Dalton Transactions

RSC Publishing

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Dalton Trans.*, 2013, **42**, 12844

Received 15th June 2013,
Accepted 11th July 2013

DOI: 10.1039/c3dt51591e

www.rsc.org/dalton

Selective detection and bio-imaging of Pd^{2+} with novel 'C–CN' bond cleavage of cyano-rhodamine, cyanation with diaminomaleonitrile†

Shyamaprosad Goswami,*[a] Abhishek Manna,[a] Anup Kumar Maity,[b] Sima Paul,[a] Avijit Kumar Das,[a] Manas Kumar Das,[a] Partha Saha,[b] Ching Kheng Quah[c] and Hoong-Kun Fun[c,d]

A new rhodamine based chemosensor, cyano-rhodamine, has been designed and synthesized with a green approach which shows a specific 'C–CN' bond breaking with the action of the Pd^{2+} ion to produce the specific color and fluorescence of rhodamine 6G itself in solution and in HeLa cells.

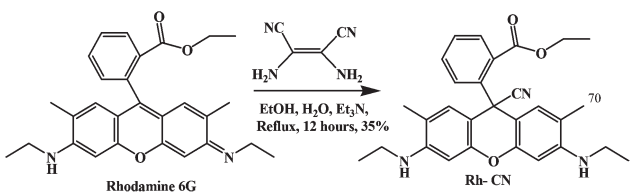
Recently, a great deal of attention has been paid to the determination of palladium (Pd) species among the metal ions because of its extensive use in numerous materials such as alloys, jewellery, dental crowns, fuel cells and catalysts.[1] Palladium(II) salts are predominantly used as oxidizing reagents and pre-catalysts for many cross-coupling reactions.[2] However, the largest use of palladium today is in catalytic converters of automobiles that frequently release a large quantity of palladium to the environment[3] which may cause a serious health problem[4] owing to the formation of complexes between Pd and some biomacromolecules such as proteins, DNA and RNA.[5] Thus, government restrictions on the levels of residual heavy metals in end-products are very strict, and its threshold for palladium is 5–10 ppm.[6]

Hence, considerable attention has been paid to the development of methods for selective sensing of palladium. Colorimetric and fluorimetric methods for the quick detection of Pd^{2+} have been very popular in recent years because they are inexpensive and nondestructive in nature[7] compared with high cost instrumental analysis.[8]

Being a paramagnetic species, most of the reported fluorescence sensors of Pd^{2+} readily undergo fluorescence quenching.[9] Despite advances in the development of highly selective and sensitive fluorescent indicators for palladium,[10] there are still some limitations on the quantitative detection and bio-imaging including complicated synthetic procedures, poor water solubility and selectivity over metal ions including Pd(0). Furthermore, so far, few reports on the imaging of palladium in living systems have been published.[10e,h] Thus, novel cell-permeable fluorescence indicators for palladium species became our target.

Herein, we report a new cyano-rhodamine (Rh-CN) based colorimetric 'off–on' fluorescence probe that can selectively detect Pd^{2+} over other examined metal ions studied including Pd(0) in aqueous solution. In general, the rhodamine architecture provides an ideal mode for the construction of 'off–on' type fluorescence-enhanced probes based on spirolactam (colorless, nonfluorescence) to ring-open amide (pink colored, fluorescence) equilibrium,[11] but in this paper we report a novel selective 'C–CN' bond breaking approach on the rhodamine moiety to gain its color and fluorescence with the addition of Pd^{2+} in solutions and in cells.

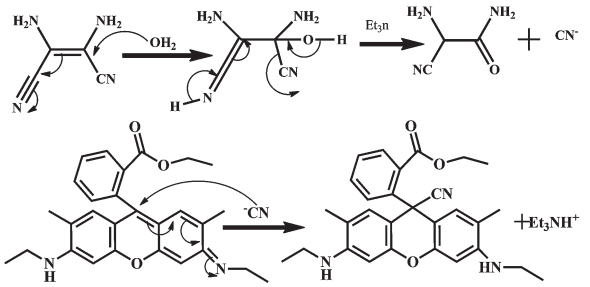
The cyano-rhodamine moiety (Ortep view: Fig. 4a) is prepared from rhodamine 6G with the help of diaminomaleonitrile, water and triethyl amine in ethanol (Scheme 1). For the preparation of nitrile compounds, conventional methods are most often used: the Sandmeyer[12] and Rosenmund-von Braun[13] procedures employ stoichiometric amounts of CuCN as the cyanating reagent. While the recent development of the



Scheme 1

[a]*Department of Chemistry, Bengal Engineering and Science University, Shibpur, West Bengal, India. E-mail: spgoswamical@yahoo.com*
[b]*Crystallography and Molecular Biology Division, Saha Institute of Nuclear Physics, Kolkata, West Bengal, India*
[c]*X-ray Crystallography Unit, School of Physics, Universiti Sains Malaysia, 11800 USM Penang, Malaysia*
[d]*Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh 11451, Kingdom of Saudi Arabia*

†Electronic supplementary information (ESI) available: Details of synthetic procedure, X-ray crystal structure and spectral data available. CCDC 934861. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c3dt51591e



Scheme 2

transition-metal-catalyzed cyanation[14] allows the use of certain metal- or metalloid bound cyanide sources,[15] but the sources of cyanation are also toxic in nature. In response to these issues, the feasibility of using nonmetallic cyanation species has been actively investigated in recent years.[16]

In fact, a range of organic precursors bearing a "CN" moiety have been examined: acetone cyanohydrin and its analogues,[17] alkyl nitriles,[18] malononitrile,[19] phenyl cyanates,[20] benzyl thiocyanates,[21] *N*-cyanobenzimidazole,[22] TMSCN,[23] nitromethane,[24] and triselenium dicyanide (TSD).[25] Herein, we report the diaminomaleonitrile as a source of cyanide in the presence of water.

The plausible mechanism for cyanation of rhodamine 6G is presented in Scheme 2. At first the diaminomaleonitrile is hydrolysed to give HCN. HCN is then neutralised by the organic base triethyl amine to give the unmask cyanide ion which readily attacks to give the product, cyano rhodamine (Rh-CN).

The photophysical properties of the receptor was investigated by monitoring the absorption and fluorescence behaviour upon the addition of several metal ions such as K^+, Mg^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Cu^{2+}, Cd^{2+}, Hg^{2+}, Fe^{2+}, Fe^{3+}, Pd^0, Pd^{2+}, Pt^{2+} in MeOH–H_2O (2 : 8, v/v) at pH 7.4 by using 20 mM HEPES buffer. When we evaluated changes in the absorbance of a receptor upon treatment with Pd^{2+} ions up to 4 equiv., the intensity of the absorption bands of the receptor at 525 nm increases rapidly and gives a sigmoidal type curve (Fig. 3a). Thus the strong coordination by the cyanide group present in the receptor to the Pd^{2+} ion breaks the 'C–CN' bond to reproduce the characteristics color of rhodamine 6G itself which is absent with the addition of other metal ions (Fig. 1a). In addition, the same group element Pt^{2+} also shows an increased absorption band at 525 nm but the intensity is less than for Pd^{2+}. This indicates that the receptor is highly selective and sensitive for Pd^{2+} which is shown in bar graph representation (Fig. 1b). The receptor is inert towards all other cations except Pd^{2+} and Pt^{2+}, *i.e.* all other cations are unable to break the 'C–CN' bond of the Rh-CN and hence show no such effect in absorption spectroscopy (ESI†). As the absorption band at 525 nm appears with a high absorption value, the "naked eye" detection of the metal ion is possible (Fig. 1b). Thus without using any instrument we can sense Pd^{2+} using aqueous solution of the receptor, Rh-CN. From the linear dynamic graph, it is clearly indicated that the receptor can easily detect 0.83 μM Pd^{2+} using the K*S/Sb1 equation[12] (ESI†). The fluorogenic response of the sensor, Rh-CN, is also in well agreement with

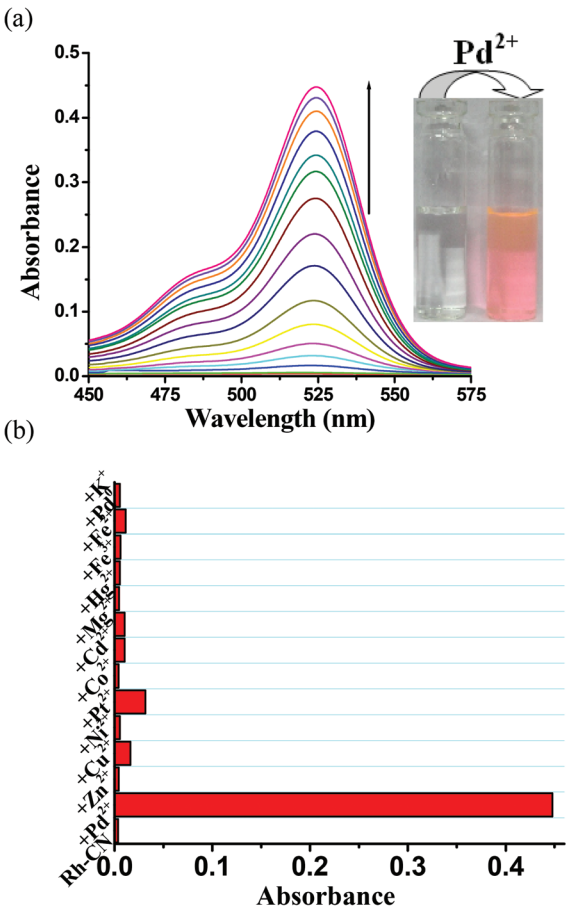


Fig. 1 (a) UV-vis absorption titration spectra of the receptor ($c = 1 \times 10^{-5}$ M) in the presence of Pd^{2+} ($c = 2 \times 10^{-4}$ M) in MeOH–H_2O (2 : 8, v/v, at pH = 7.4, 20 mM HEPES buffer) with the interval of 5 minutes (naked eye color change in the inset). (b) Absorption intensity of Rh-CN after the addition of 6.0 equiv. of each of the guest cations (except Pd^{2+}: 4 equiv.).

the bond breaking mechanism, an almost 21 fold enhancement of the emission intensity at 555 nm (Fig. 2a) observed upon gradual addition of Pd^{2+} (0–4.0 equiv.) with an 88 fold enhancement of quantum yield (ESI†). It is noteworthy to mention that for the rest of the cations except Pd^{2+}, neither pink coloration nor a large fluorescence intensity is observed demonstrating that the sensor is insensitive towards the other metal ions. Fig. 2b displays a comparative view of the emission intensity of the receptor, Rh-CN fluorophore, after the addition of 6.0 equiv. of each of the guest cations (except Pd^{2+}: 4 equiv.).

The mass spectrum (ESI MS) of the Rh-CN with the treatment of Pd^{2+} shows peaks at *m*/*z* 443.22 possibly for the rhodamine moiety, which also proves the cleavage of the 'C–CN' bond of the Rh-CN moiety, *m*/*z* 470.23, with the action of Pd^{2+} (ESI†). From ^{13}C-NMR data (ESI†) we also conclude the elimination of the cyanide group from Rh-CN (Fig. 4b).

In fluorescence spectroscopy, there is also a small enhancement occurring during the gradual addition of the Pt^{2+} ion. The reason is the same as for the absorption phenomenon. Interestingly Pd(0) which is the most interfering species[10] for the detection of Pd(II) is inert towards Rh-CN under the

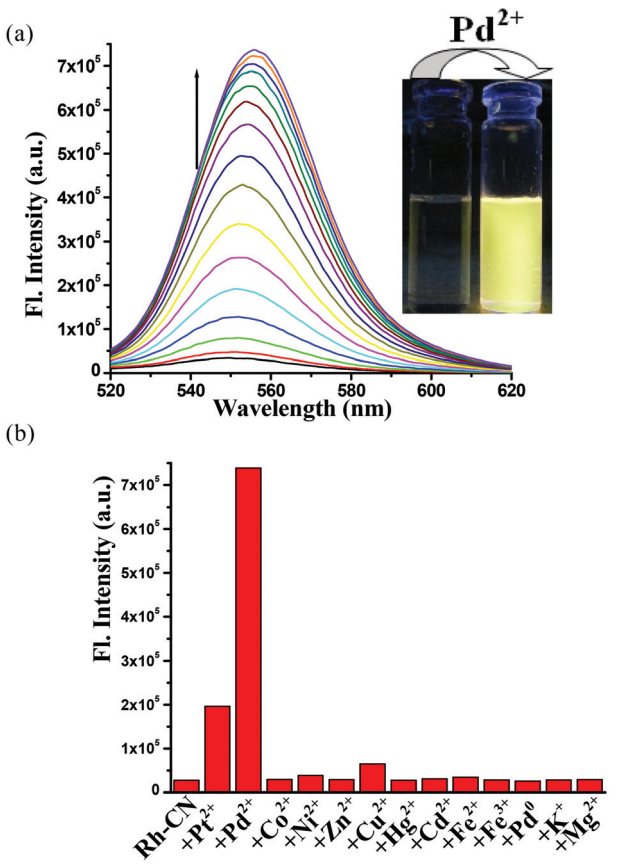


Fig. 2 (a) Fluorescence titration spectra of the receptor ($c = 1 \times 10^{-5}$ M) in the presence of Pd^{2+} ($c = 2 \times 10^{-4}$ M) in MeOH–H_2O (2 : 8, v/v, at pH = 7.4, 20 mM HEPES buffer) with the interval of 5 minutes (λ_{ex} = 505 nm) (naked eye color change of Rh-CN with the addition of Pd^{2+} under UV-light in the inset). (b) Emission intensity of Rh-CN fluorophore after the addition of 6.0 equiv. each of the guest cations (except Pd^{2+}: 4 equiv.).

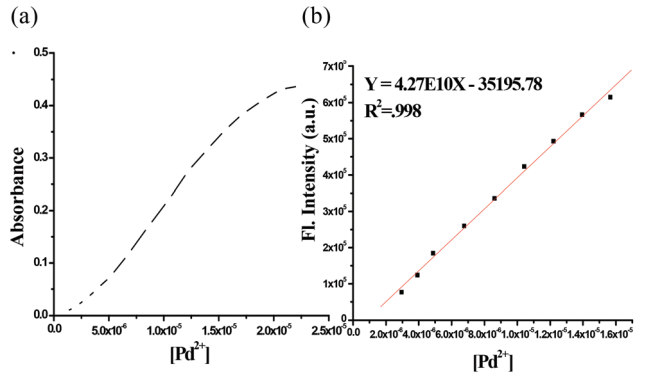


Fig. 3 (a) Absorbance *vs.* [Pd^{2+}] plot adding up to 4 equivalents of Pd^{2+} towards Rh-CN ($c = 1 \times 10^{-5}$ M) and (b) fluorescence intensity *vs.* [Pd^{2+}] plot between the addition of 3 to 16 μM of Pd^{2+}.

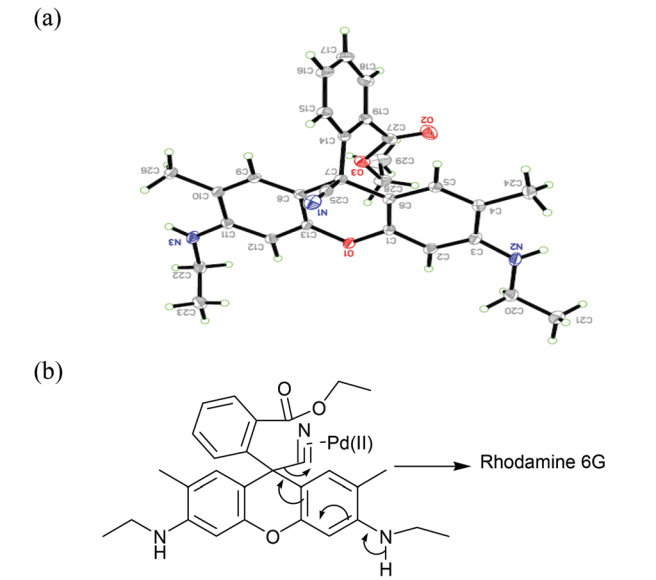


Fig. 4 (a) Ortep view of the receptor, cyano rhodamine (Rh-CN), showing 50% probability displacement ellipsoids for non-H atoms and the atom-numbering scheme. (b) Plausible mechanism of 'C–CN' bond cleavage of Rh-CN in the presence of Pd^{2+}.

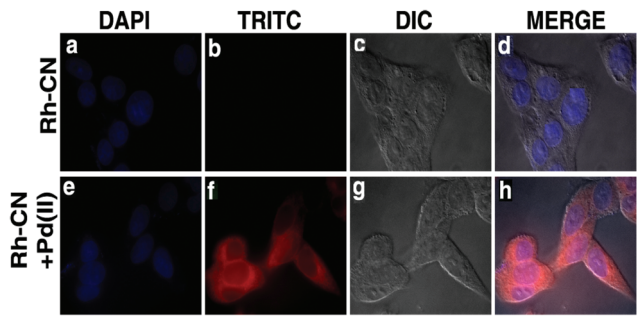


Fig. 5 Fluorescence images of HeLa cells incubated with 50 μM of the receptor (Rh-CN) in the absence (a: blue channel; and b: red channel) and in the presence (e: blue channel; and f: red channel) of 50 μM of $PdCl_2$. Corresponding differential interference contrast (DIC) images (c and g) and merged images (d and h) of the cells are shown.

mentioned condition. Further, competition experiment also reveals that Pd^{2+}-induced fluorescence enhancement remains unperturbed and does not get any interference by the co-existing metal ions (Fig. 6). The calculated detection limit from the fluorescence intensity *vs.* conc. of the Pd^{2+} curve is found to be 0.57 μM less than the detection limit observed by using absorption data calculation with the same equation.[26] Under this condition, the changes of the intensity produced an excellent linear function with the concentration of Pd^{2+} between 3 and 16 μM (Fig. 3b).

In order to determine the membrane permeability of the receptor (Rh-CN) and its specific binding ability to the Pd^{2+} in living cells, HeLa cells were first incubated with the $PdCl_2$ followed by the addition of the receptor. A controlled incubation of the cells with Rh-CN only was also carried out. As shown in Fig. 5, the cells showed intense red fluorescence, specifically in the cytoplasm in TRITC channel when they were treated with Pd^{2+} followed by the receptor. Nuclei of the cells stained with DAPI are shown in deep blue colour. In the absence of $PdCl_2$ no fluorescence was observed in cells. The result clearly established that the receptor (Rh-CN) could permeate the

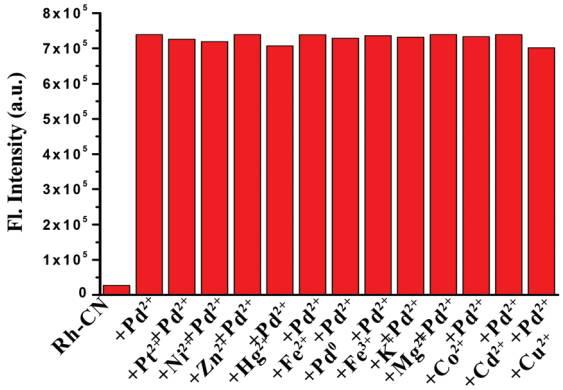


Fig. 6 Metal ion sensitivity profile of Rh-CN: change of the emission intensity of Rh-CN + 10.0 equiv. of the investigated M^{n+} + 4.0 equiv. of Pd^{2+}.

plasma membrane of the cells and give specific fluorescence in the cytoplasm only in the presence of Pd(II).

In conclusion, we have reported the first synthesis of a hitherto unknown cyano-rhodamine (Rh-CN) fluorophore with a green approach; it easily undergoes selective Pd^{2+} induced 'C–CN' bond breaking to produce the characteristics pink coloration along with fluorescence of rhodamine 6G itself. The recognition events have been successfully applied to the bio-imaging of Pd^{2+} on HeLa cells.

Acknowledgements

We thank DST and CSIR (Govt. of India) for financial support. A.M., A.K.D., K.A. and M.K.D. acknowledge CSIR and S.P. acknowledges UGC for providing fellowships. The authors extend their appreciation to The Deanship of Scientific Research at King Saud University for funding the work through research group project no. RGP-VPP-207.

Notes and references

1 (*a*) T. Iwasawa, M. Tokunaga, Y. Obora and Y. Tsuji, *J. Am. Chem. Soc.*, 2004, **126**, 6554; (*b*) M. Lafrance and K. Fagnou, *J. Am. Chem. Soc.*, 2006, **128**, 16496.

2 J. M. Baxter, D. Steinhuebel, M. Palucki and I. W. Davies, *Org. Lett.*, 2005, **7**, 215.

3 S. Rauch, H. F. Hemond, C. Barbante, M. Owari, G. M. Morrison, B. Peucker-Ehrenbrink and U. Wass, *Environ. Sci. Technol.*, 2005, **39**, 8156.

4 International Programme on Chemical Safety. Palladium; Environmental Health Criteria Series 226, World Health Organization, Geneva, 2002.

5 (*a*) J. C. Wataha and C. T. Hanks, *J. Oral Rehabil.*, 1996, **23**, 309, 2002; (*b*) J. Kielhorn, C. Melber, D. Keller and I. Mangelsdorf, *Int. J. Hyg. Environ. Health*, 2002, **205**, 417.

6 C. E. Garrett and K. Prasad, *Adv. Synth. Catal.*, 2004, **346**, 889.

7 (*a*) D. Kalny, A. M. Albrecht-Gary and J. Havel, *Anal. Chim. Acta*, 2001, **439**, 101; (*b*) R. J. T. Houk, K. J. Wallace, H. S. Hewage and E. V. Anslyn, *Tetrahedron*, 2008, **64**, 8271.

8 (*a*) C. Locatelli, D. Melucci and G. Torsi, *Anal. Bioanal. Chem.*, 2005, **382**, 1567; (*b*) K. V. Meel, A. Smekens, M. Behets, P. Kazandjian and R. V. Grieken, *Anal. Chem.*, 2007, **79**, 6383.

9 (*a*) A. Tamayo, L. Escriche, J. Casabo, B. Covelo and C. Lodeiro, *Eur. J. Inorg. Chem.*, 2006, 2997; (*b*) J. R. Matthews, F. Goldoni, H. Kooijman, A. L. Spek, A. P. H. J. Schenning and E. W. Meijer, *Macromol. Rapid Commun.*, 2007, **28**, 1809; (*c*) L. P. Duan, Y. F. Xu and X. H. Qian, *Chem. Commun.*, 2008, 6339; (*d*) B. Liu, Y. Bao, F. Du, H. Wang, J. Tian and R. Bai, *Chem. Commun.*, 2011, **47**, 1731.

10 (*a*) H. L. Li, J. L. Fan, J. J. Du, K. X. Guo, S. G. Sun, X. J. Liu and X. J. Peng, *Chem. Commun.*, 2010, **46**, 1079; (*b*) T. Schwarze, W. Mickler, C. Dosche, R. Flehr, T. Klamroth, H. G. Lohmannsroben, P. Saalfrank and H. J. Holdt, *Chem.–Eur. J.*, 2010, **16**, 1819; (*c*) F. Song, E. J. Carder, C. C. Kohler and K. Koide, *Chem.–Eur. J.*, 2010, **16**, 13500; (*d*) H. L. Li, J. L. Fan, F. L. Song, H. Zhu, J. J. Du, S. G. Sun and X. J. Peng, *Chem.–Eur. J.*, 2010, **16**, 12349; (*e*) M. Santra, S.-K. Ko, I. Shin and K. H. Ahn, *Chem. Commun.*, 2010, **46**, 3964; (*f*) M. E. Jun and K. H. Ahn, *Org. Lett.*, 2010, **12**, 2790; (*g*) S. Goswami, D. Sen, N. K. Das, H.-K. Fun and C. K. Quah, *Chem. Commun.*, 2011, **47**, 9101; (*h*) B. Zhu, C. Gao, Y. Zhao, C. Liu, Y. Li, Q. Wei, Z. Ma, B. Du and X. Zhang, *Chem. Commun.*, 2011, **47**, 8656; (*i*) B. Liu, H. Wang, T. Wang, Y. Bao, F. Du, J. Tian, Q. Li and R. Bai, *Chem. Commun.*, 2012, **48**, 2867; (*j*) X. Bu, K. Koide, E. J. Carder and C. J. Welch, *Org. Process Res. Dev.*, 2013, **17**, 108; (*k*) S. Cai, Y. Lu, S. He, F. Wei, L. Zhao and X. Zeng, *Chem. Commun.*, 2013, **49**, 822; (*l*) M. Kumar, N. Kumar and V. Bhalla, *RSC Adv.*, 2013, **3**, 1097.

11 (*a*) H. N. Kim, M. H. Lee, H. J. Kim, J. S. Kim and J. Y. Yoon, *Chem. Soc. Rev.*, 2008, **37**, 1465; (*b*) X. Chen, T. Pradhan, F. Wang, J. S. Kim and J. Yoon, *Chem. Rev.*, 2012, **112**, 1910; (*c*) S. Goswami, A. Manna, K. Aich and S. Paul, *Chem. Lett.*, 2012, 1600; (*d*) S. Goswami, K. Aich, S. Das, A. K. Das, A. Manna and S. Halder, *Analyst*, 2013, **138**, 1903; (*e*) S. Goswami, S. Paul and A. Manna, *RSC Adv.*, 2013, **3**, 10639; (*f*) A. K. Mahaptra, S. K. Manna, S. K. Mukhopadhyay and A. Banik, *Sens. Actuators, B*, 2013, **183**, 350; (*g*) K. Ghosh, T. Sarkar and A. Majumdar, *Asian J. Org. Chem.*, 2013, **2**, 157.

12 (*a*) T. Sandmeyer, *Ber. Dtsch. Chem. Ges.*, 1884, **17**, 1633; (*b*) H. H. Hodgson, *Chem. Rev.*, 1947, **40**, 251; (*c*) C. Galli, *Chem. Rev.*, 1988, **88**, 765.

13 (*a*) K. W. Rosenmund and E. Struck, *Ber. Dtsch. Chem. Ges.*, 1919, **2**, 1749; (*b*) J. Lindley, *Tetrahedron*, 1984, **40**, 1433.

14 (*a*) C. Yang and J. M. Williams, *Org. Lett.*, 2004, **17**, 2837; (*b*) H.-J. Cristau, A. Ouali, J.-F. Spindler and M. Taillefer, *Chem.–Eur. J.*, 2005, **11**, 2483; (*c*) J. Zanon, A. Klapars and S. L. Buchwald, *J. Am. Chem. Soc.*, 2003, **125**, 2890; (*d*) R. S. Jensen, A. S. Gajare, K. Toyota, M. Yoshifuji and

F. Ozawa, *Tetrahedron Lett.*, 2005, **46**, 8645; (*e*) F. G. Buono, R. Chidambaram, R. H. Mueller and R. E. Waltermire, *Org. Lett.*, 2008, **10**, 5325.

15 (*a*) A. Sekiya and N. Ishikawa, *Chem. Lett.*, 1975, 277; (*b*) M. Sundermeier, A. Zapf, S. Mutyala, W. Baumann, J. Sans, S. Weiss and M. Beller, *Chem.–Eur. J.*, 2003, **9**, 1828; (*c*) M. Sundermeier, A. Zapf and M. Beller, *Angew. Chem., Int. Ed.*, 2003, **42**, 1661.

16 (*a*) T. Schareina, A. Zapf, A. Cotte, M. Gotta and M. V, *Adv. Synth. Catal.*, 2011, **353**, 777; (*b*) K. Ouchaou, D. Georgi and F. Taran, *Synlett*, 2010, 2083; (*c*) E. J. Park, S. Lee and S. Chang, *J. Org. Chem.*, 2010, **75**, 2760.

17 F.-H. Luo, C.-I. Chu and C.-H. Cheng, *Organometallics*, 1998, **17**, 1025.

18 Z. Jiang, Q. Huang, S. Chen, L. Long and X. Zhou, *Adv. Synth. Catal.*, 2012, **354**, 589.

19 N. Sato and Q. Yue, *Tetrahedron*, 2003, **59**, 5831.

20 G.-Y. Zhang, J.-T. Yu, M.-L. Hu and J. Cheng, *J. Org. Chem.*, 2013, **78**, 2710.

21 G. Kaupp, J. Schmeyers and J. Boy, *Chem.–Eur. J.*, 1998, **4**, 2467.

22 M. Sundermeier, S. Mutyala, A. Zapf, A. Spannenberg and M. Beller, *J. Organomet. Chem.*, 2003, **684**, 50.

23 X. Chen, X.-S. Hao, C. E. Goodhue and J.-Q. Yu, *J. Am. Chem. Soc.*, 2006, **128**, 6790.

24 (*a*) P. Anbarasan, H. Neumann and M. Beller, *Angew. Chem., Int. Ed.*, 2011, **50**, 519; (*b*) Y. Yang, Y. Zhang and J. Wang, *Org. Lett.*, 2011, **13**, 5608.

25 S. Goswami, A. C. Maity, N. K. Das, D. Sen and S. Maity, *Synth. Commun.*, 2009, **39**, 407.

26 M. Zhu, M. Yuan, X. Liu, J. Xu, J. Lv, C. Huang, H. Liu, Y. Li, S. Wang and D. Zhu, *Org. Lett.*, 2008, **10**, 1481.