Organic & Biomolecular Chemistry

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/obc

Journal Name

RSCPublishing

COMMUNICATION

Visual Detection of Formaldehyde by Highly Selective Fluorophore Labeling *via* Gold(III) Complex-Mediated Three-Component Coupling Reaction

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

Kong-Fan Wong,[a] Jie-Ren Deng,[a] Xiao-Qun Wei,[b] Shi-Ping Shao,[b] Da-Peng Xiang*[b] and Man-Kin Wong*[a]

A novel method for visual detection of formaldehyde with excellent selectivity *via* a gold(III) complex-mediated three-component coupling reaction of resin-linked sterically bulky amines and fluorescent alkynes has been developed.

Formaldehyde is a reactive organic compound ubiquitously found in the environment[1] and present in food mainly due to natural occurrence and/or release from food packaging materials.[2] Common food contains formaldehyde with an average level of 1-98 mg/kg.[2] However, formaldehyde has been illegally added to food for preservation and bleaching with extreme cases of over 4,250 mg/kg.[2]

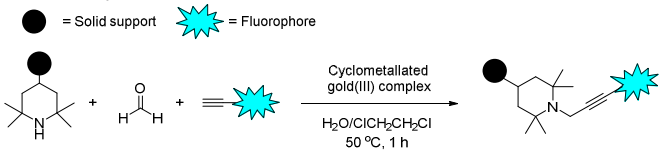
Serious exposure to formaldehyde may induce acute poisoning, dermal allergy, and irritation problems.[2] According to International Agency for Research on Cancer (IARC), formaldehyde is carcinogenic to humans in causing nasopharyngeal cancer.[2b] Chronic toxicities of formaldehyde include neurotoxicity, reproductive toxicity, hematotoxicity and genotoxicity. World Health Organization (WHO) establishes a tolerable daily intake of 0.15 mg/kg/day for formaldehyde. United States Environmental Protection Agency (USEPA) also defines an oral reference dose of 0.2 mg/kg/day.[2]

Standard analytical methods for formaldehyde measurement include gas chromatography[3] and liquid chromatography.[4] However, these methods require expensive instruments and sophisticated operational skills are not suitable for on-site detection. Significant advances have been achieved for rapid detection of gaseous formaldehyde.[5] Yet, the development of rapid formaldehyde detection for food samples remains sparse.

Without chromatographic separation and structural characterization by instruments, the key challenge of rapid detection methods is to achieve excellent specificity towards the target analytes. Hantzsch pyridine synthesis featuring multi-component coupling reaction of formaldehyde with 2,4-pentanedione and ammonium acetate affording 3,5-diacetyl-2,6-dihydrolutidine has been used as a highly selective reaction for formaldehyde detection since 1950s.[6] However, no major breakthrough in the development of highly selective chemical reactions amendable for rapid formaldehyde detection under mild reaction conditions has been made for decades.[1a]

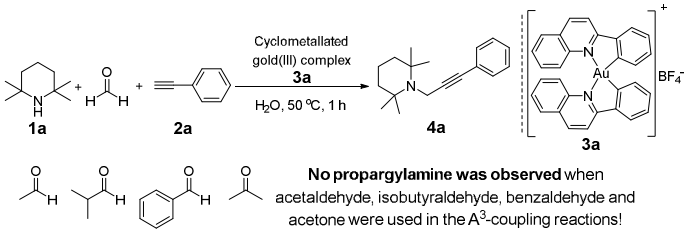
Gold-catalyzed three-component coupling reaction of aldehydes, amines, and alkynes (A^3-coupling reaction) has become a convenient approach for propargylamine synthesis.[7] Along with our ongoing efforts on the development of gold(III) catalysis for A^3-coupling reaction,[8] we have developed a bis-cyclometallated gold(III) complex with distorted square planar geometry as an efficient catalyst for propargylamine synthesis.[8e] The generally accepted reaction mechanism of the A^3-coupling reaction involves iminium ion generation from amines and aldehydes followed by gold-catalyzed alkylation of alkynes to give propargylamines. Formaldehyde is an aldehyde of the smallest size in nature. In principle, we are able to employ sterically bulky amines that could exclusively generate iminium ions with formaldehyde even in the presence of other aldehydes of larger sizes. Subsequent catalytic alkylation of the iminium ions by fluorescent alkynes would give fluorescent propargylamines. Accordingly, the amount of formaldehyde could be determined by the fluorescent intensity of the propargylamines.

Herein we first report a visual detection method with excellent selectivity for formaldehyde based on the gold(III)-mediated A^3-coupling reaction (Scheme 1). Using resin-linked sterically bulky amines, formaldehyde can be exclusively linked to fluorescent alkynes through propargylamine formation. A good linearity (R^2 = 0.9937) between the formaldehyde concentration and propargylamine formation was found by LC–MS/MS analysis. Using a hand-held UV lamp, the formaldehyde concentration (as low as 10 ppm) could be qualitatively determined by naked eyes. Excellent selectivity and tolerance have been demonstrated by application on formaldehyde detection of crude mushroom water extracts.



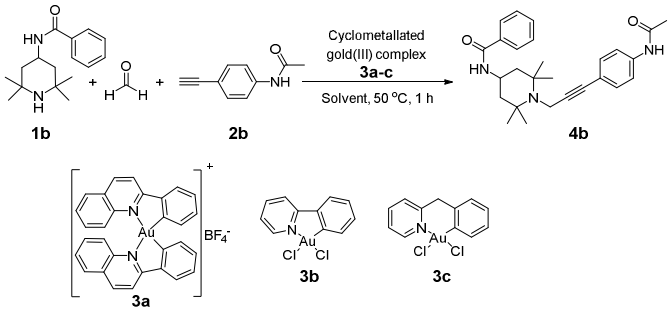
Scheme 1. Bis-cyclometallated gold(III) complex-mediated three-component coupling reaction for exclusive fluorescent detection of formaldehyde.

We hypothesized that sterically bulky amines would achieve excellent selectivity for formaldehyde in the step of iminium ion formation. Thus, sterically bulky 2,2,6,6-tetramethyl-piperidine (**1a**) bearing four alpha-methyl groups was chosen for the studies (Scheme 2). As a rapid detection method, we set out to conduct the A^3-coupling reaction for 1 h. Heating of formaldehyde (0.1 mmol), amine **1a** (0.11 mmol), phenylacetylene **2a** (0.15 mmol) and gold(III) complex **3a** (0.01 mmol) in water (0.6 mL) at 50 °C for 1 h gave propargylamine **4a** in 6% isolated yield (ESI†). Under the same reaction conditions, no propargylamine formation for acetaldehyde, isobutyraldehyde, benzaldehyde, and acetone was detected by ESI-MS analysis of the reaction mixtures (ESI†). These findings indicated the excellent formaldehyde selectivity of sterically bulky 2,2,6,6-tetramethyl-piperidine **1a**.



Scheme 2. Seletivity study of A^3-coupling reaction with different carbonyl-containing compounds.

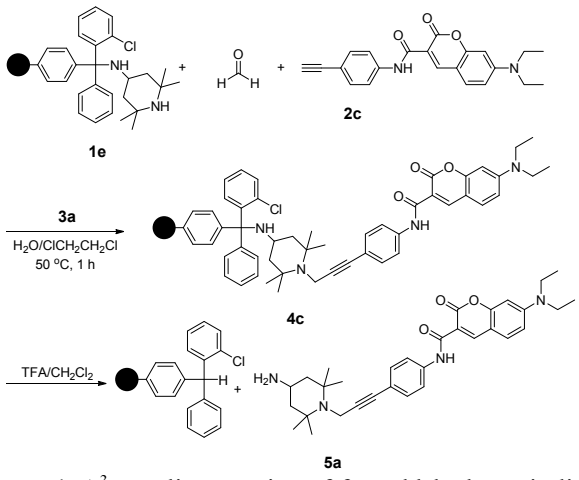
After identifying the sterically bulky amine component for exclusive formaldehyde selectivity, we studied the effects of different reaction parameters on the formation of propargylamine **4b** by HPLC analysis using formaldehyde, amine **1b**, and alkyne **2b** (Scheme 3). A calibration curve of propargylamine **4b** with excellent linearity (R^2 = 0.9996) was constructed for quantification (Figure S2, ESI†).



Scheme 3. A^3-coupling reaction of formaldehyde with amines and alkynes catalyzed by different gold(III) complexes.

Three cyclometallated gold(III) complexes **3a-c** commonly used by our research group[8d-e] were screened to study their catalytic activities on the formation of propargylamine **4b** (Scheme 3) (Table S2, ESI†). Bis-cyclometallated gold(III) complex **3a** gave the highest yield among the three catalysts that is in line with our previous work.[8e] In addition, we studied the effects of different solvents, solvent ratio, temperature and amounts of formaldehyde, amine **1b**, alkyne **2b** and bis-cyclometallated gold(III) complex **3a** on the yield of propargylamine **4b** (Tables S3-S6, ESI†). Up to 56% yield could be achieved at 50 °C in 1 h using formaldehyde (10 μmol), amine **1b** (50 μmol), alkyne **2b** (100 μmol), and gold(III) complex **3a** (50 μmol) in 1,2-dichloroethane (450 μL).

To achieve fluorescent detection of formaldehyde, we decided to attach a fluorophore to the alkyne component. The amount of formaldehyde can then be determined by measuring the fluorescent intensity of the resulting propargylamines. On the other hand, to minimize the background fluorescent signal coming from the unreacted fluorescent alkynes, excess reagents have to be removed after the A^3-coupling reaction. To facilitate the washing step, the sterically bulky amines were attached to polymer-bound 2-chlorotrityl chloride resins to give resin-linked sterically bulky amine **1e** according to literature.[9] The A^3-coupling reaction of formaldehyde, resin-linked sterically bulky amine **1e**, and coumarin-linked alkyne **2c** gave fluorescent propargylamines (Scheme 4).



Scheme 4. A^3-coupling reaction of formaldehyde, resin-linked sterically bulky amine **1e** and coumarin-linked alkyne **2c** via propargylamine formation and cleavage of propargylamine **5a** from the resin **4c**.

The A^3-coupling reactions of formaldehyde (0.02 mmol), resin-linked sterically bulky amine **1e** (15 mg), coumarin-linked alkyne **2c** (0.02 mmol) and bis-cyclometallated gold(III) catalyst **3a** (0.01 mmol) were conducted in 1,2-dichloroethane at 50 °C for 1 h to give resin-bound propargylamine **4c** (Scheme 4). To confirm the product formation, propargylamine **5a** was cleaved from the respective resins by treatment with TFA/CH_2Cl_2[9] and confirmed by ESI-MS analysis (ESI†). Upon excitation at 355 nm, propargylamine **5a** exhibited a maximum emission wavelength at 461 nm that is consistent with a coumarin chromophore (Figure 1A).

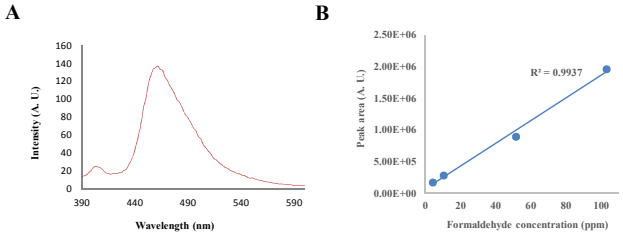


Figure 1. (A) Fluorescent spectrum of propargylamine **5a**; (B) A good linearity (R^2 = 0.9937) between formaldehyde concentration (4-103 ppm) and the peak area of the propargylamine **5a** determined by LC–MS/MS.

LC–MS/MS was used to determine the amount of the propargylamine **5a**. The peak area, which represented the amount of propargylamine **5a**, increased with the concentration of formaldehyde (from 4 to 103 ppm) (Figure 1B). A good linear relationship (R^2 = 0.9937) between the peak area and formaldehyde concentrations was observed, suggesting the readout from the LC–MS/MS can be used for quantification.

Under irradiation by a hand-held UV lamp at 365 nm, the brightness of the resin-bound propargylamine **4c** was related to the formaldehyde concentrations. The observable change of the brightness could be straightforwardly differentiated by naked eyes to qualitatively detect the formaldehyde concentration as low as 10 ppm (Figure 2).

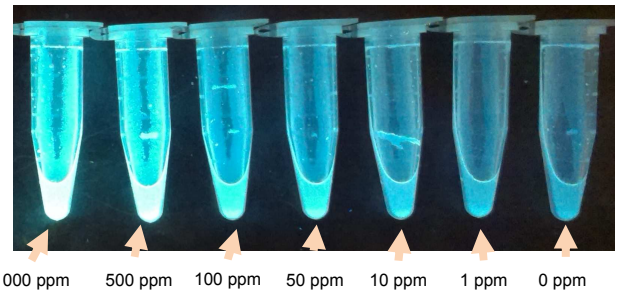


Figure 2. Resin-bound propargylamine **4c** in dichloromethane under irradiation of UV lamp at 365 nm.

The selectivity experiments were performed by using resin-linked sterically bulky amine **1e** and coumarin-linked alkyne **2c** with acetaldehyde (250 μmol, ~18,400 ppm), isobutyraldehyde (250 μmol, ~30,000 ppm) and benzaldehyde (125 μmol, ~26,500 ppm), respectively (ESI†). No propargylamine was detected after cleavage of the resins by ESI-MS analysis. As a competitive experiment, only the corresponding propargylamine **5a** derived from formaldehyde was detected by ESI-MS analysis in the A^3-coupling reaction of a mixture of 5,000 ppm of each of formaldehyde, acetaldehyde, isobutyraldehyde, and benzaldehyde. These experiments indicated the excellent formaldehyde selectivity of this method.

To study the applicability of this method for formaldehyde detection in food, crude water extract of dried Shiitake mushroom was used. It was found that formaldehyde in the mushroom water extract could be exclusively captured by the resin-linked sterically bulky amine **1e** after reaction at 50 °C for 1 h as confirmed by the detection of propargylamine **5a** in ESI-MS analysis (ESI†). In this connection, the A^3-coupling reaction is of excellent tolerance towards complex matrix in food samples.

Conclusion

In summary, a visual detection method for formaldehyde based on a gold(III) complex-mediated three-component coupling reaction of formaldehyde, amines and alkynes has been developed. No expensive instruments and sophisticated operation skills are required with fast reaction time of 1 h at 50 °C. Exclusive formaldehyde selectivity was achieved by using resin-linked sterically bulky amines. Signal readout can be achieved by using LC–MS/MS analysis or naked eyes. Excellent tolerance in complex reaction mixtures has been demonstrated by formaldehyde detection of crude mushroom water extracts. This highly selective method opens up a new direction for the development of rapid on-site detection of formaldehyde with diverse applications.

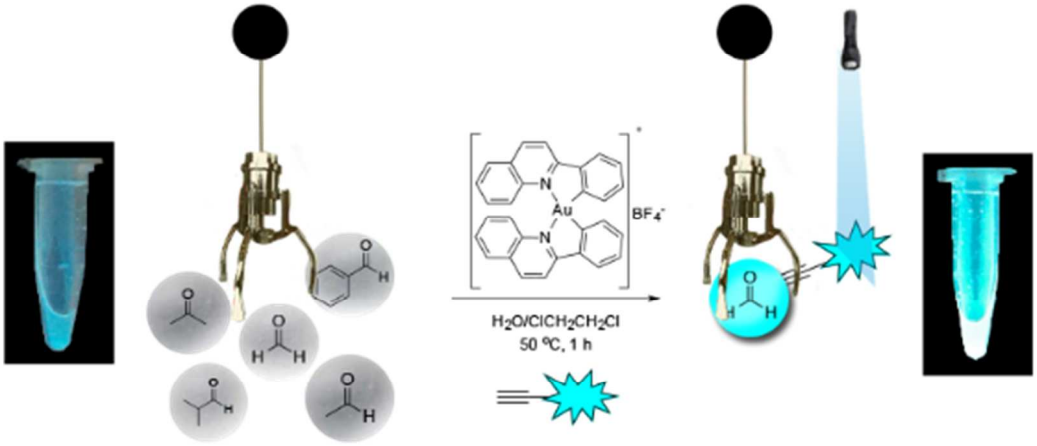
This work was supported by Guangzhou Science and Technology Fund (12S496140081), Hong Kong Research Grants Council (PolyU 5031/11p), State Key Laboratory of Chirosciences, and The Hong Kong Polytechnic University (SEG PolyU01).

Notes and references

[a] *Food Safety and Technology Research Centre, State Key Laboratory of Chirosciences, and Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Hong Kong, China. Fax: +852-2364-9932; Email: mankin.wong@polyu.edu.hk*
[b] *Guangdong Inspection and Quarantine Technology Center, Guangdong Entry-Exit Inspection and Quarantine Bureau, Tower B, 66 Huacheng Avenue, Zhujiang Xincheng, Guangzhou, China. +86-20-3829-0537; Email: xiangdp@iqtc.cn.*

† Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/c000000x/

1 (*a*) T. Salthammer, S. Mentese and R. Marutzky, *Chem. Rev.*, 2010, **110**, 2536–2572; (*b*) T. Salthammer, *Angew. Chem., Int. Ed.*, 2013, **52**, 3320–3327.
2 (*a*) P. Restani and C. L. Galli, *Crit. Rev. Toxicol.*, 1991, **21**, 315–328; (*b*) IRAC, *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, 2006, **88**, 39–325; (*c*) X. J. Tang, Y. Bai, A. Duong, M. T. Smith, L. Y. Li and L. P. Zhang, *Environ. Int.*, 2009, **35**, 1210–1224.
3 (*a*) F. Bianchi, M. Careri, M. Musci and A. Mangia, *Food Chem.*, 2007, **100**, 1049–1053; (*b*) T. S. Yeh, T. C. Lin, C. C. Chen and H. M. Wen, *J. Food Drug Anal.*, 2013, **21**, 190–197.
4 (*a*) D. J. Mason, M. D. Sykes, S. W. Panton and E. H. Rippon, *Food Addit. Contam.*, 2004, **21**, 1071–1082; (*b*) J. F. Liu, J. F. Peng, Y. G. Chi and G. B. Jiang, *Talanta*, 2005, **65**, 705–709.
5 (*a*) G. J. Mohr, *Anal. Bioanal. Chem.*, 2006, **386**, 1201–1214; (*b*) A. Allouch, M. Guglielmino, P. Bernhardt, C. A. Serra, and S. Le Calvé, *Sens. Actuators, B*, 2013, **181**, 551–558; (*c*) L. Feng, C. J. Musto and K. S. Suslick, *J. Am. Chem. Soc.*, 2010, **132**, 4046–4047; (*d*) J. Y. Kim, J. Lee, S. Hong, and T. D. Chung, *Chem. Commun.*, 2011, **47**, 2892–2894; (*e*) J. B. Zeng, S. G. Fan, C. Y. Zhao, Q. R. Wang, T. Y. Zhou, X. Chen, Z. F. Yan, Y. P. Li W. Xing and X. D. Wang, *Chem. Commun.*, 2014, **50**, 8121–8123; (*f*) Y. Yu, X. M. Zhang, J. P. Ma, Q. K. Liu, P. Wang and Y. B. Dong, *Chem. Commun.*, 2014, **50**, 1444–1446.
6 (*a*) T. Nash, *Biochem. J.* 1953, **55**, 416; (*b*) H. Tsuchiya, S. Ohtani, K. Yamada, M. Akagiri, N. Takagi and M. Sato, *Analyst*, 1994, **119**, 1413–1416; (*c*) Y. Suzuki, N. Nakano, and K. Suzuki, *Environ. Sci. Technol.*, 2003, **37**, 5695–5700; (*d*) X. Q. Zhao and Z. Q. Zhang, *Talanta*, 2009, **80**, 242–245; (*e*) X. Weng, C. H. Chon, H. Jiang, and D. Li, *Food Chem*, 2009, **114**, 1079–1082.
7 (*a*) C. Wei and C. J. Li, *J. Am. Chem. Soc.*, 2003, **125**, 9584–9585; (*b*) W. J. Yoo, L. Zhao and C. J. Li, *Aldrichimica Acta*, 2011, **44**, 43–51.
8 (*a*) V. K. Y. Lo, Y. G. Liu, M. K. Wong and C. M. Che, *Org. Lett.*, 2006, **8**, 1529–1532; (*b*) V. K. Y. Lo, K. K. Y. Kung, M. K. Wong and C. M. Che, *J. Organomet. Chem.*, 2009, **694**, 583–591; (*c*) K. K. Y. Kung, G. L. Li, L. Zou, H. C. Chong, Y. C. Leung, K. H. Wong, V. K. Y. Lo, C. M. Che and M. K. Wong, *Org. Biomol. Chem.*, 2012, **10**, 925–930; (*d*) K. K. Y. Kung, V. K. Y. Lo, H. M. Ko, G. L. Li, P. Y. Chan, K. C. Leung, Z. Zhou, M. Z. Wang, C. M. Che and M. K. Wong, *Adv. Synth. Catal.*, 2013, **355**, 2055–2070; (*e*) H. M. Ko, K. K. Y. Kung, J. F. Cui and M. K. Wong, *Chem. Commun.*, 2013, **49**, 8869–8871.
9 J. J. McNally, M. A. Youngman and S. L. Dax, *Tetrahedron Lett.*, 1998, **39**, 967–970.



Visual fluorescent detection of formaldehyde by a highly selective gold(III) complex-mediated three component coupling reaction