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A novel fluorescent photoinduced electron transfer (PET) sensor for lithium

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Abstract—The chiral diaza-9-crown-3 derivative **1** is the first example of a fluorescent PET chemosensor designed specifically for detecting lithium ions (Li^+). **1** shows 'off–on' switching of fluorescence when treated with various lithium salts in organic solvents such as CH_3CN, with binding constant $\log \beta = 5.4$, at the same time as discriminating against a variety of group I and II metal ions. In water, the emission is quenched by anions due to the heavy-atom effect.

Sensing and recognition of physiologically important species using luminescent chemosensors is of current interest in biology and medicine, since it affords a non-destructive way of obtaining real time, on-line information. Such sensing can be observed via changes in emission intensities (quantum yield), wavelength, lifetimes and chirality.[1] A particularly attractive approach has been the use of photoinduced electron transfer (PET) sensors.[2] These are designed on the *fluorophore-spacer-receptor* archetype where an ionic or molecular input at the receptor site can modulate the emission such as lifetime and quantum yield of fluorescence (Θ_F), causing the luminescent emission to be switched either '*off–on*' or '*on–off*' upon sensing.[3] We have been interested in this field and have recently developed PET sensors for anions, such as acetate and phosphate,[4] and for cations, e.g. for the selective detection of sodium in blood or serum.[5] We have also developed several lanthanide-based luminescent sensors, switches and logic gate mimics using cations and neutral molecules.[6] Even though many elegant PET sensors have been developed for cations over the years, there are, to the best of our knowledge, no reports on the development of fluorescent PET designed chemosensors for selective sensing of lithium.[7] Selective detection of Li^+ in biological samples is of great importance since Li^+ is used for the treatment of manic-depressive psychosis and other related illnesses.[8] Currently, the determination of Li^+ in serum (at the therapeutic level in blood in the range of 0.5–1.5 mM, which also contains 140 mM concentration of Na^+) is carried out by employing Li^+ selective electrodes.[9] Li^+ is also important from an industrial point of view because of its use in batteries. Because of its small size and high charge density, selective detection of Li^+ without interference from Na^+ is a challenging task. de Silva et al. recently reported the synthesis of Na^+ selective fluorescent PET sensors for intracellular Na^+ detection ($\log \beta = 3.1$).[10] Whilst these sensors showed no significant response to other physiologically active cations, the sensor displayed an order of magnitude fluorescence enhancement in the presence of a high concentration of Li^+ ($\log \beta = 2.2$). However, selective Li^+ detection was not possible. We have also seen similar effects in our attempts to develop Na^+ selective sensors for blood Na^+.[5] A few other reports on the use of internal charge transfer (ICT) based sensing of Li^+ have also recently been reported.[8] Inspired by these results, and the fact that no PET selective chemosensors designed for Li^+ detection has to best of our knowledge been reported, we set out to develop the PET Li^+ chemosensor **1**, with the aim of achieving selective Li^+ detection over other alkali and alkali earth metal ions, preferably in the physiological concentration range.

1 was designed as a *fluorophore-spacer-receptor* system.[2,5] The small diaza-9-crown-3 (1-oxo-4,7-diazacyclononane) was chosen as the Li^+ receptor with the aim of reducing Na^+ affinity which is often associated with larger crown ethers such as 12-crown-4. The receptor was further modified using amide-based side arms, to aid higher Na^+ discrimination. Such modification has previously been suggested for competitive Li^+ recognition and transport over a liquid membrane.[11] The receptor (unit **2** in **1**) was connected to a naphthalene fluorophore via the chiral methyl substituted spacer, which was chosen with the aim of introducing steric hindrance to further impose Li^+ selectivity.

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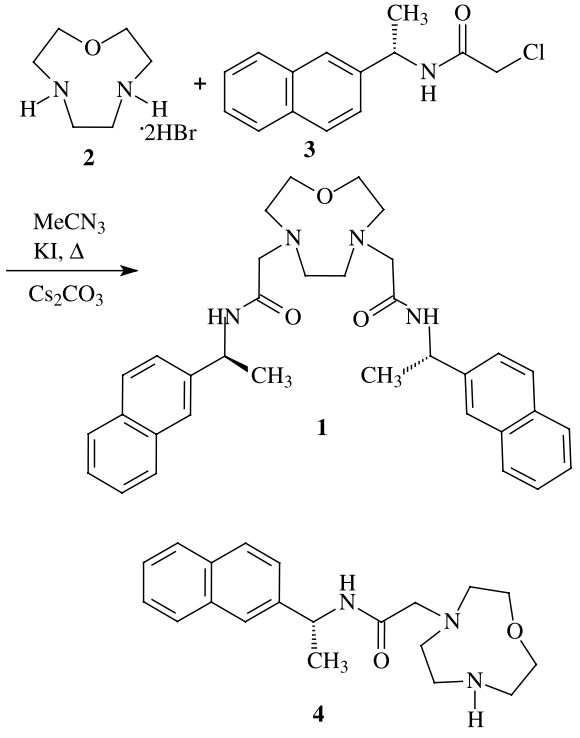
Sensor **1** was synthesized as described in Scheme 1 from 1-oxo-4,7-diazacyclononane·2HBr **2**[12] and the chiral 2-chloro-*N*-[(*S*)-2-naphthyl]ethylethanamide **3** (which was synthesized in one step using a modified literature procedure from *S*-1-(naphthyl)ethylamine and chloroacetic acid using HOBt and EDCl peptide coupling procedures in $CHCl_3$, followed by aqueous $KHCO_3$ work up and recrystallization from ether)[13] in 37% yield using 4.1 equiv. of Cs_2CO_3 and 2.1 equiv. of KI in refluxing freshly distilled CH_3CN under an inert atmosphere at room temperature followed by a column chromatographic workup on neutral flash silica.[14] The use of 100% CH_2Cl_2 eluted the mono substituted crown ether **4**, in >10% yield, but the stepwise addition of MeOH to 95:5 CH_2Cl_2:MeOH as eluent gave the desired product **1** in ca. 70% yield. However, we found it was necessary to triturate **1** with diethyl ether and wash the resulting solution with 1 M NaOH after the column chromatography to ensure the crown ether was not protonated. When the reaction was carried out in dry DMF the overall yields were somewhat smaller, and **4** was formed in ca. 30% yield. The ^{1}H NMR of **1** showed well-dispersed signals for the 14 aromatic protons, a quartet for the stereogenic center and several multiplets in the region of 1.4–3.4 ppm assigned to the aza-crown protons; appearing as several diastereotropic centers as assigned using 2D ^{1}H and ^{1}H–^{13}C NMR. The electro-spray mass spectrum of **1** showed a single peak at 552.5 for the molecular ion.

The photophysical properties of **1** were evaluated in water, MeOH, CH_3CN, and in 50:50 MeOH:CH_3CN solutions in the presence of several metal cations from group IA and IIA. In water, using 160 mM NaCl to maintain constant ionic strength, the pK_a of **1** was determined by the changes in the fluorescence emission spectrum of **1** at λ_F 337 nm, when excited at 280 nm. A pK_a of 7.2±0.1 was determined from these changes (Fig. 1), with the emission reversibly '*switched on*' upon addition of acid to an alkaline solution of **1**, with an order of magnitude enhancement in fluorescence without any other substantial spectral shifts. No changes were observed in the absorption spectra of **1** because the covalent spacer separates the naphthalene fluorophore from the receptor and thus minimizes any π–n orbital interactions. The switching also occurs over ca. two pH units, an indication of 1:1 binding and a simple equilibrium. These fluorescent '*off–on*' changes indicate that the protonation of the amino-crown moiety suppresses any PET process from the amine crown ether to the naphthalene fluorophore.



Scheme 1. The synthesis of Li^+ chemosensor **1**.

The effects of titrating **1** with a series of cations from group IA and IIA was also investigated in water at pH 7.4 (partly protonated) and at pH 8.5 (deprotonated crown ether). As expected, the absorption spectra of **1** were unaffected upon titration using LiCl, NaCl, KCl and $CaCl_2$ salts at pH 7.4. However, substantial quenching, rather than fluorescent enhancement, was observed in the emission spectra upon titration of **1** using these ions. This indicated that the PET mechanism was not active, or at least not observed, due to other active quenching mechanisms. Further investigation showed that the quenching was most likely due to a heavy-atom effect by the halide counter ion, since it was largely noticeable for spherical anions such as Br^- and I^- with 50 and 83% reduction in fluorescence, respectively, in the concentration range of 0–2.5 mM. In the case of Cl^-, less quenching was observed. It is possible that in the case of Cl^-, the excited state was quenched by an electron transfer mechanism. We are currently investigating this. Anions such as $CH_3CO_2^-$ and ClO_4^- showed relatively small quenching (~5%) even at high concentrations (~25 mM). The effect of anion quenching of **1** was further investigated by evaluating the photophysical properties of the α-chloroamide **3** under the same conditions as above. At pH 7.2 and pH 8.5 the fluorescence emission was quenched by these anions, but to a lesser extent than that seen for **1**.

The PET sensing ability of **1** was then investigated in several organic solvents with the aim of incorporating **1**

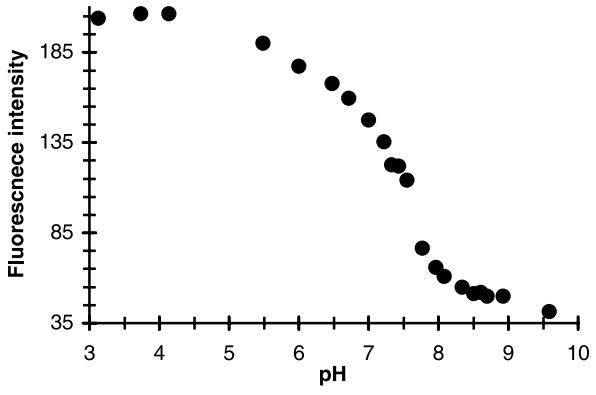


Figure 1. pH-titration profile in 100% water for **1**.

into polar membranes.[15] These measurements showed that **1** was acting as a PET Li^+ chemosensor in non-aqueous solutions. In CH_3CN, a ca. fivefold enhancement in the fluorescence emission intensities were observed upon Li^+ titration using $LiClO_4$ indicating that the electron transfer from the crown ether amines to the fluorophore was substantially reduced upon Li^+ complexation (Fig. 2). No other changes were seen in the emission spectra, e.g. no changes were seen in the λ_{max}, and no excimer emission was observed at longer wavelength. The fluorescence quantum yield, Φ_F for the fully complexed sensor was measured to be 0.11, whereas in the free sensor (no Li^+) it was measured to be 0.022. The sensor was also highly selective towards Li^+ over other physiologically active cations such as Na^+, K^+, Ca^{2+} and Mg^{2+}, which showed much less of an effect, with only 5–10% fluorescence enhancement at high concentrations ($\sim$25 mM). Analyses of the Li^+ selectivity of **1** is shown in Fig. 3, as a ratio of I_F/I_{Fo} as a function of pM ($-\log[M+]$), where I_{Fo} is the fluorescence emission of the free sensor. From these changes, fluorescence emission is switched '*on*' over two pM units between 6 and 4. This is an indication of 1:1 binding and simple equilibrium. The binding constant $\log\beta$ was measured to be 5.4($\pm$0.1) for **1** with lithium, but the changes for other cations were too small to evaluate their binding constant. This indicates that the receptor is extremely sensitive to the presence of Li^+ in the media. Similar results were observed in MeOH, but the binding values and switching factors were different. Fig. 3 shows that the chemosensor is extremely selective for Li^+ with a fluorescence switching factor of ca. 5. This is a somewhat smaller factor than observed for many other PET cation chemosensors[2] and that observed in the pH titration of **1**.[5] We believe that the reason for this is twofold. Firstly, for the pH titration, the amine forms a strong covalent bond with the proton, which increases the oxidation potential of the amine, making ΔG_{ET} unfavorable. We believe that this interaction is relatively much weaker in the case of Li^+. By carrying out a Li^+ titration and observing the changes in the ^1H NMR (CD_3CN) spectrum we saw that upon Li^+ complexation, both the crown ether protons and the α-CH_2 protons of the pendent arms were effected, being shifted upfield and broadened after the addition of 10 equiv. of Li^+. The amide protons and the chiral centers were also substantially broadened. From these results, we predict that the Li^+ binding is most likely underneath the crown ether, where the amide also participates in the binding. Preliminary molecular modeling studies also support our suggestion. If this is the case, the excellent selectivity for Li^+ over other group I and II cations is due to size discrimination. Secondly, the rate of electron transfer falls off with distance ($\sim 1/r^6$), which in our case also contributes to the relatively higher fluorescence switching since the receptor is separated from the fluorophore by four atoms. Similar observations have been made in systems with 2–3 carbon atom spacers.[16] We are currently investigating these features in more detail as well as looking at the effect of using other fluorophores.

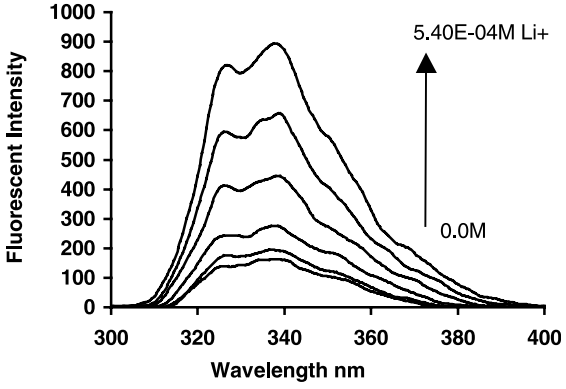


Figure 2. Changes in the fluorescence emission spectra of **1** upon addition of Li^+, when excited at 280 nm in MeCN.

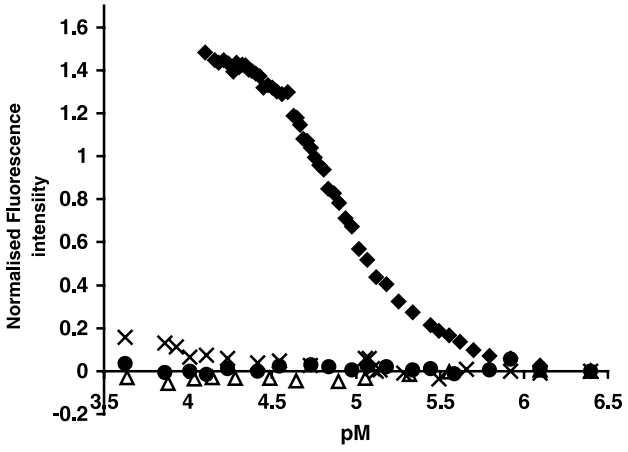


Figure 3. Titration profiles for **1** using perchlorate salts in CH_3CN. ◆, Li^+; ×, Ca^{2+}; ●, K^+; △, Na^+.

In summary, we have designed and synthesized a highly Li^+ selective fluorescent PET chemosensor. The fluorescence emission of the naphthalene fluorophores is switched on with an enhancement factor of ca. 5 upon addition of Li^+ in CH_3CN whereas the emission is hardly affected by other group I and II cations. To the best of our knowledge, this is the first example of a highly selective Li^+ PET chemosensor.

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References

1. (a) Spichiger-Keller, U. S. *Chemical Sensors and Biosensors for Medical and Biological Applications*; Wiley-VCH: Weinheim, Germany, 1998; (b) *Chemosensors of Ion and Molecular Recognition*; Desvergne, J. P.; Czarnik, A. W., Eds.; Kluwer Academic Publishers: Dordrecht, Netherlands, 1997; (c) *Fluorescent Chemosensors for Ion and Molecular Recognition*; Czarnik, A. W., Ed.; ACS Books: Washington, DC, 1993; (d) Hartley, J. H.; James, T. D.; Ward, C. J. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3155; (e) Czarnik, A. W. *Acc. Chem. Res.* **1994**, *27*, 302.
2. (a) de Silva, A. P.; Fox, B. D.; Huxley, A. J. M.; Moody, T. S. *Coord. Chem. Rev.* **2000**, *205*, 41; (b) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515; (c) Cooper, C. R.; Spencer, N.; James, T. D. *Chem. Commun.* **1998**, 136.
3. (a) Fabbrizzi, L.; Licchelli, M.; Pallavicini, P. *Acc. Chem. Res.* **1999**, *32*, 846; (b) Czarnik, A. W. *Acc. Chem. Res.* **1994**, *27*, 302; (c) Fabbrizzi, L.; Poggi, A. *Chem. Soc. Rev.* **1995**, 197; (d) Dickins, R. S.; Gunnlaugsson, T.; Parker, D.; Peacock, R. D. *Chem. Commun.* **1988**, 1643.
4. Gunnlaugsson, T.; Davis, A. P.; Glynn, M. *Chem. Commun.* **2001**, 2556.
5. (a) Gunnlaugsson, T.; Nieuwenhuyzen, M.; Richard, L.; Thoss, V. *J. Chem. Soc., Perkin Trans. 2* **2002**, 141; (b) Gunnlaugsson, T.; Nieuwenhuyzen, M.; Richard, L.; Thoss, V. *Tetrahedron Lett.* **2001**, *42*, 4725.
6. (a) Gunnlaugsson, T. *Tetrahedron Lett.* **2001**, *42*, 8901; (b) Gunnlaugsson, T.; MacDónaill, D. A.; Parker, D. *J. Am. Chem. Soc.* **2001**, *123*, 12866; (c) Gunnlaugsson, T.; MacDónaill, D. A.; Parker, D. *Chem. Commun.* **2000**, 93; (d) Reany, O.; Gunnlaugsson, T.; Parker, D. *Chem. Commun.* **2000**, 473; (e) Reany, O.; Gunnlaugsson, T.; Parker, D. *J. Chem. Soc., Perkin Trans. 2* **2000**, 1819; (f) Gunnlaugsson, T.; Parker, D. *Chem. Commun.* **1998**, 511.
7. There has been a recent report on the use of Li^+ complexes that are photoactive in alkaline methanol solutions, the authors describe this as 'chelation enhancement in fluorescence (CHEF)': Ciampolini, M.; Formica, M.; Fusi, V.; Saint-Mauricec, A.; Micheloni, M.; Nardi, N.; Pontellini, R.; Pina, F.; Romani, P.; Sabatini, A. M.; Valtancoli, B. *Eur. J. Inorg. Chem.* **1999**, 2261.
8. A few examples of fluorescent or optically based Li^+ sensors that are not PET active have recently been published: (a) Obare, S. O.; Murphy, C. J. *New J. Chem.* **2001**, *25*, 1600; (b) Ajayaghosh, A.; Chenthamarkshan, C. R. *Tetrahedron Lett.* **1998**, *39*, 1795; (c) Tsuchiya, S.; Ogawa, S. *Chem. Lett.* **1996**, 709; (d) Bocheñska, M. *J. Incl. Phenom.* **1995**, *22*, 269; (e) Blackburn, C.; LeCompte, M. B. K.; Langmuir, M. E. *Tetrahedron Lett.* **1994**, *35*, 1994; (f) McCarrick, M.; Wu, B.; Harris, S. J.; Diamond, D.; Barrett, G.; McKervey, M. A. *J. Chem. Soc., Perkin Trans. 2* **1993**, 1963; (g) Hiratani, K.; Nomoto, M.; Sugihara, H.; Okada, T. *Analyst* **1992**, *117*, 1491; (h) Sholl, A. F.; Sutherland, I. O. *J. Chem. Soc., Chem. Commun.* **1992**, 1716; (i) Hiratani, K. *Analyst* **1988**, *113*, 1065; (j) Nakashima, K.; Nagaoka, Y.; Nakatsuji, S.; Kaneda, T.; Tanigawa, I. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 3219.
9. Birch, N. J. *Chem. Rev.* **1999**, *99*, 2659.
10. de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Nieuwenhuizen, M. *Chem. Commun.* **1996**, 1967.
11. Faulkner, S.; Kataky, R.; Parker, D.; Teasdale, A. *J. Chem. Soc., Perkin Trans. 2* **1995**, 1761.
12. Ewin, G.; Hill, J. *Chem. Res.* **1985**, 3501.
13. Dickens, R. S.; Howard, J. A. K.; Maupin, C. L.; Moloney, J. M.; Parker, D.; Peacock, R. D.; Riehl, J. P.; Siligardi, G. *New J. Chem.* **1998**, *22*, 891.
14. The bis hydrobomide salt of the diaza-9-crown-3-ether **2** (0.5 g, 1.2×10^{-3} mol), $CsCO_3$ (1.4 g, 7.3×10^{-3} mol) and KI (0.75 g, 4.8×10^{-3} mol) were stirred in 30 ml of dry MeCN under an inert atmosphere. The α-chloroamide 2-chloro-*N*-[(*S*)-2-naphthyl]ethylethanamide **3** (2.52×10^{-3} mol), in 20 ml MeCN was added via a pressure equalizing dropping funnel over 20 min. This was left to reflux at 80°C overnight under an inert atmosphere. The reaction was filtered and the solvent evaporated in vacuo. The yellow residue was taken up in $CHCl_3$ and washed twice with 10% K_2CO_3 (3×20 ml) and once with water. The solvent was dried over $MgSO_4$ and evaporated in vacuo. After purification by flash silica chromatography with DCM: 0→5% MeOH, the product was triturated from diethyl ether yielding a white precipitate in 68% 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. ES MS: m/z 553 (M^{+1}); ^{1}H NMR δ: 1.677 (d, 6H, $J=7.04$); 2.570 (m, 8H); 3.047 (s, 4H); 3.240 (m, 4H); 5.802 (m, 2H, $J=7.04$), 7.530 (m, 8H, $J=8$); 7.815 (d, 2ArH, $J=8$); 7.910 (d, 2ArH, $J=7.52$); 7.815 (d, 2ArH, $J=8.52$); ^{13}C NMR δ: 19.8, 43.7, 55.1, 56.3, 60.2, 71.6, 122.2, 122.8, 124.8, 125.3, 125.8, 127.2, 128.2, 130.5, 133.4, 139.1; ε (λ [nm], MeCN), 10 427 (260.8), 11 801 (281.6), 6801.5 (293.2). ν/cm^{-1} (KBr disc) 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).
15. (a) Suzuki, K.; Yamada, H.; Sato, K.; Watanabe, K.; Hisamoto, H.; Tobe, T.; Kazuya, K. *Anal. Chem.* **1993**, *65*, 3404; (b) Metzger, E.; Aeschimann, R.; Egli, M.; Suter, G.; Dohner, R.; Ammann, D.; Dobler, M.; Simon, W. *Helv. Chem. Acta* **1996**, *69*, 1821.
16. de Silva, A. P.; Gunaratne, H. Q. N. N.; Gunnlaugsson, T. *Tetrahedron Lett.* **1998**, *39*, 5077.