hexafluorophosphate) (5H³⁺).

3,5-Di-*tert*-butylbenzyl-4-[(4,4'-pyridylpyridinium)methyl] benzylammonium bis(hexafluorophosphate)¹⁶ (50 mg, 0.065 mmol) was dissolved in CH₃CN (5 mL) and then methyl iodide (12 μL, 0.194 mmol, 3 eq.) was added. The mixture was heated under reflux for 3 d. The solvent was removed *in vacuo* and the crude mixture subjected to column chromatography (eluent CH₂Cl₂/MeOH 9:1 then 7:1 and finally MeOH/H₂O/NH₄Cl 2M 7:2.5:0.5). Methanol was removed *in vacuo* and the solution was then treated with NH₄PF₆ aqueous solution. The precipitate was filtered and washed with water to afford title compound as a white solid (34 mg, 56%) ¹H-NMR (400 MHz, (CD₃)₂CO) δ 1.30 (s, 18 H), 4.62 (s, 2 H), 4.69 (s, 2 H), 4.73 (s, 3 H), 6.22 (s, 2H), 7.45 (s, 2 H), 7.56 (s, 1 H), 7.71-7.77 (m, 4H), 8.77 (m, 4 H), 9.35 (m, 2 H), 9.45 (m, 2 H). ¹³C NMR (101 MHz, (CD₃)₂CO) δ = 152.54, 151.59, 147.77, 146.92, 135.56, 133.76, 132.21, 131.21, 130.68, 128.48, 127.75, 125.08, 124.37, 65.09, 53.25, 51.92, 35.50, 31.59, 29.84. ESI-MS (*m/z*) 784 [M-PF₆]⁺.

EPR Spectroscopy. The EPR spectra were recorded on a Bruker ELEXYS spectrometer equipped with an NMR gaussmeter for field calibration and Bruker ER033M field-frequency lock. The temperature was controlled with a standard variable temperature accessory and was monitored before and after each run with a copper-constantan thermocouple. The instrument settings were as follows: microwave power 0.79 mW, modulation amplitude 0.4 G, modulation frequency 100 kHz, scan time 180 s, 2 K data points. The hyperfine splittings were determined by computer simulation using a Monte Carlo minimisation procedure.¹⁷

Nitroxides **2** and **4** are generated by mixing a solution of the corresponding amine (4-30 mM) with a solution of 3-chloroperbenzoic acid (Aldrich, technical grade, 0.5-5 mM). After, aliquots from a concentrated cation solution are added to the solution of nitroxide to yield the required concentrations. Samples are then transferred in capillary tubes (1 mm i.d.) and the EPR spectrum is immediately recorded. Radical concentrations were measured with respect to a solution of DPPH of known concentration using the signal from a ruby crystal as internal standard.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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Notes and references

‡ It must be considered that nitroxide can also be re-formed by oxidation of hydroxylamine **2b**.

§ Because diamagnetic crown ethers derivatives are in large excess if compared to nitroxide **2**, the amount of M⁺ complexed by **2** has been omitted in equation 2.

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