The final publication is available at

http://doi.org/10.1039/c7cc01214d

Additional Information

Journal Name

COMMUNICATION

A new class of silica-supported chromo-fluorogenic chemosensors for anion recognition based on a selenourea scaffold.

Arianna Casula,[a] Antoni Llopi- Lorente,[b,c,d] Alessandra Garau,[a] Francesco Isaia, [a] Maciej Kubicki,[e] Vito Lippolis,[a] Félix Sancenón, [b,c,d] Ramón Martínez-Máñez, [b,c,d*] Agata Owczarzak,[e] Claudio Santi,[f] M. Andrea Scorciapino,[g] and Claudia Caltagirone[a*]
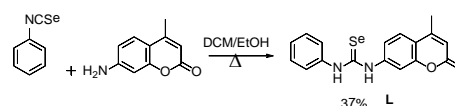
The first example of a chemosensor (L) containing a selenourea moiety is here described. L is able to colorimetrically sense the presence of CN^- and S^{2-} in $H_2O:MeCN$ (75:25, v/v). Moreover, when L is loaded in functionalised mesoporous silica nanoparticles an increase in the selectivity towards S^{2-} occurs via a selective fluorescent response.

The development of optical chemosensors for neutral and charged species recognition has been one of the most active research field in Supramolecular Chemistry in the last 20 years.[1-6] A vast library of chemosensors has been proposed so far, but only recently organoselenium and organotellurium compounds have been used for this purpose.[7, 8,9] Heavy chalcogen-containing derivatives, and in particular those containing selenium, are of great interest because selenium compounds play an important role as enzymatic antioxidants participating in the conversion of hydrogen peroxides in water in biological systems.[10] In the particular case of selenium-containing chemosensors, the formation of highly stable selenoxides is often the event that triggers the optical recognition of analytes such as reactive oxygen species (ROS) superoxide,[11] hypochlorite[12] or reactive nitrogen species (RNS, for instance peroxynitrate).[13] However, very few examples of selenium-containing optical chemosensors for "classic" anions such as fluoride[14] or carboxylates[15] have been described. In

reported selenium-containing probes, the sensing mechanism of fluoride is based on a deprotonation event of an amino group, while carboxylate recognition depends on the formation of hydrogen bonds between the guest and pyrimidine and amide protons of the receptor. However, in both cases, selenium is contained in a selenodiazole moiety and do not actively participate to the sensing event.

One of the most common strategy for the design of anion chemosensors is the use of hydrogen-bond donor groups such as urea or thiourea. In the case of thiourea-based chemosensors, due to the more acidic nature of the NHs protons (compared to those of the analogous urea derivatives), the recognition event is often caused by the deprotonation of the receptor that usually leads to a remarkable modification in the electronic properties of the system resulting in colour or emission changes. However, in this scenario, and, to the best of our knowledge, examples of selenourea-based anion sensors have never been reported in the literature to date despite selenoureas are interesting organoseleno compounds that show anticancer activity, antioxidant properties, enzyme inhibition, and DNA binding properties.[16-20] Taking these concepts into account and following our interest in anion recognition and sensing[21-25] we decided to explore the potential use of selenoureas as a new scaffold for the design of anion chemosensors.

In particular, here, we describe the ability of the asymmetric 1-(4-methyl-2-oxo-2H-chromen-7-yl)-3-phenylselenourea (L) to act as a colorimetric and/or fluorescent sensor for S^{2-} and CN^- recognition. L was obtained according to the reaction shown in Scheme 1.

a. Dipartimento di Scienze Chimiche e Geologiche, Università degli Studi di Cagliari, S.S. 554 Bivio per Sestu, 09042 Monserrato (CA), Italy. ccaltagirone@unica.it
b. Instituto Interuniversitario de Investigación de Reconocimiento Molecular y Desarrollo Tecnológico (IDM), Universitat Politècnica de València, Universitat de València, rmaez@qim.upv.es
c. Departamento de Quìmica, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain
d. CIBER de Bioingenierìa, Biomateriales y Nanomedicina (CIBER-BBN)
e. Faculty of Chemistry Adam Mickiewicz University Umultowska 89b, 61-614 Poznań, Poland
f. Università degli Studi di Perugia, Dipartimento di Scienze Farmaceutiche, Via del Liceo, 1, 06123 Perugia, Italy
g. Dipartimento di Scienze Biomediche, Università degli Studi di Cagliari, S.S. 554 Bivio per Sestu, 09042 Monserrato (CA), Italy

† Footnotes relating to the title and/or authors should appear here.
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

Scheme 1. Synthesis of 1-(4-methyl-2-oxo-2H-chromen-7-yl)-3-phenylselenourea (L).

Phenylisoselenocyanate was synthesised following a literature procedure.[26] Reaction with 7-amino-4-methylcoumarin in DCM/EtOH (1:1, v/v) at 40°C for 3 hours under N_2 atmosphere

afforded the desired selenourea **L** in 37% yield (see ESI for synthetic details). The colorimetric and sensing properties of **L** towards a set of anions (S^{2-}, CN^-, AcO^-, SO_3^{2-}, F^-, HCO_3^-, HSO_4^-, $H_2PO_4^-$, NO_3^-, Br^-, I^-, ClO_4^-, BzO^-, OH^-, SCN^-, and Cit^{3-}) were firstly tested in MeCN. Upon addition of one equivalent of S^{2-}, CN^-, AcO^-, F^-, HCO_3^-, or BzO^- to a MeCN solution of **L** (see ESI, Fig. S1), the formation of a new absorption band at around 360 nm with the presence of an isosbestic point at 333 nm was observed. Moreover, addition of an excess of anions (up to 50 equivalents, see ESI Fig. S2) caused severe changes in the absorption spectrum of **L**, suggesting the presence of multiple equilibria in solution. With the other anions tested (i.e. SO_3^{2-}, HSO_4^-, $H_2PO_4^-$, NO_3^-, Br^-, I^-, ClO_4^-, OH^-, SCN^-, and Cit^{3-}) no relevant changes in the absorption properties of **L** were observed. Upon excitation of the free receptor at 333 nm an emission band at 414 nm arose. However, upon addition of all the above-mentioned anions, no specific response in terms of changes of the fluorescence emission was found. In order to further investigate the sensing properties of **L**, we decided to repeat the measurements in a more competitive solvent mixture in terms of anion solvation properties and we moved towards the use of H_2O/MeCN 75:25 (v/v). In this solvent mixture **L** shows two absorption bands at 281 nm (ε = 16050 $M^{-1}cm^{-1}$) and at 324 nm (18330 $M^{-1}cm^{-1}$). Interestingly, in these conditions, we observed changes in the absorption spectrum of **L** only in the presence of S^{2-} (Fig. 1) and CN^- (see ESI Fig. S3). In particular, upon addition of a small aliquot of S^{2-} a dramatic change was observed (also visible to the naked eye, see ESI Figure S4) with the loss of the bands from free **L** and the formation of a new absorption band at 353 nm (ε = 25150 $M^{-1}cm^{-1}$). By fitting the experimental data using Hyspec[27] we were able to calculate the stability constant for the formation of a 2:2 complex (Log K_{22} = 15.5 (3) and Log K_{22} = 16.0 (4) for sulphide and cyanide, respectively). The Limit of Detection (LOD) in these experimental conditions was $1.0 \cdot 10^{-5}$ M and $9.8 \cdot 10^{-6}$ M for sulphide and cyanide, respectively. Addition of all the other anions did not cause any change in the UV-Vis spectrum of **L** in H_2O/MeCN 75:25 (v/v) (see ESI, Figs. S4 and S5). In addition, the emission properties of **L** in H_2O/MeCN 75:25 (v/v) were affected only by the presence of a large excess of S^{2-} (up to 100 equivalents) that caused a partial quenching (see ESI, Fig. S6) of the fluorescence emission of the free ligand. No emission changes were observed upon addition of CN^-.

We believed that the dramatic changes observed in such a competitive solvent mixture could not be merely ascribed to a simple hydrogen bond interaction between the anions and the NH groups of the neutral receptor. Therefore, in order to understand the behaviour of **L** in the presence of CN^- and S^{2-} in solution, we performed ^{1}H-, ^{13}C-, ^{77}Se-NMR spectroscopy and ESI-MS spectrometry experiments. NMR titrations could not be performed in H_2O/MeCN (75:25 v/v) due to low solubility of **L** at the concentration required for the experiments. NMR experiments of **L** in the presence of CN^- and S^{2-} were, therefore, performed in DMSO-d_6/0.5% H_2O (it was previously verified that absorption changes in H_2O/MeCN (75:25 v/v) upon the addition of anions were very similar to those

observed in DMSO, thus suggesting an analogous behaviour of **L** in these two different solvent media).
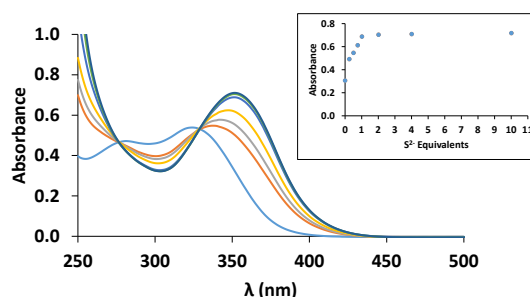

Figure 1. Changes in the absorption spectrum of **L** ($3.0 \cdot 10^{-5}$M) in H_2O/ MeCN (75:25 v/v) upon addition of increasing amounts (up to 10 equivalents) of Na_2S (0.1 M). Inset: Plot of absorbance at 353 nm *vs*. equivalents of S^{2-} added.

In the ^{1}H-NMR titration of **L** (0.05 M) with TBACN, upon addition of increasing amounts of CN^- (see ESI, Fig. S7) we observed the disappearance of the signal of the selenourea protons (green box in Fig. S7) at 10.2 ppm and the formation of two new peaks (red box in Fig. S7) at 4.2 ppm) presumably ascribable to amino NHs. In the ^{13}C spectrum ([**L**] = 0.2 M), we observed the disappearance of the signal at 179.01 ppm of the C=Se carbon and the formation of a signal at 61.92 ppm (attributed to the C-Se-Se-C carbon), and at 111.66 ppm (attributed to nitrile functional group CN) already upon addition of one equivalent of CN^- (see ESI, Fig. S8). These diagnostic signals were not affected by the addition of a second equivalent of CN^- (data not shown). In the case of the ^{77}Se spectrum ([**L**] = 0.2 M, see ESI, Fig. S9) an upfield shift from 314 to 243 ppm of the selenium signal of the free receptor was observed in the presence of one equivalent of CN^-. No further changes in the ^{77}Se spectrum were observed upon addition of a second equivalent of CN^-. This behaviour is in accordance with the formation of a Se-Se bond and a change of the hybridization of the selenium atom from sp^2 to sp^3.[28] On the basis of these results the mechanism shown in Scheme 2a could be proposed for the formation of the 2:2 **L**/CN^- complex **1** in DMSO/H_2O 95:0.5 (v/v), and in H_2O/MeCN 75:25 (v/v).

In the attempt to isolate the diselenide **1** (Scheme 2a), single crystals suitable of X-ray diffraction analysis were obtained by slow evaporation at room temperature of a solution of **L** in H_2O/MeCN 75:25 (v/v) in the presence of an excess of TBACN (see Scheme 2b and ESI, Fig. S10, Tables S2 and S3). Surprisingly, the structure shows the formation of the carbonocyanamidic amide **3**. Presumably, this compound originates from **1**, after an elimination reaction with the formation of black elemental selenium which was visible by naked-eye in the bottom of the vial where the crystals of **3** formed. It is worth noticing that this is the first crystal structure of a carbonocyanamidic amide, to the best of our knowledge.

The LR-ESI-MS spectrum of a 2:2 mixture of **L** and CN^- showed a peak at m/z 357 attributed to [**L**-H]$^-$ and a peak at m/z 715 attributed to the fragment [**1**-2HCN-H]$^-$ (see ESI, Fig. S11). The

isotopic pattern fits for the presence of one and two selenium atoms, respectively.
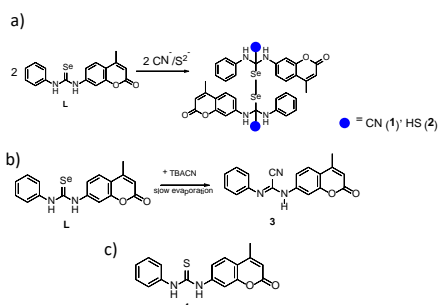
Scheme 2 a) Proposed detection mechanism of **L** for CN⁻ and S²⁻ in solution with the formation of the diselenides **1** and **2** in the presence of CN⁻ and S²⁻, respectively ; b) reaction scheme for the carbonocyanamidic amide **3**; c) 1-(4-methyl-2-oxo-2*H*-chromen-7-yl)-3-phenylthiourea **4**.

Interestingly, the elimination process was faster in the case of S²⁻. Indeed, we immediately observed the loss of the selenium signal (see ESI, Fig. S12) in the ^{77}Se spectrum ([**L**] = 0.2 M after the addition of one equivalent of S²⁻ with the formation of black elemental selenium (visible in the bottom of the NMR tube). The ^{1}H-NMR spectrum of the same NMR tube used for the ^{77}Se experiment, was found to be very similar to that of the thiourea **4** (see Scheme 2c) suggesting that the elimination reaction from the diselenide **2** leads to the formation of the corresponding 1-(4-methyl-2-oxo-2*H*-chromen-7-yl)-3-phenylthiourea (see ESI Fig. S13).

In addition, by LR-ESI-MS spectrometry, which allows for faster experiments at lower concentration with respect to ^{1}H- and ^{77}Se-NMR spectroscopy, we were able to observe in the spectrum of a solution of **L** and S²⁻ in 2:2 molar ratio (see ESI, Fig. S14) a peak at m/z 357 attributed to [**L**-H]⁻ and a peak at m/z 745 attributed to the fragment [**2**-H₂S-H]⁻. The isotopic pattern fits for the presence of one and two selenium atoms, respectively.

The tendency of selenium containing compounds such as selenoamides to form diselenide species in the presence of dihalogens and inter-halogens or even air is well documented in the literature[29] and it has been reported to have implications in the mechanism of action of thioamide and selenoamide-based antithyroid drugs.[30, 31] The presence of strong nucleophiles such as CN⁻, and S²⁻ appears to induce and facilitate this process in the case of **L**.

Due to the promising results obtained in H₂O/MeCN 75:25 (v/v) in terms of the selective optical sensing of CN⁻ and S²⁻, we were interested in evaluating the potential use of **L** as anion chemosensor in pure water. However, **L** proved to be insoluble in water, and therefore, no changes in optical properties were observed for **L** in H₂O in the presence of anions. As an alternative, we decided to use modified mesoporous silica nanoparticles (MSNs) as a support to host **L** in hydrophobic pores.[32] It was expected that the incorporation of **L** in "hydrophobic pockets" in mesoporous scaffolds would result in the possible use of the receptor for the selective sensing of certain anions in pure water. The prepared nanoparticles contained hydrophilic PEG groups in the external surface (to get stable suspensions) and hydrophobic trimethylsilyl groups in the inner pores (see ESI, Scheme S1).[33] Loading of the chemosensor in the functionalised MSNs was achieved by simply suspending the hybrid nanoparticles (60 mg) in a MeCN solution of **L** (1.3 mM) at room temperature for 12 h. Using thermogravimetric and elemental analysis we were able to calculate that 0.44 mmol of **L** per g SiO₂ were incorporated to the MSNs through this simple adsorption process and, as a consequence, a naked eye emission of the material was observed (Fig. 2).
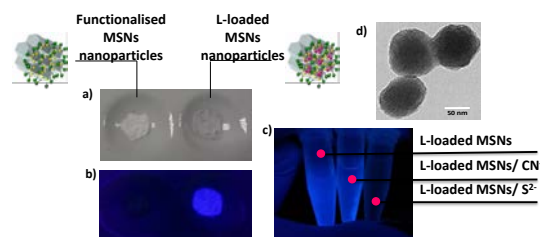

Figure 2. Differences with the empty MSNs (a) and the **L**-loaded nanoparticles (b) at light room and under a UV-Vis lamp (λ_{exc} = 365 nm); c) naked-eye fluorescence emission changes of **L**-loaded MSNs in water in the presence of CN⁻ and S²⁻ anions; d) TEM image of the functionalized MCM-41 S₄. Scale: 50 nm.

The different solids (nude, functionalised and **L**-loaded) were characterized by PXRD, thermogravimetric and elemental analysis, Porosimetry and TEM technique (see ESI, Figures S15-S16 and Table S3 and discussion therein).

In order to evaluate the anion sensing properties of **L** embedded in the silica nanoparticles, an excess of CN⁻ and S²⁻ was added into a dispersion of the **L**-loaded MSNs in pure water. Differently to what observed in H₂O/MeCN 75:25 (v/v) (see above), an almost complete quenching of the emission of the **L**-loaded nanoparticles was observed to the naked eye after the addition of S²⁻ (see Fig. 2c) whereas no emission changes were found in the case of cyanide.
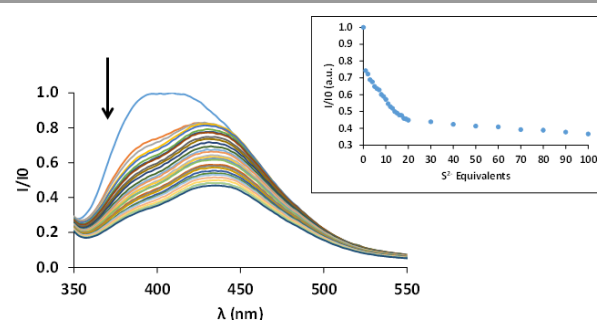

Figure 3. Changes in the emission spectrum of **L**-loaded silica nanoparticles (12mg nanoparticles/800μl of water correspondent to 4.7 10⁻³ mmol of **L**) in H₂O (λ_{exc} = 330 nm) upon addition of increasing amounts (up to 200 equivalents) of Na₂S (0.05M). Inset: Plot of emission (λ_{em} = 430 nm) vs. equivalents of S²⁻.

In order to further quantify the quenching effect caused by S²⁻, a fluorescence titration was carried out by adding this anion (0.05 M in water) into a water dispersion of the **L**-loaded nanoparticles (12mg nanoparticles/800μl of water corresponding to 4.7 10⁻³ mmol of **L**). After the addition of S²⁻

(up to 20 equivalents) a drastic decrease of the emission intensity of the sensing material at 394-427 nm (λ_{exc} = 330 nm) was observed as reported in Figure 3. This fluorescence response confirms a particular S^{2-} affinity for **L**, when the latter is loaded in MSNs, also supporting the concepts that encapsulation of **L** in in "hydrophobic pockets" in mesoporous supports is a suitable strategy to obtain a selective fluorogenic probes for S^{2-} sensing in water.

Conclusions

In conclusion, we have here described the first example of a simple selenourea **L** as a suitable probe for the chromo-fluorogenic sensing of CN^- and S^{2-}. ^{1}H-, ^{13}C-, ^{77}Se-NMR spectroscopy, and LR-ESI-MS spectrometry results support a sensing mechanism based on the formation of a diselenide derivative. Moreover, we have been able to observe an enhanced optical selectivity towards S^{2-}, with respect to CN^-, when **L** is embedded into functionalised MSNs containing hydrophobic pores. In this case, the selenourea worked as an ON-OFF fluorescent chemosensor for S^{2-} recognition in pure water. Encouraged by these interesting preliminary results, we are currently developing a family of fluorescent selenoureas for anion binding and sensing.

Notes and references

‡. Crystallographic data: CCDC 1521357. Formula: $C_{18}H_{13}N_3O_2$. Unit Cell Parameters: a 18.832(3) b 3.8846(6) c 20.977(3) P21/n.

1. S. Lee, K. K. Y. Yuen, K. A. Jolliffe and J. Yoon, *Chemical Society Reviews*, 2015, **44**, 1749-1762.
2. J. F. Zhang, Y. Zhou, J. Yoon and J. S. Kim, *Chemical Society Reviews*, 2011, **40**, 3416-3429.
3. X. Zhou, S. Lee, Z. Xu and J. Yoon, *Chemical Reviews*, 2015, **115**, 7944-8000.
4. Y. Zhou and J. Yoon, *Chemical Society Reviews*, 2012, **41**, 52-67.
5. N. Busschaert, C. Caltagirone, W. Van Rossom and P. A. Gale, *Chemical Reviews*, 2015, **115**, 8038-8155.
6. P. A. Gale and C. Caltagirone, *Chemical Society Reviews*, 2015, **44**, 4212-4227.
7. S. Panda, A. Panda and S. S. Zade, *Coordination Chemistry Reviews*, 2015, **300**, 86-100.
8. S. T. Manjare, Y. Kim and D. G. Churchill, *Accounts of Chemical Research*, 2014, **47**, 2985-2998.
9. D. Wu, L. Chen, N. Kwon and J. Yoon, *Chem*, 2016, **1**, 674-698.
10. A. J. Mukherjee, S. S. Zade, H. B. Singh and R. B. Sunoj, *Chemical Reviews*, 2010, **110**, 4357-4416.
11. S. T. Manjare, S. Kim, W. D. Heo and D. G. Churchill, *Organic Letters*, 2014, **16**, 410-412.
12. Y. Kim, M. Choi, S. T. Manjare, S. Jon and D. G. Churchill, *RSC Advances*, 2016, **6**, 32013-32017.
13. F. Yu, P. Li, G. Li, G. Zhao, T. Chu and K. Han, *Journal of the American Chemical Society*, 2011, **133**, 11030-11033.
14. C. Saravanan, S. Easwaramoorthi, C. Y. Hsiow, K. Wang, M. Hayashi and L. Wang, *Organic Letters*, 2014, **16**, 354-357.
15. S. Goswami, A. Hazra, R. Chakrabarty and H. K. Fun, *Organic Letters*, 2009, **11**, 4350-4353.
16. R. A. Hussain, A. Badshah and A. Shah, *Applied Organometallic Chemistry*, 2014, **28**, 61-73.
17. R. A. Hussain, A. Badshah, M. N. Tahir, T. U. Hassan and A. Bano, *Journal of Biochemical and Molecular Toxicology*, 2014, **28**, 60-68.
18. R. A. Hussain, A. Badshah, M. N. Tahir, B. Lal and I. A. Khan, *Australian Journal of Chemistry*, 2013, **66**, 626-634.
19. R. A. Hussain, A. Badshash, M. Sohail, B. Lal and A. A. Altaf, *Inorganica Chimica Acta*, 2013, **402**, 133-139.
20. H. Takahashi, A. Nishina, R. H. Fukumoto, H. Kimura, M. Koketsu and H. Ishihara, *Life Sciences*, 2005, **76**, 2185-2192.
21. C. Caltagirone, C. Bazzicalupi, A. Bencini, F. Isaia, A. Garau and V. Lippolis, *Supramolecular Chemistry*, 2012, **24**, 95-100.
22. C. Caltagirone, C. Bazzicalupi, F. Isaia, M. E. Light, V. Lippolis, R. Montis, S. Murgia, M. Olivari and G. Picci, *Organic and Biomolecular Chemistry*, 2013, **11**, 2445-2451.
23. A. Casula, C. Bazzicalupi, A. Bettoschi, E. Cadoni, S. J. Coles, P. N. Horton, F. Isaia, V. Lippolis, L. K. Mapp, G. M. Marini, R. Montis, M. A. Scorciapino and C. Caltagirone, *Dalton Transactions*, 2016, **45**, 3078-3085.
24. M. Olivari, R. Montis, L. E. Karagiannidis, P. N. Horton, L. K. Mapp, S. J. Coles, M. E. Light, P. A. Gale and C. Caltagirone, *Dalton Transactions*, 2015, **44**, 2138-2149.
25. M. A. Tetilla, M. C. Aragoni, M. Arca, C. Caltagirone, C. Bazzicalupi, A. Bencini, A. Garau, F. Isaia, A. Laguna, V. Lippolis and V. Meli, *Chemical Communications*, 2011, **47**, 3805-3807.
26. J. G. Fernández-Bolaños, Ó. López, V. Ulgar, I. Maya and J. Fuentes, *Tetrahedron Letters*, 2004, **45**, 4081-4084.
27. P. Gans, A. Sabatini and A. Vacca, *Talanta*, 1996, **43**, 1739-1753.
28. H. Deddek, in *Progress in NMR Spectroscopy*, Pergamon, Oxford, 1995, vol. 27, pp. 1-323.
29. F. Bigoli, F. Demartin, P. Deplano, F. A. Devillanova, F. Isaia, V. Lippolis, M. L. Mercuri, M. A. Pellinghelli and E. F. Trogu, *Inorganic Chemistry*, 1996, **35**, 3194-3201.
30. F. Isaia, M. C. Aragoni, M. Arca, F. Demartin, F. A. Devillanova, G. Floris, A. Garau, M. B. Hursthouse, V. Lippolis, R. Medda, F. Oppo, M. Pira and G. Verani, *Journal of Medicinal Chemistry*, 2008, **51**, 4050-4053.
31. G. Roy, K. P. Bhabak and G. Mugesh, *Crystal Growth & Design*, 2011, **11**, 2279-2286.
32. E. Climent, R. Martínez-Máñez, F. Sancenón, M. D. Marcos, J. Soto, A. Maquieira and P. Amorós, *Angewandte Chemie - International Edition*, 2010, **49**, 7281-7283.
33. L. E. Santos-Figueroa, C. Giménez, A. Agostini, E. Aznar, M. D. Marcos, F. Sancenón, R. Martínez-Máñez and P. Amorós, *Angewandte Chemie - International Edition*, 2013, **52**, 13712-13716.