Detection of human lung carcinoma cell using quartz crystal microbalance amplified by enlarging Au nanoparticles *in vitro*

Zhanfang Ma,[a] Jinglian Wu,[b] Tianhong Zhou,[c] Zhihua Chen,[c] Yonggui Dong,[b] Jintian Tang[c] and Sen-fang Sui*[a]

[a] *Department of Biological Sciences and Biotechnology, Tsinghua University, Beijing 100084, P. R. China. E-mail: suisf@mail.tsinghua.edu.cn; Fax: + 86 10 6278 4768; Tel: + 86 10 6278 4768*
[b] *Department of Precision Instruments and Mechanology, Tsinghua University, Beijing 100084, P. R. China*
[c] *Institute of Clinical Medical Science, China-Japan Friendship Hospital, Beijing 100020, P. R. China*

Received (in Montpellier, France) 28th June 2002, Accepted 3rd September 2002
First published as an Advance Article on the web 30th October 2002

A new amplification route for human lung carcinoma cell detection based on the detection of gold deposition on an Au-antibody conjugate label acting as a ''seeding'' catalyst is reported. Quartz crystal microbalance (QCM) was employed to measure the catalyzed deposition of gold on the piezoelectric crystals. The gold-coated quartz crystal was modified by polystyrene films, which provided a substrate for antibody immobilization by physical adsorption. Atomic force microscopy (AFM) was used to evaluate the morphologies and smoothness of the polystyrene films obtained by dip coating. The polystyrene films significantly improve the surface smoothness of the electrode. Compared to the conventional Enzymelinked Immunosorbent Assay (ELISA), the method described herein is a less time consuming procedure and gives comparable detection sensitivity of human lung carcinoma cells, *ca.* 100 cells mL^{-1}. This will be of great significance in the diagnosis of this disease.

The development of simple, rapid and sensitive approaches to detect biomolecules is a topic of significant interest because of its potential applications in the diagnosis of disease.[1–5] The use of gold nanoparticles as a signal amplifier has attracted increasing interest in biosensor development.[6–10] For example, in surface plasmon resonance and QCM based on protein binding assay and DNA hybridization assay, mass coupling of functional Au nanoparticles results in a great increase of sensitivity.[11–16] Although immunogold silver staining is commonly used to visualize protein-, antibody-, and DNA-conjugated gold nanoparticles in histochemical electron microscopy studies,[17] this amplification strategy has been adopted in a pioneering study of scanometric DNA array quantification.[18] The incorporation of Au nanoparticle labeling and silver staining amplification allows a 100-fold increase in sensitivity over conventional fluorescent DNA detection.

QCM is a very sensitive mass-measuring device in the gas phase and in aqueous solution.[19,20] Free of using specific indicators, QCM is a promising candidate in biosensor applications.[21] In conventional solid phase immunoassays, polystyrene has been frequently used as a solid support or reaction carrier for biological interactions.[22] Antibodies and antigens can be attached easily through passive adsorption or hydrophobic interactions. Therefore, covering the gold electrode of QCM with a polystyrene film will enable the use of standard ELISA-type protocols for antibody attachment.

We present herein a QCM biosensor, in which a polystyrene film is used to modify the QCM crystal and the primary Au nanoparticle-amplified sandwiched-immunoassay is followed by the deposition of gold using Au nanoparticles as a ''seeding catalyst''. Human lung carcinoma cells (LCC) as a model were detected using the present method.

Experimental

Materials and apparatus

LCC and anti-surface antigen on LCC monoclonal antibody (ALT04) were kind gifts from the Institute of Clinical Medical Science, China-Japan Friendship Hospital (Beijing, China). Hydrochloroauric acid and hydroxylamine hydrochloride were purchased from Beijing Chemical Reagents Company (Beijing, China). BSA and tris base were obtained from Sigma (USA). Water used in the study was Milli-Q water (18.2 $M\Omega\ cm^{-1}$). The reaction carriers used were AT-cut, 5 MHz quartz crystals with gold electrodes purchased from Beijing Chenguang Electronics Ltd. Co. (Beijing, China). The quartz wafer diameter was 8.8 mm and the gold electrode diameter was 5 mm.

Preparations

Au nanoparticles. Au nanoparticles were prepared according to the literature[23] with slight modification. All glassware used in these preparations was thoroughly cleaned, rinsed with water, and oven dried prior to use. The following stock solutions were made fresh as needed using water. In a 1 L round-bottom flask 500 mL of 1 mM $HAuCl_4$ was brought to a rolling boil with vigorous stirring. 40 mL of 38.8 mM sodium citrate was rapidly added to the vortex of the solution. Boiling was continued for 30 min; the heating mantle was then removed, and stirring was continued for an additional 15 min.

Samples for particle sizing by TEM were prepared by drop coating 10 μL of the colloid onto a Formvar-coated Cu grid and following the sample to dry. The average diameter of the Au colloid was approximately 10 nm with a standard deviation of ~2 nm.

Colloidal Au-ALT04 antibody conjugates. The conjugates were synthesized by the addition of 400 μg ALT04 antibody to 10 mL of colloidal Au solution (~40 nM, pH 8.2) followed by incubation on ice with periodic gentle mixing for 1 h. The conjugate was centrifuged at 12 500 g for 45 min. The supernatant was removed and the soft pellet resuspended in a 40 mM phosphate buffer (pH 7.0). The conjugates were stored at 4 °C.

LCC culture. LCC was cultured in Dulbecco's modified Eagles medium (DMEM) containing 10% fetal calf serum (FCS). Cells were grown in a 37 °C incubator with 5% CO_2 atmosphere. Cells were then washed, trypsininzed, and resuspended in DMEM with 10% FCS.

Preparation and characterization of polystyrene films

The quartz crystals covered with gold electrode were cleaned with "piranha solution", a 30:70 mixture (by volume) of 30 g L^{-1} H_2O_2 and concentrated H_2SO_4. ***Caution: such a solution should be handled with extreme care!*** The cleaned QCM plate was thoroughly rinsed in water, absolute ethanol, acetone, and water with sonication for 10 min. The plate was then dried in a stream of nitrogen. Polystyrene was dissolved in toluene to form solutions with different concentrations. Polystyrene films were prepared by dipping a cleaned QCM plate into polystyrene solution for 10 min. The excess of polystyrene solution on the QCM plate was carefully removed by filter paper from the edge of the plate. The QCM plate was then thoroughly dried in air atmosphere for about 2 days. AFM imaging of the surface was carried out using a commercial AFM SPA-400 (Seiko Instruments Inc., Japan). The instrument was operated in non-contact AFM mode using silicon cantilevers oscillating with an amplitude of 0.992 V. The scanning rate selected was 1 Hz. All of the images presented here were obtained repeatedly and were stable under the experimental conditions. The surface roughness was calculated after horizontal leveling.

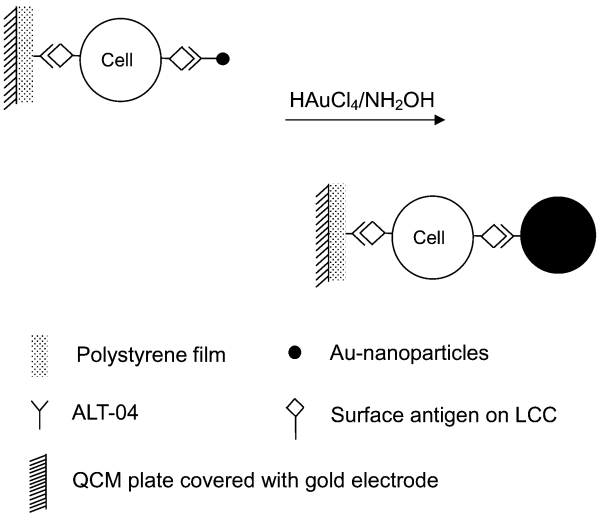
Detection of LCC using QCM amplified by enlarging Au nanoparticles *in vitro*

A QCM plate covered with a polystyrene film was incubated in ALT04 solution at a concentration of 0.3 mg mL^{-1} in PBS buffer (pH 7.4, 0.15 M NaCl) at 37 °C for 1 h. This QCM plate was treated subsequently with 3% BSA, LCC suspension, and Au-ALT04 conjugate. Finally, this QCM plate was treated with mixture of 0.01% $HAuCl_4$ and 0.4 mM NH_2OH at 30 °C for 2 min. After each step, the QCM plate was fully rinsed with PBS buffer and water. Prior to measuring the frequency decrease, the QCM plate must be thoroughly dried. The frequency changes were measured at 25 °C in air atmosphere.

Results and discussion

Principle of the method

While NH_2OH is thermodynamically capable of reducing Au^{3+} to bulk metal,[24] the reaction is dramatically accelerated by Au surfaces.[25] As a result, no new particle nucleation occurs in solution and all added Au^{3+} goes into production of larger particles. In this case, Au nanoparticles will be specifically enlarged by $HAuCl_4$ and NH_2OH. The enlarging reaction is usually complete within 2 min,[26] therefore a time of 2 min was used for the enlarging step. A model immunochemical molecular recognition event between ALT04 and surface antigen on LCC is chosen to illustrate the sandwich procedure (Scheme 1). First, the gold electrode was modified by a polystyrene film (from a 5 mg mL^{-1} toluene solution), which provides a substrate for the immobilization of ALT04 antibodies. Second, the sensor surfaces were exposed to ALT04 solution.



Scheme 1 Schematic diagram of the detection of LCC with QCM amplified by enlarging Au nanoparticles *in vitro* by 0.01% $HAuCl_4$ and 0.4 mM NH_2OH.

The ALT04 molecules were firmly immobilized on the polystyrene films through passive adsorption and hydrophobic interaction. After blocking the non-occupied binding sites with BSA, the modified sensor surface was exposed to a LCC suspension and then to the conjugate of 10 nm Au nanoparticles and ALT04. As a result, the analyte LCC was sandwiched between bound ALT04 and Au-conjugated ALT04, and the Au nanoparticles were immobilized on the surface of the LCC. Finally, the mixture of $HAuCl_4$ and NH_2OH was applied to the sensor surface. Upon formation of the sandwiched immunocomplex, the sensor surfaces are coated with Au nanoparticles, which serve as nucleation sites to catalyze gold ion reduction. The deposition of gold ions on the surface of the bound Au nanoparticles will result in the dramatic frequency decrease.

Preparation and characterization of polystyrene films

Conventional polymer coating techniques include spin coating, drop coating, and dip coating.[27] For a well-fabricated quartz crystal, spin coating is difficult because of its irregular shape. In this study, therefore, dip coating was adopted due to its ease of operation. The microscopic structure of the electrode surface plays an important role in the adsorption of antibody or other proteins. To determine the optimum film flatness, the morphology and surface analysis before and after polystyrene modification with three solutions (2.5, 5, and 10 mg mL^{-1}) were studied using AFM.

Fig. 1 shows representative AFM images of the gold electrode with and without the polystyrene film. The corresponding surface analysis is also indicated. For a bare gold electrode, the AFM image reveals that there are many gold particle domains and holes as shown in Fig. 1(A). The average surface roughness was 0.833. Compared to the bare gold electrode, the smoothness of the gold electrode covered with polystyrene film has been improved. Nevertheless, for 2.5 mg mL^{-1} polystyrene solution, there are still many obvious gold particle domains and holes [Fig. 1(B)]. The corresponding average surface roughness was 0.441. In contrast, for 5 and 10 mg mL^{-1} polystyrene solutions, the morphologies of the gold electrode covered with polystyrene film were fairly uniform and smooth as shown in Fig. 1(C) and 1(D). Their corresponding average surface roughness was 0.226 and 0.221. The surface analysis data were consistent with the results obtained from the AFM images (traces a–d in Fig. 1).

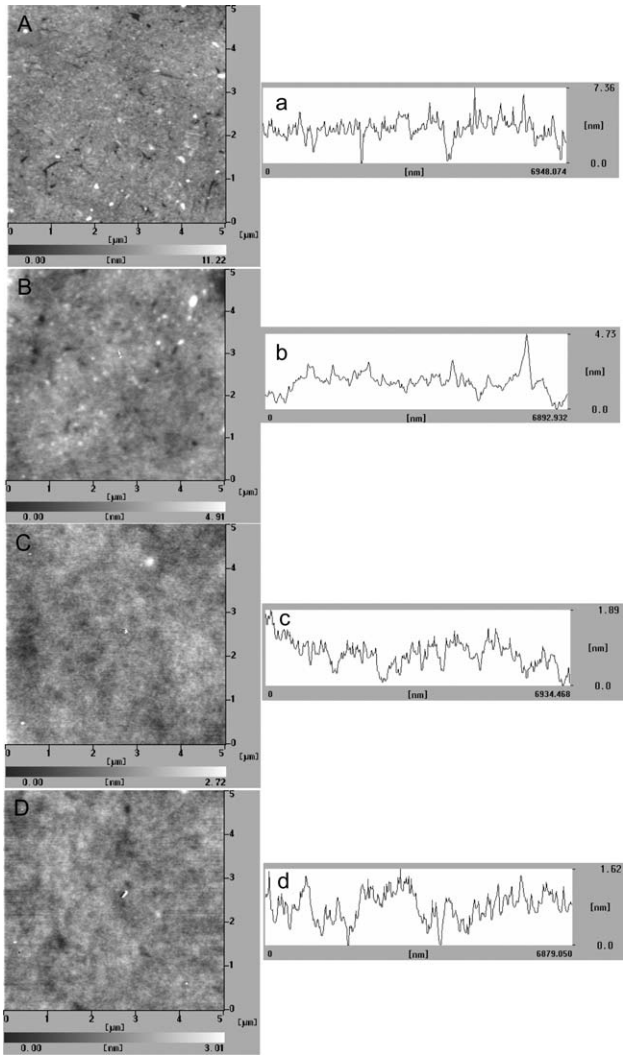


Fig. 1 AFM images of the gold electrode treated with polystyrene toluene solution with different concentrations: (A/a) bare gold electrode, (B/b) 2.5, (C/c) 5, and (D/d) 10 mg mL^{-1}. All of the corresponding cross-sectional analyses were carried out along a line extending from the top right corner to the bottom left corner.

These results demonstrate that for the concentration of polystyrene toluene solution used to modify the gold electrode by dip coating, there are no significant differences when using 5 or 10 mg mL^{-1}. Both solutions can provide smooth, uniform, and ideal polystyrene films. Therefore, the 5 mg mL^{-1} polystyrene solution was adapted to prepare the polystyrene film in the following experiments.

To address the nonspecific enlargement on the gold electrode covered with polystyrene film, the following control experiment was performed. The gold electrode covered with polystyrene film was treated directly with 0.01% $HAuCl_4$ and 0.4 mM NH_2OH. No frequency decrease was detectable. This implies that the gold electrode can be fully covered by the polystyrene film and no new gold nucleation occurs on the polystyrene film, showing that no significant nonspecific gold deposition occurs. This is the precondition for the present assay.

Detection of LCC using QCM amplified by 0.01% $HAuCl_4$ and 0.4 mM NH_2OH

Fig. 2 shows the frequency decrease caused by treatment with LCC suspension. The binding of LCC with ALT04 bound on the surface of polystyrene film will lead to a frequency decrease. For 1×10^3 and 1×10^4 cells mL^{-1}, the frequency decrease was approximately 50 and 100 Hz, respectively. The

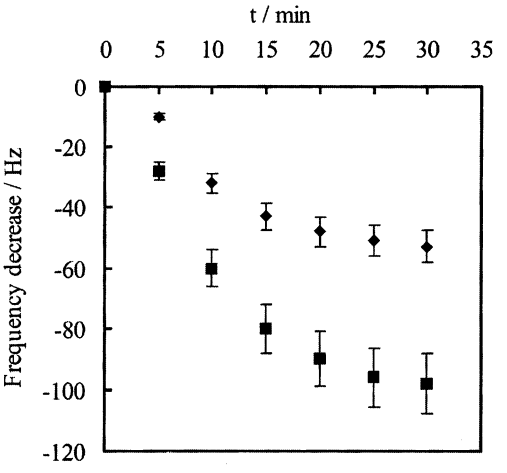


Fig. 2 LCC adsorption curves. Cell concentration: (◆) 1×10^3 and (■) 1×10^4 cells mL^{-1}.

complete binding was achieved after 30 min. These results show that the frequency decrease is dose-dependent to some extent. The following Au conjugate application results in a further frequency decrease. For 1×10^3 and 1×10^4 cells mL^{-1}, the frequency decrease caused was approximately 30 and 60 Hz, respectively, after binding of the Au-ALT04 conjugates to the surface antigens on LCC as shown in Fig. 3. As a control, the sensor surface without LCC was treated directly with conjugates of Au-ALT04. The frequency decrease caused was within *ca.* 5 Hz, showing that there is no nonspecific adsorption of Au-ALT04 on the sensor surface.

Fig. 4 shows the frequency responses of the sandwiched-immunocomplex-coated surfaces upon the enlargement of the immobilized Au nanoparticles *in vitro* by the mixture of 0.01% $HAuCl_4$ and 0.4 mM NH_2OH. The deposition of metallic gold on the surface of bound Au nanoparticles boosts the further dramatic frequency decrease. When the concentration of LCC is *ca.* 100 cells mL^{-1}, the frequency decrease is *ca.* 100 Hz after enlarging the immobilized Au nanoparticles *in vitro* by the mixture of 0.01% $HAuCl_4$ and 0.4 mM NH_2OH. This result was comparable to that of ELISA.[28] In order to address the nonspecific enlargement and nonspecific adsorption, the corresponding control experiment was performed. After the polystyrene film was treated subsequently with ALT04, BSA, and LCC, the obtained sensor surface was then treated with the mixture of 0.01% $HAuCl_4$ and 0.4 mM NH_2OH. The frequency decrease was within 20 Hz, which is much lower than that caused by *in vitro* enlarging the Au nanoparticles pre-adsorbed on the surface of LCC. This result

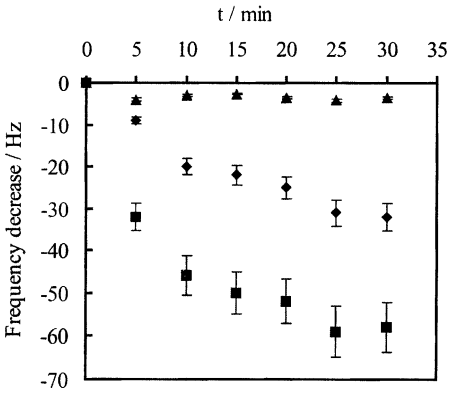


Fig. 3 Conjugate of Au and ALT04 adsorption curve. LCC concentration: (▲) control without LCC, (◆) 1×10^3 and (■) 1×10^4 cells mL^{-1}.

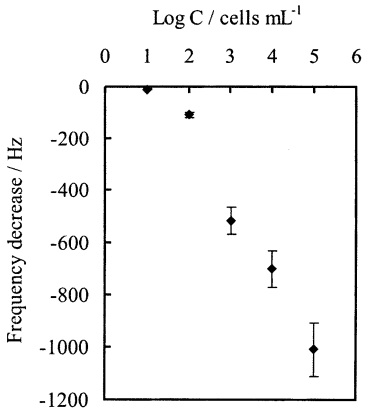


Fig. 4 Frequency response *versus* cell concentration after treatment with the mixture of 0.01% $HAuCl_4$ and 0.4 mM NH_2OH.

suggests that the detection sensitivity of LCC by the present method is *ca.* 100 cells mL^{-1}. No frequency decrease was detectable using Hela cell instead of LCC to treat the sensor surface immobilized ALT04, showing the sensitivity of the technique.

Although the QCM sensitivity was probably decreased by the polystyrene films on the QCM electrode to some extent, this effect may be neglected due to the same conditions for preparing the polystyrene films. The decrease of sensitivity can be overcome by the electrodeless deposition presented here. Moreover, the sensitivity will be significantly increased because the Au nanoparticles can be dramatically and specifically enlarged by the mixture of $HAuCl_4$ and NH_2OH. Therefore, the present method provides a new, simple and sensitive technique for biosensor applications.

Conclusions

The enlargement of Au nanoparticles *in vitro* by the mixture of 0.01% $HAuCl_4$ and 0.4 mM NH_2OH was successfully applied to amplify the sensitivity of the detection of human lung carcinoma cells using QCM. The results show that the detection sensitivity of this present method is 100 cells mL^{-1}, which is comparable to that of ELISA. These current results suggest a new method for immunoassays and will be of great importance in early and rapid clinical diagnosis.

Acknowledgements

This research was financed by grants from the National Natural Science Foundation of China and Tsinghua University.

References

1 D. H. Charych, J. O. Nagy, W. Spevak and M. Bednarski, *Science*, 1993, **261**, 585.
2 Z. F. Ma, J. R. Li, M. H. Liu, J. Cao, Z. Y. Zou, J. Tu and L. Jiang, *J. Am. Chem. Soc.*, 1998, **120**, 12 678.
3 R. Elghanian, J. J. Storhoff, R. C. Mucic, R. L. Lestinger and C. A. Mirkin, *Science*, 1997, **277**, 1078.
4 Z. F. Ma and S.-F. Sui, *Angew. Chem., Int. Ed.*, 2002, **41**, 2176.
5 J. E. Geswicki, L. E. Strong and L. L. Kissling, *Angew. Chem., Int. Ed.*, 2000, **39**, 4567.
6 L. He, M. D. Musick, S. R. Nicewarner, F. G. Salinas, S. J. Benkovic, M. J. Natan and C. D. Keating, *J. Am. Chem. Soc.*, 2000, **122**, 9071.
7 T. A. Taton, R. C. Mucic, C. A. Mirkin and R. L. Letsinger, *J. Am. Chem. Soc.*, 2000, **122**, 6305.
8 J. J. Storhoff and C. A. Mirkin, *Chem. Rev.*, 1999, **99**, 1849.
9 R. A. Reynolds, C. A. Mirkin and L. L. Lestinger, *J. Am. Chem. Soc.*, 2000, **122**, 3795.
10 S. -J. Park, A. A. Lazarides, C. A. Mirkin, P. W. Brazis, C. R. Kannewurf and R. L. Lestinger, *Angew. Chem., Int. Ed.*, 2000, **39**, 3845.
11 L. A. Lyon, M. D. Musick and M. J. Natan, *Anal. Chem.*, 1998, **70**, 5177.
12 L. A. Lyon, M. D. Musick, P. C. Smith, B. D. Reiss, D. J. Pena and M. J. Natan, *Sens. Actuators, B*, 1999, **54**, 118.
13 F. Patolsky, K. T. Ranjit, A. Lichtenstein and I. Willner, *Chem. Commun.*, 2000, 1075.
14 L. Lin, H. Q. Zhao, J. R. Li, J. A. Tang, M. X. Duan and L. Jiang, *Biochem. Biophys. Res. Commun.*, 2000, **274**, 817.
15 S. B. Han, J. Q. Lin, M. Satjapipet, A. J. Baca and F. M. Zhou, *Chem. Commun.*, 2001, 609.
16 Z. F. Ma, J. R. Li, L. Jiang, M. S. Yang and S.-F. Sui, *Chem. Lett.*, 2002, 328.
17 M. A. Hayat, *Colloid Gold: Principles, Methods and Applications*, Academic Press, San Diego, 1989, vol. 1.
18 T. A. Taton, C. A. Mirkin and R. L. Letsinger, *Science*, 2000, **289**, 1757.
19 H. Ebato, J. N. Herron, W. Muller, Y. Okahata, H. Ringsdorf and P. Suci, *Angew. Chem., Int. Ed. Engl.*, 1992, **31**, 1087.
20 Y. Okahata, K. Kimura and K. Ariga, *J. Am. Chem. Soc.*, 1989, **111**, 9190.
21 A. Janshoff, H.-J. Galla and C. Steinem, *Angew. Chem., Int. Ed.*, 2000, **39**, 4004.
22 J. R. Crowther, *ELISA Theory and Practice*, Institute for Animal Health Working, UK., Humana Press, Totowa, New Jersey, 1995, p. 63.
23 K. C. Grabar, R. G. Freeman, M. B. Hommer and M. J. Natan, *Anal. Chem.*, 1995, **67**, 735.
24 $E = 1$ V *versus* NHE(Au) and $E_{1/2} = -0.4$ V *versus* NHE-(NH_2OH). A. J. Bard, *Encyclopedia of Electrochemistry of the Elements*, Marcel Dekker, Inc., New York, 1975, vol. 4.
25 G. Stremsdoerfer, H. Perrot, J. R. Martin and P. J. Clechet, *J. Electrochem. Soc.*, 1988, **135**, 2881.
26 K. R. Brown and M. J. Natan, *Langmuir*, 1998, **14**, 726.
27 J. S. Schultz, *Handbook of Chemical and Biological Sensors*, Institute of Physics Publishing, Bristol and Philadephia, 1996, p. 155.
28 Z. F. Ma, R. Liang, W. L. Jiang, T. H. Zhou, Z. H. Chen, M. X. Duan, J. T. Tang and S.-F. Sui, *Chem. Lett.*, 2002, 570.