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A novel optically based chemosensor for the detection of blood Na^+

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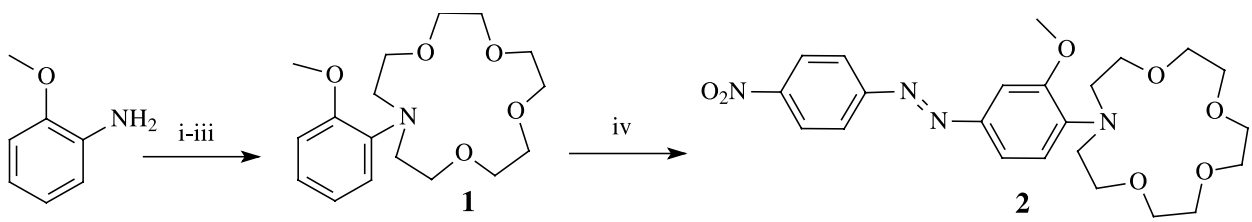
Abstract—The azo dye based chemosensor **2** displays extremely good sensitivity and selectivity for Na^+ over other physiologically important alkali and alkali earth metal ions in the $0.3–1.0\times10^{-4}$ M concentration range as well as being pH independent above pH 3.9. This sensitivity coincides with the Na^+ concentration range found in blood. The sensor exhibits almost 110 nm hypsochromic shift in the absorption spectrum upon Na^+ detection, with a strong colour change from red to yellow in aqueous solution which is clearly visible to the naked eye even at low concentrations.

The recognition and sensing of physiologically important ions and molecules has been one of the most active areas of research within the field of supramolecular chemistry.[1] Systems responding to concentration variations by displaying changes in their optical properties, such as absorption, fluorescence, phosphorescence and metal based luminescence upon analyte recognition, are very attractive for in vivo applications.[2] Such sensors need to fulfil the criteria of high selectivity and high sensitivity including good solubility in water or alternatively being easily incorporated into hydrophobic membranes, and they should absorb and/or emit at long wavelength to overcome undesirable side effects such as autofluorescence and light scattering in vivo.

We have recently synthesised several kinetically robust Eu(III) and Tb(III) chemosensors for H^+, O_2 and Zn^{2+} where the line-like time delayed emission of the lanthanide ion, emitting between 550–730 and 500–650 nm, respectively, was modulated upon ion recognition in 100% water.[3] We have also employed this detection mode to demonstrate the function of molecular logic gates.[4] Concurrently we have been interested in the detection of intra- and extracellular Na^+ concentration by employing such lanthanide based time-delayed detection.[5] Our initial goal was to design a Na^+ selective macrocyclic receptor for blood Na^+ that would exhibit good selectivity for Na^+ over K^+ as well as being easily incorporated into the lanthanide emitting moieties. With this in mind, we synthesised the pivot crown ether **1**, previously designed by Gokel et al. for Na^+ detection in MeOH.[6] By simple modification, the receptor can be easily incorporated into lanthanide-based macrocyclic systems, or it could be functionalised to



Scheme 1. (i) Na_2HPO_4, KI, $ClCH_2CO_2CH_3$ and CH_3CN; (ii) $LiAlH_4$, THF; (iii) NaH, THF, $(TsOCH_2CH_2OCH_2)_2$; (iv) a. $NO_2C_6H_6NH_2$, $NaNO_2$, HCl, THF/H_2O (1:1), b. 1.

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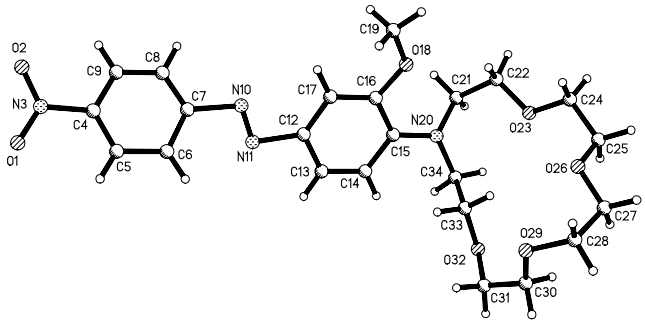
yield a chromogenic receptor/ionophore that would absorb and emit at long wavelengths.[7] In both cases interference from the physiological system would be overcome. In this letter we describe the synthesis and incorporation of this receptor into an azo dye, yielding **2**, that displays both large changes in the absorption spectra upon Na^+ recognition, while discriminating against K^+, Ca^{2+} and Mg^{2+}.

The synthesis of **1** was achieved in good yield by modification of the procedure by Gokel et. al.,[6] Scheme 1. The sensor **2** was obtained in 52% yield in a one-pot reaction by first carrying out diazoniation on *p*-nitroaniline in aqueous THF solution, followed by the addition of **1**.[8] The resulting red residue was purified by flash-column chromatography on neutral alumina (30:70 EtOAc:hexane) giving **2** as a red powder. Interestingly, when other diazonium salts such as *p*-chloro, *p*-sulfonic or *p*-carboxylic acids were used, no coupling was observed. All the products and intermediates were analysed by ^1H and ^{13}C NMR, ESMS, accurate mass determination and IR. The ^1H NMR of **2** showed five aromatic signals resonating between 8.3 and 7.0 ppm, a singlet for the methoxy group at 3.91, a multiplet and a triplet at 3.68 and 3.79 ppm, respectively, corresponding to the crown ether protons. In the ESMS only two major peaks were observed at 475.5 and 497.5 corresponding to the molecular ion and the Na^+ complex of **2**. We were able to obtain suitable crystals of **2** for crystallographic evaluation by slow evaporation from ethanol. The X-ray crystal structure of **2** is shown below, showing the compound in its *trans* conformation, with a dihedral angle of 23.3° for the N11–N10–C7–C8 and −163.3° for the C6–C7–N10–N11 atoms. The dihedral angle for C18–C16–C15–N20 was measured to be −4.5°, whereas the C21–N20–C15–C16 and C34–N20–C15–C14 angles were measured to be −44.0 and −12.7°, respectively.[9–11] These values indicated that the nitrogen lone pair is not fully conjugated with the aromatic π-system due to the steric affect of the *o*-methoxy group, but the group should provide an extra chelation site upon complexation to Na^+. We are presently undertaking further studies of the solid-state properties of **2** in the presence of group I and II ions.



The Na^+ sensing of **1** and **2** was measured at pH 7.5 in Tris buffered (0.01 M) 50:50 MeOH:H_2O solution, and in the presence of 0.1 M tetramethyl ammonium chloride to maintain constant ionic strength. The pK_a for **1** and **2** was also determined by absorption titration. The absorption spectra of **1** showed two bands at 245 and 285 nm in alkaline solution, which reduced in intensity upon addition of acid. A similar effect was observed using sodium acetate, with ca. 15% reduction in the absorption intensities. From these changes a pK_a of 5.8 (±0.05) and a log β_{Na} of 2.4 (±0.05) was determined, which is in good agreement with that observed by Gokel et al. in MeOH.[6]

The changes in the absorption spectra of **2** were more dramatic as shown in Fig. 1a. The compound displayed a broad absorption band at 487 and 285 nm in the absence of Na^+, whereas upon addition of Na^+, the 487 nm band hypsochromically shifted to 380 nm ($\Delta\lambda \sim 110$ nm) with the formation of an isosbestic point at 410 nm. These changes were also clear to the naked eye where the deep-red colour solution of **2** changed to a yellow after addition of Na^+ ($[Na^+]=1.0\times10^{-4}$ to 0.3 M). These changes are shown in Fig. 1b, where the Na^+ titration profile is sigmoidal over 2 pM units, from pK_a = 0.5–2.5, consistent with a simple ion-binding equilibrium. A similar effect was seen for the pH titration of **2**, with the exception that the 487 nm band completely disappeared upon addition of acid to an alkaline solution of **2**. From these changes a log β_{Na} of 1.25 (±0.05) and pK_a of 3.9 (±0.05) was determined. This Na^+ sensitivity conveniently overlaps with the Na^+ concentration range found in blood, which has an upper limit of 145 mM (extracellular concentration ranges from 120–450 mM in animal

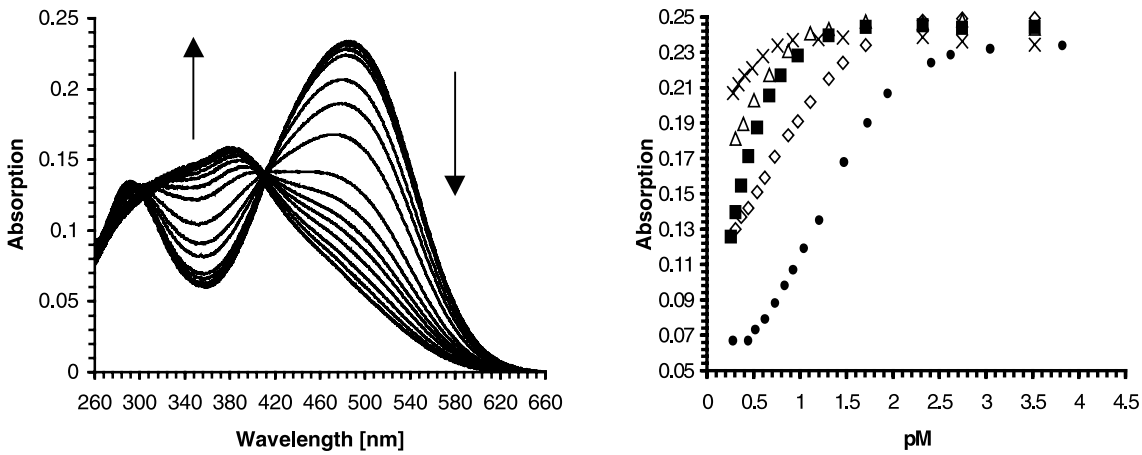


Figure 1. (a) The absorption spectra of **2** upon addition of sodium acetate [Na] = 0→0.5 M; (b) absorption versus pM (–log[M], M = Li^+ (△), Na^+ (●), K^+ (◇) Ca^{2+} (■) Mg^{2+} (×)) profile.

cells), to a background of ca. 4.8–3.5 mM and 2.6–2.2 mM of K^+ and Ca^{2+}, respectively (in blood).[12] Furthermore, the sensor is pH independent at physiological pH as required for blood analysis. However, we do recognise that Na^+ determination in 100% water would yield lower binding values that call upon some modification of **2** for such practical application. The spectral changes and the binding values are substantially different from those seen for **1**, because in **2** the nitrogen lone pair of the anisole moiety is engaged in a charge push–pull mechanism with the nitro group. This gives rise to internal charge transfer excited state (ICT), which gives **2** its intense red colour prior to the Na^+ complexation. Upon Na^+ complexation, the lone pair is engaged in Na^+ binding reducing its ability to participate in the ICT mechanism and causing shifts in the absorption spectra to shorter wavelengths.[13] Furthermore, the strongly electron withdrawing nitro substituents reduce the ability of the nitrogen lone pair to interact with the ion, yielding weaker binding than that seen for **1** and shifting the binding constant towards the desired range for blood Na^+ detection.

The sensitivity of **2** was measured by carrying out analogous experiments using Li^+, K^+, Ca^{2+} and Mg^{2+} acetate salts. The results are shown in Fig. 1b as the changes in absorption intensity versus pM, indicating the high sensitivity of **2** for Na^+ over the other cations. This high Na^+ selectivity is possible to the size of the crown moiety and the steric effect enforced by the *o*-methoxy group, which is also directly involved in the binding of the Na^+.[14] The fluorescence emission spectrum of **2** was also monitored upon addition of Na^+, showing 60% reduction in intensity after the addition of a 0.5 M concentration of sodium acetate.

In conclusion, the β_{Na}, the large colour change (red to yellow) and its resistance to protonation at physiological pH makes **2** an ideal candidate for the evaluation of blood Na^+ and serum, especially because it displays such a high selectivity for Na^+, while discriminating against any other physiologically active group I and II cations. To the best of our knowledge, there are no such chromogenic chemosensors in the literature. We are in the process of evaluating these properties by incorporating **2** into optodes and membranes for online and real-time monitoring in vivo.

Acknowledgements

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References

1. (a) *Chemical Sensors and Biosensors for Medical and Biological Applications*; U.S. Spichiger-Keller, Wiley-VCH: Weinheim; Germany, 1998; (b) *Chemosensors of Ion and Molecular Recognition*; Desvergne, J. P.; Czarnik, A. W., Eds.; Kluwer Academic Publishers: Dordrecht, 1997; (c) *Fluorescent Chemosensors for Ion and Molecular Recognition*; Czarnik, A. W., Ed.; ACS Books: Washington, 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) Parker, D. *Coord. Chem. Rev.* **2000**, *205*, 109; (b) de Silva, A. P.; Fox, B. D.; Huxley, A. J. M.; Moody, T. S. *Coord. Chem. Rev.* **2000**, *205*, 41; (c) 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; (d) Fabbrizzi, L.; Licchelli, M.; Pallavicini, P. *Acc. Chem. Res.* **1999**, *32*, 846; (f) Fabbrizzi, L.; Poggi, A. *Chem. Soc. Rev.* **1995**, 197.
3. (a) Gunnlaugsson, T.; Parker, D. *Chem. Commun.* **1998**, 511; (b) Reany, O.; Gunnlaugsson, T.; Parker, D. *Chem. Commun.* **2000**, 473; (c) Reany, O.; Gunnlaugsson, T.; Parker, D. *J. Chem. Soc., Perkin Trans. 2* **2000**, 1819.
4. Gunnlaugsson, T.; MacDónaill, D. A.; Parker, D. *Chem. Commun.* **2000**, 93.
5. (a) Tahri, A.; Cielen, E.; Van Aken, K. J.; Hoornaert, G. J.; De Schryver, F. C.; Boens, N. *J. Chem. Soc., Perkin Trans. 2* **1999**, 1739; (b) Yoshida, K.; Mori, T.; Watanabe, S.; Kawai, H.; Nagamura, T. *J. Chem. Soc., Perkin Trans. 2* **1999**, 393.
6. Schultz, R. A.; White, B. D.; Dishong, K. A.; Arnold, W.; Gokel, G. *J. Am. Chem. Soc.* **1985**, *107*, 6659.
7. (a) Oguz, U.; Akkaya, E. U. *Tetrahedron Lett.* **1997**, *38*, 4509; (b) Sauve, G.; Kamat, P. V.; Thomas, K. G.; Thomas, K. J.; Das, S.; George, M. V. *J. Phys. Chem.* **1996**, *100*, 2117; (c) Mitewa, M.; Mateeva, N.; Antonov, L.; Deligeorgiev, T. *Dyes Pigments* **1995**, *27*, 219; (d) Li, H.; Yao, Z.; Liu, R.; Tan, G.; Yu, X. *Chem. Lett.* **1998**, 1205.
8. (a) Dix, J. P.; Vögtle, F. *Chem. Ber.* **1980**, *113*, 457; (b) Lohr, H. G.; Vögtle, F. *Acc. Chem. Res.* **1985**, *18*, 65; (c) Dix, J. P.; Vögtle, F. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 857.
9. X-Ray crystallographic data for $C_{23}H_{30}N_4O_7$ (**2**) were collected using a Bruker SMART diffractometer with graphite-monochromated Mo-Kα radiation. The crystal stability was monitored and there was no significant decay (±1%). Cell parameters were obtained from 215 accurately centred reflections. Data were collected at ca. 120 K in a dinitrogen stream. Phi and omega scans were employed for data collection and Lorentz and polarisation corrections were applied. The structure was solved by direct methods and all non-hydrogen atoms were refined with anisotropic atomic displacement parameters. Hydrogen atom positions were added at idealised positions and a riding model was used in the subsequent refinement ($U_{eq}=1.2U_{ij}$ of the parent atom). The atomic displacement parameters indicated that the oxygen atom O30 of the crown moiety was disordered. This disorder has been modelled over two sites and refined to 75(1)% for the major component. The function minimised for wR_2 was $\Sigma[w(|F_o|^2-|F_c|^2)]$ with reflection weights $w^{-1}=[\sigma^2|F_o|^2+(g_1P)^2+g_2P]$ where $P=[\max|F_o|^2+2|F_c|^2]/3$ for all F^2 and the function minimised for R_1 was $\Sigma[w(|F_o|-|F_c|)]$. The SAINT[10] and SHELXTL[11] PC packages were used for data collection, reduction, structure solution and refinement. Additional material available from the Cam-

bridge Crystallographic Data Centre includes atomic coordinates, thermal parameters, remaining bond lengths and angles and structure factors (CCDC number 158714). Crystal data for $C_{23}H_{306}N_4O_7$ (**2**): $M=474.51$, monoclinic, space group $P2_1/c$, $a=14.161$ (5), $b=7.521(3)$, $c=22.967(9)$ Å, $\beta=105.703(8)°$, $U=2354.9(15)$ Å^{-3}, $Z=4$, D_c 1.338 Mg m^{-3}, $F(000)=1008$, $\mu=0.100$ mm^{-1}, crystal dimensions $=0.28\times0.23\times0.10$ mm. A total of 14543 reflections were measured for $3<2\theta<45$ and 3075 unique reflections were used in the refinement, the final parameters were $wR_2=0.2130$ and $R_1=0.0695$ [$I>2\sigma(I)$], $S=1.210$, 318 parameters.

10. *SMART Software Reference Manual*, *version 5.054*, Bruker Analytical X-Ray Systems, Madison, WI, 1998.
11. Sheldrick, G. M. SHELXTL, An Integrated System for Data Collection, Processing, Structure Solution and Refinement, Bruker Analytical X-Ray Systems, Madison, WI, 1998.
12. Blinks, J. R.; Wier, W.; Hess, P.; Prendergast, F. G. *Prog. Biophys. Mol. Biol.* **1982**, *40*, 1.
13. (a) Létard, J.-F.; Delmond, S.; Lapouyade, R.; Braun, D.; Rettig, W.; Kreissler, M. *Recl. Trav. Chim. Pays-Bas.* **1995**, *114*, 517; (b) Létard, J.-F.; Lapouyade, R.; Rettig, W. *Pure Appl. Chem.* **1993**, *68*, 1705.
14. We have observed that the OCH_3 signal in the ^{1}H NMR is shifted by 0.16 ppm upon addition of 5 equiv. of $NaPF_6$ in CD_3OD. A similar effect has been seen in a related Na^+ receptor for intracellular Na^+ detection: de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson T.; Nieuwenhuizen, M. *Chem. Commun.* **1996**, 1967.