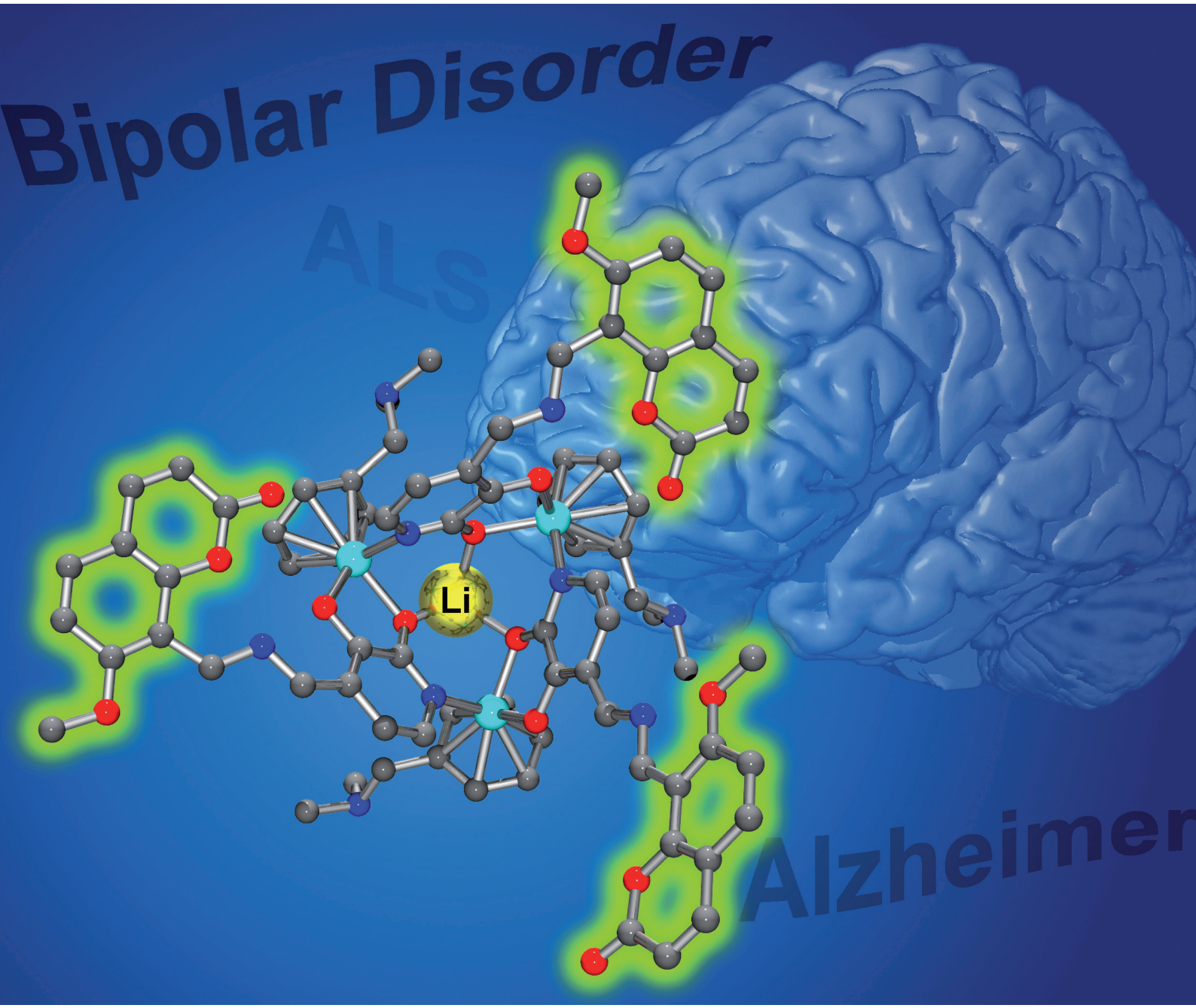
Organic & Biomolecular Chemistry

www.rsc.org/obc

Volume 7 | Number 6 | 21 March 2009 | Pages 1029-1232



ISSN 1477-0520

RSC Publishing

Ruthenium-based metallacrown complexes for the selective detection of lithium ions in water and in serum by fluorescence spectroscopy

Sébastien Rochat, Zacharias Grote and Kay Severin*

Received 18th November 2008, Accepted 12th December 2008
First published as an Advance Article on the web 27th January 2009
DOI: 10.1039/b820592b

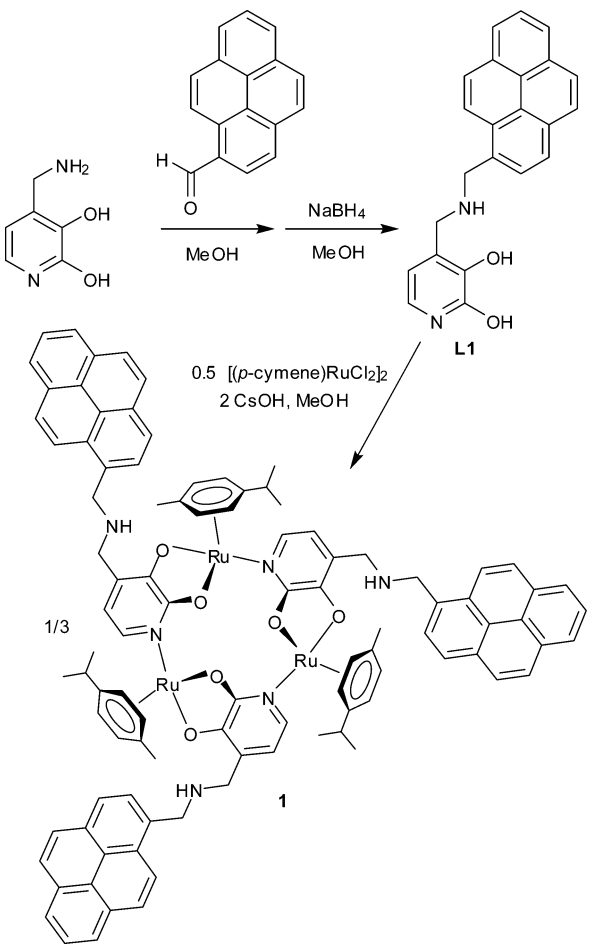
Fluorescent dihydroxypyridine ligands were prepared by attaching pyrene-, dansyl-, and methoxycoumarin-fluorophores *via* dimethyleneamine linkers. The reaction of these ligands with $[(p\text{-cymene})RuCl_2]_2$ or $[(C_6H_5CH_2NMe_2H)RuCl_2]_2Cl_2$ resulted in the formation of 12-metallacrown-3 complexes, which possess strong affinity for lithium ions. By a judicious choice of the fluorophore and the arene π-ligand, a macrocycle was obtained that could be used in aqueous solution to selectively and quantitatively detect lithium ions by fluorescence spectroscopy.

Introduction

Lithium salts are important drugs for patients suffering from bipolar disorder.[1] Recent studies suggest that lithium salts could also be of interest for the treatment of amyotrophic lateral sclerosis (ALS)[2] and of Alzheimer's disease.[3] It was even speculated whether lithium could become the "aspirin of the brain".[4] Apart from applications in the field of neurology and psychiatry, lithium salts have been proposed to treat skin diseases and certain viral infections.[1] In view of their pharmacological relevance, it is not surprising that considerable efforts have been devoted towards the development of sensors for lithium ions.[5] But the selective detection of the well-solvated lithium ion in an aqueous environment containing an excess of competing ions such as Na^+ or Mg^{2+} is a challenging task. In fact, a recent review about the pharmacological action of lithium salts concludes that "... progress in lithium research has been hampered by the unavailability of a sensitive and specific Li^+ spectroscopic tool."[6] A fluorescent sensor for lithium ions would be particularly appealing in this context. Although there are several publications about fluoroionophores for Li^+,[7,8] only a few were shown to work in homogeneous aqueous solution.[8] Below we report efforts to address this challenge. In particular, we will describe a fluorescent chemosensor that allows the detection of low millimolar concentrations of lithium in water or serum with excellent selectivity over Na^+ or Mg^{2+}.

Results and discussion

As the recognition unit for our lithium sensor, we decided to use a 12-metallacrown-3 complex.[9] This structural motif can easily be obtained by reaction of half-sandwich complexes of Ru^{II}, Rh^{III}, or Ir^{III} with 2,3-dihydroxypyridine ligands.[10] Previous work by us and by others have shown that these complexes can display a very high affinity and selectivity for lithium ions, even in polar solvents such as water.[10,11] Our strategy was to attach a fluorophore to the 2,3-dihydroxypyridine ligands. In a first attempt, this was accomplished by reductive amination of 1-pyrenecarboxaldehyde with 4-aminomethyl-2,3-dihydroxypyridine (Scheme 1). The latter was prepared by a Mannich reaction of commercial 2,3-dihydroxypyridine with dibenzylamine, followed by hydrogenolysis over Pd/C.



Scheme 1

Complex **1** was then obtained by reaction of ligand **L1** with $[(p\text{-cymene})RuCl_2]_2$ in methanol in the presence of a base (CsOH). After filtration, the product was separated from the salts by

Institut des Sciences et Ingénierie Chimiques, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland. E-mail: kay.severin@epfl.ch; Fax: +41(0)21 6939305; Tel: +41(0)21 6939302

washing with methanol. Macrocycle **1**, which contains the highly hydrophobic pyrene fluorophore, was only moderately soluble in benzene and dichloromethane. To evaluate the affinity of **1** for lithium cations, we studied the complexation of Li^+ using ^1H NMR spectroscopy. 12.5 μL of a 100 mM LiCl stock solution in CD_3OD were added to 487.5 μL of a solution of complex **1** in C_6D_6. (Final concentrations: [**1**] = 5.0 mM; [Li^+] = 2.5 mM.) After stirring for 5 minutes, the ^1H NMR spectrum of the solution showed a 1:1 mixture of complex **1** and a new species, which we attribute to the Li^+ adduct (Fig. 1). The peaks of the Li^+ adduct were slightly shifted and in some cases (aromatic cymene signals) strongly broadened compared to those of complex **1**. More detailed complexation studies were not performed due to the poor solubility of the macrocycle in polar solvents. However, it was concluded that the presence of a bulky fluorophore does not prevent Li^+ from reaching the binding site.

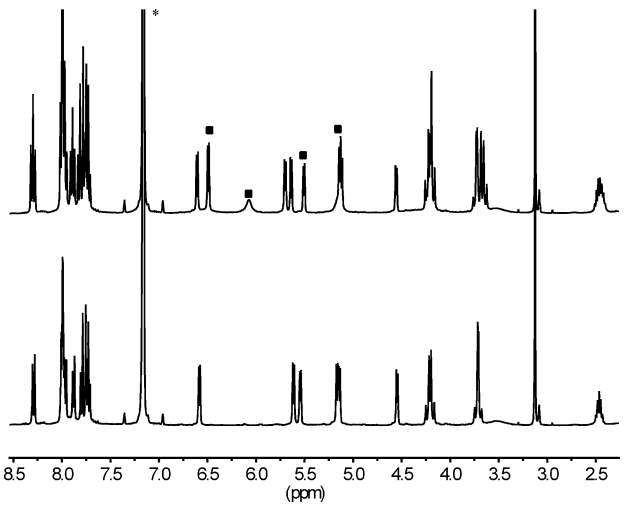
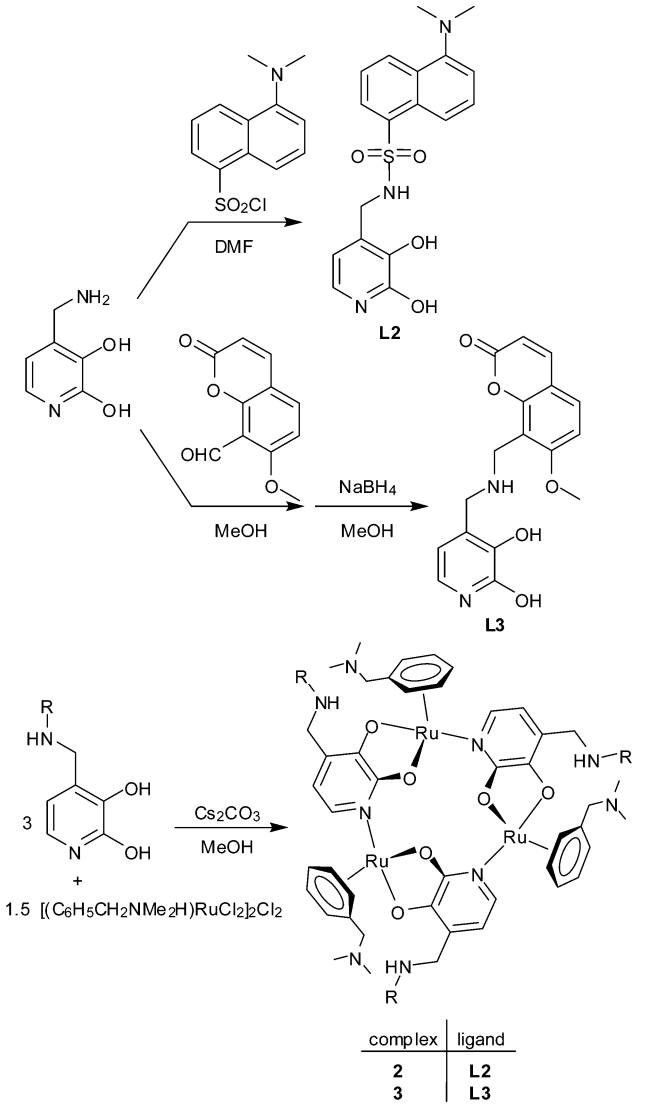


Fig. 1 Bottom: part of the ^1H NMR spectrum of the receptor **1** (5.0 mM) in C_6D_6 containing 2.5 vol% CD_3OD. Top: receptor **1** in the presence of 0.5 equivalent (2.5 mM) of LiCl. The peaks labeled with ■ indicate the presence of signals corresponding to the pyridine and/or *p*-cymene of the macrocycle–Li^+ adduct. The asterisk indicates the solvent peak.

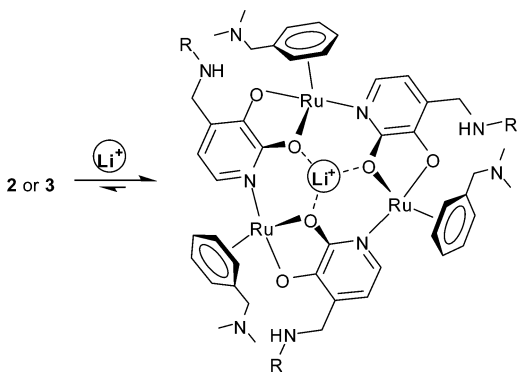
In order to obtain less lipophilic ligands, the pyrene group was exchanged for dansyl and methoxycoumarin fluorophores. The syntheses of the corresponding ligands **L2** and **L3** was accomplished by coupling 4-aminomethyl-2,3-dihydroxypyridine with dansyl chloride or by reductive amination with 8-formyl-7-methoxycoumarin,[12,13] respectively (Scheme 2). To further increase the polarity, the standard $[(p\text{-cymene})RuCl_2]_2$ was replaced with the amino-substituted $[(C_6H_5CH_2NMe_2H)RuCl_2]_2Cl_2$.[14] The tertiary amine groups of the arene π-ligand were expected to enhance the solubility of the resulting macrocycles in water.

Complex **2** was synthesized by the reaction of **L2** with $[(C_6H_5CH_2NMe_2H)RuCl_2]_2Cl_2$ in methanol in the presence of Cs_2CO_3 (Scheme 2). After removal of the solvent, the complex was extracted from the residual salts with dichloromethane and isolated by precipitation. Macrocycle **2** turned out to be soluble in aqueous methanol (containing up to 30% H_2O) but not in pure water.

The ^1H NMR spectra of solutions containing **2** and various concentrations of LiCl unequivocally indicated a strong affinity between the macrocycle and Li^+ (Scheme 3). The association



Scheme 2



Scheme 3

constant was determined by NMR measurements: a two-fold excess of LiCl (10.0 mM) was added to a solution of the macrocycle **2** (5.0 mM) in CD_3OD/D_2O (9:1, pD 7.0, 10 mM phosphate buffer). After stirring for 5 minutes, the ^1H NMR spectrum was recorded. A new set of signals, corresponding to the Li^+ adduct, was visible together with the signals corresponding

to uncomplexed receptor **2**. The ratio of the two species was determined by integration of selected signals. From that, the association constant was calculated as $K_a(Li^+) = 5.0\ (\pm\ 0.5)\ 10^3\ M^{-1}$. The water content of the solution could be increased to 30% if a HEPES buffer was used since phosphate salts show a limited solubility in $MeOH/H_2O$.

The fluorescence of complex **2** was strongly reduced compared to that of the free ligand **L2**. This was an expected effect of the Ru^{II} centers, which are able to quench the fluorescence by electron transfer. Still, solutions of complex **2** displayed a pronounced fluorescence signal centered at 547 nm when excited at 360 nm. This signal increased in intensity when LiCl was added to the solution (Fig. 2). We propose that the 'turn-on' response of receptor **2** upon the binding of Li^+ is due to the reduced ability of the Ru centers to quench the fluorescence of the dansyl groups. This assumption is supported by previous electrochemical measurements, which have shown that Ru-based metallacrown complexes are significantly more difficult to oxidize in the presence of Li^+.[11k,l]

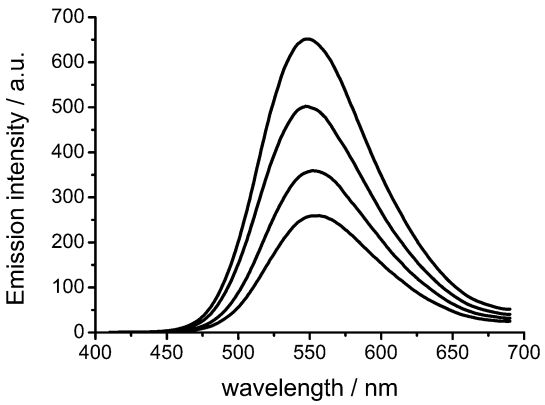


Fig. 2 Increase of the emission signal of the macrocycle **2** (2.0 mM) in methanol/water (9:1, pH 7.0, 10 mM phosphate buffer) upon the addition of LiCl (0.0, 0.5, 1.5 and 3.0 mM). Excitation wavelength: 360 nm.

Complex **3** was synthesized by the base-assisted reaction of ligand **L3** with $[(C_6H_5CH_2NMe_2H)RuCl_2]_2Cl_2$ in methanol, followed by extraction with dichloromethane (Scheme 2). In contrast with **2**, complex **3** was soluble in water at neutral pH. It was therefore possible to generate the macrocycle **3** by simply mixing two equivalents of the ligand **L3** with $[(C_6H_5CH_2NMe_2H)RuCl_2]_2Cl_2$ in water (pH 8.0, 100 mM phosphate buffer). The *in situ* reaction is essentially quantitative and further host-guest experiments were performed without prior isolation of macrocycle **3**.

When 2 equivalents of LiCl were added to an aqueous solution of complex **3**, the ^1H NMR spectrum showed new signals for the Li^+ adduct along with small signals for the remaining **3** (Fig. 3). From the ratio of the two species, a value of $K_a(Li^+) = 8.3\ (\pm\ 0.3)\ 10^2\ M^{-1}$ was calculated. This value is similar to what had been observed for water-soluble (*p*-cymene)Ru-derived metallacrown complexes.[11e] The amine side chains of the π-ligands are thus a way to enhance the solubility without compromising the Li^+ affinity. The same experiment performed with a large excess of NaCl instead of LiCl allowed us to estimate the binding constant for sodium as $K_a(Na^+) = 6\ (\pm\ 3)\ 10^{-1}\ M^{-1}$, thus indicating a remarkable Li^+:Na^+ selectivity of 3 orders of magnitude. Previous investigations had shown that organometallic 12-metallacrown-3

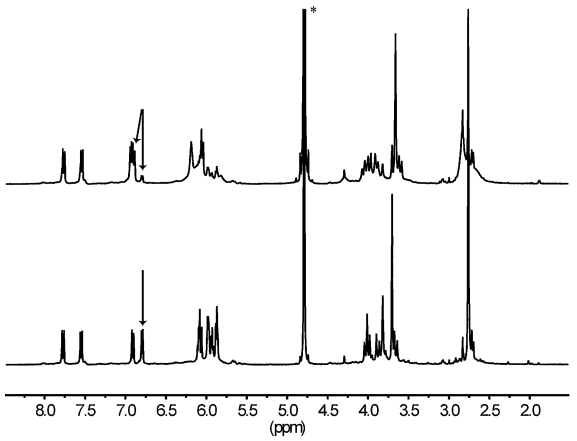


Fig. 3 Bottom: ^1H NMR spectrum of receptor **3** (5.0 mM) in D_2O (pD 8.0, 100 mM phosphate buffer). Top: receptor **3** in the presence of 2 equivalents of LiCl. The arrows indicate signals of the pyridine ring; in the presence of Li^+ these signals are slightly downfield shifted, and some "empty" receptor is still visible. The asterisk indicates the water peak.

complexes have a negligible affinity for K^+. Binding studies with K^+ were therefore not performed.

Due to its solubility in water, complex **3** was selected for more detailed fluorescence investigations. Solutions of complex **3** displayed a fluorescence signal at 480 nm when excited at 382 nm. Upon incremental addition of LiCl to an aqueous solution of **3**, an increase in the fluorescence was observed (Fig. 4). Kinetic measurements showed that the response was very quick and that a stable signal was obtained after 1 minute.[15] The data were fitted to a 1:1 binding model[16] with the help of the non-linear least square curve-fitting program WinEQNMR.[17] The obtained value of $K_a(Li^+) = 7.4\ (\pm\ 0.6)\ 10^2\ M^{-1}$ was in good agreement with the NMR studies.

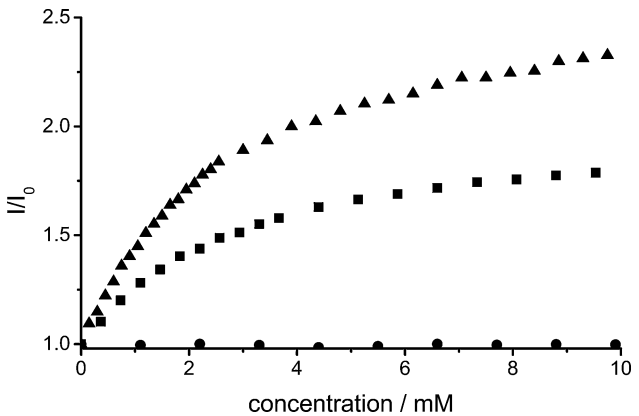


Fig. 4 Relative fluorescence intensity at 480 nm (excitation at 382 nm) for solutions containing the metallacrown complex **3** (2.0 mM) and variable amounts of LiCl (▲ and ■) or NaCl (●). The data labeled with ■ and ● were obtained in water (100 mM phosphate buffer, pH 8.0), whereas the data labeled with ▲ were obtained in serum after removal of proteins.

In biological samples such as serum, the Na^+ concentration can be more than 100 times higher than the pharmacologically relevant Li^+ concentration of around 1.0 mM. For potential applications, it is therefore of prime importance that the sensor displays a very good selectivity for Li^+ over Na^+. Fluorescence titration

experiments with NaCl instead of LiCl showed that complex **3** is indeed highly specific (Fig. 4). Only a minor increase (less than 15%) in fluorescence was observed for Na^+ concentrations of up to 140 mM. Mg^{2+} is another potentially interfering ion,[6] but sensor **3** showed a negligible response for concentrations of up to 3.0 mM, which is much higher than the biological relevant concentration.

Encouraged by the results obtained in water, we investigated whether the metallacrown sensor **3** could be used in a more complex environment such as human serum. Blood serum contains numerous salts, glucose, hormones, and proteins (with albumin, transferrin and immunoglobulins representing ~90% of the total weight).[18] The latter were expected to interfere with our sensor due to autofluorescence and competitive coordination to the (arene)Ru complexes. Consequently, large proteins were removed prior to analysis by a simple precipitation procedure.[19] Fluorescence measurements were then performed with samples that contained different concentrations of LiCl. The results were similar to what was observed for water: the presence of Li^+ resulted in a concentration-dependent increase in fluorescence (Fig. 4). The signal to noise ratio was sufficient for quantitative measurements in the biologically interesting concentration range of 0–3 mM. Fitting of the binding isotherm gave an association constant of $K_a(Li^+) = 8.3\ (\pm\ 0.6)\ 10^2\ M^{-1}$, and the increase in fluorescence was slightly more pronounced than for the measurements performed in water.

Conclusion

We have described the synthesis and host-guest chemistry of fluorescent (arene)Ru-based 12-metallacrown-3 complexes. The solubility of the complexes was found to depend strongly on the arene π-ligand, as well as on the appended fluorophore. A water-soluble complex (**3**) was obtained by combining a Ru complex with an amino-substituted π-ligand with methoxycoumarin fluorophores. This complex acts as a 'turn-on' fluorescence sensor, which can be used to quantify low millimolar concentrations of the pharmacologically important lithium ion. The sensor can be formed *in situ* from $[(\eta^6\text{-}C_6H_5CH_2NMe_2H)RuCl_2]_2Cl_2$ and ligand **L3**, both of which are straightforward to synthesize. A key advantage is its high affinity and selectivity for Li^+, which allows working in purely aqueous solution in the presence of an excess of Na^+, or in a complex matrix such as serum. A current limitation is the modest increase in signal intensity (up to 2.5-fold) and the low overall fluorescence. It should be possible, however, to overcome these limitations by changing the nature of the (π-ligand)M fragment and the fluorophore. Investigations in this direction are ongoing in our laboratory.

Experimental

General details and procedures

Commercial reagents were purchased from Acros or Fluka. $RuCl_3(H_2O)_n$ was obtained from Precious Metals Online, and human serum from Sigma-Aldrich. All chemicals were used as received. The compounds $[(p\text{-cymene})RuCl_2]_2$,[20] $[(\eta^6\text{-}C_6H_5CH_2NMe_2H)RuCl_2]_2Cl_2$,[14] 4-(dibenzylamino)methyl-2,3-dihydroxypyridine,[21] 8-formyl-7-hydroxycoumarin,[13] and 8-formyl-7-methoxycoumarin[12] were prepared as described in the literature. The reactions involving ruthenium compounds were carried out under an atmosphere of dry dinitrogen using standard Schlenk techniques. All reactions and titrations in H_2O were performed in Millipore water. The ^1H and ^{13}C spectra were recorded on a Bruker Advance DPX 400 spectrometer using the residual protonated solvents (^1H, ^{13}C) as internal standards. ^{13}C NMR spectra in D_2O were recorded using dioxane as an internal standard. All spectra were recorded at room temperature. High resolution mass spectra were obtained with a Waters CapLC-coupled Micromass Q-ToF Ultima ESI-instrument. Purification by reversed phase HPLC was carried out using a Waters system consisting of a Waters 600 controller unit, a Waters Delta 600 pump and a Waters 2487 dual wavelength absorbance detector. A Sunfire preparative C_{18} column (5 μm, 10 × 250 mm) was used as stationary phase, and Millipore water and acetonitrile (HPLC grade) as mobile phase. Fluorescence spectra were recorded on a Varian Cary Eclipse spectrophotometer equipped with a thermostatted cell holder. The binding isotherms were fitted to a 1:1 binding model using the program WinEQNMR.[17]

4-Aminomethyl-2,3-dihydroxypyridine

4-(Dibenzyl-amino)methyl-2,3-dihydroxypyridine[21] (1.00 g, 3.12 mmol) was dissolved in degassed methanol (200 mL). Pd/C (0.332 g, 0.312 mmol, 10 mol%) was added and the mixture was stirred under a hydrogen atmosphere for 8 h. After filtration through a pad of Celite, the solvent was evaporated under reduced pressure. The residue was suspended in MeOH (10 mL), and the resulting solid was filtered off, and dried in vacuum. Yield: 305 mg (2.18 mmol, 70%). ^1H NMR (DCl 0.1 M in D_2O): δ (ppm) 4.14 (s, 2 H, CH_2), 6.44 (d, $^3J = 6.8$ Hz, 1 H, pyridine), 7.11 (d, $^3J = 6.8$ Hz, 1 H, pyridine). $^{13}C\{^1H\}$ NMR (DCl 0.1 M in D_2O): δ (ppm) 38.36 (CH_2), 109.65 (C-H), 124.78 (*C*-CH_2), 125.34 (C-H), 145.45 (C-OH), 159.08 (C-OH). *m/z* (ESI) 141.0665 ($M + H^+$. $[C_6H_9N_2O_2]^+$ requires 141.0659).

Ligand L1

4-Aminomethyl-2,3-dihydroxypyridine (200 mg, 1.43 mmol) and 1-pyrenecarboxaldehyde (329 mg, 1.43 mmol) were stirred in anhydrous methanol (75 mL) for 2 h. The resulting imine **L1a** was filtered off, washed with MeOH, and dried under vacuum. Yield: 372 mg (1.06 mmol, 74%). ^1H NMR (d6-DMSO): δ (ppm) 4.98 (s, 2 H, NCH_2), 6.30 (d, $^3J = 7.0$ Hz, 1 H, pyridine), 6.90 (d, $^3J = 7.0$ Hz, 1 H, pyridine), 8.13 (t, $^3J = 8.0$ Hz, 1 H, pyrene), 8.23 (d, $^3J = 9.0$ Hz, 1 H, pyrene), 8.29 (d, $^3J = 9.0$ Hz, 1 H, pyrene), 8.31–8.41 (m, 4 H, pyrene), 8.59 (d, $^3J = 10.0$ Hz, 1 H, pyrene), 9.55 (s, 1 H, CHN). $^{13}C\{^1H\}$ NMR (d6-DMSO): δ (ppm) 58.19 (NCH_2), 106.57, 123.01, 123.20, 123.80, 124.08, 125.11, 125.91, 126.21, 126.49, 126.66, 127.30, 127.48, 128.43, 128.75, 128.84, 129.42, 130.17, 130.85, 132.46, 143.56 (pyrene, pyridine, CHN), 157.96, 161.99 (pyridine). *m/z* (ESI) 353.1287 ($M + H^+$. $[C_{23}H_{17}N_2O_2]^+$ requires 353.1290).

A suspension of the imine **L1a** (250 mg, 0.71 mmol) and $NaBH_4$ (96 mg, 2.48 mmol) in anhydrous methanol (50 mL) was stirred for 2 h. The off-white precipitate was filtered off, washed with MeOH, and dried in vacuum. Yield: 161 mg (0.45 mmol, 64%). ^1H NMR (d6-DMSO): δ (ppm) 3.76 (s, 2 H, NCH_2), 4.40 (s, 2 H, NCH_2), 6.30 (d, $^3J = 7.0$ Hz, 1 H, pyridine), 6.86 (d, $^3J = 7.0$ Hz, 1 H, pyridine), 8.07 (t, $^3J = 8.0$ Hz, 1 H, pyrene), 8.10 (d, $^3J = 8.0$ Hz,

1 H, pyrene), 8.15 (m, 2 H, pyrene), 8.21 (d, ^3J = 9.0 Hz, 1 H, pyrene), 8.26 (d, ^3J = 8.0 Hz, 1 H, pyrene), 8.29 (d, ^3J = 8.0 Hz, 1 H, pyrene), 8.44 (d, ^3J = 9.0 Hz, 1 H, pyrene), 11.57 (s, br, 1 H, OH). $^{13}C\{^1H\}$ NMR (d6-DMSO): δ (ppm) 46.44, 50.18 (NCH_2), 106.37, 122.96, 123.70, 124.00, 124.10, 124.66, 125.01, 125.08, 126.18, 126.87, 127.21, 127.87, 128.62, 129.97, 130.37, 130.81, 134.21, 144.25, 157.86 (pyrene, pyridine). *m/z* (ESI) 355.1444 (M + H^+. $[C_{23}H_{19}N_2O_2]^+$ requires 355.1447).

Ligand L2

Dansyl chloride (400 mg, 1.47 mmol) was added to a suspension of 4-aminomethyl-2,3-dihydroxypyridine (1150 mg, 7.40 mmol) in anhydrous DMF (20 mL). The mixture was stirred overnight at room temperature. The excess of 4-aminomethyl-2,3-dihydroxypyridine was filtered off and washed with CH_2Cl_2. The organic solutions were poured into 30 mL H_2O, and evaporated under vacuum. The solid was purified by flash chromatography on silica gel ($MeOH/CH_2Cl_2$ 2:98 to 10:90) to afford the title compound. Yield: 356 mg (953 μmol, 65%). ^1H NMR (d6-DMSO): δ (ppm) 2.82 (s, 6 H, $N(CH_3)_2$), 3.88 (s, 2 H, NCH_2), 5.97 (d, 1 H, ^3J = 6.8 Hz, pyridine), 6.59 (d, 1 H, ^3J = 6.1 Hz, pyridine), 7.24 (d, 1 H, ^3J = 7.6 Hz, dansyl), 7.54–7.60 (m, 2 H, dansyl), 8.09 (d, 1 H, ^3J = 7.4 Hz, dansyl), 8.29 (d, 1 H, ^3J = 8.6 Hz, dansyl), 8.41 (d, 1 H, ^3J = 8.6 Hz, dansyl). $^{13}C\{^1H\}$ NMR (d6-DMSO): δ (ppm) 39.42 (CH_2), 45.09 (CH_3), 105.46, 115.05, 119.12, 122.25, 123.44, 124.95, 127.79, 128.33, 128.97, 129.04, 129.42, 135.85, 143.70 (pyridine, dansyl), 151.33, 157.80 (pyridine). *m/z* (ESI) 374.1156 (M + H^+. $[C_{18}H_{20}N_3O_4S]^+$ requires 374.1175).

Ligand L3

8-Formyl-7-methoxycoumarin[12,13] (408 mg, 2.00 mmol) and 4-aminomethyl-2,3-dihydroxypyridine (280 mg, 2.00 mmol) were stirred for 3 h in anhydrous methanol (20 mL). The resulting imine **L3a** was filtered off, washed with methanol, and dried under vacuum. Yield: 490 mg (1.50 mmol, 75%). ^1H NMR (d6-DMSO): δ (ppm) 3.93 (s, 3 H, CH_3), 4.68 (s, 2H, CH_2), 6.29 (d, ^3J = 6.8 Hz, 1H, pyridine), 6.33 (d, ^3J = 9.5 Hz, 1H, coumarin), 6.83 (d, ^3J = 6.8 Hz, 1H, pyridine), 7.17 (d, ^3J = 8.8 Hz, 1H, coumarin), 7.78 (d, ^3J = 8.8 Hz, 1H, coumarin), 8.02 (d, ^3J = 9.5 Hz, 1H, coumarin), 8.67 (s, 1 H, CHN), 8.83 (s, br, 1 H, OH), 11.55 (s, br, 1 H, OH). $^{13}C\{^1H\}$ NMR (d6-DMSO): δ (ppm) 58.6 (CH_2), 58.9 (CH_3), 106.2, 108.5, 112.2, 112.8, 112.9, 122.9, 127.13, 131.1, 143.3, 144.5, 152.5, 155.6, 157.9, 159.9, 160.9 (pyridine, coumarin, CHN). *m/z* (ESI) 327.0979 (M + H^+. $[C_{17}H_{15}N_2O_5]^+$ requires 327.0975).

Excess $NaBH_4$ (166 mg, 4.39 mmol) was added to a suspension of the imine **L3a** (409 mg, 1.25 mmol) in anhydrous methanol (50 mL). After stirring the mixture for 3 h, the solvent was evaporated. The crude product was purified by reversed phase HPLC (H_2O/CH_3CN, gradient 88:12 to 76:24 in 16 minutes, λ_{max} = 254 nm, sample: 1 mL, 20 mg crude material, R_f = 14.5 min). Yield: 197 mg (0.60 mmol, 48%). ^1H NMR (DCl 0.1 M in D_2O): δ (ppm) 3.88 (s, 3H, CH_3), 4.11 (s, 2H, CH_2), 4.43 (s, 2H, CH_2), 6.11 (d, ^3J = 6.8 Hz, 1H, pyridine), 6.26 (d, ^3J = 9.5 Hz, 1H, coumarin), 6.83 (d, ^3J = 6.9 Hz, 1H, pyridine), 6.99 (d, ^3J = 9.1 Hz, 1H, coumarin), 7.60 (d, ^3J = 8.8 Hz, 1H, coumarin), 7.87 (d, ^3J = 9.5 Hz, 1H, coumarin). $^{13}C\{^1H\}$ NMR (DCl 0.1 M in D_2O): δ (ppm). 39.57 (CH_2), 45.59 (CH_2), 57.06 (CH_3), 106.17, 109.11, 109.62, 112.75, 113.62, 122.42, 124.89, 132.40, 145.73, 146.64, 153.39, 158.50, 161.79, 163.91 (pyridine, coumarin). *m/z* (ESI) 329.1121 (M + H^+. $[C_{17}H_{17}N_2O_5]^+$ requires 329.1132).

[(*p*-cymene)Ru(L1–2H$^+$)]$_3$ (1)

A mixture of ligand **L1** (100 mg, 282 μmol), [(*p*-cymene)$RuCl_2$]$_2$ (86.3 mg, 141 μmol), and CsOH (94.7 mg, 564 μmol) in 10 mL of degassed methanol was stirred for 2 h. The precipitate was filtered off, washed with cold methanol and dried under vacuum. Yield: 132 mg (74.9 μmol, 80%). ^1H NMR (C_6D_6): δ (ppm) 1.07 (d, ^3J = 6.8 Hz, 9 H, $CH(CH_3)_2$), 1.10 (d, ^3J = 7.1 Hz, 9 H, $CH(CH_3)_2$), 1.76 (s, 9 H, CH_3), 2.63 (sept, ^3J = 6.8 Hz, 3 H, $CH(CH_3)_2$), 3.91 (dd, ^2J = 13.2 Hz, 6 H, CH_2), 4.17 (dd, ^2J = 13.4 Hz, 6 H, CH_2), 4.58 (d, ^3J = 5.6 Hz, 3 H, cymene), 5.13 (d, ^3J = 5.6 Hz, 3 H, cymene), 5.25 (d, ^3J = 5.6 Hz, 3 H, cymene), 5.61 (d, ^3J = 5.6 Hz, 3 H, cymene), 5.90 (d, ^3J = 6.1 Hz, 3 H, pyridine), 6.96 (d, ^3J = 6.1 Hz, 3 H, pyridine), 7.70–7.75 (m, 9 H, pyrene), 7.83–7.90 (m, 12 H, pyrene), 8.00 (d, ^3J = 7.8 Hz, 3 H, pyrene), 8.28 (d, ^3J = 9.3 Hz, 3 H, pyrene). *m/z* (ESI) 882.7197 (M + 2H$^+$. $[C_{99}H_{92}N_6O_6Ru_3]^{2+}$ requires 882.7131).

[(η^6-$C_6H_5CH_2NMe_2H$)Ru(L2–2H$^+$)]$_3$ (2)

A mixture of ligand **L2** (100 mg, 268 μmol), [(η^6-$C_6H_5CH_2NMe_2H$)$RuCl_2$]$_2Cl_2$ (92 mg, 134 μmol), and Cs_2CO_3 (329 mg, 1.01 mmol) in degassed methanol (15 mL) was stirred for 3 h. The solvent was removed in vacuum, and the solid residue was extracted with CH_2Cl_2 (2 × 15 mL). Hexane (60 mL) was added to the orange solution, and the volume was reduced to 30 mL. The precipitate was filtered off, washed with hexane, and dried under vacuum. Yield: 129 mg (70.8 μmol, 79%). ^1H NMR (MeOD): δ (ppm) 2.34 (s, 18 H, CH_3), 2.90 (s, 18 H, CH_3), 3.35 (dd, ^2J = 3.92 Hz, 6 H, CH_2), 3.74 (s, 6 H, CH_2), 5.44–5.58 (m, 15 H, arene and pyridine), 5.69 (t, ^3J = 5.6 Hz, 3 H, arene), 6.44 (d, ^3J = 6.4 Hz, 3 H, pyridine), 7.27 (d, ^3J = 7.4 Hz, 3 H, dansyl), 7.59 (t, ^3J = 8.1 Hz, 3 H, dansyl), 7.75 (t, 3 H, ^3J = 7.8 Hz, dansyl), 8.22 (d, ^3J = 7.3 Hz 3 H, dansyl), 8.54 (d, ^3J = 8.5 Hz, 3 H, dansyl), 8.59 (d, ^3J = 8.6 Hz, 3 H, dansyl). *m/z* (ESI) 912.6633 (M + 2H$^+$. $[C_{81}H_{92}N_{12}O_{12}S_3Ru_3]^{2+}$ requires 912.6647).

[(η^6-$C_6H_5CH_2NMe_2$)Ru(L3–2H$^+$)]$_3$ (3)

The synthesis was performed as described for complex **2**, using ligand **L3** instead of **L2**. Yield: 65%. ^1H NMR ($CDCl_3$): δ (ppm) 2.25 (s, 18 H, NCH_3), 3.18 (d, ^2J = 13.4 Hz, 3 H, CH_2), 3.25 (d, ^2J = 13.4 Hz, 3 H, CH_2), 3.35 (d, ^2J = 13.0 Hz, 3 H, CH_2), 3.51 (d, ^2J = 13.0 Hz, 3 H, CH_2), 3.81 (s, 9 H, OCH_3), 3.95 (d, ^2J = 12.2 Hz, CH_2), 3.99 (d, ^2J = 12.2 Hz, CH_2), 5.33 (d, ^3J = 4.8 Hz, 3 H, arene), 5.52 (d, ^3J = 6.0 Hz, 3 H, arene), 5.62 (d, ^3J = 5.6 Hz, 3 H, pyridine), 5.63 (t, ^3J = 5.2 Hz, 3 H, arene), 5.75 (t, ^3J = 5.0 Hz, 3 H, arene), 6.16 (t, ^3J = 5.8 Hz, 3 H, arene), 6.23 (d, ^3J = 9.6 Hz, 3 H, pyridine), 6.46 (d, ^3J = 6.4 Hz, 3 H, coumarin), 6.83 (d, ^3J = 8.8 Hz, 3 H, coumarin), 7.35 (d, ^3J = 8.8 Hz, 3 H, coumarin), 7.65 (d, ^3J = 9.2 Hz, 3 H, coumarin).*m/z* (ESI) 845.1574 (M + 2H$^+$. $[C_{78}H_{83}N_9O_{15}Ru_3]^{2+}$ requires 845.1588).

In situ synthesis of the water-soluble complex 3

A mixture of $[(\eta^6\text{-}C_6H_5CH_2NMe_2H)RuCl_2]_2Cl_2$ (7.5 μmol, 5.2 mg) and the ligand **L3** (15 μmol, 4.9 mg) was stirred in degassed water (1.0 mL, pH 8.0, 100 mM phosphate buffer) for 3 h. The concentration of the receptor in such an experiment was 5.0 mM. For experiments with lower receptor concentration (2.0 mM), the amounts were varied accordingly. It should be noted that aqueous solutions of **3** are not particularly sensitive towards oxygen and can be handled for hours without a protective inert atmosphere. ^{1}H NMR (D_2O, pD 8.0, 100 mM phosphate buffer): δ (ppm) 2.76 (s, 18 H, N-$(CH_3)_2$), 3.66 (d, ^3J = 13.5 Hz, 3 H, CH_2), 3.70 (s, 9 H, O-CH_3), 3.78–3.90 (m, 9 H, CH_2), 3.98–4.04 (m, 6 H, CH_2), 5.86–5.88 (m, 6 H, aromatic), 5.93 (t, ^3J = 5.7 Hz, 3 H, π-ligand), 5.96–6.00 (m, 6 H, aromatic), 6.06–6.11 (m, 6 H, aromatic), 6.79 (d, ^3J = 6.4 Hz, 3 H, pyridine), 6.91 (d, ^3J = 8.9 Hz, 3 H, coumarin), 7.55 (d, ^3J = 8.8 Hz, 3 H, coumarin), 7.77 (d, ^3J = 9.5 Hz, 3 H, coumarin).

Fluorescence measurements with complex 2

A 2.0 mM solution of complex **2** was prepared in a mixture of MeOH/H_2O (9:1, pH 7.0, 10 mM phosphate buffer). Fluorescence spectra were recorded (λ_{ex} = 360 nm) 5 minutes after each addition of LiCl (μL amounts of a 1.0 M solution in MeOH).

Fluorescence measurements with complex 3 in water

A 2.0 mM solution of complex **3** was prepared in H_2O (pH 8.0, 100 mM phosphate buffer). The solution was filtered and an aliquot of 3.0 mL was placed in a cuvette for fluorescence measurements. The solution was stirred and its temperature equilibrated to 20 °C. The fluorescence was measured (λ_{ex} = 382 nm; λ_{em} = 480 nm) every 4 seconds during one minute, or until the signal was stabilized. The solution was then titrated with μL amounts of a 1.10 M solution of LiCl (respectively NaCl or $MgCl_2$) in H_2O. After each addition, the solution was equilibrated for 1 minute, and fluorescence was recorded for one minute every 4 seconds (15 measurements that were averaged) before the next analyte addition took place. The data were fitted with the non-linear least square curve-fitting program WinEQNMR[17] using a 1:1 binding model to calculate the association constant $K_a(Li^+)$.

Fluorescence measurements with complex 3 in serum

Serum preparation:[19] 5.0 mL of HPLC-grade acetonitrile were added to 2.5 mL serum. The suspension was vigorously mixed for 5 min at room temperature, then centrifuged at 4000 rpm for 30 minutes. 6.0 mL of the supernatant were removed and lyophilized. The residue was dissolved in 2.0 mL H_2O (pH 8, 100 mM phosphate buffer) to obtain reconstituted serum.

Titration in reconstituted serum: the reconstituted serum solution was filtered and 1.5 mL was added to 0.5 mL of a solution of the receptor **3** (8.0 mM in H_2O pH 8.0, 100 mM phosphate buffer). The resulting mixture was equilibrated (as indicated by a stable fluorescence signal), and titrated with LiCl (μL doses of a 0.30 M stock solution) while fluorescence was recorded under the same conditions as in plain water. The final concentration of **3** was 2.0 mM, whereas all serum constituents were diluted to 75% of their initial value.

Acknowledgements

The work was supported by the EPFL and by the Claude & Giuliana Foundation.

Notes and references

1 (*a*) N. J. Birch, in *Metal Ions in Biological Systems* (Eds. A. Sigel, H. Sigel), Vol. 41, Marcel Dekker, Basel, 2004, p. 325; (*b*) N. J. Birch, *Chem. Rev.*, 1999, **99**, 2659.

2 (*a*) F. Fornai, P. Longone, L. Cafaro, O. Katsiuchenka, M. Ferrucci, M. L. Manca, G. Lazzeri, A. Spalloni, N. Bellio, P. Lenzi, N. Modugno, G. Siciliano, C. Isidoro, L. Murri, S. Ruggieri and A. Paparelli, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 2052; (*b*) F. Fornai, P. Longone, M. Ferrucci, P. Lenzi, C. Isidoro, S. Ruggieri and A. Paparelli, *Autophagy*, 2008, **4**, 527.

3 (*a*) J. Zhong and W.-H. Lee, *Exp. Opin. Drug Safety*, 2007, **6**, 375; (*b*) C. J. Phiel, C. A. Wilson, V. M.-Y. Lee and P. S. Klein, *Nature*, 2003, **423**, 435.

4 H. R. Pilcher, *Nature*, 2003, **425**, 118.

5 (*a*) G. D. Christian, *Sensors*, 2002, **2**, 432; (*b*) R. A. Bartsch, V. Ramesh, R. O. Bach, T. Shono and K. Kimura, in *Lithium Chemistry* (Eds.: A.-M. Sapse, P. von Ragué Schleyer), John Wiley & Sons, New York, 1995, p. 393.

6 D. M. De Freitas, M. M. C. A. Castro and C. F. G. C. Geraldes, *Acc. Chem. Res.*, 2006, **39**, 283.

7 (*a*) F. Cheng and N. Tang, *Inorg. Chem. Commun.*, 2008, **11**, 400; (*b*) N. Wanichecheva, J. S. Benco, C. R. Lambert and W. G. McGimpsey, *Photochem. Photobiol.*, 2006, **82**, 268; (*c*) K. Hiratani, M. Kaneyama, Y. Nagawa, E. Koyama and M. Kanesato, *J. Am. Chem. Soc.*, 2004, **126**, 13568; (*d*) J. S. Benco, H. A. Nienaber and W. G. McGimpsey, *J. Photochem. Photobiol. A*, 2004, **162**, 289; (*e*) T. Gunnlaugsson, B. Bichell and C. Nolan, *Tetrahedron*, 2004, **60**, 5799; (*f*) T. Gunnlaugsson, B. Bichell and C. Nolan, *Tetrahedron Lett.*, 2002, **43**, 4989; (*g*) J.-A. Chen, J.-L. Lai, G. H. Lee, Y. Wang, J. K. Su, H.-C. Yeh, W.-Y. Lin and M.-k. Leung, *Org. Lett.*, 2001, **3**, 3999; (*h*) S. O. Obare and C. J. Murphy, *Inorg. Chem.*, 2001, **40**, 6080; (*i*) M. Ciampolini, M. Formica, V. Fusi, A. Saint-Mauricec, M. Micheloni, N. Nardi, R. Pontellini, F. Pina, P. Romani, A. M. Sabatini and B. Valtancoli, *Eur. J. Inorg. Chem.*, 1999, 2261; (*j*) Ç. Erk, A. Göçmen and M. Bulut, *Supramol. Chem.*, 1999, **11**, 49; (*k*) C. R. Chenthamarakshan and A. Ajayaghosh, *Tetrahedron Lett.*, 1998, **39**, 1795.

8 (*a*) D. Citterio, J. Takeda, M. Kosugi, H. Hisamoto, S.-i. Sasaki, H. Komatsu and K. Suzuki, *Anal. Chem.*, 2007, **79**, 1237; (*b*) W. Liu, J.-H. Lu, Y. Ji, J.-L. Zuo and X.-Z. You, *Tetrahedron Lett.*, 2006, **47**, 3431; (*c*) A. Caballero, R. Tormos, A. Espinosa, M. D. Velasco, A. Tárraga, M. A. Miranda and P. Molina, *Org. Lett.*, 2004, **6**, 4599.

9 G. Mezei, C. M. Zaleski and V. L. Pecoraro, *Chem. Rev.*, 2007, **107**, 4933.

10 (*a*) K. Severin, *Chem. Commun.*, 2006, 3859; (*b*) K. Severin, *Coord. Chem. Rev.*, 2003, **145**, 3.

11 (*a*) S. Katsuta, Y. Iwabe, Y. Kato, Y. Kudo and Y. Takeda, *Inorg. Chim. Acta*, 2008, **361**, 103; (*b*) L. Mimassi, C. Cordier, C. Guyard-Duhayon, B. E. Mann and H. Amouri, *Organometallics*, 2007, **26**, 860; (*c*) Z. Grote, H.-D. Wizemann, R. Scopelliti and K. Severin, *Z. Anorg. Allg. Chem.*, 2007, **633**, 858; (*d*) I. Saur and K. Severin, *Chem. Commun.*, 2005, 1471; (*e*) Z. Grote, R. Scopelliti and K. Severin, *J. Am. Chem. Soc.*, 2004, **126**, 16959; (*f*) L. Mimassi, C. Guyard-Duhayon, M. N. Rager and H. Amouri, *Inorg. Chem.*, 2004, **43**, 6644; (*g*) Z. Grote, M.-L. Lehaire, R. Scopelliti and K. Severin, *J. Am. Chem. Soc.*, 2003, **125**, 13638; (*h*) M.-L. Lehaire, A. Schulz, R. Scopelliti and K. Severin, *Inorg. Chem.*, 2003, **42**, 3576; (*i*) M.-L. Lehaire, R. Scopelliti, H. Piotrowski and K. Severin, *Angew. Chem., Int. Ed.*, 2002, **41**, 1419; (*j*) M.-L. Lehaire, R. Scopelliti and K. Severin, *Inorg. Chem.*, 2002, **41**, 5466; (*k*) H. Piotrowski and K. Severin, *Proc. Natl. Acad. Sci.*, 2002, **99**, 4997; (*l*) H. Piotrowski, G. Hilt, A. Schulz, P. Mayer, K. Polborn and K. Severin, *Chem.–Eur. J.*, 2001, **7**, 3196; (*m*) H. Piotrowski, K. Polborn, G. Hilt and K. Severin, *J. Am. Chem. Soc.*, 2001, **123**, 2699.

12 P. W. Chow, A. M. Duffield and P. R. Jefferie, *Aus. J. Chem.*, 1966, **19**, 483.

13 M. Black, J. I. G. Cadogan, H. McNab, A. D. MacPherson, V. P. Roddam, C. Smith and H. R. Swenson, *J. Chem. Soc., Perkin Trans. 1*, 1997, 2483.

14 C. Scolaro, T. J. Geldbach, S. Rochat, A. Dorcier, C. Gossens, A. Bergamo, M. Cocchietto, I. Tavernelli, G. Sava, U. Röthlisberger and P. J. Dyson, *Organometallics*, 2006, **25**, 756.
15 Depending on the nature of the (π-ligand)M fragment, 12-metallacrown-3 complexes may display very slow Li^+ binding kinetics. See for example ref. 11c and 11h.
16 Due to steric reasons, the binding site of a 12-metallacrown-3 complex can only accommodate one cation.
17 M. J. Hynes, *J. Chem. Soc. Dalton Trans.*, 1993, 311.
18 *Geigy Scientific Tables* (Ed.: C. Lentner,), Ciba-Geigy, Basel, 8th edn, 1984, Vol. 3, p. 65.
19 A. J. Alpert and A. K. Shukla, *ABRF 2003: Translating biology using proteomics and functional genomics*, Poster n° P111-W, Denver, 2003.
20 M. A. Bennett, T.-N. Huang, T. W. Matheson and A. K. Smith, *Inorg. Synth.*, 1982, **21**, 74.
21 C. Olivier, E. Solari, R. Scopelliti and K. Severin, *Inorg. Chem.*, 2008, **47**, 4454.