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Supporting Information

# EPR Sensing of a Cation Species by Aza-Crown Ethers Incorporating a Persistent Nitroxidic Radical Unit

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# Supporting Information

## *General information*

EPR spectra has been recorded on Bruker-ELEXYS spectrometer by using the following instrument settings: microwave power 0.79 mW, modulation amplitude from 0.01 to 0.1 mT, modulation frequency 100 kHz, scan time 180 s, 2K data points. The hyperfine splittings were determined by computer simulation using a Monte Carlo minimisation procedure.[18]

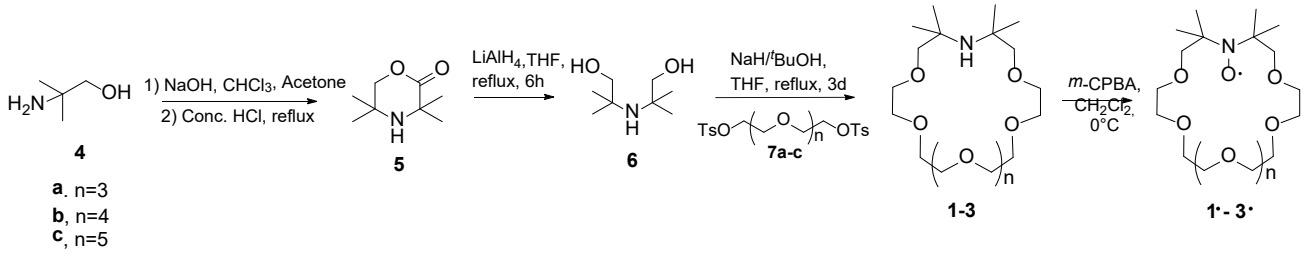
$^{1}H$ and $^{13}C$ NMR spectra were recorded at 298 K on a Varian Mercury spectrometer operating at 400 MHz in $CDCl_3$ solutions using the solvent peak as internal standard (7.26 ppm). $^{13}C$ NMR spectrum was recorded at 298 K on a Varian Mercury spectrometer operating at 101 MHz in $CDCl_3$ solutions using the solvent peak as internal standard (77.0 ppm). Chemical shifts are reported in parts per million ($\delta$ scale).

ESI-MS spectra were recorded on Waters Micromass ZQ 4000 spectrometer by using the following instrumental settings: positive ions; desolvation temp. 200° C; capillary voltage: 3.54 kV; cone voltage: 113 V.

HRMS spectrum of rotaxane **RH$^{\bullet 3+}$** was recorded on Waters Xevo G2-XS QTof by using the following instrumental settings: positive ion mode; desolvation temp. 600° C; capillary voltage: 0.8 kV; cone voltage: 30 V. (Cone gas flow: 50 L/h Desolvation gas flow: 1000 L/h)

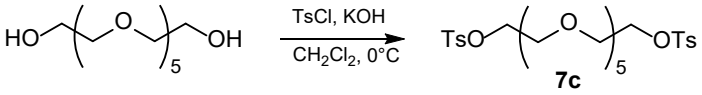
2-Amino-2-methyl-1-propanol (**4**), tetraethylene glycol di(*p*-toluenesulfonate) (**7a**), pentaethylene glycol di(*p*-toluenesulfonate) (**7b**), anhydrous tetrahydrofuran (THF), dichloromethane (DCM), *tert*-butyl alcohol, chloroform, acetone and the other reagents were purchased from Sigma Aldrich and used without further purification. All reactions were monitored by TLC.

3,3,5,5-tetramethyl-2-oxomorpholine (**5**) and 2,2'-azanediylbis(2-methylpropan-1-ol) (**6**) were prepared using the method of Lai[12] with modifications as described in literature.[19]




**Scheme S1.** Synthetic steps of macrocycles **1$^{\bullet}$-3$^{\bullet}$**.

*Synthesis of hexaethylene glycol di(p-toluenesulfonate) (**7c**)*




**7c**

To an ice-cooled stirred solution of hexaethylene glycol (2.0 g, 7 mmol) in $CH_2Cl_2$ (15 mL), KOH powder (3.13 g, 56 mmol) was gradually added. Then *p*-toluenesulfonyl choride (TsCl, 5.34 g, 28 mmol) dissolved in $CH_2Cl_2$ (30 mL) was added dropwise to the suspension over a period of 30 min. The reaction mixture was stirred for 2 h at 0°C whereupon it was washed with 100 mL of distilled water, extracted with $CH_2Cl_2$, dried ($MgSO_4$), and the solvent evaporated. The crude product was chromatographed over silica gel column eluting with cyclohexane/ethyl acetate 1:1 and then 1:9. The desired product was collected and concentrated to give 3.2 g (77% yield) of ditosylate as an oil. $^1$H NMR: $\delta$ 7.75 (d, *J* = 8.0 Hz, 4H), 7.30 (d, *J* = 8.0 Hz, 4H), 4.09-4.13 (m, 4H), 3.62-3.66 (m, 4H), 3.57 (s, 8H), 3.53 (s, 8H), 2.40 (s, 6H). ESI-MS: *m/z* 613.5 $(M+Na)^+$.

## *Synthesis of compounds* **1-3**

Compounds **1-3** were prepared modifying the synthetic procedure reported in ref. [12].
In a 250 mL three-necked round bottom flask, fitted with a reflux condenser and flashed with $N_2$, NaH (0.100 g, 2.48 mmol) was added portionwise to the mixture over a period of 30 minutes to a solution of *t*-BuOH (0.183 g, 2.48 mmol) in 20 mL of THF. The mixture was left under stirring for 2 h. After this period a solution of aminodiol **6** (0.200 g, 1.24 mmol) in THF (20 mL) was added dropwise and left reacting for 20 minutes. Then, ditosylate **7a-c** (1.24 mmol) in 40 mL of THF was added dropwise to the solution and left stirring under reflux (65 °C) for 3 days. The solvent was evaporated and water was added (100 mL). After extraction with $CH_2Cl_2$, the organic phase was dried over $MgSO_4$ and evaporated *in vacuo*. The crude was purified by chromatography over $SiO_2$ column ($CH_2Cl_2$:MeOH 95:5 until 60:40), to obtain **1-3** as pale yellow oils. Yields were not optimized.

Compound **1**: 0.150 g, 30% yield; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 3.64-3.72 (m, 20H), 3.59-3.63 (m, 4H), 3.21 (s, 4H), 1.16 (s, 12H); $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$: 81.68, 71.19, 70.94, 70.80, 70.76, 70.69, 70.48, 54.10, 27.34; HRMS: *m/z* 408.2896 $(M+H)^+$.

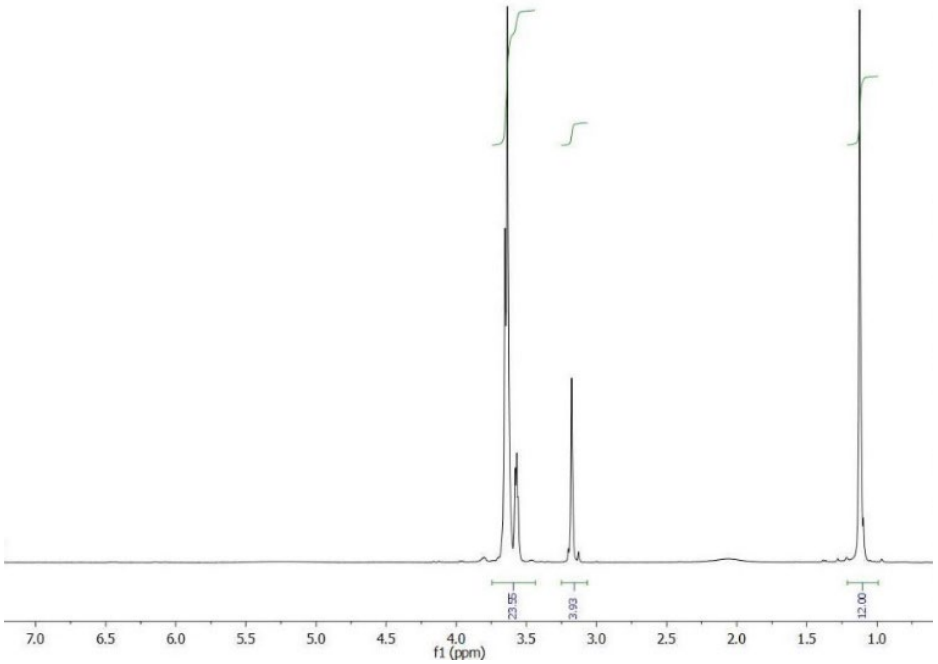



**Figure S1.** $^{1}H$ NMR spectrum (400 MHz, $CDCl_3$) of **1**.

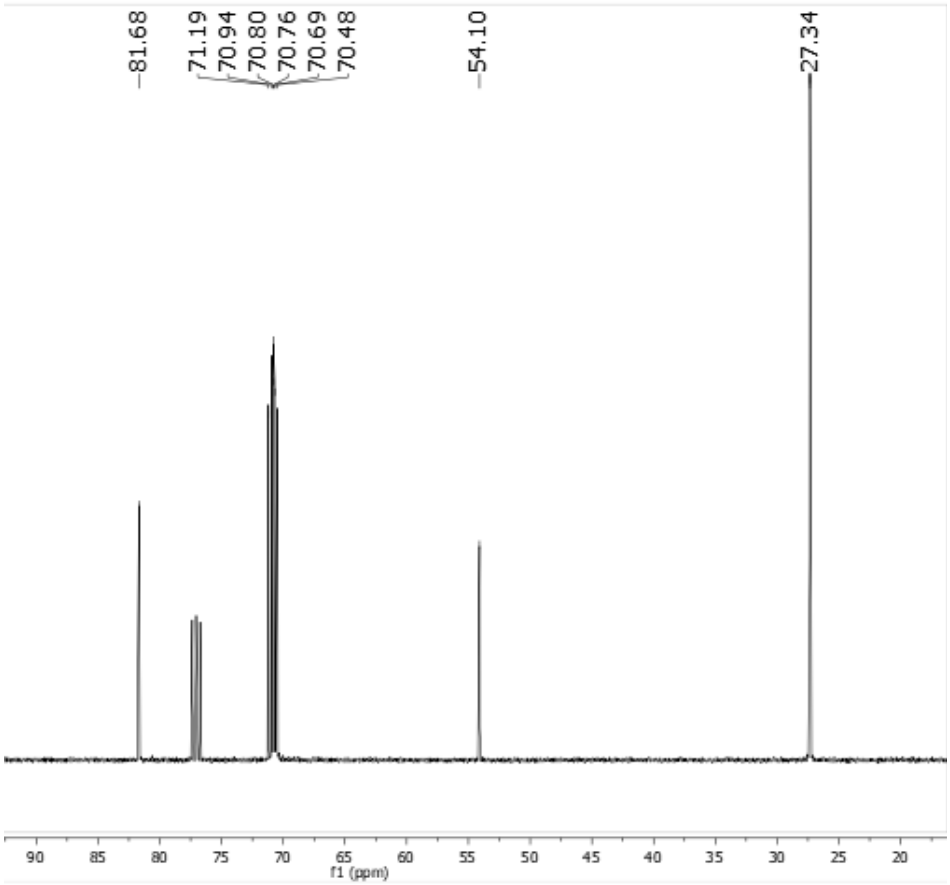



**Figure S2.** $^{13}C$ NMR spectrum (400 MHz, $CDCl_3$) of **1**.

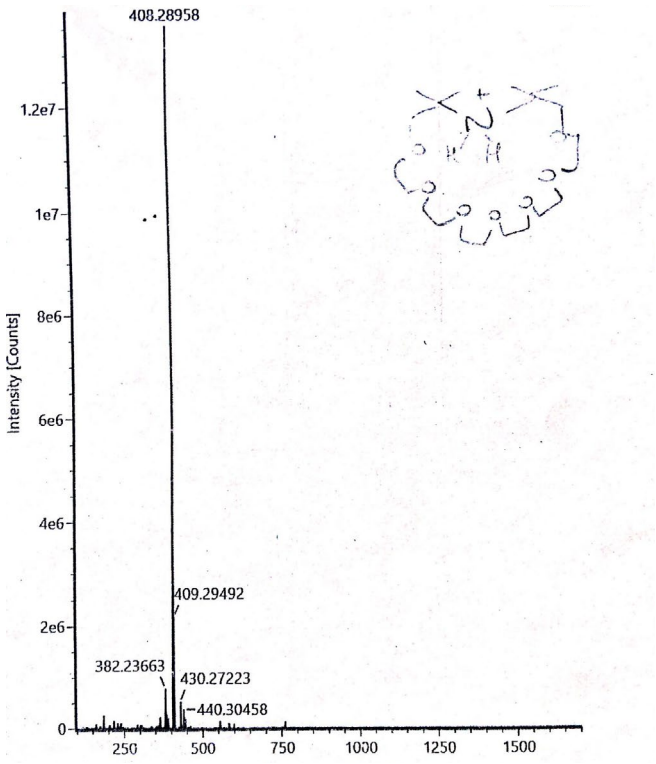
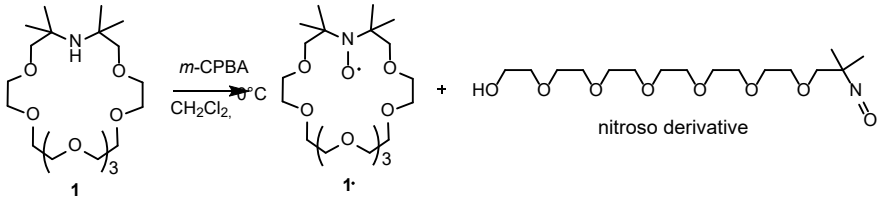



**Figure S3.** HRMS spectrum of **1** in MeOH.

Compound **2**: 0.123 g, 27% yield; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 3.64-3.73 (m, 16H), 3.58-3.64 (m, 4H), 3.20 (s, 4H), 1.16 (s, 12H); ESI-MS: *m/z* 364.36 $(M+H)^+$.

Compound **3**: 0.138 g, 35% yield; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 3.68-3.71 (m, 12H), 3.58-3.61 (m, 4H), 3.21 (s, 4H), 1.17 (s, 12H); ESI-MS: *m/z* 320.32 $(M+H)^+$.

*Synthesis of compounds* **1•-3•**




**Scheme S2.** Oxidation step of macrocycles **1•** and formation of the nitroso derivative.

In a 50 mL three-necked round bottom flask, containing **1** (0.130 g, 0.32 mmol) in $CH_2Cl_2$ (6 mL) cooled by an ice bath, a solution of *m*-CPBA (0.038 g, 0.22 mmol) in 6 mL of DCM was added by a syringe-pump over a period of 30-60 minutes. In order to minimize the formation of the open nitroso compound (bright blue oil), the formation of nitroxide **1•** was monitored by EPR following the signal intensity of the radical until a *plateau*. Then, after treatment with a solution of $Ba(OH)_2$, extraction

with $CH_2Cl_2$, drying over $MgSO_4$ and evaporation of the organic phase *in vacuo*, the crude was purified by chromatography over $SiO_2$ ($CH_2Cl_2$:MeOH 97:3 until 95:5) to obtain **1•** as an orange sticky oil (0.041 g) in 30% yield.

Similar procedures were carried out for the preparation of crown ethers **2•** and **3•** (30-35 % yield)

In the case of **1•**, the $^1H$ spectrum was acquired before and after addition of $PhNHNH_2$[20] in order to reduce the radical **1•** to the corresponding diamagnetic hydroxylamine **1-OH** (see Figure S6).

Compound **1•**: EPR (ACN) $a_N$ = 15.24 G, $g_{factor}$ = 2.00594; ESI-MS: *m/z* 423.2771 $(M+H)^+$, 440.3097 $(M+NH_4)^+$ 445.2613 $(M+Na)^+$

Compound **1-OH**: $^1H$-NMR (400 MHz, $CD_3CN$) $\delta$: 3.52-3.75 (m, 24H), 3.38 (s, 4H), 1.23 (s, 12H).

Compound **2•**: EPR (ACN) $a_N$ = 15.36 G, $g_{factor}$ = 2.00582; ESI-MS: *m/z* 396.2878 $(M+NH_4)^+$, 401.2404 $(M+Na)^+$

Compound **3•**: EPR (ACN) $a_N$ = 15.42 G, $g_{factor}$ = 2.00606; ESI-MS: *m/z* 357.2177 $(M+Na)^+$

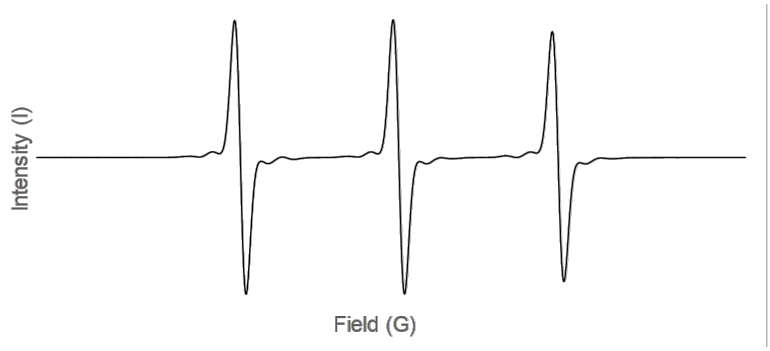



**Figure S4.** EPR spectrum of **1•** in acetonitrile at room temperature.

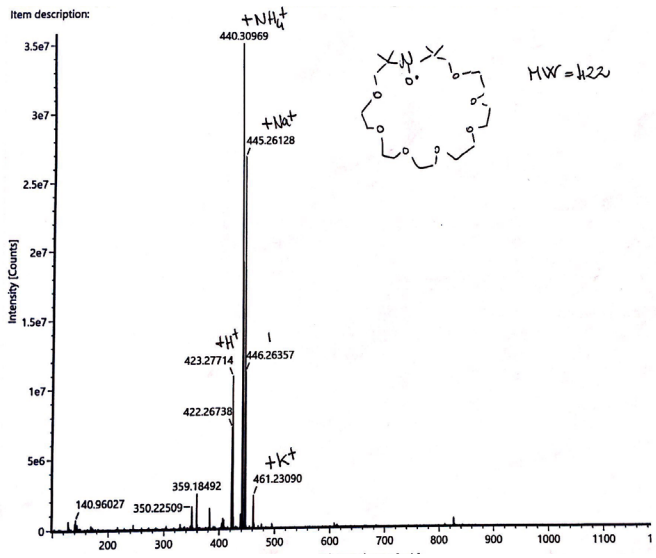


**Figure S5.** HRMS spectrum of **1•** in MeOH.

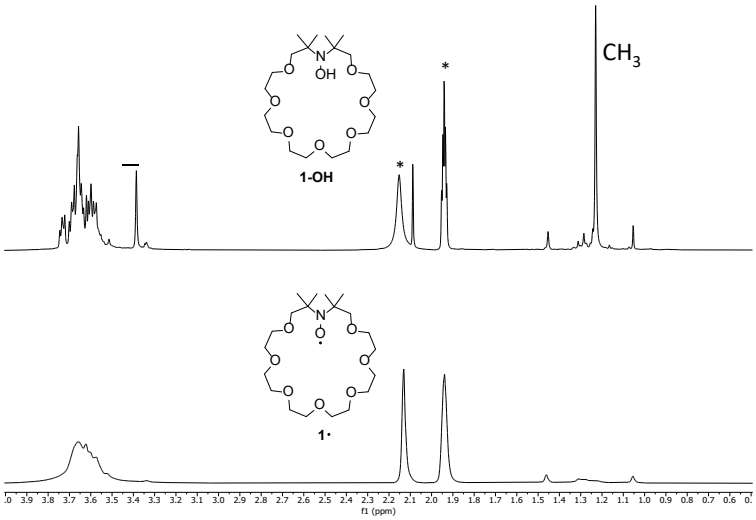
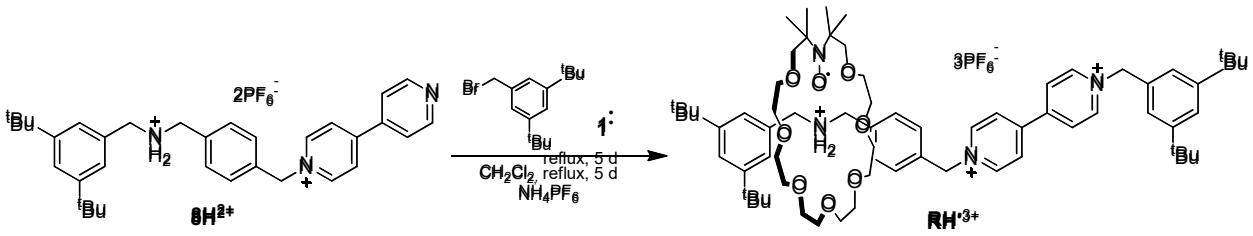



**Figure S6.** $^1$H-NMR spectra in $CD_3CN$ of a) **1•**; b) the corresponding hydroxylamine **1-**OH detected after reduction of **1•** with phenylhydrazine. Stars are referred to solvent and water peaks.

*Synthesis of rotaxane* **RH•3+**




**Scheme S3**. Preparation of the rotaxane **RH•3+**.

To a titrated solution (see EPR measurements for details) of radical macrocycle **1•** (0.030 g, 0.07 mmol) in anhydrous $CH_2Cl_2$ (8 mL) was added the half thread **8H$^{2+}$** (0.077 g, 0.1 mmol) and the mixture was stirred for 2 h under nitrogen atmosphere, until it assumes a pale yellow colour. The EPR analysis of the solution reveals an increase of the coupling constant value of 0.8 G (from 15.24 G to 16.01 G) corresponding to the formation of the pseudorotaxane **1•@8H$^{2+}$**. After this period 1-(bromomethyl)-3,5-di-tert-butylbenzene (0.070 g, 0.25 mmol) was added and the reaction mixture was stirred under reflux for 5 days. After cooling the solution was concentrated *in vacuo* and the reaction mixture was purified by silica gel column (from pure $CH_2Cl_2$ to $CH_2Cl_2$-$CH_3OH$ 9:1).

The concentrated fractions of the chromatographic column containing the product were dissolved in Acetone/$H_2O$ 1:2 and treated with a satured solution of $NH_4PF_6$. The resulting precipitated solid was collected by filtration, washed with water to remove the excess of $NH_4PF_6$ and dried to afford the rotaxane **RH•3+** in a not optimized 20% yield.

EPR (ACN): $a_N$=16.08 G, $g$ = 2.00587.

HRMS: calcd. (%) for $C_{68}H_{104}F_{12}N_4P_2O_8^{3+}$ 1394.6307, obs. 1394.71.

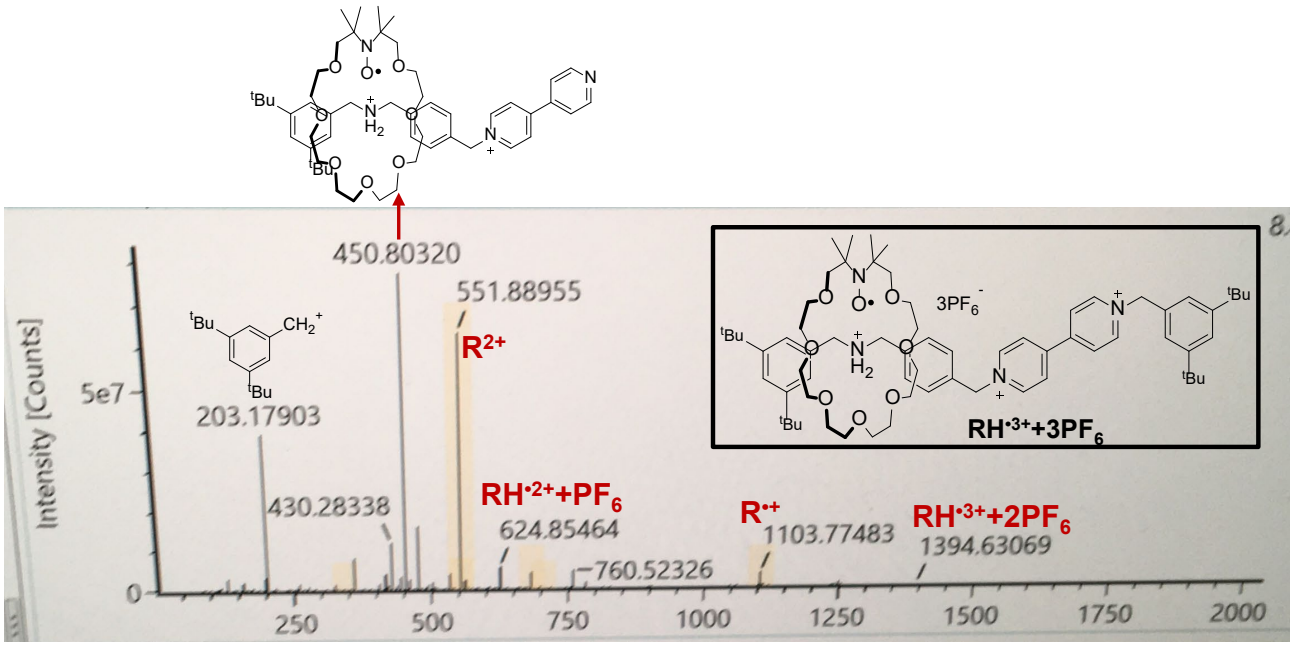



**Figure S7.** HRMS spectrum of **RH**$^{\bullet 3+}$ in acetonitrile a room temperature.

## Shuttling of rotaxane

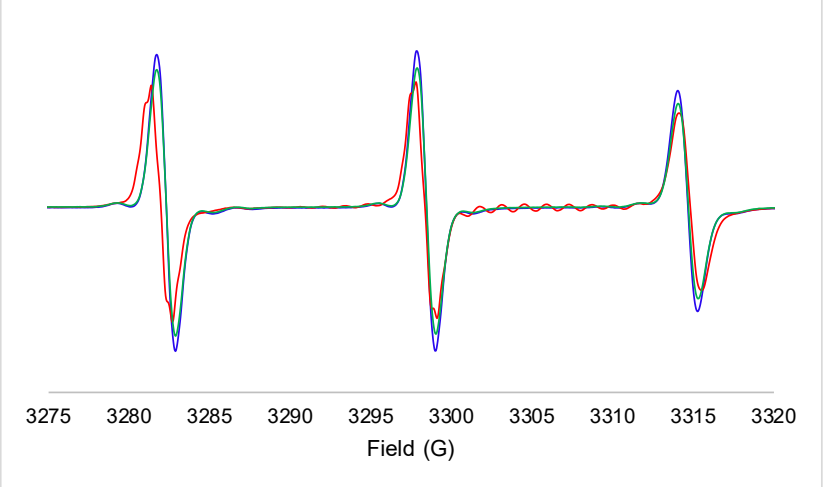



**Figure S8.** EPR spectra (amplitude modulation 1 mT) of rotaxane **RH**$^{\bullet 3+}$ (blue line), **RH**$^{\bullet 3+}$ after addition of DIPEA (red line) e **R**$^{\bullet 2+}$ after addition of TFA (green line), in acetonitrile a 298 K.

## EPR measurements.

EPR spectra were collected using a Bruker ELEXYS spectrometer equipped with an NMR gaussmeter for field calibration. The sample temperature was controlled with a standard variable

temperature accessory and monitored before and after each run using a copper-constantan thermocouple. The instrument settings were as follows: microwave power 5.0 mW, modulation amplitude 0.05 mT, modulation frequency 100 kHz, scan time 180 s. Digitized EPR spectra were transferred to a personal computer for analysis using digital simulations carried out with a program developed in our laboratory and based on a Monte Carlo procedure. The input data for the program are the number of non-equivalent nuclei, the hyperfine splitting constants, the intrinsic linewidth of the free and complexed nitroxide and their relative amounts.[18] Additional nitroxide species were considered in the simulation when a mixture of different cations was present in solution.

The samples for the EPR analysis were prepared by dissolving constant quantities of macrocycle, (0.5-1.0 x $10^{-4}$ M) for each series considered, and variable quantities of guests, in a concentration range optimized according to the affinity shown, in different solvents, depending on the investigation, and in a total volume of 50 µl, inside a narrow diameter tube then inserted into the EPR sample tube. The exact concentrations of radical macrocycles were determined by integration using as external standard a known concentration solution of TEMPO. Each sample was degassed using a nitrogen flow to minimize the presence of oxygen within the mixture, optimizing the resolution of the recorded EPR spectra.

The $K_a$ were calculated using the mathematical equations described in the paper.

When using concentrations of guest comparable to those of ligand **L•**, the $K_a$ were obtained considering the complete mathematical treatment:

$$K_a = \frac{[\boldsymbol{L}\cdot@M^{n+}]}{[\boldsymbol{L}\cdot][M^{n+}]} =$$

$$= \frac{[\boldsymbol{L}\cdot@M^{n+}]}{([\boldsymbol{L}\cdot]_0 - [\boldsymbol{L}\cdot@M^{n+}])([M^{n+}]_0 - [\boldsymbol{L}\cdot@M^{n+}])}$$

If $\left[\boldsymbol{L}\cdot@M^{n+}\right] = x$, $K_a$ becomes:

$$K_a = \frac{x}{([\boldsymbol{L}\cdot]_0 - x)([M^{n+}]_0 - x)}$$

$$K_a([\boldsymbol{L}\cdot]_0 - x)([M^{n+}]_0 - x) = x$$

$$K_a[\boldsymbol{L}\cdot]_0[M^{n+}]_0 - K_a[\boldsymbol{L}\cdot]_0 x - K_a[M^{n+}]_0 x + K_a x^2 - x = 0$$

$$K_a x^2 + x(-K_a[\boldsymbol{L}\cdot]_0 - K_a[M^{n+}]_0 - 1) + K_a[\boldsymbol{L}\cdot]_0[M^{n+}]_0 = 0$$

If $-K_a[\boldsymbol{L}\cdot]_0 - K_a[M^{n+}]_0 - 1 = b$

$$x = \frac{K_a[\boldsymbol{L}^{\cdot}]_0 + K_a[M^{n+}]_0 + 1 \pm \sqrt{b^2 - 4K_a^2[\boldsymbol{L}^{\cdot}]_0[M^{n+}]_0}}{2K_a}$$

From the simulation of the spectra that allows to determine the percentages of macrocycle respectively occupied (%bound) and free (%free), the value of $x$ can be obtained simply considering the following relation:

$$x = \frac{[\boldsymbol{L}^{\cdot}]_0}{\%bound + \%free} * \%bound$$

The complexation of $K^+$ cation in the presence of **1•** does not show spectral variations. However, it is plausible to assume that the inclusion takes place, but that the nitroxide function is not significantly involved in the complexation process, but rather the glycolic oxygens more distant from the nitroxide that coordinate the potassium cation. The proof that $K^+$ is coordinated by **1•** is found in the fact that if increasing concentrations of $K^+$ are added to samples containing constant concentrations of **1•** and $Ca^{2+}$ (the latter at the minimum concentration, but sufficient to occupy the entire macrocycle) we observe the progressive displacement of the $Ca^{2+}$ cation leaving the host system pushed out by the entering $K^+$ ion. Assuming that the macrocycle released from $Ca^2$ is occupied by $K^+$, the complexation constant for $K^+$ ($K_{K^+}$) can be derived using the following equation:

$$\frac{[\mathbf{1}^{\cdot}@\mathrm{Ca}^{2+}]}{[\mathbf{1}^{\cdot}@\mathrm{K}^{+}]} = \frac{K_{\mathrm{Ca}^{2+}}}{K_{\mathrm{K}^{+}}} \frac{[\mathrm{Ca}^{2+}]}{[\mathrm{K}^{+}]}$$

Here $\frac{[\mathbf{1}^{\cdot}@\mathrm{Ca}^{2+}]}{[\mathbf{1}^{\cdot}@\mathrm{K}^{+}]}$ was obtained by EPR spectra simulation, $K_{\mathrm{Ca}^{2+}}$ was known from the previous titrations, $[\mathrm{Ca}^{2+}]$ was kept constant, while $[\mathrm{K}^{+}]$ was approximated $[\mathrm{K}^{+}]_0$. A linear regression plot was extrapolated by plotting $\frac{[\mathbf{1}^{\cdot}@\mathrm{Ca}^{2+}]}{[\mathbf{1}^{\cdot}@\mathrm{K}^{+}]}$ value as a function of $[\mathrm{K}^{+}]_0$ for obtaining the accurate value of $K_{\mathrm{K}^{+}}$.

As an example Table S1 reports the method used to determine the $K_a$ of **1•**@$M^{n+}$ in acetonitrile and the relative used concentrations for ligand and guests.

**Table S1**. Calculated $K_a$ of **1•**@$M^{n+}$ for the association between **1•** and different guests in acetonitrile, method and concentrations used.

| Salt | Cation | $K_a/M^{-1}$ | [Host]($10^{-3}$ M) | [Guest]($10^{-3}$ M) | Method |
|---|---|---|---|---|---|
| $LiClO_4$ | $Li^+$ | 536 | 0.045 | 0.4 - 9.0 | Linear |
| $NaClO_4$ | $Na^+$ | 9000 | 0.1 | 0.04 – 8.19 | Non linear |
| Na(Picr) | $Na^+$ | 9000 | 0.055 | 0.036 – 6.4 | Non linear |
| K(Picr) | $K^+$ | 11000 | 0.037 | 0.056 – 6.4 | Linear |
| Cs(Picr) | $Cs^+$ | 5042 | 0.045 | 0.144 – 1.3 | Linear |

| | | | | | |
|---|---|---|---|---|---|
| $Mg(ClO_4)_2$ | $Mg^{2+}$ | 2921 | 0.05 | 0.24-2.4 | Linear |
| $Ca(Picr)_2$ | $Ca^{2+}$ | 59600 | 0.045 | 0,01- 0.2 | Non linear |
| $Sr(Picr)_2$ | $Sr^{2+}$ | 100000 | 0.05 | 0.005 – 0.125 | Non linear |
| $NH_4PF_6$ | $NH_4^+$ | 12334 | 0.045 | 0.04-20 | Non linear |
| $(PhCH_2)_2NH_2PF_6$ | $(PhCH_2)_2NH_2^+$ | 117 | 0.08 | 1.2-38 | Linear |

As an example Figure S9 reports the linear regression obtained analysing the behaviour of ligand **1•** in the presence of CsPicr in acetonitrile.

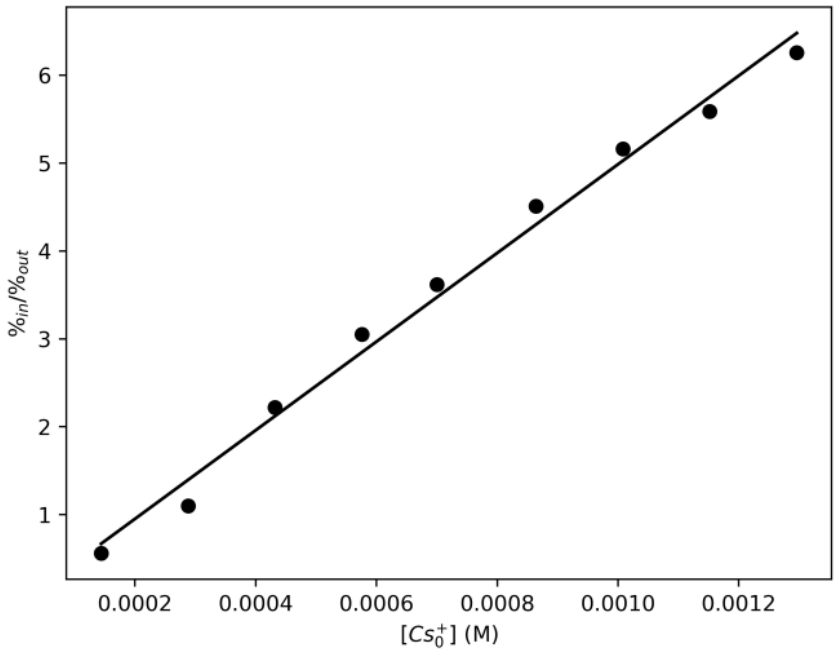



**Figure S9**. Linear regression obtained reporting $\%_{bound}/\%_{free}$ (here indicated as $\%_{in}/\%_{out}$) in the function of $[Cs^+]_0$ in the presence of **1•** in acetonitrile. $K_a$ results 5042 $M^{-1}$ and $r^2$=0.99.

As an example Figure S10 reports the non-linear regression obtained analysing the behaviour of ligand **1•** in the presence of $NH_4PF_6$ in acetonitrile.

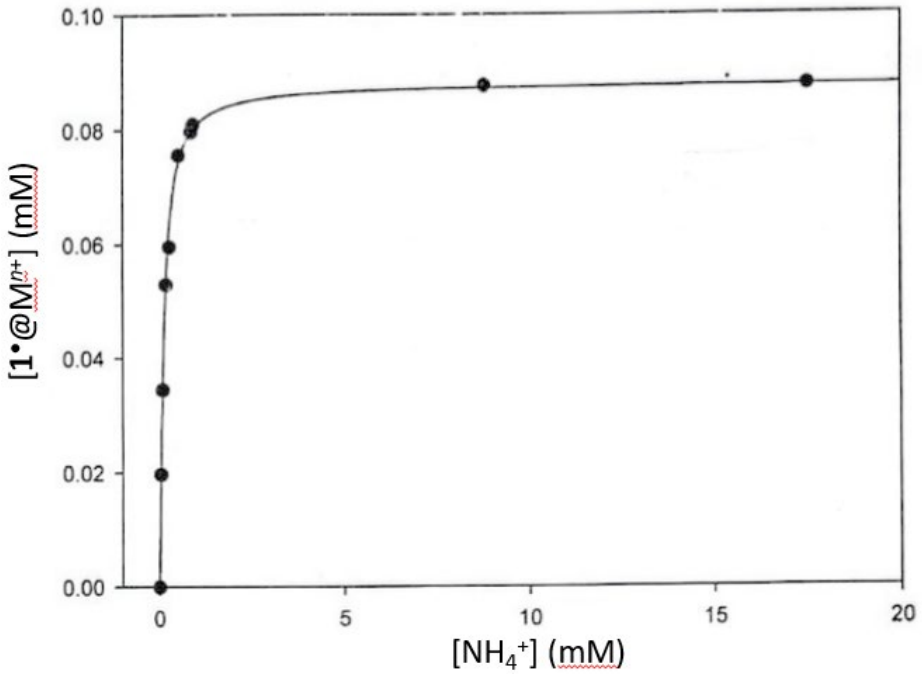



**Figure S10**. Non-linear regression obtained reporting [**1•**@$M^{n+}$] in the function of [$NH_4^+$] in the presence of **1•** in acetonitrile. $K_a$ results 12334 $M^{-1}$.

Table S2 reports the irregular variation of the ratio between the bound and free form obtained by simulation of the spectra of the ligand **2•** in the presence of different $Sr^{2+}$ concentrations.

**Table S2**. Calculated parameters for the association between **2•** and $Sr^{23}$ (from $Sr(ClO_4)_2$)in acetonitrile

| $[Sr^{2+}]_0$ (mM) | $[Sr^{2+}]_{free}$ (mM) | $a_N$ free (G) | $a_N$ bound (G) | % free | % bound | $[2•]_0$ (mM) | $[2•@Sr^{2+}]$ (mM) |
|---|---|---|---|---|---|---|---|
| 0,08 | 0,068 | 15,36 | 16,32 | 260,93 | 100 | 0,043 | 0,0191 |
| 0,18 | 0,153 | 15,36 | 16,32 | 56,21 | 100 | 0,043 | 0,0275 |
| 0,30 | 0,263 | 15,36 | 16,32 | 16,39 | 100 | 0,043 | 0,0369 |
| 0,48 | 0.44 | 15,36 | 16,32 | 5,6 | 100 | 0.043 | 0,0407 |

Note: The concentration of metal free ($[Sr^{2+}]_{free}$) is obtained by subtracting the concentration of complexed species ([**2•**@$Sr^{2+}$]) from the initial one ($[Sr^{2+}]_0$). The association constant was found by considering $K_a$ = %bond/$\sqrt{}$%free $[Sr^{2+}]_{free}$

Table S3 reports further results obtained in the presence of some cations and ligands in different ACN/$H_2O$ mixtures.

**Table S3.** Further EPR spectroscopic parameters and calculated $K_a$ for the association between **1•**, **2•** and **3•** and different cations in mixtures ACN/$H_2O$

| Cation | Mixture ACN/$H_2O$ | Crown ether 1• $K_a/M^{-1}$ | Crown ether 2• $K_a/M^{-1}$ | Crown ether 3• $K_a/M^{-1}$ | *a*/G *1• free* **1• bound** | *a*/G *2• free* **2• bound** | *a*/G *3• free* **3• bound** |
|---|---|---|---|---|---|---|---|
| $Na^+$ (*I* = 3/2) (*r* = 1.02 Å) | 98/2 | 345 | 1269 | | *$a_N$=15.42* **$a_N$=15.56** **$a_{Na}^+$=2.38** | *$a_N$=15.48* **$a_N$=15.51** **$a_{Na}^+$=2.50** | |
| $Na^+$ (*I* = 3/2) (*r* = 1.02 Å) | 96/4 | 193 | | 4302 | *$a_N$=15.44* **$a_N$=15.63** **$a_{Na}^+$=2.11** | | *$a_N$=15.58* **$a_N$=16.11** **$a_{Na}^+$=2.01** |

| | | | | | |
|---|---|---|---|---|---|
| $Na^+$ ($I$ = 3/2) ($r$ = 1.02 Å) | 92/8 | 65 | | *$a_N$=15.56*<br>**$a_N$=15.91**<br>**$a_{Na^+}$=2.11** | |
| $Ca^{2+}$ ($I$ = 3/2) ($r$ = 1.02 Å) | 98/2 | 3124 | 3642 | *$a_N$=15.42*<br>**$a_N$=16.78** | *$a_N$=15.53*<br>**$a_N$=16.74** |
| $Ca^{2+}$ ($I$ = 3/2) ($r$ = 1.02 Å) | 97/3 | | 1136 | | *$a_N$=15.59*<br>**$a_N$=16.66** |

## References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