Heavy metal analysis using a Heck-catalyzed cyclization to create coumarin

Qiaoyin Wu and Eric V. Anslyn*

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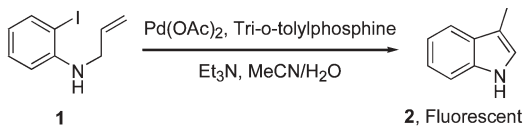
This paper describes a new method for heavy metal analysis *via* catalytic signal amplification. This signal amplification protocol relies upon an exogenous inhibitor for deliberate deactivation of an organometallic reaction that catalytically creates a fluorophore. When the deactivation process is performed in the presence of the analyte, competitive binding of the inhibitor with the analyte and the catalyst occurs. A Heck reaction creating a coumarin fluorophore with a high quantum yield was studied. 1,4,7,10,13-Pentaaza-cyclopentadecane was chosen as the inhibiting ligand to selectively coordinate pre-catalyst Pd(II) and analytes Cd(II), Ni(II), Co(II). Monitoring product fluorescent intensity in real time revealed the concentration of analyte.

Introduction

Over the last few years, optical chemical sensors[1] and biosensors[2] have received increasing attention for chiral recognition,[3] biological analyses,[4] food analyses,[5] as well as environment protection.[6] The detection of specific organic molecules, ions, DNAs and antibodies, can benefit from signal amplification[7] to enhance the readout to detectable levels. Recently, many biosensors have been reported to use signal enhancement. For example, using a specific enzyme (AlaDH) catalyzed dehydrogenation, alanine was measured down to 7.2 μM in 2 seconds *via* an amperometric method that monitored the consumption rate of O_2.[8] In addition, catalytic RNA cleavage has been used to give both detection and quantification of RNAs *in situ*.[9] Due to its generality in industry, Enzyme-Linked Immunosorbent Assay (ELISA) remains a well renowned signal amplification technique regardless of the requirement for different levels of antibodies and multiple washing steps.[10] In addition to enzyme catalyzed reactions, a polymer based method is a well established amplifying approach. The "Molecule wire" approach results from energy migration through conjugated semi-conducting polymer backbones.[11] This technique has found applications in toxic ion[12] and explosive compound detection.[13] As a last example, discrimination of chirality can be achieved *via* conformational changes in helical polymers as the result of chiral molecular interactions.[14]

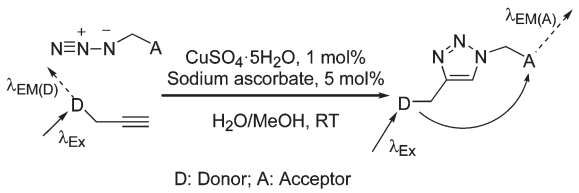
Very recently, we reported a signal amplification protocol using organometallic reactions for transition metal detection and measurement.[15] This signal amplification protocol relies upon deliberate deactivation by an inhibitor of an organometallic reaction that catalytically creates a fluorophore or chromophore. When the deactivation process is performed in the presence of the analyte, competitive binding of the inhibitor with the analyte and the catalyst occurs. The extent that the deactivating ligand is sequestered by the analyte directly affects the rate of the catalytic reaction, and the concentration of the analyte can be deciphered through monitoring of the fluorescence. In one example, reproducible results for Cu(II) were reported for both qualitative detection and quantitative measurement (Scheme 1).[15a] Fluorophore 3-methylindole (**2**) was generated through a Pd(0) catalyzed Heck reaction of 2-iodo-*N*-allylaniline (**1**). Cyclam was used as the inhibitor, which possesses higher affinity to Cu(II) compared to other metals (Cd(II), Co(II), Ni(II)). This study showed that Cu(II) can successfully restore the Heck reaction, and the limit of detection was 30 nM. As an extension of this protocol, FRET induced by a Cu(I) catalyzed Huisgen cycloaddition was exploited[15b] for investigation of other metal analytes (*e.g.* Zn(II), Pb(II), Mg(II)). This study showed that the inhibitor EDTA has preferential coordination with Zn(II) and Pb(II), thus releasing free Cu(II) to the catalytic cycle (Scheme 2).

In this paper, we report a Heck reaction that is faster than that of Scheme 1, producing 7-diethylaminocoumarin (**4**), which has a higher quantum yield of fluorescence than **2**. We use this system for general toxic transition metals detection, specifically, Cd(II). The Heck reaction of acrylic acid 5-diethylamino-2-iodo-phenyl ester (**3**) produces the fluorophore **4** (Scheme 3). The electron withdrawing carbonyl group adjacent to the terminal alkene in **3** makes it more electrophilic, therefore increasing the Heck coupling rate.[16] 1,4,7,10,13 Pentaaza-cyclopentadecane (**5**) is a classic ligand for complexation of Cd(II), the coordination complex crystal structure of which has been previously reported.[17] As reported here, the method is faster than that reported for our original study, and the increased quantum yield of the fluorophore



Scheme 1 Fluorophore 3-methylindole (**2**) is generated from the non-fluorescent compound 2-iodo-*N*-allylaniline (**1**).

*anslyn@ccwf.cc.utexas.edu



Scheme 2 A FRET system constructed through an *in situ*-generated Cu(I) catalyzed regiospecific Huisgen cycloaddition.

enhances sensitivity, but the new reaction is hampered by a slow initiation step at low concentrations of analyte.

Experimental

All reactions were run under an atmosphere of argon unless otherwise indicated. Reaction vessels were oven or flame-dried and allowed to cool in a dry box or desiccator prior to use. Solvents and reagents were purified by standard methods.[18] The chemicals were obtained from Aldrich and Fischer, and no further purification was done unless otherwise noted. Acetonitrile (CH_3CN) was chromatography grade; triethylamine (Et_3N) was distilled from calcium hydride right before usage. ^1H NMR and ^{13}C NMR were obtained from a Varian Unity Plus 300 or 400 spectrometer. For ^1H NMR, chemical shifts are reported in parts per million (ppm) and referenced to the residual proton resonance peaks: δ 7.24 for $CHCl_3$. Emission spectra were obtained on a PTI fluorimeter. A Finnigan VG analytical ZAB2-E spectrometer was used to obtain high resolution mass spectra.

Acrylic acid 3-diethylamino-phenyl ester (7)

To a 25 mL round bottom flask was added 3-diethylamino-phenol **6** (343 mg, 2.1 mmol), Et_3N (0.28 mL, 4 mmol), 4-dimethylaminopyridine (DMAP, 6 mg, 0.1 mmol.), and CH_2Cl_2 (7 mL). The resulting solution was cooled to 0 °C followed by slow addition of acryloyl chloride (0.1 mL, 2.4 mmol). After 1.5 h, the reaction solution was poured into aqueous saturated $NaHCO_3$ (8 mL) and the resulting mixture was extracted with chloroform (3 × 5 mL). The combined organic solution was washed with H_2O (2 × 2 mL), brine (3 mL) and dried ($MgSO_4$). After filtration through a small pad of celite, volatiles were removed under reduced pressure. The product was purified by chromatography on silica gel using 9 : 1 hexanes–EtOAc for elution to give the title compound as a light yellow oil (0. 27 g, 61%). ^1H NMR (400 MHz, $CDCl_3$): δ 7.18 (dd, J = 8.8, 2.4 Hz, 1H), 6.58 (dd, J = 16.8, 0.8 Hz, 2H), 6.52 (d, J = 7.6 Hz, 1H), 6.37 (d, J = 6.8 Hz, 1H), 6.30 (dd, J = 17.2, 10.4 Hz, 2H), 5.97 (dd, J = 10, 1.2 Hz, 1H), 3.32 (q, J = 14, 6.8 Hz, 4H), 1.14 (t, J = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.7, 151.9, 149.0, 132.1, 129.8, 128.2, 109.2, 107.9, 104.5, 44.4, 12.5.

Acrylic acid 5-diethylamino-2-iodo-phenyl ester (3)

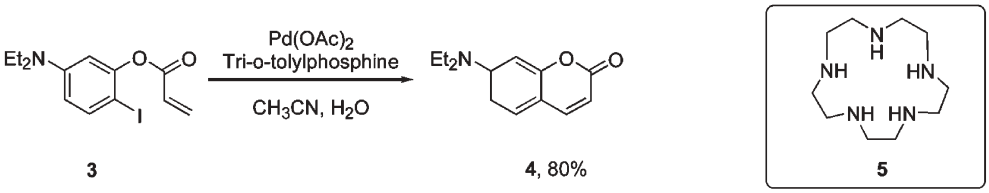
Acrylic acid 3-diethylamino-phenyl ester **7** (37 mg, 0.17 mmol) was dissolved in 2 mL dry CH_2Cl_2 in a 25 mL round bottom flask. To the resulting solution was added thallium(I) acetate (53.3 mg, 0.203mmol) and a CH_2Cl_2 (5 mL) solution of iodine (48.2 mg, 0.19 mmol). The mixture was allowed to stir at room temperature for 48 h. After filtration through a small pad of celite, the solution was concentrated under reduced pressure and the residue was diluted in Et_2O (6 mL). The resulting solution was washed sequentially by water, 5% $Na_2S_2O_3$, brine and dried ($MgSO_4$). The product was purified by chromatography on silica gel using 9 : 1 hexanes–EtOAc for elution to give the title compound as a light yellow oil (53.8 mg, 82%). ^1H NMR (400 MHz, $CDCl_3$): δ 7.49 (d, J = 8.8 Hz, 1H), 6.66 (dd, J = 17.6, 1.6 Hz, 1H), 6.41 (d, J = 2.4 Hz, 1H), 6.37–6.30 (m, 2H), 6.03 (dd, J = 10.4,1.2 Hz, 1H), 3.29 (q, J = 14 Hz, 4H), 1.126 (t, J = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.8, 151.9, 149.0, 132.9, 133.0, 127.9, 111.69, 109.7, 106.2, 44.5, 12.3; HRMS m/z calcd for $C_{13}H_{16}NIO_3$ $[M]^+$ 345.0226, found: 345.0224.

7-Diethylaminocoumarin (4)

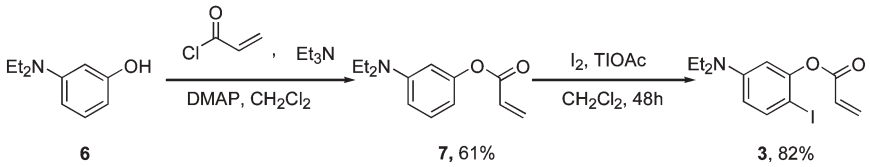
Acrylic acid 5-diethylamino-2-iodo-phenyl ester **3** (0.0398 g, 0.115 mmol) was dissolved in a mixed solvent of CH_3CN–H_2O (5 mL : 0.5 mL) in a 25 ml round bottom flask under argon. To the flask was sequentially added $Pd(OAc)_2$ (0.64 mg, 0.0029 mmol), Et_3N (30 μL, 0.23 mmol), and tri-*o*-tolylphosphine (1.75 mg, 0.0057 mmol). The solution was stirred at 70 °C for 1 h. The reaction mixture was partitioned with Et_2O and H_2O, and the organic layer was dried over anhydrous $MgSO_4$. After filtration through a small pad of celite, volatiles were removed under reduced pressure. The product was purified by chromatography on silica gel using 7 : 1 hexanes–EtOAc for elution to give the title compound as a light yellow solid (20 mg, 80%). Mp 90–94 °C; ^1H NMR (400 MHz, $CDCl_3$): δ 7.51 (d, J = 9.2 Hz, 1H), 7.22 (d, J = 8.8 Hz, 1H), 6.54 (dd, J = 8.8,2.4 Hz, 1H), 6.47 (d, J = 2.4 Hz, 1H), 6.01 (d, J = 9.2 Hz, 1H), 3.39 (q, J = 14 Hz, 4H), 1.19 (dd, J = 6.8, 2.8 Hz, 6H); HRMS m/z calcd for $C_{13}H_{15}NO_2$ $[M + H]^+$ 218.1181, found: 218.1174.

Measurements

Solutions of acrylic acid 5-diethylamino-2-iodo-phenyl ester (**3**, 0.5 mM), tri-*o*-tolyphosphine (0.05 mM), triethylamine (1 mM) were prepared in CH_3CN–H_2O (10 : 1) in 3.5 mL



Scheme 3 Synthesis of **4**.



6 7, 61% 3, 82%

Scheme 4 Synthesis of **3**.

quartz cuvettes, followed by the addition of 1,4,7,10,13-pentaaza-cyclopentadecane (**5**, 0.025 mM) and the analyte. After stirring at rt for 5 min, $Pd(OAc)_2$ (0.025 mM) was added into the solution. The product was excited at 377 nm (absorption maximum), emission was monitored at 450 nm (emission maximum) in real time at 60 °C, under argon.

Results and discussion

The substrate acrylic acid 5-diethylamino-2-iodo-phenyl ester (**3**) was prepared from commercially available 3-diethylaminophenol (**6**) through acylation[19] with acryloyl chloride followed by regioselective iodination[20] (Scheme 4). The non-fluorescent product (**3**) was subjected to an intramolecular Heck coupling condition under the mediation of $Pd(OAc)_2$ to afford fluorophore product 7-diethylaminocoumarin (**4**) (Scheme 3). Ligand 1,4,7,10,13-pentaaza-cyclopentadecane (**5**) was prepared according to the literature procedure.[21]

Control experimental analysis

The Heck reaction is a Pd(0) catalyzed chemical process, and it has not been reported to be influenced by Cd(II). As demonstrated in Fig. 1, curve (a) represents a Heck reaction without exogenous Cd(II) and inhibiting ligand. In the presence of Cd(II), the reaction curve (b) perfectly overlapped with the former, indicating no interference with the Heck reaction. Theoretically, when an equal amount of Pd(II) and the inhibiting ligand are present in the solution, complete complexation would be established and no active palladium would flow into the Heck reaction cycle. However, in this case the Heck reaction was not completely shut down. As represented by curve (e), very slow product formation was observed. The observed result could be attributed to a thermodynamic binding equilibrium. In our previous study, the fluorescence was monitored once per 10 minutes to minimize possible side photo reactions.[15a] Curve (d) in Fig. 1 indicates that photo reactions are negligible in the current Cd(II) detection study.

Cd(II) kinetic study by monitoring the fluorescence of coumarin (4)

As anticipated, introducing one equivalent of Cd(II) and an equivalent of inhibiting ligand does not change the Heck reaction rate (curve (c) *vs.* (a), Fig. 1). This result reveals that one Cd(II) releases one Pd(II), and by thus monitoring the fluorescence intensity one can obtain the Cd(II) concentration in the system. Hence, a series of reactions were performed in the fluorimeter. The reaction progress was monitored in real time by tracking the fluorescence intensity of coumarin. Various reaction rates were collected for concentrations of Cd(II) varying from 25 μM to 0 μM (Fig. 2). If the conversion of starting compound into product is monitored over only the first 10% of reaction, pseudo-first order initial rate kinetics can be applied. By dividing the rate of the reaction by the Cd(II) concentration, the observed rate constant was obtained. A plot of this rate constant as a function of [Cd(II)] is given in Fig. 3. The rate constants were measured between 300 seconds and 700 seconds in Fig. 2. It is well established that Pd(II) has to be reduced by tri-*o*-tolyphosphine into Pd(0) to catalyze the coupling reaction, and this initiation process was complete

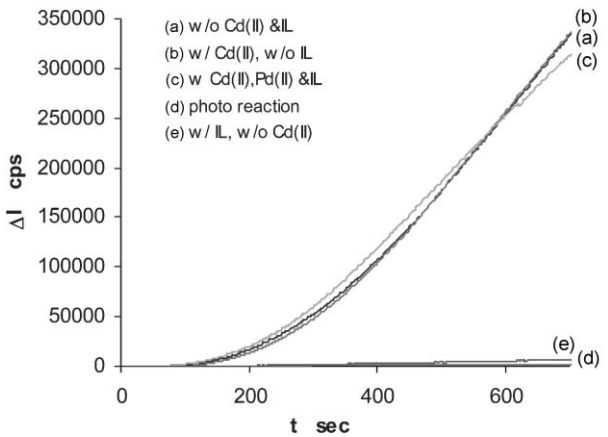


Fig. 1 Control experiments. Coumarin was excited at 377 nm, emission was monitored at 451 nm. Reaction conditions: [**3**] = 0.5 mM, [Pd(II)] = 0.025 mM, [IL] = 0.025 mM, at 60 °C, CH_3CN–H_2O (10 : 1 v/v), Ar.

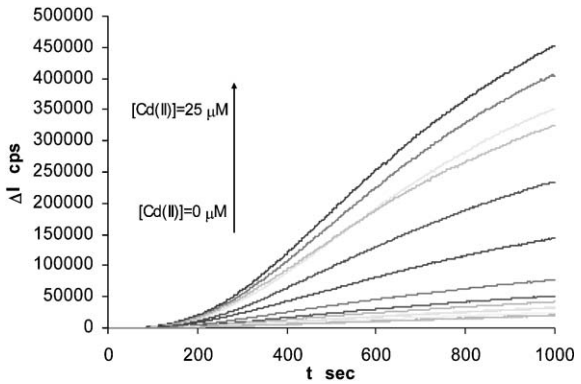


Fig. 2 Kinetic study of Cd(II). Real time monitoring of Pd(0) catalyzed Heck reactions with various concentrations of Cd(II) being subjected to the system. Coumarin was excited at 377 nm, emission was monitored at 451 nm. Reaction conditions: [**3**] = 0.5 mM, [Pd(II)] = 0.025 mM, [IL] = 0.025 mM, at 60 °C, under Ar. Co-solvent CH_3CN–H_2O (10 : 1 v/v).

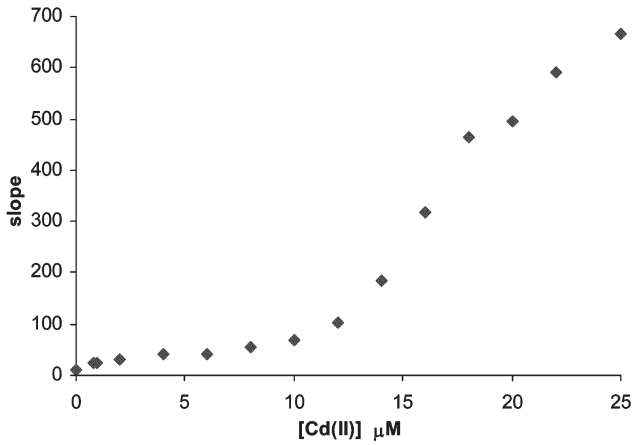


Fig. 3 Plot of slope *vs.* [Cd(II)].

within the first 300 seconds for the more concentrated Cd(II) solutions. However, by inspection of the kinetic plots for the lower concentrations of Cd(II), it does not appear that the initiation step is complete by 300 s.

In the proposed mechanism, the relationship between the initial rate and the corresponding analyte (Cd(II)) concentration should be constant. In practice, however, this was not observed. As shown in Fig. 3, when the Cd(II) concentration is lower than 10 μM the rate is much slower than that with higher concentration. This appears to be due to the incomplete initiation of Pd(II) to Pd(0), as required in the catalytic Heck cycle, when lower Cd(II) concentrations are used. Initiation is a second order process, and hence with a large fraction of Pd(II) sequestered by the cyclam the free Pd(II) is negligible, resulting in very slow initiation by the added tri-*o*-tolylphosphine. In the lowest concentration samples, the initiation does not appear complete even after 1000 seconds. Therefore, the initial rates plotted in Fig. 3 near or below 10 μM do not correspond to the same steps that are plotted at the higher concentrations. This limits the sensitivity of the method we report here, and indicates that future directions for this strategy will focus on catalytic cycles that do not require initiation steps.

Selectivity analysis

Many transition metals have high affinities to poly-aza macrocyclic ligands. We were interested in testing the selectivity of this sensing protocol. Hence, the transition metals Co(II) and Ni(II) were analyzed using the same procedure as applied to Cd(II). Fig. 4 shows that the catalytic production of fluorophore **4** tracks the affinities of the inhibiting ligand for Ni(II), Co(II), Cd(II). The higher the binding affinity, the greater the initial rate observed.

Conclusion

In conclusion, a new signal amplification protocol for Cd(II) recognition has been developed. This methodology involves a Pd(II) catalyzed Heck transformation of acrylic acid 5-diethylamino-2-iodo-phenyl ester (**5**) to fluorescent product 7-diethylaminocoumarin (**6**). This reaction shortens the detection time compared to the former one shown in Scheme 1, but the slow

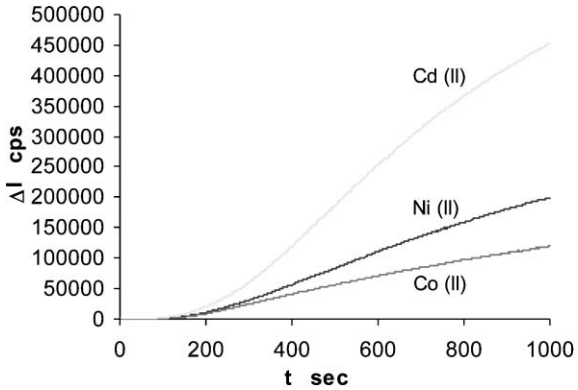


Fig. 4 Sensitivity analysis. Reaction conditions: [**5**] = 0.5 mM; [Pd(II)] = 0.025 mM; [IL] = 0.025 mM; [Ni] = [Co(II)] = [Cd] = 0.025 mM; 60 °C; Solvent: CH_3CN/H_2O (V:V/10:1). Coumarin **6** was excited at 377 nm, emission was monitored at 451 nm.

initiation associated with the Heck catalyst limits the sensitivity. The general protocol is associated with easy reagent accessibility, and convenient operation. As we and others expand the general principle, we foresee high potential for practical applications.

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Qiaoyin Wu and Eric V. Anslyn*
Department of Chemistry and Biochemistry, The University of Texas at Austin, Austin, TX, USA. E-mail: anslyn@ccwf.cc.utexas.edu

References

1 (*a*) K. R. A. S. Sandanayake and I. O. Sutherland, *Sens. Actuators B: Chem.*, 1993, **B11**, 331; (*b*) D. R. Johnson and L. G. Bachas, *Anal. Bioanal. Chem.*, 2003, **376**, 328; (*c*) M. Ando, *Chem. Sens.*, 2001, **17**, 123; (*d*) B. Mizaikoff, *Anal. Chem.*, 2003, **75**, 258A.
2 (*a*) I. Willner and E. Katz, *Angew. Chem., Int. Ed.*, 2000, **39**, 1180; (*b*) D. R. Thévenot, K. Toth and G. S. Wilson, *Pure Appl. Chem.*, 1999, **71**, 2333; (*c*) G. S. Wilson and Y. Hu, *Chem. Rev.*, 2000, **100**, 2693; (*d*) T. Chen, K. A. Friedman, I. Lei and A. Heller, *Anal. Chem.*, 2000, **72**, 3757.
3 (*a*) E. Yashima, k. Maeda and T. Nishimura, *Chem. Eur. J.*, 2004, **10**, 4; (*b*) L. Zhu and E. V. Anslyn, *J. Am. Chem. Soc.*, 2004, **126**, 3676–3677; (*c*) Z.-B. Li, J. Lin, H.-C. Zhang, M. Sabat, M. Hyacinth and L. Pu, *J. Org. Chem.*, 2004, **69**, 6284.
4 A. Miyawaki, J. Llopis, R. Helm, J. M. McCaffery, J. A. Adams, M. Ikura and R. Y. Teisen, *Nature*, 1997, **388**, 882.
5 (*a*) A. K. Deisingh, D. C. Stone and M. Thompson, *Int. J. Food Sci. Technol.*, 2004, **39**, 587–604; (*b*) W. Khampha, V. meevootisom and S. Wiyakrutta, *Anal. Chim. Acta*, 2004, **520**, 133.
6 (*a*) R. Pohl, D. Aldakov, P. Kubát, K. Jursíková, M. Marquez and P. Anzenbacher, Jr., *Chem. Commum.*, 2004, 1282; (*b*) T. Vo-Dinh, *Sens. Actuators B*, 1995, **29**, 183.
7 (*a*) J. S. Hartig, I. Grüne, S. H. Najafi-Shoushtari and M. Famulok, *J. Am. Chem. Soc.*, 2004, **126**, 722; (*b*) B. Liu and G. C. Bazan, *Chem. Mater.*, 2004, in press; (*c*) J. J. Harvey, S. P. Lee, E. K. Chan, J. H. Kim, E.-S. Hwang, C.-Y. Cha, J. R. Knutson and M. K. Han, *Anal. Biochem.*, 2004, **333**, 246; (*d*) T. M. Swager, *Acc. Chem. Res.*, 1998, **31**, 201; (*e*) A. Ehret, M. T. Spitler and L. S. Stuhl, *Comments Inorg. Chem.*, 2002, **23**, 275; (*f*) W. J. Blaedel and R. C. Boguslaski, *Anal. Chem.*, 1978, **50**, 1026; (*g*) T. Shibata, J. Yamamoto, N. Matsumoto, S. Yonekabo, S. Osanai and K. Soai, *J. Am. Chem. Soc.*, 1998, **120**, 12157.
8 R. C. H. Kwan, P. Y. T. Hon and R. Renneberg, *Anal. Chim. Acta*, 2004, **523**, 81.

9 J. S. Hartig, I. Grüne, S. H. Najafi-Shoushtari and M. Famulok, *J. Am. Chem. Soc.*, 2004, **126**, 722.
10 (*a*) H. C. Hang, C. Yu, M. R. Pratt and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2004, **126**, 6; (*b*) P. Geymayer, N. Bahr and J.-L. Reymond, *Chem. Eur. J.*, 1999, **5**, 1006; (*c*) Y. Weizmann, F. Patolsky, E. Katz and I. Willner, *J. Am. Chem. Soc.*, 2003, **125**, 3452; (*d*) M. P. Robertson and A. D. Ellington, *Nat. Biotechnol.*, 1999, **17**, 62.
11 Y. Kim, Z. Zhu and T. M. Swager, *J. Am. Chem. Soc.*, 2004, **126**, 452.
12 T. Kim and T. M. Swager, *Angew. Chem., Int. Ed.*, 2003, **42**, 4803.
13 K. Kuroda and T. M. Swager, *Macromol. Symp.*, 2003, **201**, 127.
14 E. Yashima, K. Maeda and T. Nishimura, *Chem. Eur. J.*, 2004, **10**, 42.
15 (*a*) Q. Wu and E. V. Anslyn, *J. Am. Chem. Soc.*, 2004, **126**, 14682; (*b*) L. Zhu, V. M. Lynch and E. V. Anslyn, *Tetrahedron*, 2004, **60**, 7267.
16 G. T. Crisp, *Chem. Soc. Rev.*, 1998, **27**, 427.
17 G. W. Franklin, D. P. Riley and W. L. Neumann, *Coord. Chem. Rev.*, 1998, **174**, 133.
18 W. L. F. Armarego and D. D. Perrin, *Purification of Laboratory Chemicals*, 4th edn., Butterworth Heinemann, Oxford and Boston, 1996.
19 K. Hiroya, N. Suzuki, A. Yasuhara, Y. Egawa, A. Kasano and T Sakamoto, *J. Chem. Soc., Perkin Trans. 1*, 2000, 4339.
20 R. C. Cambie, P. S. Rutledge, T. Smith-Palmer and P. D. Woodgate, *J. Chem. Soc., Perkin Trans. 1*, 1976, 1161.
21 Z. Kovacs, E. A. Archer, M. K. Russell and A. D. Sherry, *Synth. Commun.*, 1999, **29**, 2817.