Organic & Biomolecular Chemistry

PAPER

View Article Online
View Journal

Cite this: DOI: 10.1039/c6ob00468g

Crowned spiropyran fluoroionophores with a carboxyl moiety for the selective setection of lithium ions†

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Received 1st March 2016,
Accepted 10th March 2016

DOI: 10.1039/c6ob00468g

www.rsc.org/obc

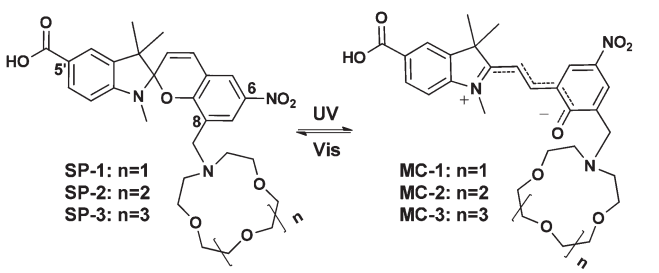
The absorbance and fluorescence spectra of carboxylated spiropyrans containing methyl-1-aza-12-crown-4, methyl-1-aza-15-crown-5, methyl-1-aza-18-crown-6 moieties are compared. Characteristic changes in spectra after addition of the alkali metal salts of Li^+, Na^+, K^+ and Cs^+ were observed. Chromism induced by the binding of the metal cations was observed as an increase in absorbance and fluorescence. Of these metal cations, the Li^+ ion produced the largest change in all three spiropyran systems. Reversible photoswitching of the spiropyran-metal complexes was observed on irradiation with alternating 352 nm UV and white light. This results in reversible fluorescence based sensing of lithium ions with potential for use in a biological sensor device.

Introduction

An ability to selectively detect metal ions in biological samples is an important area of current biosensor research.[1] This is especially true of the lithium cation (Li^+), a trace metal of unknown biological function found in mammalian tissues at levels of 0.001–0.01 mM.[2–5] Li^+ is also of interest as a therapeutic to treat neurological diseases such as manic-depressive illness. However, dosage is critical, as Li^+ is only effective within a narrow therapeutic window (0.6–1.2 mM); too low a dose has no effect while too high a dose (>2 mM) is toxic and lethal.[1–3,6]

Despite the importance of Li^+ in biology there are only a few reports of fluorescence-based sensors for its detection.[1,6–11] Most of these are either non-functional at relevant biological concentrations, or they do not display sufficient selectivity over other metal cations, in particular Na^+. New biologically compatible fluorescent sensors and sensing devices that are selective for Li^+ are needed to provide a greater understanding of the biological role of Li^+. Spiropyran-based photoswitchable sensors offer some potential in this area. These structures reversibly switch between a non-fluorescent spiropyran form (SP) and a charge delocalized fluorescent merocyanine isomer (MC) when exposed to a stimulus; such as UV light, change in local environment (polarity), or when interacting with a charged metal ion (see Scheme 1). The charged MC state can be exploited to provide a strong ion interaction site.[12–14] For example, incorporating a cation binding domain at the 8 position of the spiropyran *ortho-* to the phenolate group (Scheme 1) is known to enhance ion affinity and selectivity of the ion binding domain.[15–17]

We have previously reported a methyl-1-aza-15-crown-5 modified spiropyran (Scheme 1, compound 2) attached to a microstructured optical fibre surface *via* a 5′-carboxyl group for fluorescence based detection of Li^+.[11] Here we compare the influence on the fluorescence spectra produced by the photoswitching and binding of a range of biologically relevant alkali metal ions to spiropyrans containing differing sized azacrown ether rings. This then provides a fluorescence based structure affinity profile with potential to develop a more selective regenerable sensor for Li^+ that will have applications as a dye in fluorescence microscopy, for example.[18] Three carboxylated



Scheme 1 Structures of the crowned spiropyrans, **SP-1**, **-2**, and **-3** and the corresponding photoswitched merocyanine isomers, **MC-1**, **-2**, and **-3** respectively.

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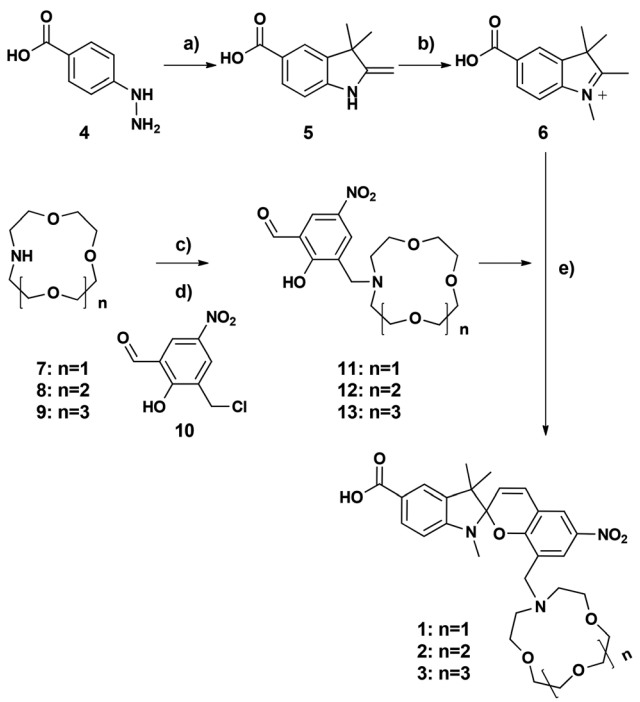
†Electronic supplementary information (ESI) available. See DOI: 10.1039/c6ob00468g

spiropyrans, each with a different aza-crown at the 8-position were prepared; methyl-1-aza-12-crown-4 (**1**), methyl-1-aza-15-crown-5 (**2**) and methyl-1-aza-18-crown-6 (**3**). The incorporation of a 5′-carboxyl group to the spiropyran sensor provides increased aqueous solubility for biological based studies as well as a site for potential functionalization.[19] Previous studies have modified this 5′ site with electron withdrawing CF_3 or NO_2 groups to enhance the control of reverse photoswitching by stabilising the spiropyran isomer, the more weakly electron withdrawing CO_2H is expected to have a similar effect, whilst providing the before mentioned benefits.[20,21]

Results and discussion

Compounds **1**, **2**, and **3** were prepared using methodology previously reported for **2**, see Scheme 2.[11] 4-Hydrazinobenzoic acid **4** was reacted with 2-methyl-2-butanone (Fischer indole reaction)[22,23] to give indoline **5**, which was alkylated with iodomethane to give the methylindole **6**. The aza-1-crownethers **7–9** were separately alkylated with the chloride **10** to give 3-methyl(azacrownether)-2-hydroxy-5-nitrobenzaldehydes **11–13**. A condensation reaction between these benzylaldehydes and the methylindole **6** in refluxing ethanol, followed by purification by reverse-phase liquid chromatography gave the desired spiropyrans **1–3**.

Influence of the aza-1-crownether group on absorbance and fluorescence of the spiropyran base-unit.



Scheme 2 Synthesis of spiropyrans **1**, **2** and **3**. (a) 2-methyl-butanone, sulphuric acid, EtOH, 85 °C, 18 h, 73%; (b) iodomethane, 2 : 1 toluene : MeCN, 95 °C, 24 h, 72%; (c) triethyl amine, THF, 0 °C, 1 h; (d) THF, 75 °C, 17 h; (e) EtOH, 85 °C, 3 h, 18–20% (3 steps).

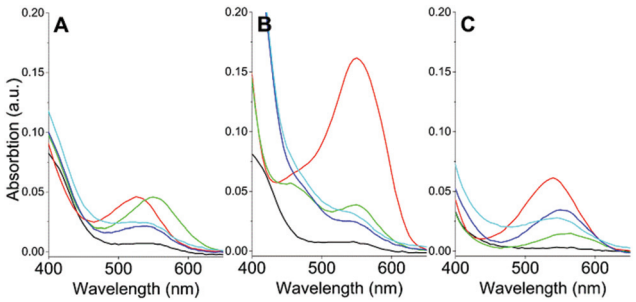


Fig. 1 Absorption spectra of crowned spiropyrans in acetonitrile (A) **SP-1**, (B) **SP-2**, (C) **SP-3** in the presence of no ions (black), 100× excess of lithium perchlorate (red), sodium perchlorate (green), potassium perchlorate (navy), caesium sulphate (cyan).

The effect that the size of the aza-1-crown ether ring has on absorbance and fluorescence spectra of **SP-1**, **SP-2**, and **SP-3** and the corresponding UV photoswitched **MC** states was investigated. Specifically, **SP-1**, **SP-2**, and **SP-3** were separately dissolved in acetonitrile (50 μM) and the respective UV-vis absorbance and fluorescence (λ_{ex} = 532 nm) spectra were recorded using a Synergy H4 Hybrid Microplate Reader. Acetonitrile was used as solvent since it is known to give rise to slow thermal switching of a spiropyran providing a reduced background signal,[24] and we have shown that it gives comparable fluorescence in the presence of Li^+ in H_2O : acetonitrile solutions.[11] **SP-1**, **SP-2** and **SP-3** gave a common absorbance in the UV region at 360 nm, with **SP-1** and **SP-2** giving an additional peak at 400 nm. All three solutions showed a weak absorbance peak at 545 nm (Fig. 1, black) possibly due to the presence of small amounts of the **MC** isomer. Irradiation of these three solutions, with a filtered UV black lamp (352 nm) for 10 min, induced photoswitching to give the **MC** enriched photostationary state. This resulted in an increase in the absorbance at 545 nm in each case. Photoswitching caused an increase in the intensity of the fluorescence of **MC-1** and **MC-2** with a maximum intensity observed around 627 nm, and for **MC-3** with a slightly red shifted peak at around 632 nm (Fig. 2, black). **MC-1** and **MC-3** had comparable emission intensities; however, **MC-2** showed a 3–4 fold higher emission intensity. Irradiation of the solutions with white light resulted in reversal of the absorbance and fluorescence spectra associated with the ring closed spiropyran isomer (Fig. 2B, black). Thus the ring size of the azacrownether rings does not appear to have a significant effect on the spectral and photoswitching characteristics of the spiropyran.

Absorbance changes upon binding alkali metal ions

The absorbance spectra of compounds **SP-1**, **SP-2**, and **SP-3** in the presence of 100 fold excess of perchlorate salts of Li^+, Na^+ and K^+ and Cs_2SO_4 in acetonitrile (as per literature on similar sensing systems)[11,20,24,25] were next measured to investigate spectral changes induced by ion binding, as well as the influence of the 5′-carboxylic acid group on the observed spectra. An excess of metal ions was used in order to give the

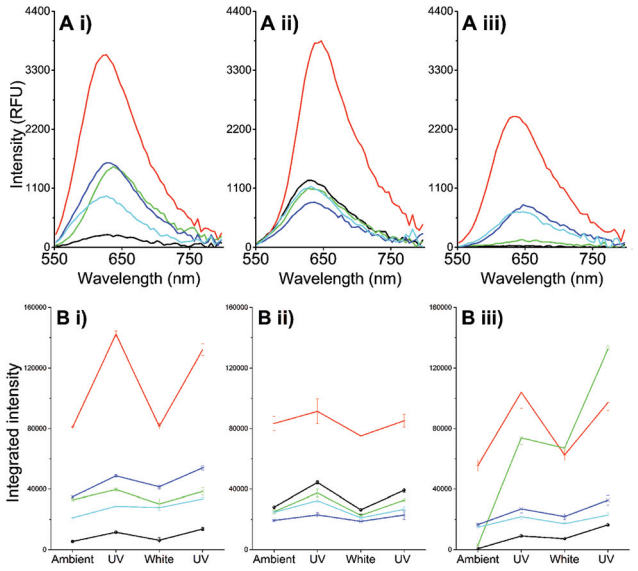
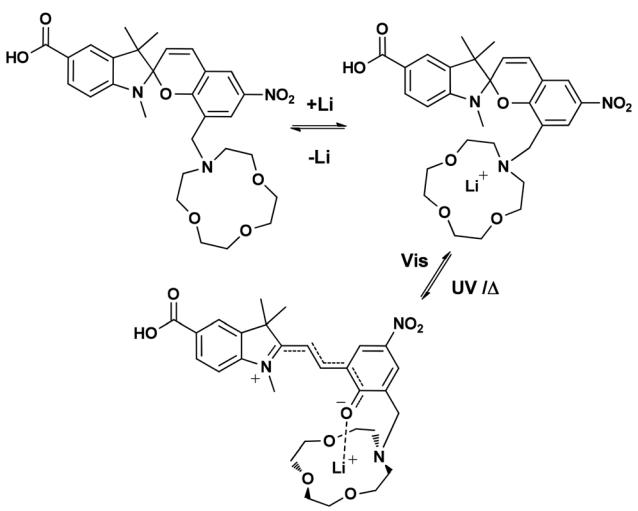


Fig. 2 (A) The fluorescence spectra of crowned spiropyrans (i) **1**, (ii) **2**, (iii) **3** in the presence of no ions (black), and 100× excess of lithium perchlorate (red), sodium perchlorate (green), potassium perchlorate (navy), and caesium sulphate (cyan). B) Integrated fluorescence intensities in the presence of metals during photocycling with UV black light and white light.

maximum signal from the spiropyran complex's, despite the optimal binding stoichiometry of 1 : 1 spiropyran to metal ion (as determined by a Jobs plot, see ESI Fig. S4†). **SP-1** showed a 2 fold greater absorbance in the visible region in the presence of Li^+ and Na^+ compared to K^+ and Cs^+ (Fig. 1A). This increase in absorbance is the result of metal ion induced thermal switching to the more coloured merocyanine state, see Scheme 3.[24] Binding of **SP-1** to Li^+ (**SP-1-Li**$^+$) caused a blue shift compared to the unbound form, with a peak maximum at 530 nm (Fig. 1A, red). Interestingly this peak is bathochromically shifted compared to a structurally similar crowned spiropyran lacking a carboxylic acid group, which is reported to have a peak at 514 nm with Li^+.[24] **SP-1-Na**$^+$ gave a similar absorbance intensity compared to **SP-1-Li**$^+$, however it was significantly red shifted to 550 nm. UV induced photoswitching to the **MC-1** isomer caused a further 3–4 fold increase in the absorbance of the resulting **MC-1-Li**$^+$ and **MC-1-Na**$^+$. **MC-1-K**$^+$ and **MC-1-Cs**$^+$ showed only a small increase, giving an absorbance spectrum similar to **MC-1** without metal ions (see ESI Fig. S1†). **SP-2** showed a 4 fold greater increase in absorbance at 550 nm in the presence of Li^+ compared to Na^+, Cs^+, and K^+ (Fig. 1B). Again, this **SP-2-Li**$^+$ absorbance peak is bathochromically shifted compared to the 533 nm peak reported for a structurally similar crowned spiropyran lacking a carboxylic acid group at 5′.[24] Photoswitching on irradiation with 352 nm UV light caused a 2 fold increase in the signal intensity for **MC-2-Li**$^+$, and a 3 fold increase for **MC-2-Na**$^+$. The absorbance intensity of **MC-2-K**$^+$ and **MC-2-Cs**$^+$ underwent only a small increase at 550 nm, to give a signal similar to **MC-2** in the absence of metal ions. **SP-3** displayed an increase in absorbance at 540 nm in the presence of Li^+ (Fig. 1C). **SP-3** with K^+ and Na^+ underwent a smaller increase in absorbance, with peaks at 550 nm and 565 nm respectively. After UV induced photoswitching to give the **MC-3** metal complexes the **MC-3-Li**$^+$ system underwent a 4.5 fold increase in absorbance, much greater than the 2–3 fold increase observed in the **MC-3-Na**$^+$ and **MC-3-K**$^+$ solutions. Therefore, unlike for the **SP-3** isomeric form in which there was only a small difference between its absorbance with Li^+ and K^+, **MC-3** showed an improved selectivity profile with a much larger, 3 fold greater, absorption intensity in the presence of Li^+ compared to the other metal ions (Fig. S1iii†). Reverse photoswitching with visible light reduced the absorbance intensity of each system to near that of the non-irradiated system, demonstrating reversibility of the photoswitch (see ESI Fig. S1B†). This shows, however, that the carbonyl at the 5′ position does not sufficiently stabilise the spiropyran form to shift the spiropyran : merocyanine equilibrium towards the spiropyran under visible irradiation, unlike previously observed for the CF_3 group.[20,21] However, formation of the spiropyran-metal complex had sufficient time to reequilibrate between irradiation and spectral measurements.



Scheme 3 Model of lithium ion binding and ion induced switching of spiropyran **1**.

These absorbance profiles indicate that, regardless of the ring size, all compounds (**1**, **2**, and **3**) undergo metal ion induced chromism in the presence of lithium perchlorate in acetonitrile; with the largest and most selective change observed for **2-Li**$^+$. Binding of the different metal cations produced varying shifts in the maximum absorbance wavelength dependent on the size of the ring and the metal cation species. Importantly, this difference was significant enough for **SP-1** to distinguish between Li^+ and Na^+. This observation may reflect the exact nature of the interactions between the metal ion and the phenolate of the merocyanine. The crown ether ring size also influences selectivity of binding to the other alkali metal ions. The smaller rings of **1** and **2** show some selectivity for Na^+ over K^+ while the larger ring in **SP-3** showed increased selectivity for K^+. However, selectivity of

binding is also dependent on the photoswitched state, with **SP-2** only showing a strong response to Na^+ in the **MC-2** isomeric form.

The absorption spectra of **1–3** with Li^+, Na^+, and K^+ in a 1 : 1 mixture reveal similar absorption profiles compared to other related spiropyrans.[24,26,27] Thus the 5′ carboxyl substituent does not interfere with ion binding and sensing (Fig. S2†). This group does, however, cause an approximate 15 nm bathochromic shift in the merocyanine absorbance peak. This needs to be considered in the design of other spiropyran sensors that employ such modification.

Fluorescence detection of alkali metal ion binding

The fluorescence emissions of the three crowned spiropyrans **1**, **2**, and **3**, in the presence of perchlorate salts of Li^+, Na^+ and K^+ and Cs_2SO_4, were observed (Fig. 2). Binding to Li^+ ions caused the largest increase in the fluorescence for all three spiropyrans, producing emission maxima at 627, 647, and 637 nm for **1**, **2** and **3** respectively (Fig. 2A red). A 15 fold increase in fluorescence intensity was observed for **SP-1** (Fig. 2i) bound with Li^+ (**SP-1-Li$^+$**) compared to **SP-1** alone, a change significantly greater than the 6 fold increase observed in the absorbance. **SP-1-Na$^+$** and **SP-1-K$^+$** had a similar fluorescence of half the intensity of **SP-1-Li$^+$**, despite **SP-1-Na$^+$** having a similar absorbance intensity to **SP-1-Li$^+$**. **SP-1-Na$^+$** and **SP-1-K$^+$** have emission maximum at around 642 and 632 nm, respectively, red shifted compared to the peaks of **SP-1**, **SP-1-Li$^+$**, and **SP-1-Cs$^+$** at 627 nm. Irradiation with UV black light for 10 min, to promote photoswitching to **MC-1**, caused a large increase in emission intensity of **MC-1-Li$^+$**, compared to the smaller increases in intensity when in the presence of Na^+, K^+, Cs^+, or just **MC-1** alone (Fig. 2Bi). Irradiation with white light, to photoswitch back to the **SP-1** isomer, resulted in a decrease in fluorescence back to the non-photoswitched intensities, demonstrating photo-reversibility of the systems. **SP-2-Li$^+$** produced, a 3 fold greater fluorescence signal compared to **SP-2** alone, which in turn was slightly greater than that in the presence of Na^+, Cs^+, and K^+ (Fig. 2ii). This contrasts the greater absorbance observed for **SP-2-Na$^+$**, **SP-2-K$^+$**, and **SP-2-Cs$^+$** compared to **SP-2**. UV induced photoswitching of all the **SP-2** complexes caused only a small fluorescence increase (Fig. 2Bii) compared to the 2–3 fold increase observed in the absorbance spectra of **MC-2-Li$^+$** and **MC-2-Na$^+$**. Surprisingly, the presence of Na^+, Cs^+ and K^+ resulted in a weaker fluorescence compared to that in the absence of metal ions. **SP-3-K$^+$** and **SP-3-Cs$^+$** gave a similar fluorescence intensity with emission maxima at 652 nm and 647 nm, respectively, and with an intensity approximately one third to that of **SP-3-Li$^+$**, (Fig. 2Aiii). **SP-3-Na$^+$** had a low intensity emission around 655 nm. Interestingly photoswitching with UV light caused a large increase in emission from **MC-3-Na$^+$** with a blue shifted peak at 605 nm (Fig. 2Biii green, see spectrum in ESI Fig. S3†). This contrasts the small increase in a blue shifted absorbance spectrum (Fig. S1†).

Exposure of **MC-3-Na$^+$** to white light for 10 min, to induce photoswitching back to **SP-3**, gave no change in the fluorescence signal. This lack of photoswitching for **MC-3-Na$^+$** suggests a strong binding affinity for the Na^+ ion which stabilises the merocyanine complex, therefore reducing the ability for **MC-3** to isomerise to **SP-3**.

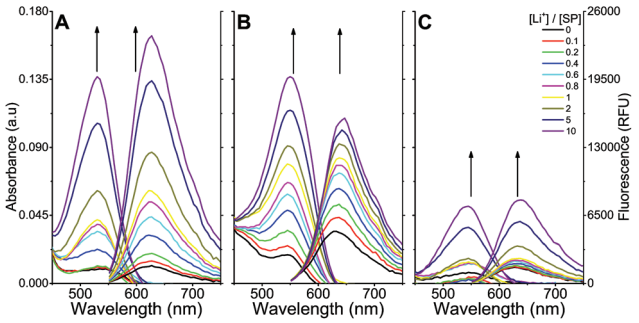


Fig. 3 The absorbance and fluorescence spectra of crowned spiropyrans (A) **SP-1**, (B) **SP-2**, (C) **SP-3** 50 μM in the presence of increasing concentrations of $LiClO_4$ 0, 10, 20, 30, 40, 50, 100, 250, 500 μM in acetonitrile.

These results demonstrate that, although ion induced switching from the spiropyran to merocyanine isomers in **1–3** can be observed as an increase in the absorbance spectra, the same relative increase in signal intensity is not observed in the fluorescence spectra. That is, the binding of the different metal ions appears to alter the fluorescence yields of each spiropyran. Regardless, based on fluorescence spectroscopy all three crowned spiropyran complexes (**1–3**) were able to selectively detect Li^+ over the other alkali metal ions investigated. **SP-2** appeared to show the greatest difference in fluorescence intensity between the binding to Li^+ and the other meatal ions, hence demonstrating the best selectivity for Li^+.

Finally the concentration dependence of Li^+ on the absorbance and fluorescence was investigated. Fig. 3 shows the increase in absorbance and fluorescence signal of spiropyrans **1–3** on increasing Li^+ concentrations. In these conditions an increase in the **SP-2** spectrum was observed for Li^+ at 0.1 molar equivalents, the lowest concentration ratio investigated. Spiropyrans **SP-1** and **SP-3** likewise showed a typical concentration dependant sigmoidal increase between 0.1 and 1 molar equivalents.

Conclusions

Three photoswitchable spiropyrans **SP-1**, **SP-2**, **SP-3** were synthesized, each with a different sized azacrown ether attached at the 8-position. Absorbance and fluorescence responses to binding of alkali metal cations Li^+, Na^+, K^+, and Cs^+ in acetonitrile were determined. Each spiropyran gave a strong absorbance and fluorescence response in the presence of Li^+, irrespective of the size of the crown ether ring. A weaker response was observed in the binding of all spiropyrans to the other alkali metal ions, with the selectivity of ion binding defined by the size of the crown ether ring. As such the 1-aza-15-crown-5 containing spiropyran (**SP-2**) showed the most

selective response to Li^+ over the other metal ions, exhibiting a stronger relative absorbance and fluorescence spectra. Therefore in conclusion, these azacrownether spiropyrans have potential as new reversible fluorescent probes for investigating the concentration of Li^+ in biological systems, which will lead to a greater understanding of Li^+'s role in diseases such as manic-depressive illness.

Experimental

Materials and methods

All ^{13}C NMR and ^1H NMR spectra were recorded on an Agilent Technologies 500 MHz NMR with DD2 console in $CDCl_3$ or DMSO-d6 (Cambridge Isotope Laboratories, Cambridge, MA). Chemical shifts (δ) are reported in ppm, with $CDCl_3$ (δ_C = 77.1 ppm), DMSO-d6 (δ_C = 39.52 ppm) or TMS (δ_H = 0.0 ppm) used as internal standards. High resolution mass spectrometry was performed on the Agilent 6230 TOF LC-MS. All commercially available chemicals were reagent grade and used without further purification.

Synthesis of azacrownetherspiropyrans

3,3-Dimethyl-2-methyleneindoline-5-carboxylic acid (5). 4-Hydrazinobenzoic acid (**4**) (5.0 g, 33 mmol) was suspended in ethanol (20 mL) and to this was added 2-methyl-2-butanone (4 mL, 37 mmol) followed by conc. sulphuric acid (1 mL). The mixture was stirred at reflux for 18 h. After cooling to r.t. the precipitate was removed by filtration and washed with acetonitrile. The filtrate was quenched with saturated sodium bicarbonate solution and washed with DCM 2 × 60 mL. The aqueous layer was carefully acidified to pH 5 (universal indicator paper) with 2 M aqueous hydrochloric acid solution and red product was extracted with DCM (3 × 60 mL), dried with $MgSO_4$ and solvent removed *in vacuo* to give **5** as a dark red solid (4.8 g) in 73% yield. mp. 198–202 °C, ^1H NMR (500 MHz, $CDCl_3$) δ 8.16 (d, J = 8.1 Hz, ^1H), 8.07 (s, 1H), 7.67 (d, J = 8.1 Hz, 1H), 2.38 (s, 3H), 1.38 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 192.4 (s), 171.3 (s), 157.6 (s), 145.6 (s), 130.9 (s), 126.6 (s), 123.2 (s), 119.7 (s), 53.9 (s), 22.9 (s), 15.6 (s). MS (m/z) for $C_{12}H_{13}NO_2$ + H ($[M + H^+]^+$) calcd 204.1025; found 204.1025.

1,3,3-Trimethyl-2-methyleneindoline-5-carboxylic acid (6). 3,3-Dimethyl-2-methyleneindoline-5-carboxylic acid (**5**) (3.9 g, 19 mmol) was dissolved in a solution of 2 : 1 toluene : acetonitrile (100 mL). To this was added iodomethane (1.3 mL, 21 mmol) and the solution was stirred at 95 °C for 24 h. The solution was cooled to r.t. and the precipitate was collected by filtration and washed with acetonitrile to give **6** (3.0 g) in 72% yield. mp. 154–157 °C, ^1H NMR (500 MHz, DMSO-d6) δ 8.36 (s, 1H), 8.17 (d, J = 8.1 Hz, 1H), 8.02 (d, J = 8.2 Hz, 1H), 4.00 (s, 3H), 2.82 (s, 3H), 1.57 (s, 6H); ^{13}C NMR (126 MHz, DMSO-d6) δ 199.0 (s), 166.5 (s), 145.2 (s), 141.9 (s), 131.6 (s), 130.3 (s), 124.2 (s), 115.4 (s), 54.3 (s), 35.2 (s), 21.5 (s), 14.8 (s). MS (m/z) for $C_{13}H_{16}NO_2$ ($[M]^+$) calcd 218.1181; found 218.1184.

Aza-12-crown-4-ether spiropyran (1). To a solution of 1-aza-12-crown-4-ether (**7**) (90 mg, 0.51 mmol) in dry THF (3 mL) was added triethylamine (80 μL, 1.1 mmol) and the solution cooled in an ice bath. To this mixture was added a solution of 3-(chloromethyl)-2-hydroxy-5-nitrobenzaldehyde (**10**) (0.11 g, 0.51 mmol) in dry THF (5 mL). The solution was allowed to warm to r.t. over 1 h followed by reflux for 17 h. The precipitate was removed by filtration and the solvent was removed *in vacuo* to give **11** as a yellow solid (0.19 g). ^1H NMR (500 MHz, DMSO-d6) δ 10.19 (d, J = 0.6 Hz, 1H), 8.27 (dd, J = 0.5, 3.0 Hz, 1H), 8.09 (d, J = 3.0 Hz, 1H), 4.28 (s, 2H), 3.83–3.75 (m, 5H), 3.68–3.51 (m, 11H); ^{13}C NMR (126 MHz, DMSO-d6) δ 190.1 (s), 178.9 (s), 130.7 (s), 129.9 (s), 126.4 (s), 123.7 (s), 122.3 (s), 70.1 (s), 69.8 (s), 64.6 (s), 56.5 (s), 54.3 (s). MS (m/z) for $C_{16}H_{22}N_2O_7$ + H ($[M + H^+]^+$) calcd 355.1505; found 355.1507. A sample of **11** (0.18 g) and **6** (0.18 g, 0.51 mmol) were dissolved in ethanol (10 mL) and the solution refluxed for 3 h. The solvent was removed *in vacuo* and the resulting purple solid (0.36 g) was purified twice by C18 reverse phase silica chromatography eluting with a gradient of acetonitrile in water to give **1** (50 mg) in a yield of 18% (based on **7**). mp. 118–121 °C, ^1H NMR (500 MHz, DMSO-d6) δ 12.31 (s, 1H), 8.35 (d, J = 2.6 Hz, 1H), 8.13 (d, J = 2.6 Hz, 1H), 7.81 (d, J = 8.2 Hz, 1H), 7.69 (s, 1H), 7.25 (d, J = 10.4 Hz, 1H), 6.70 (d, J = 8.2 Hz, 1H), 6.01 (d, J = 10.4 Hz, 1H), 3.58–3.36 (m, 18H), 2.73 (s, 3H), 1.25 (s, 3H), 1.14 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d6) δ 167.4 (s), 156.5 (s), 151.2 (s), 140.3 (s), 135.9 (s), 130.8 (s), 128.7 (s), 127.1 (s), 125.9 (s), 122.9 (s), 121.5 (s), 121.3 (s), 120.3 (s), 118.4 (s), 106.3 (s), 105.8 (s), 70.9 (s), 69.5 (s), 69.3 (s), 54.4 (s), 52.4(s), 51.3 (s), 28.4 (s), 25.5 (s), 19.5 (s). MS (m/z) for $C_{29}H_{35}N_3O_8$ + H ($[M + H^+]^+$) calcd 554.2502; found 554.2517

Aza-18-crown-6-ether spiropyran (3). To a solution of 1-aza-18-crown-6-ether (**9**) (0.25 g, 0.95 mmol) in dry THF (5 mL) was added triethylamine (0.16 mL, 2.2 mmol). The solution was cooled in an ice bath and to this was added dropwise a solution of 3-(chloromethyl)-2-hydroxy-5-nitrobenzaldehyde (**10**) (0.21 g, 0.97 mmol) in dry THF (7 mL). The solution was allowed to warm to r.t. over 1 h followed by reflux for 17 h. The precipitate was removed by filtration and the solvent was removed *in vacuo* to give **13** as a thick orange oil (0.48 g). ^1H NMR (500 MHz, DMSO-d6) δ 10.23 (s, 1H), 8.27 (d, J = 3.1 Hz, 1H), 8.13 (d, J = 3.1 Hz, 1H), 4.38 (s, 2H), 3.84–3.79 (m, 4H), 3.56 (s, 8H), 3.53 (s, 8H), 3.33–3.27 (m, 5H); ^{13}C NMR (126 MHz, DMSO-d6) δ 189.9 (s), 178.6 (s), 130.7 (s), 130.6 (s), 126.0 (s), 123.8 (s), 122.2 (s), 69.9 (s), 69.8 (s), 69.7 (s), 69.4 (s), 64.6 (s), 56.0 (s), 52.0 (s). MS (m/z) for $C_{20}H_{30}N_2O_9$ + H ($[M + H^+]^+$) calcd 443.2030; found 443.2009. A sample of **13** (0.47 g) and **6** (0.24 g, 1.1 mmol) were dissolved in ethanol (15 mL), and the solution refluxed for 18 h. Solvent was removed *in vacuo* to give purple crude solid (0.68 g, 1.1 mmol) of which 0.34 g was purified by C18 reverse phase silica chromatography eluting with a gradient of acetonitrile in water to give **3** (120 mg) in a yield of 20% (based on **10**). mp. 95–99 °C, ^1H NMR (500 MHz, DMSO-d6) δ 12.30 (s, 1H), 8.15–8.11 (m, 2H), 7.81 (d, J = 8.2 Hz, 1H), 7.68 (s, 1H), 7.24 (d, J = 10.3 Hz, 1H), 6.69 (d, J = 8.2 Hz, 1H), 6.00 (d, J = 10.3 Hz, 1H), 3.57–3.46 (m, 12H), 3.46–3.42 (m, 5H), 3.41 (s, 2H), 3.37–3.33 (m, 2H), 3.29–3.26 (m, 5H), 2.71 (s, 3H), 1.26 (s, 3H),

1.14 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d6) δ 167.9 (s), 157.1 (s), 151.6 (s), 140.6 (s), 136.4 (s), 131.2 (s), 129.2 (s), 127.4 (s), 126.4 (s), 123.3 (s), 122.1 (s), 121.9 (s), 120.8 (s), 118.9 (s), 106.8 (s), 106.2 (s), 70.4 (s), 70.4 (s), 70.3 (s), 70.0 (s), 69.3 (s), 53.7 (s), 52.1 (s), 51.7 (s), 28.8 (s), 26.1 (s), 19.8 (s). MS (*m*/*z*) for $C_{33}H_{43}N_3O_{10}$ + Na ($[M + Na^+]^+$) calcd 664.2846; found 664.2868.

Comparative assay procedure

Stock solutions of spiropyran **1–3** (100 μM) and metal ion salts (100 μM and 10 mM) were prepared in HPLC grade acetonitrile. Salt solutions were prepared from dried $LiClO_4$, $NaClO_4$, $KClO_4$ and Cs_2SO_4. On the same microplate tray, each spiropyran 100 μL was mixed separately with each of the metal solutions 100 μL (1 : 1 or 1 : 100 ratio spiropyran : metal ion) in triplicate. The absorbance and fluorescence spectra were recorded between 300 and 800 nm, and 552 and 802 nm, respectively, at 25 °C using a BioTek Synergy H4 Hybrid Multi-Mode Microplate Reader scanning with a resolution of 5 nm. Fluorescence excitation was at 532 nm with bandgap of 9 nm. The assay tray was then removed and repetitive photoswitching was performed by exposing to 352 nm UV light from a filtered 8 W Hg lamp (UVP), or halogen white lamp for 10 min each, with the absorbance and fluorescence spectra obtained after each irradiation.

Li^+ concentration dependence assay procedure

Stock solutions of spiropyran **1–3** (100 μM) and $LiClO_4$ (100 μM and 1 mM) were prepared in HPLC grade acetonitrile. On the same microplate tray in triplicate each spiropyran 100 μL was mixed separately with the $LiClO_4$ solutions and acetonitrile to make a total volume of 200 μL per well with spiropyran 50 μM and Li^+ 0, 5, 10, 20, 30, 40, 50, 100, 250, 500 μM. The solutions were left in the dark for 30 min. The absorbance and fluorescence spectra were recorded between 300 and 800 nm, and 552 and 802 nm, respectively, at 25 °C using a BioTek Synergy H4 Hybrid Multi-Mode Microplate Reader scanning with a resolution of 5 nm. Fluorescence excitation was at 532 nm with bandgap of 9 nm and gain of 100.

Acknowledgements

This research was supported by an Australian Research Council (ARC) grant to the Centre of Excellence for Nanoscale BioPhotonics (CNBP). This work was performed in part at the OptoFab node of the Australian National Fabrication Facility (ANFF) utilising Commonwealth and SA State Government funding.

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