NJC

LETTER

View Article Online
View Journal | View Issue

Cite this: *New J. Chem.*, 2019, 43, 548

Received 31st August 2018,
Accepted 13th October 2018

DOI: 10.1039/c8nj04438d

rsc.li/njc

A new turn-on fluorescent probe for the detection of palladium(0) and its application in living cells and zebrafish†

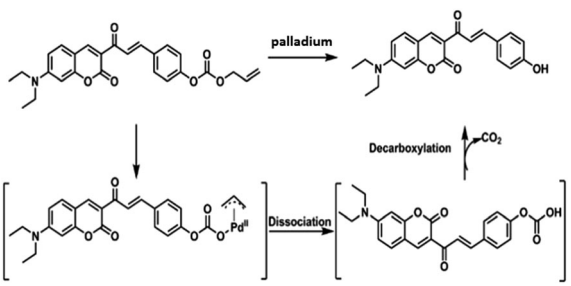
Ming Jin,[a] Lihong Wei,[a] Yutao Yang,*[b] Mingtao Run[a] and Caixia Yin *[c]

A new "turn-on" fluorescent probe 1 for the detection of Pd^0 has been designed and synthesized. The probe possesses potent selectivity to Pd^0 and can respond quickly to changes in Pd^0. In addition, the mechanisms of the reactions were studied and the product of the probe with Pd^0 was separated. Moreover, the probe can be used to sense Pd^0 in BT-474 cells and zebrafish.

Palladium, an important noble metal, is of current interest in catalytic converters, dental crowns, jewellery, and fuel cells in view of its excellent photophysical and chemical properties.[1–4] Importantly, palladium-catalyzed reactions are increasingly appealing owing to its unique advantages in catalyzing the formation of difficult bonds and it also serves as an irreplaceable catalyst involved in the synthesis of multiple pharmaceutical drugs.[5–9] However, in sharp contrast to its favorable effects, palladium, which is capable of binding tightly to sulfur-containing amino acids, proteins, RNA, DNA, and other biomolecules, can also disturb various physiological processes and thus can bring about diverse health hazards.[10–15] Worryingly, with the increasingly wide use of palladium, the resulting high level of Pd residue has raised great concern. Hence, governments are imposing increasingly severe limits on the level of residual palladium in final products, with the threshold being strictly controlled to 5–10 ppm and the daily intake being no more than 1.5–15 μg per person per day according to the European Agency for the Evaluation of Medicinal Products (EMEA).[16–18] Hence, it is essential to develop sensitive and selective methods for the detection and determination of palladium in the environment as well as in living systems.

To date, some traditional analytical methods, including X-ray fluorescence,[19] atomic absorption spectroscopy (AAS),[20] coupled plasma mass spectrometry (ICP-MS)[21] and so on, have been widely used to detect palladium species. But all of these methods require expensive instrumentation, rigorous experimental conditions and highly skilled individuals. Contrary to these traditional methods, fluorescent probes, with the unique ability to generate a florescence reporter *in situ* upon reaction with palladium species, possess several appealing advantages such as simplicity in operating, low cost, high selectivity and sensitivity, non-destructive and *in situ* analysis, and so on.[22–39] Moreover, to the best of our knowledge, a variety of fluorescent probes for Pd^0 have been synthesized mainly based on the Pd^0-catalyzed Tsuji–Trost allylic oxidative insertion reaction.[40–42]

Herein, we developed a new fluorescent probe **1** for the selective detection of Pd^0, with an allyl carbonate moiety as the recognition site for Pd^0 and a coumarin derivative as the fluorescent group. On the basis of previously reported research,[38–40] the reaction mechanism was attributed to the high specificity of the Pd^0-triggered cleavage process and the detailed reaction mechanism is shown in Scheme 1. The free probe is non-fluorescent and immediately reacted with Pd^0, and this probe underwent Pd^0-triggered elimination, resulting in the release of



Scheme 1 Proposed sensing mechanism.

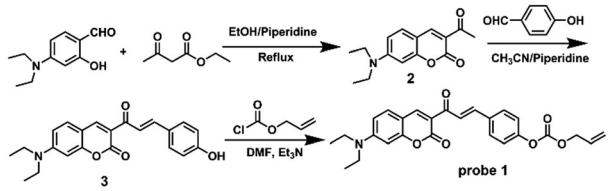
[a] *National Experimental Teaching Demostration Center of Chemistry, College of Chemistry & Environmental Science, Hebei University, Baoding 071002, P. R. China*
[b] *Key Laboratory of Medicinal Chemistry and Molecular Diagnosis of the Ministry of Education, College of Chemistry & Environmental Science, Hebei University, Baoding 071002, P. R. China. E-mail: yutaoyang2016@163.com*
[c] *Key Laboratory of Chemical Biology and Molecular Engineering of Ministry of Education, Key Laboratory of Materials for Energy Conversion and Storage of Shanxi Province, Institute of Molecular Science, Shanxi University, Taiyuan 030006, China. E-mail: yincx@sxu.edu.cn*

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c8nj04438d

the fluorophore and thus recovery of the orange fluorescence. And the reaction mechanism was confirmed by the ^{1}H NMR spectrum and HR-MS data of an isolated reaction product of probe **1** with Pd^0 (Fig. S1, ESI†). UV-vis absorption and fluorescence spectra were further used to investigate its optical properties and the results indicated that probe **1** possessed a turn-on fluorescence response to Pd^0 (6-fold). Moreover, the probe can penetrate cell membranes and react with Pd^0 within BT-474 cells and zebrafish.

Methods/experimental section

All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals at the Academy of Military Medical Science animals (SYXK-2014-001) and experiments were approved by the Animal Ethics Committee of Academy of Military Medical Science.



Scheme 2 Synthesis of probe **1**.

The synthetic methods associated with the probe are summarized in Scheme 2, and the details are in the ESI† (Fig. S1). We investigated the optical sensing properties of probe **1** for Pd^0 in EtOH-PBS buffer solution (2 : 3, v/v, 10 mM PBS, pH = 7.4). Initially, the reaction time of probe **1** with Pd^0 was investigated (Fig. 1). Pd^0 (40 μM) was added to an EtOH–PBS buffer (2 : 3, v/v, 10 mM PBS, pH = 7.4) solution containing probe **1** (5 μM), leading to a gradual increase of the absorption at 348 nm and the enhancement of the fluorescence intensity at 560 nm. The UV absorption and fluorescence spectrum reached a plateau within 10 minutes, implying that the reaction rate of probe **1** towards Pd^0 was very fast (Fig. 1).

Subsequently, the UV-vis and fluorescence titration experiments were performed. As shown in Fig. 1, the UV absorption peaks were mainly concentrated at 473 nm and 324 nm. With the addition of Pd^0 (0–40 μM), an ultraviolet absorption peak was almost unchanged at 473 nm and the appearance of a new absorption peak gradually increased at 348 nm, accompanying the color of the solution changing from light yellow to yellowish brown. Correspondingly, probe **1** is almost non-fluorescent (fluorescence quantum yield: 0.0095), the addition of Pd^0 also caused the enhancement of the fluorescence intensity of 560 nm (the fluorescence quantum yield of the product: 0.28) and the detection limit was 4.18 μM based on the IUPAC method (Fig. 2 and Fig. S2, ESI†).

Moreover, the selectivity and competition of probe **1** towards Pd^0 were also investigated (Fig. 3). By the addition of various metal ions (200 μM), including Ag^+, Ce^{3+}, K^+, Ni^{2+}, Na^+, Mn^{2+},

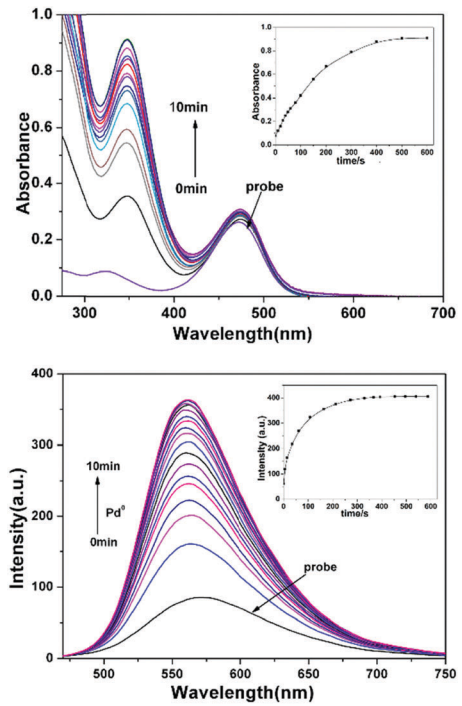


Fig. 1 Top: Time dependent UV-vis absorbance spectra upon addition of Pd^0 in ethanol–PBS buffer solution (2 : 3, v/v, 10 mM PBS, pH = 7.4) (inset: time dependent absorbance spectra of probe **1** (5 μM) upon addition of Pd^0 (40 μM) at 348 nm); bottom: time dependent fluorescence spectra upon addition of Pd^0 in ethanol–PBS buffer solution (2 : 3, v/v, 10 mM PBS, and pH = 7.4) (λ_{ex} = 450 nm, slit: 5 nm/5 nm) (inset: time dependent fluorescence intensity of probe **1** upon addition of Pd^0 (40 μM) at 560 nm).

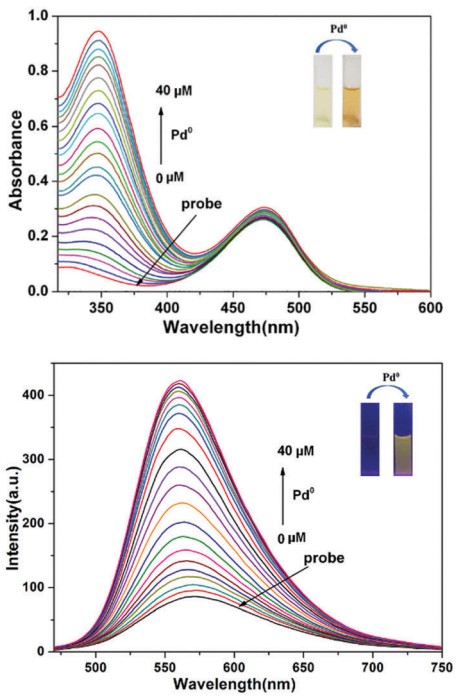


Fig. 2 Top: Absorption spectra of probe **1** (5 μM) upon addition of Pd^0 (40 μM) in ethanol–PBS buffer solution (2 : 3, v/v, 10 mM PBS, and pH = 7.4) (inset: color changes of the solution of 1 upon addition of Pd^0); bottom: fluorescence spectra of probe **1** (5 μM) upon addition of Pd^0 (40 μM) in ethanol–PBS buffer solution (2 : 3, v/v, 10 mM PBS, and pH = 7.4) (inset: fluorescence changes of the solution of 1 upon addition of Pd^0).

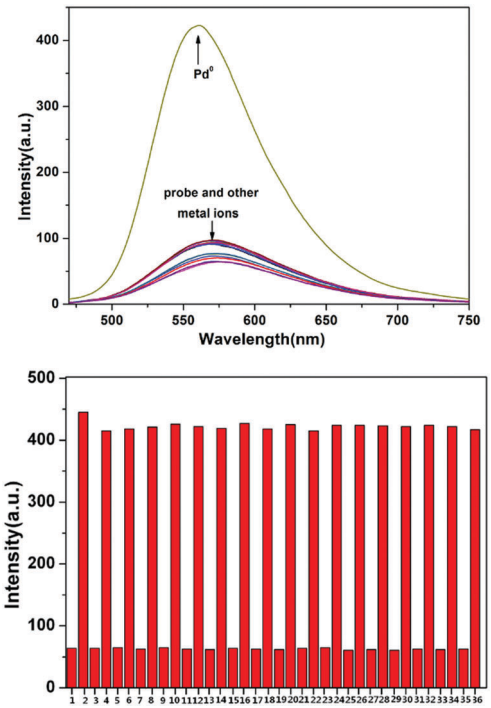


Fig. 3 Top: Fluorescence spectra of probe **1** (5 μM) in the presence of Pd^0 (40 μM) and other metal ions (Ag^+, Ce^{3+}, K^+, Ni^{2+}, Na^+, Mn^{2+}, Mg^{2+}, Ca^{2+}, Hg^{2+}, pb^{2+}, Fe^{3+}, Cd^{2+}, Zn^{2+}, Cr^{3+}, Al^{3+}, Cu^{2+} and Co^{2+}) (200 μM) in ethanol–PBS buffer solution (2 : 3, v/v, 10 mM PBS, and pH = 7.4) (λ_{ex} = 450 nm, slits: 5 nm/5 nm); bottom: fluorescence spectra of probe **1** (5 μM) in ethanol–PBS buffer solution with various relevant species (200 μM): (1) none; (2) Pd(0); (3) Ag^+; (4) Ag^+ + Pd(0); (5) Ce^{3+}; (6) Ce^{3+} + Pd(0); (7) Mn^{2+}; (8) Mn^{2+} + Pd(0); (9) Mg^{2+}; (10) Mg^{2+} + Pd(0); (11) Ca^{2+}; (12) Ca^{2+} + Pd(0); (13) Hg^{2+}; (14) Hg^{2+}+ Pd(0); (15) K^+; (16) K^+ + Pd(0); (17) Na^+; (18) Na^+ + Pd(0); (19) Fe^{3+}; (20) Fe^{3+} + Pd(0); (21) Zn^{2+}; (22) Zn^{2+} + Pd(0); (23) Cd^{2+}; (24) Cd^{2+} + Pd(0);(25) Ni^{2+}; (26) Ni^{2+} + Pd(0); (27) Pb^{2+}; (28) Pb^{2+} + Pd(0); (29) Cr^{3+}; (30) Cr^{3+} + Pd(0); (31) Al^{3+}; (32) Al^{3+} + Pd(0); (33) Cu^{2+}; (44) Cu^{2+} + Pd(0); (35) Co^{2+}; (36) Co^{2+} + Pd(0).

Mg^{2+}, Ca^{2+}, Hg^{2+}, pb^{2+}, Fe^{3+}, Cd^{2+}, Zn^{2+}, Cr^{3+}, Al^{3+}, Cu^{2+} and Co^{2+}, there was no obvious change in the fluorescence spectrum. However, the fluorescence intensity was increased observably at 560 nm after the addition of Pd^0 (40 μM). Accordingly, absorption spectra have been studied (Fig. S4, ESI†). Meanwhile, anions did not interfere with the detection of Pd^0 (Fig. S5, ESI†). These results showed that probe **1** has good selectivity for Pd^0.

Besides, the effect of pH was also studied; Fig. 4 shows that probe **1** was stable in the pH range of 3–12. Upon addition of Pd^0, the fluorescence intensity at 560 nm was increased significantly at pH values ranging from 5 to 10, suggesting that probe **1** can be applied to the detection of Pd^0 in biological systems.

Encouraged by the results of the above research, the applicability of probe **1** to image Pd^0 was further investigated. MTT assay results indicated that 20 μM of probe **1** had little effect on BT-474 cell growth after 12 h (Fig. S6, ESI†). Hence, we studied the detection of Pd^0 in living cells. BT-474 cells were incubated with probe **1** (10 μM) for 0.5 h and weak fluorescence was observed (Fig. 5A). When BT-474 cells were incubated with 10, 40, and 100 μM of Pd^0 for 0.5 h, and then incubated with 10 μM of probe **1** for another 0.5 h, a remarkable enhancement of fluorescence was respectively observed (Fig. 5B–D). The results showed that probe **1** could detect Pd^0 in living cells.

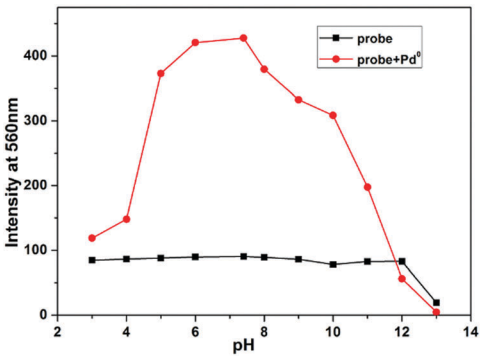


Fig. 4 Effect of pH on the fluorescence intensity at 560 nm of probe **1** upon addition of Pd^0.

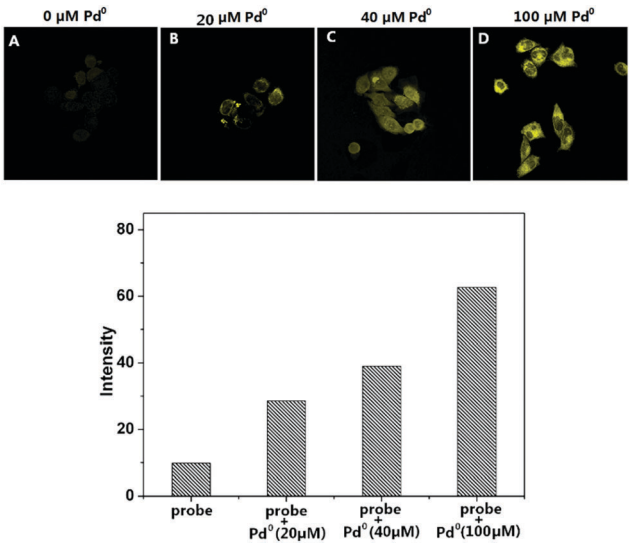


Fig. 5 Confocal fluorescence images of probe **1** in BT-474 cells. Top: (A) BT-474 cells were incubated with probe **1** (10 μM) for 0.5 h, (B–D) BT-474 cells were incubated with Pd^0 (20, 40, and 100 μM) for 0.5 h and then with probe **1** (10 μM) for another 0.5 h; (emission was observed at 493–591 nm (excited at 458 nm, scale bar: 40 μm)); bottom: fluorescence intensity of A, B, C, and D.

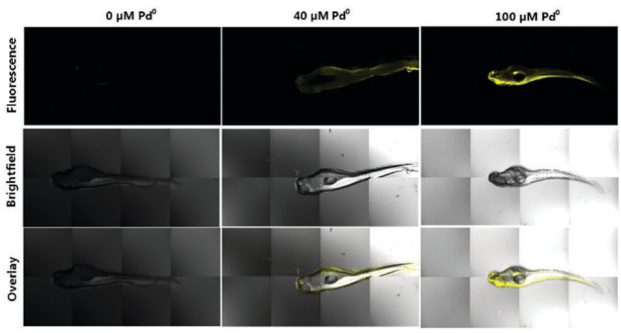


Fig. 6 Confocal fluorescence images of the probe with different concentrations of Pd^0 (0, 40, and 100 μM) in zebrafish (emission was observed at 493–591 nm (excited at 458 nm, scale bar: 500 μm)).

To test the ability of the probe to image Pd^0 *in vivo*, 3-old-day zebrafish were incubated with Pd^0 (0, 40, and 100 μM) for 0.5 h, and following incubation with the probe (10 μM) for another 0.5 h, an image of each zebrafish was obtained using a fluorescence microscope (Fig. 6).

Conclusions

To sum up, we have developed a new "turn-on" fluorescent probe for the detection of Pd^0. The experimental results showed that the probe exhibits good selectivity and high sensitivity for Pd^0. The probe could respond quickly to changes of the concentration of palladium ions. Furthermore, the probe could also be successfully applied to the imaging of Pd^0 in BT-474 cells and zebrafish.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (21807021, 21672131, 21775096, and 21705102), the Hebei University Science Foundation for High-level personnel (1081-801260201024) and the Hebei Province Natural Science Foundation for Youths (No. B2017201093). We also acknowledge the Teaching Reform Project of Hebei University (HBUHXJG2016-16).

References

1 T. Schwarze, H. Muller, C. Dosche, T. Klamroth, W. Mickler, A. Kelling, H. G. Lohmannsroben, P. Saalfrank and H. J. Holdt, *Angew. Chem., Int. Ed.*, 2007, **46**, 1671.
2 T. Schwarze, H. G. Lohmannsroben, P. Saalfrank, E. Cleve, H. J. Buschmann and H. J. Holdt, *Chem. Commun.*, 2010, **46**, 2034.
3 W. Liu, J. Jiang, C. Y. Chen, X. L. Tang, J. M. Shi, P. Zhang, K. M. Zhang, Z. Q. Li, W. Dou, L. Z. Yang and W. S. Liu, *Inorg. Chem.*, 2014, **53**, 12590.
4 W. Su, B. Gu, X. J. Hu, X. L. Duan, Y. Y. Zhang, H. T. Li and S. Z. Yao, *Dyes Pigm.*, 2017, **137**, 293.
5 M. Lafrance and K. J. Fagnou, *J. Am. Chem. Soc.*, 2006, **128**, 16496.
6 T. W. Lyons and M. S. Sanford, *Chem. Rev.*, 2010, **110**, 1147.
7 P. Wang, J. X. Xia and Y. Q. Gu, *Tetrahedron Lett.*, 2015, **56**, 6491.
8 I. S. Turan, O. Yilmaz, B. Karatas and E. U. Akkay, *RSC Adv.*, 2015, **5**, 34535.
9 J. S. Carey, D. Laffan, C. Thomson and M. T. Williams, *Org. Biomol. Chem.*, 2006, **4**, 2337.
10 K. Wang, G. Q. Lai, Z. F. Li, M. M. Liu, Y. J. Shen and C. Y. Wang, *Tetrahedron*, 2015, **71**, 7874.
11 J. Y. Wang, Z. R. Liu, M. G. Ren, B. B. Deng and W. Y. Lin, *J. Photochem. Photobiol., A*, 2016, **317**, 108.
12 W. F. Luo and W. S. Liu, *Dalton Trans.*, 2016, **45**, 11682.
13 K. Q. Xiang, Y. C. Liu, C. J. Li, B. Z. Tian and J. L. Zhang, *RSC Adv.*, 2015, **5**, 52516.
14 X. L. Zheng, R. X. Ji, X. Q. Cao and Y. Q. Ge, *Anal. Chim. Acta*, 2017, **978**, 48.
15 L. C. Li, R. F. Guan, M. M. Guo, P. Ning, R. Shao and X. M. Meng, *Sens. Actuators, B*, 2018, **254**, 949.
16 S. Sanda, S. Parshamoni, S. Biswas and S. Konar, *Chem. Commun.*, 2015, **51**, 6576.
17 C. E. Garrett and K. Prasad, *Adv. Synth. Catal.*, 2004, **346**, 889.
18 H. Li, J. Fan, J. Du, K. Guo, S. Sun, X. Liu and X. Peng, *Chem. Commun.*, 2010, **46**, 1079.
19 K. Van Meel, A. Smekens, M. Behets, P. Kazandjian and R. Van Grieken, *Anal. Chem.*, 2007, **79**, 6383.
20 C. Locatelli, D. Melucci and G. Torsi, *Anal. Bioanal. Chem.*, 2005, **382**, 1567.
21 B. Dimitrova-Koleva, K. Benkhedda, E. Ivanova and F. Adams, *Talanta*, 2007, **71**, 44.
22 W. R. Kitley, P. J. S. Maria, R. Cloyda and L. M. Wysocki, *Chem. Commun.*, 2015, **51**, 8520.
23 X. Wang, Z. Guo, S. Zhu, H. Tian and W. Zhu, *Chem. Commun.*, 2014, **50**, 13525.
24 S. Sun, B. Qiao, N. Jiang, J. Wang, S. Zhang and X. Peng, *Org. Lett.*, 2014, **16**, 1132.
25 S. Cai, Y. Lu, S. He, F. Wei, L. Zhao and X. Zeng, *Chem. Commun.*, 2013, **49**, 822.
26 H. Chen, W. Lin and L. Yuan, *Org. Biomol. Chem.*, 2013, **11**, 1938.
27 W. Chen, B. D. Wright and Y. Pang, *Chem. Commun.*, 2012, **48**, 3824.
28 B. Zhu, C. Gao, Y. Zhao, C. Liu, Y. Li, Q. Wei, Z. Ma, B. Du and X. Zhang, *Chem. Commun.*, 2011, **47**, 8656.
29 J. Jiang, H. Jiang, W. Liu, X. Tang, X. Zhou, W. Liu and R. Liu, *Org. Lett.*, 2011, **13**, 4922.
30 S. Goswami, D. Sen, N. K. Das, H. K. Fun and C. K. Quah, *Chem. Commun.*, 2011, **47**, 9101.
31 H. Li, J. Fan, F. Song, H. Zhu, J. Du, S. Sun and X. Peng, *Chem. – Eur. J.*, 2010, **16**, 12349.
32 M. E. Jun and K. H. Ahn, *Org. Lett.*, 2010, **12**, 2790.
33 T. Schwarze, C. Dosche, R. Flehr, T. Klamroth, H. G. Löhmannsröben, P. Saalfrank, E. Cleve, H. J. Buschmann and H. J. Holdt, *Chem. Commun.*, 2010, **46**, 2034.
34 M. Santra, S. K. Ko, I. Shin and K. H. Ahn, *Chem. Commun.*, 2010, **46**, 3964.
35 A. L. Garner and K. Koide, *Chem. Commun.*, 2009, 86.
36 H. S. Hewage and E. V. Anslyn, *J. Am. Chem. Soc.*, 2009, **131**, 13099.
37 L. Duan, Y. Xu and X. Qian, *Chem. Commun.*, 2008, 6339.
38 A. L. Garner, F. Song and K. Koide, *J. Am. Chem. Soc.*, 2015, **131**, 5163.
39 J. Qin, X. M. Li, Z. Z. Chen and F. Feng, *Sens. Actuators, B*, 2016, **232**, 611.
40 X. Q. Chen, H. D. Li, L. Y. Jin and B. Z. Yin, *Tetrahedron Lett.*, 2014, **55**, 2537.
41 J. W. Yan, X. L. Wang, Q. F. Tan., P. F. Yao, J. H. Tan and L. Zhang, *Analyst*, 2016, **141**, 2376.
42 L. Cui, W. P. Zhu, Y. F. Xu and X. H. Qian, *Anal. Chim. Acta*, 2013, **786**, 139.