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COMMUNICATION

Simple pyrazoline and pyrazole "turn on" fluorescent sensors selective for Cd^{2+} and Zn^{2+} in MeCN

Alexander Ciupa,[a] Mary F. Mahon,[b] Paul A. De Bank*[a] and Lorenzo Caggiano*[a]

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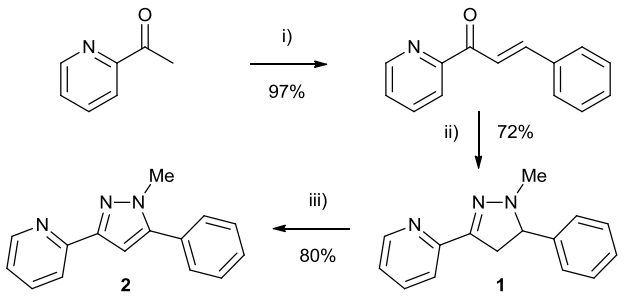
An efficient two-step synthesis of pyrazoline ligand 1 is described which is an effective "turn on" fluorescent sensor for Cd^{2+} in MeCN. Oxidation to the corresponding pyrazole ligand 2 creates a "turn on" fluorescent sensor now selective for Zn^{2+} and able to distinguish it from Cd^{2+}.

Cadmium is a toxic element widely distributed throughout the environment originating from natural sources and as a pollutant from industrial and agricultural use.[1] Cadmium can accumulate in the body over a period of years and has been linked to various diseases including cancer, even at low-levels of exposure.[1-2] Therefore the facile detection and monitoring of cadmium in various media is of great importance.[3]

Zinc is chemically similar to cadmium due to its position in the periodic table, yet it has a vital role in many biological processes.[4] The detection of zinc in biological systems has become a rapidly emerging field as excess free zinc is toxic.[5] Many Cd^{2+} and Zn^{2+} probes are based on fluorescent sensors which upon chelation to the cation result in a decrease ("turn off") or increase ("turn on") in fluorescence intensity. A large variety of complex and sophisticated Zn^{2+} fluorescent sensors have been reported.[5-6] Various Cd^{2+} sensors have also been reported,[3, 6l, 7] some of which are able to distinguish cadmium from zinc ions.[6l, 7c-f] We report herein a simple two-step synthesis of a "turn on" fluorescent sensor selective for Cd^{2+} which can be readily oxidised in high yield to afford a "turn on" fluorescent sensor selective for Zn^{2+} and able to differentiate it from Cd^{2+}.

The ligands are based on the pyrazoline motif as it has been previously reported to possess favourable photophysical properties[8] and chelate a variety of metals,[6a-c, 9] including Zn^{2+}.[6a-c, 9a, 9b] It is an attractive and versatile scaffold due to the ease of synthesis from a large range of commercially available acetophenones and benzaldehydes.

Pyrazoline **1** and related pyrazole ligand **2** were synthesised as outlined in Scheme 1. Following previous literature precedent,[10] 2-acetylpyridine underwent Claisen-Schmidt condensation with benzaldehyde in the presence of catalytic NaOH to afford the corresponding chalcone in 97% yield. Treatment of the chalcone with an excess of methylhydrazine gave the pyrazoline ligand **1** as the sole product in 72% yield. Similar transformations suggest the reaction proceeds *via* 1,2-addition followed by cyclisation and not initial 1,4-addition, which could generate a different isomer.[8c] Following a procedure for the oxidation of a 1,2,3,4-tetrahydro-β-carboline,[11] pyrazoline **1** was readily oxidised with Pd/C to afford



Scheme 1 Synthesis of pyrazoline **1** and pyrazole **2**. i) PhCHO, 10% NaOH(aq), 24h, ii) H_2NNHMe, EtOH, 3h, iii) 10 mol% Pd/C, 200 °C, 4h

pyrazole **2** in 80% yield. This simple synthesis is highly scalable as it uses common commercially available starting materials and all the products are highly crystalline.

The pyrazoline ligand **1** has been previously reported complexed with ruthenium, although no other metals were described.[9f] Similarly, the synthesis of pyrazole **2** was recently reported by an alternative route although no chelation properties were reported.[12] We now describe the investigation of pyrazoline **1** and pyrazole **2** with various cations‡ analysed by UV/Vis, NMR and fluorescence spectroscopy.

UV/Vis spectroscopy was performed in MeCN, as similar ligands were investigated in this solvent,[6a] and in the presence of Group 1 and 2 metals produced negligible results with both ligands. Upon the addition of various transition metals, however, both ligands **1** and **2** showed spectral changes consistent with chelation. The effect of Zn^{2+} and Cd^{2+} with pyrazoline **1** is given in Figure 1 and is representative of the various metals examined with ligands **1** and **2** (the complete list is available in the Electronic Supplementary Information). The absorbance at 320 nm (ε = 14800 $M^{-1}cm^{-1}$) is due to the pyrazoline ligand **1** only and disappears upon the addition of Zn^{2+} or Cd^{2+}. The formation of a new band at 360 nm with Zn^{2+} (ε = 8650 $M^{-1}cm^{-1}$, Figure 1A) and 350 nm with Cd^{2+} (ε = 7650 $M^{-1}cm^{-1}$, Figure 1B) is evident, which increased proportionally up to 1.0 equivalent of the metal ion and levelled off thereafter, suggesting a 1:1 ratio of the metal to ligand. Job plot analysis also suggests the formation of a 1:1 complex with a 0.5 molar ratio of pyrazoline **1** and Zn^{2+} or Cd^{2+} (Figure 1).

This is consistent with a crystal structure of a similar pyrazoline ligand in a 2:2 complex with Zn^{2+}, with chloride atoms acting as bridging ligands.[9a] Attempts to obtain a crystalline

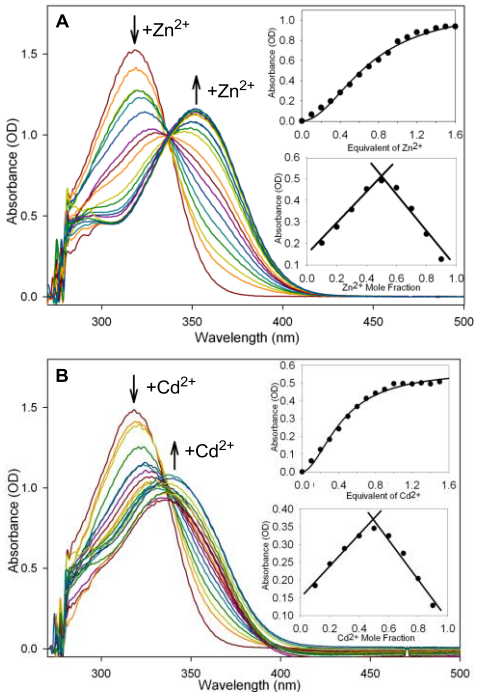


Figure 1 Absorbance spectra of pyrazoline **1** (MeCN, 500 μM) with the addition of 0-1.5 equiv. (0.1 increments) of Zn^{2+} (**A**) and Cd^{2+} (**B**). Insets at λ_{em} = 370 nm. Lower inset Job plot

complex of pyrazoline **1** with Zn^{2+} using the previously reported conditions (EtOH/H_2O, reflux, 24 h),[9a] instead resulted in a Zn^{2+} complex with the *pyrazole* ligand **2** (Figure 2). This is presumably the result of aerobic and/or zinc mediated oxidation during the harsh recystalisation process. Interestingly, the asymmetric unit was seen to contain four independent Zn(pyrazole)Cl_2 motifs. Three of these are 1:1 monomers but the fourth, located proximate to a crystallographic inversion centre, forms a 2:2 dimer, with bridging chloride atoms (ESI, Figure 2 only shows one of the monomeric 1:1 structures for clarity).

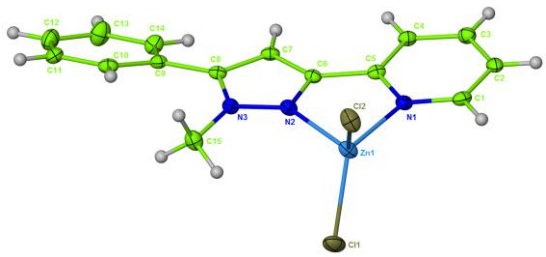


Figure 2 Ortep-3 representation of one of the three 1:1 monomers containing pyrazole ligand **2** and Zn^{2+} in the asymmetric unit of the crystal structure, with the ellipsoids represented at 30% probability[13]

The interaction of the pyrazoline **1** and pyrazole **2** ligands with Zn^{2+} and Cd^{2+} was also analysed by ^{1}H NMR spectroscopy. The addition of Cd^{2+} to pyrazole **2** is representative of the results obtained and is shown in Figure 3 (complete study shown in ESI). Significant chemical shifts of the pyridine and pyrazole protons were observed upon the addition of the metal, broadening and moving downfield as previously reported for other sensors upon chelation to Zn^{2+} [6g-k, 7c] and Cd^{2+} [6j, 6l, 7a-c] (Figure 3).

Pyrazoline **1** and pyrazole **2** were examined by fluorescence

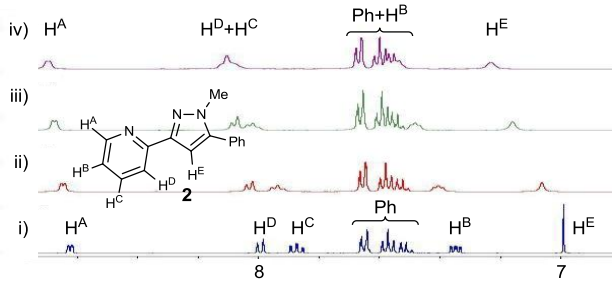


Figure 3 Partial ^{1}H NMR spectra of i) pyrazole **2** (DMSO-d_6, 63 mM) with ii) 0.9, iii) 2.0 and iv) 3.0 equiv. Cd^{2+}

spectroscopy in MeCN at 20 μM, as previously reported with similar ligands.[6a] No change in fluorescence was observed with various Group 1, 2 and transition metals, while exposure to Zn^{2+} or Cd^{2+} produced a large increase in fluorescence for both ligands **1** and **2** (Figure 4 and ESI). This result is particularly pleasing as it demonstrates that although there is no selectivity in the absorbance spectroscopy of either ligand with various metals, they are however only fluorescent in the presence of either Zn^{2+} or Cd^{2+}. Moreover, with pyrazole **2** they fluoresce at different wavelengths providing a "turn on" fluorescent sensor that can distinguish between Zn^{2+} and Cd^{2+} in MeCN. The effect of different solvents on fluorescence was investigated with ligand **1** and **2** with Zn^{2+} and gave variable results, with complete fluorescence quenching observed in the presence of water (ESI).

The addition of 5 equivalents of Zn^{2+} to pyrazoline **1** resulted in an 8 fold increase in fluorescence at 460 nm, whereas 5 equivalents of Cd^{2+} gave a 14 fold increase in fluorescence also at 460 nm with a Stokes shift of 100 nm (Figure 4A and 5). As previously observed in the UV/Vis study, Job plot analysis is consistent with a 1:1 ratio of metal to ligand for both cations

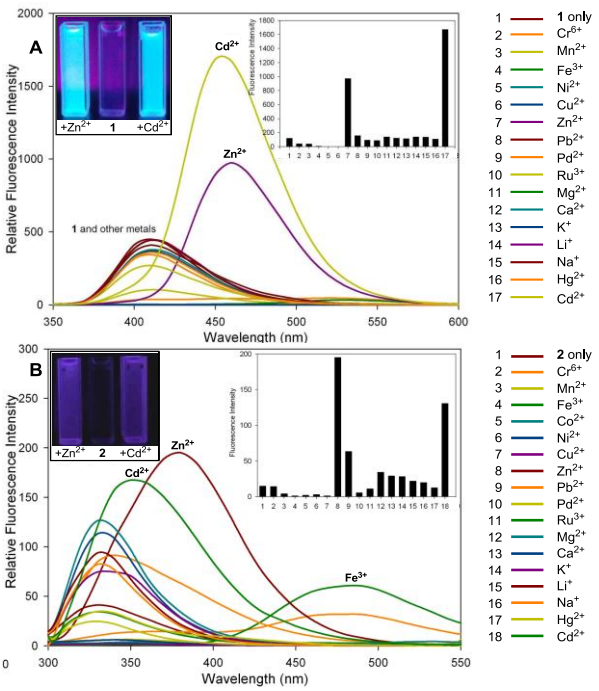


Figure 4 Fluorescence spectra of pyrazoline **1** (**A**, λ_{ex}=320 nm) and pyrazole **2** (**B**, λ_{ex}=285 nm, MeCN, 20 μM), upon addition of 5 equiv. of metal. Metal screen at λ_{em}=460 nm (**A**) and 380 nm (**B**). Inset shows fluorescence with Zn^{2+} and Cd^{2+} at 313 nm (**A**) and 254 nm (**B**)

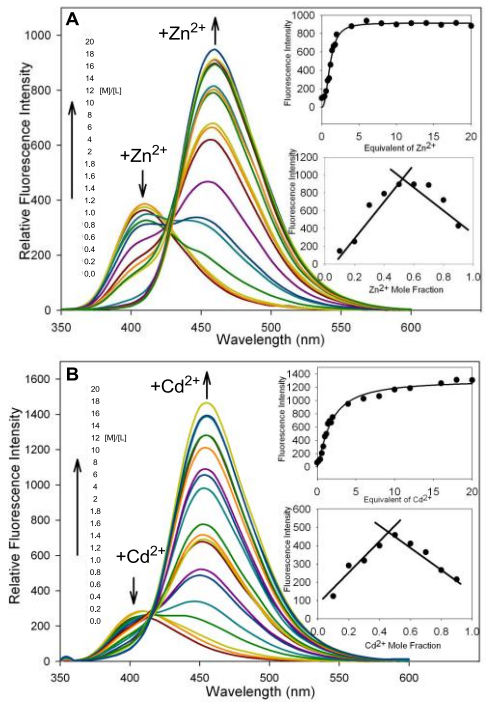


Figure 5 Fluorescence spectra of pyrazoline **1** (MeCN, 20 μM, λ_{ex}=320 nm) upon addition of 0-20 equiv. Zn^{2+} (**A**) and Cd^{2+} (**B**). Insets at λ_{em}=460 nm upon addition of cation. Lower inset Job plot

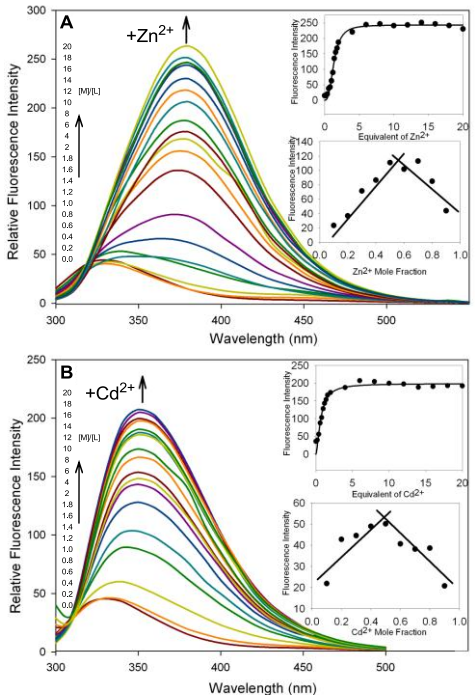


Figure 6 Fluorescence spectra of pyrazole **2** (MeCN, 20 μM, λ_{ex}=285 nm) upon addition of 0-20 equiv. Zn^{2+} (**A**) and Cd^{2+} (**B**). Insets at λ_{em}=380 nm (**A**) and 350 nm (**B**) upon addition of cation. Lower inset Job plot

(Figure 5 insets). Similar pyrazolines have been previously reported as ligands[9a, 9b] or sensors[6a-c] for Zn^{2+}. The data presented here however shows that the pyrazoline ligand **1** is more sensitive towards Cd^{2+} than Zn^{2+} and could be a useful "turn on" fluorescent sensor for cadmium.

Conversely, the related pyrazole ligand **2** displays increased fluorescence in the presence of Zn^{2+} than Cd^{2+} (Figure 4B and 6). A 13 fold increase in fluorescence at 380 nm is observed with Zn^{2+} with a Stokes shift of 90 nm, whereas Cd^{2+} only exhibits a 5 fold increase in fluorescence at 350 nm with a Stokes shift of 60 nm. Titration with Zn^{2+} and Cd^{2+} is again consistent with the 1:1 stoichiometry previously observed by the UV/Vis analysis and X-ray crystallography of pyrazole **2** with Zn^{2+} (Figure 6 insets). Although it is challenging to selectively distinguish Zn^{2+} from Cd^{2+} ions due to similar physical properties, the 30 nm difference between the Zn^{2+} (380 nm) and Cd^{2+} (350 nm) emission maxima enables the pyrazole ligand **2** to distinguish these cations. In addition, the pyrazole ligand **2** exhibits increased sensitivity for Zn^{2+} providing an effective "turn on" fluorescent sensor for Zn^{2+}.

Following previous reports,[6c, 14] the detection limits of Zn^{2+} and Cd^{2+} by the ligands **1** and **2** were calculated and show that the pyrazoline **1** has a detection limit of Zn^{2+} 0.20 μM and Cd^{2+} 0.12 μM (ESI). Pyrazole **2** has a detection limit of Zn^{2+} 0.24 μM and Cd^{2+} 0.34 μM, again highlighting that pyrazoline **1** is a more effective fluorescent sensor for Cd^{2+} and pyrazole **2** a more effective sensor for Zn^{2+}.

Competition assays were performed with ligands **1** and **2** to investigate the effect of detecting Zn^{2+} or Cd^{2+} in the presence of other cations (Figure 7 and ESI). The addition of Mn^{2+}, Pb^{2+}, Ru^{3+}, Mg^{2+}, Ca^{2+} and Hg^{2+} to a mixture of pyrazoline **1** and Cd^{2+} results in only minor decreases in fluorescence (Figure 7). The ability to detect Cd^{2+} even in the presence of heavy metals such as Pb^{2+} and Hg^{2+} is of particular interest.

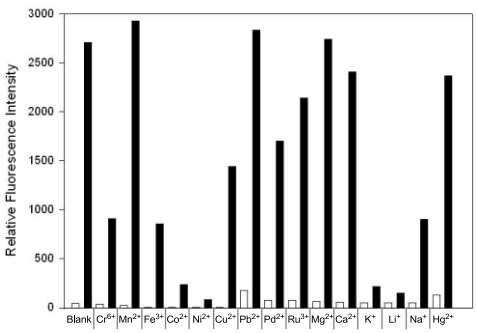


Figure 7 Competition experiments. The white bar represents pyrazoline **1** (MeCN, 20 μM, λ_{ex}=350 nm, λ_{em}=460 nm) and 5 equiv. of the cation; the black bar is the same plus 5 equiv. Cd^{2+} after equilibrating for 3 min.

Significant fluorescence quenching was observed upon the addition of the paramagnetic metals such as Fe^{3+}, Co^{2+} and Ni^{2+} as previously reported with other sensors.[6c-f, 7c] A similar trend was observed for competition experiments with pyrazoline **1** with Zn^{2+}, although fluorescence quenching was more pronounced (ESI). Combined with the increased sensitivity for Cd^{2+} over Zn^{2+} (Figure 3), these results suggest that the pyrazoline ligand **1** is more suitable as a Cd^{2+} fluorescent sensor.

Competition assays were also performed with pyrazole **2** in the presence of either Zn^{2+} or Cd^{2+} (ESI). In both cases large variations were observed, although heavy metals such as Hg^{2+} and Pb^{2+} had little or no effect on fluorescence in competition with Zn^{2+} in the presence of pyrazole **2**.

In summary, pyrazoline **1** and pyrazole **2** were prepared in an efficient synthesis and are effective “turn on” fluorescent sensors in MeCN for Cd^{2+} and Zn^{2+} respectively. In addition, the pyrazole

ligand **2** can distinguish between these ions in MeCN and therefore complements other sensors which can differentiate these ions in aqueous media.[6j-l, 7c-f] The ligands are also able to detect Zn^{2+} or Cd^{2+} in the presence of other heavy metals, which is of great importance for industrial applications. Although these simple ligands do not operate in aqueous media and suffer from competition with some biological ions, the modular design and molecular framework of the ligands allows for further functionalisation to fine-tune desirable physicochemical properties, which will be investigated and reported in due course.

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Notes and references

[a] *Medicinal Chemistry, Department of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath, BA2 7AY, UK. Fax: +44 1225 386114; Tel: +44 1225 385709; E-mail: l.caggiano@bath.ac.uk*
[b] *X-ray Crystallography Unit, Department of Chemistry, University of Bath, Claverton Down, Bath BA2 7AY, UK.*

† Electronic Supplementary Information (ESI) available: Experimental procedures, characterisation data, ^{1}H NMR and ^{13}C NMR spectra are provided. Data from UV/Vis and fluorescence spectroscopy, NMR titration experiments, competition experiments, X-ray crystallography, calculations of detection limits and extintion co-efficients are also provided. See DOI: 10.1039/b000000x/

‡ The metal complexes used in this study were all metal chlorides (*i.e.* MCl_x), except LiBr, CrO_3, $Pd(OAc)_2$, $Hg(OAc)_2$ and $Cd(OAc)_2.2H_2O$. The complete list is provided in the ESI.

§ Crystal data for pyrazole **2** complexed with $ZnCl_2$, CCDC 885728. Formula: $C_{60}H_{52}Cl_8N_{12}Zn_4$. M = 1486.22, monoclinic. Unit cell parameters: a = 14.2600(2) Å, b = 26.1400(4) Å, c = 16.8220(2) Å. α = 90°, β = 101.476(1)°, γ = 90°, V = 6145.15(15) Å^3, T = 150(2) K, space group $P2_1/c$, Z = 4, 99 814 reflections collected, 14032 independent reflections [R(int) = 0.0737]. Final R indices [I>2σ(*I*)] R_1 = 0.0452 and wR_2 = 0.1015. R indices (all data) R_1 = 0.0782 and wR_2 = 0.1156.

1. L. Järup and A. Åkesson, *Toxicol. Appl. Pharmacol.*, 2009, **238**, 201-208.
2. (a) A. Hartwig, *BioMetals*, 2010, **23**, 951-960; (b) K. S. Abrahim, N. B. Abdel-Gawad, A. M. Mahmoud, M. M. El-Gowaily, A. M. Emara and M. M. Hwaihy, *Toxicol. Ind. Health*, 2011, **27**, 173-179; (c) C. T. McMurray and J. A. Tainer, *Nat. Gen.*, 2003, **34**, 239-241.
3. H. N. Kim, W. X. Ren, J. S. Kim and J. Yoon, *Chem. Soc. Rev.*, 2012, **41**, 3210-3244.
4. (a) C. J. Frederickson, J.-Y. Koh and A. I. Bush, *Nat. Rev. Neurosci.*, 2005, **6**, 449-462; (b) S. L. Sensi, P. Paoletti, A. I. Bush and I. Sekler, *Nat Rev Neurosci*, 2009, **10**, 780-791.
5. (a) E. Kimura and S. Aoki, *BioMetals*, 2001, **14**, 191-204; (b) P. Jiang and Z. Guo, *Coord. Chem. Rev.*, 2004, **248**, 205-229; (c) K. Kikuchi, K. Komatsu and T. Nagano, *Curr. Opin. Chem. Biol.*, 2004, **8**, 182-191; (d) D. W. Domaille, E. L. Que and C. J. Chang, *Nat. Chem. Biol.*, 2008, **4**, 168-175; (e) Z. Xu, J. Yoon and D. R. Spring, *Chem. Soc. Rev.*, 2010, **39**, 1996-2006; (f) M. D. Pluth, E. Tomat and S. J. Lippard, *Annu. Rev. Biochem*, 2011, **80**, 333-355.
6. Selected examples include: (a) P. Wang, N. Onozawa-Komatsuzaki, Y. Himeda, H. Sugihara, H. Arakawa and K. Kasuga, *Tetrahedron Lett.*, 2001, **42**, 9199-9201; (b) Z.-L. Gong, B.-X. Zhao, W.-Y. Liu and H.-S. Lv, *J. Photochem. Photobiol., A*, 2011, **218**, 6-10; (c) Z.-L. Gong, F. Ge and B.-X. Zhao, *Sens. Actuators, B*, 2011, **159**, 148-153; (d) A. E. Dennis and R. C. Smith, *Chem. Commun.*, 2007, 4641-4643; (e) C.-H. Hung, G.-F. Chang, A. Kumar, G.-F. Lin, L.-Y. Luo, W.-M. Ching and E. Wei-Guang Diau, *Chem. Commun.*, 2008, 978-980; (f) H. Chen, W. Gao, M. Zhu, H. Gao, J. Xue and Y. Li, *Chem. Commun.*, 2010, **46**, 8389-8391; (g) M. Natali, L. Soldi and S. Giordani, *Tetrahedron*, 2010, **66**, 7612-7617; (h) E. Manandhar, J. H. Broome, J. Myrick, W. Lagrone, P. J. Cragg and K. J. Wallace, *Chem. Commun.*, 2011, **47**, 8796-8798; (i) Z. Li, L. Zhang, L. Wang, Y. Guo, L. Cai, M. Yu and L. Wei, *Chem. Commun.*, 2011, **47**, 5798-5800; (j) Z. Xu, K.-H. Baek, H. N. Kim, J. Cui, X. Qian, D. R. Spring, I. Shin and J. Yoon, *J. Am. Chem. Soc.*, 2010, **132**, 601-610; (k) Z. Xu, X. Liu, J. Pan and D. R. Spring, *Chem. Commun.*, 2012, **48**, 4764-4766; (l) Y. Pourghaz, P. Dongare, D. W. Thompson and Y. Zhao, *Chem. Commun.*, 2011, **47**, 11014-11016.
7. Selected examples include: (a) M. Mameli, M. C. Aragoni, M. Arca, C. Caltagirone, F. Demartin, G. Farruggia, G. De Filippo, F. A. Devillanova, A. Garau, F. Isaia, V. Lippolis, S. Murgia, L. Prodi, A. Pintus and N. Zaccheroni, *Chem. Eur. J.*, 2010, **16**, 919-930; (b) L. Xue, G. Li, Q. Liu, H. Wang, C. Liu, X. Ding, S. He and H. Jiang, *Inorg. Chem.*, 2011, **50**, 3680-3690; (c) L. Xue, C. Liu and H. Jiang, *Org. Lett.*, 2009, **11**, 1655-1658; (d) Y. Bao, B. Liu, H. Wang, F. Du and R. Bai, *Analytical Methods*, 2011, **3**, 1274-1276; (e) J. L. Vinkenborg, S. M. J. van Duijnhoven and M. Merkx, *Chem. Commun.*, 2011, **47**, 11879-11881; (f) Z. Liu, C. Zhang, W. He, Z. Yang, X. Gao and Z. Guo, *Chem. Commun.*, 2010, **46**, 6138-6140.
8. (a) C. J. Fahrni, L. Yang and D. G. VanDerveer, *J. Am. Chem. Soc.*, 2003, **125**, 3799-3812; (b) J. Cody, S. Mandal, L. Yang and C. J. Fahrni, *J. Am. Chem. Soc.*, 2008, **130**, 13023-13032; (c) Z.-L. Gong, L.-W. Zheng, B.-X. Zhao, D.-Z. Yang, H.-S. Lv, W.-Y. Liu and S. Lian, *J. Photochem. Photobiol., A*, 2010, **209**, 49-55; (d) M. Verma, A. F. Chaudhry, M. T. Morgan and C. J. Fahrni, *Org. Biomol. Chem.*, 2010, **8**, 363-370; (e) M. Barceló-Oliver, A. Terrón, A. García-Raso, N. Lah and I. Turel, *Acta Crystallogr. Sect. C: Cryst. Struct. Commun.*, 2010, **66**, o313-o316.
9. (a) M. Barceló-Oliver, A. Terrón, A. García-Raso, I. Turel and M. Morell, *Acta Crystallogr. Sect. Sect. E: Struct. Rep. Online*, 2010, **66**, m899-m900; (b) H.-h. Zhang, W. Dou, W.-s. Liu, X.-l. Tang and W.-w. Qin, *Eur. J. Inorg. Chem.*, 2011, **2011**, 748-753; (c) A. Satake and T. Nakata, *J. Am. Chem. Soc.*, 1998, **120**, 10391-10396; (d) V. Montoya, J. Pons, J. García-Antón, X. Solans, M. Font-Bardía and J. Ros, *Organometallics*, 2007, **26**, 3183-3190; (e) M. Verma, A. F. Chaudhry and C. J. Fahrni, *Org. Biomol. Chem.*, 2009, **7**, 1536-1546; (f) C. Marzin, F. Budde, P. J. Steel and D. Lerner, *New J. Chem.*, 1987, **11**, 33-41.
10. (a) S. Otto, F. Bertoncin and J. B. F. N. Engberts, *J. Am. Chem. Soc.*, 1996, **118**, 7702-7707; (b) E. B. Mubofu and J. B. F. N. Engberts, *J. Phys. Org. Chem.*, 2004, **17**, 180-186.
11. J. McNulty and I. W. J. Still, *J. Chem. Soc., Perkin Trans. 1*, 1994, 1329-1337.
12. A. Tinarelli, P. Righi, G. Rosini, D. Andreotti, R. Profeta and S. Spada, *Tetrahedron*, 2011, **67**, 612-617.

13. L. J. Farrugia, *J. Appl. Crystallogr.*, 1997, **30**, 565.
14. B. P. Joshi, J. Park, W. I. Lee and K.-H. Lee, *Talanta*, 2009, **78**, 903-909.