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Fluorescent PET chemosensors for lithium

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Abstract—The synthesis of the chiral diaza-9-crown-3 derivatives **1** (*S*,*S*) and **2** (*R*,*R*) is described. These sensors were designed as luminescent chemosensors for lithium where the fluorescence emission from the naphthalene moieties was 'switched on' upon Li^+ recognition by the crown ether moiety in organic solvents, showing excellent selectivity over other group I and group II cations. Even though the recognition of Li^+ was not achieved in water (pH 7.4) or aqueous alcohol solution, the fluorescence (which was switched on at pH 7.4) was substantially modulated by spherical anions, where the fluorescence emission was quenched in the presence of Br^- and I^- but less by Cl^- and not by acetate. This indicates that the emission was quenched by heavy-atom affect. The recognition of Li^+ was also investigated by ^1H NMR in CD_3CN and by observing the changes in the circular dicromism spectra. For the former, the resonances for the crown ether moiety and α-methyl protons adjacent to the ring were sifted upfield and broadened, whereas for **1** the intensity of the CD signal for the π–π transition was substantially modulated upon Li^+ recognition.

1. Introduction

The design and synthesis of chemosensors for ions and neutral molecules has been an area of immeasurable study in recent years.[1] Chemical sensors have non-invasive and non-destructive properties, which has made them an important diagnostic tool in medicine and industry.[2] Many are based on the use of the 'fluorophore–spacer–receptor' model, where the fluorescence emission at the fluorophore site is modulated by ion or molecular recognition at the receptor site, which usually results in the suppression of photo-induced electron transfer (PET) quenching, operating between the two moieties.[3] Although PET sensors have been developed for a wide range of analytes such as neutral molecule, zwitterions, cations, Na^+, K^+, Ca^{2+}, Mg^{2+}, Cd^{2+} and Zn^{2+} anions such as acetates, halides, phosphates and biologically active bis-carboxylates and pyrophosphate,[5] then to the best of our knowledge, no Li^+ selective PET sensors have been reported in the literature prior to our investigation.[6,7] Li^+ is unusual as it is one of the smallest and lightest solid elements and has important clinical, pharmacological and biochemical properties.[8] Its beauty lies in its simplicity, activating brain cells to regulate abnormal mood cycles for the treatment of mentally ill patients, such as manic-depressives.[8,9] New uses of Li^+ include the treatment of skin diseases (such as dermatitis) and autoimmune and immunological diseases. It is usually administered orally as Li_2CO_3, at a total dose up to 30 mmol (approximately 2 g) per day for the treatment of mental illness. The therapeutic index for Li^+ is narrow and should lie between 0.4 and 0.8 mM in serum, 12 h after the dose has been administered. If the serum concentration is too high (around 1.5 mM), shaking, dizziness, drowsiness, vomiting and diarrhoea are experienced by the patient. These syndromes indicate serious toxicity effects and are usually seen 4 h after the drug has been administered. Long-term side effects include dermatological disorders, weight gain and some problems with kidney and thyroid functions.[8] In medicine, Li^+ determination in blood samples was traditionally carried out using atomic absorption spectroscopy and flame emission spectroscopy. The impracticalities of measuring serum samples on site using these methods led to the development of ion-selective electrodes, and ionophores which are more practical.[10–12] They work by measuring the activity in Li^+ solutions and are active within the clinical range (0.4–0.8 mM serum). This technique provides immediate feedback without long delays, high operation, instrumentation costs and bulkiness of instruments. Determination of Li^+ levels in serum must also be monitored in the presence of 140 mM sodium, 4.3 mM potassium and 1.26 mM calcium. Furthermore, Li^+ is important from a synthetic as well as an industrial point of view because of its use in batteries, but currently there is real momentum away from cadmium-based batteries towards the use of lithium in mobile phones. Lithium is thus also a potential environmental hazard in the future.

We have developed both fluorescent and lanthanide luminescent devices as luminescent switches,[13] sensors[14] and logic gate mimics.[15] We have focused our efforts particularly on the development of chemosensors for

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biologically relevant targets such as ions, small molecules and nucleic acids.[16] Here we describe our efforts towards developing novel PET sensors for Li^+.[6] We demonstrate that by using structurally simple motifs, whereby a small crown ether and two amide moieties are combined in a receptor, highly selective Li^+ chemosensing can be achieved in organic solvents such as CH_3CN. Although we were unable to achieve such sensing in an aqueous environment, this work achieved the objectives outlined, i.e. the selective sensing of Li^+ by employing PET chemosensing.

2. Results and discussion

2.1. Synthesis

The lithium-selective PET sensors **1** and **2** were designed using the PET principle by combining a small crown ether moiety with two naphthalene fluorophores, giving rise to a 'fluorophore–spacer–receptor–spacer–fluorophore'[3–7] model as developed by de Silva (Fig. 1). With the aim of maximizing the Li^+ selectivity over other competitive cations, the rather small diaza-9-crown-3 (1-oxo-4,7-diazacyclononane)[17] crown ether was chosen, which was functionalized with two amide pendent arms which could participate directly in the coordination of the ion. Such modifications have previously been demonstrated for competitive Li^+ recognition and transport across a liquid membrane.[18] We proposed that this design would ensure that Li^+ would not be complexed directly within the macrocycle cavity, but just below it, where Li^+ recognition would be assisted by the amide arms, i.e. by direct coordination to the oxygens of the carboxyamides. This would ensure enhanced Li^+ selectivity over Na^+. To achieve this additional chelating effect we selected the chiral amides **3** and **4**, synthesized from the chiral *S*- and *R*-1-(naphthyl)ethylamine, respectively.

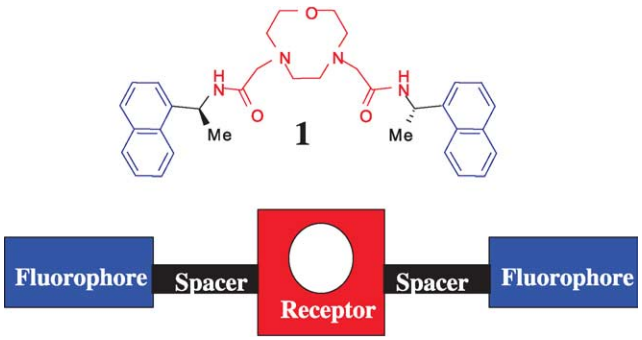


Figure 1. Diagram illustrating the 'fluorophore–spacer–receptor–spacer–fluorophore' model and the corresponding structure of **1**.

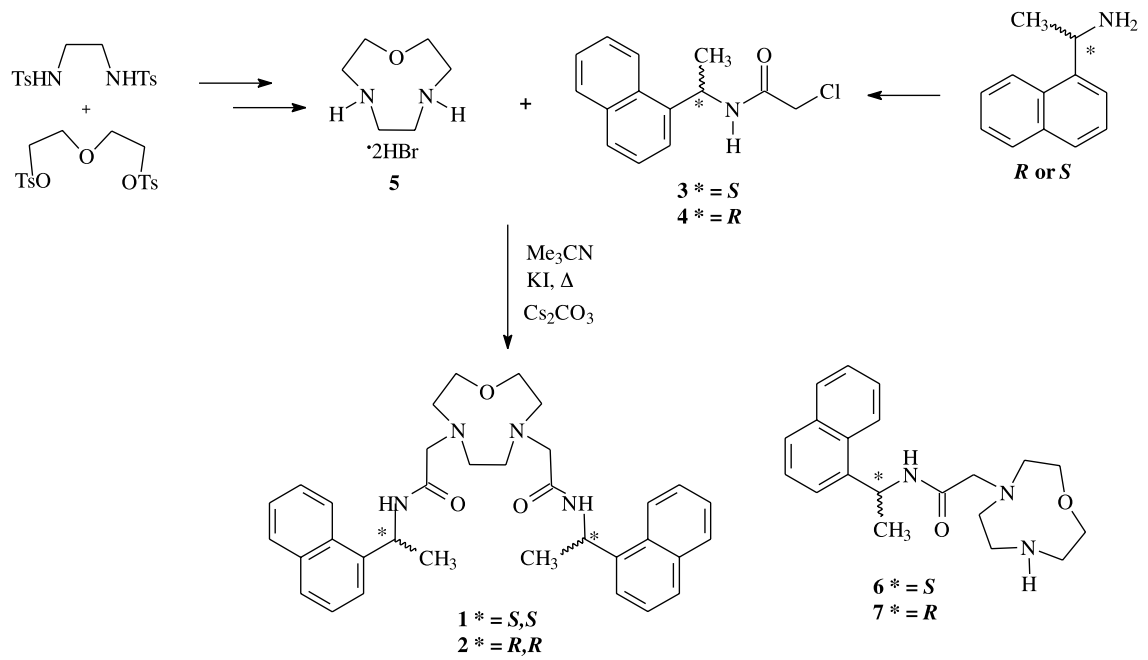
The synthesis of **1** and **2** is shown in Scheme 1.[†] The synthesis of both was achieved in two sequences. The first sequence was the synthesis of the diaza-9-crown-3-ether receptor **5**, which is a known compound, using conventional Williamson ether synthesis, from *N*,*N*-ditosyl diaminoethane and diethylene glycol ditosylate, which gave the *N*,*N*′-ditosyl-1,4-diaza-9-crown ether. This product was detosylated by refluxing in 48% HBr–AcOH solution for four days, yielding the HBr salts **5** in 87% yield. The α-chloroamide arms **3** and **4** were made in a single step by peptide coupling of chloroacetic acid with either *S*- or *R*-1-[1-naphtyl]ethylamine using EDCI and HOBt as reactants, giving approximately 87% yield for each. This compound and other related chiral derivatives have previously been reported by Parker et al. using a different synthesis.[19] The final step in the above synthesis involved the coupling of either **3** or **4** to the crown ether **5** in MeCN at 80 °C, giving **1** and **2**, initially in good yields of ca. 70% as a crude material, but in 33 and 30% yield respectively, after final workup. An increase in the reaction times did not improve the original yields nor prevent the one-armed systems **6** and **7** being formed in a small amount, ca. 10% (as crude material). No advantages were achieved by refluxing the reaction in DMF, in fact this resulted in lower yields of (~45%) with increased yield of the one-armed side product **6**. Both the *R*,*R*- and *S*,*S*- sensors had to be purified via alumina chromatography using DCM and 0⟶1% MeOH as an eluent. When flash silica chromatography was used the sensor became protonated. Consequently, it was necessary to treat the resulting fractions with 1 M NaOH to yield the free sensor. The final products were isolated as semi solids, and titurated from ether to give both products as powders. Both sensors were fully characterized by, ^1H and ^{13}C NMR, ES-MS, IR and CHN or accurate mass analysis using HRMS.

The ^1H NMR spectrum of either the *S*,*S*-isomer **1** or the *R*,*R*-isomer of **2** showed that the axial and equatorial positions of the aza-crown ring were not magnetically equivalent, with several multiplets occurring around 2.3 and 3.6 ppm. The diagnostic peak is a multiplet at 5.38 ppm representing the chiral CH group of the spacer. It was expected that this resonance would be a quartet, so its appearance as a multiplet suggests that the environments around this proton are non-equivalent. The electro-spray mass spectrum of **1** and **2** showed a single peak at 552.5 *m*/*z* for the molecular ion.

2.2. Ground and excited state evaluation of 1 and 2

The photophysical properties of **1** and **2** were evaluated in water, MeOH, in 50:50 MeOH/CH_3CN mixture and in CH_3CN solutions in the presence of several metal cations from groups IA and IIA. In water, using 160 mM NaCl to maintain constant ionic strength, the pK_a of both sensors was determined by observing the changes in the fluorescence emission spectrum at λ_F 337 nm, when excited at 280 nm. However, the changes in the absorption spectrum, which displayed typical naphthalene absorption bands with fine structure at 271, 281 and 293 nm, over the same pH range were only minor and not significant enough for accurate binding constant determination. This is due to the covalent spacers that separate the two naphthalene fluorophores from the crown ether receptor and thus minimize any π–n orbital interactions between the two moieties. In alkaline solution the fluorescence of the naphthalene unit of **1** was almost fully 'switched off', signifying the quenching

[†] In our earlier communication (Ref. 6), the naphthalene part of **1**, was shown to be connected through position two on the aromatic ring. However, the description of the synthesis in the text was correct. We apologize for this mistake.



Scheme 1. Synthesis of chemosensors **1** and **2** from the α-chloroamides **3**, **4** and the diaza-9-crown-3 (1-oxo-4,7-diazacyclononane) crown ether **5**. The mono products **6** and **7** were also observed in about 84–10% yields.

of the naphthalene excited state by efficient electron transfer from the amino moieties of the crown ether receptor to the naphthalene-excited state. This is typical PET quenching, as on protonation of the amino moiety, the oxidation potential of the receptor was increased, removing the thermodynamic pathway for the excited state quenching.[1,4] The corresponding changes in the fluorescence emission of **1** at 337 nm as a function of pH are shown in Figure 2. As the spacer between the amino moiety and the naphthalene moiety is made of four carbon bond lengths, which is significantly

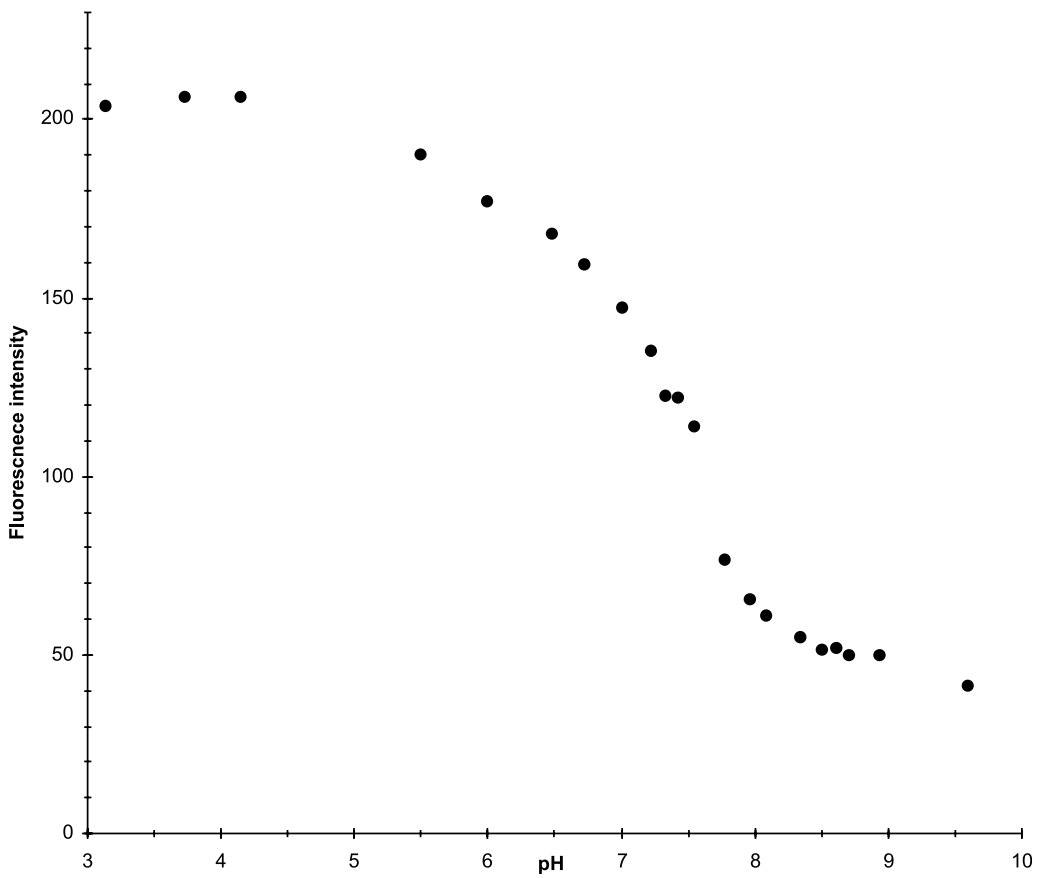


Figure 2. The changes in the fluorescence emission spectra of **1** at 337 nm upon pH titration in water. Excitation at 280 nm.

larger then the classical one or two carbon spacers usually used for PET sensors,[1,4] the efficiency of the quenching process is not ideal as the PET is distance dependent (function of $1/r^6$). Hence the emission is not fully switched off in alkaline solution. However, upon protonation of the amino receptor the emission was 'switched on' with a large order of magnitude enhancement in fluorescence, without any other substantial spectral shifts. This process was fully reversible, as upon addition of base to **1** in acidic solution, the emission was switched off. The changes in Figure 2 occur over ca. two pH units indicating that the protonation is due to 1:1 binding and a simple equilibrium.[4] We were unable to determine the second protonation of the amino crown ether, which suggests that it is either extremely low, due to repulsive effects by the first protonation, or that the small crown ether is operating like a pseudo proton sponge, where the proton is shared between the two amino moieties. It could also be the case that the second protonation simply does not affect the fluorescent properties of the molecule. We were however, unable to prove this explanation. From the above change, a pK_a of 7.2±0.1 was determined (Fig. 1). Similarly, upon carrying out a pH titration of **2**, the same pK_a value of 7.2 was determined. From these changes it is clear that pK_a's are too high for determination of Li^+ in water at pH 7.4, as the partial protonation of the amino crown ether would prevent the Li^+ sensing due to charge repulsion. This was indeed found to be the case as upon titration of **1** or **2** at this pH using LiCl, NaCl, KCl and $CaCl_2$ salts. The emission of these two sensors was not enhanced, which would have signified the suppression of the PET process. However, substantial quenching was observed. This indicated that the PET mechanism was not active, or at least not observed, due to other active quenching mechanisms. To investigate this further we carried out a series of titrations at pH 7.4 and 8.5, where the receptor would be expected to be in its 'not fully protonated' or 'free-form' respectively, using a range of lithium salts.

The changes in the fluorescence emission for LiBr, LiI and LiOAc are shown in Figure 3, for the changes in **1** at pH 7.4 (0.1 M buffer and 0.1 M ionic strength). No major changes were observed when LiOAc, NaOAc or KOAc were used. However, when using the above LiCl, LiBr or LiI solutions, substantial quenching was observed. As acetate did not give rise to any significant quenching, we propose that the quenching by Cl^-, Br^- and I^- was most likely due to a heavy-atom effect by these halide counter ion, since it was largely noticeable for spherical anions, in the concentration range of 04–2.5 mM. This is strengthened by the fact that other anions, such as perchlorate ClO_4- or sulfate, did not give rise to such quenching. To investigate this further we also carried out anion titrations using the α-chloronaphthalene ligand **4**. They strongly suggested that the anion was quenching fluorescence due to the heavy atom effect even in the absence of the crown ether, suggesting that the anion was not binding to the receptor. However, these changes were somewhat smaller than those seen for **1**, suggesting that some cooperative effect by the crown ether moiety. When the fluorescence emission was evaluated at pH 8.5, using the acetate salt of Li^+, Na^+ and K^+, no fluorescence enhancement were observed, signifying that the receptor was unable to extract the Li^+ from the highly solvated aqueous environment. Because of this, the Li^+ recognition was evaluated in less polar protic and aprotic solvents.

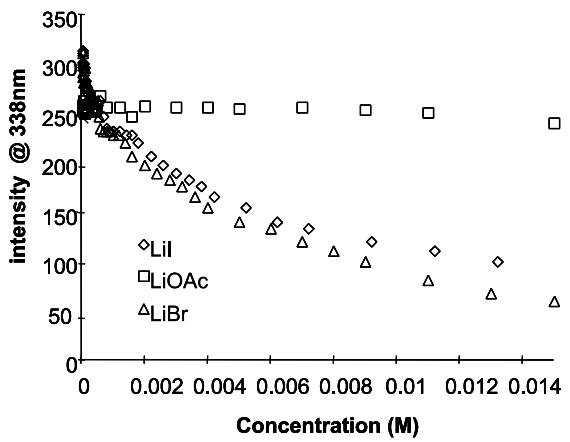


Figure 3. The changes in the fluorescence emission spectra of **1** at 338 nm in pH 7.4 titration in water.

The recognition of Li^+ using **1** was evaluated in MeOH, MeCN and in a mixture of both. In MeOH there were minor changes in fluorescence when titrated with Li^+ acetate. Again, no significant changes were seen in 50:50 MeCN/MeOH solution. However, in 80:20 MeCN/MeOH a slight increase in fluorescence was observed but this was insignificant, giving mere 0.8-fold enhancement in fluorescence. The above titrations were repeated in 100% MeCN using both lithium acetate and lithium perchlorate salts. Unlike previously observed, the fluorescent emission was greatly enhanced upon titration with Li^+, leading to a 9-fold increase in fluorescent intensity, as shown in Figure 4. The fluorescence quantum yield, Φ_F at high Li^+ concentration was measured to be 0.11, whereas in the absences of Li^+ it was 0.022. This is an order of magnitude fluorescent enhancement upon ion recognition, which is quite significant given the fact that the crown ether receptor (i.e. the electron donor) is separated form the naphthalene fluorophore by a four-carbon spacer. This would be expected to reduce the efficiency of the PET quenching, which is highly distance dependent, as previously discussed. Moreover, no significant spectral changes were observed in the fluorescence spectra when titrated with any other group I and II cations, as is evident from Figure 5, which shows the changes in the fluorescence emission of **1** at 337 nm as the function of the concentration of these ions. From Figures 4 and 5, it is clear that the fluorescence emission of **2** is highly dependent on the Li^+ concentration, which upon recognition by the receptor increases the oxidation potential of the receptor in a similar manner to that observed for the pH titration earlier. Furthermore, no other significant spectral changes were seen in the emission spectra (Fig. 4), e.g. no changes were seen in the λ_{max}, and no excimer emission was observed at longer wavelength. One can thus conclude that **1** is behaving like an ideal PET sensor.[1,4]

From the changes in the 337 nm wavelength we were able to determine the binding constant log β as 5.4 (±01) for **1** using the equation:

$$\log \beta = \log[(I_{max} - I)/(I - I_{min})] - \log[Li^+]$$

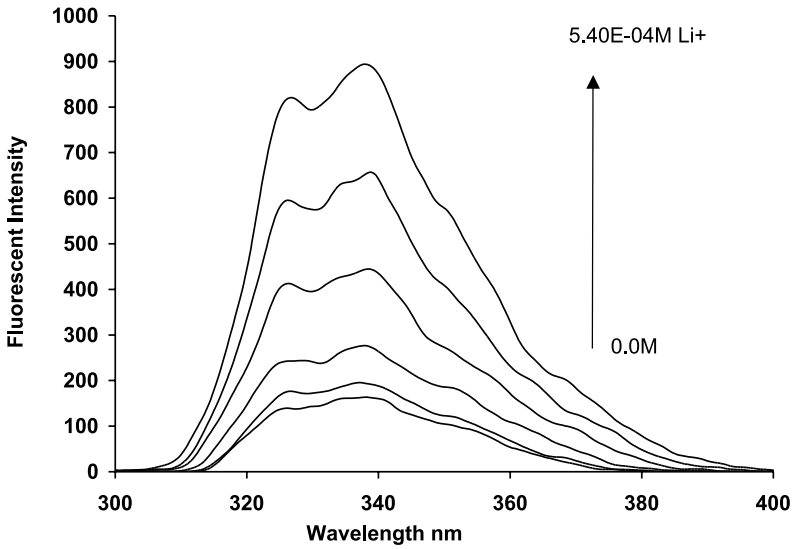


Figure 4. The changes in the fluorescence emission spectra of **1** upon titration with Li^+ in CH_3CN. Excitation at 280 nm.

where I is the fluorescent intensity at 337 nm, I_{max} is the maximum intensity observed at 337 nm and I_{min} is the minimum intensity observed at 337 nm. As can be seen from Figure 5, the selectivity of **1** towards Li^+ is very good, as only at very high concentration of other competitive group I and group II ions is the emission modulated. It is thus clear that **1** is highly selective and sensitive to Li^+ recognition in a non-aqueous environment. To the best of our knowledge, this is the first example of such a highly selective and sensitive PET sensor for Li^+. When these titrations were repeated for **2**, similar results were observed, as can be seen in Figure 6. Again, the fluorescence emission spectra were switched on, with no other major spectral changes. The quantum yield for the free sensor **2**, and with Li^+ was determined to be 0.11 and 0.22, respectively, mirroring that of **1** earlier. Furthermore, these changes occurred over two logarithmic units, indicating that the recognition was due to 1:1 binding and a simple equilibrium.

We also investigated the changes in the ^1H NMR of **1** upon titration with Li^+ in CD_3CN. We foresaw that the largest changes would be expected to occur for the resonances of the crown ether moiety and the α-position of the pendent arm. Indeed this was found to be the case, as upon titration of **1** with Li^+, the largest changes were encountered for the ring protons, causing minor upfield shift and broadening of these resonances. It was also noticeable that the aromatic resonances became broadened, which might suggest some minor interactions between the two naphthalene moieties upon Li^+ recognition or perhaps some contribution from cation−π type interactions.

To investigate the Li^+ recognition further we observed the changes in the circlar dicromism (CD) spectrum as a function of Li^+ concentration. Since the two pendent arms are chiral they could cause an enhancement in the CD spectra, where Li^+ recognition gives rise to significant changes in the CD intensity. We investigated these features

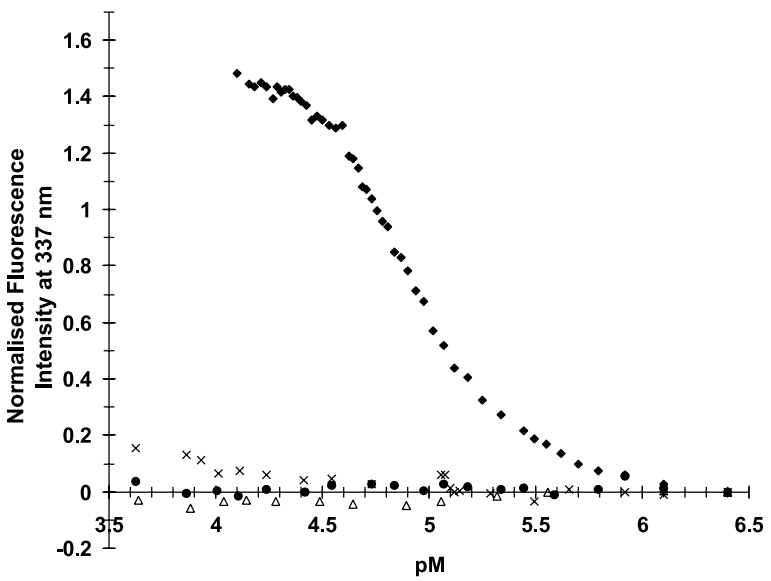


Figure 5. Titration profiles for **1** using perchlorate salts in CH_3CN. ◆=Li^+, ×=Ca^{2+}, ●=K^+, △=Na^+. Excitation at 280 nm.

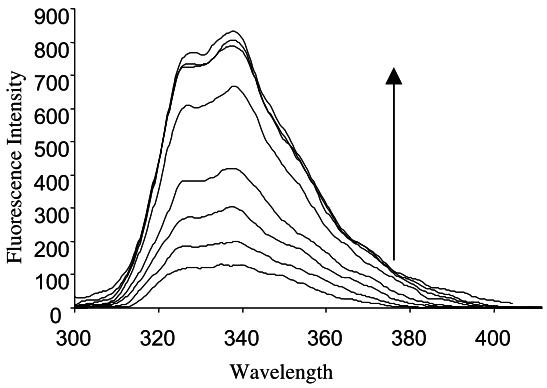


Figure 6. The changes in the fluorescence emission spectra of **2** (being switched on) upon titration with Li^+ in CH_3CN. Excitation at 280 nm.

for both **1** and **2**. The CD analysis of **1** and **2**, gave opposite spectra, where **1** had a negative absorption band and **2**, positive absorption for the $\pi \rightarrow \pi^*$ transition. The results for **1** are shown in Figure 7. Here it can be seen that **1** gave rise to three transitions in the CD spectra, centered at 230, 250 and 280 nm, respectively. Of these the 225 nm and the 280 nm transitions were substantially affected by the Li^+ recognition. Hence, the long 280 nm transition doubled in intensity and the short wavelength transition reduced in intensity by half. These changes were not investigated any further, but they do strongly suggest that upon Li^+ sensing, which is clearly observed by the fluorescence enhancements discussed above, conformational changes occur in **1**. Similar, but not identical changes were observed for **2**, where enhancements were seen in the 280 nm absorption band, indicating that the Li^+ complexation might be give rise to slightly different conformation within the molecule. Even though we are unable to support this further, then these results indicate that the amide functions are participating in the Li^+ coordination. We deduce this from the fact that the two chromophores in **1** are separated at a relatively long distance from the crown ether moiety to be actively affected if the ion was only coordinating to the nitrogens and the oxygens of the ring. Consequently, if the lithium ion was coordinating to the two oxygen of the carboxylic amides such a conformational effect would more likely to be observed strongly by the chromophores. However, we were unable to support this theory by X-crystallographic evidence, as various attempts to obtain crystals of **1** or **2** suitable for X-ray diffraction, or their corresponding Li^+ complexes failed.

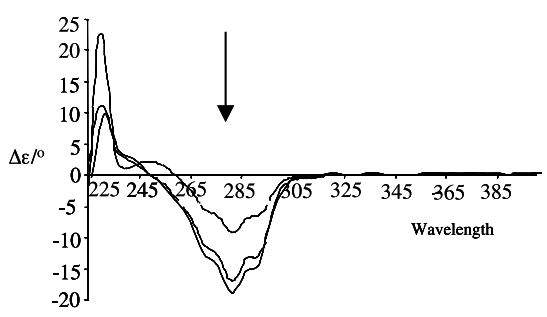


Figure 7. The changes in the CD spectrum of **1** upon titration with 0, 1 and 5 equiv. of Li^+ in CD_3CN.

3. Conclusion

In summary, we have synthesized two amide based crown ether ligands **1** and **2** for the detection of Li^+. Both were synthesized in two step synthesis form the corresponding *S* or *R* 1-(-1-naphtyl)ethylamine, and the 1,4-diaza-9-crown-3-ether that was made in short step synthesis. The ^1H NMR of the resulting sensors showed that the axial and the equatorial crown ether protons were magnetically inequivalent. The ability of these two sensors to detect Li^+ was investigated in various solutions. In water, the pK_a of 7.2 was determined for both from the changes in the fluorescence emission changes upon pH titration. Unfortunately, we were unable to show Li^+ detection in aqueous solution. However, in CH_3CN the fluorescence emissions were significantly modulated upon Li^+ recognition, where the emission was switched on with almost an order of magnitude enhancement in the fluorescence. In aqueous solutions, the emission was quenched, most likely due to vibrational heavy atom affect quenching by spherical anions such as Cl^-, Br^- and I^-. However, this quenching was only very minor using acetate or perchloride salts. The recognition was also evident from the ^1H NMR and the CD spectra which indicated that ion recognition was most likely involving the crown ether nitrogen and the oxygen moieties as well as the two oxygens of the carboxylic amides. We are currently initiating a research programme into the developments of PET sensors for Li^+ for use in aqueous solution.

4. Experimental

4.1. General

Starting materials were obtained from Sigma Aldrich, Strem Chemicals and Fluka. Solvents were used at GPR grade unless otherwise stated. Infrared spectra were recorded on a Mattson Genesis II FTIR spectrophotometer equipped with a Gateway 2000 4DX2-66 workstation. Oils were analyzed using NaCl plates, solid samples were dispersed in KBr and recorded as clear pressed discs. ^1H NMR spectra were recorded at 400 MHz using a Bruker Spectrospin DPX-400 instrument. Tetramethylsilane (TMS) was used as an internal reference standard, with chemical shifts expressed in parts per million (ppm or δ) downfield from the standard. ^{13}C NMR were recorded at 100 MHz using a Bruker Spectrospin DPX-400 instrument. Mass spectroscopy was carried out using HPLC grade solvents. Mass spectra were determined by detection using Electrospray on a Micromass LCT spectrometer, using a Shimadzu HPLC or Water's 9360 to pump solvent. The whole system was controlled by MassLynx 3.5 on a Compaq Deskpro workstation.

4.2. Modified peptide synthesis of 2-chloro-*N*-(2-naphthyl)ethylethanamide 3 and 4

1-(-1-Naphtyl)ethylamine (1 g, 0.63 mmol) and HOBt (0.8 g, 0.63 mmol), chloroacetic acid (0.6 g, 0.63 mmol) were stirred in DCM (25 mL) at −10 °C for 20 min under inert atmosphere. EDCl (1.2 g, 0.63 mmol) was then added and the reaction stirred overnight at room temperature under inert atmosphere. The solution was washed with 1 M

$NaHCO_3$ and brine. The organic layer was collected, dried over $MgSO_4$, filtered and evaporated to give a white solid.

4.2.1. 2-Chloro-*N*-[(*S*)-1-naphthyl]ethylethanamide 3. 0.58 g, 86.9% yield. Mp 140 °C; calcd for $C_{14}H_{14}NOCl$: C 67.88; H, 5.70; N, 5.65. Found C 67.63, H 5.77, N 5.86; ^1H NMR (400 MHz, CD_3CN): δ 8.16 (1H, d, Ar-H, J=8.5 Hz), 7.96 (1H, d, Ar-H, J=7.5 Hz), 7.87 (1H, d, Ar-H, J=7.5 Hz), 7.59 (1H, m, Ar-H), 7.25 (1H, sb, Ar-H,), 5.83 (1H, m, J=7.0 Hz), 4.04 (2H, s), 1.63 (3H, d, J=7.0 Hz); ^{13}C NMR (100 MHz, CD_3CN): 164.8, 138.8, 133.4, 130.2, 128.31, 127.3, 125.0, 125.3, 125.0, 122.6, 122.1, 42.2, 20.1; ES-MS: m/z 247.9 (M+); ν/cm^{-1} (KBr) 3295 (N–H), 1648 (C═O), 1541 (C═C), 780 (C–Cl).

4.2.2. 2-Chloro-*N*-[(*R*)-1-naphthyl]ethylethanamide 4. 0.60 g, 87.3% yield. Mp 140 °C; calcd for $C_{14}H_{14}NOCl$: C, 67.88;H, 5.70; N, 14.31. Found: C; 67.67;H, 5.81; N, 5.83; ^1H NMR (400 MHz, $CDCl_3$): δ 8.11 (1H, d, J=8.5 Hz), 7.92 (1H, d, J=8.6 Hz), 7.86 (1H, d, J=7.6 Hz), 7.56 (4H, m), 6.81 (1H, s, N–*H*), 4.15 (2H, q), 1.74 (3H, d, J=7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): 164.4 ($_qC$), 137.0 ($_qC$), 133.5 ($_qC$), 130.5 ($_qC$), 128.5 (Ar-H), 128.2 (Ar-H), 126.2 (Ar-H), 125.5 (Ar-H), 124.8 (Ar-H), 122.6 (Ar-H), 122.1 (Ar-H), 44.8 (C H_2), 42.2 (C H_2), 20.4 (C H_3); ES-MS: m/z 247.9 (M^+); ν/cm^{-1} (KBr) 3290 (N–H), 1652 (C═O), 1540 (C═C), 782 (C–Cl).

4.3. General synthesis of the sensors 1 and 2

1,4-Diaza-9-crown-3-ether (1 equiv.), Cs_2CO_3 (6 equiv.) and KI (0.1 equiv.) were stirred in MeCN under inert atmosphere. The appropriate chromophore (2.1 equiv.) in MeCN was added via a pressure equalized dropping funnel. The mixture was left to reflux at 80 °C overnight under inert atmosphere. The reaction was filtered and the solvent evaporated. The yellow residue was dissolved in $CHCl_3$ and washed with 10% K_2CO_3 (3×20 mL). The organic layer was collected, dried over $MgSO_4$, filtered and evaporated to give a white solid. After purification by alumina chromatography with DCM: 0→5% MeOH, the product was washed and recrystallized from diethyl ether to yield a white solid.

4.3.1. Compound 1 (*S*,*S*). 0.22 g, 32.7% yield. Mp 107 °C; calcd for $C_{34}H_{40}N_4O_3$: C 73.88; H, 7.29; N, 10.14. Found C 73.39, H 7.14, N 9.66; ^1H NMR (400 MHz, CD_3CN): δ 8.12 (2H, d, Ar-H, J=8.5 Hz), 7.91 (2H, d, Ar-H, J=7.5 Hz), 7.81 (2H, d, Ar-H, J=8.0 Hz), 7.53 (8H, m, J=8.0 Hz), 5.80 (2H, m, J=7.0 Hz), 3.24 (4H, m), 3.05 (4H, s), 2.57 (8H, m), 1.68 (6H, d, J=7.0 Hz); ^{13}C NMR (100 MHz, CD_3CN): 139.2 133.4, 130.5, 128.2, 127.2, 125.8, 125.3, 124.9, 122.8, 122.2, 71.7, 60.2, 56.3, 55.1, 43.7, 19.8; ES-MS: m/z 553.5 (M^+), $\Delta\varepsilon/°$=+25 (at OD=0.1, 225 nm); λ_{max}/nm (CH_3CN) 260.8 (ε/dm^3 mol^{-1} cm^{-1} 10427) 281.6 (11801) 293.2 (6801.5); ν/cm^{-1} (KBr) 3287 (N–H); 2525, 2554 (ar C═H); 1650 (C═O amide); 1602, 1511 (ar C═C); 1450 (C–N amide); 1357 (C–N crown ether); 1126 (C–O–C crown ether).

4.3.2. Compound 2 (*R*,*R*). 0.18 g, 30.1% yield. Mp 107 °C. Accurate mass: calcd for $C_{34}H_{40}N_4O_3$: found $C_{34}H_{41}N_4O_3$; ^1H NMR (400 MHz, $CDCl_3$): δ 8.02 (2 ArH, d, J=8.4 Hz), 7.67 (5 ArH, dd, J=8.0 Hz), 7.53 (4H, d, Ar-H, J=8 Hz), 7.03 (2H, d, N–H), 5.59 (2H, d, C H(CH_3)NH), 3.22 (4H, m), 2.77 (4H, q), 2.40 (8H, m), 1.67 (6H, d, J=6.5 Hz, CH(CH_3)NH); ^{13}C NMR (100 MHz, CD_3CN): 169.8 (C═O), 137.7 (Ar-H), 133.4 (Ar-H), 130.9 (Ar-H), 128.3 (Ar-H), 128.0 (Ar-H), 126.1 (Ar-H), 125.5 (Ar-H), 124.7 (Ar-H), 123.1 (Ar-H), 122.3 (Ar-H), 71.6, 60.2, 56.0, 54.8, 43.5, 19.7 (CH(*C* H_3); ES-MS: m/z 552.9 (M^+); $\Delta\varepsilon/°$=−25 (at OD=0.1, 225 nm) λ_{max}/nm (CH_3CN) 260.7 (ε/dm^3 mol^{-1} cm^{-1} 10399) 281.6 (11750) 293.2 (6889.5); ν/cm^{-1} (KBr) 3272 (N–H); 2499, 2554 (ar C═H); 1656 (C═O amide); 1607, 1528 (ar C═C); 1468 (C–N amide); 1357 (C–N crown ether); 1132 (C–O–C crown ether).

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